Standing Committee of Analysts

The Microbiology of Recreational and Environmental Waters (2015) – Part 1 – Water quality, epidemiology and public health

Methods for the Examination of Waters and Associated Materials

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This bluebook updates and replaces section 7.1 of the earlier version of The Microbiology of Recreational and Environmental Waters published in 2000.

Whilst specific commercial products may be referred to in this document, this does not constitute an endorsement of these products but serves only as illustrative examples of the types of products available. Equivalent products may be available and it should be understood that the performance of the method might differ when other materials are used and all should be confirmed by validation of the method.

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About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, waste water and effluents as well as sewage sludges, sediments, soils (including contaminated land) and biota. In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets within the series "Methods for the Examination of Waters and Associated Materials" and their continuing revision is the responsibility of the Standing Committee of Analysts (established 1972 by the Department of the Environment). At present, there are seven working groups, each responsible for one section or aspect of water quality analysis. They are

1 General principles of sampling and accuracy of results
2 Microbiological methods
3 Empirical, Inorganic and physical methods, Metals and metalloids
4 Solid substances
5 Organic impurities
6 Biological, biodegradability and inhibition methods
7 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with these methods are listed at the back of this booklet.

Publication of new or revised methods will be notified to the technical press. If users wish to receive copies or advanced notice of forthcoming publications or obtain details of the index of methods then contact the Secretary on the Agency's web-page (http://standingcommitteeofanalysts.co.uk/) or by post.

Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary. Users should ensure they are aware of the most recent version they seek.

Robert Carter
Secretary
June 2015

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 2002 (SI 2002/2677). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.
Numerous publications are available giving practical details on first aid and laboratory safety. These should be consulted and be readily accessible to all analysts. Amongst such resources are; HSE website HSE: Information about health and safety at work ; RSC website http://www.rsc.org/learn-chemistry/collections/health-and-safety
"Safe Practices in Chemical Laboratories" and "Hazards in the Chemical Laboratory", 1992, produced by the Royal Society of Chemistry; "Guidelines for Microbiological Safety", 1986, Portland Press, Colchester, produced by Member Societies of the Microbiological Consultative Committee; and "Biological Agents: Managing the Risks in Laboratories and Healthcare Premises", 2005 and "The Approved List of Biological Agents" 2013, produced by the Advisory Committee on Dangerous Pathogens of the Health and Safety Executive (HSE).

1 Introduction

1.1 Objectives and Scope

The use of rivers, lakes and the sea for recreational bathing, boating and water sports is well established and growing in the United Kingdom. This activity presents a potential and significant risk of disease transmission where the recreational water is a receiving environment for treated wastewater effluent and wastewater storm overflows. Rivers may also contain zoonotic and anthropogenic pathogens[1]. In addition, various surface waters are used as private water supplies which are often subjected to only rudimentary, or no, treatment and, therefore, may be of dubious microbiological quality[2,3]. Marine waters are used for shellfish cultivation which presents a potential risk of pathogen 'concentration' within these filter feeders[4]. Swimming, hydrotherapy and spa pools are used extensively throughout the developed world for recreation and for therapeutic use by convalescent patients. These have also been associated with disease transmission often caused by chlorine resistant organisms such as *Cryptosporidium* spp. or viral pathogens[5,6].

There is, therefore, a public health rationale for the integrated management of microbiological fluxes which impact on these environments. This is recognised by recent EU legislation in the form of the Water Framework Directive (WFD)[7]. This Directive sets a structure for water management throughout Europe in which the principal aim is the achievement of '*good ecological status*' and '*good chemical status*' in controlled waters. EU Member States are required to identify '*river basin districts*' which are defined as '*the main unit for the management of river basins for the purposes of the Directive and being made up of a river basin or neighbouring river basins, together with associated groundwater, transitional waters and coastal water*'.

In England and Wales, the Environment Agency is required to secure the objectives of the Directive in the defined River Basin Districts. It is specifically charged with developing a '*River Basin Management Plan'* for each district containing a '*programme of measures*' designed to achieve good ecological status and defined water quality criteria within the River Basin Districts. The Scottish Environmental Protection Agency and the Department of Environment for Northern Ireland fulfil the same functions in Scotland and Northern Ireland respectively.

Bathing and shellfish harvesting water are defined as 'protected areas' under Annex 4 of the WFD[7]. This has been incorporated into law in England and Wales[8]. The Environment Agency is charged with the design of a 'programme of measures' under Article 11, involving point and diffuse source control to ensure compliance with the microbiological standards outlined in daughter directives such as the revised Bathing Water Directive[9]. This will require a catchment scale assessment, and possibly remediation, of diffuse agricultural pollution and anthropogenic point source loadings of microbial flux. Several examples of UK studies of this type have been published [10, 11, 12, 13].

1.2 Epidemiology and evidence based standards

The epidemiological evidence base for the microbiological standards specified in the revised Bathing Water Directive[9] derive from UK randomised controlled trials using healthy volunteers at marine bathing waters[14] and the implementation of the same protocol at German fresh water recreation sites[15]. The derivation of the numerical guidelines has been outlined[16] (Table 1) and presented in the 2006 EU Council Directive on Bathing Waters [9].

Table 1 Microbiological parameters contained in Annex 1 of the revised Bathing Water Directive[9]

Inland waters

A	B	C	D	E
Parameter	Excellent quality	Good quality	Sufficient	Reference methods of analysis
Intestinal enterococci (cfu/100 ml)	200 (*)	400 (*)	330 (**)	ISO 7899-1 or ISO 7899-2
Escherichia coli (cfu/100 ml)	500 (*)	1000 (*)	900 (**)	ISO 9308-3 or ISO 9308-1

(*) Based on a 95-percentile evaluation. See Annex II of the Directive
(**) Based on a 90-percentile evaluation. See Annex II of the Directive
cfu – colony forming units

Coastal and transitional waters

A	B	C	D	E
Parameter	Excellent quality	Good quality	Sufficient	Reference methods of analysis
Intestinal enterococci (cfu/100 ml)	100 (*)	200 (*)	185 (**)	ISO 7899-1 or ISO 7899-2
Escherichia coli (cfu/100 ml)	250 (*)	500 (*)	500 (**)	ISO 9308-3 or ISO 9308-1

(*) Based on a 95-percentile evaluation. See Annex II of the Directive
(**) Based on a 90-percentile evaluation. See Annex II of the Directive
cfu – colony forming units

Treated swimming pool waters and spa pool waters, including whirlpool waters and hydrotherapy pool waters, are not covered by the Bathing Water Directive. In addition, there are no legislative or regulatory standards in the UK for these types of water. Guidance on some of these waters is given in the Swimming Pool Water Treatment and Quality Standards for Pools and Spas[6] and in Management of Spa Pools: Controlling the Risk of Infection[18].

Table 2 presents microbiological criteria which are based on the principle that most waters of this type have been subjected to some form of disinfection. Properly maintained pool water with the correct treatment regime (i.e. correct pH and adequate residual disinfectant) should satisfy these microbiological criteria.

