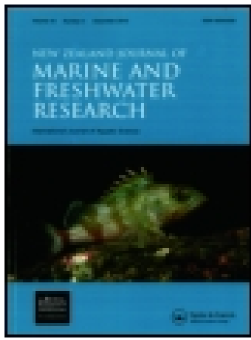

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Review

Distinguishing human from animal faecal contamination in water: a review

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Abstract Management of faecal contamination of water would be improved if sources could be accurately identified through water analysis. Human faeces are generally perceived as constituting a greater human health risk than animal faeces, but reliable epidemiological evidence is lacking. United States waterborne disease data suggest that human-specific enteric viruses account for over half the documented outbreaks. However, in New Zealand, where there is a high grazing animal:human ratio (increasing the relative importance of water-transmissible zoonoses), it seems prudent to assume that human and animal faecal pollution both constitute a risk to human health. Irrespective of the relative risks, the ability to identify sources would assist in overall management of microbial water quality. Faecal streptococci do not appear to provide reliable faecal source identification. Human and animal sources, respectively, may be distinguishable by two tests on *Bifidobacterium* spp.—growth at 45°C in trypticase phytone yeast broth and sorbitol fermentation. Different species of *Bacteroides* tend to be present in humans and animals, but poor survival in water is a problem. Phages of the *Bacteroides fragilis* strain HSP40 appear to be human specific, but low counts in effluent in some countries, including New Zealand, may limit their usefulness. Different F-RNA phage subgroups appear to be associated with human and animal faecal sources. The actinomycete *Rhodococcus coprophilus* has potential as a grazing animal indicator but it is persistent, and existing culturing techniques are time consuming. The development of DNA-based techniques, such as polymerase chain reaction (PCR), may assist in the assay of some microbial faecal source indicators. Various faecal sterol isomers offer the possibility of distinguishing between human and animal sources, and even between different animals. Washing powder constituents such as fluorescent whitening agents, sodium tripolyphosphate and linear alkyl benzenes, offer useful human source identifiers. It is unlikely that any single determinand will be useful in all situations, but statistical analysis of appropriate "baskets" of microbial and chemical determinands offers the possibility of identifying and apportioning human and animal faecal inputs to natural waters.

Keywords faecal source identifiers; faecal streptococci; bifidobacteria; *Bacteroides fragilis*; bacteriophages; *Rhodococcus coprophilus*; faecal sterols; fluorescent whitening agents; sodium tripolyphosphate; linear alkyl benzenes

INTRODUCTION

Faecal contamination of aquatic environments can degrade them for uses such as contact recreation, shellfishing, and drinking water supply, principally because of the possible introduction of pathogenic micro-organisms. Management and mitigation of this form of pollution would be more cost-effective if the correct sources could be identified and apportioned.

An important factor behind the need for water managers to identify faecal sources is the perception that there is a higher health risk associated with

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human exposure to waters polluted with human, as opposed to animal, faeces. This issue assumes particular significance in New Zealand, where there is a high grazing animal:human ratio, and where animal processing industries are often subject to similar discharge conditions as those imposed for urban sewage. Thus, the first part of this review briefly examines the issue of the relative health risks associated with human and animal faecal material. However, it is important to note that, irrespective of the relative risks involved, improved methods for identifying and apportioning faecal sources would assist water managers in lowering overall faecal pollution levels.

Often, the probable sources of faecal inputs (e.g., sewer outfalls, stormwater drains, or run-off from grazed pasture) can be identified from geographical surveys. However, this approach will be unsatisfactory in some situations, particularly where it is important to quantify or apportion the inputs. For example, small rural communities, or semi-rural areas on the outskirts of large cities, will frequently contain a wide variety of potential faecal sources, including grazing animals, animal containment areas, and small, scattered sewage treatment and disposal systems, often serving single households.

Faecal source identification is particularly difficult for contaminated groundwater. In New Zealand, groundwater quality surveys of some unsewered communities have shown that up to 33% of household drinking water bores contain faecal bacteria (Sinton 1982; Ayrey & Noonan 1983). Although septic tank effluent was the suspected source of this contamination, animal sources could not be discounted because of uncertainties over groundwater flow directions and velocities. In these situations, source identification through water analysis would offer considerable advantages.

Unfortunately, the most commonly used faecal indicator micro-organisms—coliforms, faecal coliforms, *Escherichia coli*, and (in recreational waters) enterococci—are found in both human and animal faeces, and don't allow sources to be differentiated. Thus, since the early 1900s a wide variety of both microbial and chemical approaches to faecal source identification have been investigated. In the second and third parts of this review, we review the main microbial and chemical determinands that have been suggested as potential faecal source indicators. The literature is specifically reviewed from a New Zealand perspective, but much of the information should have wider relevance.

In addition to the factors which render the microbial and chemical indicators specific to their human or animal origins, their utility is obviously also strongly dependent on the same set of factors which determine their value as general faecal indicators (i.e., irrespective of source)—ease of assay, concentrations or counts in faeces and sewage, and rates of disappearance in receiving waters. Thus, for each of the potential faecal source indicators, we have briefly reviewed methodologies, levels in faeces, sewage, and natural waters, and rates of degradation or inactivation in receiving waters, as well as faecal source specificity.

RELATIVE HEALTH RISKS ASSOCIATED WITH HUMAN AND ANIMAL FAECAL SOURCES

Epidemiological evidence

Although there have been some controlled studies of waterborne disease transmission via tap waters (e.g., Payment 1996), most epidemiological evidence appears to have been derived from studies carried out in recreational waters. The illness risk associated with bathing in water polluted primarily with human faecal material has been investigated in studies in the United States (Cabelli 1983; Dufour 1984), Canada (Seyfried et al. 1985a,b), Israel (Fattal et al. 1983, 1986, 1987), Egypt (El-Sharkawi & Hassan 1982; Cabelli 1983), Spain (Mujeriego et al. 1982), France (Foulon et al. 1983; Ferley et al. 1989); the United Kingdom (Brown et al. 1987; Jones et al. 1989, 1991), Hong Kong (Holmes 1989), and Australia (Corbett et al. 1993; Harrington et al. 1993). Most of these studies showed a positive correlation between gastrointestinal illness and faecal indicator density, although some (e.g., Corbett et al. 1993) showed that increasing counts were associated more with respiratory and other symptoms.

However, there is little equivalent evidence from waters polluted primarily with animal faeces. Animal inputs were likely in the waters studied by Ferley et al. (1989) and Holmes (1989), but the animal-associated results would not have been distinguishable from those associated with the human inputs. It appears that the only study specifically designed to address swimming-associated illness in animal-impacted waters was that of Calderon et al. (1991). They found no association between gastrointestinal illness and faecal indicator bacteria densities (i.e., suggesting no impact on human health from animal

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inputs). However, the study catchment was largely impacted by non-domestic animals, such as squirrels, rabbits, and wild deer. In addition, McBride (1993) questioned some of the statistical interpretations in this study.

In an epidemiological survey of New Zealand marine beaches, Bandaranayake et al. (1995) could find no "substantial difference" in illness risks between two "rural" and two oxidation pond-impacted beaches. However, bacterial counts in this survey were low overall, and there was a general lack of statistical significance between all the indicators and most illness types (gastrointestinal and respiratory) in each of four microbial water quality categories ("quartiles"). Of eight statistically significant indicator/illness associations, only one (between enterococci and respiratory illness) was regarded as valid by the authors. The other seven (involving faecal coliforms or *E. coli*) were described as "anomalous", because of a lack of correspondence with microbial counts in the associated quartiles. In addition, human inputs to the rural beaches were possible, and there appeared to be substantial risk differences between the rural and "control" beaches, even though the microbial water quality was similar.

In the absence of reliable epidemiological evidence, it is necessary to seek information from two other sources—waterborne disease incidence data, and the likely incidence of zoonoses in New Zealand. Unfortunately, this exercise also provides conflicting information.

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Incidence of waterborne disease

There is no reliable documentation of water-transmissible disease in New Zealand. However, the Ministry of Health (MoH 1995) produced a list of water transmissible pathogens they considered to be potential problems in New Zealand drinking waters. Bacterial pathogens included: (1) *Campylobacter* spp.—an important enteric pathogen in New Zealand (Lane & Baker 1993), which has been linked with New Zealand waterborne disease outbreaks (Briesman 1994); (2) *Salmonella* spp.—although isolated from New Zealand waters, there are no published accounts of waterborne outbreaks in this country; (3) *E. coli* — although some strains of this organism cause enteric infections (Levine 1987), there are no data on their prevalence in New Zealand; (4) *Shigella* spp.—there are, as yet, no reports of water transmission in New Zealand, where the incidence is low; (5) *Vibrio* spp.—there appears to be no published information on isolation of vibrios from New Zealand waters, and all reported instances of *V. cholerae* (which causes cholera) in New Zealand have been imported; and (6) *Yersinia* spp.—there is a growing awareness of the importance of *Yersinia* spp. as a source of gastroenteritis in New Zealand, but there appears to be no published information on incidence in New Zealand waters.

