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Bacteria of potential health concern

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5.1 INTRODUCTION

Organisms detected by the heterotrophic plate count (HPC) are ubiquitous in drinking-water. In order to assess the basis of confidence in existing measurements, particularly of the HPC, we have to re-examine the current role of potential waterborne bacteria in human disease. In this chapter, we therefore consider the following groups of bacteria of concern:

- commensal bacteria;
- recognized pathogens;
- emerging bacteria; and
- bioterrorist threat agents.

The key factor in assessing the utility of the HPC will be whether it will provide a trigger for the successful investigation for these agents.

This chapter addresses only the bacteria of concern; there are, of course, viruses and protozoa that fall into the same categories. Furthermore, *Cryptosporidium* oocysts and *Giardia* cysts and virus particles are not incorporated into biofilms permanently and of course do not go through growth cycles.

5.2 COMMENSAL BACTERIA

Many bacteria live within our gastrointestinal tract, on our bodies or in the environment with which we come into daily contact without there being any resulting disease. In these situations, the bacteria are non-pathogenic and are called commensal bacteria, which means “eating at the same table.” It is recognized, however, that many of these organisms can cause infections such as wound infections or septicaemia if they are introduced into body tissues, particularly if the person is immunocompromised.

Although many genera and species of heterotrophic bacteria have been isolated from water and have been found to colonize distribution systems, no outbreaks of associated human disease have been conclusively reported. Suspicions have been raised about several organisms, such as *Klebsiella* spp. and *Citrobacter* spp., but their frequent isolation and lack of involvement in human gastrointestinal disease make them very unlikely candidates. There are concerns about the potential for *Aeromonas* spp. and *Yersinia enterocolitica* to cause diarrhoeal disease.

5.2.1 *Aeromonas*

Species of *Aeromonas* are ubiquitous in the environment and commonly occur in soil, marine (Kaper *et al.* 1981) and freshwater habitats (Rhodes and Kator 1994). Marine recreational waters pose a potential source of human infection. In a study in southern Italy, many of the isolated strains produced several virulence factors, and all isolates produced cytotoxin and haemolysin. Three isolates produced enterotoxin, and all isolates bound to human intestinal cells in varying degrees (Krovacek *et al.* 1994). A survey of chlorinated water in which 286 samples were taken from taps and storage tanks in nine London and Essex boroughs and nine local hospitals revealed the presence of *Aeromonas hydrophila* in 25% of samples during the summer months and in 7% during the winter months (Millership and Chattopadhyay 1985).

Aeromonas spp. have been isolated from supplies of drinking-water throughout the world and are able to grow in drinking-water. Their growth is associated with the accumulation of biofilm on internal surfaces and is influenced by temperature, the availability of organic carbon and the degree of

stagnation. Biofilm can accumulate in the presence of chlorine at a concentration of 0.8 mg/litre.

In Swedish water distribution systems, sampling demonstrated counts of up to 300 cfu/100 ml in raw water and up to 750 cfu/100 ml in tap water samples (Kuhn *et al.* 1997). The significance of *Aeromonas* in drinking-water is not fully understood. It is recognized that, on occasions, the ingestion of *Aeromonas* spp. may lead to diarrhoeal disease, and this is associated with an enterotoxin (Janda and Duffey 1988). There are numerous reports of *Aeromonas* isolates from patients with diarrhoea, but also reports of *Aeromonas* strains that produce a heat-labile cytotoxin, have enterotoxin activity (Ljungh *et al.* 1977; Turnbull *et al.* 1984) and possess other pathogenic characteristics. It is suggested that when all are present in a strain, enteric infection may result. Human volunteer challenge trials using five enteropathogenic strains of *Aeromonas hydrophila* demonstrated diarrhoea in only 2 of 57 persons with administered doses ranging from 10^4 to 10^5 cfu (Morgan *et al.* 1985). A number of factors, such as age, immunocompetence, previously developed immunity, exposure and infective doses of the organisms, as well as the possession of virulence factors, could affect the ability of *Aeromonas* to establish overt infection.

