

Chapter 7

SURVEILLANCE AND INVESTIGATION OF CONTAMINATION INCIDENTS AND WATERBORNE OUTBREAKS

*P.R. Hunter, Y. Andersson, C.H. Von Bonsdorff, R.M. Chalmers, E. Cifuentes,
D. Deere, T. Endo, M. Kadar, T. Krogh, L. Newport, A. Prescott and
W. Robertson*

7.1 Introduction

This chapter examines the investigation of possible waterborne outbreaks (due to drinking water) and, in particular, the role of laboratory analyses in the investigation. Outbreaks are the most obvious manifestation of waterborne disease, though not all such disease is associated with outbreaks. The detection and investigation of outbreaks provides some of the best insights into the microbial aetiology and the types of process failures that lead to waterborne disease. As such, they provide essential information for hazard analysis and risk assessment associated with drinking water (see Chapter 3). Because of this, it is essential that outbreaks are adequately investigated so that the appropriate lessons can be learned and preventative measures applied to mitigate against future outbreaks and to improve the microbial safety of water generally.

The World Health Organization's (WHO) definition of a food - or waterborne - outbreak is when two or more persons experience a similar illness after ingestion of the same type of food or water from the same source and when the epidemiological evidence implicates the food or the water as the source of the illness (Schmidt, 1995). Unfortunately, in the early stages of an outbreak it is usually far from clear whether cases are linked or related to drinking water. This is a particular problem for common infections transmitted through various different routes. A small number of cases associated with a water supply may not be detectable against the general background of infection.

Instead, existing surveillance systems only detect general changes in the incidence of infectious disease.

A more useful definition of a waterborne outbreak, for the purposes of active surveillance, is when more cases than would be expected are clustered, geographically and in time. In other words, are more cases being reported from a particular geographical location than would be considered normal? Clearly, in order to make this judgement, there has to be a system in place for the detection of cases of infection and an understanding of the expected frequency of reporting.

An outbreak needs to be in progress to be detected by a public health surveillance system. Preventing outbreaks occurring in the first place is the focus of the authorities responsible for supplying drinking water. A combination of a study of outbreaks combined with theoretical risk analysis can be used to predict scenarios that are likely to lead to water becoming unsafe. After examining waterborne outbreaks of illness in general, this chapter includes an overview of the role of indicator parameters in providing early warning of possible outbreak scenarios and the importance of having contingency plans in place to expedite corrective action. It then goes on to examine waterborne outbreak investigation in more detail.

Table 7.1. Outbreaks of infectious illness linked to drinking water in the UK, 1991-2000

(Adapted from Percival *et al.*, 2000)

Water system	Disease	Number of outbreaks	Number of cases
Public supplies ¹	Cryptosporidiosis	24	>2955
	Campylobacteriosis	1	281
	Total	25	>3236
Private supplies ²	Gastroenteritis of unknown cause	2	81
	Campylobacteriosis	7	162
	Giardiasis	1	31
	Cryptosporidiosis	3	74
	Enterohaemorrhagic <i>E. coli</i>	1	14
	Mixed campylobacteriosis and cryptosporidiosis	1	43
	Total	13	405

1. Public supplies are owned by commercial water utilities.

2. Private supplies are not owned by commercial water utilities and vary from supplies providing water to single dwellings up to some quite large supplies.

7.2 Waterborne outbreaks

We have very little idea how many outbreaks of waterborne disease there are in the world as few countries, even in Western Europe and North America, have surveillance systems in place that can reliably detect such outbreaks (WHO, 1999). Two countries that do have good quality disease surveillance systems are the United States of America and the United Kingdom, both of which produce regular reports of the number of detected outbreaks associated with water.

Table 7.1 shows the number of outbreaks reported in England and Wales for the years 1991 to 2000, while Table 7.2 shows outbreaks for the USA for 1991 to 1998. From these two tables it is clear that outbreaks of illness associated with drinking water are common even in affluent nations and can be a cause of substantial illness. Furthermore, it can be seen that a relatively small number of pathogens have been implicated in these outbreaks.

Table 7.2. Outbreaks of infectious illness (1991-1998) linked to drinking water in the USA

(Moore *et al.*, 1993; Kramer *et al.*, 1996a; Levy *et al.*, 1998; Barwick *et al.*, 2000)

Disease	Water system ¹					
	Community		Non-community		Independent	
	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
Acute gastroenteritis of unknown cause	5	10 105	35	4 673	3	51
Giardiasis	10	1 986	3	128	2	12
Cryptosporidiosis	6	407 637	2	578	2	39
Norwalk-like virus	2	742				
Campylobacteriosis	1	172	2	51		
<i>Salmonella typhimurium</i>	1	625				
Non-O1 <i>Vibrio cholerae</i>	1	11				
<i>E. coli</i> O157	1	157	3	39	1	3
<i>Shigella sonnei</i>	1	83	5	484	1	5
<i>Shigella flexneri</i>					1	33
<i>Plesiomonas shigelloides</i>			1	60		
Hepatitis A			1	46	1	10
Total	28	421 518	52	6 059	11	153

1. Community and non-community water systems are public water supplies that serve ≥ 15 service connections or an average of ≥ 25 residents for ≥ 60 days/year. A community water system serves year-round residents of a community, subdivision or mobile home park. A non-community water system can be non-transient or transient. Non-transient systems serve ≥ 25 of the same persons for >6 months of the year (e.g. factories or schools), whereas transient systems do not (e.g. restaurants, highway rest stations or parks). Independent systems are small systems not owned or operated by a water utility serving <15 connections or <25 persons.

In addition to those cases of illness associated with outbreaks, there remain an uncertain number of sporadic cases. A sporadic case is a single case of infection that is not obviously linked to other cases. In most sporadic cases of disease, it is usually impossible to state with certainty where that individual acquired his/her infection. Indeed, for most potentially waterborne diseases it is difficult to estimate the proportion of such sporadic cases that are associated with drinking water. What evidence there is comes from case-control and other epidemiological studies and these are reviewed elsewhere (Hunter, 1997).

Outbreaks of disease from drinking water supplies often result from chance events (Deere *et al.*, 2001). Table 7.3 provides an illustration of the diversity of scenarios that can result in drinking water outbreaks. This has significant implications for the design and operation of drinking water supplies. The water suppliers need to have preventative and emergency response procedures in place to ensure safe water delivery in the event of a variety of circumstances.