Protozoans in the MoH (1995) list include: (1) *Cryptosporidium parvum*—although periodic isolations of *Cryptosporidium* spp. and instances of cryptosporidiosis have been reported (Carter 1984, 1986), there is limited information on incidence in New Zealand, and (2) *Giardia intestinalis* (*lamblia*)—according to MoH (1995), cases of giardiasis are frequent in some parts of New Zealand (three outbreaks have been reported), and some water surveys have returned high proportions of positive samples (Brown et al. 1992).

The principal viral groups reported as significant waterborne pathogens overseas are also listed by MoH (1995) as potential problems in New Zealand—the adenoviruses, rotaviruses, Norwalk virus, hepatitis A, astroviruses, and enteroviruses. However, only the enteroviruses appear to have been reported in New Zealand effluents, receiving waters and sediments (e.g., Lewis et al. 1986), primarily because of the methodological difficulties associated with the detection and enumeration of the other types in environmental samples.

The above list of pathogens is similar to that compiled by Craun (1991) from North American data. In the United States, from 1971 to 1988, the most important waterborne bacterial pathogens were *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., and *Yersinia* spp., although enterohaemorrhagic *E. coli* 0157:H7 was the cause of a possible waterborne outbreak in Missouri. Also among the identified agents were the protozoans *Giardia* spp. and *Cryptosporidium* spp. Most of the illnesses identified as having a viral aetiology were described only as viral gastroenteritis, although hepatitis A was separately identified. Significantly, about half of United States waterborne outbreaks tend to be of unknown aetiology, although Rao & Melnick (1986) suggest that most of these are probably viral in origin. About 44% of the United States outbreaks reported by Craun (1991) were associated with contaminated groundwater.

Although the above United States findings do not define the relative risks associated with human and animal sources, some information may be deduced from them. First, a large proportion (probably more

than half) of the illnesses are likely to be of viral origin. Enteric viruses tend to be highly host specific, which suggests that a large proportion of the water-transmitted illnesses documented by Craun (1991) were of human origin. Of the remaining (mostly bacterial and protozoan) agents, less can be deduced about their sources because of the possibility of zoonotic infections.

Zoonoses

The strict definition of a zoonose is an organism which causes disease in both man and animals. However, the term is becoming more loosely applied, and now often includes those organisms which are commensal in, or in passage through, the animal gut. For the purposes of this review, we will adopt the wider definition and define a zoonose as any organism which can be harboured in animals (where it may or may not cause disease), and can be transmitted to humans, where it can cause disease.

The incidence of zoonoses in New Zealand has been comprehensively reviewed by Blackmore & Humble (1987), and a review of the zoonoses associated with pigs in this country has been conducted by Fairley (1996). Of the more than 150 zoonoses recorded throughout the world, less than 30 have been reported in New Zealand. However, because the number of domestic stock per unit of population in New Zealand is very high, the few endemic zoonoses may create a greater hazard than in other countries where these diseases occur.

Blackmore & Humble (1987) list 2 viral, 11 bacterial, 1 mycotic, 2 protozoan, and 9 helminthic zoonoses, plus 4 zoonotic (arthropod) ectoparasites. *Giardia* sp. (which comes within the definition of a zoonose in the present review) is listed as a non-zoonotic disease common to both man and animals. Another important pathogen listed as a non-zoonotic infection by these authors is *Listeria monocytogenes*. In this review, we will consider that five micro-organisms in the Blackmore & Humble review are potentially important water-transmissible pathogens. These are certain serotypes of the bacteria, *Campylobacter jejuni*, *Salmonella typhimurium*, and *Yersinia enterocolitica*, and the protozoans *Cryptosporidium* sp. and *Giardia* sp.

As noted above, there is a high incidence of campylobacteriosis in humans in New Zealand (Lane & Baker 1993), and *C. jejuni* is a common intestinal infection in a wide range of domestic stock, poultry, and wildlife. However, the incidence of animal to human transfer is unknown. The salmonellae are also ubiquitous organisms, causing infections in most terrestrial vertebrates, and asymptomatic infections in 1–5% of sheep and cattle in New Zealand (Blackmore & Humble 1987). Although this theoretically means that the potential reservoir in New Zealand is enormous, the number of salmonellae passed in the faeces of these carriers is far less than those of clinically affected animals, which means that carrier animals are not necessarily a significant risk (Blackmore & Humble 1987). *Yersinia* spp. are frequently isolated from the intestinal tract of healthy animals and birds, and from the environment. Intestinal prevalence rates of up to 30% have been demonstrated in clinically normal cattle, sheep, and deer in New Zealand, and cattle can remain infected for more than 7 months. Clinical infections are more common in young animals and deer (Blackmore & Humble 1987).

Although studies have also shown the presence of *Giardia* sp. cysts in a wide range of New Zealand domestic and wild animals, including cattle, horses, sheep, and birds (Marino et al. 1991; Tonks et al. 1991), it has not yet been demonstrated that these are human strains (Brown 1993). However, there is strong evidence of animal to human transmission of *Giardia* sp. from North America (Faubert 1988), and it would seem prudent to assume that this form of transmission can also occur in New Zealand. *Cryptosporidium* sp. causes infection (cryptosporidiosis) in humans and animals. It is found in a wide variety of animals, including cattle, sheep, goats, deer, pigs, horses, dogs, cats, rodents, and poultry. Infection in humans can be life-threatening (Smith 1993), but in most animals it is usually inapparent. All ages of animals may show clinical signs of disease, but younger animals, particularly calves, are most often affected, showing classic enteritis symptoms. Worldwide, animals appear to be regarded as an important reservoir for *Cryptosporidium* sp. (Carrington & Smith 1995), and it seems reasonable to assume that this situation also occurs in New Zealand.

In the spring of 1996 there were 70 million sheep/lambs and 9.2 million beef/dairy cattle/calves (Anon. 1996). Based on conservative faecal excretion rates (R. McFarlane, Lincoln University pers. comm.), an annual deposition on New Zealand pastures of up to 20×10^6 t of sheep faeces and up to 25×10^6 t of cattle faeces could occur. The entry of even a small proportion of this material into rural waterways would provide a high non-point source microbial load, and a potentially important reservoir for zoonotic pathogens.

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MICROBIAL INDICATORS OF FAECAL SOURCES

Introduction

A wide range of micro-organisms have been investigated as faecal source indicators. The idea of using microbes for this purpose was first suggested in the early 1900s. For example, Gordon (1905) reported that streptococci from human faeces fermented mannitol but not raffinose, whereas the opposite was found for isolates from cows and horses.

Investigations into the use of microbes for faecal source identification have tended to involve four basic approaches: (1) **speciation**, based on findings that particular species may be indicative of human or animal sources; (2) **biochemical reactions** (often linked to speciation), whereby simple biochemical tests have been claimed to differentiate sources; (3) **assemblages and ratios**, these have been considered useful in some situations; and (4) **DNA profiles**, genotype is considered by some authors to be more reliable than phenotypic biochemical reactions.

Each of the above approaches has attendant problems. Many micro-organisms have multiple hosts, and, not surprisingly, different enteric organisms exhibit similar biochemical responses to their environment. In addition, inter-species gene transfer occurs, so even genomic differentiations have to be treated with caution. Other limitations to some microbial indicators include cumbersome assay procedures, and low counts in the environment. Thus, many organisms proposed as faecal source identifiers have been found to be unreliable on subsequent investigation, whereas others continue to show promise. This review focuses on the latter group, but also includes the faecal streptococci, because they have been extensively investigated as source indicators. The following approaches are not discussed in detail in this review:

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Those based on DNA analysis systems

These include: (1) restriction endonuclease analysis (REA), which involves cleavage of DNA at specific sites and separation (usually by gel electrophoresis) of the resulting fragments; and (2) polymerase chain reaction (PCR), which involves the biochemical amplification of selected DNA gene sequences. For example, REA, in combination with a dichotomous key, has been used to differentiate between *E. coli* strains associated with different faecal inputs to estuarine waters in Virginia, United States (Simmons et al. 1995; Simmons pers. comm.). Applications of PCR, have included that of Turner et al. (1996), who considered that a randomly amplified polymorphic DNA (RAPD) marker, localised to the glycine decarboxylase gene (gcvP), showed promise as a molecular marker for *E. coli* isolates of human origin. DNA-based approaches show considerable promise for source identification, but we consider that this area of work is too new to allow a broad review. Nevertheless, DNA-based taxonomy and some specific applications of PCR are referred to in the discussion below.