In the United Kingdom study of infectious intestinal disease in England, the percentage isolation rates were the same in diarrhoeal cases and in matched controls (Food Standards Agency 2000).

The absence of defined outbreaks and the low levels of infectivity in human volunteer experiments suggest that people have a relatively high degree of resistance to infection with *Aeromonas*.

The significance of *Aeromonas* in drinking-water in the Netherlands has been reviewed (van der Kooij 1988), and the health authorities in the Netherlands have defined maximum values for *Aeromonas* present in drinking-water: i.e., 200 cfu/100 ml in water distribution systems and 20 cfu/100 ml in water leaving the production plant. However, there have not been any outbreaks of disease in the United Kingdom, even though blooms of *Aeromonas* occur in some distribution systems during the summer months.

There have been reports suggesting associations between the presence of heterotrophs in water in the distribution system and illness in the consumers of that water. In a study reported from Egypt, 9 out of 10 samples analysed from the district of Cairo were positive for *Aeromonas* strains, of which 56% were reported to be enterotoxigenic (Ghanen *et al.* 1993). *Aeromonas* was isolated from diarrhoeic and non-diarrhoeic faeces of children. Typing of the isolates was not performed. There have been two other reports of *Aeromonas* colonization of distribution systems (Havelaar *et al.* 1992; Moyer *et al.* 1992).

In the latter, sophisticated typing systems did not reveal any correlation between isolates made from drinking-water and those made from patients.

Additional information on *Aeromonas* may be found in WHO (2002).

5.2.2 *Yersinia*

Yersinia is a genus of heterotrophic bacteria with 11 recognized species, some of which cause disease in humans, and both pathogenic and non-pathogenic strains of *Yersinia* have been found in surface water and unchlorinated drinking-water (Lassen 1972; Caprioli *et al.* 1978; Cafferkey *et al.* 1993). The source of the organism is the environment or non-human hosts, such as wild animals and birds. However, only certain serotypes of *Yersinia enterocolitica* that occur in the environment are considered to be pathogenic for humans. This depends on the possession of virulence factors associated with pathogenesis of infection. Serotypes O:3, O:4,32, O:5,27, O:6,30, O:6,31, O:8, O:9 and O:21 are thought to be pathogenic for humans and cause diarrhoea or mesenteric adenitis, a disease that often mimics appendicitis. Other serotypes have been isolated from patients with infection, but their role is uncertain. The most common serotype of *Yersinia enterocolitica* associated with human infection is serotype O:3. The significance of *Yersinia* in patients with diarrhoea is uncertain, however; on occasion, it can cause mesenteric adenitis and reactive arthritis with an antibody response and is clearly pathogenic. On other occasions, most isolates from patients with mild diarrhoea do not contain the full set of virulence markers found in isolates from systemic infections.

5.2.3 *Klebsiella*

A study of *Klebsiella* species isolated from water in Germany identified so-called virulence factors such as pili, serum resistance and siderophore production in isolates from surface waters and compared them with clinical isolates (Padschun *et al.* 2001). Fifty-three per cent of surface water samples were positive for *Klebsiella pneumoniae*. The surface water isolates resembled the clinical isolates in the expression of these virulence factors, and it was suggested that further studies should be carried out to determine the public health implications. *Klebsiella* is ubiquitous in nature and is a commensal organism of the gastrointestinal tract, where it does not cause disease. It may be involved in urinary tract infection, particularly in females, where it is transferred across the perineum to the urethra, and it may be involved in wound infections, particularly following bowel surgery.

5.2.4 *Pseudomonas*

There are many species of *Pseudomonas* that are widespread in the environment and commonly occurring in soil and water. They are capable of growth in low-nutrient situations and can grow in water in distribution systems if they gain access and on materials used in domestic plumbing situations. They may colonize taps and grow on surfaces, such as plastic pipes in drink vending machines. *Pseudomonas aeruginosa* is the most important species for public health considerations, although it does not cause any effects if it is ingested. It is resistant to many antibiotics and can produce serious nosocomial infections if it gains access to the body through wounds or intravenous lines. Hospital control of infection procedures that limit the use of tap water is an effective measure to prevent disease. In the community, *P. aeruginosa* may readily colonize spa pools and lead to wound infections if persons with open wounds or sores use them. Care must also be taken in the care of contact lenses and contact lens solutions to prevent contamination by *P. aeruginosa* on taps, leading to eye infections from water contact.

