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Human Viruses in Water
Albert Bosch (Editor)

DOI 10.1016/S0168-7069(07)17001-4

Chapter 1

Overview of Health-Related Water Virology

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Historical perspective

Viruses play an important role in biological mechanisms by which nature maintains a balance among living organisms on earth. The role of viruses in controlling numbers of human beings is illustrated by the impact of variola viruses (smallpox) which killed an estimated 10–15 million people per year until as recently as 1967. Others include influenza viruses, which reduced numbers of humans by some 20 million during the 1918–1919 pandemic, and measles and hepatitis viruses. Eventually mankind rose above all other living organisms with intelligent and innovative resistance to biological mechanisms for controlling its proliferation at the cost of other lives on earth. The role of smallpox was finally eliminated in 1977 by eradication of the virus, and the impact of viruses like influenza, poliomyelitis and measles was restricted by vaccination. Today we have reached the point where man has severely disrupted the balance of living organisms on the planet. It is almost sad to note how nature keeps fighting a seemingly lost battle by bringing in reinforcements in the form of new viruses like the human immunodeficiency virus (HIV) and mutants of old stalwarts like influenzaviruses with new battle strategies.

Viruses affect all forms of life, from single cellular plants like bacteria and single cellular animals (protozoa), to the highest forms of plants and animals, including man. Remarkable features of virus–host relationships include the variety of mechanisms by which different viruses are transmitted from one host to the next. Some viruses are highly host specific, like HIV, while others are less host specific, like influenzaviruses. Different viruses are designed for specific modes of transmission. For instance, viruses such as HIV, rabies and haemorrhagic fever viruses, are designed for direct inoculation of contaminated body fluids from an infected host

into the tissue or blood stream of a new host. Viruses like influenza and measles are designed for airborne transmission and inhalation of air containing the viruses by a new host. Then there is the large group of enteric viruses that primarily infect the intestinal tract and are typically transmitted by the faecal-oral route often involving the ingestion of food and water contaminated with the viruses. However, there are no rules and regulations cast in concrete. There are exceptions to all these principles, allowing viruses to exploit alternative options when it serves their best interest.

The earliest record of diseases caused by enteric viruses may well be the report in the Babylonian Talmud that hepatitis was common in the fifth century BC (Zuckerman, 1983). It would appear that the most likely cause of the hepatitis referred to was hepatitis A and/or E viruses, both of which enteric viruses typically transmitted by food and water (Grabow, 2002; Chapter 3).

Impact of human viruses in water

Health impact

The global impact of waterborne and water-related diseases is difficult to assess. This is due to a lack of data, many variables and shortcomings in epidemiological studies and the interpretation of results. Additional reasons include the difficulty to confirm the source of infections, sub-clinical infections and secondary transmission of infections, increased susceptibility to infections in certain communities due to undernourishment and immune incompetence, reduced susceptibility in others due to immunity and geographical and seasonal distribution of diseases (Gerba et al., 1996a).

Consequently estimates of the health impact of these diseases vary. The following gives an indication.

Infectious diarrhoea or gastroenteritis is the most frequent, non-vector, water-related health outcome, in both the developed and developing world. Diarrhoea causes approximately 2.2 million deaths per year, mostly among children under the age of five, and while water is not solely responsible, water sanitation and hygiene are extremely important factors in this death toll (Prüss-Üstün and Fewtrell, 2004). The World Health Organization (WHO) estimated that every year there are 1.7 million deaths related to unsafe water, sanitation and hygiene, mainly through infectious diarrhoea. Some 4 billion cases of diarrhoea annually account for over 82 million disability adjusted life years (DALYs), representing 5.7% of the global burden of disease and placing diarrhoeal diseases as the third highest cause of morbidity and sixth highest cause of mortality (Prüss and Havelaar, 2001). In addition, waterborne disease is a major threat to millions who live in underdeveloped and informal conditions, or are displaced or otherwise affected by conflicts and disasters. Although the developing world is hardest hit by waterborne diseases, developed countries are also affected. For instance, the largest outbreak of a waterborne disease on record with some 403,000 cases of cryptosporidiosis occurred in

1993 in Milwaukee, a highly developed modern city in the USA (MacKenzie et al., 1994).

There is reason to believe that the health impact of waterborne diseases, and particularly those caused by viruses, tends to be underestimated (Regli et al., 1991; Gerba et al., 1996a). For instance, mortality data do not reflect the large number of infected individuals who suffer from clinical manifestations that range from mild unreported discomfort to non-fatal severe illness, with far-reaching socio-economic implications (Pegram et al., 1998). Waterborne and water-related diseases are associated with exposure to water environments in many ways. These include treated waters like those used for drinking and recreation in swimming pools and related facilities, and in food processing and other industrial activities, as well as untreated waters used for drinking, recreation and agricultural purposes such as crop irrigation and animal husbandry.

Expressed in terms easier to understand, data on waterborne diseases have been calculated for purposes of comparison as equivalent to a jumbo jet with 400 children and 100 adults on board crashing with no survivors every half hour around the clock (see Grabow, 1996). This illustration is based on authentic estimates that some 50,000 people die each day in the world due to waterborne and water-related diseases.

Viruses are a major cause of waterborne and water-related diseases. Extreme examples include the outbreak of 300,000 cases of hepatitis A and 25,000 cases of viral gastroenteritis in 1988 in Shanghai caused by shellfish harvested from a sewage-polluted estuary (Halliday et al., 1991). In 1991, an outbreak of 79,000 cases of hepatitis E in Kanpur was ascribed to polluted drinking water (Ray et al., 1991).

Socio-economic impact

Although the mortality of many waterborne diseases is relatively low, the socio-economic impact even of non-fatal infections is immense. Undetected diarrhoeal illnesses are common but generally not severe; their significance is often unrecognised and many illnesses are unreported. The societal cost of the so-called ''mild gastrointestinal illnesses'' is several orders of magnitude higher than the costs associated with acute hospitalised cases. In the US, the annual cost to society of gastrointestinal infectious illnesses was estimated as $19,500 million dollars (1985 US dollars) for cases with no consultation by physician, $2750 million dollars for those with consultations, and only $760 million dollars for those requiring hospitalisation. From the data collected during the Canadian studies and based on reported symptoms in the US (population of 300 million individuals) the estimate of the cost of waterborne illness ranges from US$269 to 806 million for medical costs and US$40 to 107 million for absences from work. These figures illustrate the enormous economic costs of endemic gastrointestinal illnesses, even in societies where waterborne disease is not perceived to be a problem (Payment, 2006b).