Use of Pseudomonas aeruginosa

This organism is present in c. 11–16% of adult humans (Ringer & Drake 1952; Sutter et al. 1967; Hoadley 1977), but appears to be rarely recovered from the faeces of lower animals (Ringer & Drake 1952; Wheater et al. 1979). Cabelli et al. (1976) suggested that faecal coliform : *P. aeruginosa* ratios could be useful in determining the nature and proximity of faecal pollution in water. However, although the findings of Wheater et al. (1980) tended to support this theory, a number of factors significantly reduce the potential value of the organism as a source indicator. These include the rapid die-off of *P. aeruginosa* in aquatic environments (Hoadley 1977) which would diminish the value of the ratio approach, possible growth in warm, organically enriched waters (Hoadley 1968), and presence on insects, plants and in soils, which means that it is present in surface run-off following rainfall (Hoadley 1977). For these reasons, the organism appears to have received little attention as a source indicator in the last 10–15 years.

Use of human enteric viruses

These include enteroviruses, adenoviruses, and small round structured viruses (SRSVs), including the Norwalk virus. Because of a high degree of host specificity, these are theoretically human source indicators. However, they are intermittently present in human effluents (with the possible exception of enteroviruses), and the methodologies involved in their detection and enumeration tend to be costly and time consuming. In a field evaluation of a range of microbial faecal source indicators, Jagals et al. (1995) detected no enteric viruses, and concluded that better concentration and enumeration procedures would be required for their practical use as human faecal indicators. Similar considerations probably apply to animal viruses.

Miscellaneous approaches

These include the use of host (human or animal) DNA, from cells sloughed off the walls of the intestines during digestion and excretion. This approach does not appear to have been investigated in natural waters, probably because quantities of host DNA in these environments are likely to be very low. The eggs of helminths (intestinal worms) are a more likely research direction. Helminths tend to be host specific, are present in most animal herds, and shed large numbers of eggs. Although complex extraction and enumeration techniques exist for wastewaters (Ayres et al. 1989; Gaspard & Schwartzbrod 1995), typical counts in natural waters appear to be unknown, and they have not been systematically investigated as faecal source indicators. Again, PCR techniques, which open the possibility of more rapid assays, may make the use of heminth eggs feasible for faecal source identification, including the differentiation of different animal sources.

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Faecal streptococci

General

The faecal streptococci are probably the group of microbes most intensively investigated as faecal sources indicators. Their use in this role has been reviewed by a number of authors, including Geldreich & Kenner (1969), Clausen et al. (1977), and Sinton et al. (1993a,b).

The characteristics of the faecal streptococci have been summarised in Sinton et al. (1993a). The group is generally considered to comprise the enterococci—*Enterococcus faecium*, *E. faecalis*, *E. durans*, *E. avium*, *E. gallinarum* (these species have been placed in the genus *Enterococcus* largely on the basis of common biochemical characteristics)—together with two non-enterococci, *Streptococcus bovis* and *S. equinus*.

Methods for recovery and enumeration

The history of selective media development for faecal streptococci and enterococci is summarised in Sinton et al. (1993a). Media for the recovery and enumeration of faecal streptococci are usually based on their ability to grow in the presence of azide, and their fermentation of carbohydrates to produce lactic acid. Media for the recovery and enumeration of enterococci are usually based on the ability of the genus to hydrolyse the complex carbohydrate esculin in the presence of high concentrations of bile salts. The most commonly-used enterococcus enumeration method is probably the membrane filtration (MF) procedure recommended in Standard Methods (APHA 1995), which uses the mE-EIA media combination of Levin et al. (1975). Media have also been developed for the recovery of non-enterococci, particularly *S. bovis* (Switzer & Evans 1974; Oragui & Mara 1981, 1984).

Presence in faeces and effluents, and survival in water

Streptococcal concentrations in human faeces (at c. 10^6 per g) are generally less than faecal coliforms. In sewage, faecal streptococci tend to be present in concentrations 10–100 times less than faecal coliforms (Sinton et al. 1993b; Sinton & Donnison 1994). In contrast, faecal streptococci in animal faeces generally outnumber faecal coliforms—up to 10^7 per g in sheep faeces and 10^6 in cow faeces (Mara 1974; Sinton et al. 1993b; Sinton & Donnison 1994).

Faecal streptococcal counts in receiving waters are generally correlated with faecal coliform counts, although there is a shift in the ratio with time/distance from the faecal source (Sinton et al. 1994; Sinton & Donnison 1994). Although the inactivation of faecal streptococci in water has not been intensively investigated, most studies have indicated that they are more persistent than faecal coliforms in receiving waters (Evison & Tosti 1980; Fujioka & Narikawa 1982; Evison 1988). More recently, Davies-Colley et al. (1994) and Sinton et al. (1994) have shown that greater amounts of cumulative solar radiation are required to inactivate enterococci in seawater compared to faecal coliforms. Faecal streptococci also appear to survive longer than faecal coliforms in groundwater (Keswick et al. 1982; Bitton et al. 1983; Sinton & Donnison 1994).

Use as faecal source indicators

There have been three approaches to the use of faecal streptococci as faecal source indicators:

The faecal coliform : faecal streptococci (FC : FS) ratio

The FC : FS ratio has been reported as >4 in human faeces and <0.7 in animal faeces (Geldreich & Kenner 1969; Mara 1974; Feachem 1975). It is therefore theoretically possible to ascribe a human or animal source to faecal pollution based on the evidence of FC : FS ratios of >4 or <0.7. However, application of this ratio to environmental samples is accompanied by so many qualifications that the approach is now considered to be too unreliable to

be useful in characterising pollution sources (APHA 1995).

Species identification

The ratio of enterococci to other streptococci in faeces differs among vertebrate species. Human faeces and sewage are characterised by a predominance of enterococci, whereas animal sources also contain significant numbers of non-enterococci (Geldreich & Kenner 1969; Wheater et al. 1979; Sinton & Donnison 1994). *E. durans* and *S. bovis* were found to collectively comprise c. 50% of the faecal streptococci isolates from New Zealand sheep, and c. 75% of the isolates from cattle (Sinton & Donnison 1994). However, because enterococci are also present in animals, and are more persistent than other faecal streptococci in the environment (Geldreich & Kenner 1969; McFeters et al. 1974; Sinton & Donnison 1994), the species identification approach to source identification is generally regarded as unreliable (APHA 1995).

Ratio shift

This approach, which was suggested by Feachem (1975), based on the findings of McFeters et al. (1974), involves interpretation of the differential die-off of faecal coliforms and faecal streptococci in stored samples. The theory is that a predominantly human source (dominated by enterococci, which are more persistent than faecal coliforms) should exhibit an initially high FC : FS ratio (>4) which falls on storage, whereas non-human sources (dominated by *S. bovis* and *S. equinus*, which are less persistent than faecal coliforms), exhibit an initially low FC : FS ratio, which subsequently rises.

Application of the ratio shift approach has produced variable results. Burman et al. (1978) found that most stored sewage or sewage-polluted samples exhibited steady or increasing FC : FS ratios, in contrast to the predictions of Feachem (1975). However, Wheater et al. (1979) found an overall decrease in FC : FS ratios down stream of an outfall, but an overall increase up stream. Nevertheless, these authors recommended caution in using FC : FS ratios in receiving waters, because of the many unquantifiable factors involved.

Bifidobacteria

General

The bifidobacteria are a group of strictly anaerobic, Gram positive, enteric bacteria (Sneath et al. 1986). Depending on nutritional conditions, they adopt various morphologies, including bifurcated Y forms, from which their name is derived (Levin 1977; Sneath et al. 1986).

Bergey's Manual (Sneath et al. 1986) lists 24 species in the genus *Bifidobacterium*. A more recent description of the genus by Gavini et al. (1991) increased the number of species to 28. These authors listed some important species as *B. adolescentis*, *B. dentium*, *B. breve*, *B. infantis*, and *B. longum*. Bifidobacteria are present in high numbers in the faeces of humans and some animals, and have been found in sewage and polluted waters, but not in unpolluted environments, such as springs, uncontaminated soil, or garden compost (Evison & James 1975). However, although they were first described in 1899 (noted in Levin 1977), their potential as faecal indicators appears to have first been noted by Mossel (1958). As a result, bifidobacteria are probably the least studied of all faecal indicator organisms.

Early work with bifidobacteria was reviewed by Levin & Resnick (1981). Since then, interest in the group has increased, for two reasons. First, they may have potential as faecal indicators in tropical waters (Carillo et al. 1985). These environments contain a significant number of non-faecal bacteria that can ferment lactose and produce indole at 44°C—reactions which are typical of faecal coliforms. These "false positive" results are eliminated with bifidobacteria, because the lactose-fermenting strains are exclusively of faecal origin. Second, some bifidobacteria appear to have potential as faecal source indicators. The evidence for this is discussed later.

Methods for recovery and enumeration

A major difficulty in the selective isolation of bifidobacteria from environmental samples is the inhibition of other anaerobic, Gram-positive organisms, such as various *Streptococcus*, *Peptostreptococcus*, and *Lactobacillus* species, which may occur in higher numbers than bifidobacteria (Evison & Morgan 1978). The first selective medium was developed by Gyllenberg & Niemelä (1959), and was later modified by Evison & James (1975), who added kanamycin to suppress background organisms. Higher recoveries, particularly of species of human origin, were reported by Resnick & Levin (1981a) for another formulation, YN-6, which contained neomycin and nalidixic acid. However, subsequent studies with YN-6 have produced unsatisfactory results. For example, Carrillo et al. (1985) found that it failed to inhibit background organisms in tropical waters, producing c. 22% false positives. Conversely,

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Mara & Oragui (1983) earlier found YN-6 to inhibit some species of bifidobacteria. They found that a modified formulation, YN-17, incorporating kanamycin, produced higher recoveries and greater (although incomplete) suppression of background faecal streptococci.

