Fact Sheets on Emerging Waterborne Pathogens

Final Report to the Department of the Environment
FACT SHEETS ON EMERGING WATERBORNE PATHOGENS

Final Report to the Department of the Environment

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EXECUTIVE SUMMARY

The possibility always exists for pathogenic organisms to contaminate water supplies and cause outbreaks of waterborne illness. The threat which is posed by an organism, in terms of its occurrence and distribution in water sources and amenability to removal or inactivation during conventional water treatment, is often poorly understood or completely unknown.

Government Departments, Local Authorities, Water Utilities, Managers of water treatment processes, and others with responsibility for consumer and media relations may be expected to give assurances about the effectiveness of water treatment processes in protecting public health against emerging pathogens. In such situations it would be important to have access to concise, accurate information on which to base an assessment of the threat to the health of water consumers.

The fact sheets which are included as Appendix A to this report are intended to provide this kind of information, by summarising the state of existing knowledge of the public health significance of selected organisms, the likelihood of their occurrence in water sources, and whether conventional water treatment processes could be expected to give adequate protection against them.

This first series of twenty-two fact sheets includes information on bacteria (Aeromonas, Helicobacter pylori, Campylobacter, Yersinia enterocolitica, the Mycobacterium avium complex, the enterovirulent strains of Escherichia coli, Vibrio, Arcobacter, Salmonella and Legionella), protozoa (Microsporidia, Cyclospora, Isospora, Toxoplasma, and Acanthamoeba) and viruses (Norwalk-like viruses, Coxsackieviruses, Adenoviruses, Astroviruses, Non-Group A Rotaviruses, Hepatitis E virus, and Caliciviruses). The fact sheets have been produced in a standardised format, and each has been restricted to a single sheet of A4-sized paper, printed on both sides.

The fact sheets have been jointly prepared by the Public Health Laboratory Service and WRe.
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1. INTRODUCTION

An “emerging” pathogen may be defined as a micro-organism whose ability to cause disease has only recently been identified. This may be as a result of new technologies or improved isolation methods which make it easier to detect specific pathogens, so that they can be associated with outbreaks of illness when this was previously impossible. On the other hand “emerging” pathogens may arise from changed environmental or living conditions providing new habitats where they may multiply. Examples would be the increased use of some types of air conditioning systems which may become colonised by Legionella, or the extensive use of ophthalmic contact lenses, where organisms such as Acanthamoeba have been found to contaminate cleaning and conditioning fluids.

The potential always exists for pathogenic organisms to contaminate water supplies and cause outbreaks of waterborne illness. For many such organisms efficient methods of detection do not exist, or if they do they may be too technically specialised for general use. When an emerging pathogen is discovered, the threat which is posed by the organism, in terms of its occurrence and distribution in water sources and amenability to removal or inactivation during conventional water treatment, is often poorly understood or completely unknown.

Government Departments, Local Authorities, Water Utilities, Managers of water treatment processes, and others with responsibility for consumer and media relations may be expected to give assurances about the effectiveness of water treatment processes in protecting public health against emerging pathogens. In such situations it would be important to have access to concise, accurate information on which to base an assessment of the threat to the health of water consumers.

The fact sheets which are included as Appendix B to this report are intended to provide this kind of information, by summarising the state of existing knowledge of the public health significance of selected organisms, the likelihood of their occurrence in water sources, and whether conventional water treatment processes could be expected to give adequate protection against them. The list of topics to be covered by the fact sheets is given in the next section.
2. **COMPILATION OF FACT SHEETS ON EMERGING PATHOGENS**

The objective of this project was to produce a series of fact sheets on selected existing, emerging, or re-emerging pathogenic organisms, including bacteria, protozoa and viruses. Each fact sheet would occupy no more than two sides of A4-sized paper.

The supplied list of emerging pathogens to be considered was as follows:--

**Protozoa:**
- Microsporidia
- *Cyclospora*
- *Isospora*
- *Toxoplasma*
- *Acanthamoeba*

**Bacteria:**
- *Aeromonas*
- *Helicobacter pylori*
- *Campylobacter*
- *Yersinia enterocolitica*
- *Mycobacterium avium* complex
- Enterovirulent *Escherichia coli*
- *Vibrio*
- *Arcobacter*
- *Salmonella*
- *Legionella*

**Viruses:**
- Enteric viruses with serious secondary sequelae
- Norwalk-like viruses
- Coxsackieviruses
- Adenoviruses
- Astroviruses
- Non-Group A Rotaviruses
- Hepatitis E virus
- Caliciviruses

Fact sheets have been produced on all of these, with the exception of "enteric viruses with serious secondary sequelae". On this subject, the PHLS virologist, Mrs Jane Sellwood of the Public Health Laboratory, Reading, Berkshire, has made the following comments:--

"Hepatitis B virus may be considered as an enteric virus as it infects the liver but as virus is released into the blood stream and not into the gut it is not relevant to this Fact Sheet. Except for bloodstained stool and other bloodstained human body fluids there will be little shedding of virus into sewage.

"Wild type polioviruses are enteric viruses with potential for severe paralysis in a small number of those infected. However these viruses have been eradicated from North and South America by vaccination. Wild types do still circulate in some parts of eastern
Europe but vaccination campaigns in Africa, Asia and China as part of the WHO Poliomyelitis Eradication Programme have reduced the incidence substantially. Wild type poliovirus is not an emerging pathogen but a well-understood established pathogen which may soon be eradicated.

“Echoviruses are part of the enterovirus group and have similar characteristics to coxsackieviruses. They are established pathogens and well-understood so are not emerging pathogens. Some serotypes may cause meningitis or transient paralysis in a minority of affected patients.”

“There are no enteric viral emerging pathogens with serious sequelae which have not been discussed under the other virus headings.”

A fact sheet of this title has therefore not been produced, since the topic appears to be covered by the other fact sheets concerning enteric viruses.

The following topic headings were specified for the fact sheets:—

Morphology
Biochemical characteristics
Detection methods
Cultural characteristics
Health effects
Routes of transmission
Occurrence in water sources
Sources of exposure
Susceptibility to removal or inactivation by conventional water treatment processes.

In addition, a paragraph has been added at the beginning of each sheet, giving a brief description or definition of the organisms under consideration. The topic headings listed above have been used in all the fact sheets on bacterial pathogens. Slight modifications have been made for the others. For example, “biochemical characteristics” is not an appropriate subject heading for viruses, and that section has been replaced with “physicochemical properties” on the virus sheets. The same section has also been removed from the sheets on parasitic protozoa (though not from Acanthamoeba, which is free-living), and the “morphology” section has been re-named “morphology and life cycle” for all the protozoa.
3. **SUBJECT AREAS IN WHICH INFORMATION IS LACKING**

3.1 **Bacteria**

In general there is more information available on bacterial pathogens than on viruses or protozoa. This is no doubt because they are generally easier to detect, enumerate and study. However, one could always wish for better information about removal of pathogens by water treatment processes, and the effectiveness of different disinfectants for inactivating them. That said, information of this type has been reported in the literature for many years, but because of factors such as different analytical methodology and experimental design, information from one source can rarely be combined or directly compared with information from another.

Of the bacteria included in the fact sheets, some are well-understood, while knowledge of some others is comparatively rudimentary. Some organisms, such as *Vibrio*, have been included not because they are emerging pathogens in the strict sense, but because circumstances could allow them to re-emerge as significant pathogens in developed countries where their occurrence is rare at present. The following sub-sections summarise where significant gaps in knowledge exist.

3.1.1 **Arcobacter**

*Arcobacter butzleri* has only been associated with illness in humans relatively recently, and its role as an enteric pathogen has not been clearly established. There is virtually no information about the incidence of human *Arcobacter*-associated infections in the United Kingdom nor on the likely routes of infection. It is not known how humans become infected with arcobacters. The organisms have been found in various types of water, but the significance of a waterborne route of transmission is unknown. As with *Helicobacter pylori* no direct evidence is available on the effectiveness of water treatment processes against arcobacters, but assumptions can be made on the basis of the similarities between these organisms and campylobacters. Basic studies are needed to develop and evaluate current isolation methods, especially with regard to drinking water. The health significance and epidemiology of *Arcobacter* infections need to be established.

3.1.2 **Campylobacter**

The outstanding issues with regard to *Campylobacter* relate to a clearer understanding of the epidemiology of human infection, improvement of isolation and detection methods, and clarification of the importance of campylobacters in biofilms, particularly in the viable non-culturable state. The ecological distribution, host range, seasonal variation and transmission routes of individual strains have not been determined. Also the mechanisms of pathogenicity have not been established.
3.1.3 *Helicobacter pylori*

While information continues to accumulate on *H. pylori*, its mode of transmission is not entirely understood. The association of this organism with gastritis and gastric and duodenal ulcers is now well established, but its public health significance in the environment is still unknown. *H. pylori* has only been indirectly detected in water, and the significance, if any, of this route of transmission has not been determined. No direct evidence is available on the effectiveness of water treatment processes against *H. pylori*, but some assumptions can be made on the basis of the similarities between this organism and campylobacters.

3.1.4 *Enterovirulent Escherichia coli*

The pathogenic strains of *E. coli* have been studied in some detail, and considerable medical knowledge has accumulated about the various types. However, there is little information on their occurrence and distribution in environmental and treated waters. Without improved methods for distinguishing the pathogenic strains from others in environmental samples, their significance is difficult to determine.

One of the main areas for further knowledge and research is that of Vero cytotoxigenic *E. coli* (VTEC). Further clinical and microbiological studies are required to provide a better understanding of the epidemiology of infections caused by VTEC, and of haemolytic uraemic syndrome caused by VTEC. The importance of VTEC of serogroup O157 in human disease has been clearly established but that of VTEC belonging to non-O157 serogroups is unclear. Studies are required to determine the incidence and association with human disease and to establish the sources and vehicles of infection. The role of virulence factors other than Vero cytotoxin production needs further research. This includes studies of adhesion mechanisms and haemolysin production.

One of the newly-recognised classes of enterovirulent *E. coli*, the enteroaggregative *E. coli*, appears to be a significant cause of diarrhoeal disease in Britain, but relatively little is known about these organisms. Research is needed to develop suitable diagnostic tests for the enteroaggregative *E. coli*. Once developed these tests would lead to studies on the sources and vehicles of infection, including water. Further studies are needed to examine the pathogenesis of these organisms and to investigate the role of different virulence factors such as adhesins, toxins and haemolysins.

3.1.5 *Yersinia enterocolitica*

Epidemiological studies are needed to determine the true level of infection with *Yersinia enterocolitica* in the community. Improved cultural and non-cultural methods are required for the rapid detection of pathogenic strains from water and food, particularly as the currently-used cold enrichment techniques are time consuming. Polymerase chain reaction (PCR) methods may help with rapid detection. The role of the enterotoxin Yst in pathogenicity of *Yersinia* needs to be clarified. Strains of biotype 1A do not harbour the 70-kb virulence plasmid and are routinely described as non-pathogenic, but yet are
isolated from cases of diarrhoeal disease. Further studies are required to determine the role of these and other virulence factors among Yersinia strains from cases of human infection and from water and food.

3.1.6 Legionella

Although 42 species of Legionella have been described, very few other than L. pneumophila are ever isolated from environmental samples by the existing methods. The use of PCR suggests that some samples contain legionellae that are not culturable, and other data suggest that the growth of legionellae can be suppressed by the background flora on artificial media. The methods used for clinical diagnosis are aimed primarily at L. pneumophila and so it is possible that infections due to other species may go undetected. Work is therefore required to develop methods for the detection of species other than L. pneumophila from both clinical and environmental samples.

The interaction of Legionella species with protozoa is well described but the interaction with other bacteria is less well understood. Improved knowledge in this area should lead to a better understanding of how legionellae grow and survive in water systems, and might lead to the development of methods of biological control. Further work is required to develop effective methods of controlling Legionella in water systems. Ionisation (electrolytically generated silver and copper ions) and stabilised chlorine dioxide are two promising alternatives to the current practices of temperature control and chlorination, but there is a need for further field trials. Further work is also required to establish whether ionisation can be used effectively in cooling water systems and spa pools, and to develop reliable methods of feed-back control for ionisers. The further development of alternative control methods should be encouraged.

3.1.7 Mycobacterium avium complex

Organisms of the Mycobacterium avium complex (MAC) have received increasing attention in recent years, since their identification as a cause of infection in immune-compromised individuals, particularly AIDS patients. Nevertheless, the means by which the infection is transmitted to man remains unclear. Studies have detected MAC organisms in many types of aquatic habitats, but the public health significance of these findings is not known. Work is required to investigate the link between M. avium complex infection and possible water sources. This should include the development of improved methods for isolating and typing these organisms from human and environmental sources. This would allow more exacting studies of the occurrence of these organisms in aquatic environments. The possibility that mycobacteria, including organisms of the M. avium complex, gather in biofilms within water systems has not been adequately investigated.

3.1.8 Vibrio

There is little information on the use of buffers to control the pH of liquid enrichment media used for isolation of vibrios. These are used largely unbuffered at the present time.
V. cholerae O1 (non-toxin producing) was isolated in the UK in 1976 from a drainage ditch, but since that time it would appear that we have no up-to-date information on the occurrence of vibrios in untreated waters in this country. Improved isolation techniques are required for the detection of vibrios in water. Development of media which would allow quantitative detection by membrane filtration would be a distinct advantage. However, with effective water treatment and disinfection, there is little risk of Vibrio infections being transmitted by drinking water, and the low incidence of clinical cases of Vibrio gastroenteritis (almost all travel-related) in this country also reduces the possibility of significant water contamination. Tests for vibrios would only be rarely needed in this country for drinking water. However, the procedures in Report 71 may be needed for imported bottled waters, and their efficiency should be determined for this purpose.

With possible increased use of river and other surface waters as sources of drinking water, surveys may be needed for the presence of vibrios to assess any public health risk. Surveys for the presence of V. vulnificus in estuarine waters in this country may be needed as little information is available.

3.2 Viruses

Of the viruses included in the fact sheets, there is generally more information available on those which can be grown relatively easily in culture (adenovirus groups A to E and coxsackieviruses). The existence of reasonably practical detection and enumeration methods means that some information exists on the environmental occurrence and distribution of the viruses, and on their removal by treatment processes.

Little is known about the occurrence in water of the viral pathogens which cause gastroenteritis, nor of hepatitis E virus. Satisfactory culturing methods are not currently available for the “classic” caliciviruses, Norwalk-like viruses, hepatitis E virus, non-Group A rotaviruses or adenovirus types 40 and 41, and those for astroviruses have not yet been widely employed. Some indirect evidence of occurrence in water sources is available for rotaviruses and Norwalk-like viruses from epidemiological studies. No direct information is available on removal of any of these viruses by water treatment processes, and very little on the effectiveness of disinfectants such as chlorine, ozone and ultraviolet light.

Norwalk-like viruses (also known as “small round structured viruses” or SRSV’s) are the most important cause of adult gastroenteritis, so the highest priority should be given to the development of concentration techniques and detection methods such as PCR for these viruses. Rotaviruses cause gastroenteritis in children under 5 years old on a massive scale each winter. Although less likely to cause waterborne outbreaks, since most adults are immune, these are significant pathogens, and their survival in water is poorly understood. Effective concentration and detection methods are needed. The other viral pathogens cause only a minority of the reported cases of gastroenteritis and so are of lower priority.

Epidemiological investigation of outbreaks of disease have produced at least some information on the routes of transmission of most of the viruses under consideration, but
there is a lack of unequivocal evidence of the role of the "classic" caliciviruses in gastrointestinal illness, and of the way in which calicivirus infection is spread.

3.3 Protozoa

With the exception of Acanthamoeba, where its role in causing keratitis in humans has been established, there is a general lack of detailed information on the occurrence and environmental distribution of the other four protozoa included in the fact sheets (microsporidia, Cyclospora, Isospora and Toxoplasma). Also little is known about their removal by treatment processes, or their susceptibility to inactivation by disinfectants. A certain amount can be inferred from the size of the oocysts or spores which are produced, as physical removal by filtration or coagulation-sedimentation will be important, rather than biological treatment. The resistance to disinfection of oocysts of Cryptosporidium leads to the suspicion that the oocysts of other protozoa may be similarly robust, but there is little direct evidence to support this theory. Culture methods are not available for Cyclospora or Isospora, and the lack of good analytical techniques will limit progress in this area. Toxoplasma and some microsporidia have been grown in tissue cultures and rodents, but these techniques are as yet of little use for environmental studies or monitoring of treatment processes.

There is information on the routes of transmission of Toxoplasma, but little is known about how humans become infected with microsporidia, Cyclospora or Isospora, or whether there are animal reservoirs of infection.

