

# All faeces are not equal:

## microbial source tracking as a health protection tool

● Testing our water supplies and recreational waters for faecal indicator organisms has made a significant contribution to preventing infectious human diseases. More recently, scientists have developed techniques that not only demonstrate the presence of faecal matter, but also suggest whether the source of contamination is human or non-human, a factor of potentially considerable health significance. **HUW TAYLOR** and **JAMES EBDON** review recent developments.

**John Snow's observation in the early 1850s that a cluster of cholera victims in the Soho district of London obtained their drinking water from a particular pump in Broad Street did not immediately convince the health practitioners of the time that the disease could be water-borne. However, the impact of his community observations, combined with the findings of the early microbiologists (such as Robert Koch and Louis Pasteur), led most in society to dismiss finally the 'miasma theory' of disease transmission and to appreciate that protection of our drinking water sources was fundamental to protecting human health.**

In the early 21st century, a safe supply of clean drinking water is considered a basic human right, but it is one as yet denied to one sixth of the

world's population. In a world in which approximately 2.2 million people die each year from diarrhoea associated with lack of access to safe drinking water, inadequate sanitation and poor hygiene, water microbiology is by no means a redundant science and epidemiology has not become an academic backwater.

### Reducing water-borne disease

So how do we decide whether drinking a glass of water or swimming at our local bathing beach will not cause us the inconvenience of a few days of diarrhoea and sickness, or much worse? We could test these waters for the pathogenic organisms that are known to make us ill, but which of the several hundred should we test for, and if these organisms are not detected can we conclude that the water is indeed safe?

Since the early days of the 20th century, the provision of reliable and

acceptably safe drinking water (and more recently recreational waters) has depended on regular monitoring for faecal indicator organisms (FIOs). The ideal FIO would be easily and safely enumerated, be of strictly faecal origin, and would exist in greater numbers than any disease-causing organisms present. In practice, of the many candidate microbial groups, the most commonly used FIO are the faecal coliforms (the majority of which, on further analysis, are usually found to be members of the bacterial species *Escherichia coli*).

We should not underestimate the benefits to human health of monitoring *E. coli* in water, even though levels of this organism in drinking and recreational waters have rarely demonstrated a strong linear correlation with levels of proven gastro-intestinal disease. The bottom-line is, perhaps, that a drinking water in which a bacterium of normally faecal origin is not detected, or is at least detected at very low levels, is better than one in which it is detected at high levels.

Preventing faecal material from entering our water supplies and recreational waters has remained a key concern of the global water industry. But are some faecal sources more dangerous than others and could the ability to distinguish between them further protect us from disease? The answer must be yes, and recent research has produced methods that may help us make the distinction. In this article we suggest that the newly emerging science of microbial source tracking may improve microbial risk management and thereby reduce human contact with pathogens throughout the water cycle.

### Microbial source tracking

Why distinguish between human and non-human faeces? Disease-causing microorganisms exist in a complex and as yet little-understood symbiosis with their host. Certain microbes have gained evolutionary advantages by their specific association with a human host: as a result, human faeces are more likely than those of other animals to contain the organisms that make us ill.

This is one reason why greater investment has been made into

### Case study: Health effects of the 'Bosccastle flood', Cornwall, England, 2004

Television images of cars being carried on flood waters through the village of Bosccastle following sudden torrential rainfall in August 2004 led to fears of mortality from drowning amongst villagers and holiday-makers in the Cornish village.

Thankfully, these fears were unfounded, but this extreme weather event did lead to serious ill health in seven young children. All had played in a beach stream at nearby Watergate bay. Investigations demonstrated the causative agent of disease to be *Escherichia coli* O157. A rapidly convened outbreak investigation unsurprisingly demonstrated the presence of *E. coli* O157 in the faeces of cattle from the local river catchment.

The flood event had clearly led to rapid overland flow of cattle faeces from pastures into surface waters. A hope is that microbial source tracking and improved river catchment management may allow prediction of such pollution 'hotspots'. A better understanding of where and when faecal pollution may present a significant risk to water resources and recreational water users, and an indication of its source, would support improved water resource management and could at least provide accurately focused warnings of water-borne health hazards.



(Pic: Reuters / Martin Bennett ASA)

diverting flows of human excrement away from areas of human habitation than the non-human excrement produced in massive quantities by modern agricultural practices. In parts of the world in recent years, point sources of municipal wastewaters containing human excrement have routinely undergone biological treatment prior to discharge. More thorough and effective municipal wastewater treatment has led to cleaner rivers and bathing beaches, but in some cases the improvements to water quality have been less than had been predicted.

This is often because the contribution to water pollution by the faeces of reared and wild animals has been underestimated. The parasites, bacteria and viruses found in diffuse runoff from agriculture may, overall, pose a relatively lower risk to human health than untreated municipal wastewaters but they still present an often unquantified hazard because of the presence of zoonotic microorganisms.

These can cause disease in humans but are more usually found to cause disease in (or to at least co-exist with) other non-human animals. Examples of recent concern include *Cryptosporidium parvum*, an organism that is potentially lethal if swallowed in sufficient numbers by immunosuppressed humans; and a toxigenic strain of *E. coli* known as O157:H7, which can cause renal failure, especially among vulnerable groups such as the elderly and young children.