5.2.5 Virulence factors

Pathogenic bacteria produce a variety of virulence factors — e.g., adherence factors, so that the organisms can attach to intestinal cells; enzymes, including haemolysin, that facilitate cell invasion; exotoxins; and several other factors that produce immunomodulation. The successful pathogen will possess a whole range of these factors, but some are critical; an example is *Vibrio cholerae* with and without cholera toxin gene, the former producing cholera and the latter being avirulent. It is important to appreciate that the possession of a single virulence factor by an organism not normally considered to be pathogenic may not be significant. The assessment of virulence should therefore include detection systems for a whole range of virulence factors. There are no simple tests available; although haemolysis on blood agar by heterotrophs (Payment *et al.* 1993) and cytopathic effects on Y1 and renal cell overlays (Lye and Dufour 1991) have been put forward as assessment methods, they will not indicate which organisms are potential human pathogens.

Virulence factors enable bacteria to survive in hostile environments. The approach to a better understanding of them should be to identify disease states where the organisms are involved through epidemiological studies, investigate the pathogenic mechanisms in detail, examine the host responses and then look at possible transmission routes, appropriate interventions and protection of the public.

5.2.6 Hospital-acquired infection

Many different types of heterotrophic bacteria occur in hospital distribution systems, and counts may increase because of stagnation caused by the many “dead ends” that result from previously modified systems. All wet areas in wards, such as sluices, showers and baths, become colonized with Gram-negative bacteria such as *Pseudomonas*, *Klebsiella*, *Citrobacter* and *Acinetobacter*. These areas also provide ecological niches for highly resistant organisms, which can be transmitted to patients and cause infection problems. The heterotrophic bacteria in the water distribution systems have not caused infection in patients by ingestion; however, in clinical areas or in special situations such as home care, where water is used to provide humidification in incubators, nebulizers and ventilators, the use of tap water has led to respiratory tract colonization and infections. These problems have been largely eliminated by policies for infection control. It has been recognized that tap water is not sterile and should not be used in situations where organisms from tap water or taps may initiate infection. Instead, the policies recommend the use of sterilized or boiled water in all situations that could pose a risk to patients. Any harmful effects of heterotrophic bacteria are therefore eliminated. Patients are, however, encouraged to drink tap water, as the heterotrophic bacteria present pose no risk unless the patient is significantly immunocompromised, in which case boiled water is recommended.

5.3 RECOGNIZED WATERBORNE PATHOGENS

Many of the organisms that cause gastroenteritis can be transmitted by the waterborne route when there is faecal contamination from humans or other animals. The human pathogens that can be transmitted orally via drinking-water are listed in Table 5.1 (compiled from data provided by the Communicable Disease Surveillance Centre), together with a summary of their health significance and main properties.

Table 5.1. Orally transmitted waterborne pathogens and their significance in water supplies

Pathogen	Health significance	Persistence in water supplies ¹	Resistance to chlorine ²	Relative infective dose ³	Important animal reservoir
Bacteria					
<i>Campylobacter jejuni</i> , <i>C. coli</i>	High	Moderate	Low	Moderate	Yes
Pathogenic <i>Escherichia coli</i>	High	Moderate	Low	High	Yes