3.3.1 Acanthamoeba

Studies are required to determine the source and incidence of Acanthamoeba in domestic tap water and their association with keratitis. Laboratory models are required to study factors which favour the presence, growth and persistence of Acanthamoeba in domestic water supplies. A priority is the development of a selective medium, inhibitory to other faster-growing but non-pathogenic amoebae, for the isolation of Acanthamoeba from the environment. An evaluation of contact lens hygiene practices in relation to tap water should also be carried out. Acanthamoeba keratitis should be considered a preventable disease. The education of contact lens wearers would be a highly cost-effective measure, and would help prevent a disease which costs the British health service over £1 million each year in medical and surgical treatment.
4. CONCLUSIONS

The investigators have identified areas of further scientific investigation for the emerging pathogens dealt with in the fact sheets collected together in Appendix B of this report. In priority terms the organisms which should be dealt with first are the entrovirulent *Escherichia coli*, the *Mycobacterium avium* complex and the viruses which may survive in water.

Work on the detection of entrovirulent *E. coli* could transform current knowledge on the role of unchlorinated water in the transmission of infection by these organisms.

There is a real concern that water in distribution may be a route of transmission for the *Mycobacterium avium* complex. Although cases of human infection are usually restricted to the immune-compromised, infection may be life-threatening.

A number of pathogenic viruses pass through sewage treatment plants and may be discharged into rivers or other watercourses in large numbers. Viruses which are resistant to chlorination may also be present in distribution systems. So that there can be better understanding of both of these classes of viruses, it is essential that detection and isolation procedures are improved, and that systems are developed to establish the role of waterborne viruses as a cause of human infection.
APPENDIX A  LIST OF AUTHORS

In addition to the named authors, a number of PHLS staff were involved in drafting sections of the fact sheets and advising on appropriate areas for further work. They are listed below, with the fact sheets to which they contributed:

Dr Eric Bolton (Campylobacter)
Dr David Casemore (Cyclospora, Isospora and microsporidia)
Dr Owen Caul (all viruses)
Mr Tom Cheasty (Yersinia)
Dr Terry Donovan (Vibrio)
Dr Ed Guy (Toxoplasma)
Dr Peter Hawtin (Helicobacter)
Dr Tom Humphrey (Salmonella)
Dr Simon Kilvington (Acanthamoeba)
Dr John Lee (Legionella)
Dr John Magee (Mycobacterium avium complex)
Dr Gordon Nicholls (Aeromonas)
Dr Bob Owen (Arcobacter)
Mrs Jane Sellwood (all viruses)
Dr Henry Smith (enterovirulent E. coli).
## APPENDIX B  FACT SHEETS ON EMERGING WATERBORNE PATHOGENS

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Aeromonas

This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

The genus Aeromonas
There are eleven named species of Aeromonas and two of these have subspecies: A. allosaccharophila, A. caviae, A. eucrenophila, A. hydrophila, A. jandaei, A. media, A. schubertii, A. sobria, A. trota, A. salmonicida subsp. achronogena, A. salmonicida subsp. masoucida, A. salmonicida subsp. salmonicida, A. salmonicida subsp. smithia, A. veronii subsp. veronii, A. veronii subsp. sobria.

Morphology
Straight rods or coco-bacilli (0.3-1.0μm by 1.0-3.5μm) appearing singly, in pairs or short chains. Gram negative and motile with a single polar flagellum (A. media and A. salmonicida are non motile).

Biochemical characteristics
Chemo-organotrophic facultative anaerobes having both respiratory and fermentative types of metabolism. They are oxidase, catalase, gelatinase and DNase positive, reduce nitrates, and most strains ferments carbohydrates. Urease and phenylalanine tests are negative and they are resistant to 2,4-diamino-6,7-di-isopropylpteridine (vibriostatic agent O129). The species and subspecies can be differentiated biochemically but such identifications are less reliable than methods based around genetic hybridisation groups. For most purposes within the water industry identification to the level of Aeromonas species is sufficient, and this can be achieved using API 20E test strips or an equivalent biochemical system.

Cultural characteristics
Most Aeromonas species grow well at 37°C although their optimal growth temperature is 22-28°C, and their range is 4-42°C. A. salmonicida has a growth temperature range of 5-30°C with an optimum of 20°C. The optimum pH range for Aeromonas species is 5.5 to 9, and they are not halophilic. All species grow well on Columbia blood agar, nutrient agar and MacConkey agar, but selective media are required for their isolation from contaminated food and water.

Detection methods
Aeromonas species grow on a wide range of culture media, and can be isolated from water on membrane filters of the type used for the enumeration of coliforms and E. coli. A number of cultural techniques have been used to selectively isolate aeromonads from water or sewage. Ampicillin dextrin agar has been recommended for the isolation of Aeromonas species from water, and Ampicillin blood agar or Ryan's medium (based on modified XLD medium with ampicillin) have also been used. Media containing ampicillin will be unable to isolate A. trota which is sensitive to this antibiotic.

Health effects
Aeromonas species can cause wound infections and septicaemia, and have been associated with diarrhoeal illness throughout the world. In the UK infections are most frequent in late summer, and are often acquired through foreign travel. Wound infections are relatively uncommon but can progress rapidly if not treated, to become severe and life threatening. They can occur in previously healthy people of all ages, but are more common in people with an underlying immune deficiency. The main species causing human infection are A. hydrophila, A. caviae, A. veronii subsp. sobria and A. media. Some species are pathogenic for fish and amphibians.
Aeromonas

Routes of transmission
Aeromonas species are common in fresh water and sewage, and can be frequently isolated from food. Point source outbreaks of diarrhoeal illness from a single source do not occur, but in some countries an association has been made between human infections and water contamination. Person to person infection does not occur and experimental evidence suggests that the majority of adults are resistant to the consumption of relatively high numbers of A. hydrophila. Their presence in water in distribution systems can result from seasonal blooms during periods when there is a temperature above 15°C, a raised organic carbon level and a reduction in residual chlorine. Water and food can contain a variety of Aeromonas species and their speciation, typing and virulence are complex. Because of this and the fact that point source outbreaks of infection do not occur, clear evidence of food or water borne infection has been difficult to obtain. Contaminated food and water remain the most likely sources of human infection, but low counts of aeromonads in water are unlikely to lead to outbreaks of waterborne infection.

Occurrence in water sources
The normal habitat of Aeromonas species is the aquatic environment, and they are very widely distributed. Their presence has been reported in sewage and sewage effluents, surface waters (fresh, estuarine and marine), fish ponds, soils, natural mineral springs, stagnant water, and chlorinated and unchlorinated drinking water.

Sources of exposure
Exposure to waterborne aeromonads may be through ingestion of contaminated water or by contact with it. Most recorded cases of the latter follow accidents during swimming or diving, and include skin or wound infections due to impact injuries, lacerations or punctures. There have also been descriptions of pneumonia after near-drowning incidents, and cases of bone infections after contact of water with open fractures and wounds.

However, it should be noted that food may also be an important source of exposure, and the possibility this should be borne in mind if water is implicated as a source of infection. Aeromonads have been isolated from meat (particularly when packaged under modified atmospheres), poultry, sausages, fish and shellfish, raw milk, ice cream and vegetables. The psychrophilic nature of some aeromonads allows them to grow well at refrigerator temperatures.

Susceptibility to removal or inactivation by conventional water treatment processes
Most drinking water treatment processes appear to be able to reduce numbers of aeromonads to low levels (i.e. below 1 cfu per 100 ml). However, treated water can contain larger numbers as a result of regrowth in storage reservoirs or distribution systems. The size of the population will depend on many factors, including the temperature and organic content of the water, the residence time in the distribution system, and the presence or absence of residual disinfectant.

Water treatment should be aimed at reducing the possibility of regrowth, principally by removing organic compounds which could be used as carbon and energy sources. Distribution systems should be designed with short residence times if possible. Measures may be needed to prevent accumulation of sediments in sections of pipework with low water velocities.

Free available chlorine residuals of 0.2–0.5 mg.l⁻¹ are considered sufficient to control aeromonad densities in water distribution systems. Lower residuals may not prevent regrowth if other conditions are favourable for it to occur.
This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

The genus *Arcobacter*
Four species of similar general morphology are recognised: *Arcobacter cryaerophilus* (containing two subgroups, and formerly called *Campylobacter cryaerophilus*), *A. butzleri* (also formerly *C. cryaerophilus*), *A. skirrowii*, and *A. nitrofigilis* (formerly *C. nitrofigilis*).

Morphology
Members of the genus *Arcobacter* are gram-negative, non-sporeforming rods (width 0.2 to 0.9 μm; length 1 to 3 μm). Unusually long cells are occasionally seen. The cells are usually curved or s-shaped when viewed by conventional light microscopy, and are motile with a darting, corkscrew-like motion by means of a single polar, unshelled flagellum.

Biochemical characteristics
Arcobacters are metabolically inactive. D-glucose and other carbohydrates are neither fermented nor oxidized. Strains have oxidase and catalase activities, hydrolyse indoxyl acetate but are negative in hydrolysis of hippurate, reduction of nitrate, and production of indole and hydrogen sulphide (rapid test and triple sugar iron agar). Nearly all strains are urease negative with the exception of *A. cryaerophilus*, and susceptible to nalidixic acid, but susceptible to cephalothin (30-μg disc) is variable. Negative to weak catalase activity, reduction of nitrate and growth on MacConkey agar and in 8% glucose are the key distinguishing features of *A. butzleri*. *A. skirrowii* differs from *A. butzleri*, the most closely related species, by its inability to grow on MacConkey agar and by the fact that most *A. skirrowii* strains do not grow in the presence of 1.5% NaCl. *A. cryaerophilus* strains are distinctive in not reducing nitrate. *A. nitrofigilis* can be differentiated from other arcobacters by its nitrogenase activity and from *A. cryaerophilus* and *A. skirrowii* in particular by its ability to grow in the presence of 1.5% NaCl. Generally, accurate differentiation between species on conventional phenotypic tests is difficult. A PCR-based identification assay (targeted at 16S ribosomal RNA sequences) has been developed to differentiate *Arcobacter* species from other closely related campylobacters, including *Campylobacter* and *Helicobacter*. Biotyping of *A. butzleri* can be performed using four biochemical tests and serotyping by slide agglutination based on heat-labile antigenic factors of live bacteria using absorbed and unabsorbed antisera.

Cultural characteristics
*Arcobacter* strains grown on blood agar for 24 h under microaerobic conditions at 37°C form small, flat, watery, translucent beige to yellow, irregular colonies with a campylobacter-like appearance. *A. nitrofigilis* has a slightly different appearance with typical colonies that are white and round. Growth occurs at 15°C and 30°C but only slightly at 42°C. Hydrogen is not required for microaerobic growth. The distinctive feature of *Arcobacter* is aerotolerance indicated by the ability to grow in atmospheric oxygen optimally at 30°C. Growth is slight under anaerobic conditions at 37°C. It has been observed that most aerotolerant strains, except *A. butzleri*, grow weakly on the common blood agar bases, although recently Brain Heart Infusion agar containing 0.6% yeast extract and 10% blood agar has been used successfully for routine culturing.

Detection methods
Arcobacters are seldom isolated by the routine culture methods developed for campylobacters. Their primary isolation was first achieved by two-step motility enrichment in a semisolid medium at 30°C followed by culture on blood agar containing a selective agent such as carbenicillin. Direct filtration (continued)
Detection methods (continued)
onto enrichment media as well as non-selective media has been used. A primary isolation procedure based on swarming in a semisolid blood-free selective medium at 24°C has recently been developed for the examination of meat products. The selective agents used were piperacillin, ceftazidime, trimethoprim, and cycloheximide, and these were incorporated in a basal medium comprising Mueller-Hinton broth and 0.25% agarose (Arcobacter Selective Medium). Pure cultures of Arcobacter are then isolated from the swarming zones which may extend 30-40 mm.

Health effects
A. butzleri is the species most commonly associated with illnesses in humans and has been isolated from stools of patients with abdominal pain and/or diarrhoeal illness. Occasionally this species is isolated from blood and peritoneal fluid of patients following acute appendicitis. The role of A. butzleri as an enteropathogen is not yet clearly established. Strains of Arcobacter were originally isolated from aborted livestock foetuses (pigs and cattle) and have since been extensively studied in relation to abortion in pigs. Even so, the prevalence of such organisms in abortion and enteritis in other species of livestock is unknown and their pathogenic role has yet to be defined. Arcobacters have been isolated from plant material, from various animal and human sources, particularly faeces, from reservoir water, and from retail meat products including poultry and ground pork.

Routes of transmission
It is not known how humans become infected by Arcobacters but consumption of or contact with potentially contaminated water associated with travel has been suggested as a possible risk. Recently, A. butzleri-like organisms have been isolated from retail meat products, in particular poultry meat, indicating that food-borne infection may be another possible route of transmission.

Occurrence in water sources
The epidemiology of Arcobacter infections is not well understood. However, A. butzleri has been isolated from urban drinking water, river water, faeces and sewage. Isolation from a drinking water reservoir in Germany has also been documented.

Sources of exposure
It is likely that consumption of contaminated water is a source of exposure to Arcobacters, but there is no direct evidence of this. Recent findings have shown that several biotypes and serogroups of A. butzleri which have been associated with human disease have also been isolated from fresh poultry carcasses. Food may therefore be a possible source of exposure.

Susceptibility to removal or inactivation by conventional water treatment processes
There is no direct evidence on removal of Arcobacters by treatment processes, though they are likely to be removed to a similar extent as other bacteria. On the basis of their similarity to campylobacters, they should be susceptible to inactivation by disinfectants such as chlorine and ozone.
Drinking Water Inspectorate Fact Sheet

Campylobacter

Fact Sheet No 3: Issue No 1

March 1997

This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

The genus Campylobacter

The genus Campylobacter includes fourteen species: C. coli, C. concisus, C. curvus, C. fetus, C. gracilis, C. helveticus, C. hyointestinalis, C. jejuni, C. lari, C. mucosalis, C. rectus, C. showae, C. sputorum, and C. upsaliensis. Some of these are pathogens of man and other animals with the most important human pathogens being the thermophilic Campylobacter species which include C. jejuni, C. coli and C. upsaliensis. Occasionally C. fetus subspecies fetus and C. lari cause human infections. The following information relates particularly to the thermophilic campylobacters.

Morphology

Campylobacters are slender, spirally curved, Gram-negative rods. The cells are 0.2 to 0.5 μm wide and 0.5 to 5 μm long and appear as ‘S’ or ‘gull-wing’ shaped in stained smears. They do not produce spores and are motile with a flagellum at one or both ends of the cell and exhibit a typical darting, corkscrew-like motility.

Biochemical characteristics

Campylobacter species are oxidase positive, reduce nitrate to nitrite and most are catalase positive. Several species hydrolyse indoxyl acetate but only one species, C. jejuni, hydrolyses sodium hippurate. Some strains produce deoxyribonuclease, hydrogen sulphide or hydrolyse urea. Campylobacters do not utilise carbohydrates, produce indole or show proteinase or lipase activity.

Cultural characteristics

Campylobacters are microaerophilic and grow optimally in the presence of 5 - 10% oxygen and 10% carbon dioxide. All species grow at 37°C but the thermophilic species C. jejuni, C. coli and C. lari also grow well at 42°C. Most species require 48 hours to produce typical colonies on primary isolation media. The thermophilic species produce flat colonies which have a metallic sheen and tend to swarm on moist media.

Detection methods

A variety of media are available for culture of Campylobacter species from human, food and environmental samples. Campylobacters are detected in faecal samples by direct culture onto selective agars but recovery from foods, milk or water normally requires enrichment in selective broth followed by subculture onto agar. Some Campylobacter species will not grow on selective agar media and a method of passive filtration onto non-selective culture media is recommended. This is important for detection of C. upsaliensis which has emerged as a new pathogen. Alternative methods of detection include immunoassay, impedance and the polymerase chain reaction (PCR).

Health effects

Campylobacter jejuni is a major cause of gastrointestinal disease which is clinically indistinguishable from other forms of bacterial enteritis. The disease typically presents as a ‘flu-like’ illness followed by profuse watery diarrhoea and abdominal pain which may last for several days. Most infections resolve without treatment but occasionally complications occur, and these include septicaemia, meningitis, reactive arthritis, haemolytic uraemic syndrome (anaemia, other blood disorders and kidney failure) and Guillain-Barré syndrome (a disorder of peripheral nerves causing progressive muscular weakness, and even paralysis of the muscles of respiration).
**Campylobacter**

**Routes of transmission**
*Campylobacter* enteritis is a zoonosis and occurs as consequence of direct or indirect contact with pets, farm animals and poultry. Large outbreaks of enteritis have been associated with contaminated milk and potable water. The majority of cases are sporadic and may be associated with consumption or handling of poultry meat, drinking milk delivered to the doorstep which has been contaminated by bird pecking, and exposure to recreational water. Person-to-person spread is rare. Campylobacters do not multiply on food at room temperature but cross contamination from raw foods and inadequate cooking are major risk factors for *Campylobacter* disease.