The socio-economic costs of epidemics and outbreaks of diseases with more severe illness and higher mortality rates such as cholera, typhoid fever and shigellosis are much higher (Pegram et al., 1998).

Viruses associated with waterborne transmission

Viruses predominantly associated with waterborne transmission are members of the group of enteric viruses that primarily infect cells of the gastrointestinal tract, and are excreted in the faeces of infected individuals. The viruses concerned are highly host specific, which implies that their presence in water environments is sound evidence of human faecal pollution. In some cases different strains of a viral species, or even different species of a viral genus, may infect animals. The extent of the host specificity of enteric viruses is such that it is used as a valuable tool to distinguish between faecal pollution of human and animal origin, or to identify the origin of faecal pollution. The hepatitis E virus may be the only meaningful exception to this rule, having strains which seem to infect both humans and certain animals, complying with the definition of a zoonosis (Grabow, 2002; Maluquer de Motes et al., 2004; Chapter 3). The following is a summary of typical human enteric viruses:

Adenoviruses

The family Adenoviridae consists of the genus *Mastadenovirus* associated with mammals, and three other genera associated with a spectrum of animals including birds and reptiles. The 51 antigenic types of human adenoviruses (HAds) consist of a double-stranded DNA genome in a non-enveloped icosahedral capsid with diameter about 80 nm and unique fibres. HAds cause a wide range of infections with a spectrum of clinical manifestations in the gastrointestinal, respiratory and urinary tracts, as well as the eyes. Relevant examples include types 40 and 41, which are an important cause of childhood gastroenteritis, and types 3, 4 and 7 associated with pharyngo-conjunctival fever commonly known as ''swimming pool conjunctivitis''. HAds are excreted by infected individuals in high numbers (enteric HAds in numbers of $10^{11}\,g^{-1}$ of faeces) and occur in relatively large numbers in faecally polluted waters, often outnumbering cytopathogenic enteroviruses. They are relatively resistant to unfavourable conditions, notably ultraviolet light. Apart from types 40 and 41, most HAds are readily detectable by cell culture propagation. In view of these features they have been suggested as useful indicators for enteric viruses. The US EPA has included HAds in its drinking water Candidate Contaminant List (CCL) as a group of high-priority viruses for water research, together with the groups of entero-, rota- and caliciviruses. For further details see EPA (1989), Muniain-Mujika et al. (2003), WHO (2004), Van Heerden et al. (2005a,b).

Astroviruses

The family Astroviridae contains eight types of human astroviruses (HAstVs) consisting of a single-stranded RNA genome in a 28 nm diameter non-enveloped icosahedral capsid, which displays a characteristic Star of David surface image under the electron microscope. HAstVs are excreted in substantial numbers in the faeces of infected individuals and are readily detectable in polluted water environments. The virus is a common cause of gastroenteritis, predominantly in children. HAstVs do not readily produce a cytopathogenic effect (CPE) in cell cultures, but their nucleic acid is detectable by molecular techniques after amplification in cell cultures. For further details see Nadan et al. (2003), WHO (2004).

Caliciviruses

The family Caliciviridae contains the genera *Norovirus* (Norwalk-like viruses) and *Sapovirus* (Sapporo-like viruses), which typically infect humans (HuCVs), as well as two other genera associated with infections of a wide variety of animals including mammals, fish, reptiles and insects. HuCVs consist of a single-stranded RNA genome in a non-enveloped 35–40 nm diameter icosahedral capsid, which under optimal conditions displays 32 calicle-like (cup-like) structures on the surface. HuCVs are exceptionally difficult to detect. They do not even seem to infect available cell culture systems since no viral RNA is detectable in cell cultures exposed to the viruses. Much of the initial research on the viruses was, therefore, carried out in human volunteers. Today progress is accomplished by means of molecular techniques. Noroviruses (NoVs) may be excreted in numbers of $10^{10}\,g^{-1}$ of stool or more. They are highly infectious and the most common cause of gastroenteritis associated with water and food in all age groups. However, the most frequent routes of transmission are person-to-person contact and the inhalation of contaminated aerosols and dust particles, as well as airborne particles of vomitus. Although clinical symptoms typically including vomiting, abdominal cramps, headache and muscular pain are relatively mild and rarely last for more than 3 days, the socio-economic impact is enormous. HuCVs are notorious for outbreaks on cruise ships, at holiday resorts, hotels, schools and hospitals, causing interruptions with far-reaching implications. Only about 40% of infected cases present with diarrhoea. Since cases with vomiting in the absence of diarrhoea are common, the infection is also known as ''winter-vomiting disease'', even though there is no meaningful seasonal trend. The immune response is poor and immunological protection short-lived, many infections are sub-clinical, and reinfection of individuals by the same HuCV strain is common. Secondary spread occurs often. For further details see Graham et al. (1994), Monroe et al. (2000), Duizer et al. (2004), Maunula et al. (2004), WHO (2004), Chan et al. (2006), Chapter 2.

Enteroviruses

The family Picornaviridae includes the genus *Enterovirus* of which the following species infect humans: poliovirus (PV1-3), coxsackievirus A (CVA1-24 with no type 23), coxsackievirus B (CVB1-6), echovirus (EV1-35 with no types 10 and 28), and enterovirus (EV68-71). Other species of the genus infect animals, for instance, the bovine (ECBO) group of enteroviruses. Enteroviruses are among the smallest known viruses and consist of a single-stranded RNA genome in a non-enveloped icosahedral capsid with a 20–30 nm diameter. Some members of the genus are readily detectable by CPE in cell cultures, notably polio-, coxsackie B-, echo- and enteroviruses. Members of the genus *Enterovirus* are among the most common causes of human infection, with an estimated 30 million infections per year in the USA. The viruses are associated with a broad spectrum of diseases ranging from mild febrile illness to myocarditis; meningoencephalitis; poliomyelitis; haemorrhagic conjunctivitis; herpangina; Bornholm disease; hand, foot and mouth disease; diabetes mellitus and neonatal multi-organ failure. Chronic infections are associated with conditions such as polymyositis, dilated cardiomyopathy and chronic fatigue syndrome. There is reason to believe that the health implications of enterovirus infections are not fully understood, particularly in terms of the long-term effects of chronic infections that are not readily evident from epidemiological data. Most infections, particularly in children, are asymptomatic, but still result in excretion of large numbers of the viruses that may cause clinical disease in others. Poliomyelitis, which caused severe mortality and suffering for a long time, has almost been eradicated by vaccination, but mutants of live vaccine strains are of concern. Since enteroviruses are excreted in large numbers by many people, they are detected in large numbers in raw and treated water supplies worldwide by techniques commonly used for water analysis. They tend to be outnumbered, at least at times, only by adenoviruses. In view of their common presence, resistance to treatment and disinfection processes, and easy detection of some members, they are widely used in water quality assessment, control and monitoring.