Table 7.3. Scenarios affecting drinking water implicated in disease outbreaks

(from Deere *et al.*, 2001)

Causal event(s)	Aetiology	Water type	Cases	Reference
Pre abstraction and treatment				
Surface run off from contaminated catchment after heavy rain. Increased chlorine demand due to turbidity	<i>Campylobacter</i>	Chlorinated surface water	3 000	Vogt <i>et al.</i> , 1982
Contaminated surface run off from melt water and heavy rain entering municipal wells	<i>Campylobacter</i>	Untreated ground water	241	Millson <i>et al.</i> , 1991
Drought followed by heavy rain agricultural surface run off and poor coagulation and mixing	<i>Cryptosporidium</i>	Chlorinated and package filtered river water	34	Leland <i>et al.</i> , 1993
Poor mixing and flocculation with filters started up without backwashing	<i>Cryptosporidium</i>	Surface water (CT)	13 000	Rose <i>et al.</i> , 1997
Increase in turbidity, poor coagulation and backwash recycling	<i>Cryptosporidium</i>	Surface water (CT)	403 000	Rose <i>et al.</i> , 1997
Catchment contaminated by higher than realised population, chlorine dosage too low	<i>Giardia</i>	Chlorinated surface water	350	Shaw <i>et al.</i> , 1977

Table 7.3. Scenarios affecting drinking water implicated in disease outbreaks (continued)

Causal event(s)	Aetiology	Water type	Cases	Reference
Post abstraction and treatment				
Backflow of farm contaminated river water due to low mains pressure	<i>Campylobacter</i>	Sand filtered groundwater	2 000	Mentzing, 1981
Agricultural run-off entering unsealed supply	<i>Cryptosporidium</i>	Surface water (CT)	27	Badenoch, 1990
Deliberate contamination of water storage tank	<i>Giardia</i>	Municipal supply	9	Ramsay and Marsh, 1990
Cross connection between pressure dropped potable and wastewater lines at pump wash	<i>Giardia</i> & <i>Entamoeba</i>	Surface water (CT)	304	Kramer <i>et al.</i> , 1996b
Sewage overflow entering pipes after repairs of ice breaks made without post chlorination	<i>E. coli</i> O157	Municipal supply	243	Swerdlow <i>et al.</i> , 1992
Birds entering water storage tank	<i>Salmonella</i>	Untreated ground water	650	Angulo <i>et al.</i> , 1997

CT: conventionally treated.

7.3 Preventing outbreaks

The variety of scenarios that can lead to outbreaks from drinking water has been illustrated in Table 7.3. Each water supply system is unique and, therefore, the scenarios that could lead to an outbreak can differ between supplies. The relevant authorities need to assess the risk of outbreaks from a range of scenarios for each specific supply, and then controls should be put in place to prevent such outbreaks occurring.

A ‘water safety plan’ can be developed to detail both the design of controls and the operating practices that would theoretically lead to the consistent provision of safe water (see Box 1.3). Such a plan would consider both nominal operating conditions and unusual events. A detailed discussion of a water safety plan is outside the scope of this chapter, however, an overview of how the responsible authorities would manage ‘incidents’ of suspected unsafe drinking water is given here.

7.3.1 *Incident management*

For the purposes of this section of the chapter the term ‘incident’ will be used to refer to any situation in which there is reason to suspect that water being supplied for drinking is, or is about to become, unsafe. Such a broad definition means that a variety of triggers can lead to an incident being declared.

Judicious use of indicator parameters can provide the earliest practical warning of the possibility that water may become unsafe. In other cases an incident might not be declared until health authorities detect an increase in disease and begin to question the safety of the drinking water supply. Incident triggers could include:

- Process indicators:
 - Inadequate performance of a sewage treatment plant discharging to source water.
 - Inadequate performance of drinking water treatment plant.
- Notification of chance events:
 - Spillage of a hazardous substance into source water.
 - Failure of power supply to a critical asset.
- Non-microbial indicator parameters:
 - Extreme rainfall in a catchment.
 - Detection of unusually high turbidity (source or treated water).
 - Unusual taste, odour or appearance of water.
- Microbial indicator parameters:
 - Measurement of unusually high faecal indicator densities (source or treated water).
 - Measurement of unusually high pathogen densities (source or treated water).
- Public health indicators:
 - Disease outbreak for which water is a suspect vector.

For the purposes of this chapter, two categories of incident will be discussed separately, namely:

- Specified incidents involving a pre-determined response to a nominated and routinely measured indicator trigger.
- Unspecified incidents involving a more general response, which is not fully pre-determined, to a range of possible triggers.

7.3.2 *Response to specified incidents*

Indicators of potentially unsafe water can be selected and systematically monitored throughout the water supply chain or cycle. Such indicators should yield information in good time to enable corrective action to prevent unsafe water being supplied. Alert levels can be set against which to compare observations. Alert levels would typically be just within critical limits of operation, outside of which confidence in water safety would be lost. Pre-determined corrective actions can be implemented once alert levels are exceeded. The corrective action (contingency) plans form part the specified aspects of the incident preparedness program.

Incident plans can have a range of alert levels. These can be minor, early warning, necessitating no more than additional investigation by a designated team, through to full emergency, requiring all available personnel and equipment. Major emergencies are likely to require the resources of organisations beyond the authority primarily responsible for supplying drinking water, particularly the health authorities.

Incident plans typically consist of items such as:

- Accountabilities and contact details for key personnel, often including several organisations and individuals.
- Lists of measurable indicators that might trigger incidents along with a scale of alert levels.
- Clear description of the actions required in response to alerts.
- Location and identity of the detailed standard operating procedures and required equipment.
- Location of backup equipment.

- Relevant logistical and technical information.
- Checklists, proformas and quick reference guides.

The plan may need to be followed at very short notice so standby rosters, effective communication systems and up to date training and documentation are required

Case study: Incident response to turbidity indicator levels

The incident preparedness program of the Sydney Catchment Authority includes detailed contingency plans for responding to indicators of poor source water quality. For example, the Authority monitors turbidity at many points in the source water supply system. It has developed an integrated bulk water supply system that provides a number of source water supply options. The turbidity of water entering critical reservoirs is monitored continuously. If there is an increase of > 5 NTU within three hours then an incident is declared and an alternative source water may be used. Filtered water turbidity is also monitored continuously and if it exceeds 1 NTU, an alternative source water will be selected.

7.3.3 Response to unspecified incidents

Some scenarios that lead to water being considered potentially unsafe might not be specifically identified within incident plans. This may be either because the events were unforeseen, or because they were considered too unlikely to justify preparing detailed corrective action plans. To allow for such events, a generalised water safety incident response plan can be developed. The plan would be used to provide general guidance on identifying and handling of incidents along with specific guidance on responses that would be applied to many different types of incident.

Rather than alert-level categories being pre-determined, a protocol for situation assessment and declaring incidents would be provided that includes personal accountabilities and categorical selection criteria. The selection criteria may include:

- Time to effect.
- Population affected.
- Nature of the suspected hazard.

Alert levels can vary, as they do for specified incidents, from minor through to full-scale emergencies. The preparation of clear procedures, accountabilities and equipment for the sampling and storing water in the event of an incident can be valuable for follow up epidemiological or other investigations, and the sampling and storage of water from early on during a suspected incident should be part of the response plan.

The success of unspecified incident responses depends on the experience, judgement and skill of the personnel operating and managing the drinking water supply systems. However, generic activities that are common to many suspected contamination events can be incorporated within general unspecified incident preparedness programs. For example, for piped systems, emergency flushing standard operating procedures can be prepared, and tested, for use in the event that contaminated water needs to be flushed from a piped system. Similarly, standard operating procedures for rapidly changing or by-passing reservoirs can be prepared, tested and incorporated. The development of such a 'toolkit' of supporting material limits the likelihood of error and speeds up responses during incidents.

Case study: General incident response involving emergency flushing

Sydney Water has developed a detailed incident preparedness program. If it is suspected that water may be contaminated for whatever reason, an incident is declared. Among the emergency standard operating procedures available for use during incidents are systematic emergency flushing plans. These have been developed to provide standard operating procedures for the most rapid practical removal of suspect water from the distribution system. The plans have been prepared in manageable sections in a ready-to-use format for supply direct to operations officers.

7.3.4 Water avoidance and boil water orders

In most water supply scenarios it is possible to:

- Terminate the supply of water.
- Advise (some or all) consumers to avoid consuming water.
- Advise (some or all) consumers to treat water, usually by boiling.