**Occurrence in water sources**
Studies have shown that thermophilic campylobacters are frequently present in sewage. Sewage pollution of water sources therefore carries the risk that campylobacters may be present. They can survive for several weeks in aquatic environments at low temperatures, suggesting that water may be an effective vehicle of transmission. Campylobacters have been detected in surface waters, but their occurrence seems to depend on weather conditions and water temperature. The presence of roosting birds and waterfowl makes their presence more likely.

**Sources of exposure**
Thermophilic campylobacters are transmitted by the oral route. The most important reservoirs are wild birds and poultry, but others include pigs, cattle, dogs and cats. Meat, particularly poultry products, and unpasteurised milk are important sources of infection. Several waterborne outbreaks of campylobacteriosis have been reported. The consumption of unchlorinated or inadequately chlorinated surface waters is an important risk factor. In one outbreak the main source of infection was faecal contamination of water storage reservoirs by wild birds. In developing countries contact with faeces of infected animals may be a risk factor for agricultural workers.

**Susceptibility to removal or inactivation by conventional water treatment processes**
Water treatment processes can effectively reduce numbers of campylobacters to very low levels. Chlorination at the levels normally employed in water treatment is sufficient to inactivate campylobacters and prevent spread through distribution systems.
Enterovirulent *Escherichia coli*

**Morphology**
*E. coli* are defined as Gram-negative rod-shaped bacteria, 1-1.5 μm x 2-6 μm. Strains are usually motile by possession of flagella.

**Biochemical characteristics**
*E. coli* are oxidase-negative facultative anaerobes which usually produce gas from fermentable carbohydrates. Lactose is fermented promptly by most strains and other carbohydrates usually fermented include arabinose, glycerol, maltose, mannitol, rhamnose, sorbitol and xylose. Most *E. coli* produce indole but do not hydrolyse urea or produce hydrogen sulphide. Both lysine and ornithine are usually decarboxylated and most strains can utilise acetate, but not citrate, as a sole carbon source. EIEC are biochemically atypical often being anaerogenic, lactose non-fermenting and lysine decarboxylase-negative, thus resembling *Shigella*. With very few exceptions, VTEC of serotype O157 do not ferment sorbitol.

**Cultural characteristics**
*E. coli* have an optimum growth temperature of 37°C, usually with a range from 15°C to 45°C. Most strains grow well on solid media such as MacConkey, blood or nutrient agar and form smooth, circular, low convex colonies of 2-3 mm diameter after incubation at 37°C for 18 h.

**Detection methods**
There are very few culture methods available that distinguish enterovirulent *E. coli* from other strains. However, techniques based on the detection of virulence factors or the genes encoding them have been developed for each class of enterovirulent *E. coli*. EPEC are detected using antisera prepared with strains of the traditional enteropathogenic serogroups. However, these will not detect strains with properties of EPEC that do not belong to these serogroups, and many strains within the traditional EPEC serogroups do not possess the characteristic virulence factors. "Classical" EPEC show adhesion in a localised pattern to tissue culture cells such as Hep-2 cells. They form attaching and effacing lesions which can be tested *in vitro* using the fluorescence actin-staining test. Alternative methods include ELISA immunoassay and DNA-based methods using hybridisation or polymerase chain reaction (PCR) amplification. ETEC produce heat-labile (LT) or heat-stable (ST) enterotoxins or both. Toxin production was originally demonstrated in animal models but alternative methods have been developed. An ELISA for detection of ST is satisfactory and commercially available. LT produces cytotoxic effects on certain tissue culture cells. ELISA and latex agglutination tests for LT are commercially available. DNA hybridisation and PCR assays have been developed for the ST and LT genes. The invasive ability of EIEC was originally detected in guinea pigs, but can also be detected in tissue culture using Hep-2 or HeLa cells. ELISA, DNA probes and PCR amplification provide alternative methods. Most clinical laboratories only test for VTEC strains belonging to serogroup O157. Samples are plated direct onto MacConkey agar containing D-sorbitol (SMAC) instead of lactose. Sorbitol non-fermenting colonies are tested for agglutination with an O157 antiserum or latex kit. A modification of SMAC agar containing cefixime and tellurite is now recommended. VTEC belonging to all serogroups can be detected by assays for VT production or for the presence of VT genes using latex agglutination, ELISAs, DNA probes or by PCR amplification.
Enteroxirulent *Escherichia coli*

**Health effects**

Enteroxirulent *E. coli* can cause a wide range of symptoms. EPEC infection usually results in a watery diarrhoea accompanied by vomiting and fever but in some cases there is a protracted chronic enteritis. EPEC are traditionally associated with outbreaks in maternity units and nurseries but have also caused outbreaks in adults. ETEC are a major cause of travellers' diarrhoea and infantile enteritis in the tropics. The diarrhoea is watery, accompanied by abdominal cramps, fever and vomiting; in severe cases the symptoms may resemble those of cholera. EIEC infection produces a disease similar to that caused by *Shigella*. The diarrhoea is initially acute and watery, accompanied by fever and abdominal cramps and then may progress to the colonic phase with bloody and mucoid stools.

Infection with VTEC can cause a mild watery diarrhoea but in many cases it is severe and bloody accompanied by abdominal pain usually without fever. It may be followed by the development of haemolytic uraemic syndrome (HUS). HUS is characterised by microangiopathic haemolytic anaemia, thrombocytopenia and acute renal failure. In the clinical syndrome of thrombotic thrombocytopenic purpura the features of HUS are seen together with neurological complications.

**Routes of transmission**

EPEC infections are frequently spread from person to person. This also occurs with ETEC although these infections can result from ingestion of contaminated foods or water. Outbreaks of EIEC infection have also been traced to contaminated foods and water as well as spread from person to person. The reservoir of EIEC, as well as EPEC and ETEC, appears to be human and not animals. In contrast, the reservoirs of VTEC are cattle and sheep: foods of animal origin are amongst the most common vehicles of infection. In particular, contaminated beef products and milk have caused large outbreaks of infection with O157 VTEC. Occasional water-borne outbreaks have also been reported. Person to person spread is important in the large number of small family outbreaks of O157 VTEC. A further mode of transmission of infection is by direct contact with animals.

**Occurrence in water sources**

The presence of *E. coli* in water sources is used to indicate faecal contamination. *E. coli* may therefore occur in any type of water subject to pollution of a faecal nature. Enteroxirulent *E. coli* will be present in sewage if the organisms are being excreted by members of the contributing population. The types and numbers will depend on the state of infection in the community. Because of the animal reservoir of VTEC, these organisms may also enter water sources through runoff from agricultural land, or other situations where water becomes contaminated with animal faeces. The detection of the pathogenic strains of *E. coli* has seldom been attempted in water supplies, although it may be necessary in epidemiological studies.

**Sources of exposure**

In developing countries contaminated water is a principal vehicle for transmission of *E. coli* infections, either by direct consumption or by contamination of foods which are irrigated, washed or prepared with contaminated water. This is less common in developed countries with higher standards of general hygiene. However, drinking water contaminated by sewage or animal wastes has been implicated in outbreaks, and water in a paddling pool has been suspected as the means of spreading infection from an index case to other children. As food and person-to-person contact are concurrent alternative sources of exposure the role of water may be difficult to prove.

**Susceptibility to removal or inactivation by conventional water treatment processes**

As the absence of *E. coli* is used to indicate the satisfactory bacteriological quality of drinking water, it follows that treatment processes, including biological and physico-chemical treatment and disinfection, will reduce the numbers of these organisms to undetectable levels in standard tests. The presence of *E. coli* in treated drinking water supplies indicates that treatment processes are faulty or inadequate, or that contamination has occurred after treatment.

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This fact sheet has been jointly prepared by the Public Health Laboratory Service and WRc plc (formerly the Water Research Centre).
This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

The genus *Helicobacter*
The genus *Helicobacter* has grown steadily in the past few years with new species, and some have been reassigned from *Campylobacter*. The genus now includes *H. pylori*, *H. helium*, *H. muridarum*, *H. nemestrinae*, *H. mustelae*, *H. felis*, *H. acinonyx*, *H. pamatensis*, *H. bilis*, *H. canis*, *H. hepaticus*, *H. pullorum*, *H. cinaedi*, and *H. fennelliae*. However, the only human pathogenic species described so far are *H. pylori* and *H. heilmanni*. This may change as detection systems and discriminatory tests are improved.

**Morphology**
The morphology of this Gram negative spiral bacterium is so characteristic that its recognition in gastric biopsy material is possible by light microscopy using routine histological stains. The bacterium is about 3 μm long and between 0.5 and 1μm wide with usually a single “twist” in its length. Electron microscopy reveals between 4 and 6 flagella positioned on one pole. As with *Campylobacter* and *Vibrio* spp. a coccoid form of *H. pylori* has been described, though speculation continues as to the function, if any, and indeed the actual viability of these forms. They may mimic the so-called non-culturable but viable forms of *Campylobacter* and *Vibrio* spp.

**Biochemical characteristics**
*H. pylori* is oxidase positive but non-saccharolytic and does not reduce nitrate. Two enzymes of *H. pylori*, urease and catalase, are unusual among medically important bacteria because of their high activity and can be used in the identification of *H. pylori*.

**Cultural characteristics**
*H. pylori* has been cultured from both the oral cavity and from faeces but the typical site is the gastric mucosa. It can be isolated from the stomach in individuals whose faecal and oral cultures are negative, but is always found in the stomach when the other two sites are positive. Samples can be simply smeared on to blood agar containing an antifungal agent (which is required to avoid fungal overgrowth) and incubated in a humid atmosphere. The culture conditions are similar to those used for *Campylobacter*, i.e. a reduced oxygen atmosphere of about 5% with 10% carbon dioxide and the balance either nitrogen or hydrogen. Hydrogen may offer better recovery of injured organisms. Cultures should be incubated at 37°C for a minimum of 5 days.

**Detection methods**
Detection of *H. pylori* ranges from relatively simple microscopical, cultural and immunological methods to sophisticated molecular and biochemical methods. Urease activity in *H. pylori* can be used diagnostically in two ways. Firstly, it can used as a bedside test by applying a gastric biopsy sample to a simple medium containing urea and phenol red indicator. In the presence of *H. pylori* urease the ammonia released by splitting urea raises the pH of the medium, and this is detected by a colour change of the phenol red. The speed with which this reaction occurs in *H. pylori* is highly unusual, and results are obtained in minutes at room temperature. Secondly, an alternative also uses *in situ* detection of urease activity. The patient drinks a solution containing urea where the carbon is labelled as either stable C13 or radioactive C14 isotope. If *H. pylori* is present the label is released as carbon dioxide and eventually exhaled. Samples of breath are analysed by either mass spectrometry or scintillation counting, depending on the isotope used, and the presence of isotope is indicative of *H. pylori* infection. *H. pylori* produces an immune response which, in the overwhelming majority of cases, fails to eradicate the infection. This means the identification of specific antibodies is a highly accurate method of detecting infection and is not evidence of past exposure as is the case in some other bacterial and viral infections.
**Helicobacter pylori**

**Health effects**

*H. pylori* has been found to be associated with gastritis, and with gastric and duodenal ulcers. The major focus of infection is the gastric antrum and also in the duodenum when the normal type of duodenal epithelium is replaced with antral type mucosa. *H. pylori* will only infect this antral type of mucosa. However, it seems that diseases of the stomach are multifactorial and infection by *H. pylori* is probably one part of a sequence of events required to produce disease. The clearest evidence for the crucial role *H. pylori* plays in gastroduodenal disease comes from treatment trials. If a patient suffering a duodenal ulcer is cured of the infection, the chances of a recurrence of the ulcer is almost zero, whereas without cure the risk of ulcer relapse is extremely high. Similarly, low grade B cell gastric lymphoma appears to regress, in some cases disappears, when *H. pylori* is eradicated. The epidemiological evidence of an association between *H. pylori* and gastric cancer is strong, especially in Japan where it has a high rate of gastric cancer. *H. pylori* may act as an initiator or promoter of a sequence of events, possibly irreversible, which then leads to gastric cancer. *H. pylori* may be absent in the tumour tissue probably displaced by inappropriate epithelia. Therefore, *H. pylori* is a serious infection with major health and economic burdens. Epidemiological studies have found that *H. pylori* is possibly the most prevalent bacterial infection in man. In the developed world, up to about 50% of the population will be infected by the age of 50 years, with many more in the developing world. However, only a small proportion will develop any symptoms. When asymptomatic volunteers have been investigated they all appear to have inflammation of the stomach.

**Routes of transmission**

The mode of transmission is not entirely understood. The reservoir of *H. pylori* is the digestive tracts of humans and some primates, and transmission is considered to be from person-to-person. Although there is some evidence for faecal-oral transmission, the most common route is probably oral-oral. *H. pylori* is shed in the faeces after turnover of the gastric mucosa, and has been detected by PCR in sewage in Peru. Sewage pollution is therefore a possible though not proven route of transmission.

**Occurrence in water sources**

Environmental sources of *H. pylori* remain unclear. *H. pylori* has only been indirectly detected in water by molecular methods and not cultured from the same samples. Therefore, doubt remains because, although the molecular detection had proven specificity for *H. pylori* in the laboratory, related naturally-occurring cross reacting species cannot be excluded. Furthermore, the detection of specific DNA does not indicate whether the target bacteria are viable. The water quality was not established by standard methods in these studies to determine whether any treatment failure had occurred.

**Sources of exposure**

Indirect evidence of the sources of exposure to *H. pylori* infection has come from epidemiological studies. Risk factors include bedroom sharing in childhood, and whole families can become infected. Rates of infection have been falling since the second world war as living conditions have improved. In South America consumption of raw vegetables fertilised with human faeces has been found to be a risk factor for infection in Chile, and consumption of water from a municipal supply has been suggested as a risk factor for children in Peru. *H. heilmanni* infection appears to be associated with pet ownership.

**Susceptibility to removal or inactivation by conventional water treatment processes**

There is no direct evidence on the effectiveness of treatment processes for removal of *H. pylori*, though they are likely to be removed to a similar extent as other bacteria. On the basis of their similarity to campylobacters, they should be susceptible to inactivation by disinfectants such as chlorine and ozone.
Legionella

Fact Sheet No 6: Issue No 1
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This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

The genus Legionella
Forty two species of Legionella have been described and 17 have been associated with disease in man. The type species is L. pneumophila which is the species most commonly detected both in the environment and in patients. Legionella pneumophila is divided into at least 16 serogroups of which serogroup 1 is the most common cause of disease.

Morphology
All species described so far are straight rods with non parallel sides tapering to rounded ends. The cells are 2-3 μm long by 0.3-0.9 μm wide. Long forms of up to 20 μm or more are relatively common when grown on artificial media. Cells are Gram-negative and motile by one or two or more polar or lateral flagella. Non-motile strains are occasionally seen.

Biochemical characteristics
Legionellae are aerobic chemo-organotrophs using amino acids as carbon and energy sources. They require L-cysteine and iron salts for growth on primary isolation. The oxidase test is negative or weakly positive. They are catalase-positive and most strains possess gelatinase. They do not reduce nitrates, are urease-negative and neither ferment nor oxidise carbohydrates. Some species are autofluorescent. Identification to species level is not easy, but it is normally only necessary to differentiate legionellae into the following broad divisions: L. pneumophila serogroup 1, L. pneumophila serogroups 2-14 and Legionella species other than L. pneumophila. This identification can be performed relatively easily using phenotypic tests and serological methods. Further accurate differentiation is outside the scope of routine laboratories.

Cultural characteristics
Legionellae grow between 25°C and 43°C with an optimum of about 37°C. The optimum pH is 6.8 to 7.0. They are fastidious and are grown on buffered charcoal yeast extract agar (BCYE) which contains charcoal, L-cysteine, ferric pyrophosphate and ACES buffer, in a humidified incubator. One species, L. germanii, requires CO₂ for growth. For this reason legionellae are commonly grown in an atmosphere enriched to 2.5% CO₂, but it is not strictly necessary for most species. They will not grow on simple laboratory media such as nutrient agar or blood agar.