The family Picornaviridae also includes the genus *Hepatovirus* with only one species, the hepatitis A virus (HAV). This virus, of which there is only one antigenic type, shares all the basic features of other picornaviruses, including primary infection of cells of the gastrointestinal tract. From here HAV readily spreads via the blood stream to the liver where it may cause serious damage known as acute hepatitis with jaundice a typical clinical symptom. HAV is highly infectious and one of the best-known waterborne diseases with well-defined records of outbreaks and cases. As with many other picornaviruses, up to 90% of infected individuals, particularly children, display no clinical symptoms of infection, but they do excrete the virus, which may cause clinical disease in others. Although the mortality is generally less than 1%, recovery is a slow process that may keep patients incapacitated for 6 weeks or longer, which has substantial burden of disease implications. Immunity acquired by natural infection is typically lifelong, but not vaccine-derived immunity, which may constitute risks for immunised individuals later in

life. The virus is not detectable by conventional cell culture systems, but molecular detection of the viral RNA is well established. For further details see Bosch et al. (1991), Grabow et al. (1999, 2001), Grabow (2002), WHO (2004), Pavlov et al. (2005), Chapters 3 and 4.

Hepatitis E virus

The hepatitis E virus (HEV) has some unique genetic and epidemiological properties, which rendered classification into existing families and genera inappropriate. After various efforts of classification over many years failed, the virus with one antigenic type only has eventually been classified into its own exclusive genus *Hepevirus*, in its own family Hepeviridae. HEV and HAV share many clinical and epidemiological features, to the extent that they were identified as different viruses only relatively recently. HEV causes acute hepatitis and typical waterborne outbreaks very much like HAV, but there are differences. For instance, the incubation period is longer for HEV. Particularly important is that HEV has a mortality rate of up to 25% in pregnant women. Although HEV seems to occur in most parts of the world at least in animals, clinical disease and outbreaks in humans tend to have a specific geographical distribution, with high incidence in developing countries of India, Pakistan, Mexico and some parts of Africa. As mentioned earlier, outbreaks with tens of thousands of cases are on record for these areas. HAV, on the other hand, causes unprecedented clinical disease in non-immune populations all over the world. Immunity derived by natural infection is lifelong. One of the unique features of HEV is that it appears to be the only known enteric virus that is a typical zoonosis. There seems to be meaningful evidence that the same strains of HEV infect both humans and at least certain animals, notably swine, cattle, goats and rodents. This unfortunately implies that some animals may serve as reservoir for HEV strains that infect humans. For further details see Grabow (2002), WHO (2004), Chapter 3.

Rotaviruses

The genera *Rotavirus* and *Orthoreovirus* of the family Reoviridae are associated with water quality. The family has other genera that are irrelevant. Viruses belonging to this family consist of a double-stranded RNA genome in a non-enveloped icosahedral capsid with a diameter of 60–80 nm. The capsid has a characteristic double layer with spikes between the layers giving it the appearance of a wheel (Latin "rota").

Species of the genus *Rotavirus* are known as rotaviruses (RVs). They are divided into seven antigenic groups, A–G, each of which is subdivided into a number of subgroups. Certain members of groups A–C, predominantly group A, are associated with human infections (HRVs), while the rest infect a variety of animals including calves, swine, dogs, mice and monkeys. The stool of infected individuals may contain HRVs in numbers of $10^{12}\,g^{-1}$. No other enteric viruses are excreted in

numbers this high. In addition, HRVs are highly infectious, and if infections are not treated in time, the mortality rate is high. Consequently it is not surprising that HRVs are the most important single cause of infantile death in the world. Typically, HRVs are the cause of 50–60% of hospitalised cases of children with acute gastroenteritis. The burden of disease of HRV infections is, therefore, extremely high. Despite faecal excretion in exceptionally high numbers, and confirmation that waterborne transmission may occur, the predominant route of transmission is by personal contact, droplets, aerosols and airborne particles. HRVs are not readily detectable by CPE in cell cultures. HRVs recovered from water have successfully been identified by infection of cell cultures followed by detection of the replicated RNA by molecular techniques. Some RVs, notably monkeys strains, are readily detected by CPE in cell cultures, and are used as models for research on HRVs with regard to behaviour in water treatment and disinfection, as well as HRV vaccine production.

Species of the genus *Orthoreovirus* are known as reoviruses. The name is derived from ''Respiratory Enteropathogenic Orphan'' virus. These are typical ''orphan'' viruses, referring to viruses that have not been associated to meaningful extent with any disease. There seem to be indications of an association with gastroenteritis under circumstances. The three antigenic types infect humans and a variety of animals, including cattle, mice, chimpanzees and monkeys, typically without indications of disease. They seem to replicate in the respiratory tract of healthy individuals and are excreted in large numbers in the faeces of many humans and animals. Reoviruses are, therefore, commonly detected in water environments, often outnumbering other viruses. They are readily detected by CPE in cell cultures, although it takes longer for the CPE to become visible than with enteroviruses. Since reoviruses are readily detectable, occur in relatively high numbers in faecally polluted water and constitute no health risk, they are often used as indicators for other viruses. For further details see Hopkins et al. (1984), Gerba et al. (1996b), WHO (2004), Van Zyl et al. (2006), Chapters 2–4.