Pathogen	Health significance	Persistence in water supplies ¹	Resistance to chlorine ²	Relative infective dose ³	Important animal reservoir
<i>Salmonella typhi</i>	High	Moderate	Low	High ⁴	No
Other salmonellae	High	Long	Low	High	Yes
<i>Shigella</i> spp.	High	Short	Low	Moderate	No
<i>Vibrio cholerae</i>	High	Short	Low	High	No
<i>Yersinia enterocolitica</i>	High	Long	Low	High (?)	No
<i>Pseudomonas aeruginosa</i> ⁵	Moderate	May multiply	Moderate	High (?)	No
<i>Aeromonas</i> spp.	Moderate	May multiply	Low	High (?)	No
Viruses					
Adenoviruses	High	?	Moderate	Low	No
Enteroviruses	High	Long	Moderate	Low	No
Hepatitis A	High	?	Moderate	Low	No
Enterically transmitted non-A non-B hepatitis, hepatitis E	High	?	?	Low	No
Norwalk virus	High	?	?	Low	No
Rotavirus	High	?	?	Moderate	No (?)
Small round viruses	Moderate	?	?	Low (?)	No
Protozoa					
<i>Entamoeba histolytica</i>	High	Moderate	High	Low	No
<i>Giardia intestinalis</i>	High	Moderate	High	Low	Yes
<i>Cryptosporidium parvum</i>	High	Long	High	Low	Yes
Helminths					
<i>Dracunculus medinensis</i>	High	Moderate	Moderate	Low	Yes

? - not known or uncertain

¹ Detection period for infective stage in water at 20 °C: short, up to one week; moderate, one week to one month; long, over one month.

² When the infective stage is freely suspended in water treated at conventional doses and contact times. Resistance moderate, agent may not be completely destroyed.

³ Dose required to cause infection in 50% of health adult volunteers: may be as little as one infective unit for some viruses.

⁴ From experiments with human volunteers.

⁵ Main route of infections is by skin contact, but can infect immunosuppressed or cancer patients orally.

The number of recognized outbreaks is low compared with reports of outbreaks due to other routes of transmission. Details of outbreaks of these potential waterborne pathogens in the United Kingdom are given in Table 5.2.

Table 5.2. Outbreaks of bacterial pathogens associated with infectious intestinal disease in the United Kingdom

Causative agent	Incubation period	Duration of symptoms	Laboratory reports, UK, 2001	Waterborne outbreaks, UK, 1991–2000
<i>Campylobacter</i> spp.	2–5 days	4–6 days	56 420	20
<i>E. coli</i> (enteropathogenic)	12–72 h	<2 weeks	n.a.	
<i>E. coli</i> (enterotoxigenic)	12–72 h	3–5 days	n.a.	
<i>E. coli</i> (verocytotoxigenic)	1–6 days	4–6 days	768	6
Salmonellas (non-enteric fever)	12–72 h	<3 weeks	16 465	1
Salmonellas (typhi, paratyphi)	1–3 weeks	10–14 days	17*	0
<i>Shigella</i> spp.	1–7 days	<2 weeks	966*	0
<i>Vibrio cholerae</i> (O1, O139)	2–3 days	<7 days	30*	0
<i>Vibrio</i> spp. (not <i>V. cholerae</i> O1, O139)	12–18 h	<7 days	n.a.	0

* Provisional figures.

Source: Communicable Disease Reports, Public Health Laboratory Service.

5.3.1 *Campylobacter*

Campylobacter are the most common cause of human bacterial gastroenteritis in the United Kingdom, with *Campylobacter jejuni* being the predominantly isolated species. They are widespread in the environment and occur very commonly in the intestinal tracts of animals, including birds. Ninety-five per cent of ready-prepared chickens are contaminated with *Campylobacter*, and poultry meat is thought to be an important source of infection. Wild birds also have a high intestinal colonization rate. *Campylobacter* can easily be isolated from surface waters, and a number of outbreaks in the United Kingdom have been associated with private water supplies. An outbreak of gastroenteritis

associated with contamination of a public water supply occurred in Wales in September 2000. Two hundred and eighty-one people out of a population of 1215 served by the supply developed gastroenteritis following an incident of influx of surface water into a holding tank for treated water. Fifteen of the cases were positive for *Campylobacter*, but it was not isolated from the water; indicator organisms were detected.