An incident preparedness program should include a thorough evaluation of the basis for calling such orders. The objective of the order should be taken in the public interest and typically involves a final decision by health authorities.

Even where drinking water contamination is suspected, the public interest is not always best served by making avoidance or disconnection orders. Research has shown that many people do not follow advice to boil their water, in part because of confusion over what to do (Angulo *et al.*, 1997; O'Donnell *et al.*, 2000; Willocks *et al.*, 2000). Furthermore, there is also evidence that boil water notices can have negative public health consequences through causing anxiety and also burns and scalds (Mayon-White and Frankenberg, 1989; Willocks *et al.*, 2000). If advice to boil water is issued then the incident team must be convinced of an ongoing risk to health of drinking tap water, which outweighs any risk from the boil water notice itself (Hunter, 2000a). Disability-adjusted life years can be used to provide a common currency to assist in this type of health-based decision-making (Murray, 1994). Financial considerations are also likely to be important. Turning off water supplies can have major economic consequences due to lost production and damaged equipment.

The relevant authorities should have a clear understanding of the accountabilities, circumstances and criteria regarding the calling of such orders. In addition, practical operating procedures should be in place. For example, procedures for rapid shutdown, or for alerting the public in the event of a water avoidance or boil order, should be thoroughly planned. Additionally, any incident management team intending to issue advice to boil water should be very clear at the outset about the criteria that will be used to lift the advice.

Emergency water supplies, such as the use of water tankers, can be maintained on standby at all times, or powers can be put in place to enable commandeering. Rapid notification procedures such as media, mail-drops and public address system vehicles need to be practical and available at any time. Systems that enable tracing of water from source to consumer can assist in better targeting of these types of responses to minimise the extent of their impacts.

7.4 Outbreak investigation

This section outlines the steps typically taken in the investigation of an outbreak of suspected waterborne illness in a developed country. The timely discovery of an outbreak and its cause normally involves a long series of events with different agencies involved. Consequently, the most effective outbreak investigations follow a sequence of activities, outlined below:

- **Planning.** Planning should address key issues about who should be involved in the investigation and what their roles should be. Outbreak plans should also address who will have lead responsibility for implementing the outbreak plan and who will have leadership of the management group.
- **Outbreak detection and confirmation.** Normally an increase in reports of illness or detection of particular pathogens in human samples is the first sign of an outbreak. Rarely, the first sign of a waterborne outbreak can also be a technical problem with the water source or in the water treatment or distribution. An important first step in any outbreak investigation is the confirmation of an apparent outbreak. Before an outbreak is officially declared, possible causes of error should be considered and excluded. Such causes of apparent outbreaks include laboratory false positives, the introduction of new laboratory methods and sudden changes in reporting behaviour (Casemore, 1992).
- **Outbreak description.** The first step in outbreak description is the derivation of the 'case definition'. A case definition is necessary to identify those cases that should and should not be included in subsequent analyses. The case definition should contain the presence of key symptoms and/or laboratory results, geographical location and date of onset or notification. There may be several case-definitions in use at a single time (*e.g.* one for a possible case and one for a confirmed case). In the beginning of the outbreak investigation a fairly wide definition is needed in order not to lose cases. Later during the investigation, when more information is revealed, the case definition can often be narrowed. When the case definition has been agreed, the next step is to identify how many people meet the case definition, by a process of 'case finding'. This may involve reviewing existing laboratory or other notification records, or involve proactive searching by contacting doctors, or possible cases themselves to identify cases that may not have been formally notified. It is important to find out when the outbreak started and identify the first case (primary or index case). The date at which each case fell ill (and sometimes even the hour) plotted as a graph (epidemic curve) gives valuable epidemiological information, and can provide a picture of the outbreak (*e.g.* a point source or a continuous outbreak). The geographical spread might give an idea about the cause of the outbreak. Cases can be plotted on a map to examine the possibility of clustering (*e.g.* households with the same community water or households situated on just one part the water distribution system or a private well). Age, sex and other socio-economic data may also give information about the likely causes.

- **Hypothesis generation.** Once sufficient information has been collected, a preliminary hypothesis as to the cause of the outbreak can be generated. Based on this, various remedial control measures may be suggested.
- **Hypothesis confirmation.** When the outbreak team has a hypothesis as to the cause of the outbreak, efforts are directed at proving or disproving this suggestion. There are three strands to this part of the investigation: further epidemiological investigations, further microbiological analyses of human and environmental samples and, in the case of a suspected waterborne outbreak, a sanitary inspection of the water treatment and distribution system. Epidemiological investigations at this stage will normally be of the case-control or cohort type. In these types of study, cases and controls (other individuals who were not ill) are interviewed and the responses analysed statistically to identify differences between the two groups (Hunter, 1997). The further microbiological analyses during outbreak investigation may include additional collection of human or environmental samples or more detailed characterisation of those samples. The sanitary inspections of the water treatment plant and distribution system are undertaken to collect evidence of failure in, or inadequate design of, the water treatment system. Such information is helpful in confirming the hypothesis of a water source for the outbreak. Evidence of what went wrong is also essential for informing the water supplier on how such failures and the consequent risk to public health can be prevented in future. The entire treatment and distribution system should be surveyed. Evidence of failure may be available in existing routinely collected data (Section 7.5) or become obvious only after enhanced monitoring or after surveying the treatment and distribution system.
- **Strength of association.** When all the evidence has been collected, the outbreak management team has to come to a conclusion about whether or not the suspect water supply was indeed the cause of the outbreak. Both the UK and USA have developed a form of scoring system that attempts to define the reliability of the conclusion of any association between water and disease. However, the two systems are not compatible as the UK classifies the strength of association between water and disease whilst the US system classifies the completeness of the investigation. There is a need for an internationally agreed system of classifying the strength of association between drinking water and disease in outbreaks. Both of these categorisations give considerable weight to analytical epidemiological studies, most commonly the case-control study, although recent evidence has suggested that these studies may be highly biased in those outbreaks where the possible cause has been made public. Such bias

can lead to drinking water being falsely associated with an outbreak (Hunter, 2000b; Hunter and Syed, 2002).

7.5 Reviewing existing data

In any outbreak investigation where drinking water is suspected as the cause, one of the key sources of information are the records of the routine analyses of water quality, typically already held by the water supplier. Such a retrospective review of routine water quality data will seek evidence of reduction in source water quality, failure in water treatment and distribution and, rarely, evidence of the presence of the suspect pathogen in the treated water supply.

Routine bacteriological tests of drinking water are, in most countries, concentrated on parameters like *E. coli*, thermotolerant coliforms, total coliforms and heterotrophic plate-count bacteria which have simple analysis techniques. In some countries faecal streptococci (enterococci) and spores of sulphite-reducing clostridia are also included in the routine tests. Recently, some countries have started to include tests for *Cryptosporidium* in samples originating from, or influenced by, surface water but these involve rather expensive sampling procedures and analytical techniques, and only a few countries demand such tests to be done.

The most commonly available microbial results will normally be *E. coli* or thermotolerant coliforms. These species are used as an index of relatively fresh faecal contamination. In addition to the microbial tests, physicochemical water parameters such as turbidity, pH, chlorine residual, colour and organic matter may be monitored. Additionally, registration of failures in water treatment units, filters, dosing equipment, water pumps, distribution system, intake pipelines and so on, is of utmost importance for later investigation and determining the cause of the outbreak of illness.