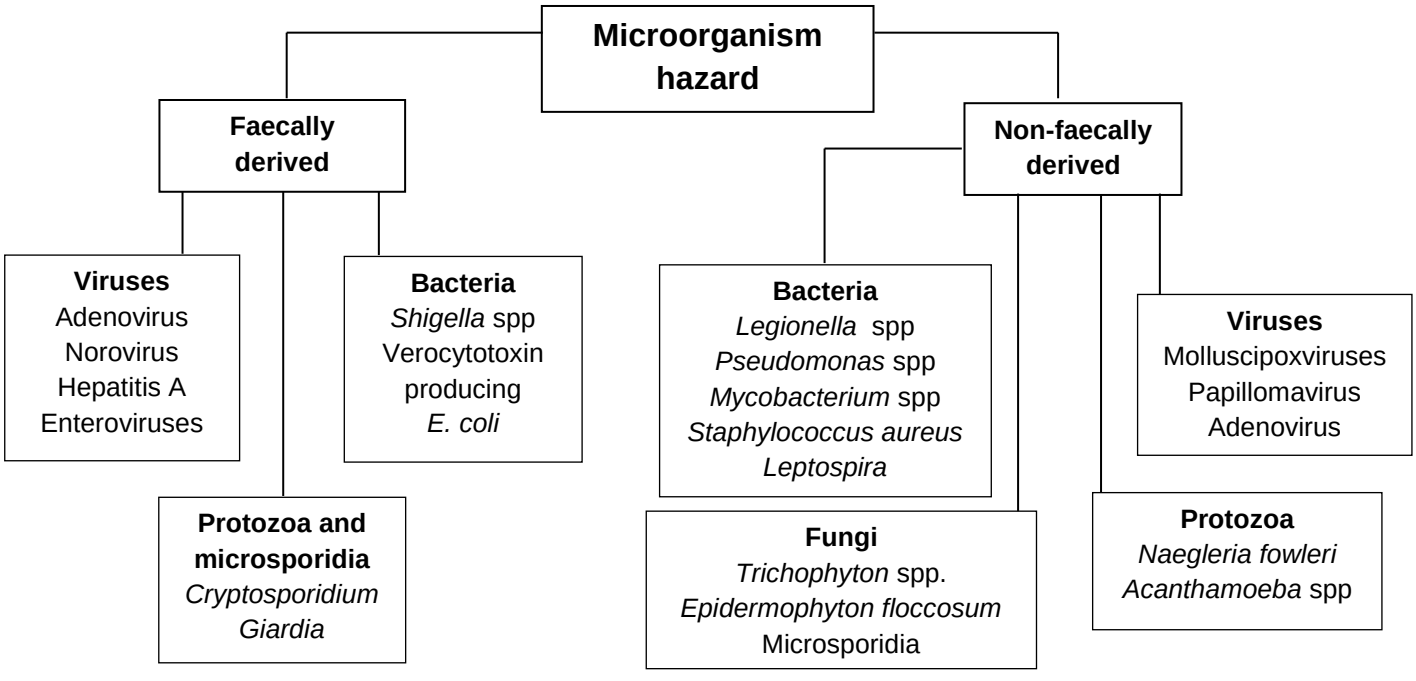
Table 2 **Microbiological levels for swimming pool waters and spa pool waters** (WHO 2006[5] ; PWTAG, 2009[6])

Parameter	Recommended value
Coliform organisms	0 / 100 ml
Escherichia coli	0 / 100 ml
Total viable count (37°C)**	0 - 10 cfu* / ml
Pseudomonas aeruginosa	0 / 100 ml

* cfu - colony forming units.
** Pool waters with a heavy bathing load may have colony counts in the range of 10 - 100 cfu/ml. Values above 100 cfu/ml would indicate that operating conditions are unsatisfactory.

A variety of micro-organisms can be found in swimming pools and these can be introduced in several ways. Faecal contamination may be introduced through accidental faecal release by bathers or as a result of residual faecal material on bathers bodies. Non-faecal human shedding, for example from vomit, mucous or skin can contaminate pool waters and surroundings. Other non-faecal bacteria and amoebae may multiply in the water environment also. Contamination may also come from the use of equipment normally used away from the pool, for example, canoes. In outdoor pools, micro-organisms (including pathogens) may come from animals. The principle micro-organisms of concern are set out in Figure 1. Most will not be present in well operated pools. Their significance and incidence of these micro-organisms is detailed in the following sections. Generally, their presence in pool waters is not routinely monitored. Pool water quality relies on the maintenance of an acceptable pH and disinfectant residual. Monitoring for microbiological quality is carried out less frequently and a number of tests may be applied. Heterotrophic plate count at 37°C, coliform bacteria, *E. coli* and *Pseudomonas aeruginosa* are the basic tests for microbiological quality based on the standards presented in Table 2.

Figure 1 **Microbial hazards associated with swimming pools [5, 6]**



2 Microbial indicators of water quality

A wide range of pathogens may be present in recreational surface waters and swimming pool water environments. For surface recreational waters these may be derived from human or animal faecal material either in the form of wastewater, agricultural or industrial waters. Routine monitoring for a wide range of microbial pathogens would be expensive and time consuming. The alternative is to select indicator micro-organisms which are absent in uncontaminated waters but are present when, for example, faecal contamination occurs and there is the possibility of pathogens being present. The indicators should not multiply in the environment and should be present in larger numbers than the pathogens whose potential presence they may indicate. Initially, indicators should respond in the environment in a similar manner to the pathogens and there should be an inexpensive, rapid method for their enumeration.

There are three principal indicators for faecal contamination:- *Escherichia coli*, intestinal enterococci and bacteriophages.

2.1 *Escherichia coli*

The 1976 Bathing Water Directive[17] used the term 'faecal coliforms' as an indicator of faecal contamination. By definition, these are coliform bacteria which are able to grow and ferment lactose at 44°C. Many coliforms are able to grow at 44°C but they are not necessarily faecal in origin. Interpretation of faecal coliform data as being indicative of the presence of *E. coli* and hence faecal contamination may be erroneous. The revised Bathing Water Directive[9] replaced the term 'faecal coliform' with *E. coli*, which is recognised as being associated specifically with the faeces of humans and warm-blooded animals. Of equal significance is the fact that chromogenic and fluorogenic substrates can be used to specifically target the identification of *E. coli* without the need for additional confirmatory tests. Confirmed data can now be available within 24 hours of a sample being taken and this, although not in 'real time,' provides a rapid and reliable indicator of water quality. The target enzyme for *E. coli* is β-glucuronidase and there are now a number of commercially available media containing a substrate which when cleaved either produce a coloured colony on a membrane or a colour change or fluorescence in a liquid medium[19].

The numbers of *E. coli* in untreated wastewater can be around 10^7 colony forming units (cfu) per 100 ml of sample. Wastewater treatment and dilution in surface water will reduce levels to around 10^4 cfu/100 ml. Contamination of recreational water with wastewater, either untreated or treated, can result in high levels of *E. coli* being detected. Details of methods for the detection of *E. coli*, including *E. coli* O157:H7 are described elsewhere[20] in this series.

2.2 Intestinal enterococci

This group were originally designated 'faecal streptococci' belonging to Lancefield's Group D. They were reclassified in 1984 as enterococci and include those which are faecal in origin such as *Enterococcus faecalis*, *Ent*. *faecium*, *Ent. durans* and *Ent. hirae* and those which are environmental such as *Ent*. *casseliflavus*, *Ent*. *flavescens* and *Ent*. *mundtii*. All the members of this group can grow in the presence of 400 mg/l of sodium azide, reduce triphenyltetrazolium chloride to the red pigment formazan and possess the enzyme β-glucosidase. The standard medium for the isolation of

enterococci is membrane enterococcus agar. For confirmation, this is usually followed by the demonstration of β-glucosidase caused by the hydrolysis of aesculin. As such, the current isolation and confirmatory media used for recreational waters will identify both intestinal and environmental groups and speciation may be necessary to differentiate them.

Enterococci may be found in wastewaters in similar numbers to *E. coli* but, in general terms they are better environmental survivors. Enterococci are, therefore, better indicators of more remote or long-term faecal contamination. Details of methods for the detection of enterococci are described elsewhere[21] in this series.

2.3 F-specific coliphage

F-specific coliphage (phage) are RNA bacteriophages which attach to the host bacterium (*E. coli*) using the F+ or sex pilus. Replicating in the host bacterium, they cause lysis of the cell releasing bacteriophage particles. They are rarely excreted in the faeces of humans, dogs, cattle and horses but are more frequently excreted by pigs and poultry, sheep and calves.

The use of F-specific coliphages in the demonstration of the potential presence of enteric viruses in surface water, sea water, wastewater and sludge has been demonstrated[22]. They are also useful as an indicator of virus contamination in shellfish flesh.