5.3.2 *Escherichia coli*

Most *E. coli* are not pathogenic and are part of the normal human bowel flora. Some types possess virulence factors and cause gastroenteritis in humans by several different mechanisms. Seven such groups have been defined, of which three may be waterborne (Food Standards Agency 2000):

- Enteropathogenic *E. coli* have been associated with outbreaks in children in nurseries and hospital wards. These strains belong to particular “O” serotypes.
- Enterotoxigenic *E. coli* are a common cause of diarrhoea in travellers. They are identified by the production of a heat-stable toxin and a heat-labile toxin.
- Verocytotoxigenic *E. coli* (VTEC) cause serious diarrhoeal disease, with bloody diarrhoea and painful abdominal cramps. In 10–15% of cases, haemolytic uraemic syndrome develops as a complication, which can result in kidney failure or even death. The most frequent serotype isolated is O157, but other serotypes, such as O139, have been reported. The organism is common in cattle and sheep and other farm animals, in which it behaves as a commensal organism and does not cause any recognized disease. The infectious dose for VTEC is very low, about 10–100 organisms, which explains their potential to cause waterborne outbreaks when animal faeces-contaminated material gains access to water supplies past treatment or where treatment is inadequate.

In North America, there have been two outbreaks of waterborne VTEC gastroenteritis. In August 1999 at the Washington County Fair in New York State, USA, contaminated well water infected over 1000 people and resulted in two deaths (Anonymous 1999). In May 2000, a waterborne outbreak occurred in Ontario, Canada, where 1286 people were infected. Six people died, and 65 patients were admitted to hospital. The source of the contamination was manure

runoff accelerated by high-density farming and flooding, which probably had occurred over two months (Anonymous 2000).

Indicator organism tests will indicate the potential for the presence and survival of pathogenic *E. coli* in water, but it should be remembered that conventional analytical methods may not detect VTEC, as they do not all grow at 44 °C. Limitations in indicator organism detection systems highlight the need for water safety management. Fortunately, these organisms are highly susceptible to water disinfection techniques.

5.3.3 *Salmonella*

The salmonellas cause two distinct types of disease. One group of two species, *Salmonella typhi* and *Salmonella paratyphi*, is the cause of the enteric fevers, typhoid and paratyphoid. The other group, consisting of over 2000 serotypes of what is now considered to be one species, *Salmonella enterica*, causes gastroenteritis. These serovars were previously considered to be separate species and were named after the city or animal from which the organism was initially isolated. Transmission of salmonellas is by the faecal oral route and often involves food and sometimes water. The enteric fever salmonellas are associated only with humans and human disease and remain important causes of waterborne disease worldwide, but nowadays very rarely in developed countries. The gastroenteritis salmonellas are widespread in animals and are often found in poultry, eggs and meat products. Food is the major vehicle of infection, but transmission via water does occur, even though the bacteria survive for only a few hours or days in surface water. Normal water treatment processes are adequate to remove the organism from drinking-water. The organisms are susceptible to chlorine disinfection. The infectious dose for humans for the enteric fever salmonellas is about 10^2 – 10^3 organisms, whereas the infectious dose for humans for the gastroenteritis salmonellas is about 10^6 – 10^8 organisms, mainly because of their susceptibility to gastric acid.

The enteric fevers are systemic infections presenting with high fever (40–41 °C), headache, malaise and rigors. Diarrhoea does not usually occur, and patients often experience constipation in early enteric fever.

A massive epidemic of typhoid fever occurred in Tajikistan in 1997, resulting in 8901 cases and 95 deaths (Mermin *et al.* 1999). Investigations revealed inadequate treatment of faecally contaminated water, and tap water samples showed a mean faecal coliform level of 175 cfu/100 ml. The outbreak was controlled after installing coagulation and chlorination at the water treatment plants. Tank water contaminated with *Salmonella enterica* serovar Saintpaul caused an outbreak of 28 cases among 200 workers on a construction site in 1999 (Taylor *et al.* 2000). The contamination was believed to have been caused

by frogs or mice. The HPC would have indicated a developing problem and could have triggered further investigation.

5.3.4 *Shigella*

Species of *Shigella* are the causative organisms of dysentery and are almost entirely human pathogens; no other animal species play a role in maintenance or spread of infection in the community. Occasionally, higher primates become infected by human-to-animal transmission. Shigellas are transmitted by the faecal–oral route and sometimes, because the infectious dose is low, around 10^2 organisms, by person-to-person spread. Patients excrete large numbers of organisms, between 10^5 and 10^8 per gram of faeces. Point source outbreaks associated with infected food handlers are reported from time to time (Jewell *et al.* 1993). Occasionally, waterborne outbreaks occur, although the organism does not survive for more than a few hours or days in surface water, and normal water treatment processes are adequate to remove it from drinking-water.