Other useful parameters that may be monitored by the water supplier, or may be obtained from other sources include meteorological data (*e.g.* rainfall) and data on incidents that might affect water flow or water quality (*e.g.* floods, droughts, avalanches etc.).

Among data that are not usually monitored by suppliers on routine basis, but may be helpful if available are:

- Leakage from sewers or storm overflows affecting the water source.
- Traffic or industrial accidents with an effluent causing water pollution.
- Incidents that create low or negative pressure inside the drinking water pipelines (which may allow the ingress of polluted water).

Case study: A pressure drop and cross connection

This case study relates to an outbreak of illness in Hungary in 1986. Over the course of the outbreak (which lasted two weeks) about 350 cases were detected, with 14 different pathogens detected from clinical samples (11 serotypes of *Salmonella*, 2 of *Shigella flexneri* and *E. coli* O124). When the outbreak first came to light, interviews revealed that all the cases had consumed drinking water at Szolnok railway station. Although no breakdown or failure in the drinking water system had been noted (other than a pressure drop that had affected the whole area), microscopic examination of water from the station showed the presence of large amounts of diatomaceous algae. The algae were identical to those previously detected in the river, indicating that untreated river water (containing the town's sewage) was present in the station drinking water. Later, bacteriological analysis of the same samples confirmed the microscopic examination, with 75% containing at least 80/100 ml thermotolerant coliforms. The fault causing the contamination was eventually shown to be a cross connection with an industrial water system using river water, with the valve connection probably being opened in response to the drop in pressure.

7.6 Enhanced monitoring including pathogen detection

Following the detection or suspicion of an outbreak, it may be appropriate to increase the amount of sampling over that normally undertaken for a particular supply. The reasons for this are two-fold: in order to provide further evidence that the water supply is the source of the outbreak and to identify the failure in treatment or distribution that led to the outbreak. Enhanced monitoring may involve:

- Taking more samples than normal from the same sites.
- Taking samples from elsewhere in the distribution system.
- Undertaking microbiological analyses that would not normally be undertaken (this may include monitoring for pathogens).

Increased sampling using standard methods at the routine sites may be useful to detect short-lived transient events. Small supplies may only be sampled infrequently, once a month or less. If such a small supply is implicated in an outbreak, sample frequency may be increased to one or more samples daily.

Increasing the number of sites where samples are taken may be useful for detecting localised problems within the distribution system and the greater number of samples may also improve the chances of detecting transient events. In this context, it may be appropriate to extend sampling to include:

- Livestock and potential sources of human pollution from within the catchment area.
- Source water, including wells that may not be currently used for extraction and sediment from storage reservoirs.
- Various critical points in the treatment plant, including backwash water from filter beds.
- Water and sediment from various points in the distribution system, including service reservoirs, pipelines and consumers taps.
- Stored water such as container water, ice, or filters if these are available.

One of the most powerful pieces of evidence implicating a water supply as the cause of an outbreak of infectious disease is the demonstration of the causative agent in the supply, especially in water pre-dating the event. Therefore, during most suspected waterborne outbreaks efforts will be made to isolate the pathogen from the water.

Case study: Additional sampling and catchment investigations

In the UK, an outbreak of cryptosporidiosis was linked to Thirlmere reservoir a surface water source that was chlorinated but not filtered prior to distribution (Hunter and Syed, 2001). An increase in the cases of illness in the areas served by Thirlmere, followed the detection of oocysts in a sample of treated water (34/10 litres). Oocysts isolated from the clinical samples were found to be type 2 *Cryptosporidium* (a zoonotic strain). A subsequent investigation revealed oocysts in sheep faeces within the reservoir catchment, which supported the hypothesis that the sheep were the ultimate source of the outbreak. However, the following year an outbreak affecting people resident in Glasgow was associated with another unfiltered surface water source. As in the Thirlmere outbreak, genotyping of clinical cases was type 2 and oocysts were

detected in sheep faeces from around the catchment. However, when the sheep oocysts were typed they were found to be a novel genotype not previously found in man, suggesting that sheep may not have been the source (Chalmers *et al.*, 2002a). These two studies illustrate the value of linking molecular methods to the investigation of outbreaks.

Case study: Analysis of stored water

An outbreak of illness implicated a supply zone serving about 12 000 people. During the course of the outbreak 1 267 cases were identified (an attack rate of over 10%). Clinical sampling found a range of pathogens, but the dominant one was *Salmonella hadar*. Water sampling indicated heavy faecal contamination, although in most cases pathogens could not be detected, however, salmonellae were isolated from two water samples, one of which was from bottled tap water that had been stored in a patient's refrigerator. In both cases *S. hadar* (*i.e.* the strain implicated in the outbreak) was identified. The source of the outbreak was traced to ingress of sewage-contaminated groundwater through a poor weld on a new water main. Although the construction works on the new trunk main was suspected very early on and the ingress identified, isolation of *S. hadar* from both patients and water samples confirmed the waterborne nature of the outbreak.

7.6.1 Pathogen detection

There are several pathogens for which there are well proven methods available for detection in water in the international literature and in national or international standards (Anon, 1994). This is the case with several enteric bacteria, such as thermotolerant *Campylobacter spp.*, *Salmonella spp.* and *Vibrio cholerae*.

Although a fairly uncommon cause of waterborne outbreaks (Hunter, 1997), salmonellae seem to be quite easily isolated from suspect water sources during outbreaks. *Shigella spp.*, however, are a common cause of waterborne outbreaks world-wide but the detection of *Shigella spp.* from water using traditional methods is difficult because of the lack of methods of appropriate selectivity. The detection of pathogenic *E. coli* in implicated water is not usually attempted because of the difficulty in distinguishing them from non-pathogenic *E. coli*. The exception to this is during outbreaks of enterohaemorrhagic *E. coli*, which is a much more severe disease and has certain cultural characteristics to help distinguish *E. coli* O157 from other types.

Of the viruses shown to be present in faecally contaminated drinking water, the enterovirus group can be most easily detected but, in contrast with the name of this group, they do not generally cause enteric disease and are rarely involved in overt outbreaks. The exception being polioviruses, these were the cause of large outbreaks in the past when a waterborne mechanism was often supposed, although only one proven case is known (Farley *et al.*, 1984). Their role in causing low-level transmission through drinking water, however, is widely speculated upon. Although waterborne hepatitis A (HAV) outbreaks have frequently been reported, the detection of the virus in the water is generally not attempted because of the lack of available technique. Only since 1979 have techniques been developed for the propagation of HAV in cell culture and isolation from water samples (Provost and Hilleman, 1979). The only known example of successfully culturing HAV in parallel with unconventional methods from water that caused waterborne outbreak was described in the early 1990s (Divizia *et al.*, 1993).

Case study: Isolation of Salmonella sp.

Clinical samples from an outbreak of illness uniformly showed *S. typhimurium* (phage type 4, biotype 2) to be the causative agent. Food samples were negative and no common food source could be identified. The waterborne route was suggested by the exclusion of other possible routes and also some of the descriptive epidemiology. Water sampling during the outbreak was found to be acceptable in terms of coliform content and plate counts. As a result of the absence of faecal indicators the isolation of the outbreak strain of *Salmonella* in two water samples was initially dismissed as being due to faulty sampling technique. The public health authority took action and a boil water order and increased chlorination ended the outbreak. Final proof of the waterborne nature of the outbreak was not made until several months after it had ended. It was realised that shortly before the outbreak a family (served by a pit latrine rather than sewerage connection), living close to the pipeline connecting one of the supply wells (that provided unchlorinated water to the network) to a water tower had experienced illness caused by *S. typhimurium*. Investigations revealed that the pipeline close to the pit latrine had a crack in it, allowing the small-scale intrusion of contaminated groundwater. This example demonstrates the importance of not ignoring data and it also highlights the fact that pathogens may be present in disinfected drinking water in the absence of faecal indicators.

