Waterborne diseases: Update on water quality assessment and control

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Abstract

Water-borne diseases are the most important concern about the quality of water. The pathogens involved include a wide variety of viruses, bacteria and protozoan parasites. Due to differences in size, structure, composition and excretion by humans and animals, their incidence and behaviour in water environments differ. This constitutes difficult challenges for testing the safety of water and the efficiency of treatment processes. Further complications are that many water-borne pathogens, notably the great majority of viruses as well as protozoan cysts and oocysts, are not readily detectable. In addition, the prevalence of various water-borne pathogens changes as selective pressures change. In view of the diverse and variable goalposts, new epidemiological data, and progress in technology and expertise, the methods and strategies for quality monitoring and control of water-borne diseases are continually being revised and updated. This paper reviews the latest approaches to water quality monitoring using indicators of human and animal faecal pollution, and new methods for the detection of viruses. The importance of simple, economic and rapid methods for high frequency basic monitoring of water quality and the efficiency of treatment systems is emphasised. Reference is made to the fundamental need for microbiological quality data in the management of national and regional water resources and supplies.

Introduction

In a keynote address at the prestigious 1993 Stockholm Water Symposium, international authority Hillel Shuval illustrated the impact of water-borne diseases by comparing it to a 747 jumbo jet carrying 400 children and 100 adults crashing with no survivors every half hour around the clock (Editorial, 1993). This illustration is based on authentic estimates that some 50 000 people die each day in the world due to water-borne and water-related diseases (Ince, 1990; Schalekamp, 1990; Catley-Carlson, 1993). Extreme examples include the outbreak of 300 000 cases of hepatitis A and 25 000 cases of viral gastroenteritis in Shanghai caused in 1988 by shellfish harvested from a sewage-polluted estuary (Halliday et al., 1991). In 1991 an outbreak with 79 000 cases of hepatitis E in Kanpur was ascribed to polluted drinking water (Ray et al., 1991; Grabow et al., 1994), and in 1993 some 403 000 cases of cryptosporidium diarrhoea were caused by a conventional drinking water supply in Milwaukee, USA (MacKenzie et al., 1994). Although the mortality of many waterborne diseases is relatively low, the socio-economic impact even of non-fatal infections is phenomenal (Avendano et al., 1993; Payment, 1993). Further details on the public health and socio-economic implications of pathogenic micro-organisms in water, and the extent to which they outweigh the impact of diseases associated with the chemical quality of water, have been reviewed elsewhere (Bern and Glass, 1994; Craun et al., 1994a; b).

Little information is available on water-borne diseases in South Africa. This is probably due to the absence of an infrastructure for the detection and recording of such infections. The lack of information tends to create a false sense of security. There is no reason to believe that risks of water-borne diseases are any different from those in the rest of the world. In terms of escalating demands and pollution of the limited water sources, particularly in rural and developing communities, the risk may even be relatively high. This possibility is supported by data which show correlations of enteric infections in various communities to levels of sanitation, standard of living and education. The data reflect the incidence and public health impact of these diseases in the country (Von Schirnding et al., 1993). Anecdotal data and unpublished findings also point towards water-borne diseases.

The water industry has a long history of research and development aimed at supplying safe water and controlling water-borne diseases. In modern times certain principles for the treatment and disinfection of water have become established. However, evidence is mounting that drinking-water supplies which have been treated by processes generally accepted as sufficient and meeting conventional guidelines for bacterial indicators of faecal pollution, may play a meaningful role in the transmission of pathogens (Hejkal et al., 1982; Zmirou et al., 1987; Gerba and Haas, 1988; Bosch et al., 1991; Payment et al., 1991; Regli et al., 1991; MacKenzie et al., 1994). According to Payment et al. (1991) conventionally treated drinking water may be responsible for as much as 35% of household infectious gastroenteritis. The great majority of infections associated with drinking water which met criteria based on faecal bacteria, were caused by viruses and protozoan parasites (Grabow, 1991; Regli et al., 1991; Moore et al., 1994). These observations disclose shortcomings in quality surveillance programmes often used.

Since world-wide there would not seem to be a meaningful decline in the significance of water-borne diseases, research on fail-safe treatment technology and reliable quality monitoring continues (Grabow, 1986; 1990; Gerba and Haas, 1988; Regli et al., 1991; Bellamy et al., 1993; Sobsey et al., 1993; Craun et al., 1994b). The challenges to accomplish these goals increase in complexity as populations of humans and domestic animals increase with concomitant escalation in demand for potable water and pollution of limited water resources. Special efforts are required to control water-borne diseases in developing communities and countries, which are most vulnerable to these diseases (Feachem, 1980; Feachem et al., 1983; Catley-Carlson, 1993).