Detection methods
Legionellae are rarely present in high numbers in water and are usually outnumbered by at least 1000:1 by other less fastidious, faster growing aquatic bacteria. Whatever detection method is used the microorganisms are first concentrated from water by filtration or centrifugation and then resuspended in a smaller volume. Biofilm samples are suspended in a small volume of water or an appropriate buffer. Detection is usually by culture. The background flora may be suppressed by heating the concentrate or biofilm suspension at 50°C for 30 minutes or reducing the pH to 2.2 for 5 minutes. The pretreated suspension is then usually inoculated onto GVPC (BCYE containing glycine, polymyxin, vancomycin and cyclohexamide). The colonies of Legionella are recognised by their characteristic morphology but growth can be slow and isolation and simple identification can take up to 14 days. Direct immunofluorescence on the suspension can give a result within 24 hours but is really only suitable for L. pneumophila, is labour intensive and does not reveal whether the organisms are dead or alive. A commercial kit is available that utilises the polymerase chain reaction (PCR) for the detection of L. pneumophila and other Legionella species and can also provide a result within 24 hours. The PCR kit also does not permit the differentiation of living from dead organisms and there can be inhibitors present in the sample which prevent the test from working.
Health effects
Legionella species can cause an acute pneumonia called " legionnaires' disease", or a febrile illness termed "Pontiac fever". Legionnaires' disease has an incubation period of 2 to 10 days, has a low attack rate and is fatal in 10 to 20% of cases. Predisposing factors are being male (ratio 3 males : 1 female), heavy smoking, immunosuppression, alcohol abuse and being over 50 years of age. In contrast, Pontiac fever has a high attack rate, short incubation period, is not fatal and is normally a mild self limiting disease. In some outbreaks of Legionnaires' disease there have been other patients with Pontiac fever like symptoms and it is possible that the two syndromes represent extremes of a spectrum of symptoms. The most common cause of legionnaires' disease is L. pneumophila serogroup 1. What causes individuals to contract Pontiac fever rather than legionnaires' disease is not clearly understood. It has been suggested that Pontiac fever may be caused by the inhalation of dead rather than living organisms or that inhalation of an amoebal cyst containing viable legionellae may have a different clinical outcome to inhaling legionellae by themselves.

Routes of transmission
Infection is contracted by the inhalation of the organisms which have been aerosolised from the water source containing them. An aerosol can be created from water by any process which causes water surfaces to be broken. The smallest droplets so created can rapidly dry to particles that are small enough (less than 5μm diameter) to remain suspended in the air for prolonged periods and to be inhaled deep into the lungs. Such aerosols may be created by running a tap, bubbles rising through water in a spa pool or spraying water in a domestic shower or cooling tower. Legionnaires' disease is also believed to have been contracted by aspiration of contaminated water. There is no transmission from person to person.

Occurrence in water sources
Legionella species are ubiquitous in aquatic environments throughout the world, particularly if the temperature is between 30°C and 45°C. They are widespread in natural water sources, and occur commonly in man-made systems, especially hot water and cooling-water installations.

Sources of exposure
Sources of exposure to Legionella which have been incriminated in outbreaks include cooling towers and evaporative condensers, hot and cold water systems particularly in hotels and hospitals, clinical humidifiers, spa pools, natural hot spas, cutting fluids for machine tools, a decorative fountain, a grocery display mist machine and potting compost. In addition contaminated water used to make gastric feeds and contaminated ice are believed to have caused infection by aspiration.

Susceptibility to removal or inactivation by conventional water treatment processes
The principal problem with legionellae is their proliferation within water systems. Once an artificial water system has become colonised, legionellae can be very difficult to eradicate completely, either by heat or chemicals. They have a complex relationship with other micro-organisms, and it has been found that legionellae can be ingested by certain amoebae, and become incorporated in their cysts, which may be a factor in their persistence. Water distribution systems should be designed and maintained so as to minimise colonisation. Particular aspects are prevention of the accumulation of sediments and slimes, maintaining hot water systems above 60°C and cold water systems below 20°C, and the use of materials which do not release nutrients into the water which could support growth of legionellae. Biocides are less effective in controlling legionellae in water distribution systems in buildings, but are useful in air-conditioning systems using wet evaporative cooling towers.
This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

The genus *Mycobacterium*

There are seventy-one validly named species of *Mycobacterium* and an additional three sub-species. The principal pathogens in the genus are *M. bovis*, *M. leprae* and *M. tuberculosis* but, in all, thirty-two species are known to be pathogenic to humans or animals. Species of *Mycobacteria* other than those above are often referred to as "atypical mycobacteria". The most commonly encountered pathogens among the atypical mycobacteria are species of the *Mycobacterium avium* complex. The *M. avium* complex (MAC) is considered to contain *M. avium*, *M. avium* subspecies *paratuberculosis*, *M. avium* subspecies *silvaticum* and *M. intracellulare*. However, poorly identified strains which show some similarity to *M. avium* are also frequently, and incorrectly, allocated to the complex. There are over 20 recognised serotypes within the *M. avium* complex.

**Morphology**

Straight or slightly curved, non motile rods, 0.2-0.6 x 1.0 μm. Although difficult to stain, rods are Gram positive. After staining with basic fuchsin, cells resist decolouration with acidic-ethanol and are therefore termed acid-alcohol-fast bacilli (AFB). This characteristic is due to the high level of lipid in mycobacterial cell walls.

**Biochemical characteristics**

*M. avium* complex organisms are aerobic and usually non-pigmented, although pigmented strains may be found. Sugars are attacked by oxidation.

**Cultural characteristics**

Mycobacteria can be assigned to two broad groups on the basis of growth rates. Strains of the *M. avium* complex are defined as slow growing since they require at least seven days or more to produce visible growth on solid media under controlled conditions. Most strains of the *M. avium* complex grow well at 25°C and many strains will grow at 42°C and 45°C. *M. avium* and *M. intracellulare* strains grow well on solidified egg media and on albumin supplemented agar media. Growth may be faster in liquid media, particularly media based on the Middlebrook formulation. *M. avium* subspecies *paratuberculosis* requires media supplemented by mycobactin. *M. avium* subspecies *silvaticum* strains have a preference for media based on pyruvate and only grow well at low pH. The subspecies of *M. avium* can be differentiated from other species and each other by their cultural characteristics. *M. avium* and *M. intracellulare* cannot easily be separated from each other on the basis of phenotypic properties. Similarly, the differentiation of these species from other slow-growing mycobacteria can be difficult and relies on colonial morphology, temperature range and antibiotic susceptibility. Confirmation of identity is usually based on genetic probes targeting RNA fractions. However, strains do occur which fail to react with such probes and require more complex genetic profiles to confirm their identity. These difficulties have lead to the *M. avium* complex being used as a depository for poorly identified strains with a consequent confusion of cultural and epidemiological data.

**Detection methods**

Strains of the *M. avium* complex require dedicated growth media and a prolonged period of incubation. The isolation of these organisms is complicated by their overgrowth by other microorganisms which may be present in the sample. For this reason, samples are usually treated to remove other organisms. This is done using chemical agents to which mycobacteria are relatively resistant. Samples are usually concentrated by centrifugation or filtration to improve isolation rates. The application of these techniques may affect the viability and recovery of *M. avium* complex strains.
Health effects
Members of the *M. avium* complex can cause both pulmonary and non-pulmonary infections. Strains causing non-pulmonary or dissemination infections occur primarily, but not exclusively, in immune-compromised individuals. MAC strains are the most frequent mycobacterial isolates from patients with AIDS. MAC strains may cause pulmonary diseases in the elderly and lymphadenitis in children.

*M. avium* subspecies *paratuberculosis* causes Johne's disease, a chronic enteritis of cattle. The organism is also suspected of involvement in inflammatory conditions of the bowel in humans, but the evidence for this is inconclusive. *M. avium* subspecies *silvaticum* causes tuberculosis in birds and paratuberculosis in mammals, especially deer, but has not been implicated as a human pathogen.

Routes of transmission
The transmission of *M. avium* complex strains from human to human has not been convincingly demonstrated and all infections are thought to be of environmental origin. Although the association of MAC strains with disease in immunocompromised patients has led to many investigations into the environmental occurrence of this organism, the route of transmission remains unproven. Despite this, since MAC strains are commonly found in a range of environmental samples, the mode of transmission to humans is commonly thought to be via contaminated food and water. Most studies support the view that exposure to these organisms can occur via mains water.

Occurrence in water sources
Strains of the *M. avium* complex have frequently been isolated from natural water systems, including marine waters, rivers, lakes, streams, ponds and springs, though some evidence suggests that soil, rather than water, is the primary reservoir of MAC organisms. Mycobacteria have been widely isolated from piped water supplies, though species other than MAC organisms are most common, particularly *M. kansasi* and *M. xenopi*. MAC organisms seem to be more common in natural waters. However, one study found MAC organisms in 69% of hospital hot water systems which were tested. MAC organisms have also been isolated from aquaria.

Source of exposure
Despite the amount of information available, it is almost impossible to pinpoint the exact source of most mycobacterial infections in man. It is postulated that infection by environmental strains usually involves inhalation of aerosolised organisms. Ingestion in food or water, or prolonged contact of the skin with contaminated water. One study has suggested that sea waves were responsible for generating aerosols containing MAC organisms after observing high isolation rates of the organisms from sputum in coastal areas. Another suggestion is that aerosols from shower heads may disseminate MAC organisms which have colonised hot water systems.

Susceptibility to removal or inactivation by conventional water treatment processes
Water treatment processes, particularly sand filtration and coagulation-sedimentation, appear to reduce the numbers of contaminating MAC organisms to a low level, but organisms may be concentrated once more by multiplication in old pipe-work, storage tanks and poorly maintained outlet systems. The organisms are more resistant to disinfection than conventional indicator organisms such as coliforms. The levels of chlorine routinely used in drinking water treatment are unlikely to be effective against MAC organisms, and may account for them being found in distribution systems.
Salmonella

This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

The genus Salmonella
Salmonellae are members of the family Enterobacteriaceae. They are subdivided on the basis of biochemical reactions into seven sub-species. Most of the Salmonella responsible for human infections belong to subspecies 1. Other subspecies are more commonly found in cold-blooded animals, although they can infect humans. Salmonella can be differentiated into over 2000 serotypes. In recent years taxonomists have suggested several different nomenclatures for Salmonella but because of their complexity none has been generally accepted. Thus the current nomenclature has been retained for public health usage, e.g. Salmonella typhimurium or S. typhimurium.

Morphology
Salmonellae are small Gram-negative rod-shaped bacteria, 0.7-1.5 μm x 2-5 μm. Most serotypes are motile by peritrichous flagella.

Biochemical characteristics
Salmonellae are facultative anaerobes. They are catalase positive, oxidase negative and ferment glucose to produce acid or acid and gas. They usually produce hydrogen sulphide, reduce nitrates to nitrites, utilise citrate, and decarboxylate ornithine and lysine. Salmonellae cannot deaminate tryptophan or phenylalanine and are usually urease and indole negative. The above, and other, biochemical tests can be used to presumptively identify Salmonella but it is normal to serotype isolates, especially for epidemiological purposes. The typing scheme is based on the recognition of bacterial surface antigens - the thermostable polysaccharide cell wall or somatic (‘O’) antigens and the thermostable flagella proteins or ‘H’ antigens. It is possible to sub-divide Salmonella serotypes still further on the basis of phage typing. Sub-division of Salmonella is also possible by such genomic methods as plasmid profiling, ribotyping or pulsed field gel electrophoresis (PFGE) of DNA fragments.

Cultural characteristics
Salmonellae are capable of growing in a wide range of laboratory media from the simple to the complex. Growth has been reported over the temperature range of 7-46°C, although some strains may be capable of slow growth at lower temperatures, and the pH range 4-10. Optimum growth temperatures are between 35-37°C.

Detection methods
Although Salmonella can be recovered readily from water, sewage and related environments they may be present in low numbers and may be sub-lethally injured by exposure to such environments. It is normal practice for water samples to be incubated in 'pre-enrichment' media such as Buffered Peptone Water (BPW) or lactose broth for 18-24 hours at 37°C, although some studies using naturally contaminated sewage samples have shown 43°C to be superior. Volumes of pre-enrichment media are added to a selective broth such as Rappaport-Vassiliadis (RV), selenite or tetrahionate broths. Many studies have shown HV broth to be generally superior to the others although S. typhi grows poorly in this medium. Following selective culture the broths are plated onto selective/diagnostic agars. As with the liquid media, there are many agars commercially available. It is common practice for two media to be used and most laboratories will choose two from Xylose Lysine Deoxycholate (XLD), Deoxycholate Citrate (DCA) or Brilliant Green (BG) agars. Work continues on Salmonella isolation methodologies and newer plating media such as Rambach and XLT4 agars have been shown to improve isolation rates in some studies. Salmonella-like colonies on selective agars need to be confirmed by biochemical testing, and serology and phage-typing where appropriate. It may take up to five days to complete sample examination. This delay has led to the investigation of more rapid methods of Salmonella detection. These tend to be genome-based and make use of techniques such as the Polymerase Chain Reaction (PCR) where specific sections of Salmonella DNA are targeted.
Salmonella

Health effects
The incubation period for human salmonellosis is usually between 12 and 36 hours but where people have consumed a large number of cells incubation periods of 6 hours or less have been recorded. In some outbreaks as long as 12 days have elapsed between consumption of contaminated material and clinical illness. There are usually three possible manifestations of salmonellosis - gastroenteritis, systemic infection or an asymptomatic carrier state. The most common is the first. In the UK there is a peak of cases in late summer. There are 70-100 Salmonella-associated deaths in the UK each year. Asymptomatic carriers are common in salmonellosis, and excretion of salmonellae can last for more than a year after symptoms have declined.

Routes of transmission
With the exception of host-specific salmonellae such as S. typhi in man and S. gallinarum in poultry, salmonellosis is regarded as a zoonosis, i.e. a disease acquired by man from animals. Human illness is usually the result of the consumption of contaminated foods or milk although contaminated drinking and recreational waters have been implicated. Salmonella outbreaks have also occurred when contaminated water was used to cool cans of food after heat treatment. The number of bacteria required to cause infection in man will vary depending on the individuals at risk, their age, general health and the presence of underlying disease.

Occurrence in water sources
Salmonellae are associated with faecal pollution, and may be found in any water source subject to such contamination. They have been isolated from sewage-polluted surface waters (fresh, estuarine and marine) and groundwaters. A significant factor in some outbreaks has been contamination of well water (subsequently consumed untreated) by sewage, runoff from agricultural land, leaking domestic drains, and seepage from septic tanks. Most Salmonella serotypes are capable of prolonged survival in water and may be able to growth in heavily polluted water in the warmer months.

Sources of exposure
With the exception of S. typhi, water is not often implicated as the vehicle in outbreaks of human infection. Nevertheless, faecal contamination of groundwater or surface waters, and insufficient treatment or inadequate disinfection of drinking water have been identified as causes of waterborne outbreaks of salmonellosis. However, food is a much more important source of exposure. Salmonellae are commonly found in poultry and livestock, and foods prepared from them, and may be found in all kinds of food grown in faecally-contaminated environments, including shellfish grown in polluted waters. Contaminated water may be important indirectly when it is used in food processing or preparation. Natural water courses are also important in the transmission of infection between herds of food animals and are therefore a factor in zoonotic transmission.

Susceptibility to removal or inactivation by conventional water treatment processes
Water treatment processes should remove salmonellae to a similar extent to indicator bacteria such as coliforms. Standard disinfection procedures are effective against salmonellae, although there is some evidence that they are more resistant to inactivation than coliforms. However, absence of coliforms and E. coli from treated drinking water is adequate assurance that salmonellae will also be absent. Distribution systems should be designed to minimise the deposition of sediments, since contaminated sediments have been implicated in waterborne outbreaks of salmonellosis. Regrowth of salmonellae in distribution is thought to be possible, especially if levels of assimilable organic carbon are high. Salmonellae in drinking water, whether there by contamination or regrowth, may survive for long periods (reported survival times range from a few days to several months).

This fact sheet has been jointly prepared by the Public Health Laboratory Service and WRc plc (formerly the Water Research Centre).
**Vibrio**

Fact Sheet No 9: Issue No 1  
March 1997

*This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.*

**The genus Vibrio**

There are a large number of species now described in this genus. The main important species in relationship to public health are *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. Other vibrios, which are usually regarded as non-pathogens, are common in the coastal environment, e.g. *V. alginolyticus* and *V. metschnikovii*.

**Morphology**

Gram negative, rod shaped, motile bacteria, 1.4-2.6 µm long by 0.5-0.8 µm wide, which show a curvature when examined under optimum conditions. The curvature is not always demonstrated, particularly in older cultures or from solid media.