F-specific phages have the advantage in that they are cheaper and easier to assay than enteric viruses. However using *E. coli* as a specific host can give rise to the problem that somatic coliphage which are also present in wastewaters can give false positive results. This has been overcome by placing the F-specific plasmid into a strain of *Salmonella enterica* serovar Typhimurium (WG49) along with markers for lactose fermentation and resistance to the antibiotic nalidixic acid. Incorporation of this antibiotic into the assay helps to reduce the growth of unwanted background bacteria and select for host bacteria containing the plasmid.

The effect of wastewater treatment processes, for example UV disinfection, is similar on enteric viruses as it is on F-specific coliphages. On this basis, it may be inferred that a treatment process which removes or inactivates all or a proportion of F-specific coliphages will remove or inactivate all or a proportion of enteric viruses. Bacteriophage MS2 is a well characterised F-specific phage and is used particularly for demonstrating the efficacy of UV disinfection. Details of methods for the detection of F-specific coliphages are described elsewhere[23] in this series.

2.4 Bacteriophage specific to *Bacteroides fragilis*

Bacteroides fragilis is a Gram-negative obligate anaerobe which can be found in high numbers in the intestines of humans and animals. One strain of *B. fragilis*, designated HSP40, has been found in 10% of human faeces but not in the faeces of animals and are, therefore, human specific. It also prompted the thought that bacteriophages specific to this strain would indicate human faecal pollution. Further studies did not find *B. fragilis* HSP40 in wastewater from abattoirs or water contaminated by wildlife.

In order to increase the sensitivity of the test, new *B. fragilis* strains have been sought which are more sensitive to the phage. In a study of 115 strains of *B fragilis,* one strain, designated RYC2056[24], was found to detect higher numbers of bacteriophages in wastewater containing domestic effluent than HSP40, detecting

between 10 and 1,000 plaque forming units per ml in urban wastewater. The same strain can detect phages in animal faeces although the numbers were significantly lower in wastewater containing animal faeces than in urban wastewater. This finding makes it a good candidate for detecting human faecal contamination. Details of a method for the detection and enumeration of bacteriophages specific to *B. fragilis* RYC2056 are described elsewhere[23] in this series.

2.5 Microbial source tracking

The use of microbiological indicators of faecal pollution is well established in water quality monitoring. Microbial source-tracking (MST) techniques are being developed to help determine the source of such pollution. MST has advanced considerably beyond the assessment of point source inputs, and their contribution to total bacterial numbers. Efforts are now being made to use it to analyse diffuse pollution inputs and to determine the original host species of faecal contamination. Any MST approach requires the identification and measurement of a parameter that can be associated to a particular host animal group. An ideal MST parameter would show absolute specificity (found only in the host animal group of interest, and no other) and sensitivity (found in every individual animal of that host group). In addition, an ideal MST parameter would enable estimation of the concentrations of all pathogens, or of all indicator bacteria (e.g. would have the same pattern of survival and movement through a catchment) to enable quantification of each source of faecal pollution. Target parameters with these ideal characteristics may not exist, and certainly have yet to be identified for any of the array of host animal groups that would be of interest.

However, even if ideal MST targets may never be found, many possibilities have been investigated, and at least some useful information has been obtained for environmental management. The reader is referred to two special issues of the journal *Water Research* for further information (*Water Research* 2007, volume 41 issue 16 and *Water Research* 2009, volume 43 issue 19). Candidate micro-organisms for source-tracking have included *Bacteroides* spp. and *E. coli* in addition to other biological (e.g. bacteriophage, human and animal- specific viruses) and chemical (e.g. caffeine, faecal sterols and stanols) approaches. The two main microbiological approaches have been split into library-dependent and library-independent methods. Library-dependent methods use a general approach of cross-referencing an unknown sample with that of a library of samples collected and characterised from known host groups (e.g. antibiotic resistance-profiles). Library-independent methods use a general approach of measuring a parameter from a sample that has been previously validated in terms of host animal specificity and sensitivity (e.g. bacteria from the *Bacteroides-Prevotella* group; F-specific RNA bacteriophage). Many studies report varying degrees of success with each approach; many field trials report at least some success and appear to provide at least some useful information. It seems likely at the current time that a multi-technique approach may be required to assist understanding of pollution sources at many recreational water sites.

Until the performance characteristics, strengths and limitations of each approach are more fully understood, the information provided by MST techniques must be evaluated carefully. Given these uncertainties, the current approach adopted by the Environment Agency is that MST information is a useful investigative tool, but should not be the sole evidence-base for environmental management decisions. MST is, however, a rapidly developing area, and seems likely to become a useful tool for such decisions.

3 Water-borne pathogens

Microbial pathogens in recreational surface waters can potentially be transmitted to people by three routes; contact with the water itself, by ingestion or by inhalation. Swimming pool infections may arise from bather faecal release or growth of pathogens in the water or filter. Some pathogens may also originate from the bodies of bathers which may be picked up by others.

The following groups represent the most frequently detected pathogens in environmental waters:

3.1 Bacteria

3.1.1 Campylobacter

Bacteria of the genus *Campylobacter* are members of the family Spirillaceae and are the most common cause of human bacterial gastro-enteritis in the UK. Campylobacteriosis occurs most frequently in the summer months, primarily due to foodborne infection, and the most commonly isolated species is *Campylobacter jejuni*. The organism can be carried asymptomatically by cattle, sheep, poultry and other birds, and is also isolated from surface and waste waters. Occurrence in surface waters is strongly linked to rainfall, temperature and contamination by waterfowl. Most reported waterborne outbreaks in the UK have been associated with private water supplies because of a lack of protection from contamination, and absent or inadequate treatment and disinfection[25]. *Campylobacter* species can survive in water for some days, but compete poorly with other microflora and do not survive well in summer periods with higher ambient temperatures and UV exposure. However they are known to enter a viable-nonculturable state, which may enhance survival in the environment. *Campylobacter* are highly susceptible to chlorination and ultraviolet disinfection at the doses typically used in water treatment and should, therefore, not be a risk in treated drinking water or swimming pools, unless subject to significant post treatment contamination. Private water supplies without adequate disinfection represent a greater risk of infection. *E. coli* is not an adequate indicator for the presence of *Campylobacter* in water, but is appropriate for demonstrating adequacy of water treatment. Details of methods for the detection of *Campylobacter* species are described elsewhere[26] in this series.

3.1.2 *Arcobacter*

The genus *Arcobacter* was proposed in 1991 to accommodate two species of *Campylobacter* which were aerotolerant. The genus has now been increased to 12 species. Of the two original species, *A. cryaerophilus* is associated with gastro-enteritis but *A. nitrofigilis* is a nitrogen fixing bacterium associated with plants growing in salt marshes. Two additional species, *A. butzleri,* and *A. skirrowii*, have been isolated from humans and animals with diarrhoea and aborted foetuses of sheep, pigs and cattle.

Arcobacter species, unlike *Campylobacter*, are not a major cause of gastroenteritis in humans but are the cause of a newly emerging zoonotic disease. They have been isolated from wastewater, surface water, ground water and sea water. The potential routes of infection are person to person, food, including shellfish, water and companion animals.

Unlike *Campylobacter*, not all species of *Arcobacter* are thermotolerant and although the use of the selective and enrichment media for *Campylobacter* described in this series may isolate *Arcobacter*, raising the incubation temperature to 42°C will restrict the numbers of *Arcobacter* that can be isolated. *Arcobacter* will grow under micro-aerophilic conditions.

The colonial morphology and Gram stain reaction of *Arcobacter* are similar to those of *Campylobacter*, the major difference being that *Arcobacter* will grow aerobically ideally at 30°C but also at 37°C.

A recently published review of *Arcobacter* describes the taxonomy, clinical significance, isolation and differentiation in detail[27].