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This paper highlights progress in technology and approaches to quality monitoring and assessment of treatment efficiency, and outlines strategies to ensure the safety of water supplies.

Water purification and disinfection technology

A wide variety of water treatment systems and disinfection processes is available (Grabow, 1990; Bellamy et al., 1993; WHO, 1993; 1996; Schutte, 1995). At the low technology and inexpensive end of the range are methods such as the boiling or simple sand filtration of water, the addition of household bleach to a bucket of drinking water, storage of water, and the exposure of water to sunlight. At the sophisticated end of the range are multiple-barrier treatment trains capable of the direct reclamation of drinking water from waste water (Grabow, 1991; Regli et al., 1991).

Principally and theoretically all of these systems are capable of producing safe water supplies. However, without exception they are subject to breakdown and human failure in operation, supervision and quality monitoring (Grabow, 1990; Regli et al., 1991; Bellamy et al., 1993; Sobsey et al., 1993). Despite all efforts, there is no indication that we shall ever have treatment systems or disinfection processes which ultimately are not subject to failure of some kind or another. This implies that reliable quality monitoring of water sources and treated supplies will remain of fundamental importance in the control of water-borne diseases.

Water-borne and water-related pathogens

Water-borne diseases are typically caused by enteric pathogens which belong to the group of organisms basically transmitted by the faecal-oral route. In other words, they are mainly excreted in faeces by infected individuals, and ingested by others in the form of faecally contaminated water or food. Some of the pathogens may be of animal origin. Some may also be transmitted by personal contact, droplet transfer, or inhalation of contaminated aerosols. Water may also play a role in the transmission of pathogens which are not faecally excreted. These include opportunistic pathogens which are members of the normal flora of the external human body. Some of these pathogens are released into water from wounds, lesions or ulcers. Some opportunistic pathogens are natural inhabitants of certain water environments.

Assessment of the public health impact of water-borne diseases is complicated by factors such as:

- Many infections are not readily diagnosed, and detection of the aetiological agents in water is even more difficult. Water-borne transmission and assessment of the public health impact are, therefore, difficult to prove. The same applies to infections which have long incubation periods or manifest in long-term effects (Grabow, 1990; Craun, 1991; Craun et al., 1994a). For instance, viruses are estimated to play a role in about 20% of all cancer cases, and well-known oncogenic viruses such as papilloma, polyoma and hepatitis B are excreted in urine and other body fluids. However, epidemiological association of the diseases with water-borne transmission would be difficult (Grabow, 1990). Proving the water-borne transmission of pathogens is as difficult as proving the reverse, in other words, proving where people with infectious diseases got their infections from. Evaluation of available data and new information leave little doubt that the role of water in the transmission of pathogens and the public health impact of the infections are underestimated (Gerba and Haas, 1988; Payment et al., 1991; Moore et al., 1994; Gerba et al., 1995). These conclusions are supported by recent data on complications and manifestations of infections by coxsackie and other enteroviruses which are not readily associated with water-borne transmission (Clements et al., 1995; Gerba et al., 1995).
- Many water-borne infections, particularly viral infections in children, do not cause clinical disease. However, the pathogens are replicated and excreted by the infected individuals, which constitutes a risk of infection to others (Gerba and Haas, 1988).
- The impact of infectious water-borne diseases is aggravated by infected individuals who transmit the pathogens to other people by routes such as personal contact. Secondary and even tertiary transmission of water-borne diseases has been confirmed epidemiologically (Morens et al., 1979). Obviously, it is difficult to detect this indirect transmission of pathogens by water, and it becomes virtually impossible when the primary infection contracted from water was sub-clinical.
- The prevalence of different water-borne pathogens changes as selective pressures in various communities and parts of the world change (Hughes and La Montagne, 1994). For instance, in the USA the transmission of most bacterial pathogens by water decreased extensively in recent years due to more efficient treatment of water. However, the relative role of viruses, protozoan parasites and some bacteria such as *Escherichia coli* O157:H7 has increased due to factors like higher resistance to treatment processes (Craun, 1991; Moore et al., 1994; Craun et al., 1994a; Gerba et al., 1995). *Cryptosporidium parvum* was not generally accepted as a human pathogen until about 1976; it was associated with water-borne transmission for the first time in 1985; and in 1993 it caused the Milwaukee outbreak referred to earlier, which is the largest water-borne disease outbreak on record (Sobsey et al., 1993; MacKenzie et al., 1994).
- The incidence and prevalence of water-borne pathogens is subject to geographical factors. Most of the pathogens are distributed world-wide, but outbreaks of some, for instance cholera and hepatitis E, tend to be regional (Grabow et al., 1994). Dracunculiasis is restricted to rural areas in India and Pakistan, and Nigeria and certain other sub-Saharan countries (WHO, 1993). The complexity is illustrated by recent findings that the hepatitis E virus is endemic in South Africa, but clinical cases or outbreaks common in some parts of the world have so far been extremely rare in the country (Grabow et al., 1994; 1996).
- Individuals in various communities are not equally susceptible to water-borne infections. Persons with increased risk of infection as well as severity of disease include the very young and the elderly, pregnant women, the immunocompromised (e.g. organ transplant, cancer and AIDS patients), those predisposed to other illnesses (e.g. diabetes), and those with a chemical dependancy (e.g. alcoholism). In the USA these individuals at increased risk of water-borne diseases constitute almost 20% of the total population (Haas et al., 1993). This percentage is growing, and in some other countries, notably the developing world, the percentage may be considerably higher.