**Biochemical characteristics**

Vibrios are facultatively anaerobic chemo-organotrophs. They ferment glucose and are oxidase positive. They produce a wide range of extracellular enzymes including DNase, gelatinase, amylase and chitinase. The main pathogens *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* decarboxylate lysine and ornithine, with no alkaline reaction produced from arginine. Most vibrios are sensitive to the vibriostatic agent O129 (2,4-diamino-6,7-di-isopropyl-pteridine) which is used as a diagnostic test. Sensitivity to O129 has been used as a clear differential test between *Aeromonas* (O129 resistant) and *Vibrio* (O129 sensitive). However, some recent isolates of *V. cholerae* O139, which has been identified as causing cholera, are resistant to O129. This finding therefore limits the value of the O129 test, although the majority of vibrios remain sensitive to O129.

**Cultural characteristics**

Vibrios grow well on most non-selective culture media. The absence of sodium from media will inhibit growth. Electrolytes, in particular sodium, will stimulate the growth of all vibrios. Some species are described as halophilic (salt requiring) as they will not grow in the absence of sodium chloride (e.g. *V. parahaemolyticus*). Vibrios grow at neutral and alkaline pH values up to pH 9.0. This characteristic is used in both selective and enrichment media where alkaline pH values of 8 to 8.8 are used. Vibrios are acid sensitive. Vibrios of public health significance will grow at temperatures between 20°C and 40°C, with rapid growth occurring at 37°C. Environmental vibrios may not grow at 37°C. The majority of vibrios will grow at 30°C.

**Detection methods**

The methods for the detection of vibrios in water, food and environmental samples are very similar. To detect the presence of vibrios in a given volume or weight of sample, enrichment using a liquid alkaline peptone medium containing 1% sodium chloride or electrolyte supplement (1% sodium chloride, 0.4% magnesium chloride and 0.4% potassium chloride) is used. Incubation at 37°C is followed by subculture to a selective plating medium. The medium of choice is Thiosulphate-Citrate-Bile salt-Sucrose Agar (TCBS). This medium has been used world-wide for over 20 years. It is commercially available and requires no autoclaving for preparation. In addition it differentiates between sucrose-fermenting vibrios (e.g. *V. cholerae*) and non sucrose-fermenting vibrios (e.g. *V. parahaemolyticus* and *V. vulnificus*). Membrane filtration with direct incubation of the membrane on TCBS is not satisfactory due to overgrowth by non-vibrio bacteria. Enumeration therefore requires an MPN method using alkaline peptone water, or in the case of large numbers of vibrios, direct inoculation onto TCBS. There is a wide range of other media described in the literature for detection of vibrios, but these are mainly for the detection of specific species, e.g. *V. parahaemolyticus*.
Vibrio

Health effects
The main human disease caused by vibrios is cholera. Cholera is a disease that is characterised by acute diarrhoea and dehydration. This is usually associated with epidemic outbreaks. Historically there have been cholera pandemics, which have been documented, from 1817. Until 1991 these were caused by the O1 serotype of *V. cholerae* which produces a cholera toxin. In 1991 a new serotype, *V. cholerae* O139 was identified as causing cholera in South Asia with epidemic potential. Other serotypes of *V. cholerae* can also cause gastro-enteritis by toxin production or other enteropathogenic mechanisms. *V. parahaemolyticus* can cause gastro-enteritis associated with under-prepared seafoods in areas with warm sea water, as the vibrio is present in sea water. Infection of wounds with *V. vulnificus* may result from contact with contaminated sea water. Some other vibrios have been associated with gastro-enteritis (e.g. *V. fluvialis* and *V. mimicus*) or localised superficial infections (e.g. *V. alginolyticus*).

Routes of transmission
Recent research has shown that the pathogenic vibrios can be free living in the aquatic environment in the absence of clinical cases. Clinical cases do lead to an increase of pathogenic vibrios in the environment because of the large numbers of vibrios excreted in the faeces. Cholera remains the classical water borne disease, but recent observations in the South American pandemic indicate food-borne transmission can be a significant factor in outbreaks. Other diseases caused by vibrios remain water-borne or are food-borne from sea foods, particularly those which are eaten raw or only lightly cooked. Laboratory studies have also shown that non-toxigenic strains of *V. cholerae* may acquire the ability to produce the cholera toxin by genetic transfer from bacteriophages. Studies have also shown that *V. cholerae* may undergo a change to a viable non-culturable form. Neither the genetic transfer of toxin production nor the non-culturable phenomenon have yet been proved to be significant in the epidemiology of cholera.

Occurrence in water sources
During outbreaks *V. cholerae* will be present in sewage, and may be isolated from rivers and water sources, though rarely in high numbers. It has been suggested that *V. cholerae* may persist in an aquatic reservoir, and that this may be a mechanism by which cholera is maintained in endemic areas, though much of the evidence for this is circumstantial. Endemic areas are often estuarine or coastal, and *V. cholerae* can survive for long periods in saline water, although the ecological niche occupied by vibrios in such environments is uncertain. There is no evidence for a reservoir in animals or fish, and persistence of the organisms in chronic carriers over long periods is thought unlikely. There is evidence that cholera vibrios may be part of the normal flora of natural waters in some circumstances.

Sources of exposure
Contaminated drinking water is well-established as a source of exposure to *V. cholerae*, and numerous waterborne outbreaks have been documented, although none in the UK or US in recent years. Foodborne outbreaks have been identified, and person-to-person transmission may occur under conditions of extreme overcrowding and poor hygiene. Contaminated shellfish (especially filter-feeders which are eaten raw or only lightly cooked) have also been found to be a source of exposure.

Susceptibility to removal or inactivation by conventional water treatment processes
Water treatment and disinfection are highly effective for removal and inactivation of vibrios. Transmission of disease by drinking water is usually accompanied by serious breakdowns in treatment or by gross contamination of untreated supplies. Chlorine and ozone are effective disinfectants.
This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

Serotypes of *Yersinia enterocolitica*

Strains of *Yersinia enterocolitica* are found world-wide and cause disease in man and animals. Pathogenicity is associated with carriage of a 70 kb plasmid, non-pathogenic and environmental strains do not harbour this plasmid. A serotyping scheme recognises 76 serogroups of which the following are considered to be pathogenic: O:3, O:5, 27, O:8, O:9 and to a lesser extent O:6, 30, O:10, O:11, O:12, O:17, O:18, O:20, O:21 and O:22.

**Morphology**

Short gram-negative rods or cocco-bacilli, 0.5-0.8 μm by 1-3 μm. Motile with peritrichous flagella at 22°C but non-motile at 37°C.

**Biochemical characteristics**

*Y. enterocolitica* is a facultative anaerobe and produces acid from glucose and a variety of carbohydrates. Some strains produce small amounts of gas from glucose. All strains are nitrate and catalase positive, hydrolyse urea, decarboxylate ornithine and are methyl red positive. Strains of *Y. enterocolitica* are negative in tests for oxidase, arginine dihydrolase, lysine decarboxylase, hydrogen sulphide, malonate, phenylalanine deaminase, gelatinase and utilisation of citrate as a sole carbon source. A biotyping scheme (Wauters revised scheme) separates *Y. enterocolitica* into 7 biotypes, 1A, 1B, and 2 to 6. Strains of biotype 1A and 6, are considered to be non-pathogenic whereas the others can be pathogenic. A combination of biotype and serogroup may indicate the likely pathogenicity of an isolate.

**Cultural characteristics**

*Y. enterocolitica* will grow at temperatures between 4° and 42°C with an optimal growth temperature of 28°C. After 24 hours incubation primary cultures of *Y. enterocolitica* appear as pin point colonies on all solid media, growing to 1-2 mm in diameter after 48 hours. On nutrient agar colonies are circular, smooth, and low convex with an entire edge. On MacConkey agar colonies are normally yellow in colour with lactose positive variants forming red colonies. On cefsulodin-irgasan-novobiocin (CIN) agar colonies appear as dark red bulls-eyes surrounded by a transparent border, between 1 and 1.5 mm in diameter and low convex in shape. They can be smooth with an entire edge or rough with an irregular edge.

**Detection methods**

*Y. enterocolitica* can be isolated from faeces by plating directly onto commonly used selective media such as MacConkey or deoxycholate citrate agar (DCA). CIN agar is a highly selective medium and is recommended for the isolation of *Y. enterocolitica* from all clinical, food and environmental samples, but strains can be isolated from blood using standard blood culture media. Cold enrichment in buffered saline pH 7.6, at 4°C for 2-3 weeks followed by plating onto selective and non-selective media, can also be used. Strains carrying the 70 kb pathogenicity plasmid are able to take up the dye Congo red (a property associated with invasive intestinal bacteria) and if grown on a Congo red containing agar medium devoid of calcium, at 37°C, plasmid positive colonies appear as pin-point in size and magenta red in colour, whereas plasmid-negative colonies are colourless and 1-3 mm in size. Pathogenic strains of *Y. enterocolitica* can also be detected using gene probes for specific virulence determinants. DNA probes are available for detection of the invasin gene (inv), responsible for cellular penetration and for the attachment invasion locus (ail) gene which has a function in both adhesion and invasion along with resistance to serum killing. Clinical isolates of *Y. enterocolitica* have been shown to produce a heat stable enterotoxin (Yst) which has similar properties to that produced by *E. coli*. A DNA probe is available to detect this.
Yersinia enterocolitica

Health effects
The predominant disease caused by pathogenic strains of Y. enterocolitica is diarrhoea, but there may be secondary complications such as reactive arthritis, erythema nodosum and Reiter’s syndrome. Enterocolitis, an acute febrile diarrhoea, is the most common form, particularly in young children, and is frequently accompanied by abdominal pain. Symptoms typically last for 1-2 weeks, but can be prolonged. Another manifestation is a syndrome which mimics appendicitis (often in older children and young adults). This may be complicated by erythema nodosum (occurring in 10% of adults, predominantly in females). Acute reactive arthritis can accompany Y. enterocolitica infections, and joint pain is reported in half of infected adults. Arthritic symptoms can accompany the gastrointestinal illness or occur weeks to months later; the symptoms usually persist for several months and chronic arthritis can develop. Reiter’s syndrome and reactive arthritis associated with Yersinia infection occur more frequently in patients with the HLA-B27 genetic type. Septicaemia caused by Y. enterocolitica is a severe illness with fatality rates of 7-50%. Predisposing factors such as immunosuppression, iron-overload, cirrhosis, diabetes and malnutrition nearly always play a part in such cases. Y. enterocolitica septicemia has resulted from the transfusion of contaminated blood products with a reported fatality rate of greater than 50%. Donors of the implicated contaminated blood were healthy at the time of donation but frequently reported a history of diarrhoea in the four weeks prior to donation.

Routes of transmission
Animals are the main reservoir for Y. enterocolitica with the pig as the predominant host for pathogenic Y. enterocolitica belonging to serogroups O:3 and O:9. The consumption of under-cooked pork plays a major role in the epidemiology of Y. enterocolitica infections. The mode of transmission is primarily by consumption of contaminated food or water. Infection from treated mains water has not been reported in the UK. Person to person spread also occurs. Transmission by transfusion of contaminated blood has been reported. Contact with infected animals is another source of infection.

Occurrence in water sources
Studies have shown that human pathogenic serotypes of Y. enterocolitica can be found in sewage and polluted surface waters, and there is some evidence that these strains can enter drinking water via pollution from these sources. Some non-pathogenic strains have been isolated from drinking water, but these are considered to be of environmental origin and of no public health significance. A particular feature of Y. enterocolitica is its ability to grow at low temperatures and to survive for long periods in the aquatic environment (survival has been shown under experimental conditions for 18 months in water at 4°C). Such long survival can make it difficult to determine the origin of contamination when Yersinia organisms are detected.

Sources of exposure
Y. enterocolitica infection is nearly always acquired by the oral route, so exposure to waterborne Yersinia organisms would be by ingestion of contaminated water. However, available evidence suggests that the major sources of exposure for humans are foods, particularly meat, meat products, milk and dairy products.

Susceptibility to removal or inactivation by conventional water treatment processes
The normal disinfection procedures used in water treatment are sufficient to avoid the transmission of Yersinia if the water is of low turbidity. Studies have shown the complete inactivation of these bacteria by free chlorine levels of 0.2-0.5 mg.l⁻¹ applied for 10 minutes at pH 7.0, and by 0.05 mg.l⁻¹ of ozone after 1 minute of contact regardless of pH.
Acanthamoeba

This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

The genus Acanthamoeba
Acanthamoeba are environmental amoebae found in most soil and water habitats, and there are at least 18 reported species divided into three morphological groups. The organism can infect a variety of mammals, including humans, producing severe and often fatal disease. Acanthamoeba species are not parasites as they do not require the infection of a host organism to complete their life-cycle. They are therefore referred to as “free-living”. The main species reported as causing human disease are A. polyphaga, A. castellani, and A. culbertsonii.

Morphology
Acanthamoeba is characterised by a feeding and replicating trophozoite which, under adverse conditions, can form a dormant cyst stage. Trophozoites measure 25 to 40 μm in length and the cysts 15 to 28 μm depending on the species.

Biochemical characteristics
Acanthamoeba is an aerobic organism and as such cannot exist as the trophozoite stage in environments with a low oxygen content. However, Acanthamoeba cysts have been isolated from anaerobic material such as faeces, sewage and other habitats low in oxygen. The cysts have been recovered from sea water although the trophozoites are killed by saline concentrations of greater than 1% (w/v).

Cultural characteristics
Acanthamoeba trophozoites have a growth temperature range of 12°C to 45°C depending on the species. All pathogenic species will grow at 35°C to 37°C with an optimum of about 36°C. The cysts will retain viability from -20°C to 56°C. Acanthamoeba cysts are highly resistant forms capable of withstanding extremes of temperature, disinfection and desiccation. This accounts for the presence of the organism in virtually all soil, natural or man-made aquatic sites and even the atmosphere. When favourable conditions occur, such as a ready supply of bacteria and a suitable temperature, the cysts hatch (excyst) and the trophozoites emerge to feed and replicate.

Detection methods
Acanthamoeba can be easily isolated and cultured on non-nutrient agar culture plates covered with a dense lawn of the bacterium Escherichia coli (NNA-E. coli). Trophozoites feed and replicate on the bacterial food source. If cysts are present in the sample, these hatch (excyst) and the emergent trophozoites grow on the bacteria. With experience, Acanthamoeba can be identified from other free-living amoebae on NNA-E. coli medium or in microscope slide preparations by their characteristic morphology. However, biochemical and molecular methods are necessary for the identification of the various Acanthamoeba species.

Health effects
Certain species of Acanthamoeba are pathogenic to humans causing two clinically distinct diseases, affecting the brain and the cornea respectively. Although Acanthamoeba is common in most environments, human contact with the organism rarely leads to infection. Granulomatous amoebic encephalitis (GAE) is a chronic disease which may be found in people who are immunosuppressed as a result of chemotherapy, or drug or alcohol abuse. Cases of GAE in patients with acquired immune deficiency syndrome (AIDS) have also been reported. GAE is sub-acute or chronic and invariably fatal. The route of infection in GAE is unclear although invasion of the brain may be via the blood following a primary infection elsewhere in the body, possibly the skin or lungs. GAE is extremely rare with only 50 cases reported worldwide including one in the United Kingdom. A. culbertsoni is the species most commonly associated with GAE.

(continued)
Health effects (continued)

Acanthamoeba keratitis is a severe and potentially blinding infection of the cornea which affects previously healthy persons. Patients typically have inflammation of the eye and are in considerable pain, which is made worse by exposure to bright light. Infection is initially of the corneal surface, with point lesions, but later ulceration may occur. An alternative clinical appearance is of microscopic abscesses which may be in a circular pattern or as a ‘snow-storm’ effect. If Acanthamoeba is not identified as the cause of such symptoms, the disease will progress remorselessly. The protozoa can penetrate into the area behind the cornea, causing deep-seated abscesses, and the cornea may be perforated, leading to loss of vision. Treatment is possible in the early stages of infection, but if the disease is allowed to progress, corneal grafting may be needed. Acanthamoeba cysts may remain in the eye and re-infect the new graft, thus continuing the disease. Wearers of contact lenses are most at risk from infection and account for 90% of reported cases in the United Kingdom. The remaining cases are caused by direct injury to the eye. Approximately 250 cases of Acanthamoeba keratitis have occurred in the United Kingdom since the disease was first recognised in 1976. Although rare, the incidence of Acanthamoeba keratitis in this country has risen sharply in recent years with 3 cases reported in 1989 and 80 in 1995. A. polyphaga and A. castellanii are the two species most commonly reported in cases of Acanthamoeba keratitis.