7.6.2 *Molecular techniques*

The chance of detecting a pathogen from an implicated drinking water source is often much improved using novel microbiological methods (especially molecular techniques – see Chapter 8 for more details), this is particularly true for viruses with no readily available or rapid cultural method. This group includes rotaviruses, astroviruses, caliciviruses, hepatitis A virus, Norwalk virus and other small round viruses (West, 1991). Traditional methods for the detection of viruses are based on tissue culture techniques that can take several weeks to perform. Direct polymerase chain reaction (PCR) methods although faster than conventional cell culture techniques are less sensitive than culture techniques with low levels of viral particles undetectable in environmental samples. Combined tissue culture and PCR methods offer major advantages over each individual method in that the detection of infectious virus is maximised and PCR inhibitors are removed. The assay greatly reduces the time needed to detect these organisms with times reduced to a few days. New developments in PCR technology may provide faster more sensitive detection and quantification of viral particles in the future.

Alternative methods for the identification/detection of potentially pathogenic bacteria include the use of *in situ* hybridisation and species-specific probes (Prescott and Fricker, 1999). This powerful technique enables organisms to be detected *in situ* within a few hours and can be adapted for use with any organism. With the advancement of micro-array and technologies several different probes targeting many different pathogens can be processed together. This could be invaluable for sample analysis during outbreak conditions.

Whether to undertake such a demanding examination has to be decided in each situation. In most cases, success is dependent on the ready availability of personnel with the relevant skills and resources. For many pathogens the best results are likely to be obtained by a national or regional reference laboratory specially practised in the detection of certain pathogens. It is most useful if an action plan for outbreak investigation is available, containing the necessary steps to be taken and laboratories to be contacted in case of emergency. In turn, reference laboratories should also have a contingency plan in order to deal with urgent requests to participate in waterborne outbreak investigations.

Case study: Virus identification

In Finland a waterborne outbreak of Norwalk-like virus in Heinävesi was attributed to an outbreak of illness (affecting 500 people) three months earlier in Kuopio, a town 70 kilometres upstream (Kukkula *et al.*, 1999). The sewage

from Kuopio is discharged to a lake (from which Heinävesi takes its raw water), which at the time of the outbreak was iced over. Reverse transcriptase polymerase chain reaction analysis revealed virus in tap water samples in Heinävesi and also demonstrated that the virus was identical to those isolated from clinical samples in both outbreaks (Maunula *et al.*, 1999).

7.6.3 *Negative results*

Although pathogen detection is important in outbreak investigation, recovery of pathogens from drinking water is often unsuccessful even when a supply is strongly associated with an outbreak. Probably the most common cause of failure to detect an implicated pathogen is the time between contamination and subsequent infection and the time that the outbreak is detected and investigations commenced. A transient contamination event may lead to only temporary contamination of the supply. The chance of finding the pathogenic agent is also dependent on the method used, the organism's robustness in the water environment in general and its resistance to water disinfectants. Additionally, the ability to detect a pathogen in a water supply may be hampered by the common practice of performing a preventative super-disinfection, which is sometimes conducted prior to ensuring that appropriate sample(s) are taken for examination of the water, so destroying any remaining pathogens that may have been present.

Even if pathogenic agents are detected in the implicated drinking water, this may not always correlate with the clinical picture. In one outbreak, for example, both echo- and coxsackie viruses were isolated from water samples but the clinical picture implicated a different type of viral infection (Stenström, 1994), clearly where sewage contamination has occurred the detection of mixed pathogens is unsurprising. In this situation, isolation of a pathogen different to the one causing the outbreak could only be taken as evidence of inadequate water management.

7.6.4 *Pathogen typing and strain characterisation*

Even where it has been possible to detect a pathogen, in some cases it may be insufficient to identify the causative organism in human or environmental samples only down to the species level. Further characterisation may be vital in determining the source of contamination and a number of properties can be utilised, such as antibiotic resistance profiles. These can differentiate for example between human and non-human faecal sources as the bacteria infecting humans and livestock are often resistant to different antibiotics.

The traditional use of typing is to enable the investigators to determine whether strains isolated from different sources are indistinguishable or not. Another use is in the determination of virulence (*i.e.* if different strains within a species vary in their ability to cause illness). Finally, sometimes different strain types have different epidemiology as in the case of *Cryptosporidium parvum* where type 2 strains are zoonotic and type 1 are largely restricted to causing infection in humans.

When choosing any typing method there are a number of criteria that need to be considered (Hunter, 1991). These include:

- Typability (the proportion of strains that can be typed by that method).
- Reproducibility (the probability that if the same strain was re-tested it would give the same result).
- Discriminatory power (the ability of a method to distinguish between unrelated strains).

In addition, cost, ease of use and timeliness are important factors. There are many different typing methods described in the literature and the optimal method depends on the organism under investigation, the reasons for typing (whether as an aid to characterise a few strains associated with a hospital outbreak or an aid to surveillance within a country) and the resources available to the typing laboratory (both financial and technical expertise).

This section focuses principally on modern molecular typing methods (with more details in Chapter 8), which have been used increasingly since the early 1980s. However, typing methods have been used by microbiologists long before then. One of the most important 'traditional' techniques is serotyping, which is still the primary typing method used in the categorisation of a number of microorganisms including: *Salmonella* spp., *Shigella* spp., *E. coli*, and the enteroviruses (Threlfall and Frost, 1990; Hinton, 1985; Wenner, 1982). It works by discriminating between strains on the basis of their surface antigens. For bacterial pathogens the method usually involves mixing the strain under investigation with various sera and looking for agglutination. For viruses, the technique usually involves demonstrating loss of the ability to infect tissue culture cells after mixing with sera.

Other traditional typing methods include (Aber and Mackel, 1981):

- Bacteriophage typing where strains are discriminated according to their susceptibility to killing by bacteriophages. This method is still commonly used in typing of *Staphylococcus aureus* and various serotypes of *Salmonella* (Threlfall and Frost, 1990).
- Biotyping that discriminates on the basis of the requirements for selected nutrients to grow. Although widely used in the past for many different pathogens including *E. coli* (Hinton, 1985), biotyping has largely fallen out of favour. However, it may still have a role to play in laboratories with few resources.
- Resistotyping distinguishes between strains on the susceptibility to various antibiotics and other antimicrobial agents, usually known as antibiograms. Resistotyping based on antibiotic sensitivity patterns has a considerable advantage in that antimicrobial susceptibility testing is frequently undertaken to guide therapy and so the data is usually to hand. As a typing method, resistotyping comes into its own for the rapid identification of strains with unusual antibiograms.
- Bacteriocin typing is based on the production of, or susceptibility to, various bacteriocins (compounds produced by bacteria that inhibit the growth of other strains). This method was commonly used in the typing of *Pseudomonas aeruginosa* when it was known as pyocin typing (Pitt, 1988), it has now, however, been largely superseded by other methods.