One implication of the above is that data on the incidence of water-borne disease, the importance of various pathogens potentially transmissible by water, and the risk of infection, cannot be extrapolated from one part of the world, country or community to another. The wide variety of pathogens which may be transmitted by water has been reviewed (Casemore, 1991; Sobsey et al., 1993; WHO, 1993; 1996; Grabow et al., 1994). The following is a summary of typical representatives and examples:

Enteric pathogens typically transmitted by the faecal-oral route

Bacteria

Salmonella spp., *Shigella* spp., pathogenic *Escherichia coli*, *Campylobacter* spp., *Vibrio cholerae* and *Yersinia enterocolitica*.

Viruses

Rotaviruses, enteric adenoviruses, caliciviruses (including "small round structured viruses" such as Norwalk, Snow Mountain, Hawaii, SA Christmas, SA Congress, etc.), astroviruses, "small round viruses", enteroviruses, coronaviruses, hepatitis A and E viruses, etc.

Protozoa

Entamoeba histolytica, *Giardia intestinalis*, *Cryptosporidium parvum*.

Opportunistic pathogens

Infections of the skin and mucous membranes of the eye, ear, nose and throat:

Pseudomonas aeruginosa, and species of *Mycobacterium*, *Aeromonas*, *Flavobacterium*, *Acinetobacter*, *Klebsiella* and *Serratia*.

Infections contracted by the inhalation of contaminated aerosols:

Legionella spp. (legionellosis), *Naegleria fowleri* (primary amoebic meningo-encephalitis) and *Acanthamoeba* spp. (amoebic meningitis, pulmonary infections).

Larval stages of parasites

Infections contracted by exposure to, or ingestion of, infectious larval stages of human parasites released by specific snails or water fleas:

Schistosoma spp. (schistosomiasis, bilharziasis) and *Dracunculus medinensis* (dracunculiasis).

The latter is not faecally excreted but typically transmitted by water and of major public health importance in restricted geographical areas (WHO, 1993).

Toxins from cyanobacteria

Toxins released by blooms of cyanobacteria (blue-green algae) such as *Microcystis aeruginosa* may adversely affect the health of animals and possibly also humans.

Nuisance organisms

A variety of non-pathogenic micro-organisms, and small plants and animals, may under undesirable conditions thrive in water supplies and cause turbidity, taste and odour, or visible animal life, which are aesthetically objectionable.

Indicator organisms

Commonly used indicators

The detection of many water-borne and water-related pathogens requires expensive and time-consuming techniques, while others are not detectable by conventional methods at all. It would, therefore, hardly be possible to include tests for all, or even a meaningful representation, of these pathogens in routine quality surveillance. Water quality monitoring programmes are, therefore, usually based on tests for indicator organisms. The primary objective of indicators commonly used is to indicate faecal pollution. The following are some of the most important requirements of faecal indicators (Grabow, 1986; 1990; WHO, 1993):

- Present whenever pathogens are present
- Present in the same or higher numbers than pathogens
- Specific for faecal or sewage pollution
- At least as resistant as pathogens to conditions in natural water environments, and water purification and disinfection processes
- Non-pathogenic
- Detectable by simple, rapid and inexpensive methods.

Ideally, various other properties are desirable, such as counts which are directly related to those of pathogens. However, in view of major differences in features such as structure, composition, size, resistance, and excretion by humans and animals, no such correlations exist. These differences also explain shortcomings of faecal bacteria as indicators for pathogens such as viruses and protozoan cysts or oocysts. At best commonly used indicators such as coliform bacteria can, therefore, be expected to indicate relatively recent faecal pollution, which reflects the potential presence or absence of enteric pathogens.

Many micro-organisms have attractive indicator features. However, they all have advantages and disadvantages, and there is no single indicator that universally meets all requirements. The reliability of indicators is evaluated by comparison of their incidence and survival in water environments and treatment processes to that of pathogens, as well as epidemiological studies on the consumers of water supplies, calculations based on the minimal infectious dose of pathogens, and experiments using human volunteers (Grabow, 1990; Payment et al., 1993; Sobsey et al., 1993; Graham et al., 1994).