Routes of transmission

Contact lens-related Acanthamoeba keratitis usually arises from contamination of the contact lens storage case. The primary source is thought to be tap water. If the contact lens case is not cleaned and disinfected adequately (and some lens wearers admit to washing lenses or cases in non-sterile water from the tap), conditions may be favourable for survival and replication of Acanthamoeba trophozoites. The presence of bacteria, yeasts and other organisms will provide them with a ready source of nutrition, and since they can adhere to some polymers used in the manufacture of contact lenses, transfer to the eye can occur, and infection may be initiated.

Occurrence in water sources

Acanthamoeba can be found in all aquatic environments, including chlorinated swimming pools and drinking water. Acanthamoeba have been more frequently isolated from bathroom cold water supplied from roof storage tanks than from mains water. Water in the tanks can be open to airborne contamination, and accumulation of sludge can provide suitable conditions for many microbes, including Acanthamoeba, to multiply. No studies have yet been undertaken to establish whether Acanthamoeba enter the tanks direct from the mains water supply or from the atmosphere.

Sources of exposure

The primary source of Acanthamoeba infection of the cornea in contact lens wearers is thought to be tap water in the home or the workplace, which is used to clean storage cases or to prepare solutions. However, one cannot discount the fact that Acanthamoeba has also been found in dust and dirt around wash-basins, and in hot-tubs and swimming pools (even when chlorinated). The wide environmental distribution of Acanthamoeba thus presents a constant challenge to the contact lens wearer.

Susceptibility to removal or inactivation by conventional water treatment processes

Acanthamoeba cysts are fairly large, and would be efficiently removed by filtration processes. The cysts, but not the trophozoites, are resistant to the levels of chlorine which would be present in adequately treated mains water supplies.
Drinking Water Inspectorate Fact Sheet

Cyclospora cayetanensis

Fact Sheet No 12: Issue No 1  March 1997

This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

The genus Cyclospora
There are a number of species of Cyclospora but only one, C. cayetanensis has been found to infect man. C. cayetanensis has previously been described variously as cyanobacteria-like, blue green algae-like or Cryptosporidium-like bodies.

Morphology and life cycle
Cyclospora are protozoan parasites, related to Cryptosporidium. They are small single celled protozoa which have a life cycle consisting of multiplying (proliferative) asexual and sexual stages within host cells, with the production of resistant oocysts. Following ingestion, the oocysts release banana shaped motile sporozoites. The asexual stages produce motile merozoites which spread the infection within the gut tissues. They complete their life cycle within a single host. The mature (sporulated) oocysts have two motile forms (sporozoites) in each of two bodies (sporocysts) within the spherical oocyst. On excretion, in the faeces, the immature oocysts appear in unstained wet-mount preparations as smooth, round bodies of 8–10 μm diameter, containing an inner, greenish-tinged, mulberry-like structure of small (1–2 μm) membrane-bound spheres. C. cayetanensis has not been found in any animal other than man. However, it remains unclear whether humans are the only definitive host for C. cayetanensis, and the possibility that an intermediate host might play a part in the life cycle cannot yet be discounted.

Cultural characteristics
Cyclospora organisms have not yet been grown in tissue cultures or laboratory animal models.

Detection methods
The oocysts of the parasite can be detected in clinical samples (mainly faeces) by phase-contrast or differential interference contrast (DIC) microscopy of wet-mount preparations. The oocyst wall fluoresces blue under ultraviolet light. Oocysts may be stained in dried smears on slides, using modified Ziehl-Neelsen (MZN) acid-fast staining. By this method, oocysts appear variably stained from deep red to pink, with a mottled pattern of staining, or may remain unstained. In the latter case often showing a shiny or frosted glass-like appearance. These methods are often restricted to samples from patients giving a history of foreign travel, and cases without a history of such travel would therefore tend to be missed. Detection of oocysts in environmental samples has not been reported. Limited attempts have been made using the techniques currently available for detection of Cryptosporidium oocysts, but have so far been unsuccessful. Suitable reagents for specific stains such as fluorescent tagged monoclonal antibody stains (IFAT), and molecular methods (e.g. PCR) are not yet available. No animal or tissue culture model has yet been reported.

Health effects
After the incubation period, thought to be about a week, the symptoms include severe watery diarrhoea, abdominal cramps and nausea, and there may be mild fever, weight loss and prostration. After the initial phase the illness may relapse for a period. As with most newly-recognised infectious diseases, this picture reflects the findings in the more severe cases, and wider awareness may increase the clinical spectrum associated with this organism. Asymptomatic infection has been noted in the population of developing countries. The parasite is believed to develop intracellularly, within the mucosal cells lining the small bowel (enterocytes). Microscopically, the mucosal tissues may show changes typical of other gastrointestinal infection. Little is known of the life cycle or pathogenesis of Cyclospora cayetanensis. Treatment with cotrimoxazole appears to be effective in symptomatic cases.
Drinking Water Inspectorate Fact Sheet

Cyclospora cayetanensis

Routes of transmission
C. cayetanensis has been reported in indigenous subjects and travellers staying in or returning from Nepal, SE Asia, South and Central America and a number of holiday areas (Turkey, Dominican Republic). These reports suggest that transmission is associated mainly with poor hygiene and contaminated food and water. Sporadic cases and outbreaks have been reported in the USA, in the UK infections are most often seen in people who have travelled abroad. Infection has also been found in AIDS patients. Cyclospora are unable to grow or multiply in the environment although the oocysts allow the parasite to be transmitted through the environment. Like Cryptosporidium, Cyclospora is transmitted by means of oocysts which are found in the stools of infected patients. However, unlike Cryptosporidium, the oocysts are excreted in an immature or unsporulated form (i.e. non-infectious). The oocysts require a period of about a week in the environment, depending on conditions, to mature and to be infective if swallowed. Although infection is thought to be derived from other infected humans, the requirement for a period of maturation in the environment implies that the route of transmission is unlikely to be directly faecal-oral. Thus the majority of infections are likely to be transmitted by an indirect route. Some reports have suggested the possibility of transmission via food, such as salad vegetables and soft fruits, or by water. However, limited studies have failed to recover oocysts from the environment, including water samples.

Occurrence in water sources
Little direct information is available, but Cyclospora organisms have been detected in stored, chlorinated drinking water during an outbreak of illness in a military establishment in Nepal. Contaminated drinking water and recreational bathing waters have been implicated in other epidemiological studies, but without direct demonstration of the presence of Cyclospora organisms.

Sources of exposure
As noted above, the main source of exposure is infected humans, though the requirement for the oocysts to mature in the environment seems to rule out direct person-to-person contact as a source. Contaminated water is thought to be a source of exposure, but compelling evidence for this has so far only been obtained in one outbreak. Consumption of undercooked meat has been considered as a source. Cyclospora has been identified as the cause of some large foodborne outbreaks in the USA associated with consumption of soft fruit (raspberries) imported from an underdeveloped country. The likely infective dose for man is not known, but is probably low. The risk of spread from imported infections within the UK is probably low.

Susceptibility to removal or inactivation by conventional water treatment processes
No specific information is available on the effectiveness of water treatment processes for removal of Cyclospora. However, since Cyclospora oocysts are slightly larger than those of Cryptosporidium, for example, they should be efficiently removed by coagulation-sedimentation and filtration. There is indirect evidence that the oocysts are resistant to disinfectants, since they have been found in a drinking water supply containing 0.3-0.8 mg/l of free chlorine, which was implicated in an outbreak of Cyclospora infection.
Drinking Water Inspectorate Fact Sheet

**Isospora belli**

Fact Sheet No 13: Issue No 1

March 1997

This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

**The genus Isospora**

There are many species of *Isospora* but only *I. belli* infects humans.

**Morphology and life cycle**

The *Isospora* are coccidian parasites related to *Cryptosporidium* and *Cyclospora*. They are single celled protozoan parasites which have a life cycle consisting of multiplying (proliferative) asexual and sexual stages growing within host cells. Following ingestion of mature oocysts, motile sporozoites are released which infect the mucosal cells of the small bowel. The asexual developmental stages produce motile merozoites which spread the infection within the gut tissues. They complete their life cycle within a single host. The morphological distinction from other coccidia is usually by the oocysts which are found in the faeces, or on the developmental stages within gut tissues. The oocysts are translucent, elliptical bodies (22-33 x 12-15 μm) which are excreted, mainly undeveloped, but sometimes partially or fully developed. The immature oocysts contain a single spherical body (sporoblast). This subsequently divides into two bodies (sporocysts) which mature to produce four motile banana-shaped stages (sporozoites) in each.

**Cultural characteristics**

*Isospora*, like other coccidia, is an obligate parasite, and cannot be cultured *in vitro*.

**Detection methods**

Diagnosis is most often through microscopical examination of faeces, usually after concentration of the specimen. Detection of oocysts in environmental samples has not been reported. Detection of the oocysts in faeces may be by wet-film preparations, either unstained or following incubation with carbol-fuchsin. Alternatively, dried faecal smears can be stained using acid-fast stains such as the modified Ziehl-Neelsen method. The oocysts appear as variably red-staining spherical inner bodies (sporoblasts or sporozoites) within the unstained transparent oocyst wall. Suitable reagents for specific stains such as fluorescent tagged monoclonal antibody stains (IFAT), and molecular methods (e.g. PCR) for detection are not yet available.

**Health effects**

Infection with *I. belli* may cause colicky pain and, in some cases, diarrhoea, malabsorption and fever. In those with normal immunity, the symptoms are usually mild and self-limiting although in some patients symptoms may be recurrent or even chronic. Infection is associated with AIDS patients, particularly in Africa, Central and South America and the US. In these cases, the infection may be more severe and likely to be recurrent or chronic. The infection can be controlled with trimethoprim-sulphamethoxazole, although symptoms may recur when treatment is stopped.

**Routes of transmission**

*Isospora*, like the other coccidia, are unable to grow or multiply in the environment although the oocysts allow the parasite to be transmitted through the environment. Infection follows ingestion of mature oocysts by the faecal-oral route. It is likely also to be transmitted directly through poor hygiene, and by contaminated food and water. The infection is found primarily in warmer regions of the world such as Africa and Central and South America, and is occasionally reported in the UK in people returning from abroad, and from AIDS patients. Significant secondary spread is thought unlikely, except perhaps through homosexual contact.
Isospora belli

Occurrence in water sources
No direct information is available. Transmission of Isospora belli to humans from contaminated water and infected animals is suspected but has not been clearly demonstrated.

Sources of exposure
Transmission appears to be from person to person or animal to person through contaminated food or water, or by hand to mouth. Close contact in barracks or institutions, the presence of other intestinal disease, and poor hygiene appear to favour transmission.

Susceptibility to removal or inactivation by conventional water treatment processes
No specific information is available on the effectiveness of water treatment processes for removal of Isospora. However, since Isospora oocysts are considerably larger than those of Cryptosporidium, for example, they should be efficiently removed by coagulation-sedimentation, and especially by filtration. No direct information is available on susceptibility to disinfectants, but the oocysts are likely to be more resistant than indicator bacteria such as coliforms.
Microsporidia

This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

Genera of microsporidia
Microsporidia are distinct from other protozoa and are widely distributed in nature, with more than 100 genera and approximately 1000 species. They have been described as parasites in most classes of invertebrates and vertebrates, and have been recognised as pathogens of insects, fish, birds and mammals. A number of genera of microsporidia can infect man, most of which have only recently been recognised as human pathogens. The species found in humans are Encephalitozoon cuniculi, E. hellem, E. intestinalis (Septata intestinalis), Enterocytozoon bieneusi, Nosema conneri, Pleistophora spp, Trachipleistophora hominis, and Vittaforma corneum.

Morphology and life cycle
Microsporidia are small unicellular protozoon parasites which have a life cycle consisting of multiplying (proliferative) and spore-forming stages, both of which take place within host cells. Microsporidia are able to complete their life cycle within a single host. The spores are much smaller in size than those of other enteric protozoa. The spores of microsporidia have a double-layered wall, a protein outer spore, and an inner wall of chitin. Following ingestion of the spore by a suitable host the spore's nucleus and cytoplasm is "inoculated" into a suitable host cell by means of a polar tube structure. During the proliferative stages the organisms are generally amorphous, rounded, irregular or elongated with a very simple structure containing one or many nuclei within the cytoplasm. When examined by electron microscopy spores contain a tightly coiled polar tube, the number of coils differing with the species, this being a defining characteristic. The parasite may be in direct contact with the cell cytoplasm or be surrounded by a host-cell derived membrane (a parasitophorous vacuole). The parasite appears to absorb pre-formed nutrients from the host cell. As the cells develop they multiply, by binary or multiple fission or by producing many nuclei followed by division of the cytoplasm, each new parasite containing approximately half of the nuclei (plasmodomy).

Cultural characteristics
Microsporidia are obligate intracellular parasites, so cannot be cultured in vitro. Some of the human microsporidia have been grown in tissue cultures, and others in immunosuppressed mice.

Detection methods
The stages of the parasite described above are not easily detected in tissues, except when special staining methods are used. Diagnosis generally depends upon detection of the tissue stages using modified or conventional histological stains or by electron microscopy. Spores which have been excreted in faeces or body fluids, depending on the site of multiplication, can be detected by conventional light microscopy or by electron microscopy. Spores in faeces are hard to distinguish from the many bacteria, bacterial spores, fungi and other elements also present. The microsporidia causing human disease have not been detected in environmental samples, in food, or in surface or potable waters. By light microscopy the spores can be detected in faeces by using polychrome or chitin stains but these are non-specific and require considerable expertise. More specific stains such as fluorescent tagged monoclonal antibody stains and molecular methods are not yet available. Within the spores the tightly packed coils of the polar tube provide a diagnostic feature using electron microscopy. Continuous flow centrifugation and flow cytometric analysis have been used to detect environmental microsporidial spores in water, but these methods are unlikely to be useful for the species causing human infection.
Microsporidia

Health effects
Microsporidia have emerged as human pathogens in recent years, predominantly in people infected with HIV, and particularly in the late stage of AIDS. Increasing awareness and the increase in availability of expertise may increase the clinical spectrum associated with, and the frequency of, detection of these infections. Microsporidia are opportunistic pathogens, usually requiring sub-optimal immunity in the host to cause disease. The effects on health differ with the different parasite species. It is possible that people with normal immunity may be infected subclinically with the potential for this to develop into clinical disease if immunity becomes compromised. Little if anything is known about infectivity but the infective dose is probably low. There is little evidence on the pathogenetic mechanisms or the relative pathogenicity of different species. The infection may be generalised or localised. In enteric infections microsporidia can be found in the duodenum, small intestine and the biliary tree and sometimes also in the lamina propria (i.e. deeper tissues). Infected intestine may show non-specific changes to villous architecture and there is some evidence of malabsorption. Microsporidia may also be found in pulmonary, ocular, muscular, renal, nasopharyngeal, and CNS tissues.

Routes of transmission
Little is known about the routes of transmission of infection in man, or the possibility of animal reservoirs of infection. Microsporidial spores enable the parasite to be passed either to an adjacent cell in the same host or to another host if the spore has been excreted in faeces or body fluids. To date they have not been isolated from potable water. Large numbers of spores may be excreted in faeces or urine by infected hosts. Transmission of excreted spores may be direct (faecal-oral, including homosexual transmission) or, probably, via the environment, for example by water. Some species may be spread by other means including aerosols. Spores may also be transmitted to new hosts by ingestion of infected tissues. Some species share characteristics with species known to infect animals and may thus be zoonotic (transmitted between animals and man) although further evidence is needed to confirm this. Some human clinical infections may result from endogenous reactivation when immunity is compromised.

Occurrence in water sources
At present the microsporidia causing human disease have not been detected in environmental samples, in food, or in surface or potable waters. However, this may be due to the lack of efficient detection methods.

Sources of exposure
Little information is available, though contact with infected individuals seems the most likely source of exposure. Spores may be excreted in faeces or urine, so sewage pollution or other faecal contamination may be another source, though this remains conjectural. There is some evidence of acquisition of infection during travel to underdeveloped countries and these parasites may represent a form of travellers’ diarrhoea. There have not been any reports of large outbreaks, and there is no firm evidence of waterborne infection. Hygienic precautions are needed to limit secondary (person-to-person) transmission.