The above six groups of enteric viruses are the best known and commonly associated with water quality because they are excreted in faeces and their detection in sewage-polluted water environments is well established. However, a variety of other viruses are also excreted in faeces. These include parvoviruses (Family Parvoviridae) and corona- and toroviruses (Family Coronaviridae), all of which are at least suspected of being associated with gastroenteritis under circumstances. Viruses excreted in urine, notably polyomaviruses (Bofill-Mas et al., 2001), are also relevant.

Viruses excreted in faeces: transmission by water

The excretion in faeces and detection in water does not necessarily imply that viruses are predominantly, typically or even to meaningful extent transmitted by water. For enteric adeno-, all entero- and astroviruses there is little if any meaningful evidence of waterborne transmission. Waterborne transmission of

rotaviruses has been confirmed, but it is not a common route of transmission. Likewise, more than 30 years ago coxsackievirus infections have once been associated with bathing in polluted lake water, but water and food are not recognised as important vehicles for the transmission of these viruses. The same may apply to polyomaviruses excreted in urine, for which waterborne transmission has not yet been confirmed. Even noroviruses, the most common cause of waterborne disease, are not predominantly transmitted by water. The same applies to hepatitis A and E viruses. Virtually without exception viruses transmitted by the faecal-oral route are predominantly transmitted by routes other than food and water. The most important mechanisms involve personal contact and the transfer of viruses in droplets or the inhalation of viruses in aerosols or airborne particles. For instance, at the height of poliomyelitis epidemics some decades ago, swimming pools were closed not for fear of waterborne transmission of the viruses, but to restrict transmission among bathers by other routes. Likewise, to this day schools and related gatherings are closed at times of outbreaks to prevent the spread by personal contact of typical enteric viruses such as coxsackievirus A16 and enterovirus 71, the aetiological agents of hand, foot and mouth disease.

Obviously, the exposure to any viable viruses in water constitutes a certain risk of infection. Although in most cases the risk may be considered negligible, under circumstances it may take on catastrophic dimensions. Appropriate caution is therefore essential, and recommended guidelines to control the risk of waterborne viral infections should be strictly adhered to at all times (WHO, 2004). It should be noted that without exception the absence of commonly used faecal indicator bacteria, such as coliforms, is not reliable evidence of the absence of any enteric viruses, notably in water treated and disinfected for human consumption.

Avian influenza and SARS

It is feared that new strains of influenzaviruses may cause pandemics similar to the one that killed some 20 million people in 1918–1919. For a number of reasons, the global impact may be much larger this time (Webster, 1994). The mutation rate of influenzaviruses is exceptionally high because the single-strand RNA genome consists of eight segments that facilitates recombination among different strains (antigenic shift) in addition to point mutations (antigenic drift). Another important factor is that influenzaviruses have animal hosts, notably birds such as waterfowl and domestic chickens, and pigs, all of which occur in exceptionally large numbers in certain parts of the world. This promotes high-rate multiplication of influenzaviruses with abundant opportunities for mutations and recombinations among human and animal strains. Currently, concerns are that the highly virulent avian influenza A strain H5N1, which recently emerged, may undergo a mutation or recombination changing the host specificity to also infect humans. Human influenzaviruses replicate primarily in the respiratory tract, but avian strains primarily in the gastrointestinal tract of birds. This implies that the viruses are faecally excreted in large numbers into the water on which dense populations of waterfowl

occur. Consequently it is feared that water may play an important role in the transmission of influenzaviruses among waterfowl and potentially also from waterfowl to humans. In assessment of the risks involved it should be taken into account that viruses excreted in faeces are not necessarily transmitted to meaningful extent by water as has been explained earlier. There is little information on the mode of transmission of influenzaviruses among waterfowl. Water may possibly not play a particularly important role in the transmission of influenzaviruses among waterfowl because the virus seems to spread equally rapid among birds with restricted exposure to faecally polluted water such as poultry in breading batteries and ostriches in high-density farming units. The most important route of transmission may be direct contact and inhalation of droplets or aerosols, as is the predominant route of influenzavirus transmission among humans.

Severely acute respiratory syndrome (SARS) caused by an apparently new strain of coronavirus was diagnosed for the first time in patients in the Guangdong Province of China in November 2002. The virus spread rapidly and proved highly virulent. By 10 April 2003, 2781 cases with 111 deaths had been reported from 17 countries on 3 continents. Since the virus resembles coronaviruses known to be part of the intestinal viral flora of many people, and it was detected in the stool of patients, an association with waterborne transmission was considered possible. However, as in the case of avian influenzaviruses, waterborne transmission has not yet been confirmed.

Both influenza- and coronaviruses have a typical envelope, which is a distinct difference from naked enteric viruses typically associated with waterborne transmission. Viral envelopes have a high lipid content, which renders them vulnerable to detergents, oxidising agents such as chlorine commonly used for water disinfection and other unfavourable environmental conditions. Since the viral receptor sites are located on the envelope, any damage to the envelope renders the virus non-infectious. Consequently enveloped viruses are not as resistant as typical enteric viruses to water treatment and disinfection processes.

The potential risk of infection associated with respiratory viruses such as influenza and SARS in water environments cannot be ignored. However, there is sound reason to believe that treatment and disinfection processes recommended for the acceptable control of enteric viruses (WHO, 2004) will also accommodate enveloped viruses with a substantial safety margin.

Virological analysis of water

Virological analysis of water is required for a number of purposes. These include research on the incidence and behaviour of viruses in water environments, assessment of the presence of viruses and the risk of infection, evaluation of the efficiency of treatment and disinfection processes and routine quality monitoring to test the compliance of water quality with guidelines and specifications. These analyses generally consist of the following basic components:

- Recovery of small numbers of viruses from large volumes of water.
- Detection of the recovered viruses.
- Confirmation of the infectivity, or potential health risk, of the viruses detected.

In view of the fundamental nature of viruses, their size and composition, and their mode of replication in specific host cells, each of these components constitutes major challenges. The following is an introductory summary of these challenges, with further details in other chapters of this book.