Presence in faeces and effluents, and survival in water

Bifidobacteria can be detected in human intestines within the first 6 days of neonatal life (Poupard et al. 1973; Mitsuoka & Kaneuchi 1977). They are one of the dominant groups of anaerobes in the gut of humans, reaching densities of up to 10^{10} cells per g of faeces (Wheater et al. 1980; Carillo et al. 1985). Gyllenburg et al. (1960) and Evison & James (1975) reported that bifidobacteria counts in human faeces were typically 10–100 times greater than those of coliforms. Mara & Oragui (1985) found highest bifidobacteria counts to occur in older children and healthy adults up to middle age.

In human faecal isolates, an interesting feature of the genus is the change in species composition that appears to occur as individuals age. For example, Evison & Morgan (1978) found that *B. adolescentis* was the principal species in individuals aged <20 years, whereas *B. longum* was found to predominate in the 20–40-year age group. In adults >50 years old, only *B. adolescentis* was found.

Wheater et al. (1980) suggested that during the transformation of faecal material into sewage, an initial rapid fall in numbers of bifidobacteria occurs, followed by a further reduction at a lower rate. Although there is little published information on bifidobacteria concentrations in sewage, it appears that typical concentrations in raw sewage are c. 10^6/100 ml (Evison & James 1975; Wheater et al. 1980; Resnick & Levin 1981a).

There is conflicting information in the literature as to the incidence of bifidobacteria in animals, although this may be partly attributable to the selective media problems discussed above. In addition to detecting them in human faeces, Evison & James (1975) found bifidobacteria in pig and poultry faeces. Evison & Morgan (1978) again detected bifidobacteria in poultry faeces but also found them in the faeces of cattle and sheep. In most faecal samples, the bifidobacteria count obtained was higher than the *E. coli* count. Conversely, Resnick & Levin (1981b), using YN-6 medium, found bifidobacteria only in humans and pigs, and not in chickens, cows, dogs, horses, cats, sheep, beavers, goats, and turkeys.

Bifidobacteria are strict anaerobes, grow poorly below 30°C, and have fairly rigorous nutrient requirements, so they are unlikely to find conditions suitable for regrowth in receiving waters (Gyllenberg et al. 1960; Evison & James 1975). However, there are differing reports on the survival of bifidobacteria in water. Gyllenberg et al. (1960) and Levin (1977) found no difference between the survival of bifidobacteria and coliforms in natural waters held at a range of temperatures. Field surveys of receiving waters have also suggested that bifidobacteria counts tend to be positively correlated with (but higher than) counts of faecal coliforms, *E. coli* or faecal streptococci (Evison & James 1975; Carrillo et al. 1985).

In contrast, Resnick & Levin (1981a) found that bifidobacteria did not survive as well as *E. coli* in either fresh or marine waters. Jagals et al. (1995) also found that the ratio of faecal coliforms to bifidobacteria increased in a river 20 km down stream of a human pollution source. Notwithstanding their findings with respect to *E. coli* and faecal streptococci (above), Evison & James (1975) noted that bifidobacteria counts tended to fall relative to coliform counts in sewage and receiving waters. Similarly, although Carrillo et al. (1985) found bifidobacteria outnumbered *E. coli* in their study, in diffusion chambers seeded with pure cultures, counts of *B. adolescentis* tended to fall faster than those of *E. coli*.

Use as faecal source indicators

Early studies on the use of bifidobacteria as faecal source indicators centred on the relationship between the source of the organism and the electrophoretic mobility of the enzyme, phosphoketalase. Scardovi et al. (1971) reported that isolates from birds, cattle and humans appeared to show distinct electrophoresis patterns. However, according to Gavini et al. (1991), results based on this approach do not correlate well with phenotypic or genomic descriptions of the species. These authors carried out a numerical analysis on bifidobacteria from humans, animals, and sewage, using a large number of biochemical tests. This phenotypic analysis revealed seven main groups of bifidobacteria—three from humans, three from animals, and one from sewage. More useful, from a water management perspective, was their finding that growth at 45°C in trypticase phytone yeast broth (TPYB) seemed to provide good discrimination between human and animal strains—most of the strains that were isolated from animals grew at this temperature whereas the majority of human strains did not.

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An alternative approach was investigated by Mara & Oragui (1983, 1985). They found that some degree of faecal source discrimination was provided by their medium YN-17 (discussed above), on which bifidobacteria were consistently isolated from the faeces of man and pigs but only occasionally from the faeces of cattle and sheep. They found that a new formulation, mannitol sorbitol agar (MSA), isolated both mannitol-fermenting bifidobacteria strains (from both human and animal samples) and sorbitol-fermenting strains (from human faeces only). They then developed human bifid sorbitol agar (HBSA) which, used in conjunction with membrane filtration, was able to isolate and enumerate sorbitol-fermenting strains of *B. adolescentis* and *B. breve*. These strains were isolated from human samples only, and were therefore proposed as specific indicators of human faecal pollution.

The sorbitol fermentation approach has been tested under field conditions in Africa. Mara & Oragui (1985) found sorbitol-fermenting bifidobacteria strains only in human faeces and in water contaminated by human faeces. Jagals et al. (1995) sampled a stream and river exposed to faecal pollution of mainly animal origin (upstream samples), and the same stream and river following exposure to faecal pollution from a human settlement (downstream samples). Sorbitol fermenting bifidobacteria were generally not found in the upstream samples, but were consistently found in the downstream samples, where they were generally present in slightly lower concentrations than those of faecal coliforms.

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Bacteroides spp.

General

Members of the genus *Bacteroides* are rod shaped, Gram-negative, obligate anaerobes (Kreig & Holt 1986), and are among the most numerous bacteria in human faeces. Moore & Holdeman (1974) found that they accounted for c. 30% of all isolates, but were phenotypically so similar that most were considered to be subspecies of one organism, *B. fragilis*. However, subsequent DNA-DNA homology analyses showed that these subspecies differ from one another at the genetic level (Johnson 1978). On this basis, many of the former *B. fragilis* subspecies were elevated to species rank, and others were assigned to newly established species (Cato & Johnson 1976). Overall, the taxonomy of the *Bacteroides* genus has undergone a number of changes and remains somewhat confused. Some workers (e.g., Allsop & Stickler 1985) refer to the "*B. fragilis* group" (BFG), which is assumed to include *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, and *B. vulgatus*. However, in this review we will mostly use the term *Bacteroides* spp.

Although it is a natural inhabitant of the intestines, *B. fragilis* also causes various infections in humans and animals, which has lead to the development of PCR techniques for its detection in clinical specimens (Yamashita et al. 1994). These techniques are now being used to determine whether *Bacteroides* spp. are useful in distinguishing human from animal faecal pollution sources.

Methods for recovery and enumeration

Several media have been described for the isolation of *Bacteroides* spp. In clinical microbiology, blood agar supplemented with gentamicin, neomycin, or kanamycin (to suppress growth of facultative anaerobes) is frequently used. For sewage contaminated samples, penicillin-G may be added to inhibit clostridia (Sutter & Finegold 1971), although Allsop & Stickler (1984) found some constituents of supplemented blood agar to be unstable. They developed WCPG medium, based on the medium of Wilkins & Chalgren (1976), for use with membrane filtration. Because *Bacteroides* spp. can hydrolyse aesculin to aesculetin, which then forms a black complex with ferric ammonium citrate, these substances were also included to render the medium differential for these organisms. Although WCPG consistently underestimated the *Bacteroides* spp. population in natural waters compared to blood agar, the authors considered that it offered the advantages of stability and standard composition.

Selective media have been developed for *B. fragilis*, based on the ability of the species to grow in the presence of 20% bile and a high concentration of one or more antibiotics. However, many of these media probably allow the growth of other *Bacteroides* spp. (Kreig & Holt 1986).

Presence in faeces and effluents, and survival in water

The presence of the *Bacteroides* spp. in numbers c. 100 times greater than those of *E. coli* in human faeces lead Post et al. (1967), and later Allsop & Stickler (1985), to suggest that these anaerobes might serve as alternative faecal indicators to *E. coli*. Allsop & Stickler (1984) reported "presumptive BFG" counts from human faeces as ranging from 9.7 $\times 10^8$ to 2.8 $\times 10^9$/g. In contrast, *Bacteroides* spp. have generally been detected only at low levels, or not at all, in the faeces of animals (Kreader 1995).