Shigella sonnei caused a waterborne outbreak of gastroenteritis in Ioannina in Greece in 2000, affecting 288 persons in a community of 2213. The organism was isolated from tap water as well as patients (Alamanos *et al.* 2000).

5.3.5 *Vibrio*

The *Vibrio* genus is composed of over 30 species, of which the most important is *V. cholerae*, the cause of epidemic cholera, a predominantly waterborne infection. The species *V. cholerae* is subdivided into 140 O-serovars, of which the toxin-producing strains are O1 and O139. The epidemiological picture of cholera has changed and now has a wide distribution. The seventh pandemic that began in 1961 was caused by El Tor strains; it appeared in Peru in 1991, having been absent from South America for some considerable time. Within a year, it had spread to all other countries of South and Central America. The O139 strain appeared in Bangladesh in 1992, where it still persists. In 1998, it was isolated from 3.4% of patients with acute secretory diarrhoea admitted to hospital in Calcutta.

The O1 strain continues to occur in about 19.7% of patients (Basu *et al.* 2000). Cholera is a disease of humans, and approximately 5% of patients become carriers. The organism survives well in the environment, and viable but non-culturable organisms have been described (Colwell and Huq 1994). There is quite clearly potential for further epidemic spread.

Other *Vibrio* species, particularly *Vibrio parahaemolyticus*, have been associated with diarrhoea, often through the consumption of raw, contaminated

seafood. Vibrios are removed from raw waters by chlorination and normal water treatment processes.

5.4 EMERGING PATHOGENS

Although many of the established waterborne pathogens have been controlled by sanitation measures and water treatment processes, new diseases continue to be identified, and new discoveries present a better understanding of existing chronic diseases. Many of these discoveries raise questions about possible waterborne transmission. The bacteria that now need to be considered in this developing area are *Helicobacter pylori*, *Mycobacterium* species, *Burkholderia pseudomallei* and *Francisella tularensis*.

5.4.1 *Helicobacter pylori*

Although spiral-shaped organisms have been observed in the stomachs of humans for many years, it was not until 1982 that a *Campylobacter*-like organism was isolated from patients with gastritis and a causative relationship between a new species, *Helicobacter pylori*, and gastric disease realized (Warren and Marshall 1983). *H. pylori* is a pathogen of global proportions and is generally accepted as the cause of most gastric and peptic ulcers. These diseases may lead to gastric adenocarcinoma.

H. pylori occurs worldwide in developing and developed countries. Where low degrees of hygiene and socioeconomic problems exist, infection rates may approach 100%. In developed countries, infection rates are probably between 30 and 60%.

Transmission from person to person is not fully understood, mainly because of the difficulty in culturing the organism and identifying it outside the body. Epidemiological studies show the cluster phenomenon of *H. pylori* infection in families. It is suggested that infected mothers may play a key role in transmission within families (Rothenbacher *et al.* 1999).

H. pylori has been identified in faeces, and it is assumed that transmission is therefore oral–oral or faecal–oral.

The organism has not been isolated from the environment or from drinking-water, and waterborne transmission remains a possibility that should be investigated. The epidemiology, however, points to person-to-person transmission in early life.

5.4.2 *Mycobacterium*

The mycobacteria are a group of slow-growing organisms. The most important is *Mycobacterium tuberculosis*, the causative organism of tuberculosis, which takes about 4–6 weeks to grow in the diagnostic laboratory. *M. tuberculosis* is not a waterborne pathogen; there are, however, a number of *Mycobacterium* species that occur in the environment and can cause disease in humans. *Mycobacterium avium* and its related species cause an infection of cervical lymph nodes; it occurs in the environment and is most probably accompanied by ingestion or inhalation. *M. avium* can grow in water to which no additional nutrients have been added; although water treatment processes of coagulation and filtration appear to reduce the numbers, it is not affected by chlorine levels of 1 mg/ml. It is therefore not surprising that these organisms can regrow and colonize domestic water systems. Once ingested, *M. avium* can colonize the pharynx without causing any disease. The number of cases reported was very low, but patients with HIV/AIDS are very susceptible. [Editors' note: Because of the wide interest in the potential public health significance of some non-tuberculous mycobacteria in water, including *Mycobacterium avium* complex (MAC), this is the theme of a separate book in the same series as this volume.]