3.1.3 Verocytotoxin producing *E. coli*

Some strains of *E. coli* can cause serious diarrhoeal disease. Several classes of diarrhoeagenic *E. coli* are now recognised. They are defined by the possession of distinct virulence factors. The most important of these are the Vero-cytotoxin-producing *E. coli* (VTEC), in particular VTEC of serogroup O157, but other *E. coli* serogroups may contain VTEC members (for example O26, O45, O103, and O104). Typical symptoms of people infected with *E. coli* O157 range from mild diarrhoea, fever and vomiting to severe, bloody diarrhoea and painful abdominal cramps. In 10 - 15 % of cases, a condition known as haemolytic uraemic syndrome occurs, which can result in kidney failure. Individuals of all ages can be affected but children up to ten years old and the elderly are the most at risk. The infectious dose for *E. coli* O157 is relatively low compared with other bacterial causes of gastro-enteritis, perhaps as low as 10 organisms.

The reservoir for *E. coli* O157 appears to be healthy cattle and the bacterium can be transmitted to recreational and environmental waters via a variety of routes. *E. coli* O157 can survive for several days in the aquatic environment with similar survival characteristics as environmental *E. coli*. VTEC are susceptible to chlorination and ultra violet disinfection at the doses normally used in water treatment. Private water supplies may be at greater risk, and outbreaks associated with *E. coli* O157 (and *Campylobacter*) have been reported for bathing beaches[28], improperly chlorinated swimming pools[29] and children's paddling pools[30]. Conventional *E coli* tests are adequate indicator tests for the presence and survival of VTEC and other pathogenic *E. coli* in water.

VTEC may not be isolated or may not be recognised by the normal analytical methods for *E. coli*, and specific isolation methods are required. However, if *E. coli* is detected in a water supply it should be assumed that VTEC could also be present. Details of methods for the detection of *E. coli* O157 are described elsewhere[20] in this series.

3.1.4 *Salmonella*

Species of *Salmonella* are members of the family Enterobacteriaceae and are the causative agents of typhoid, paratyphoid fever, and milder forms of gastro-enteritis. The enteric fevers (typhoid, caused by *Salmonella* Typhi, and paratyphoid, caused by *Salmonella* Paratyphi) remain important contributors to water-borne disease world-wide, although nowadays very rarely in developed countries. Strains of *Salmonella* can be subdivided into more than 2000 serovars, most of which are associated with gastro-enteritis. *Salmonella* Typhi and *Salmonella* Paratyphi are only associated with human infection but the other serovars of *Salmonella* are found commonly in the faeces of animals and agricultural livestock, and have been recovered from poultry, eggs and meat products. Food-borne contamination is the major route of infection for these bacteria, but transmission can occur by water contaminated with faecal material. Shellfish can also become contaminated if the harvesting water contains faecal material. Survival in surface water is limited to hours or days, depending on the

amount of contamination and the water temperature. Some studies have indicated that serovars of *Salmonella enterica* are able to survive and multiply inside amoebae and other protozoa[31, 32]. Species and serovars of *Salmonella* are susceptible to normal methods of disinfection used in the water industry. Untreated private water supplies and uncovered storage tanks may, however, be at risk from avian (for example, pigeons and seagulls) faecal contamination that may contain *Salmonella*. *E. coli* is an adequate indicator for the presence and survival of *Salmonella* in water. Details of detection methods for *Salmonella* are described elsewhere[33] in this series.

3.1.5 *Shigella*

Species of *Shigella* are members of the family Enterobacteriaceae and cause bacillary dysentery (shigellosis) in humans. The *Shigella* group is divided into four main sub-groups differentiated by biochemical and serological tests. *Shigella dysenteriae, Shigella sonnei, Shigella flexneri* and *Shigella boydii* are the organisms of concern. *Shigella sonnei* is the most common species found in the UK and causes the mildest form of the disease. Person-to-person contact, faecally contaminated food and, less frequently, water are the main routes of transmission. Survival in surface water is limited to hours or days, depending on the amount of contamination and the water temperature. Shigellae are susceptible to chlorination and ultra violet disinfection at the doses used in water treatment. *E. coli* is an adequate indicator for the presence and survival of *Shigella* in water. Details of detection methods for *Shigella* are described elsewhere[33] in this series.

3.1.6 *Yersinia*

Species of *Yersinia* are members of the family Enterobacteriaceae, some species of which cause diseases in humans and other mammals. Human plague, caused by *Yersinia pestis*, is not a water-borne disease. Other species, including *Yersinia enterocolitica*, *Yersinia intermedia*, *Yersinia kristensenii*, *Yersinia frederiksenii* and *Yersinia pseudotuberculosis*, may produce symptoms ranging from subclinical and mild diarrhoeal infections to rare severe infection including septicaemia. Some serotypes of *Yersinia enterocolitica* are more frequently associated with human disease than others. *Yersinia* species can be isolated from natural waters and may be associated with farms and meat processing plants, and human pathogenic serotypes of *Yersinia enterocolitica* have been found in wild and domestic animals. There is evidence that some species of *Yersinia* can grow in water, whilst *Yersinia enterocolitica* can survive for long periods in the aquatic environment and remain metabolically active at low temperatures. *Yersinia* species are susceptible to chlorination and ultra violet disinfection at doses normally used in water treatment. *E. coli* is an adequate indicator for the presence and survival of *Yersinia* in water. Details of methods for the detection of *Yersinia* species are described elsewhere[26] in this series.

3.1.7 *Vibrio*

Species of *Vibrio* are members of the Vibrionaceae. Some species, most notably, strains of *Vibrio cholerae*, cause gastro-enteritis in humans. *Vibrio* species occur naturally in brackish and saline waters, and some can survive in fresh water systems.

Vibrio cholerae, which causes cholera, can be divided into approximately 140 O-serovars. The strains that usually produce outbreaks of epidemic cholera are toxin-producing strains of the O1 serovar and a more recently reported serovar, O139. Some other serovars of *Vibrio cholerae* can also cause gastroenteritis. The primary

route of transmission for cholera is contaminated water and outbreaks have also been reported following consumption of crops irrigated with wastewater-contaminated water. *Vibrio parahaemolyticus* also causes diarrhoea, often through the consumption of raw, contaminated seafood. *Vibrio fluvialis*, *Vibrio furnissii*, *Vibrio hollisae* and *Vibrio mimicus* are also recognised as causing diarrhoea. Other species of *Vibrio* are associated with wound infections, otitis externa or septicaemia following exposure to environmental waters. *Vibrio* species can grow in environmental waters, particularly when temperatures rise above 10°C and may be associated with sediments, plankton and cyanobacterial blooms. *Vibrio* are susceptible to chlorination and ultra violet disinfection at doses normally used in water treatment. Details of methods for the detection of *Vibrio* species are described elsewhere[26] in this series.

3.1.8 *Leptospira*

Species of *Leptospira* are members of the family Leptospiraceae. Three species are recognised, but *Leptospira interogans* (including serogroup *icterohaemorrhagiae* in rats) is the only pathogenic species. *Leptospira interrogans* strains can be subdivided into over 20 serogroups, which contain more than 200 serovars. If infected with leptospires, rodents and some domestic animals may shed them continually in their urine. Man acquires the disease through contact with urine contaminated water; the portal of entry is the skin via wounds or mucous membranes, from whence the bacteria gain access to the bloodstream. Bacteraemia then carries the organisms to sites throughout the body including the liver, kidneys, cerebral spinal fluid and eyes. In a mild form it causes fever, headache and myalgia. In the severe form of leptospirosis (Weil's disease, which can be fatal), vomiting and diarrhoea, jaundice, haemorrhages and liver enlargement can also occur. After infection humans, as well as other animals, continue to shed the bacteria in their urine for some considerable time, so contaminating the environment and permitting further spread.

Isolation of leptospires from water samples can take several months, but is seldom merited as non-pathogenic species are also common in some environmental waters.