Recovery of viruses from water

A wide variety of procedures has been described for the recovery of viruses from water. The most commonly applied techniques are based on adsorption–elution methods using negatively or positively charged filters, ultrafiltration or extraction. The efficiency of recovery (EOR) depends on a number of variables, including the volume, turbidity and pH of the water samples under investigation. In some studies, an EOR of 50% was considered optimal under the conditions concerned, while other studies claimed higher levels of efficiency. The effect of the recovery procedure on the viability of viruses is also important. A reliable indication of the EOR of recovery procedures is of fundamental importance for purposes such as monitoring the compliance of water with quality guidelines and assessment of infection risks. Available evidence confirms that currently available methods are in need of improvement with regard to efficiency, cost and meaningful data on EOR for viruses (Grabow et al., 2001; Vivier et al., 2002; Maunula et al., 2004; Chapter 9).

Detection of viruses

Research on viruses in water started in the 1940s. One of the pioneers in the field was Joseph L. Melnick in the USA. He started his work on the detection of polioviruses in the East River where it flowed through New York City. He used vervet monkeys to detect the polioviruses. After ingestion the viruses infect the epithelial cells of the gastrointestinal tract of the monkey. These cells release replicated viruses into the blood stream of the monkey and via this route they reach central nervous cells in the brain and spinal cord. These cells are highly susceptible to infection by polioviruses. Replication of polioviruses in these cells causes a typical CPE, which results in readily detectable paralysis of the monkey. In addition, the damaged cells are clearly visible by microscopic analysis of brain and spinal cord autopsy specimens. Monkeys inoculated with test samples were observed daily over a few weeks for signs of paralysis. Those that displayed paralysis were sacrificed for microscopic analysis of brain and spinal cord specimens.

Melnick expressed the detection of polioviruses in river water by this method in "monkey infectious doses" of polioviruses. Despite this very tedious, time-consuming and labour intensive procedure, in which large numbers of vervet monkeys

had to pay the ultimate toll, Melnick made some fundamentally import observations on the incidence and behaviour of viruses in water (Melnick, 1976).

The next major step forward in the development of techniques for the detection of viruses was the establishment of procedures for the laboratory cultivation of mammalian cells. This implied that cell cultures could be infected with viruses and the CPE caused by virus replication was readily detectable by microscopic analysis of the cells. During the 1950s, the cell culture detection of viruses became established as a routine procedure for the virological analysis of water. Cell cultures retained the role of fundamentally important tools in research on viruses to this day, and will probably carry on playing that role for a long time to come.

Despite attractive features, cell cultures have important shortcomings for the detection of waterborne viruses. This is due to the exceptional host specificity of enteric viruses. A small selection of these viruses, notably polio-, some coxsackie-, some echo-, some entero- and some adenoviruses, as well as reoviruses, readily infect cells in culture and cause a distinctive CPE. Monkey kidney cells are exceptionally susceptible to most of these viruses and are commonly used for their detection. A number of other cell cultures of animal and human origin, each with their own advantages and disadvantages, are also used.

Unfortunately, however, the great majority of the wide spectrum of enteric viruses fails to infect available cell cultures with the production of a detectable CPE. The reasons are not altogether clear, but are probably related to the loss of features required for viral replication under *in vitro* laboratory conditions. This is illustrated by viruses that successfully infect cell cultures, replicate their nucleic acid and produce capsid components, but fail to assemble complete virions and produce a CPE. These viruses are readily detectable by confirming the presence of their nucleic acid using molecular techniques. However, some enteric viruses seem to even fail to infect cell cultures, which may be due to the absence of viral adsorption sites on the cells. This includes the large group of noroviruses. Since noroviruses are so difficult to detect, much of the early information on these viruses was derived from research in which human volunteers were used to detect the viruses by infection and clinical symptoms of disease (Graham et al., 1994).

The next major breakthrough in the development of methods unfolded in the1960s when molecular techniques for the detection of viral nucleic acid were established (Metcalf et al., 1995). These techniques are based on the detection of the nucleic acid of viruses by a diversity of procedures including gene probe hybridisation, the polymerase chain reaction (PCR) and reverse transcriptase-PCR (RT-PCR). Important benefits of these techniques include the ability to detect any virus for which the nucleotide sequence of the nucleic acid is known. In addition, the techniques are highly specific and sensitive. Generally, they yield results in a shorter period of time than the isolation of viruses by cell culture propagation. Unfortunately, they also have shortcomings. Among these is the need for special procedures to distinguish between viable and non-viable viruses. Also, it is difficult to obtain quantitative data on the numbers of viruses detected by molecular techniques. The tests are relatively complicated and require well-trained staff and

appropriate laboratory facilities. False positive results due to contamination of test specimens, and laboratory environments contaminated with amplicons of viral nucleic acid, are major risks. Research on the improvement and modification of molecular techniques is a high priority in many laboratories worldwide. Recent progress includes assessment of viability by RT-PCR detection of m-RNA (Ko et al., 2003), and enumeration of viruses by real-time quantitative RT-PCR (Choi and Jiang, 2005; Fuhrman et al., 2005; Ko et al., 2005; Van Heerden et al., 2005b).

Confirmation of viability and infectivity

Viability of viruses is confirmed when they infect cell cultures and produce a CPE. Viability may also be accepted for viruses, which infect cell cultures and replicate their nucleic acid but fail to complete the viral multiplication cycle to release complete virions and produce a visible CPE (Pintó et al., 1996; Reynolds et al., 1997; Grabow et al., 1999; Van Zyl et al., 2006). At least sometimes these incomplete multiplication cycles even go as far as the production of capsid components. Failure to complete multiplication cycles may be due to a number of factors. Among others, it is known that viral infection of host cells and multiplication in the *in vitro* conditions is less successful than under *in vivo* conditions in the natural host. Another important factor is that the transformed cell cultures generally used for laboratory work have lost a variety of their original features, including functions required by at least some viruses for normal multiplication (Grabow et al., 1992). Apart from the detection of m-RNA mentioned earlier, confirmation of the viability of viruses detected by molecular techniques may be carried out by infecting cell cultures followed by molecular detection of the nucleic acid of viruses, which failed to produce a CPE. This offers a sensitive and highly specific procedure for the qualitative detection of viable viruses (Reynolds et al., 1997; Grabow et al., 1999, 2001; Fuhrman et al., 2005). It has been confirmed that at least some viruses detected in water environments by means of conventional molecular techniques are non-viable (Sobsey et al., 1998; Fuhrman et al., 2005).