Susceptibility to removal or inactivation by conventional water treatment processes
No information is available on removal of microsporidia by water treatment processes. The small size of spores (ca 1-4.5 μm) makes penetration of larger pore size (>1 μm) filters likely. However, as they are of a similar size to bacteria, they should be removed reasonably effectively by conventional coagulation-sedimentation and filtration processes. Little is known about the susceptibility of spores to disinfectants, or other means of controlling environmental transmission.
Toxoplasma gondii

This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

Definition
Toxoplasma gondii is a protozoon parasite of the class Sporozoa, subclass Coccidia and derives its name from the Greek word “toxon” meaning arc or bow, referring to the characteristic crescent shape of the active tachyzoite form of the organism. T. gondii was first recognised in 1908 in the blood of the north African rodent, the gondi. Since that time T. gondii has been found to affect almost all warm-blooded animals including man.

Morphology and life cycle
T. gondii has both a sexual and an asexual cycle. The latter occurs in a wide range of warm-blooded animals but the only definitive hosts in which the full sexual cycle has been observed are members of the cat family (Felidae). There are three distinct stages of the organism in the complete life cycle; sporozoite (oocyst), tachyzoite (active form) and bradyzoite (tissue cyst). After ingestion of tissue cysts from infected animals by the cat, the tissue cyst wall is lysed by the cat’s digestive enzymes and bradyzoites are released. These then penetrate the epithelial cells of the small intestine where they undergo initial asexual multiplication. This is followed by the sexual cycle in which male and female gametes migrate into the gut and combine to form a zygote which then develops into an immature oocyst. Final sporulation of the oocyst usually takes place approximately 1 to 5 days after shedding in the cat faeces. Final sporulation is dependent on environmental conditions and does not usually occur below 4°C or above 37°C. The mature oocyst measures approximately 12 µm x 11 µm and is now potentially infective to any warm-blooded animal that might ingest it. Cats often shed oocysts over a period of 2-3 weeks and peak levels of shedding may reach over a million per day. Oocysts are extremely resistant to adverse environmental conditions and may retain infectivity for several months at temperatures of -5°C. In tap water, oocysts have been reported to remain viable for up to 17 months. After ingestion of oocysts by other animals T. gondii may enter the blood and lymphatic system where it multiplies asexually to form the characteristic crescent-shaped tachyzoite (measuring approximately 2 µm x 6 µm). Tachyzoites can rapidly invade cells where, inside vacuoles, they may either continue to replicate in this life-stage or may develop into bradyzoites. Tissue cysts containing bradyzoites can vary in size from 12 µm or less in diameter up to 100 µm or more. Tissue cysts have a greater predilection for the central nervous system but are also found in heart and skeletal muscle. Tissue cysts may persist in the infected host for life and, in the immunocompetent, probably cause no harm. However, ingestion of infected tissues by another mammal may result in that infection being passed on by a mechanism similar to that of ingestion of oocysts.

Cultural characteristics
T. gondii is an obligate intracellular pathogen. The most common method for isolation and culture of the parasite is by inoculation into rodents. However, growth of T. gondii is also possible in several tissue culture systems although these are significantly less efficient.

Detection methods
Direct detection by microscopy is possible although in environmental samples this may be very difficult due to oocysts often being present at a low density. Inoculation of materials either into rodents or tissue culture systems is possible. An alternative to culture is direct detection by gene amplification using the polymerase chain reaction (PCR). Confirmation of infection in man relies on serological testing. Immunoglobulin G (IgG) can persist for many decades and is, therefore, not an indicator of recent infection. Immunoglobulin M (IgM) typically persists for 6-9 months after infection and is helpful in diagnosing acute infection. Laboratory diagnosis in certain clinical groups (pregnant women, neonates, HIV-infected individuals, organ transplant recipients, etc.) requires specialised testing.
Health effects
In the immunocompetent adult infection is subclinical in approximately 85% of cases. Where
symptoms do occur these are often a mild “flu-like” illness and sometimes swollen lymph glands
(particularly the cervical lymph nodes). Very rarely, symptoms may persist for many months or years
in a form of the disease termed chronic active toxoplasmosis. In the immunosuppressed (e.g. organ
transplant recipients, patients undergoing chemotherapy) or immunodeficient, infection is life-
threatening. Where a pregnant mother acquires infection shortly before or after conception, there is
a significant risk of transmission to the foetus. Where this occurs in early pregnancy the
outcome may be profound with either gross foetal abnormality or spontaneous abortion. Where
maternal infection occurs later in pregnancy the risk of transmission to the foetus is greater but
symptoms are often less severe, or the child may appear asymptomatic at birth (although further
complications of congenital toxoplasmosis may occur later in life). Congenital toxoplasmosis is a major
cause of morbidity and mortality in sheep, causing approximately 30% of all diagnosed abortions and
affecting up to 65% of all flocks.

Routes of transmission
Transmission may occur by the oral route, through the ingestion of either oocysts or tissue cysts.
Transmission by inhalation of aerosolised oocysts is thought to be a possibility. Transmission from
pregnant mothers to unborn offspring can occur via the placenta (in both humans and animals). It has
been suggested that toxoplasmosis can also be transmitted through the milk of lactating females,
though this has not been definitely proven.

Occurrence in water sources
There is no information on the distribution of Toxoplasma in water sources, but since resistant oocysts
are formed, these are likely to be found in water contaminated by the faeces of infected animals or
humans. There has, to date, been only a single well-documented outbreak of toxoplasmosis in man
due to contaminated drinking water. Over a 9-month period in 1995 in British Columbia, Canada, 110
acute cases of toxoplasmosis were recorded and included 42 pregnant women and 11 neonates
identified through a pregnancy-related screening programme. In the absence of this screening
programme it is probable that the outbreak would not have been identified. Thus, it cannot be
excluded that other such outbreaks do occur but, due to the non-specific nature of the symptoms
associated with toxoplasmosis and the absence of any screening programs, they are likely to go
unnoticed. It is suspected that the Canadian outbreak was due to contamination of a reservoir or its
feeder streams by the faeces of domestic, feralised or wild cats.

Sources of exposure
Exposure to oocysts may result from consumption of contaminated food such as unwashed
vegetables, or via unwashed hands after contact with pet cats, or contaminated soil or other material.
Children can become infected by playing near cat litter trays. Drinking contaminated water is a
possible source of exposure, but the extent to which infection occurs by this route is not known.
Exposure to tissue cysts is via raw or undercooked meat from an infected host. Cooks and butchers
handling raw meat are particularly at risk. Man and other animals can be infected by the congenital
route.

Susceptibility to removal or inactivation by conventional water treatment processes
Little information is available on the effectiveness of commonly-used water treatment processes.
However, based on experience with similar organisms of comparable size (e.g. Cryptosporidium
oocysts) it is likely that number of Toxoplasma oocysts will be significantly reduced by filtration and
cogulation-sedimentation. The oocysts are likely to be resistant to disinfectants such as chlorine. Due
to the obligate intracellular nature of Toxoplasma, regrowth in water will not occur.
This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

Adenovirus types
The human adenoviruses belong to the Mastadenovirus genus in the family Adenovirus. There are 47 serotypes of human adenoviruses which are grouped into six sub-groups (A-F). Within each serotype there are genetically different strains.

Morphology
Adenoviruses appear hexagonal by electron microscopy but are in fact icosahedrons, about 75 nm in diameter, with a distinct surface structure. They are non-enveloped and the outer coat (capsid) is made up of 240 capsomeres (hexons) and 12 vertex capsomeres (pentons) each with a protruding fibre.

Physicochemical properties
The viruses have a double-stranded DNA genome, a buoyant density of 1.32-1.35 g.ml\(^{-1}\) in caesium chloride, and a sedimentation constant of 795S. Infectivity is stable between pH 6.0 and 9.5, and is undiminished after 70 days at 4\(^\circ\)C. Adenoviruses are resistant to chloroform and ether, but are disrupted by acetone, and inactivated by 0.1 mg.l\(^{-1}\) of free chlorine. Heating above 56\(^\circ\)C disrupts the capsid, allowing inactivation of the virus.

Cultural characteristics
Adenovirus replication is dependent on the presence of arginine, so an adequate concentration of this amino acid is needed in tissue culture media. The readily-culturable types of adenoviruses produce cytopathic effects in susceptible tissue culture monolayers in 1-4 weeks. These are often characterised by rounding and clumping of the cells (giving the appearance of “bunches of grapes”) before they slough from the culture vessel surface.

Detection methods
All the sub-groups are found in faecal material and are indistinguishable by electron microscopy. The subgroups A-E grow readily in cell culture of human origin causing a characteristic cytopathic effect under liquid medium. These serotypes have been isolated from sewage and water. Serotypes 40/41 are fastidious in cell culture and will only grow in a limited number of novel cell lines such as Graham 293 and PLC/PRF/5 cells.

The polymerase chain reaction (PCR) has been used to assay processed water samples and sewage for the presence of group adenovirus genome as well as the adenovirus 40/41 serotypes. These early studies have not yet produced a clear picture of the occurrence in the environment. There are problems of interference with the assay by organic compounds in the water. ELISA methods to detect all adenoviruses and also specifically the 40/41 serotypes have been developed for clinical material, but these do not have the sensitivity to be useful for environmental samples.

Health effects
The A-E sub-groups may cause upper respiratory infections, conjunctivitis, febrile illness especially with sore throat and also glandular involvement. Asymptomatic infections occur sometimes with long-term shedding of virus from the pharynx or gut. Many infections occur in children although infections in adults are also common. There is little cross protection between serotypes. Adenovirus F serotypes cause gastroenteritis in children particularly those under one year old. There is a regular winter rise in incidence but the numbers are not large.
Adenoviruses

Routes of transmission
Adenoviruses can infect man via the conjunctiva or the nasal mucosa. Infection is more efficient if the viruses are administered in an aerosol of particles in the size range of 0.3-2.5 µm. The relative roles of contact and airborne transmission have not been elucidated. It has been found that adenovirus infections which are followed by persistent faecal excretion spread to a higher proportion of susceptible contacts than do purely respiratory infections. This has been taken to suggest that infection can occur by the faecal-oral route, and this is thought to be significant particularly among children.

Occurrence in water sources
Several adenovirus types, particularly subgroups B, C, D and E, and serotypes 1 to 7 and 15, have been isolated from sewage, rivers, lakes, groundwater, drinking water and recreational bathing waters. All serotypes may be shed in faeces or may be present in sewage and therefore impact on water. In some bathing water studies respiratory illness has been associated with the use of recreational water, and it has been postulated recently that these symptoms may be due to adenovirus infection. There is, at the present time no firm clinical or epidemiological evidence to support this. Neither is there any evidence of spread of adenovirus F by the water route.

Sources of exposure
The main source of exposure is thought to be contact with infected individuals, with transmission of viruses by inhalation of aerosols. Contaminated water is another possible source, with infection occurring by ingestion, inhalation of aerosols, or direct contact with the eyes. Several large outbreaks of pharyngoconjunctival fever caused by adenovirus serotypes 3 and 4 have been associated with swimming pools. As viruses only replicate in living host cells no increase in numbers will occur in the environment.

Susceptibility to removal or inactivation by conventional water treatment processes
Studies with culturable viruses and bacteriophages indicate that normal water treatment processes (coagulation-sedimentation, filtration and disinfection), if properly applied, can produce water which is essentially virus-free. Adenoviruses are susceptible to inactivation by chlorination to the doses normally employed in water treatment.
Astroviruses

This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

Astrovirus types
Animal and human strains of astroviruses have recently been placed in a separate Astrovirus family. Seven different serotypes of human astroviruses have now been described. The most commonly identified is serotype 1.

Morphology
The astroviruses are spherical particles about 28 nm in diameter with no envelope, but in a proportion of the particles a distinct star shaped structure can be seen by electron microscopy.

Physicochemical properties
These viruses have a single-stranded RNA genome with a molecular weight of about 2500 kD. Their buoyant density has been reported as within the range 1.35-1.40 g.ml⁻¹ in caesium chloride. Astroviruses are acid stable and resistant to a range of detergents and lipid solvents. They are heat resistant for short periods above 56°C and survive for long periods below -20°C.

Cultural characteristics
Astroviruses are fastidious viruses in cell culture but will grow in CaCo-2 cells and a limited range of monkey kidney lines with the addition of trypsin to the cells.

Detection methods
Electron microscopy is the usual method of identification of these viruses in clinical specimens but this is not practical for environmental samples. Culture is possible, as described in the previous section. This method has recently been used in a few environmental studies which have demonstrated the presence of virus in sewage and water. Molecular techniques using the polymerase chain reaction (PCR) method have also been utilised to detect the astrovirus genome directly from processed water samples and from cell culture after limited incubation. Seeded studies have demonstrated the potential of the techniques but application to unseeded environmental samples has been limited. ELISA tests have been developed for clinical detection of astroviruses in faeces but as significant quantities of virus are needed for a positive reaction, the test is unlikely to be useful for environmental studies.

Health effects
Astroviruses cause gastroenteritis, predominantly diarrhoea, mainly in children under five years old although it has been reported in adults. Seroprevalence studies show that more than 80% of children between 5 and 10 years old have antibodies to astroviruses. Occasional outbreaks in schools, nurseries and families have been reported. The illness is self-limiting, of short duration and has a peak incidence in the winter. Astroviruses are the cause of only a small proportion of reported gastroenteritis infections. The numbers of reports are similar to those of adenovirus F. However, the number of infections may be under-estimated, since the illness is usually mild, and many cases will go unreported.

Routes of transmission
Person to person spread by the faecal-oral route is thought to be the most common route of transmission. Water has been suggested as a possible route but there is no firm evidence to support this.
Astroviruses

Occurrence in water sources
Little information is available on the environmental occurrence of human astroviruses. However, since infected individuals may excrete large numbers of viruses in the faeces, they will be present in sewage, and occurrence in sewage-polluted water can be inferred. Since viruses only replicate in living host cells no increase in numbers will occur in the environment.

Sources of exposure
Contact with infected individuals is probably the most important source of exposure. Studies on marine bathing waters have suggested that contact with contaminated water may be a risk factor, but there is no definite epidemiological evidence to support this. Shellfish have been implicated as a source of exposure, but the evidence for this is considered to be dubious.

Susceptibility to removal or inactivation by conventional water treatment processes
No direct information is available on removal of astroviruses by water treatment processes. However, information gained using other culturable viruses and bacteriophages indicates that normal water treatment processes (coagulation-sedimentation, filtration and disinfection), if properly applied, can produce water which is essentially virus-free. No specific information is available on the susceptibility of astroviruses to disinfectants such as chlorine and ozone.
Caliciviruses

Molecular characterisation of the genome has placed the caliciviruses within the Calicivirus family and confirmed that they are distinct from the Norwalk-like viruses.

Morphology
The "classic" calivirus as seen by electron microscopy appears spherical and approximately 32-38 nm in diameter. Although the edges are indistinct, the surface morphology has characteristic cup-shaped hollows in a proportion of particles, which differentiates these viruses from Norwalk-like viruses. These cup-like hollows, of which there are 32, give the virus its name (calyx = cup), and form very characteristic patterns in electron micrographs, depending on which axis of symmetry the virus particles are viewed along.

Physicochemical properties
The caliciviruses have a single stranded RNA genome, a buoyant density of 1.36 g.ml⁻¹ in caesium chloride, and a sedimentation constant of about 189S.

Cultural characteristics
These viruses have not been successfully grown in culture.

Detection methods
Electron microscopy of faecal samples is the only reliable method of detection in clinical samples. No environmental studies have been reported.

Health effects
The human caliciviruses cause gastroenteritis, mainly in babies under 1 year old, but also in the 1 to 5 year age group. Most cases involve diarrhoea and/or vomiting. The illness usually lasts for 1 to 11 days, during which time viruses are excreted in the faeces. Studies in the UK, Japan and Saudi Arabia have shown that antibodies to caliciviruses are acquired during the first 5 years of life, and around 80% of children in the 6 to 12 year age group possess them, suggesting that exposure to these viruses is common. The frequency of recorded calicivirus infections decreases rapidly above this age group, presumably because of developing immunity in the population. A few reports of infections in the elderly are documented but infection is rarely seen in other adults. This again indicates long-lasting immunity after initial early infection, which is different to the epidemiology of Norwalk-like viruses.

Routes of transmission
The faecal-oral route of transmission is thought to be the most likely. Secondary infections are common, for example in hospitals and residential homes, indicating that person-to-person transmission is important.

Occurrence in water sources
No environmental studies on caliciviruses have been reported. However, since large numbers of viruses are excreted in the faeces, their presence in sewage can be inferred. This in turn means that the viruses may be present in sewage-contaminated water, though this has not been proved, and since young children are commonly infected and adults are immune, its public health significance is uncertain. Since viruses only replicate in living host cells, no increase in numbers will occur in the environment.
Sources of exposure
Little information is available. Contact with infected individuals is the only proven source of exposure. Shellfish (cockles, mussels and oysters) have been incriminated in outbreaks, indirectly suggesting that contaminated water may be a vehicle of transmission. The evidence for this is equivocal. Studies of outbreaks have either found caliciviruses in faeces of some victims, but not all, or have found other viruses to be present as well. The role of the caliciviruses in these outbreaks is therefore unclear.