A major problem with traditional methods is that they are frequently of low typability and discriminatory power. Furthermore, typing can often only be done within certain reference laboratories and sending strains away can lead to delay. Many of the modern molecular methods offer considerable advantages for typing and a number of DNA ‘fingerprinting’ techniques have been described, including the following:

- Restriction fragment length polymorphism (RFLP).
- Pulse field gel electrophoresis.
- Randomly amplified polymorphic DNA (RAPD).

These enable each isolate to be characterised by a unique set of banding patterns which can be used for species identification or for epidemiological purposes and are described in more detail in a case study below and in Chapter 8.

Case study: Shigella typing

In 1998, in Nagasaki Japan, there was a large outbreak of *Shigella sonnei* infection, with 470 confirmed cases and 821 epidemiological linked cases. The outbreak investigation started when six students were reported ill (five of whom were hospitalised); all of who had eaten lunch at the University cafeteria. Active case finding and a cohort study of University users (students, staff and visitors) was undertaken. This found that 25% of regular University users had symptoms that met the case definition.

Patient interviews provided no evidence of a common food, but consuming water on campus was suspected to be associated with illness. The campus was supplied from two shallow wells, with no water treatment other than chlorination. Disinfection, however, was thought to be inadequate with samples showing no evidence of residual chlorine. Additionally, microbial tests were positive for *Shigella sonnei*. The source of the contamination was traced to a leakage of raw sewage from a nearby sewerage pipeline (identified using a sodium chloride tracer). DNA fingerprinting, using pulse field gel electrophoresis revealed that the isolates of *Shigella sonnei* were identical from both clinical and water samples. The outbreak was halted by issuing instructions not to drink the campus water and then switching from the well source to a municipal supply.

Case study: Cryptosporidium identification

Recent advances in the application of molecular biological methods to *Cryptosporidium* have contributed much to knowledge of the epidemiology of cryptosporidiosis. Human disease is usually caused by *C. parvum*, in which two genotypes predominate. Genotype 1 is the anthroponotic genotype (type H) that is largely restricted to humans, and genotype 2 (type C) is the zoonotic genotype that causes both human and animal disease (Fayer *et al.*, 2000). Thus, the detection of genotype 1 is indicative of a human source of infection or contamination and genotype 2 of either an animal or a human source. Genotypes, and indeed some species, of *Cryptosporidium* cannot be differentiated microscopically. Characterisation of isolates using DNA amplification-based methods is advantageous over phenotypic methods since relatively few organisms are required (Gasser and O'Donoghue, 1999).

Molecular characterisation of *Cryptosporidium* has included analysis of repetitive DNA sequences, RAPD, direct PCR with DNA sequencing and PCR/RFLP analysis (Clark, 1999; Morgan *et al.*, 1999). The two distinct

C. parvum genotypes have been consistently differentiated at a variety of gene loci (Fayer *et al.*, 2000), including:

- *Cryptosporidium* Oocyst Wall Protein (COWP).
- Ribonuclease reductase.
- 18S rDNA (*syn.* small subunit ribosomal RNA).
- Internal transcribed rDNA spacers (ITS1 and ITS2).
- Acetyl-CoA synthetase.
- Dihydrofolate reductase-thymidylate synthase (dhfr-ts).
- Thrombospondin related adhesive proteins (TRAP-C1 and TRAP-C2).
- the α and β beta tubulin.
- 70kDa heat shock protein (hsp70).

Application of genotyping techniques has also led to the characterisation of additional *Cryptosporidium* spp. and genotypes, and it has become clear that while the majority of human cryptosporidiosis is caused by *C. parvum*, other species are also found infecting both immunocompetent and immunocompromised patients (Fayer *et al.*, 2000; Chalmers *et al.*, 2002b). It is evident that some primer pairs are species specific, such as those for TRAP-C2 which is specific for *C. parvum* (Elwin *et al.*, 2001), while others cross react with related protozoan parasites, and that some PCR/RFLPs differentiate species/genotypes more readily than others (Sulaiman *et al.*, 1999). While PCR/RFLP is widely used for characterisation, and allows many specimens to be analysed and compared, only bases at the restriction enzyme sites are examined. Sequence analysis provides the 'gold standard' since all the bases within the target sequence at the locus are examined. The importance of sequence confirmation of RFLP patterns was illustrated by Chalmers *et al.* (2002) who identified a novel RFLP pattern, similar to *C. parvum* genotype 1, in the COWP gene of isolates from sheep, but sequence data clearly differentiated the isolate. Therefore, careful primer selection and PCR product analysis is required for detection and characterisation, particularly from environmental specimens where a wide range of cryptosporidia and other organisms may be present. It must also be noted that oocyst recovery and PCR methods from environmental samples, including water, have yet to be standardised.

The further differentiation of subtypes within *Cryptosporidium* genotypes provides additional resolution for epidemiological investigations (Glaberman *et al.*, 2001), and a variety of tracking tools are being investigated and evaluated. The discovery of a number of dinucleotide and trinucleotide repeats within the *Cryptosporidium* genome has enabled the application of microsatellite typing as a method for further segregation within the two divisions of *C. parvum* (Caccio, 2000). Blasdall *et al.* (2001) have exploited an apparent fortuitous juxtaposition of two non-coding genes within the genome of *Cryptosporidium*, yielding host-level resolution of *C. parvum* in a robust method where banding patterns appear stable over a number of years within a single herd. Sequence analysis of small double-stranded extra chromosomal RNAs in *C. parvum* (Xiao *et al.*, 2001) and of a highly polymorphic gene encoding a 60KDa glycoprotein (Strong *et al.*, 2000) also offer tracking tools, while analysis of single strand conformation polymorphisms is also being investigated as a subtyping tool (Gasser *et al.*, 2001).

The methods discussed above are already having a significant impact on the investigation of outbreaks of waterborne cryptosporidiosis. The ability to distinguish between the anthroponotic and zoonotic genotypes is a significant pointer towards identifying the possible source of pollution. Patel *et al.* (1998) were able to demonstrate that two outbreaks originally thought to be due to agricultural pollution were actually due to human sewage. However, typing based only on genotyping has a low discriminatory power. In the case of an outbreak with a zoonotic strain, genotyping alone will not enable investigators to determine which herds are most likely to be responsible for the contamination events. The methods for increased strain discrimination discussed above have the potential to answer these types of question. Improved strain discrimination can also improve the epidemiological investigation of an outbreak by improving case definition. Possible cases who are infected with a strain other than the outbreak strain could be excluded from analysis, and so reduce potential for bias.

7.7 Summary

Waterborne outbreaks are the most obvious manifestation of waterborne disease. Microbiological examinations have several roles in the investigation of such outbreaks. The finding of the causative pathogen in the water supply is among the best evidence of a link between a water supply and an outbreak of disease. However, for a number of reasons it is frequently impossible to obtain this piece of evidence. Novel molecular methods may offer a better chance of identifying pathogens in the water supply than traditional cultural methods. However, sensitivity remains low and even if sensitivity were increased no test

will detect an organism that was flushed from the distribution system a week or more previously. Incident preparedness plans would ideally include provision for the appropriate collection and storage of samples of water during suspected waterborne disease outbreaks to assist with follow-up. However, investigation methods following an outbreak do not require the pathogen to be detected in the supply. The second major use of microbiological investigations is in demonstrating failure in optimal water treatment and distribution. Laboratory tests or process indicators assessed as part of the routine management of the supply may provide useful information, as may the results of increased sampling using standard coliform and thermotolerant coliform counts. Above all, microbiology is essential in the diagnosis of individual cases of infection in the human population. The human population remains the best monitor of certain threats to the water supply (*e.g.* for cryptosporidiosis). Increasingly, novel technologies are being used to type strains isolated from humans to confirm that cases are indeed part of an outbreak of infection. Such typing may also provide clues to the epidemiology of an outbreak as when strains from humans can be shown to be the same as strains isolated from the environment.