Allsop & Stickler (1985) found that the ratio of *Bacteroides* spp. to *E. coli* in animals ranged from 0.0005 in sheep to 108.1 in cats, with herbivores generally exhibiting the lowest counts.

Both Post et al. (1967) and Allsop & Stickler (1985) found that *Bacteroides* spp. had similar rates of survival to coliforms in raw sewage, but they died off more rapidly in water. Fiksdal et al. (1985) also found that viable counts of *B. fragilis* declined in canal water faster than those of *E. coli* and *S. faecalis*. However, fluorescent antisera tests for *B. fragilis* suggested higher cell counts than were obtained on the culture media.

Use as faecal source indicators

High numbers of *Bacteroides* spp. in human faeces, together with low numbers in animal faeces, suggest the potential of these organisms as source indicators. Using PCR techniques, Kreader (1995) examined faeces from 9 humans and 70 non-humans (cats, dogs, cattle, pigs, horses, sheep, goats, and chickens) for *B. distasonis*, *B. thetaiotaomicron*, and *B. vulgatus*. Depending on the primer set used, 67% of the human faecal extracts tested had high levels of target DNA, compared to 7–11% of non-human extracts. The probes for *B. vulgatus* detected high levels of their target DNA in house pets, but not in most other animals. This suggests that some transfer of these organisms may occur when humans and animals exist in close proximity, but that the approach may provide some discrimination between human and grazing animal sources.

Because conventional methods for isolating, identifying, and enumerating *Bacteroides* spp. are cumbersome and time consuming, the future development of this group as faecal source indicators will probably depend on DNA-based techniques. Kuritza et al. (1986), using a species-specific DNA hybridisation probe for *Bacteroides vulgatus*, *B. thetaiotaomicron*, *B. uniformis*, *B. distasonis*, and *B. ovatus*, were able to show that these organisms (with the exception of *B. ovatus*) are all present in high concentrations in faeces. However, this approach does not appear to have been applied to water samples, and there remains the problem of the relatively rapid inactivation of *Bacteroides* spp. cells in water, compared to faecal coliforms, including *E. coli*.

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Phages of *Bacteroides fragilis*

General

As noted in the previous section, although *B. fragilis* is one of the most numerous bacteria found in the human intestines, it tends to be a poor survivor in the environment. However, Jofre et al. (1986) recognised that, because bacteriophages tend to be more persistent in aquatic environments than their hosts, phages active against *B. fragilis* were potential faecal indicators. They experimented with 12 strains of *Bacteroides* spp., and found that one *B. fragilis* strain (HSP40) was found only in human faeces. Phages of this strain were thus recommended by these authors as human source indicators.

Phages of *B. fragilis* are tailed, somatic phages (attach directly to the host cell wall), with a regular icosahedral head (Booth et al. 1979).

Methods for recovery and enumeration

B. fragilis HSP40 phages are normally recovered by the double-layer agar technique of Adams (1959), although Tartera & Jofre (1987) found that higher plaque counts were obtained using a multiple tube fermentation method. For the overlay method, they used modified blood agar for the base layer, and modified brucella broth (MBB) plus agar for the overlay. For the MPN method, they used MBB. Cornax et al. (1990) increased phage recovery rates through the addition of kanamycin and vancomycin to the double-layer assay of Tartera & Jofre (1987). Tartera et al. (1992) reported further increases using a new host culture medium—bacteroides phage recovery medium (BPRM). They also found that sample pre-treatment, particularly a filtration-elution step using polyvinylidene difluoride (PVDF) filters, reduced interference from background anaerobes (which tends to occur in environmental samples, despite the inclusion of antibiotics), and increased the visible plaque count. Work is continuing with methods to improve phage recoveries on the HSP40 host (J. Jofre pers. comm.).

Presence in faeces and effluents, and survival in water

The percentage of humans who excrete *Bacteroides* spp. phages is low—c. 5% according to Kai et al. (1985), and c. 10% (for *B. fragilis* phages) according to Tartera & Jofre (1987). Although these individuals excrete high numbers, counts in sewage and sewage-polluted waters are significantly lower than those of total coliforms, faecal coliforms, somatic coliphages, and F-specific coliphages, at least as determined by existing methodologies (Cornax et al. 1991; IAWPRC 1991; Havelaar 1993). In a study by Armon (1993), bacteriophage distribution in sewage was found to be—somatic coliphages 10^3–10^6 plaque forming units (PFU)/100

ml, F-specific coliphages 10^3–10^5 PFU/100 ml, and *B. fragilis* phages 10^0–10^4 PFU/100 ml. *B. fragilis* phages were detected in only 5.48% of samples tested. Similar counts, ranging from 23–4.6 × 10^4 PFU/100 ml, have been reported by Tartera et al. (1989), but they found *B. fragilis* phages in all the raw sewage samples tested.

Tartera & Jofre (1987) found no phages of *B. fragilis* HSP40 in faecal specimens from cows, pigs, rabbits, mice, hens, or quail. Grabow et al. (1995), working in South Africa, confirmed these findings, by detecting phages in human stool specimens, but not in the excreta of seabirds, primates, including higher primates such as the gorilla, orang-outang, and chimpanzee.

Tartera & Jofre (1987) found that phage counts in polluted environmental samples (on *B. fragilis* HSP40) ranged from 93 to 10^5 in river water and from <3 to 10^3 in seawater. Tartera et al. (1988) also found that *B. fragilis* phages outnumbered enteroviruses both in the number of samples from which they were isolated and in the number of isolates (on the average by a factor of >10).

Jofre et al. (1986) considered that phage active against *B. fragilis* would be unlikely to replicate in the environment, because the temperature, anaerobic conditions, and host cell count present in the intestinal tract would probably never occur together. A study by Tartera et al. (1989) showed that, even under these optimal conditions, *B. fragilis* phages failed to replicate when added to environmental samples. Data from a laboratory study by Jofre et al. (1986) suggest that phage infecting *B. fragilis* HSP40 have a survival rate similar to coliphage f2, poliovirus, and simian rotavirus in both fresh water and sea water. *B. fragilis* phage levels in mussel samples collected at increasing distance from a sewage source by Lucena et al. (1994) suggested that survival rates were similar to somatic coliphages, but were superior to F-RNA phages. *B. fragilis* phages also appear to have a relatively high resistance to treatment processes (Bosch et al. 1989; Havelaar 1993; Jofre et al. 1995).

Use as faecal source indicators

The basis of the use of phages of *B. fragilis* HSP40 as faecal source indicators, is their specificity for human sources, and this specificity appears to have been reliably demonstrated in a number of studies (Tartera & Jofre 1987; Grabow et al. 1995). However, the overall value of this approach in environmental samples is in doubt because of low counts recorded in some parts of the world. Although useable counts have been obtained in waters tested in Spain, Israel, and South Africa, counts in the United States have generally been low (Havelaar 1993). Jagals et al. (1995), in their field evaluation of a range of faecal source indicators, found no *B. fragilis* phage in any of the samples tested.

The reasons for low or zero counts in some reported studies are unknown, but it is known that a phage-carrier state, or pseudolysogeny, may be entered into by many phage-host systems. This phenomenon was observed for *B. fragilis* phages by Booth et al. (1979), who noted that the presence of a thick capsule around some cells in pure culture of a host strain appeared to interfere with the attachment of phage to the cell wall, thus rendering these cells resistant to phage infection. Low counts of phages of *B. fragilis* HSP40 have been recorded for New Zealand effluents in our laboratory, and in the New Zealand Meat Industry Research Institute laboratory in Hamilton (A. Donnison, MIRINZ pers. comm.). We therefore concur with Havelaar (1993), who maintained that the use of *B. fragilis* HSP40 for phage detection may not be applicable throughout the world.

F-RNA phage subgroups

General

F-RNA phages are a group of icosahedral phages which are morphologically similar to several important human enteric virus groups, and have thus been proposed as enteric virus models (IAWPRC 1991). They are F-specific phages, i.e., they attach to the F-pili, which are filamentous structures on the cell walls of "male" bacterial strains. F-RNA phages which attach to the F-pili of coliform bacteria (or other bacteria genetically modified to produce *E. coli* F-pili) may be referred to as F-RNA coliphages.

F-RNA coliphages may be classified into four subgroups (Furuse 1987). Subgroups I and II form major group A, and III and IV form major group B. Interest in the use of the subgroups for faecal source identification stems from evidence that different subgroups predominate in human and animal faecal sources (Havelaar et al. 1986; Furuse 1987).

Methods for recovery and enumeration

F-RNA phages may be enumerated by a range of methods, such as those described in Havelaar & Hogeboom (1984), Sobsey et al. (1990), and Sinton et al. (1996). Identification of the phage subgroups may be achieved by either serotyping or genotyping. Phage serotyping methods were first described by

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Watanabe et al. (1967), and simplified by Havelaar et al. (1990). The procedure involves repeated plaquing F-RNA phages on a suitable host to ensure purity, mixing and incubating with antiphage serum, and spot testing on the host. A phage is considered to belong to a serological subgroup, if it is neutralised by that particular antiserum, and if neutralisation by other antisera is absent or weak.