The following is a summary of key features of commonly used indicators and new indicator concepts:

Total coliform bacteria

The term "total coliform bacteria", sometimes abbreviated to "total coliforms", "coliforms", "colis", etc., refers to a vaguely defined group of Gram-negative bacteria primarily identified by the ability to ferment lactose with the production of acid and gas, or aldehyde, within 24 h at 35 to 37°C. These organisms have a long history in water quality assessment, mainly because of their association with faecal pollution, and relatively easy and rapid detection. Some members of the group are almost conclusively of faecal origin, while others may also multiply in suitable water environments. Total coliforms are primarily used for assessment of the general sanitary quality of finally treated and disinfected drinking water (SABS, 1984; Grabow, 1990; WHO, 1993; 1996; *Standard Methods*, 1995).

The test method generally recommended is membrane filtration using M-Endo agar LES and incubation for 24 h at 35 to 37°C (Grabow and Du Preez, 1979; SABS, 1984; ISO, 1990; *Standard Methods*, 1995). Alternative agar media, pads saturated with liquid media, or most probable number (MPN) tube dilution procedures which yield comparable results, are acceptable for certain purposes. Confirmation (verification, differentiation) of coliform bacteria by various procedures including an oxidase test, production of acid and gas from lactose, and possession of the enzyme ß-D-galactosidase, is often recommended (ISO, 1990; *Standard Methods*, 1995). One reason for confirmation is to exclude cytochrome

oxidase-positive bacteria (mainly *Aeromonas* species) which produce coliform-like colonies on Endo and certain other media. Exclusion of cytochrome-oxidase positive bacteria is preferred primarily because most of these bacteria generally detected are members of the natural flora of water environments and not of faecal origin. Exclusion of these organisms is, however, debatable for the following reasons:

- *Aeromonas* and related bacteria are opportunistic pathogens known to cause gastroenteritis and wound infections, which implies that their presence in drinking water constitutes a potential health risk (Grabow and Du Preez, 1979; Burke et al., 1984; Moyer, 1987).
- *Aeromonas* bacteria are excreted by infected individuals which implies that their presence may indicate faecal pollution.
- Many oxidase-negative coliforms may also multiply in water environments, e.g. *Klebsiella* species (Grabow, 1990; WHO, 1993; *Standard Methods*, 1995).
- Properly treated drinking water is free of *Aeromonas* and related bacteria, which implies that their presence indicates treatment or disinfection failure, or aftergrowth in distribution networks (Grabow and Du Preez, 1979; Seidler et al., 1981; Burke et al., 1984; Grabow, 1990).
- Confirmatory procedures increase the time (additional 2 to 3 d), cost and labour of coliform tests, which implies that the attractive feature of a relatively simple, inexpensive and rapid test is lost.

In view of the above considerations it would seem advisable to use membrane filtration and incubation on M-Endo agar LES for 24 h at 35 to 37°C as a practical test for routine quality monitoring. Any colonies with the typical golden-green metallic sheen should be taken as indication of unacceptable quality and alert for more detailed investigation. This could include picking the colonies from the membrane for further testing to establish potential faecal origin, and immediate testing of another sample from the same source, including additional tests for faecal pollution. The sensitivity of analysis could be increased by filtering larger volumes of water. In the basic coliform test 100 mℓ and even 500 mℓ samples which fail to pass through 0.45 µm pore-size membranes, fail to comply with drinking water quality guidelines for turbidity or suspended matter. Overgrowth which interferes with typical coliform colonies indicates unacceptable quality due to shortcomings in treatment and disinfection, or aftergrowth (Grabow, 1990). This approach to coliform testing is substantiated by research and views of others, including Seidler et al. (1981), Burke et al. (1984), SABS (1984), Moyer (1987) and States and Sykora (1995).

Additional procedures for coliform tests are sometimes recommended, such as tests for "injured" coliforms, pre-incubation at lower temperatures, delayed coliform tests, rapid tests, etc., which have advantages for certain purposes (*Standard Methods*, 1995). Unfortunately, however, all the different media and test procedures tend to confuse the non-expert. Another important disadvantage is that the population of organisms recorded as "coliforms" by the different tests is not necessarily the same. The extent to which counts and populations differ for various water environments is not clear, but it may be meaningful (Grabow and Du Preez, 1979; Grabow, 1990; ISO, 1990; *Standard Methods*, 1995). This variation implies that coliform counts recorded by different methods may not be directly comparable. In addition, there is no meaningful evidence that any of these modifications make a significant contribution to the basic total coliform test recommended above (*Standard Methods*, 1995). In case of doubt about the sensitivity of the basic coliform test for chlorinated drinking-water supplies, the volume of test water could simply be increased to 500 mℓ or even 1 000 mℓ or more which would compensate for the modifications while the test remains simple, economic and rapid (Grabow, 1990).