3.1.8 Other bacteria

3.1.8.1 The Pseudomonas group and *Pseudomonas aeruginosa*

The family Pseudomonadaceae contains a number of genera that have been split from the genus *Pseudomonas* as the taxonomy has become more clearly understood. The members of the Pseudomonadaceae are Gram-negative rods, and are widespread in nature, occurring commonly in water and soil. Many are capable of growth in relatively low-nutrient environments. Some species can be pathogenic for humans and are particularly important as a cause of nosocomial (hospital acquired) infection because of their resistance to many antibiotics and disinfectants and their ability to colonise low-nutrient aquatic environments.

Pseudomonas aeruginosa is ubiquitous in fresh water, wastewater and soil and can also be isolated from the faeces of animals and humans, although it is not considered to be a faecal organism and its presence is probably transitory. The organism can grow in very low nutrient aqueous environments and can survive for many months in water at ambient temperatures. *Pseudomonas aeruginosa* is an important opportunistic pathogen and is particularly significant as a cause of nosocomial infections. *Pseudomonas aeruginosa* causes a wide range of infections, but the vast majority of people exposed to *Pseudomonas aeruginosa* suffer no adverse health effects. Community acquired infections arising from *Pseudomonas aeruginosa* are

often localised and some have been associated with contact with contaminated water. Typical infections due to *Pseudomonas aeruginosa* associated with recreational waters are folliculitis (an infection of the hair follicles), otitis (commonly known as swimmers ear) and infections of wounds and eyes. Many strains are resistant to reasonably high levels of disinfectant, particularly when they are associated with biofilms. Under such circumstances, these organisms may cause problems with pool water quality. Failure to maintain disinfectant levels, or to provide adequate treatment, or cleaning regimes, may result in pool waters, or treatment systems, becoming colonised with *Pseudomonas aeruginosa*. Details of methods for the detection and enumeration of *Pseudomonas aeruginosa* are described elsewhere[34] in this series.

3.1.8.2 *Aeromonas*

Species of *Aeromonas* are members of the Aeromonadaceae. They are natural inhabitants of surface water environments and, consequently, are common in source waters. Although the understanding of the taxonomy of the genus has improved it remains difficult to readily identify the species that can be defined by molecular genetic methods. The majority of species are motile and are common in natural water where they may sometimes form a large proportion of the total heterotrophic bacterial flora. Aeromonads can be present in high numbers in fresh waters both in the presence and absence of faecal pollution. High numbers are common in wastewater effluents but are usually of different species to those found in pristine waters. *Aeromonas* species are, generally, readily killed by chlorine and other commonly used water disinfectants. Details of methods for the detection and enumeration of *Aeromonas* are described elsewhere[34] in this series.

3.1.8.3 *Staphylococcus aureus*

Species of *Staphylococcus* are members of the family Micrococcaceae. The Staphylococci are divided into coagulase positive and coagulase negative strains, the former including *Staphylococcus aureus* and *Staphylococcus intermedius*, the latter including *Staphylococcus epidermidis*. Staphylococci are widely distributed and may be isolated from food as well as from faeces, skin and mucous membranes of warm blooded animals. They may be found in environmental sources, and, as they are opportunistic pathogens of man, may be transmitted via contaminated waters causing non-diarrhoeal diseases such as eye, ear, nose and skin infections. As they are common skin organisms they may be found in swimming pools, spa and hydrotherapy pools, especially where the bathing load is heavy. Details of methods for the detection and enumeration of *Staphylococcus aureus* are described elsewhere[35] in this series.

3.1.8.4 *Legionella*

The genus *Legionella* includes over 40 species of bacteria that occur naturally in the aquatic environment. Occasionally, some species of these bacteria cause infections in humans and these infections are collectively called legionellosis. The most common infection is Legionnaires' disease which is an acute severe pneumonia. The most common cause of Legionnaires' disease is *Legionella pneumophila* which can be subdivided into 16 serogroups, of which serogroup 1 is the most common type isolated from patients and the environment. In addition, several species of *Legionella* can cause a short-lived, self-limiting influenza-like illness without pneumonia (Pontiac fever or Lochgoilhead fever). At least 18 species of *Legionella* have been associated with disease in humans but *Legionella pneumophila* remains the most common cause in the UK and abroad. Infection normally results from the inhalation of an aerosol derived from water containing the bacterium. This has most often been associated with hot

and cold water systems in, for example, large buildings, cooling towers and evaporative condensers, and spa pools.

Although *Legionella* species occur naturally in water they can only grow with the assistance of other micro-organisms. They prefer warm water at temperatures between 30 - 45°C. *Legionella* species have been shown to be capable of growth within a variety of protozoa, particularly amoebae, and can grow in association with other bacteria in biofilms. Many protozoa are intrinsically more resistant to biocides (such as chlorine) than *Legionella*, so that growth within the protozoan provides protection. In normal situations, the growth of *Legionella* can be controlled by the application of good design and maintenance combined with some relatively simple precautions. Rigorous attention to cleanliness and adherence to a good biocide or disinfectant regime enables control in cooling systems and spa pools. Guidance on the control of *Legionella* in water systems is given elsewhere[36]. Further information on *Legionella* and details of methods for their detection are described elsewhere[37, 38].

3.1.8.5 Mycobacteria

Mycobacteria are a group of bacteria that are characterised by their slow growth. Apart from the obligate pathogen *Mycobacterium tuberculosis* (which causes tuberculosis), the genus *Mycobacterium* includes a number of other species which cause disease in humans. The organisms primarily occur in water and soil. The species of most concern are *Mycobacterium avium* and its close relatives *Mycobacterium intracellulare* and *Mycobacterium scrofulaceum*. These are commonly grouped together and referred to as the *Mycobacterium avium* complex. The source of *Mycobacterium avium* complex appears to be the environment and infection is thought to occur by inhalation or ingestion. There is growing evidence to indicate that untreated and treated water may be a source of infection. Other species that have been associated with outbreaks of disease in which water systems may have been the source include *Mycobacterium kansasii* (lung infections), *Mycobacterium genavense* (disseminated disease), *Mycobacterium xenopi* (lung infections), *Mycobacterium abscessus* (wound infections), and *Mycobacterium fortuitum* (various infections including skin, wound and lung). *Mycobacterium marinum* causes skin infections (swimming pool granuloma) associated with swimming pools. Spa pools may also become colonised and have been reported to be sources of infections.

Mycobacteria are ubiquitous in the environment being found in soil, house dust, water (including wastewater, surface water, groundwater and drinking water), a wide range of mammals, poultry and other vertebrates. *Mycobacterium avium* and closely related species will grow in water to which no additional nutrients have been added. Mycobacteria are relatively resistant to chlorine and many species can survive free chlorine levels of 1 mg/l and are between 100 and 330 times more resistant than *E. coli* [39]. Considering their widespread occurrence in source waters and their survival characteristics, it is not surprising that these saprophytic mycobacteria can colonise swimming and spa pool filters and biofilms.

Mycobacteria can be cultured but are relatively slow-growing. Isolation may be improved by pre-treatment of samples to reduce the presence of non-mycobacterial contaminants. Acids, alkalis or detergents are commonly used for this process.

3.2 **Viruses**

Human enteric viruses may be present in all types of water and wastewater and can be found in association with related particulate matter (soils, sediments and sludge),

and shellfish[40]. The main source of human viral contamination is wastewater which contains viruses shed in faeces. Swimming pools may also become contaminated with faecal material containing viruses. Many groups of viruses replicate in the gastro-intestinal tract; the most important of which are described in the following sections. Enteric viral infections may be asymptomatic (without causing clinical symptoms) such as with many enteroviruses but infections of norovirus and rotavirus in susceptible persons usually cause gastro-enteritis.

Viruses are obligate intra-cellular parasites and, therefore, will only replicate within the living cells of a susceptible host. No replication will occur in the environment but enteric viruses are robust and may persist for several weeks in cool, dark places. Viruses are sensitive to the effects of chlorine. Untreated wastewater may contain large numbers of virus particles while their concentration in tertiary treated effluent is usually low. The viral content of a river will largely reflect its wastewater component. The numbers and types of viruses present in wastewater will reflect the viruses circulating at any time within the local population and will vary from year to year and between seasons.