Another species, *Mycobacterium xenopi*, has been reported as the waterborne cause of spinal infections following a look-back exercise on over 3000 patients who had undergone discectomy operations some years beforehand (Astagneau *et al.* 2001).

Mycobacterium paratuberculosis causes Johne's disease in cattle. It is a chronic wasting disease with considerable economic consequences. The organism is extremely difficult to culture; when it does grow, it is very slow and dependent on an exogenous source of mycobactin, which is an iron chelating agent produced by all other mycobacteria. Transmission is by either direct or indirect contact with infected animals and occurs mainly through the faecal–oral route. Organisms are ingested in large numbers by young animals when they feed in troughs that have been contaminated by faeces of shedding animals (Chiodini *et al.* 1984).

M. paratuberculosis has recently been suggested as a cause of Crohn's disease, a non-specific chronic transmural inflammatory disease of humans that affects the intestinal tract, commonly the ileum. The disease is chronic, debilitating and of a relapsing nature; the symptoms experienced include diarrhoea with blood in the stools and abdominal pain. Complications include obstruction, fistulation and abscesses. There have been many bacteria implicated over the years, but no definite etiological agent has been found. It is thought that immunological mechanisms may play an important role.

Molecular techniques have been developed for the diagnosis of *M. paratuberculosis* infections and applied to human surgically resected tissues. *M. paratuberculosis* was detected in approximately 30% of samples, but the sets of results from different laboratories have been conflicting. Some studies were unable to detect the organism; in other studies, the organism was detected in a smaller percentage of healthy subjects.

In addition, a few Crohn's disease patients have shown clinical remission when treated with anti-tuberculosis drugs.

There is therefore much more work to be done to acquire a better understanding. *M. paratuberculosis* may be present in surface water contaminated by cattle faeces. Routine testing for indicator organisms would detect faecal pollution, and normal water treatment processes of coagulation and filtration are likely to remove mycobacteria. It is unlikely that drinking-water is a major source of *M. paratuberculosis*, and its association with Crohn's disease is still under investigation.

5.4.3 *Burkholderia pseudomallei*

Burkholderia pseudomallei is the cause of melioidosis, an acute pneumonia often followed by systemic infection with later presentations of abscesses. The organism is widespread in the environment and was originally described in Rangoon in patients compromised by severe poverty who had presumably inhaled the organism in dust when sleeping on the ground. It occurs commonly in southeast Asia and has been detected in service personnel repatriated from those areas in the past. It was also investigated as a biological weapon by several nations, to be released as an aerosol and cause pneumonia infection in those exposed. A recent study in Bologna, Italy, detected *B. pseudomallei* in 7% of 85 samples of drinking-water collected from public and private buildings. The mean count was 578 cfu/100 ml. The occurrence of the organism was found to correlate with the HPC at 22 and 36 °C (Zanetti *et al.* 2000).

5.4.4 *Francisella tularensis*

Tularaemia is a zoonosis caused by a highly infective and virulent organism *Francisella tularensis*, which occurs throughout the northern hemisphere but has never been isolated within the United Kingdom. It occurs in a wide range of animal reservoir hosts and can be isolated from the environment in water and mud. It is transmitted to humans who come in close contact with the animal reservoir, arthropods that feed on them or debris and dust associated with them. It can also be transmitted through the ingestion of contaminated water. Human epidemics sometimes occur and are associated with epizootics in the animal

populations, evidenced by die-offs. There are several presentations of tularaemia in humans, depending on the route of exposure. Ingestion usually results in oropharyngeal tularaemia, with fever, pharyngitis and cervical lymphadenitis. Other forms include ulcero-glandular, pleuropneumonic and typhoidal.