The recently introduced chromogenic substrate coliform test has attractive features for routine quality monitoring (*Standard Methods*, 1995; States and Sykora, 1995). The test consists of adding test water (100 mℓ or other volume) to a suitably selective growth medium which contains the hydrolysable chromogenic enzyme substrates ortho-nitrophenyl-ß-D-galactopyranoside (ONPG) and 4-methyl-umbelliferyl-ß-D-glucuronide (MUG). During incubation for 24 to 28 h at 35 to 37°C the enzyme ß-D-galactosidase specific for total coliforms hydrolyses ONPG with release of the yellow chromogen orthonitrophenol. At the same time the enzyme ß-glucuronidase specific for *Escherichia coli* hydrolyses MUG, releasing the fluorogen which fluoresces brightly under an ultraviolet light lamp (wavelength 365 nm). The enzymes are highly specific and no confirmation is required. Oxidase-negative bacteria such as *Aeromonas* and *Pseudomonas* fail to produce sufficient quantities of the enzyme ß-D-galactosidase to yield a yellow colour under the specified test conditions. The one test, therefore, simultaneously indicates the presence of total coliforms (yellow colour) and *E. coli* (fluorescence). The test is relatively simple and economic, yields results within 24 to 28 h, and can be carried out as a quantitative MPN tube dilution procedure or a qualitative presence-absence test, both of which have attractive features for routine quality monitoring. Suitable growth media are commercially available under trade names such as "Colilert". Despite attractive features, the method has shortcomings. In at least one study a substantial proportion of *E. coli* isolates (mean 34%, median 15%) from human faecal samples were found to be negative for the ß-D-glucuronidase enzyme (Chang et al., 1989). This implies that results may underestimate counts of *E. coli* and differ from results obtained by other methods.

Faecal coliform bacteria

This term refers to certain members of the group of total coliform bacteria which are more closely related to faecal or sewage pollution, and which generally do not readily multiply in water environments. This group of bacteria is also known as thermotolerant coliforms or presumptive *E. coli*, and outdated terminology includes faeca E. coli, faecal coli, etc. Faecal coliforms are primarily used for the assessment of faecal pollution in waste water and raw-water sources. They are detectable by simple and inexpensive tests and are widely used in routine water quality monitoring (Grabow et al., 1981; SABS, 1984; Grabow, 1990; ISO, 1990; 1994a; *Standard Methods*, 1995). The generally recommended test method is membrane filtration with M-FC agar (or alternative media which yield equivalent results) and incubation for 24 h at 44.0 ± 0.5°C. This incubation temperature is usually referred to as 44.5°C. The membrane filtration procedure has the advantage that individual colonies can be identified, and the presence of *E. coli* is a most conclusive evidence of faecal pollution.

Escherichia coli

This species is a member of the group of faecal coliform bacteria. Outdated terminology includes "*E. coli* type I" and "confirmed *E. coli*". *Escherichia coli* has the important feature of being highly specific for the faeces of humans and warm-blooded animals. For

all practical purposes these bacteria fail to multiply in any natural water environment, and they are, therefore, used as specific indicators for faecal pollution (SABS, 1984; Grabow, 1990; ISO, 1990; 1995a; WHO, 1993; 1996; *Standard Methods*, 1995). *Escherichia coli* is traditionally detected by carrying out a test for faecal coliforms, followed by testing isolates for the ability to produce indole from tryptophan within 24 h at 44.5°C. The latest trend is towards methods in which *E. coli* is identified directly by detection of the enzyme ß-glucuronidase, similar to the chromogenic substrate test described earlier for total coliforms. A neat system in which dilutions of test water (200 µℓ) are inoculated into wells of 96-well microtitre plates containing dehydrated growth medium and 4-methyl-umbelliferyl-ß-D-glucuronide (MUG) is under consideration (ISO, 1994a). The plates are incubated for 36 h at 44.5°C, positive wells identified in a UV observation chamber (ultraviolet light at 366 nm) and calculation of the MPN by computer program. Since 8 wells are used per dilution, the MPN value is relatively accurate.