Finally, microbial and other indicator parameters provide valuable tools for alerting the responsible authorities to the possibility of water becoming unsafe. Judicious use of indicator parameters within the context of a systematic water safety plan should provide early warning of potential public health incidents, enabling a planned corrective response. A more generalised state of incident preparedness should reduce the public health risk even in the event of a scenario for which a specific corrective response has not been prepared.

REFERENCES

- Aber, R.C. and Mackel, D.C. (1981) Epidemiologic typing of nosocomial microorganisms. *American Journal of Medicine* **70**, 899-905.
- Andersson, Y. and Bohan, P. (2001) Disease surveillance and waterborne outbreaks. In: *Water Quality: Guidelines, Standards and Health. Assessment of risk and risk management for water-related infectious disease*. Fewtrell, L. and Bartram, J. (Eds.) IWA Publishing, London. pp.115-133.
- Angulo, F.J., Tippen, S., Sharp, D.J., Payne, B.J., Collier, C., Hill, J.E., Barrett, T.J., Clark, R.M., Geldreich, E.E., Donnell, H.D. and Swerdlow, D.L. (1997) A community waterborne outbreak of salmonellosis and the effectiveness of a boil water order. *American Journal of Public Health* **87**(4), 580-584.
- Anon (1994) *The Microbiology of Water 1994: Part 1 – Drinking water*. Reports on Public Health and Medical Subjects, No. 71. Her Majesty's Stationery Office, London.
- Badenoch, J. (1990) *Cryptosporidium* in water supplies. Report of the Group of Experts. Department of the Environment, Department of Health. Her Majesty's Stationery Office, London.
- Barwick, R.S., Levy, D.A., Craun, G.F., Beach, M.J. and Calderon R.L. (2000) Surveillance for waterborne-disease outbreaks--United States, 1997-1998. *Morbidity and Mortality Weekly Report. CDC Surveillance Summaries* **49** (SS-4), 1-21.
- Blasdall, S.A., Ongerth, J.E. and Ashbolt, N.J. (2001) Differentiation of *Cryptosporidium parvum* subtypes by a novel microsatellite-telomere PCR with PAGE. *Proceedings of Cryptosporidium from Molecules to Disease, 7-12 October 2001*. Esplanade Hotel Fremantle, Western Australia. Murdoch University, Perth.

- Caccio, S., Homan, W., Camilli, R., Traldi, G., Kortbeek, T. and Pozio, E. (2000) A microsatellite marker reveals population heterogeneity within human and animal genotypes of *Cryptosporidium parvum*. *Parasitology* **120**, 237-244.
- Casemore, D.P. (1992) A pseudo-outbreak of cryptosporidiosis. *Communicable Disease Report. CDR Review* **2**(6), R66-R67.
- Chalmers, R.M., Elwin, K., Reilly, W.J., Irvine, H., Thomas, A.L. and Hunter, P.R. (2002a) *Cryptosporidium* in farmed animals: the detection of a novel isolate in sheep. *International Journal of Parasitology* **32**, 21-26.
- Chalmers, R.M., Elwin, K. and Thomas, A. (2002b) Unusual types of cryptosporidia are not restricted to immunocompromised patients. *Journal of Infectious Diseases* **185**, 270-271.
- Clark, D.P. (1999) New insights into human cryptosporidiosis. *Clinical Microbiology Reviews* **12**, 554-63.
- Deere, D., Stevens, M., Davison, A., Helm, G. and Dufour, A. (2001) Management Strategies. In: *Water Quality: Guidelines, Standards and Health. Assessment of risk and risk management for water-related infectious disease*. Fewtrell, L. and Bartram, J. (Eds.) IWA Publishing, London. pp. 257-288.
- Divizia, M., Gnesivo, C., Bonapasta, R.A., Morace, G., Pisani, G. and Pana, A. (1993) Hepatitis A virus identification in an outbreak by enzymatic amplification. *European Journal of Epidemiology* **9**, 203-208.
- Elwin, K., Chalmers, R.M., Roberts, R., Guy, E.C. and Casemore, D.P. (2001) The modification of a rapid method for the identification of gene-specific polymorphisms in *Cryptosporidium parvum*, and application to clinical and epidemiological investigations. *Applied and Environmental Microbiology* **67**, 5581-5584.
- Farley, K.R.J., Rutherford, G., Litchfield, P., Hsu, S.T., Orenstein, W.A., Schonberger, L.B., Bart, K.J., Lui, K.J. and Lin, C.C. (1984) Outbreak of paralytic poliomyelitis, Taiwan. *Lancet* **ii**, 1322-1324.
- Fayer, R., Morgan, U. and Upton, S.J. (2000) Epidemiology of *Cryptosporidium*: Transmission, detection and identification. *International Journal for Parasitology* **30**, 1305-1322.

- Gasser, R.B. and O'Donoghue, P. (1999) Isolation, propagation and characterization of *Cryptosporidium*. *International Journal for Parasitology* **29**, 1379-1413.
- Gasser, R.B., Zhu, X.Q., Caccio, S., Chalmers, R., Widmer, G., Morgan, U., Thompson, R.C.A., Pozio, E. and Browning, G.F. (2001) Genotyping *Cryptosporidium parvum* by single-strand conformation polymorphism analysis of ribosomal and heat shock gene regions. *Electrophoresis* **22**, 433-437.
- Glaberman, S., Moore, J., Lowery, C., Chalmers, R.M., Elwin K., Rooney, P., Millar, C., Dooley, J., Lal, A.A. and Xiao, L. (2001) Investigation of three drinking water associated outbreaks of cryptosporidiosis in Northern Ireland using genotyping and subgenotyping tools. American Society for Tropical Medicine and Hygiene meeting, Atlanta, GA.
- Hinton, M. (1985) The sub-specific differentiation of *Escherichia coli* with particular reference to ecological studies in young animals including man. *Journal of Hygiene* **95**, 595-609.
- Hunter, P.R. (1991) A critical review of typing methods for *Candida albicans* and their applications. *Critical Reviews in Microbiology* **17**, 417-34.
- Hunter, P.R. (1997) *Water-borne Disease: Epidemiology and Ecology*. Wiley, Chichester.
- Hunter, P.R. (2000a) Advice on the response to reports from public and environmental health to the detection of cryptosporidial oocysts in treated drinking water. *Communicable Disease and Public Health* **3**, 24-27.
- Hunter, P.R. (2000b) Modelling the impact of prior immunity, case misclassification and bias on case-control studies in the investigation of outbreaks of cryptosporidiosis. *Epidemiology and Infection* **125**, 713-718.
- Hunter, P.R. and Syed, Q. (2001) Community surveys of self-reported diarrhoea can dramatically overestimate the size of outbreaks of waterborne cryptosporidiosis. *Water Science and Technology* **43**, 27-30.
- Hunter, P.R. and Syed, Q. (2002) Recall bias in a community survey of self-reported gastroenteritis undertaken during an outbreak of cryptosporidiosis strongly associated with drinking water after much press interest. *Epidemiology and Infection*. In press