Viruses are, in the main, species specific so that animal viruses are unlikely to infect humans. The major route of enteric virus transmission is person-to-person contact and from contaminated foodstuffs. Worldwide, outbreaks of norovirus and Hepatitis A and E have resulted from contaminated drinking water and more rarely from contaminated recreational water.

The distribution of human enteroviruses in wastewater and environmental waters has been investigated using cell culture for many years and recent advances in molecular detection have enabled similar studies of norovirus, rotavirus and adenoviruses. The presence of enteroviruses in water is a marker for the presence of wastewater in the environment, but it is not an indicator of the other groups of human enteric viruses as the epidemiology of each group varies considerably. No direct relationship between the presence of enteroviruses or any other virus and bacterial indicator organisms has been demonstrated. Viruses may be present when indicators are not.

3.2.1 Enteroviruses

The enterovirus group is in the *Picornaviridae* family and includes the polioviruses, coxsackievirus A and B, echoviruses and more recently identified members that are given numerical designations. The enteroviruses are now classified as the following species; Hepatitis virus A (HEV-A) formerly some serotypes of coxsackievirus A and enterovirus 71; HEV-B formerly coxsackievirus A9, coxsackievirus B and echoviruses; HEV-C formerly some serotypes of coxsackievirus A, and PV for poliovirus. This group of viruses replicate in the gastro-intestinal and respiratory tracts. The infections are commonly asymptomatic, but may cause flu-like symptoms, occasionally meningitis and, rarely, paralysis. Enterovirus infection does not result in gastro-enteritis unless part of a more generalised illness. The worldwide poliovirus eradication programme has seen a shift from vaccination with live poliovirus to the use of killed vaccines. As the killed virus is not excreted in faeces, poliovirus is not present in sewage. Other enteroviruses are commonly shed throughout the year with the different serotypes of coxsackievirus B and echovirus predominating from year to year. Waterborne transmission has not been confirmed and, as so many infections are asymptomatic, it would be difficult to identify. Details of detection methods for enteroviruses are described elsewhere[41] in this series.

3.2.2 Norovirus

Norovirus is the internationally accepted name for the viruses of the *Caliciviridae* family, formally known as Norwalk viruses or small round structured viruses (SRSV). They are the major cause of sporadic and epidemic viral gastro-enteritis in adults, but are also the common cause of infections in children. Human Noroviruses are divided into two main genogroups (I and II) with many genotypes or strains. A numbering system has replaced the previous geographical names. As with enteroviruses, the dominant strains circulating in a population changes over time so that re-infections are common. Asymptomatic infections have also been recognised. Transmission is primarily via the person-to-person route (e.g. vomit aerosols or contamination of surfaces). Waterborne transmission has been identified, although rarely in the UK. Transmission also occurs through the consumption of wastewater contaminated shellfish.

Another member of the *Caliciviridae* family, Sapovirus, causes mild diarrhoea mainly in young children.

3.2.3 Rotavirus

Rotaviruses (*Reoviridae* family) comprise six serogroups and are further divided into serotypes and genotypes. Serogroup A is the most common rotavirus infection of humans, although members of Group B and C can also infect humans. Diarrhoea is the major symptom which is self-limiting although in developing countries rotaviruses are a major cause of death, particularly in children under 5 years old. The infection often first occurs below the age of one year and results in long-term immunity. A few waterborne outbreaks world-wide have been reported [(42)].

3.2.4 Adenovirus

The adenoviruses (*Adenoviridae* family) comprise over 40 different serotypes, of which 40 and 41 are known to cause gastro-enteritis. It is a mild diarrhoea occurring mostly in babies which results in long-term immunity. Other serotypes are detectable in wastewater and water as they can replicate in the gastro-intestinal tract. As these are DNA viruses they seem to be more robust to environmental pressures than other enteric viruses in the environment. The adenoviruses may develop into a significant tool for the investigation of the sewage content of environmental water. Studies have shown that adenoviruses are widespread in recreational water, can be concentrated by current methods and are readily detectable by molecular techniques[(43)] . The presence of this virus group can be related to the presence of indicator organisms[(44)] and are therefore a marker of human sewage contamination. A few swimming pool associated adenovirus outbreaks have been reported[(45)].

3.2.5 Astrovirus

Astroviruses (*Astroviridae* family) include at least eight serotypes that infect humans, causing gastro-enteritis, particularly in children. Waterborne transmission is rare.

3.3 **Protozoa and microsporidia**

In many countries, especially where drinking water supplies are chlorinated, pathogenic protozoa have become the leading cause of waterborne disease outbreaks for which the aetiologic agent has been identified. The recreational use of contaminated waters poses a potential route of transmission of several pathogenic protozoa and fungi including *Cryptosporidium, Giardia, Toxoplasma, Entamoeba*

histolytica, Cyclospora and the Microsporidia. These protozoa are faecal in origin and can enter watercourses via wastewater contamination, animal faecal contamination or agricultural land run-off. In the UK the majority of waterborne outbreaks reported to Public Health England (PHE) formally the Health Protection Agency (HPA) between 2000 and 2013, were due to *Cryptosporidium* and were mainly associated with swimming pool facilities.

The free-living amoebae *Naegleria fowleri* and *Acanthamoeba* spp. have also been associated with waterborne disease. The term 'free living' is applied to these organisms because they do not require the infection of a host organism to complete their life cycle. They are opportunistic pathogens that are ubiquitous in freshwater and soil and are associated with severe, often fatal, disease in the case of *Naegleria fowleri*. However, *N. fowleri* is a very rare infection in the UK.

3.3.1 *Cryptosporidium*

There are a number of species of *Cryptosporidium* that are infective for humans but *Cryptosporidium hominis* and *C*. *parvum,* are the main agents of human cryptosporidiosis in the UK. *Cryptosporidium hominis* is transmitted between humans; *C. parvum* has a wider host range that includes humans, cattle, sheep, goats and horses. Cryptosporidiosis is contracted by the ingestion of oocysts that are excreted by an infected host, which can peak during acute infection at a concentration of 10^6 to 10^7 per gram of faeces. The infective dose of the disease is low and, depending on the strain, may be fewer than 10 oocysts.

Infection can result in physiological and transport defects in the gut epithelium, leading to profuse watery diarrhoea, which may contain mucus. Other symptoms include abdominal pain, nausea, vomiting, fever, and anorexia resulting in dehydration and weight loss, although infection can also occur without resulting illness ("asymptomatic" carriage). The *Cryptosporidium* life cycle is "direct", requiring no intermediate host, and culminates in the shedding of large numbers of oocysts from infected hosts

Wastewater effluent, direct contamination with animal faeces and agricultural land run-off are all routes by which oocysts contaminate surface and groundwater bodies. *Cryptosporidium* oocysts are resistant to environmental conditions and many chemical disinfectants. Consequently they are able to survive for prolonged periods in water and have been detected at all stages of the water cycle from source waters such as springs, rivers and reservoirs through to treated drinking water. UV irradiation is now accepted as an effective process for inactivating oocysts in water.

In the UK waterborne disease outbreaks due to *Cryptosporidium* have been reported for drinking water and waters in recreational use, with the majority of outbreaks being associated with swimming pools. The presence of *Cryptosporidium* in swimming pools is generally attributed to accidental faecal release and inadequate treatment regimes. Since oocysts are very resistant to many disinfectants including chlorine, treatment options for swimming pools are limited. At present, filtration is considered to be the most reliable way of eliminating oocysts from pool water. However, the process can be easily overwhelmed by accidental faecal releases or wastewater intrusion and the recommended course of action in the event of such gross contamination is to close the pool and apply a long period of filtration. This is described by PWTAG Technical Note 2, www.pwtag.org. Pools with high rate filters may be more effectively decontaminated by using highly elevated chlorine levels, and small pools may be emptied, cleaned and re-filled.

3.3.2 *Giardia*

Giardia species are flagellated protozoans that parasitise the small intestines of mammals, birds, amphibians and reptiles. *Giardia duodenalis* has a global distribution and is the most common intestinal parasite of humans worldwide.