The ultimate confirmation of infectivity is by infection of the natural host and the production of clinical disease. In the case of human viruses this is not practical for routine laboratory work, although human volunteers have been used in research on some viruses (Graham et al., 1994; see Grabow, 2002). Today the use of laboratory animals such as monkeys has largely been phased out. Remaining work with live animals is restricted to, for instance, chicken embryos for influenzaviruses.

Viruses confirmed viable by cell culture infection and certain molecular techniques are, therefore, considered as infectious, at least for practical purposes. In this assumption it is taken into account that most enteric viruses infect the natural host more readily than available cell culture systems. Consequently the number of detected viruses confirmed viable by laboratory techniques is almost certainly an underestimate of the true number of viable and infectious viruses present (Grabow et al., 1999).

Although presently available techniques for the detection of viruses still have many shortcomings, they made it possible to obtain valuable information on the incidence and behaviour of viruses in water, and on the risk of infection they constitute. However, in combination the cumulative effect of all the variables in viral detection methods mentioned almost certainly results in a major underestimate of the true number of viruses in water under investigation. This has implications for water quality management, risk assessment, practical safety guidelines and specifications, and routine quality monitoring of water supplies.

Quality guidelines

Infectious diseases are the most important concern about the quality of water intended for human use (Craun et al., 1994). WHO (2004) emphasises that ''The potential health consequences of microbial contamination are such that its control must always be of paramount importance and must never be compromised.'' Over many years a traditional approach to the management of safe drinking water was established and based predominantly on guidelines and specifications for (EPA, 1989; WHO, 1996; EC, 1998; SABS, 2001):

- Raw water quality in terms of bacterial indicators of faecal pollution.
- Efficiency of treatment and disinfection.
- Final quality in terms of the absence of faecal indicator bacteria, notably coliforms.

Pathogenic micro-organisms were rarely or only vaguely referred to in these guidelines and specifications. This is largely because it would be impractical to include recommendations for the wide variety of pathogens that may be transmitted by water. Major considerations included cost, time of analysis, expertise and lack of suitable techniques. This applied in particular to viruses that required complicated and expensive techniques for detection, while many viruses of prime importance remained to be identified and characterised. In addition, not enough information was available on the wide spectrum of viruses involved to define meaningful and practical guidelines for routine quality monitoring. Some recommendations mention an unqualified and undefined ''absence of viruses'', which is basically meaningless because it is practically impossible, and unnecessary, to sterilize water supplies. Some of the recommendations are a little more specific and refer to enteroviruses or enteric viruses. In practice these terms referred to the small group of enteric viruses that cause a cytopathogenic effect in certain cell cultures, because that was the only practical virus detection technology available at the time. Tests for these viruses are basically restricted to an indicator function because very few of them are typically associated with waterborne diseases.

Despite shortcomings, this approach to quality testing has played a valuable role in water quality management and will undoubtedly carry on doing so for a long time to come (see Chapters 11 and 12). The principles concerned also played a

fundamental role in the establishment of a wide spectrum of water treatment and disinfection processes for preparing acceptably safe water supplies (see Chapter 6). Today, it is possible to even directly reclaim safe drinking water from wastewater.

However, as expertise and technology for the detection of pathogens and waterborne diseases were refined, shortcomings in the above approach to the management of drinking water safety were disclosed. For instance, according to Payment et al. (1991) at least in certain situations as much as 35% of household infectious gastroenteritis may be caused by drinking water supplies which meet conventional quality specifications. Cases and outbreaks of waterborne disease associated with conventionally treated drinking water have been reported in many epidemiological studies (Hejkal et al., 1982; Zmirou et al., 1987; Bosch et al., 1991; MacKenzie et al., 1994; Payment et al., 1997; Payment, 2006b). Shortcomings of widely accepted guidelines for the microbiological safety of drinking water are also confirmed by the detection of pathogens, notably viruses, in supplies that have been treated according to specifications and comply with limits for faecal indicator bacteria (Keswick et al., 1984; Payment et al., 1985; Rose et al., 1986; Regli et al., 1991; Moore et al., 1994; Grabow et al., 2001; Vivier et al., 2004). For instance, in routine monitoring of drinking water supplies which complied with all specifications, enteroviruses were detected in 17%, adenoviruses in 4% and hepatitis A virus in 3% of 413 samples analysed over 2 years (Grabow et al., 2001). All viruses detected were confirmed viable by replication of nucleic acid in cell cultures. There is sound reason to believe that these data, as in most other studies, represent an underestimate of the true number of viruses present. The routine monitoring included conventional tests for coliform bacteria and heterotrophic plate counts, as well as tests for somatic and F-RNA coliphages using presence–absence tests on 500 ml samples. By definition the detection of any viruses fail the widely accepted drinking water quality guidelines and specifications referred to earlier.

Further shortcomings of the above approach to drinking water quality management were disclosed when guidelines based on a quantifiable acceptable risk of infection were established (Haas et al., 1999; Chapter 8). In 1989, the US Environmental Protection Agency (EPA) defined one infection per 10 000 consumers per year as an acceptable risk for drinking water (EPA, 1989; Macler, 1993; Macler and Regli, 1993). This definition of an acceptable risk of infection has since been used worldwide at least as a guideline for drinking water quality. Another approach to the definition of an acceptable risk for drinking water has been defined by the WHO (2004). This is based on the burden of disease constituted by a water supply, and a limit of 10–6 DALYs has been suggested. Risk assessment analyses carried out on data for viable viruses in drinking water supplies which complied with all requirements for treatment and disinfection reveal that these supplies exceeded the recommended level of acceptable risk of infection (Regli et al., 1991; Haas et al., 1993; Crabtree et al., 1997; Grabow et al., 2001; Van Heerden et al., 2005b). Available data suggest that the number of drinking water supplies worldwide that comply with this level of an acceptable risk may be rather restricted (Grabow et al., 2001). The same seems to apply to the virological quality of swimming pool water

(Van Heerden et al., 2005a) and other water environments used for recreational purposes (Fuhrman et al., 2005).