- Kramer, M.H., Herwaldt, B.L., Craun, G.F., Calderon, R.L. and Juranek, D.D. (1996a) Surveillance for waterborne-disease outbreaks - United States, 1993-1994. *Morbidity and Mortality Weekly Report. CDC Surveillance Summaries* **45**(SS-1), 1-33.
- Kramer, M.H., Herwaldt, B.L., Craun, G.F., Calderon, R.L. and Juranek, D.D. (1996b) Waterborne disease – 1993 and 1994. *Journal of the American Water Works Association* **88**(3), 66-80.
- Kukkula, M., Maunula, L., Silvennoinen, E. and v. Bonsdorff, C-H. (1999) Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. *Journal of Infectious Disease* **180**, 1771-1776.
- Leland, D., Acanulyt, J., Keene, W. and Stevens, G. (1993) A cryptosporidiosis outbreak in a filtered-water supply. *Journal of the American Water Works Association* **85**(6), 34-42.
- Levy, D.A., Bens, M.S., Craun, G.F., Calderon, R.L. and Herwaldt, B.L. (1998) Surveillance for waterborne-disease outbreaks - United States, 1995-1996. *Morbidity and Mortality Weekly Report. CDC Surveillance Summaries* **47**(SS-5), 1-34.
- Maunula, L., Piiparinen, H. and v. Bonsdorff C.-H. (1999) Confirmation of Norwalk-like virus amplicons after RT-PCR by microplate hybridization and direct sequencing. *Journal of Virological Methods* **83**, 125-134.
- Mayon-White, R.T. and Frankenberg, R.A. (1989) Boil the water. *Lancet* **ii**, 216
- Mentzing, L.O. (1981) Waterborne outbreaks of *Campylobacter* enteritis in central Sweden. *Lancet* **ii**, 352-354.
- Millson, M., Bokhout, M., Carlson, J., Speilberg, L., Aldis, R., Borczyk, A.Z. and Lior, H. (1991) An outbreak of *Campylobacter jejuni* gastroenteritis linked to meltwater contamination of a municipal well. *Canadian Journal of Public Health* **82**, 27-31.
- Moore, A.C., Herwaldt, B.L., Craun, G.F., Calderon, R.L., Highsmith, A.K. and Juranek, D.D. (1993) Surveillance for waterborne disease outbreaks - United States, 1991-1992. *Morbidity and Mortality Weekly Report. CDC Surveillance Summaries* **42**(SS-5), 1-22.

- Morgan, U.M., Xiao, L., Fayer, R., Lal, A.A. and Thompson, R.C.A. (1999) Variation in *Cryptosporidium*: towards a taxonomic revision of the genus. *International Journal for Parasitology* **29**, 1733-1751.
- Murray, C.J.L. (1994) Quantifying the burden of disease: the technical basis for disability-adjusted life years. *Bulletin of the World Health Organization* **72**(3), 429-445.
- O'Donnell, M., Platt, C. and Aston, R. (2000) Effect of a boil water notice on behaviour in the management of a water contamination incident. *Communicable Disease and Public Health* **3**, 56-59.
- Patel, S., Pedraza-Diaz, S., McLauchlin, J. and Casemore, D.P. (1998) Molecular characterisation of *Cryptosporidium parvum* from two large suspected waterborne outbreaks. *Communicable Disease and Public Health* **1**, 231-233.
- Percival, S.L., Walker, J.T. and Hunter P.R. (2000) *Microbiological Aspects of Biofilms and Drinking Water*. CRC Press, Boca Raton.
- Pitt, T.L. (1988) Epidemiological typing of *Pseudomonas aeruginosa*. *European Journal of Clinical Microbiology and Infectious Diseases* **7**, 238-247.
- Prescott, A.M. and Fricker, C.R. (1999) Use of PNA oligonucleotides for the *in situ* detection of *Escherichia coli* in water. *Molecular and Cellular Probes* **13**, 261-268.
- Provost, P.J. and Hilleman, M.R. (1979) Propagation of human hepatitis A virus in cell culture *in vitro*. *Proc. Soc. Exp. Biol. Med.* **160**, 213-221.
- Ramsay, C.N. and Marsh, J. (1990) Giardiasis due to deliberate contamination of water supply. *Lancet* **336**, 880-881.
- Rollins, D.M. and Colwell, R.R. (1986) Viable but non-culturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. *Applied and Environmental Microbiology* **52**(3), 531-538.
- Rose, J.B., Lisle, J.T. and LeChevallier, M. (1997) Waterborne cryptosporidiosis: Incidence, outbreaks and treatment strategies. In: *Cryptosporidium and Cryptosporidiosis*. Fayer, R. (Ed.) CRC Press, Boca Raton.

- Schmidt, K. (1995) WHO surveillance programme for control of foodborne infections and intoxications in Europe. Sixth report, 1990-92. BgVV, Berlin, p. 14.
- Shaw, P.K., Brodsky, R.E., Lyman, D.O., Wood, B.T., Hibler, C.P., Healy, G.R., Macleod, K.I., Stahl, W. and Schultz, M.G. (1977) A community-wide outbreak of giardiasis with evidence of transmission by a municipal water supply. *Annals of Internal Medicine* **87**, 426-432.
- Stenström, T.A. (1994) A review of water-borne outbreaks of gastroenteritis in Scandinavia. In: *Water and Public Health*. Golding, A.M.B., Noah, N. and Stanwell-Smith, R. (Eds.) Smith-Gordon, London. pp.137-143.
- Strong, W.B., Gut, J. and Nelson, R.G. (2000) Cloning and sequence analysis of a highly polymorphic *Cryptosporidium parvum* gene encoding a 60-kilodalton glycoprotein and characterization of its 15- and 45- kilodalton zoite surface antigen products. *Infection and Immunity* **68**, 4117-4134.
- Sulaiman, IM, Xiao, L., and Lal, A.A. (1999) Evaluation of *Cryptosporidium parvum* genotyping techniques. *Applied and Environmental Microbiology* **65**, 4431-4435.
- Swerdlow, D.L., Mintz, E.D., Rodriguez *et al.* (1992) Severe life-threatening cholera in Peru: predisposition of persons with blood group O. Abstract 941. Program, 32nd Interscience Antimicrobial Agent Chemotherapy Conference, 267.
- Threlfall, E.J. and Frost, J.A. (1990) The identification, typing and fingerprinting of *Salmonella*: laboratory aspects and epidemiological applications. *Journal of Applied Bacteriology* **68**, 5-16.
- Vogt, R.L., Sours, H.E., Barrett, T., Feldman, R.A., Dickinson, R.J. and Witherell, L. (1982) *Campylobacter* enteritis associated with contaminated water. *Annals of Internal Medicine* **96**, 292-296.
- Wenner, H.A. (1982) The enteroviruses: recent advances. *Yale Journal of Biology & Medicine* **55**, 277-82.
- West, P.A. (1991) Human pathogenic viruses and parasites: emerging pathogens in the water cycle. *Society for Applied Bacteriology Symposium Series* **20**, 107S-114S.

Willocks, L.J., Sufi, F., Wall, R., Seng, C. and Swan, A.V. (2000) Compliance with advice to boil drinking water during an outbreak of cryptosporidiosis. *Communicable Disease and Public Health* **3**, 137-138.

WHO (1999) *Water and Health in Europe*. World Health Organization Regional Office for Europe, Copenhagen.

Xiao, L., Limor, J., Bern, C. and Lal, A.A. (2001) Tracking *Cryptosporidium parvum* by sequence analysis of small double-stranded RNA. *Emerging Infectious Diseases* **7**, 141-145.