Enterococci

Enterococci, also referred to as faecal streptococci, are related groups of bacteria which are more closely associated with faecal pollution than total coliform bacteria because members typically present in faeces of humans and animals do not readily multiply in water environments. Recently the term "faecal enterococci" (previously "intestinal enterococci") has been proposed for a group consisting exclusively of *Enterococcus faecalis*, *E. faecium*, *E. durans* and *E. hirae*, which are highly specific for human and animal faecal pollution (ISO, 1994b). These spherical Gram-positive bacteria tend to be more resistant than faecal coliforms which are Gram-negative. Enterococci are detectable by practical techniques, such as membrane filtration using selective media like M-Enterococcus agar and incubation for 48 h at 35 to 37°C (Grabow, 1990; *Standard Methods*, 1995). An attractive new method for faecal (intestinal) enterococci, similar to the chromogenic substrate MPN procedure using 96-well microtitre plates described earlier for *E. coli*, is under consideration (ISO, 1994b). Detection is based on the ability of the organisms to hydrolyse 4-methyl-umbelliferyl-ß-D-glucoside (MUD) with release of the fluorogen in the presence of thallium acetate and nalidixic acid within 36 h at 44.5°C.

Heterotrophic plate count

This test, which was previously known as the total or standard plate count, detects a wide variety of organisms, primarily bacteria, including organisms of faecal origin, as well as natural inhabitants of water environments. It is primarily used to assess the general microbiological quality of finally treated and disinfected drinking water supplies. The test is simple and inexpensive, yields results in a relatively short time, and has proved one of the most reliable and sensitive indicators of treatment or disinfection failure (Grabow et al., 1980; Grabow, 1986; 1990; WHO, 1993; 1995; *Standard Methods*, 1995). One reason is that highly resistant bacterial spores are also detected. The generally recommended test method is pour plates using a rich growth medium such as Yeast Extract Agar and incubation for 48 h at 35 to 37°C.

Clostridia

An important advantage of these Gram-positive anaerobic bacteria is that their spores are more resistant to conditions in water environments, as well as treatment and disinfection processes, than most pathogens, including viruses (Grabow, 1990; Payment and Franco, 1993; WHO, 1993; 1996). Clostridia are sometimes considered as too resistant, and their inclusion in water quality guidelines as too stringent. One of the members of the group, *Clostridium perfringens* is, like *E. coli*, highly specific for faecal pollution. According to Payment and Franco (1993) *C. perfringens* is the most reliable indicator for viruses and protozoan cysts or oocysts in treated drinking water. Clostridia generally occur in lower numbers in waste water than coliform bacteria, and detection methods are relatively expensive and time-consuming (Grabow, 1990; Payment and Franco, 1993; WHO, 1993; 1996).

Acid-fast bacteria

This term refers to extremely resistant members of the group of mycobacteria, including *Mycobacterium fortuitum* and *M. phlei*. The bacteria proved most useful for assessment of the efficiency of treatment trains for the direct reclamation of drinking water from waste water (Grabow, 1990).

Other indicators

A variety of other indicators has been used in water quality assessment, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Legionella* species, *Candida albicans*, and endotoxins. All of these have advantages for certain purposes (Grabow, 1986; 1990; *Standard Methods*, 1995).

Distinction between faecal pollution of human and animal origin

Batteries of indicator organisms have been selected for the distinction between faecal pollution of human and animal origin. The distinction is based on *Rhodococcus coprophilus* which is specific for animal faecal pollution, sorbitol-fermenting bifidobacteria and phages of *Bacteroides fragilis* HSP40 which are specific for human faecal pollution, and the ratio of faecal coliforms to enterococci which may also give an indication of the origin of faecal pollution (Jagals et al., 1995). Identification of the origin of faecal pollution may be important for various purposes. For instance, the risk of human infection is generally higher with human than with animal faecal pollution (Jagals et al., 1995).

Protozoan parasites

The cysts and oocysts of intestinal parasites such as *Giardia* and *Cryptosporidium* species are exceptionally resistant (Casemore, 1991; Jakubowski et al., 1991; Bellamy et al., 1993). They generally occur in low numbers in water environments, and are not readily detectable. However, their minimal infectious dose may be as low as a single cyst or oocyst. Since it is difficult to detect the low numbers in water which may constitute a health risk, quality control is often based on specifications for raw water quality and the efficiency of treatment processes rather than testing of the treated water (Regli et al., 1991; Bellamy et al., 1993).

Human viruses

Many viruses may potentially be transmitted by water. Most of them are exceptionally resistant to treatment processes and their minimal infectious dose may be as low as a single particle. It is, therefore, not surprising that they cause the majority of water-

borne infections (Haas et al., 1993). Their relative importance tends to increase due to water treatment and disinfection practices which remove or inactivate more sensitive pathogens and bacterial indicators of faecal pollution but not viruses (Craun, 1991; Regli et al., 1991; Sobsey et al., 1993). Another important feature of viruses is that their incidence in water environments may differ substantially from that of indicators such as coliform bacteria for reasons such as:

- Viruses are excreted only by infected individuals and coliform bacteria by virtually all humans and warm-blooded animals. Numbers of viruses in water environments are, therefore, generally lower than those of indicators such as faecal coliforms by several orders of magnitude.
- Viruses are excreted for relatively short periods in numbers of up to 10^{12}/g of faeces, while coliform bacteria are excreted fairly consistently in numbers of about 10^{8}/g of faeces.
- The structure, composition, morphology and size of viruses differ fundamentally from those of bacteria, which implies that behaviour and survival in water differ extensively.