The above data and considerations outline shortcomings and controversies in traditional water quality management practices of the past. Shortly, there is sound evidence that many drinking water supplies, which comply with specifications for raw water quality, treatment, disinfection and faecal indicators on the one hand, exceed recommendations for viruses and an acceptable risk of infection at least for viruses on the other hand. This situation is confusing to water supply utilities, water quality authorities and others concerned because now there is evidence that traditional specification for water treatment, disinfection and indicator monitoring fail to produce acceptably safe drinking water. Resolution may be approached by one or both of the following options:

- Tighten the specifications for treatment and disinfection to obtain water that complies with the recommended absence of viruses and an acceptable risk of infection. This will have major financial and practical implications for the water industry (Clark et al., 1993; Regli et al., 1993).
- Retain specifications for treatment and disinfection unchanged by relaxing recommendations for the absence of viruses and an acceptable risk of infection. This may prove unacceptable from a public health point of view (WHO, 2004).

Another approach (WHO, 2004) is summarised in the next section.

Shortcomings of faecal bacteria like *Escherichia coli* as indicators for the potential presence and survival of enteric viruses in water environments were already noted by Melnick in his pioneering work during the 1940s, and are not surprising. Reasons are based on major differences in the composition, structure, size and resistance to unfavourable conditions including water treatment and disinfection processes. Other reasons for the absence of a direct correlation in numbers of viruses and faecal indicators include differences in excretion by the general population and infected individuals in terms of numbers, seasonal incidence, epidemics and geographic distribution (Grabow, 1996; Chapter 5). The same applies to the value of faecal bacteria as indicators for many other pathogens, notably protozoan parasites.

Despite limitations as indicators for the potential presence and behaviour of viruses, faecal bacteria have a long history of valuable indicators of faecal pollution, and of the efficiency of water treatment and disinfection processes (WHO, 2004). A variety of more resistant indicators is widely used to supplement the shortcomings of faecal bacteria for selected purposes. More resistant indicators with valuable features include spore-forming bacteria and bacteriophages. The latter proved particularly useful as indicators for human viruses because they share many fundamentally relevant features (Grabow, 2001). The benefits of indicator organisms are due to be fully utilised in future water quality management strategies (Chapters 6, 7, 11, 12).

An important shortcoming of the above approach to water quality monitoring is endpoint analysis of grab samples. Basically this implies that by the time results are available for tests carried out on samples of the final product, the water is already in the distribution system and drunk by any number of consumers. It is often too late then to take remedial or preventive measures (Payment, 2006b).

Water safety plans

Despite progress in technology and expertise, waterborne diseases keep having far-reaching public health and socio-economic implications worldwide. In addition, new challenges emerge all the time (Ford and Colwell, 1996). These are due to factors such as an escalating world population of humans and domestic animals that increase faecal pollution of water resources while the demand for potable water that has to be derived from these sources increases. Also, the cycle of water reuse is getting shorter which results in a selection for organisms more resistant to treatment and disinfection processes, like viruses and protozoan parasites. This is reflected by the epidemiology of waterborne diseases (Craun, 1991). Another factor of concern is the ongoing appearance of new pathogens, mutants of pathogens and the re-appearance of pathogens due to changing conditions and selective pressures driven by closer contact in escalating populations, rapid and frequent movement of people and animals all over the globe, and changing lifestyle and standards of living (Nel and Markotter, 2006).

The challenges of keeping up with the escalating demand for water of acceptable quality, and the shortcomings of traditional approaches to water quality control outlined in the previous section, prompted research on new strategies for water quality management (Fewtrell and Bartram, 2001; Payment, 2006a). Combined inputs from experts all over the world led to the establishment of a Framework for Safe Drinking Water (WHO, 2004) based on the principles of HACCP (Hazard Assessment and Critical Control Points). Basically the strategy implies that the quality of water is controlled at a selected set of critical control points (CPs) in a multiple barrier drinking water treatment system. Typically, CPs are monitored by testing water quality using physico-chemical and microbiological parameters. However, other parameters may also be used, such as observational monitoring of livestock barriers and the integrity of groundwater sources. Raw water sources may be seen as the first barrier with its own set of control measures for quality protection. Final disinfection at a treatment plant is an important CP, but quality control commences throughout the distribution system. Risk assessment is used to define the quality of the final product, as well as the efficiency of each CP to accomplish the desired final quality. Once a functional system has been established it has benefits such as:

- Routine quality monitoring does not require complicated, expensive and time-consuming analysis of grab samples collected from the final product; routine monitoring is carried out by practical, reliable, cost-effective and rapid or

continuous physico-chemical and microbial indicator analysis of CPs; this includes the elimination of tests for viruses and other pathogens.
- Breakdown and failure at CPs is detected in time to take remedial or preventive measures before the water is released into the distribution system.
- The quality of the final product is based on quantifiable acceptable risks derived from assessment of infection risk and burden of disease data.

Although the principles of the new strategy for water quality management are clearly defined (WHO, 2004, 2006), the application in practice, referred to by terms such as ''water safety plans'', is at least in certain respects still in a developmental stage and subject to ongoing research. This includes a need for more data to reliably and effectively monitor the removal and inactivation of viruses at CPs. Available information on the efficiency of a number of treatment processes with regard to viruses may be considered sufficient to initiate the implementation of water safety plans. However, more details are required to comply with the ultimate objectives (WHO, 2004). For instance, many of the available data are based on research in which viruses were detected by CPE in cell cultures. As has been pointed out earlier, this does not take into account viruses that have been damaged to the extent that they fail to produce a CPE but remain viable and at least potentially infectious. Also, most of the tests were carried out by recovery procedures with restricted and poorly defined efficiency and detected by techniques with questionable reliability in many cases. Most of the data are restricted to readily detectable viruses such as poliovirus, which may indeed serve as reliable indicators for other viruses, but are themselves not associated with waterborne diseases to meaningful extent. Little information is available on viruses of primary importance such as caliciviruses. More accurate data on the behaviour and survival of viruses in treatment processes have to be compared to those of practical indicators in order to establish appropriate routine monitoring procedures for CPs. Definition of ultimate goals for water quality, from which the efficiency of CPs is calculated, requires assessment of risk of infection and burden of diseases at least for representative (model) viruses such as rota, coxsackie B or hepatitis A, on which meaningful data required for these estimates are available.