Hsu et al. (1995) and Beekwilder et al. (1996) considered that serotyping of phage produced ambiguous results, was too time consuming for routine assay, and that the antiphage sera were too hard to obtain. They developed genome-targeted procedures which could identify the four subgroups. These procedures involved plating out the phage on a suitable host, transferring the zones of lysis to a nylon filter, denaturing the phages to expose the nucleic acid, fixing the nucleic acid to the filter, and exposing it to oligonucleotide probes labelled with ^{32}P or digoxygenin. Each of four probes was specific to one subgroup. Hsu et al. (1995) showed that the serotyping and genotyping results were generally consistent for most environmental samples.

Presence in faeces and effluents, and survival in water

F-RNA phages appear to be rare in human faeces (Dhillon et al. 1976; Osawa et al. 1981; Havelaar et al. 1986, 1990). Paradoxically however, they have been found in significant concentrations (10^3–10^4/ml) in human wastewaters (Havelaar et al. 1984). F-RNA phages also appear to be uncommon in animal faeces—Havelaar et al. (1986) found F-RNA phages in the faeces of pigs, broiler chickens, sheep, and calves, but not in faeces from dogs, cows, and horses.

There have been relatively few studies published on the occurrence of F-RNA phages in polluted waters. However, in the Netherlands, they have been shown to be reasonably well correlated with enterovirus concentrations (Havelaar 1993; Havelaar et al. 1993). Similarly, the survival of F-RNA phages in the environment has not been extensively investigated, although they appear to be relatively resistant to inactivation by sunlight (Kapuscinski & Mitchel 1983). They have been shown to exhibit similar inactivation rates to human enteric viruses in laboratory studies (Sobsey 1989), and in a small field-based chamber, controlled by a dialysis unit (Springthorpe et al. 1993).

Use as faecal source indicators

Neither the serotypic nor genotypic approach to F-RNA subgroup identification has been subject to comprehensive evaluation in natural waters as a means of faecal source identification. However, Furuse (1987), using serotyping, concluded that subgroups II and III tend to be isolated from human faeces, subgroup I is usually isolated from the faeces of non-human mammals, and subgroup IV phages are of mixed origin. Havelaar et al. (1990), confirmed these findings, but demonstrated that subgroup II and III phages were found in sewage rather than faeces. Overall, it appears that serotyping is too expensive and time consuming for widespread evaluation of this approach. However, the work of Hsu et al. (1995) and Beekwilder et al. (1996) suggest that gentotypic identification of F-RNA phage subgroups may have potential in future source identification studies.

Rhodococcus coprophilus

General

Rhodococcus coprophilus is a nocardioform actinomycete, forming a fungus-like mycelium, which breaks up into bacteria-like elements (Sneath et al. 1986). The organism is Gram-positive, aerobic, and is a natural inhabitant of the faeces of herbivores. The bacteria-like elements contaminate grass and hay which are eaten by herbivores, can survive passage through their digestive systems, and thus contaminate the dung (Al-Diwany & Cross 1978).

R. coprophilus was recognised c. 20 years ago as a potential faecal source indicator by Rowbotham & Cross (1977), who noted its presence in the faeces of domesticated herbivores, in pasture run-off, and associated receiving waters and sediments, but its absence from human faecal wastes.

Methods for recovery and enumeration

Various agars have been proposed for the isolation of *R. coprophilus*, including Bennetts agar (Sneath et al. 1986), M3 agar (Rowbotham & Cross 1977), and modified M3 medium, which includes nalidixic acid and sodium azide (Mara & Oragui 1981). Before plating, counts of background bacteria are reduced by preheating samples at 55°C for 6 min. Incubation is at 30°C for 12–14 days, followed by exposure of the plates to sunlight for 4–7 days to enhance colony pigmentation (Sneath et al. 1986).

Enumeration of *R. coprophilus* from water samples by traditional culturing methods presents potential problems because of the possibility of break-up of actinomycete hyphae into coccoid elements. However, Oragui & Mara (1983) maintained that valid counts could be obtained by standard dilution and spread-plating on an appropriate agar medium.

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Presence in faeces and effluents, and survival in water

Mara & Oragui (1981) maintained that *R. coprophilus* had never been isolated from human faeces, and there appear to have been no subsequent findings to the contrary. However, the organism was found, together with other actinomycetes, in activated sludge scum by Sezgin et al. (1988), although whether it was actively growing there is unclear. *R. coprophilus* is hydrophobic, and has also been found in high concentrations in river foams (Al-Diwany & Cross 1978).

R. coprophilus has been consistently isolated from the dung of herbivores such as cows, donkeys, goats, horses, and sheep. It has also been isolated from poultry reared in proximity to farm animals, and from fresh waters and waste waters polluted with animal faecal material (Mara & Oragui 1981; Oragui & Mara 1983).

Al-Diwany & Cross (1978) examined the incidence of various actinomycetes in aquatic habitats, and found that *R. coprophilus* counts were reasonably well correlated with those of faecal streptococci, confirming its faecal origin. Mara & Oragui (1983) reported that *R. coprophilus* is common on grass and in soil beneath grazed pastures, where it can be washed into streams and lakes and accumulate in the sediments. They found that the organism appeared to survive considerably longer than *E. coli* and faecal streptococci in receiving waters and sediments.

Use as faecal source indicators

The presence of *R. coprophilus* in the dung of herbivores, but its apparent absence from human faeces, has been the basis of suggestions that the organism is an ideal grazing animal indicator. Rowbotham & Cross (1977) examined water samples from a number of streams for *R. coprophilus* and associated actinomycetes (*Micromonospora* spp. and *Streptomyces* spp.), and suggested that the ratio of *R. coprophilus* to these actinomycetes could provide a useful index for distinguishing human from dairy shed wastes. However, the longevity of *R. coprophilus* presents potential problems. Oragui & Mara (1983) suggested that the organism may need to be used in conjunction with the relatively short-lived *Streptococcus bovis* (also found in ruminants but not humans) to indicate the proximity of the animal pollution. They suggested that the long survival period of *R. coprophilus* means its presence alone points to contamination with animal faecal matter of remote or distant origin. However, the presence of *R. coprophilus* together with *S. bovis* would confirm recent animal faecal pollution.

The potential of *R. coprophilus* for distinguishing between human and animal faecal pollution in temperate climates was also evaluated in the two African field studies described above for bifidobacteria. Mara & Oragui (1985) found *R. coprophilus* only in animal faeces and in water contaminated by animal faeces. Jagals et al. (1995) sampled a stream and river exposed to faecal pollution of mainly animal origin, and the same stream and river following exposure to faecal pollution from a human settlement. Samples were assayed for *R. coprophilus* using the method of Oragui & Mara (1983). They found that *R. coprophilus* counts were highest in the stream and river up stream of the human settlement. Furthermore, up stream of the settlement, counts of *R. coprophilus* were higher than those of other faecal indicators—faecal coliforms, faecal streptococci, bifidobacteria, somatic coliphages, and F-specific phages. The ratio of *R. coprophilus* to faecal coliforms also increased with increasing distance down stream of the settlement, confirming the findings of Oragui & Mara (1983) that this species is resistant to inactivation in receiving waters.

CHEMICAL INDICATORS OF FAECAL SOURCES

Introduction

Chemical indicators of faecal pollution in water can be broadly divided into those present in faeces (direct indicators), and those strongly associated with faecal discharges (indirect indicators). Other chemicals, such as ammonia nitrogen (NH_3-N), are associated with the breakdown of faecal material in water, but are not reliable faecal indicators. For example, NH_3-N is also widely present in unpolluted environments, from sources such as rotting vegetation.

A very wide range of digestion products are present in faeces and may be detected in water polluted with faecal material. However, only the faecal sterols appear to have been systematically investigated as faecal indicators. Two other excretion products—uric acid and urobilin—are not included in this review. Although they are found only in excreta, there appears to be no reported evidence that they are useful in distinguishing pollution sources. Humans have a daily uric acid output of 700 mg/person (White et al. 1978), and this compound has been used to trace domestic sewage discharges in river, estuarine, and coastal waters (Brown et al. 1982). However, it is also found in non-human vertebrates, including mammals, birds, and some reptiles and amphibians (Dantzler 1978). Urobilin

has been used to estimate levels of faecal pollution in sediments (Miyabara et al. 1994a) and river waters (Miyabara et al. 1994b), but there appear to be no reported studies on levels in other animals.

Numerous non-faecal chemicals used in human activities are closely associated with discharges containing faecal material. However, this review covers only certain chemicals used in household liquid detergents and washing powders—fluorescent whitening agents, sodium tripolyphosphate, and linear alkyl benzenes—because these are likely to be associated with both large (industrial) and small (non-industrial) human settlements. Excluded from this discussion are other compounds indicative of human activity, such as polycyclic aromatic hydrocarbons (PAHs). PAHs are formed from the incomplete combustion of fossil fuels, and are present in smoke from domestic fires and vehicle exhausts (Cretney et al. 1986; Readman et al. 1986). Although they are found in sewage, they are primarily associated with urban stormwater run-off, and their principal value is in distinguishing between stormwater and sewage discharges (Nichols & Espey 1991).