Due to the above differences bacteria such as coliforms have shortcomings when being used as indicators for viruses. Ideally water quality surveillance should, therefore, include tests for viruses. Unfortunately, however, tests for viruses are relatively expensive, complicated and time-consuming, and require sophisticated facilities and expertise. In addition, the great majority of viruses concerned are not detectable by conventional virological cell culture techniques (Taylor et al., 1993; Grabow et al., 1994; Wolfaardt et al., 1995). Control of the virological safety of water is, therefore, as in the case of intestinal parasites, often based on raw water quality and specifications for purification and disinfection processes rather than testing of the treated water (Regli et al., 1991; Sobsey et al., 1993).

New developments in quality surveillance

Bacteriophages

Bacteriophages (phages) are viruses which infect bacteria. In terms of size, structure, morphology and composition they closely resemble human viruses. The behaviour of phages in water and related environments, and their resistance to unfavourable conditions, treatment systems and disinfection processes do, therefore, more closely resemble those of human viruses than bacterial indicators of faecal pollution (Grabow et al., 1984; 1993; Havelaar et al., 1993; ISO, 1995b; 1995c).

Phages only replicate in specific host bacteria, which implies that the phages of *E. coli* (coliphages) are, like their hosts, related to faecal pollution. Phages commonly used in water quality assessment include the groups of phages known as somatic and male-specific coliphages, which each have their own indicator advantages and disadvantages (Grabow et al., 1980; 1984; 1993; Havelaar et al., 1993). Phages which infect *Bacteroides fragilis* strain HSP40 are highly specific for human faeces, and can be used to distinguish between faecal pollution of human and animal origin (Tartera and Jofre, 1987; Grabow et al., 1995).

Virological analysis of water

Challenges in the virological monitoring of water quality include the recovery of small numbers of viruses from large volumes of water, the detection of a wide variety of viruses, and reduction of the cost and time of testing. The following progress has recently been accomplished:

Recovery of viruses

A filter system for the practical and economic on-site and in-line recovery of viruses from water supplies has been developed and evaluated (Grabow and Taylor, 1993). The perspex filters contain oiled sodocalcic glass wool with electrostatic and hydrophobic properties suitable for the efficient adsorption of viruses at pH levels of up to 9.0 (Vilagines et al., 1993). Dechlorination granules for the neutralisation of chlorine residuals in the water under investigation are placed over the glass wool. The filters can easily be attached to water supplies by means of conventional quick-fit domestic garden hose fittings Any desirable volume of water can on site be passed through the filters. The filters (dimensions 26 cm x 3 cm, mass 100 g) are then transported in cooler bags to the laboratory where viruses are eluted. Efficiency of recovery is at least 50%, which is better than that of much more expensive adsorption-elution systems generally used.

Detection of viruses

Viruses in water are generally detected by propagation in cell culture systems such as the BGM monkey kidney cell line (Regli et al., 1991). According to the latest evidence the detection of these cytopathogenic viruses can be increased considerably by using the BGM cell line, primary vervet kidney cells and the PLC/PRF/5 human liver cell line in parallel with three blind sub-passages (Grabow et al., 1996). However, many water-borne viruses are not cytopathogenic in presently available cell culture systems and are, therefore, not detectable by conventional cell culture propagation. Progress is now being made in the development of techniques for detecting many non-cytopathogenic viruses. These techniques are mainly based on molecular methods for the highly specific detection of viral nucleic acid. One of these techniques is the polymerase chain reaction (PCR), which can be used for the direct detection of viruses (Marx et al., 1995; Wolfaardt et al., 1995), or detection of some viruses after cell culture amplification (Grabow et al., unpublished). The latter approach increases the sensitivity of detection, and overcomes the disadvantage of molecular techniques of not being able to distinguish between viable and non-viable viruses.

The above developments imply that relatively practical, inexpensive and sensitive procedures are now available for the recovery of viruses from water, and for their detection by cell culture isolation and molecular techniques. In the past the running cost of virus tests by cell culture isolation exceeded that of coliform tests by the order of 10 to 100 (Grabow, 1986). This has now been reduced to the order of 5 to 10. In addition, virological water analysis has become much more sensitive and user friendly (Grabow and Taylor, 1993).