Giardiasis may present as an acute and self-limiting condition with diarrhoea, weight loss and abdominal cramps lasting from 7 to 10 days or as a sub-acute, chronic condition giving symptoms of chronic diarrhoea and intestinal malabsorption. Asymptomatic and mild infections are also common. The disease is contracted through the ingestion of the cyst. Experimental evidence suggests that the infective dose of *Giardia* may be as low as 10 to 25 cysts. At the peak of infection, cysts are excreted at a concentration of 10^6 cysts per gram of faeces and the duration of shedding may last for up to six months post infection, albeit at lower concentrations.

Giardia is indigenous to the UK. It is a less frequent cause of waterborne outbreaks of disease in the UK than *Cryptosporidium* but has been associated with significant, frequent outbreaks elsewhere. Waterborne disease outbreaks in the UK have mainly been associated with recreational water use in particular with swimming pool facilities, although less frequently than *Cryptosporidium*. The cyst is relatively resistant to chlorine compared with *E. coli* although it is less resistant than *Cryptosporidium* oocysts. Chlorination and filtration of swimming pool water is relied on to inactivate and remove *Giardia* cysts.

3.3.3 *Cyclospora cayetanensis*

Cyclospora cayetanensis is a coccidian parasite that causes protracted, relapsing gastroenteritic disease in humans similar to cryptosporidiosis and is contracted through the ingestion of oocysts. The incidence of the disease in the UK is very low and generally associated with travel to other countries where the disease is endemic.

Humans may be the only host for *Cyclospora cayetanensis* as an animal reservoir has yet to be confirmed. Unlike *Cryptosporidium*, *Cyclospora* oocysts are excreted in a non-infectious state by an infected host and require a period outside a host to sporulate and become infectious. The non-infectious nature of newly formed oocysts suggests that an indirect route, via contaminated water and food, rather than direct person-to-person contact is involved in the majority of infections. The use of contaminated water for drinking has been implicated as the source of *C. cayetanensis* in recorded outbreaks in Nepal and its use for crop irrigation purposes may have caused outbreaks associated with imported fresh produce in the US.

Cyclospora oocysts are believed to be as resistant to environmental and chemical degradation as are other protozoan oocysts/cysts.

3.3.4 *Toxoplasma gondii*

Toxoplasma gondii is a tissue cyst-forming coccidian parasite that causes the condition toxoplasmosis. The parasite has a complex life cycle that involves definitive and intermediate hosts. Cats are the definitive host and intermediate hosts possibly include all mammals. The parasite is shed as unsporulated oocysts in the faeces of the definitive host; they then sporulate to become infective and are transmitted to an intermediate host via the oral route.

The symptoms of toxoplasmosis in the immuno- competent ranges from benign to a glandular fever type illness to a serious condition that results in abortion in humans

and animals or congenital abnormalities. The infection can remain dormant and re-activate if the immune system is damaged. In untreated, immunocompromised AIDS patients toxoplasmosis is very serious and accounts for the death of up to 30% of these patients.

Human infections arise from the ingestion of oocysts from cat faeces, either directly or on contaminated fomites, food or water, or from the consumption of other life cycle stages in inadequately cooked meat from infected intermediate hosts. *T. gondii* has a worldwide distribution and sero-prevalence studies indicate up to 100% exposure in some populations in developing countries.

The sporulated oocysts of *Toxoplasma* are very resistant to environmental conditions and disinfectants although recent research indicates UV irradiation may be effective for the inactivation of cysts. Waterborne disease outbreaks that were the result of the contamination of raw water with cat faeces have been reported in the US, Canada and Columbia. There is no documented evidence of waterborne disease outbreaks in the UK.

3.3.5 *Entamoeba histolytica*

Entamoeba histolytica is a single-celled parasitic protozoan which is found in humans worldwide. It has a two stage life-cycle comprised of an actively feeding and dividing trophozoite and a dormant cyst.

Entamoeba parasitise all classes of vertebrates, and humans can be host to at least 6 species, but only *Entamoeba histolytica* is known to cause disease. *E. histolytica* is the third leading cause of morbidity and mortality due to parasitic disease in humans (after malaria and schistosomiasis), it is estimated to cause up to 100,000 deaths worldwide every year. Transmission of the infection is via ingestion of cysts present in faecally contaminated food and water. The presentation of the disease may range from an asymptomatic condition to profuse diarrhoea or a fatal condition resulting from the invasion of tissues by the parasite.

Entamoeba histolytica is not endemic in the UK and diagnosed infections in the UK are attributed to travel in countries where the disease is common.

3.3.6 *Acanthamoeba*

Acanthamoeba is a genus of environmental, free-living amoebae found in most soil and water habitats. *Acanthamoeba polyphaga, A. castellanii* and *A. culbertsoni* have been identified most frequently associated with human disease. In favourable conditions, *Acanthamoeba* normally exist as a trophozoite that feeds on bacteria. When conditions deteriorate the organism can form cysts that are resistant to extremes of temperature, disinfection and desiccation and account for the almost ubiquitous presence of the organism in the environment.

Human pathogenic species of *Acanthamoeba* cause two clinically distinct diseases: granulomatous amoebic encephalitis (GAE) and inflammation of the cornea (keratitis). GAE is a rare, chronic disease of the central nervous system (CNS) of the immuno-suppressed that is either sub-acute or chronic, but is invariably fatal. The precise source of GAE infection is unknown; it is believed that it is a secondary infection resulting from a primary infection occurring elsewhere in the body such as the respiratory tract or skin. *Acanthamoeba* keratitis affects previously healthy people; it occurs with far greater frequency than GAE and is a severe, potentially blinding

infection. Water is the principal route of transmission of *Acanthamoeba* keratitis and contact lens wearers are most at risk. Infection is mainly attributed to poor hygienic practices in the care of contact lenses or to lens wearers swimming or participating in water sports on ponds or lakes. In non-contact lens related keratitis, infection arises from trauma to the eye and contamination with environmental matter such as soil and water.

Acanthamoeba cysts are resistant to chlorine-based disinfectants and are removed by filtration in swimming pools and spas.

3.3.7 *Naegleria fowleri*

Naegleria fowleri is a free-living amoeba present in fresh water and soil; it is thermophilic and can tolerate temperatures of up to 45 °C. The life cycle includes an environmentally resistant encysted form. The organism occurs worldwide and has been isolated from both natural and artificial thermally enriched habitats such as natural hot springs, fresh water lakes, domestic water supplies, chlorinated swimming pools, water-cooling towers and effluent from industrial processes.

Naegleria fowleri causes primary amoebic meningoencephalitis (PAM) in humans; the disease is almost always fatal. Victims are usually healthy children and young adults who have had contact with water about 7–10 days before the onset of symptoms. Infection occurs when water containing the organisms is forcefully inhaled or splashed onto the olfactory epithelium, usually from diving, jumping or underwater swimming.

Although PAM is an extremely rare disease, cases have been associated with pools and spas. In the UK one confirmed case of PAM occurred in Bath Spa in 1978. The victim, a young girl, swam in a public untreated bathing pool fed with water from thermal springs that, as subsequent analysis confirmed, were contaminated with *N. fowleri*.

The risk of infection can be reduced by minimising the occurrence of the causative agent through appropriate choice of source water, proper cleaning, maintenance, coagulation–filtration and disinfection of pools. Transmission of PAM in managed spas has not been reported.

3.3.8 Microsporidia

Microsporidia are highly evolved fungi (they were previously believed to be a protozoan parasite) with over 1200 species that infect a wide range of animal hosts mainly arthropods and fish. *Enterocytozoon bienusi* and *Encephalitozoon intestinalis* are the microsporidia that are most commonly associated with human infection. They cause a gastroenteritic illness with symptoms of diarrhoea, weight loss, abdominal pain, vomiting and fever. Documented infections with other species of microsporidia, *Encephalitozoon cuniculi* and *Nosema* sp, include keratitis, conjunctivitis, hepatitis, peritonitis, CNS infection and renal failure. While microsporidial diseases are mainly associated with the immuno-compromised there is increasing evidence that the immuno-competent may carry the parasite asymptomatically. Disease in immuno-competent individuals is rare, generally mild and may form part of the spectrum of infections known as travellers' diarrhoea.