Following the recent war in Kosovo, over 900 suspected cases of tularaemia were identified and 327 cases confirmed serologically. The epidemiological investigation pointed to rodent-contaminated wells, and rodent carcasses found in some wells tested positive for *F. tularensis* (Reintjes *et al.* 2002).

In a waterborne outbreak reported from Spain, 19 cases who had contact with river-caught crayfish were identified (Anda *et al.* 2001). Attempts to isolate *F. tularensis* from water were unsuccessful. Drinking-water was not involved. *F. tularensis* is notoriously difficult to culture, requiring a source of cysteine.

F. tularensis was investigated and developed as a biological weapon; the infectious dose was found to be extremely low — 10 organisms.

5.5 BIOTERRORISM THREAT AGENTS

The classical biological warfare agents that were investigated and sometimes developed by certain countries in the past (Table 5.3) were intended for aerosol dissemination to cause infection in those exposed. Since the events of 11 September 2001 in the USA and the anthrax letters, awareness of the threat of bioterrorism has been raised considerably. As the consequences could be disastrous, much planning and international cooperation have occurred to prevent any future deliberate releases or to limit their effects, should they occur. One of the concerns is the deliberate contamination of drinking-water, where many people could be exposed. Drinking-water treatment processes would likely remove some contamination of the raw waters, but deliberate contamination post-treatment could pose a greater problem. There are, of course, the problems of dilution, the effects of chlorine and the survivability of the agent in a hostile environment to take into account.

The recognized waterborne pathogens described above are potential deliberate release agents in water, and each nation's planning will have to take into account the laboratory capability required to minimize the impact and even to signal that an incident is occurring. The role of water testing will need to be re-evaluated.

5.6 CONCLUSIONS

These are the bacteria of concern, and they need to be taken into account when re-evaluating the role of the HPC for monitoring the hygienic quality of water.

The list is quite considerable. Although the recognized waterborne pathogens, which are all faecal in origin, will be potentially present if the faecal indicator organisms are detected, pathogens that are non-faecal in origin will of course not be similarly signalled. The sensitivity of the faecal indicator organisms test is quite high and has stood the test of time; low numbers of faecal indicator organisms have often been detected without there being any public health consequences. There is, therefore, a margin of safety, and this probably applies to gastroenteritis-causing viruses that are also faecal in origin. It does not, however, apply to the intestinal parasites *Cryptosporidium* and *Giardia*.

Table 5.3. Examples of classical biological warfare agents

Agent	Disease
<i>Bacillus anthracis</i>	Anthrax
<i>Brucella</i> species	Brucellosis
<i>Burkholderia mallei</i>	Glanders
<i>Burkholderia pseudomallei</i>	Melioidosis
<i>Francisella tularensis</i>	Tularaemia
<i>Yersinia pestis</i>	Plague
<i>Rickettsia</i> species	Typhus
<i>Coxiella burnetii</i>	Q fever
<i>Clostridium botulinum</i> toxin	Botulism
<i>Staphylococcus aureus</i> enterotoxin B	Staphylococcal food poisoning
Smallpox virus	Smallpox

The remaining bacteria of concern are either heterotrophs that might have a role in disease or emerging pathogens that do have a role in disease and could possibly be waterborne. It is important that these organisms and diseases are kept under surveillance in order to confirm or refute the suggested associations. Many of the organisms are difficult to grow, and there is no validated trigger of when to look for them.

The HPC does not measure all organisms present, of which many will be non-culturable but viable, and indeed several of the organisms of concern described above would not grow on HPC media. The HPC, however, does give an indication of change in the flora of drinking-water, and the HPC should be evaluated as a trigger for further investigation. Many new molecular techniques for the detection of pathogens and putative pathogens in water are being described (Waage *et al.* 1999a,b,c; Lightfoot *et al.* 2001). DNA chips that have the capacity to detect up to 44 pathogens on one single chip are being developed.

These tests are very expensive when compared with the routine monitoring tests carried out in the water industry and in public health monitoring. The HPC

should be evaluated as the signal of changing events in a drinking-water supply to trigger the utilization of these new molecular tests to detect the new bacteria of concern and any associated virulence genes.

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