Susceptibility to removal or inactivation by conventional water treatment processes
No direct information is available on removal of the "classic" caliciviruses by water treatment processes. However, information gained using culturable viruses and bacteriophages indicates that normal water treatment processes (coagulation-sedimentation, filtration and disinfection), if properly applied, can produce water which is essentially virus-free. No studies on the effect of disinfectants on caliciviruses have been reported.
Coxsackieviruses

This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

Coxsackievirus Groups A and B
The Coxsackieviruses are classified in the Picornavirus family and within the human enterovirus genus. Originally two coxsackievirus groups A (with 23 types) and B (with six types) were defined, based on the neurovirulence to sucking mice but all enteroviruses are now numbered 1-72. Other human enteroviruses are the polioviruses and echoviruses.

Morphology
The coxsackievirus particle appears spherical, approximately 30 nm in diameter and with no surface structure when viewed by electron microscopy (EM). It is icosahedral, has no envelope and the protein coat or capsid is made up of 60 protomers.

Physicochemical properties
These viruses have a single-stranded RNA genome, and a buoyant density of 1.34 g.ml⁻¹ in caesium chloride. The enteroviruses are resistant to ether, detergents and alcohol (70%) and stable between pH 3 and 10. They are sensitive to ultraviolet light, formaldehyde, N HCl and a free chlorine residual of 0.3 - 0.5 mg.₁. Aggregation within organic debris will however protect virions. They are destroyed by heating above 50°C but may remain viable for months at 4°C and days at 18°C. Infectious virus has been identified from seawater, river water, sediments, sewage, digested sludge, and soils.

Cultural characteristics
Most coxsackievirus A types grow poorly in cell culture; only types 7, 9 and 16 grow well. Coxsackievirus B types will grow readily in human and monkey derived cell cultures. Under liquid medium a characteristic cytopathic effect (CPE) is produced in a few days.

Detection methods
Culturing methods can be used for those coxsackievirus types which grow readily in mammalian cell cultures. Using a cell and agar based system, "plaques" (areas of cell death) are produced enabling direct enumeration of the virus infectious units. This assay method has been used for water samples for over 25 years and is the basis of most of our knowledge of viruses in the environment. Polymerase chain reaction (PCR) based methods for direct virus genome detection have been developed for use in clinical material. These methods have been adapted to detect enterovirus, rather than specifically coxsackievirus, genome from processed water samples and other environmental material. Many problems associated with inhibitors of PCR and interference from organics in the water are only now being successfully addressed. A combined approach of inoculating cell cultures with processed water samples and then using PCR for virus genome detection at an early stage is proving to be worthwhile. The assay will only detect infectious virions and may reduce interference to the PCR from substances in the original sample.

Health effects
Coxsackieviruses circulate constantly in the community with the most prevalent serotypes changing from year to year. Although a high proportion are asymptomatic infections, all serotypes may cause a variety of symptoms. A flu-like illness with fever, malaise and muscular ache is the most common symptomatic illness. Occasionally there is also upper respiratory tract involvement, diarrhoea and vomiting. Coxsackieviruses may also cause aseptic meningitis or, more unusually, transient paralysis. Other symptoms which may be caused by coxsackieviruses include skin rashes (hand, foot and mouth), myocarditis and pericarditis. These viruses are not a cause of gastroenteritis in the absence of other generalised symptoms.
Coxsackieviruses

Routes of transmission
Person-to-person spread by respiratory droplet and faecal-oral transmission are the most common routes. As coxsackievirus B can readily be identified in sewage it has been suggested that transmission by water, particularly recreational water, may occur. Evidence to support this hypothesis is lacking.

Occurrence in water sources
Coxsackieviruses can be found in sewage, and therefore are likely to be found in any water sources subject to sewage pollution. They have been found in river and canal water, sea water, and in the US in chlorinated wastewater effluents. Since viruses only replicate in living host cells no increase in numbers will occur in the environment.

Sources of exposure
The main source of exposure is thought to be contact with infected individuals, with transmission of viruses by inhalation of aerosols, or by the faecal-oral route. Contaminated water is another possible source, with infection occurring by ingestion or inhalation of aerosols, but there is a lack of firm evidence to support this.

Susceptibility to removal or inactivation by conventional water treatment processes
Studies with culturable viruses and bacteriophages have indicated that normal water treatment processes are effective for removing viruses from source waters. Studies have found coxsackieviruses in treated water after coagulation-sedimentation and filtration. Viruses have not been detected in finished water after disinfection.
Hepatitis E virus

Hepatitis E virus (HEV) has now been classified as a member of the *Calicivirus* family, although distinct from the Norwalk-like viruses and "classic" caliciviruses. The limited research reported so far suggests that there are some strain differences between isolates but that there may only be one serotype. It was formerly known as "enterically-transmitted non-A, non-B" hepatitis virus.

Morphology
The hepatitis E virus particle is a sphere of diameter between 28 and 32 nm with no envelope and little surface structure apparent by electron microscopy, though spikes and indentations can be seen.

Physicochemical properties
The genome is single stranded RNA and the buoyant density in potassium tartrate-glycerol gradients has been reported as 1.29 g.ml⁻¹. The virus is extremely labile, and is unable to withstand high salt concentrations. Hence the buoyant density in caesium chloride has not been determined. As the virus is spread by the faecal-oral route it will travel through the stomach, and must therefore be acid stable. Under laboratory conditions it has been shown to be sensitive to a number of chemicals.

Cultural characteristics
HEV has not yet been successfully grown in culture.

Detection methods
Diagnosis of a clinical infection is by detection of HEV immunoglobulin M in serum. Particles of HEV have been seen in the faeces of infected patients by electron microscopy, but not usually in large numbers. Neither of these approaches is relevant to environmental studies. Although there are reports of growth in cell culture most have not been confirmed. The polymerase chain reaction (PCR) method has been used to detect the HEV genome directly from processed water and sewage samples. The virus was concentrated using membrane filtration, elution and centrifugation.

Health effects
HEV causes acute hepatitis. The disease has an incubation period of 2 to 9 weeks (mean 6 weeks), and a fatality rate of 1 to 2%. Pregnant women are particularly at risk, and among this group the disease is fatal in 10 to 20% of cases. The proportion of asymptomatic infections is not known. The epidemiological picture is often one of large epidemics involving a high proportion of young adults. Clinical disease is rarely seen in children. Sporadic cases are also known to occur. The immunity rate in older adults is only about one third of the population. HEV is endemic in parts of Africa, most of Asia, the Middle East and Central America. Imported cases have been reported in Europe and North America.

Routes of transmission
Person-to-person transmission of faecal material is the usual route for sporadic cases of Hepatitis A virus and would seem the most likely route for sporadic HEV infections. However, the epidemics of HEV especially involving a young section of the community suggests other factors. Waterborne transmission has been suggested as the cause of many epidemics. HEV has been detected in sewage but this would be expected at a time of high incidence. The low immunity rate amongst the adult population is difficult to explain. Further study of the disease in endemic areas with more sensitive detection methods is required.
Hepatitis E virus

Occurrence in water sources
Very little direct information is available on the environmental distribution of HEV other than that it has been detected in sewage and polluted rivers. However, numerous outbreaks have been epidemiologically linked with faecally-contaminated water supplies, so widespread occurrence in polluted water can be inferred.

Sources of exposure
Epidemiological evidence is that the disease is transmitted by faecally-contaminated drinking water or contact with a faecally-contaminated environment.

Susceptibility to removal or inactivation by conventional water treatment processes
No direct information is available on removal of HEV by water treatment processes. However, information gained using culturable viruses and bacteriophages indicates that normal water treatment processes (coagulation-sedimentation, filtration and disinfection), if properly applied, can produce water which is essentially virus-free. No information is available on the susceptibility of HEV to disinfectants, but since the virus has been found to be highly labile in laboratory studies, it is likely to be easily inactivated. The epidemiological association of HEV infection with faecally-contaminated water indicates that adequate sewage and water treatment measures are normally sufficient to prevent spread of HEV.
Norwalk-like viruses

This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

Types of Norwalk-like viruses
Molecular characterisation of the RNA genome has demonstrated the Norwalk-like viruses to be part of the *Calicivirus* family distinct from classic members described in the separate data sheet on Caliciviruses. Different strains of Norwalk-like viruses circulate in the community at any one time and have been named after the place where they were first detected. Genome characterisation enables them to be placed in two broad groupings: Norwalk, Southampton and Desert Shield viruses belong to the genogroup I and Mexico, Illawar, and Dristol viruses belong to genogroup II. Strains of the genogroup II type seem to be the most common in the UK and probably in the US.

Morphology
The Norwalk-like viruses are 32-38 nm in size, and have no envelope. When viewed by electron microscopy an amorphous surface structure is apparent (hence the alternative name for this group of viruses: SRSV - "small round structured viruses") which lacks distinct geometric morphology.

Physicochemical properties
These viruses have a single-stranded RNA genome. They have a buoyant density of 1.39 - 1.41 g.ml⁻¹ in caesium chloride and possess a single major polypeptide. Norwalk virus is resistant to ether, low pH (e.g. pH 3.7 for 3 hours at room temperature), and shows some resistance to heat, some infectivity surviving at 60°C for 30 minutes.

Cultural characteristics
These viruses have not been successfully cultured.

Detection methods
There are no animal models nor can they be grown in cell culture. The viruses can be seen in faeces by electron microscopy (EM) if at least 10⁶ per gram particles are present. In practice this usually means a faecal specimen must be taken within 48 hours of the onset of symptoms. This method of detection is not practical for environmental samples.

The polymerase chain reaction (PCR) may be used to detect small numbers of viruses as it is an effective amplification step for a region of the virus genome. It is able to detect smaller numbers of virus particles than EM. The drawback is that the DNA polymerase region of the genome used as the basis of the test does vary between Norwalk-like virus strains so that at the present time only 90% of strains will be detected as compared to electron microscopy. The PCR method has been developed for clinical material and is only now being adapted for use on water and associated material. Seeding experiments using PCR as the detection method have successfully recovered Norwalk-like viruses from shellfish, and recently batches implicated in transmission of disease have been found to contain viruses. Methods for detection of the viruses in water are still under development.

Detection of 'wild' virus strains by an ELISA method using antibodies raised against the capsid protein has recently been reported for the Mexico and Norwalk viruses. However, each assay is very specific for its homologous strain thereby limiting its usefulness as a detection method for all strains which may be circulating. The sensitivity of this type of assay is similar to that of EM and not yet applicable to environmental samples.

Some studies on the inactivation of Norwalk viruses by chlorine have used infection of human volunteers as a bioassay for the virus.
Health effects
Although subclinical infections are recognised, the usual symptoms are nausea, profuse diarrhoea and projectile vomiting in more than 50% of those affected. Symptoms usually last less than 48 hours with no long-term effects. The ages with the highest number of reported cases of infection are less than five years and more than 65 years. It is the most common viral pathogen causing gastroenteritis reported in adults. It is likely that immunity is short-lived and different strains may confer limited cross protection. Re-infections probably occur throughout life but sporadic infections are under-reported. Outbreaks of gastroenteritis are commonly identified in elderly care homes, hospital wards and schools. Adults of all ages have been involved in cruise ship outbreaks, hotel outbreaks and after social events. Infections occur throughout the year, but increase in winter.

Routes of transmission
Infection is by the faecal-oral route, though the finding of large numbers of viruses in vomit has led to this being considered an important vehicle of transmission. Person to person spread directly by vomit (including aerosolised particles) or faecal contamination is the most common route of transmission. Other routes are contact with contaminated material, and ingestion of contaminated water or foods.

Occurrence in water sources
Municipal drinking water supplies have been implicated in outbreaks of gastroenteritis, usually following contamination by sewage. Transmission has also been indicated via contaminated ice, stored water on cruise ships, borehole water and contaminated recreational bathing waters. Since viruses only replicate in living host cells no increase in numbers will occur in the environment.

Sources of exposure
Exposure is by contact with infected individuals or faecally-contaminated water or other materials. Environmental contamination as a result of uncontrolled diarrhoea and projectile vomiting may lead to a high proportion of secondary cases. Adequate cleaning of contaminated carpets, curtains, toilets, and floors is essential to prevent secondary spread. The contamination by food handlers of salads and cake frosting (icing) has been implicated by epidemiological studies. Oysters and other shellfish grown in sewage-polluted water may contain Norwalk like viruses and have been implicated in many outbreaks. Cooked shellfish are less of a hazard.

Susceptibility to removal or inactivation by conventional water treatment processes
No direct information is available on removal of Norwalk-like viruses by water treatment processes. Information gained using culturable viruses and bacteriophages indicates that normal water treatment processes such as coagulation-sedimentation and filtration can reduce virus numbers to very low levels. However, Norwalk virus has been found to be more resistant to inactivation by chlorine than poliovirus type 1, Group A rotaviruses and bacteriophages. As most waterborne outbreaks result from sewage-contaminated water in supplies with an unknown concentration of chlorine, or no chlorine at all, it is unclear whether this relative resistance to chlorine is important in causing outbreaks of disease.
Non-Group A Rotaviruses

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This series of fact sheets summarises current knowledge on organisms which are known or potential pathogens, and which may have the possibility of a waterborne route of infection.

Rotavirus groups
Rotaviruses are classified according to group antigenic determinants found on the structural proteins and detected by immunofluorescence and ELISA. Group A, B and C rotaviruses are found in humans and animals but D, E and F have only been found in animals. Different serotypes exist within each of the groups and may re- assort their genetic material between serotypes.

Morphology
Rotaviruses are spherical non-enveloped particles, 70-80 nm in diameter. When the outer capsid is missing, as is common in faecal material, a rough edged, incomplete and non-infectious virion remains.

Physicochemical properties
Rotaviruses are part of the Reovirus family and have a double stranded RNA genome composed of 11 segments. The buoyant density is 1.36 g.ml⁻¹ in caesium chloride. Rotaviruses are stable over a pH range of 3 to 10, ether and chloroform resistant, heat sensitive over 50°C and more resistant to chlorine in the presence of organic matter. Magnesium or calcium ions stabilise the activity of the virus at low temperature. Viruses may remain infectious at 4°C for months if protected by organic material.

Cultural characteristics
Satisfactory culturing methods have not yet been developed for non-Group A rotaviruses.

Detection methods
Electron microscopy is used to detect rotaviruses in clinical samples by which all groups appear similar.
Group A rotaviruses are fastidious viruses in cell culture and will only undergo an abortive replication cycle in certain monkey kidney cell lines such as MA 104 or LLCMK2. Only very few non-group A rotaviruses have yet been cultured.
Detection by ELISA is now common for group A viruses in clinical samples and newly developed ELISA assays are being evaluated for group C rotaviruses.

Health effects
Group B rotaviruses have been reported mainly from China where they cause gastroenteritis in adults, often in large scale epidemics. Group C rotaviruses have been reported from many countries and in the UK cause a small proportion of the gastroenteritis in children. The actual incidence has yet to be determined.

Routes of transmission
Rotaviruses are spread most commonly by the faecal-oral route. Waterborne transmission of rotavirus B in China has been documented.

Occurrence in water sources
Rotaviruses have been detected in sewage, rivers and lakes, estuarine and marine waters, and in treated drinking water in some countries. Infection is usually associated with sporadic cases, but several large waterborne outbreaks have been documented. Environmental studies have not been reported for group C rotaviruses. Since viruses only replicate in living host cells no increase in numbers will occur in the environment.
Sources of exposure
Exposure is by contact with infected individuals or contaminated water or other materials. Group B rotaviruses have caused large outbreaks of diarrhoeal illness in mainland China. Here the virus entered the population as a result of faecal contamination of water supplies drawn from rivers, and then spread through the population by person-to-person contact.

Susceptibility to removal or inactivation by conventional water treatment processes
No direct information is available on removal of non-Group A rotaviruses by water treatment processes. However, information gained using culturable viruses and bacteriophages indicates that normal water treatment processes (coagulation-sedimentation, filtration and disinfection), if properly applied, can produce water which is essentially virus-free. No specific information is available on the susceptibility of non-Group A rotaviruses to disinfectants such as chlorine and ozone, though there is some evidence that Group A rotaviruses are generally more resistant to disinfectants than culturable enteric viruses such as polioviruses and coxsackieviruses.