Therefore, if we know the predominant source of faecal pollution in a water body used for water supply or recreation, we can better predict the potential impacts on human health. We may also find that the solution to the problem is improved farm management practices rather than improved treatment of municipal wastewaters.

An early approach to distinguishing human from non-human faecal material was suggested by Geldreich and Kenner (1969). It was observed that the two commonly-used groups of FIO, namely faecal coliforms and faecal streptococci, were present at significantly different ratios in the faeces of humans and grazing animals. However, the use of the measured ratio of these indicator organisms in water samples was found to be limited by markedly different survival patterns of the two groups, so the approach was only of use in very recently contaminated waters.

There are many approaches to what has come to be known as microbial source tracking (MST): a recent detailed review of the topic is given by Santo-Domingo and Sadowsky (2007).

## Examples of source identifiers and focus of analysis used in MST studies (adapted from Harwood, 2007)

Source identifier	Species, pattern or marker (SPM)
<i>E. coli</i>	Antibiotic resistance pattern Ribotype Rep-PCR pattern (including BOX-PCR) PFGE pattern RAPD pattern
Genus <i>Enterococcus</i>	Antibiotic resistance pattern Ribotype Rep-PCR pattern (including BOX-PCR) <i>esp</i> gene (enterococcal surface protein)
<i>Bacteroidetes</i>	16S rRNA gene
<i>Bifidobacterium spp.</i>	16S rRNA gene (PCR) of <i>B. adolescentis</i> and <i>B. dentium</i>
<i>Campylobacter spp.</i>	RAPD pattern
<i>Salmonella spp.</i>	PFGE pattern
<i>Listeria monocytogenes</i>	Virulence genes and ribotype
F-specific RNA coliphage	Serotype Genotype
Enterovirus	Reverse transcriptase PCR
Adenovirus	Nested PCR
Human polyomavirus	PCR
<i>Saccharomyces cerevisiae</i>	RAPD pattern and microsatellite analysis
PFGE - pulsed-field gel electrophoresis	
RAPD - randomly amplified polymorphic DNA	
PCR - polymerase chain reaction	

Approaches are commonly classified as either library-dependent methods, where the library is a database of characteristics such as genetic fingerprint or antibiotic resistance profiles, or library-independent methods where host-specific characteristics are studied. The table (adapted from Harwood, 2007) lists many of the candidate organisms studied to date with brief details of some of the microbial techniques used and characteristics investigated.

Until recently, library-dependent methods have been the most widely used despite the limitations of temporal and spatial instability of libraries. However, initial comparative studies in the US (Griffiths et al, 2003) and Europe (Blanch et al, 2006) have demonstrated the benefits of library-independent approaches. These studies are by no means definitive, as new MST methods continue to emerge and protocols for effective and accurate comparison of methods continue to develop.

One particularly promising approach is the use of host-specific molecular markers in the *Bacteroides*-*Prevotella* group of gut bacteria (Bernard and Field, 2000). This method does not require culturing and, through the use of quantitative polymerase chain reaction (QPCR) methods, it potentially offers a precise and rapid way to apportion contributions to faecal contamination from a wide variety of human and non-human sources.

While survival of *Bacteroides* species in the environment is relatively poor,

these bacteria have been shown to release bacteriophages in great quantities in the environment. Testing for the presence of these phages in environmental samples by demonstrating their attack on a strain of *Bacteroides* that demonstrates host specificity is another promising and fairly straightforward procedure (Payan et al, 2006). As with some other methods, geographical differences have prevented the universal application of this approach. However, a more recently isolated strain of *Bacteroides* has consistently distinguished human faecal contamination from non-human contamination in Europe (Ebdon et al, 2007) and it is currently being evaluated for use in the US.

### Concluding remarks

Effective microbial source tracking has wide potential applications. The Walkerton Inquiry (O'Connor, 2002), which followed a serious outbreak of infection by *E. coli* O157, demonstrated that assuring drinking water safety is an exercise in risk management and that preventive rather than reactive interventions are necessary. Similarly, the WHO Guidelines for drinking water quality stress the need for adequate risk assessment and management through Water Safety Plans (WHO, 2003).

In the US, MST may have a role to play in helping environmental resource managers to comply with Total Maximum Daily Load (TMDL) regulations required by the US EPA, whereas in Europe its most obvious

current application is in the development of the 'bathing water profiles' required under the revised EU Bathing Water Directive (EC, 2006). In both these applications MST tools are most effective when combined with established tools of quantitative hydrology and GIS.

At a time of predicted climate change, there is still much to discover as to how environmental changes interact with human behaviour to influence the burden of water-related human disease. Microbial source tracking and quantitative microbial risk assessment are, as yet, newly-emerging areas of research, but if methods and protocols can be improved to provide reliable information on pollution sources and health risk, they should one day provide useful tools for the management of infectious disease. Research strives for a simple, low-cost approach that provides quantitative 'source apportionment' in all instances. We may not as yet have achieved this, but we are, at least, starting to ask the right questions. ●

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