Another important aspect of the framework for safe drinking water that seems to require attention is the recommendation to design goals on the basis of health-based targets. It is recommended that each country should have its own realistic targets designed by national authorities that take into consideration variables such as relevance to local conditions including economic, environmental, social and cultural factors. Considerations would have to include public health priorities and burden of disease, as well as financial, technical and institutional resources, and possibly also factors such as susceptibility to infection of communities with compromised immune status due to undernourishment or AIDS. Accomplishing these objectives may not prove easy in a world where over more than 100 years practices and perceptions based on specifications for treatment, disinfection and end-point monitoring for coliforms, got cast in concrete. This seems to be confirmed by slow

progress along these lines. The acceptable risk for drinking water of one infection per 10,000 consumers per year was recommended in the USA in 1989. In 2001, this same acceptable risk was accepted as a standard in The Netherlands (Netherlands, 2001) and there are indications that other countries may be moving into the same direction. However, as far as can be established, no country has yet accepted an alternative level of infection risk as an official guideline or standard for drinking water. Many countries, particularly in the developing world, may not find it easy to define their own health-based targets for drinking water based on a risk of infection or burden of disease as recommended. Reasons include lack of expertise and relevant information. It also seems unlikely that countries will readily accept a health-based target that differs from that in countries such as the USA and The Netherlands because it would basically be a political decision with implications for the image of the country, which affects international relations, trade, tourism and many other aspects.

The acceptable risk of infection for drinking water recommended by the US EPA is used as a valuable unofficial guideline worldwide, and as an indication of what such a figure might look like. However, it possibly has the disadvantage of creating false expectations in the mind of many people. The EPA recommendation may be feasible in a developed country such as the USA. However, in many parts of the world, without the financial resources and infrastructure of the USA, this may be unrealistic. In fact, the guideline may not even be particularly realistic in the USA because, as mentioned earlier, many drinking water supplies in the USA seem to fail the recommendation (Regli et al., 1991; Haas et al., 1993; Crabtree et al., 1997).

It should be noted that the guideline specifies "infection" without reference to clinical disease. This is intentional to cover sub-clinical infections, which may result in secondary infection of others in whom it may cause clinical disease (Macler, 1993). In the case of enteric viruses typically associated with waterborne transmission, the great majority of infections are sub-clinical, which are difficult to monitor because there is no reliable relation between infections with or without clinical disease. The relation also varies for different communities and situations, and different viruses. These shortcomings are largely eliminated by health-based targets based on burden of disease. However, on their part these may prove more difficult to apply in practice and to take into consideration the significance of sub-clinical infections.

Challenges regarding the implementation of the WHO framework for safe drinking water may be accomplished by taking the process forward step-by-step. This seems to be the approach followed in Australia where apparently a preventive risk management plan, including performance targets and technology targets indirectly related to health outcomes, has been introduced (Australian Drinking Water Guidelines, 2004). This seems to succeed in changing general thinking from end-point testing to a preventive risk management plan, which is a major step forward (Sinclair and Rizak, 2004). The next step might be to develop the best way of implementing appropriate health-based targets in the management strategy.

According to unconfirmed reports a number of other countries are making progress by first introducing the principles of water safety plans and management of water quality by monitoring critical control points in multiple barrier systems rather than traditional end-point analysis.

Efficiency of treatment and disinfection processes

Viruses constitute special challenges in water treatment and disinfection because of their unique structure, composition and size. Basically, viruses typically associated with waterborne transmission consist of nucleic acid neatly wrapped up in a small protein capsid, which is exceptionally resistant to unfavourable environmental conditions, including those in water treatment and disinfection processes. These particles are specifically designed for transmission by the faecal-oral route via water and food environments. Viruses can only multiply inside metabolically active host cells and not in any water environment like many bacteria. As a result of these features, the behaviour and survival of viruses in water environments differs substantially in many respects from that of much larger organisms like coliform bacteria commonly used as indicators of faecal pollution.

Details on the behaviour and survival of the wide spectrum of viruses in water environments, including water treatment and disinfection processes, are restricted because it is relatively difficult, expensive and time-consuming to detect these viruses and confirm their infectivity. WHO (2004) contains a useful summary of available data on the relative survival of bacteria, viruses and protozoa (cysts and oocysts) in selected water treatment and disinfection processes. The table clearly illustrates the difference in resistance of these groups of organisms to drinking water treatment and disinfection processes. Viruses are substantially more resistant than bacteria to disinfectants like chlorine, monochloramine, chlorine dioxide, ozone and ultravioet light, and are less readily removed by processes such as sand filtration and membrane filtration. The data for viruses in this summary refer predominantly to readily detectable viruses such as vaccine strains of poliovirus detected by CPE in cell cultures. Although polioviruses are recognised as exceptionally resistant and serve as sound indicators for the survival of other viruses, the data do not cover viruses damaged to the extent that they fail to produce a CPE in cell culture, but are still viable and at least potentially infectious, as discussed earlier. For further details see Chapter 6.

Data on the efficiency of treatment and disinfection processes confirm that it is indeed possible to accomplish goals for drinking water treatment such as a 4-log reduction in unqualified numbers of viruses (EPA, 1989) and an acceptable risk of infection or burden of disease as discussed earlier. However, in practice this requires suitable facilities, treatment processes and meticulous management.

Future challenges

Pioneer Louis Pasteur said, "It is characteristic of science and progress that they continually open new fields to our vision". This is true today as much as it was at his time more than 100 years ago. Although in recent times, particularly since the 1940s, major progress has been made in knowledge about viruses in water, many questions remain unanswered. Among these are a better understanding and appreciation of the public health impact of viruses. This includes the potential health implications of latent infections of entero- and other viruses, long-term effects of viruses such as polyoma associated with cancer and the health effects of many viruses that have not yet been disclosed. The challenges are complex and increase in complexity with the emergence of new viruses and mutants of existing viruses, a process driven by factors such as escalating populations of humans and related animals. Examples include avian influenzaviruses and other respiratory agents like SARS, which are associated with water to an extent that remains to be clearly defined. At the same time, major progress is being made with new strategies for water-quality management. However, the optimisation and application in practice of approaches such as water safety plans based on HACCP principles with health-based quality targets require more information on viruses. There is sound reason to believe that new tools for the detection of viruses such as molecular techniques, assessment of the health impact of viruses based on risk assessment and burden of diseases, and refined expertise on epidemiological surveillance and interpretation of results are due to ensure exciting progress in the future (Chapter 13).

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