Microsporidia form highly specialised, environmentally resistant spores that are excreted in very large numbers (10^{10} per gram of faeces) by infected individuals. Compared to *Cryptosporidium* and *Giardia*, little is known of reservoirs and routes of

transmission of microsporidia infections or the pathogenicity and virulence of spore isolates and their resistance to chemical disinfectants. It is speculated that direct person-person contact, animal vectors and environmental sources are all involved in the transmission of the disease.

The presence of large numbers of microsporidia spores in infected faeces, their demonstrated ability to survive in water for prolonged periods and the detection of spores in a range of surface waters strongly implicates water as a route of transmission of these organisms. However, to date, only circumstantial evidence exists for a link between intestinal microsporidiosis and recreational water use. This current lack of documented, concrete evidence of waterborne outbreaks of intestinal microsporidiosis is attributed to method limitations and lack of diagnostic expertise rather than the absence of such events.

Epidemiological evidence suggests that swimming in public pools is a risk factor for intestinal microsporidiosis for HIV-infected patients. The spores of microsporidia are relatively small compared to *Cryptosporidium* oocysts and *Giardia* cysts and are not removed by the diatomaceous earth filters commonly used to treat swimming pool water. In addition, data suggest that microsporidia spores are relatively resistant to chlorination.

4. Microbial tracers

In many instances, information on the movement of water, whether surface water, ground water or in the soil between the two, saline or wastewater is useful to determine time of travel, dilution and dispersion, movement of contaminants or connectivity between two or more points. Tracking sources of pollution is also an important issue. Such information can be derived from tracer experiments. Early tracer experiments used readily visible material such as sawdust and oranges. Sodium chloride was also used as a tracer by measuring conductivity. There are many different chemical and biological traces which can be employed to study water movement.

To be an ideal tracer, a material must have the following properties.

- It must be non- toxic and, if it is a microbiological tracer, it must be non-pathogenic to animals and humans;

- It must be inexpensive to produce and be available in large quantities if required;

- If dosed into surface water, it should be readily removed by water treatment such that it does not present in, or cannot be detected in, drinking water during routine monitoring programmes;

- The tracer must move with the water but it should not interfere with the natural flow of the water. Chemical solutions, for example, may be more dense than water and fail to mix completely;

- The tracer should be stable over the period of time of the study, particularly in sunlight. It must not be filtered out or absorbed by the matrix through which the tracer experiment is being conducted;

- It must be normally absent from the water or present only in low levels such that a significant increase in tracer concentration can be demonstrated without the use of excessive amounts of tracer;

- There must be a rapid and inexpensive method for detection of the tracer ideally in real time so that the tracer plume can be followed easily.

In practice no ideal tracer exists. Some tracers are or may be toxic, some will be absorbed to soils and many will decay in the environment. Experiments can use a combination of tracers. A microbial tracer can be used in combination with a fluorescent dye or radiotracers such that a tracer plume can be tracked by following the dye or radiotracer and this will tell samplers the locations at which to take samples. The following is a brief overview of the different types of tracers which are available.

4.1 **Chemicals**

The halides chloride, bromide and iodide have been used successfully as tracers. Lithium has also been used to trace wastewater. The use of chemical ions makes detection relatively easy through the use of ion specific electrodes. These will give 'real-time' results which can be used to follow a plume of tracer.

4.2 **Dyes**

The dyes fluorescein and rhodamine WT have been used as tracers. In higher concentrations, the dyes are visible so that plumes can be followed with relative ease. The use of a fluorimeter can give sensitive 'real-time' determinations if required. The addition of large amounts of dye may cause visible colouring and complaints about pollution.

4.3 **Microbiological tracers**

4.3.1 *Lycopodium* spores

Spores of the club moss *Lycopodium clavatum* have been used to trace groundwater. They are small particles 30 µm in diameter which can be stained different colours by biological dyes. They can therefore be used as multiple tracers. Once released into water, they can be captured by the use of plankton nets, washing the nets and concentrating particulate material for microscopy. One disadvantage is that the spores, if suspended in air, may be explosive.

4.3.2 Yeasts

Yeasts which are readily available in large quantities and easy to detect can be used in tracer experiments. Their use is however restricted to relatively clean waters. *Saccharomyces cerevisiae* is readily available in large quantities as brewer's yeast and can be detected by membrane filtration and culture on a suitable medium.

4.3.3 *Bacillus atrophaeus*

The bacterial spore is highly resistant to environmental inactivation. There are methods for easy detection but again there may be a large background count of naturally occurring spores. Spores of *Bacillus atrophaeus* (formally *Bacillus globigii* and *Bacillus subtilis* var *niger*) form characteristic orange colonies when grown on culture media. Spores are available commercially as a dried powder at a concentration

of approximately 5 x 10^{11} colony forming units (cfu) per gram of powder. They are ideal for marine tracer work, for example, following the discharge of wastewater from a sea outfall. However, their use is restricted in waters where shellfish are grown and harvested. Repeated experiments at one site may result in a considerable background count developing as they can survive for many months or even years in the environment. Allowing time for spores to disperse may be the only option. It is also relatively easy to contaminate equipment including boats used for dispersion and sampling equipment.

4.3.4 Bacteriophages

Bacteriophages have a number of advantages as tracers. They are non-toxic, infecting only their specific bacterial host. They are easily cultured in relatively high concentrations, for example 10^{14} plaque forming units (pfu) per ml for MS2 and there is a simple and inexpensive assay method for their detection[46]. They are normally absent or present in very low levels in most waters. Bacteriophages can be concentrated if necessary from large volumes of water[23] or, where there is no background; volumes that can be analysed can be increased by adding water samples to broth with the host, incubation and then looking for the phage. A number of different bacteriophages which do not cross-react with other specific hosts can be used for multiple-source tracing. Being very small particles, they provide information relative to the movement of viruses and even chemical ions. They are also one of the few tracers that can be used in waters where shellfish are cultured and harvested.

However, bacteriophages are readily absorbed to surface and ground water matrices and are inactivated by sunlight and this can limit their use.

A number of bacteriophage have been used as tracers for water including MS2, λ, φX174, and phages for *Serratia marcescens*, *Enterobacter cloacae* and *Pseudoalteromonas*. The latter host is a marine bacterium and therefore its phage is not found in surface waters.

4.4 **Restrictions on the use of microbial tracers in England and Wales**

In England and Wales, a licence is required for the release of microbial tracers into the environment. This licence should be obtained from the Environment Agency before any release commences. The mass release of any tracer must not exceed 10 kg per day within an area defined by the tidal excursion* distance in the direction of the principal axis of the tidal ebb and flood centred on the point of the tracer.

Microbial tracers are only to be used outside the tidal excursion* of shellfish beds or mariculture activity; in particular, the licence holder must ensure that *Bacillus atrophaeus* spores are not released within one tidal excursion of shellfish harvesting and/or mariculture activity.

* The tidal excursion is defined as the net horizontal distance over which a water particle moves during one tidal cycle of flood and ebb at the site in question.

The local Marine Management Organisation District Marine Office and Inshore Fisheries and Conservation Authorities must be notified of the timetable of any works/operations at least 10 days prior to any activities commencing. The District Marine Office must also be notified within 10 days of the completion of the works. For any release of tracers within a Marine Protected area, the relevant Statutory Nature Conservation Body(s) must be notified of the location, timing, nature and quantity of

release at least five days in advance of the release. In all cases, the Environment Agency should be notified of the location, timing, nature and quantity of release at least five days in advance of the release in order to avoid the need to respond if a 'deliberate pollution incident' is reported.

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