In the following sections, four groups of chemicals are briefly reviewed. One group—the faecal sterols—is a direct indicator of faecal contamination; the other three—fluorescent whitening agents, sodium tripolyphosphate, and linear alkyl benzenes—being washing powder constituents, are indirect indicators.

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Faecal sterols

General

The term "faecal sterols" is a broad term covering the various A-C27, C28, and C29 cholestane-based sterols found in faecal material. These include coprostanol, sitosterol, sitostanol, and campestanol. Coprostanol is produced exclusively in the intestines of humans and some higher mammals by bacterial biohydrogenation of cholesterol to 5ß(H)-stanol (Eneroth et al. 1964; Eyssen et al. 1973). In other animals, anaerobic bacteria either biohydrogenate sterols to other stanols or lack the ability to biohydrogenate sterols at all.

There was early recognition that cholesterol itself could not be used as a specific faecal marker, because it is also found in animal products such as eggs, milk, lard, and wool grease (Murtaugh & Bunch 1967). It was also recognised that coprostanol was the principal human faecal sterol (Rosenfeld 1964; Rosenfeld & Gallagher 1964).

Detection methods

Because faecal sterols bind strongly to particulate matter in the environment, a commonly used assay procedure for water starts with filtration of the sample through a 0.45 μm membrane filter, followed by quantitative extraction of the collected sediment by the modified one-phase CH_3–MeOH method (Bligh & Dyer 1959; White et al. 1978), conversion of the extracted sterols to their trimethylsilylether derivatives, and measurement using high resolution gas chromatography and mass spectrometry (Nichols et al. 1993).

Presence in faeces, effluents, and contaminated water

Coprostanol has been shown to be a reliable marker of faecal pollution, even when coliform bacteria may have been destroyed by processes such as chlorination and heat treatment (Tabak et al. 1972, Goodfellow et al. 1977). However, some forms of effluent treatment have been shown to reduce coprostanol concentrations (Smith et al. 1968; McCalley et al. 1981).

When faecal matter enters aquatic environments, the sterol fingerprint is diluted and mixed with sterols of algae, detritus, and other material, but it remains distinguishable. The 5ß-stanols such as coprostanol do not occur naturally in fresh or marine waters or in aerobic sediments (Leeming et al. 1994). Their presence in Antarctic sediments has been attributed to marine mammals (Venkatesan et al. 1986).

There have been numerous investigations of faecal sterol concentrations in the waters and sediments of rivers, lakes, and estuaries. Earlier studies were reviewed by Walker et al. (1982), and more recent investigations include those of Brown & Wade (1984), Düreth et al. (1986), and Nichols et al. (1993). Many of these studies focused on establishing a relationship between faecal sterol concentrations and levels of other (mostly microbiological) indicators of faecal pollution. Walker et al. (1982) considered that most of the studies they reviewed showed a close correlation between faecal sterol concentrations and overall levels of faecal pollution, but Düreth et al. (1986) maintained that these studies showed no stable and significant correlation between faecal sterol and indicator bacteria concentrations. More recently, Nichols et al. (1993) found good correlations for water and sediment samples between coprostanol and two faecal indicator organisms—faecal coliforms and *Clostridium perfringens* spores.

Use as faecal source indicators

The earlier investigations, as reviewed by Walker et al. (1982), made no attempt to differentiate between faecal sources. Murtaugh & Bunch (1967) showed that coprostanol was found in water polluted with both sewage and farm run-off. However, more recently, Leeming et al. (1994) have demonstrated that faecal sterols are potential faecal source "fingerprints". According to these authors, the potential source specificity of faecal sterols arises from three factors: (1) the animal's diet—omnivorous, carnivorous, and herbivorous diets (e.g., of humans, dogs, and sheep, respectively) each produce a distinctive sterol profile, so the proportions of sterol precursors entering the digestive tract are different; (2) irrespective of diet, many animals can biosynthesise sterols; (3) anaerobic bacteria in the digestive tract of some animals biohydrogenate sterols to stanols of various isometric configurations. The combination of these three factors determines the faecal sterol "fingerprint".

Leeming et al. (1996) examined the sterol content of human and animal faeces. They found that the 5ß stanol, coprostanol, constituted c. 60% of the total sterols found in human faeces. The faecal sterol profiles of herbivores was dominated by C_{29} sterols, and 5ß stanols were generally in greater abundance than 5α-stanols. The principal faecal biomarker of herbivores was 24-ethylcoprostanol. The sterol content of bird faeces was highly variable and largely dependent on the animal's diet. Both 5ß and 5α-stanols were in very low abundance in birds and dog faeces. Cats and pigs were the only animals with similar sterol profiles to humans. The authors maintained that the sterol fingerprints of human and animals were sufficiently distinctive to be of diagnostic value in determining whether faecal pollution in water samples was of human or animal origin.

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Fluorescent whitening agents

General

During the manufacture of washing powders, compounds known as fluorescent whitening agents (FWAs) are incorporated into the powder mixture. FWAs are designed to adsorb to fabrics and "brighten" washing by fluorescing when exposed to the UV radiation in sunlight. Some FWAs are discharged with the washing wastewater, and are therefore potential indicators of human sewage.

Detection methods

Various methods can be used for the detection of FWAs. The simplest methods involve direct fluorometric measurements, but organic matter in samples from natural waters can also fluoresce and interfere with the analysis. Abe & Yoshimi (1979) described an analytical method involving an extracting procedure and detection by thin layer chromatography. In a groundwater investigation in New Zealand, Close et al. (1989) used the method of Uchiyama (1979). This involved an extraction into 1, 2 dichlorethane to remove organic interferences, followed by a second extraction with the addition of an impairing agent to extract and concentrate the FWA, which was then measured fluorimetrically.

Presence in faeces, effluents, and contaminated water

There appear to have been relatively few studies on FWA levels in effluents and receiving waters. However, McKinney & Krause (1979) and Kerfoot & Skinner (1981) described the use of a device which measured fluorescence in lake water across a transect. This device was used with some success to identify septic tank leachate plumes in several United States lakes surrounded by holiday homes.

In New Zealand, Close (1989) found FWA levels of 169 mg m^{-3} in raw sewage, 114 mg m^{-3} in septic tank effluent, and 11–23 mg m^{-3} in groundwater contaminated by a septic tank soakage pit.

Use as faecal source indicators

In the 1980s in New Zealand, the most commonly used FWA was Hiltamine Arctic White (HAW), which exhibits peak excitation and emission wavelengths of 360 and 440 nm, respectively (currently, the most commonly used FWA is tinopal CBS-X, which exhibits peak excitation and emission wavelengths of 349 and 430 nm, respectively). Using the HAW wavelengths as a guide, Close et al. (1989) collected 86 samples from 67 wells at Yaldhurst—an unsewered area on the outskirts of Christchurch. They found detectable levels of FWAs and/or sodium tripolyphosphate (reviewed below) in 17% of the samples. These findings strongly suggested septic tank effluent contamination of the wells.

Sodium tripolyphosphate

General

Sodium tripolyphosphate (STP) is a basic component of washing powders, and comprises c. 20% of the powder mix. It has not been found in uncontaminated environments, and is not present in phosphatic fertilisers (Close et al. 1989). It therefore offers the potential of identification of sewage pollution sources. However, in receiving waters it is prone to

hydrolysis (to orthophosphate), particularly with increasing temperature and decreasing pH (Zinder et al. 1984), and adsorption to sediments, which can significantly reduce concentrations in the water column.

Detection methods

Early methods for the analysis of STP were based on the separation of phosphate species using an ion-exchange column, followed by acid hydrolysis of the STP to orthophosphate, which was then determined colorimetrically as a molybdenum blue complex (Lindenbaum et al. 1954; Grande & Beukamp 1956; Lundgren & Loeb 1961). Most of these workers were involved in the analysis of fairly high concentrations of STP. Lundgren & Loeb (1961) suggested that STP may spontaneously degrade below 4 g m^{-3}. However, Morita & Uehiro (1981) used high pressure liquid chromatography (HPLC) to analyse STP to low levels, and provided figures which suggest a concentration detection limit of c. 0.3 g m^{-3}. More recently, Close et al. (1989) adapted the method of Lundgren & Loeb (1961) for use on an autoanalyser. This adaptation gave a detection limit for STP of 1 mg m^{-3}.

Presence in faeces, effluents, and contaminated water

There appears to be very little published information on STP levels in sewage and receiving waters. Close et al. (1989) estimated STP levels in sewage at 15 mg/litre, assuming the average household washes 5 times per week and has a total water usage of 1000 litres/day, that STP comprises 20% of the washing powder mixture, and that no loss by hydrolysis or adsorption occurs.

Use as faecal source indicators

As noted above, Close et al. (1989) found detectable levels of FWAs and/or sodium tripolyphosphate in 17% of samples in the Yaldhurst groundwater quality survey, indicating that septic tank effluent contamination of the wells had occurred. The highest concentration detected was 4 mg/m^3, which, allowing for dilution in the groundwater, suggested that the above estimate of 15 mg/litre for sewage was realistic.

Long-chain alkylbenzenes

General

Long-chain alkyl benzenes (LABs) comprise a group of 26 secondary phenylalkanes with alkyl chains having 10–14 carbons. These synthetic hydrocarbons are used in the production of linear alkyl benzene sulphonates, the most widely used anionic surfactants in commercial detergents (Eganhouse et al. 1983a). The LABs have been found to be significant components of municipal wastes derived from sewage sludges (Takada & Ishiwatari 1987). They are persistent in the environment and are regarded as complimentary to the faecal sterols as waste-specific markers of urban or domestic sewage pollution (Eganhouse et al. 1983b; Murray et al. 1987).

Detection methods

LABs are relatively easily detected in wastes and associated waters. Samples are extracted using organic solvents such as dichloromethane, and the extracts cleaned up using silica gel chromatography (Takada & Ishiwatari 1985; Eganhouse 1986). The hydrocarbon fraction from this process contains the LABs whereas the alcohol fraction can be used for the analysis of faecal sterols.

The LABs are separated on capillary gas chromatography and measured using selected ion mass spectrometry. Ions m/z 91 and 105 are chosen as these ions are able to selectively detect the various isomers effectively (Eganhouse 1986).

Quantification is by the addition of suitable internal standards such as 1-phenyltridecane. Typical limits of detection using low resolution GC-MS are for sludges, 1–10 µg/litre, and for particulate (>0.6–1.0 µm) material derived from receiving waters, 20 ng/mg (calculated on a total LAB basis). Use of high resolution mass spectrometry may reduce these levels by 10–100 times.

Presence in faeces, effluents, and contaminated water

LABs have been shown in a number of studies to be present in the waters and sediments of sewage receiving water, in New York/New Jersey (Eganhouse et al. 1988), in southern California (Eganhouse et al. 1983a), in Tokyo Bay and its rivers (Takada & Ishiwatari 1987), and in Port Philip Bay (Murray et al. 1987). The profiles of the LABs in these studies have shown that, although the total LAB content is relatively consistent with respect to time and distance from source, the individual LABs are somewhat modified. In particular, the LABs with the benzene substituted at the 2- and 3-positions (sometimes called external LABs) appear to be microbiologically degraded at a faster rate that those substituted in the 5- and 6-positions (sometimes called internal LABs). If the LAB composition of the sources is known, then the changes in these profiles can give an indication

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as to the type of sewage treatment processes involved (e.g., activated sludges degrade the external LABs faster than anaerobic treatments) as well as extent of dispersion in the environment (Eganhouse 1986; Takada & Ishiwatari 1990).

Use as faecal source indicators

LABs are purely synthetic and are derived solely from direct industrial discharge and domestic wastes (Takada & Ishiwatari 1985; Eganhouse 1986). They are therefore strongly indicative of human sources. The levels they are detected at tend to be source specific, but they are generally present up to one order of magnitude lower than the corresponding faecal sterols, especially coprostanol, in human-derived wastes. LABs are complimentary to faecal sterols and their presence could be especially useful in situations where mixed wastes are present.

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SUMMARY AND CONCLUSIONS

Relative health risks

There is, as yet, no reliable epidemiological information on the relative risks to humans associated with human and animal faecal pollution. It may be inferred from United States waterborne disease outbreak data, where viral pathogens appear to be the most important, that human faeces probably constitute the greater risk. However, the existence of zoonoses, together with a high grazing animal : human ratio, suggests that animals are likely to be an important reservoir for enteric pathogens in New Zealand. This may increase the comparative risk of animal faecal material in this country. Accordingly, until scientifically defensible evidence to the contrary is available, it would seem prudent to assume that, in New Zealand, both animal and human faecal wastes constitute a risk to human health. However, whatever the relative risks involved, improved methods for identifying and apportioning faecal sources would assist water managers in lowering overall faecal pollution levels.

Microbial source indicators

Faecal streptococci

Despite of intensive investigation, the FC : FS ratio and species identification approaches to the use of faecal streptococci as faecal source indicators are generally regarded as too unreliable to be useful (APHA 1995). The ratio shift approach may be worthy of further investigation, but it has the disadvantage of requiring sequential assay of stored samples.

Bifidobacteria

Bifidobacteria have potential as both general faecal indicators, and faecal source indicators. Two simple tests show promise as providing some human versus animal source differentiation—sorbitol-fermentation (Mara & Oragui 1983) and growth at 45°C in TPYB (Gavini et al. 1991). However, there are three problems associated with their use: (1) as noted by Carrillo et al. (1985), procedures for enumerating bifidobacteria in water are still poorly developed, and involve time-consuming confirmatory procedures; (2) the group appears to demonstrate only moderate rates of survival in extra-intestinal environments, with some studies suggesting that they may be inactivated faster than faecal coliforms in receiving waters; (3) apart from the study by Jagals et al. (1995) in which only the sorbitol fermentation approach was tested, there has been little field evaluation of the faecal source identification capabilities of the group.

Bacteroides *spp.*

Although their dominance of the human faecal flora and their low abundance in animal faeces means that *Bacteroides* spp. have potential as faecal source indicators, there are problems associated with this group. These include cumbersome enumeration and speciation procedures (which may possibly be overcome with DNA-based techniques), and their poor survival rates in water.

B. fragilis *phages*

Phages of *B. fragilis* HSP40 appear to be highly human-specific. However, the low counts in effluents encountered by some workers mean that this approach may not be applicable throughout the world.

F-RNA phage subgroups

There is reasonable evidence to suggest that F-RNA phage subgroups II and III predominate in human effluents, and that subgroup I is characteristic of the faeces of non-human mammals. Low F-RNA counts in both human and animal faeces are a problem, although counts in human effluents are probably high enough to warrant further investigation of this approach.

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Rhodococcus coprophilus

The work by Jagals et al. (1995) confirms the findings of the earlier workers with respect to the potential of *R. coprophilus* as an animal pollution indicator. However, there are three problems associated with the use of this organism: (1) its presence in activated sludge scum may limit its use in waters receiving this form of discharge; (2) the longevity of the organism may require it to be used in conjunction with another herbivorous indicator, such as *S. bovis*; (3) as stated by Jagals et al. (1995), "the indicator value of this organism is restricted by the very time consuming and cumbersome detection procedure". Mara & Oragui (1981) and Oragui & Mara (1983) also noted the need for a selective medium for *R. coprophilus* which permitted a much shorter incubation period. An alternative to this approach may be the application of PCR techniques, which may offer a more rapid assay of this species.

Chemical source indicators

Faecal sterols

Assay and identification of faecal sterol isomers appears to have considerable potential for differentiating between faecal sources, including between different animal sources. However, further studies are required to determine whether useful quantities of the differentiating isomers are present in receiving waters.

Fluorescent whitening agents (FWAs)

Fluorometric techniques offer low detection limits, and FWA detection is highly specific for washing powders. Problems include the high level of dilution of FWAs in receiving waters (thereby requiring even lower detection limits) and interferences from fluorescent organic compounds. Field work to date with FWAs is promising, and further investigation of this approach is warranted.

Sodium tripolyphosphate (STP)

The Yaldhurst study by Close et al. (1989) demonstrated the potential of STP as a septic tank leachate indicator. However, the highest concentration detected (4.0 mg g^{-3}) is only 4 times the detection limit of the autoanalytical method used for the STP assay, which imposes significant restrictions on the use of this indicator. Improved analytical methods are required if STP is to be a useful as an identifier of human pollution sources.

Linear alkyl benzenes (LABs)

LABs are sewage-specific indicators (i.e., indicative of human sources). Care must be taken to understand the types of treatment processes they may have been subjected to. They have proven efficacy in detecting sediments and sludges contaminated by sewage wastes and there is good evidence to suggest that they are also suitable markers in receiving waters.

General conclusions

Despite many years of research, no single micro-organism or chemical determinand has been identified which will reliably distinguish human from animal faecal contamination in water. Although several groups of micro-organisms and chemicals continue to offer promising lines of investigation, it seems unlikely that any single determinand would be useful in all situations. Thus, a multivariate statistical approach, using appropriate "baskets" of microbial and chemical determinands may provide the best option for identifying and apportioning faecal inputs to natural waters.

Depending on advances in assay methods (such as the application of DNA-based methods to the faecal source indicator micro-organisms), and better information on concentrations in natural waters, baskets targeted at human sources could include sorbitol-fermenting bifidobacteria, F-RNA phage subgroups II and III, FWAs, and STPs. Similarly, baskets targeted at animal sources could include *R. coprophilus*, thermophilic bifidobacteria and specific faecal sterols (such as 24-ethylcoprostanol).

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