Water quality surveillance

Monitoring the safety of water supplies

Despite modern technology for efficient treatment and disinfection, the transmission of diseases by water does not only continue, but in at least some situations seems to increase or become more complicated due to selection for resistant or "new" pathogens (Grabow, 1989; Casemore, 1991; Craun, 1991; Regli et al., 1991; Hughes and La Montagne, 1994). Breakdown in treatment plants,

and human error in operation and supervision, generally take place without warning; in fact, like a thief in the night they tend to strike when least expected. Routine quality monitoring should, therefore, be carried out at the highest possible frequency in order to detect problems at the earliest possible stage. Since monitoring programmes are subject to many variables, including economic considerations, it is not possible to formulate universal sampling frequencies, and each case has to be considered on its own merit. Generally speaking it is better to run simple and inexpensive tests at high frequency, than long, complicated and expensive tests at low frequency (Haas, 1993; WHO, 1993; 1996).

No single indicator can fulfil all the needs of water quality monitoring. Each indicator has its own advantages and disadvantages. Best results are, therefore, obtained by using combinations of indicators for different purposes (Grabow, 1990). For instance, indicators selected for routine monitoring of treated drinking water supplies may include a basic 24-h test for total coliform bacteria, supplemented by tests for faecal coliforms or E. coli, and possibly somatic coliphages and the heterotrophic plate count (Grabow, 1990). These tests, supported by regular sanitary surveys and inspection of treatment procedures, would detect errors and potential risks in reasonable time, and make a major contribution to the control of water-borne diseases (Grabow, 1990; WHO, 1993; 1996). Practical methods for routine quality monitoring are particularly important for laboratories with limited financial resources, facilities and expertise. This typically includes laboratories of small communities and developing countries, which are particularly vulnerable to water-borne diseases (Feachem, 1980; Feachem et al., 1983; Catley-Carlson, 1993; WHO, 1993).

Increasing evidence of the risks of water-borne diseases and their public health impact, as well as shortcomings of commonly used indicators, justify supplementary tests on the quality of raw and treated waters, and the efficiency of treatment systems, wherever possible. It would seem logical for the water industry and health authorities to calculate expenditure on microbiological water quality monitoring as a percentage of the total budget for water supply, duly taking into account the potential price of disease and consumer perception of efforts to ensure the safety of water supplies.

Microbiological data for management of water resources and supplies

As a result of increasing demands and concomitant pollution of the environment, the regional and national management of water resources and supplies has become essential. This applies in particular to a country such as South Africa with limited and variable natural water resources, and an escalating population coupled with rapid growth in industry and agriculture. Details on microbiological quality constitute an integral part of data required for management systems. This information gives an indication of the suitability of water sources and supplies for various purposes, as well as treatment required to render water suitable for human and animal consumption. The utilisation of a conceptual framework on water quality is strongly recommended because traditional practice based on observing contamination and water-borne outbreaks of disease, and applying engineering technology for control, is clearly inadequate because the transmission of pathogens by water does not only continue, but would seem to increase in many areas (Sobsey et al., 1993).

Details on the quality of water sources and the treatment required have financial implications for the supply of safe water. These details are also essential with regard to pathogens such as viruses and protozoa which are difficult to detect in treated water, and quality assessment largely depends on information on the raw water quality and the efficiency of the treatment processes applied (Regli et al., 1991; Bellamy et al., 1993). The identification of pollution sources is required for the protection of water resources, and for the necessary pollution control. Details on the quality of water sources and supplies have major benefits for research on the epidemiology of water-borne diseases and infection risks associated with the utilisation of water for various purposes, including drinking, recreation and irrigation. The information is also essential for research on the survival of pathogens and indicators in water environments, the efficiency of treatment processes, and the development of reliable methods for water quality monitoring (Regli et al., 1991; Bellamy et al., 1993; Sobsey et al., 1993; Moore et al., 1994).

Technical details on the collection and processing of data for management systems are not discussed here. However, in terms of methods for microbiological water quality monitoring discussed earlier, it is important to note that the need for data based on practical, economic and comparable analyses has been emphasised (Sobsey et al., 1993; States and Sykora, 1995).

Management systems based on a conceptual framework of microbiological water quality and modelling of health risks are, therefore, essential for the optimum utilisation of available water resources, the protection of these resources, the economic supply of safe water, and the control of water-borne diseases. These considerations have far-reaching implications. For instance, access to safe water is considered a human right (ANC, 1994). The need for such management systems is widely recognised, and details on approaches and applications have been described elsewhere (Lloyd and Bartram, 1991; Regli et al., 1991; Leahy et al., 1993; Sobsey et al., 1993; Craun et al., 1994b; Moore et al., 1994; States and Sykora, 1995).

Water quality guidelines and standards

Water quality guidelines and standards recommended by various authorities and countries differ. This is primarily due to perceptions of acceptable risks in terms of economic considerations and technical capabilities, and the quality and quantity of available raw water sources (Feachem, 1980; Shuval et al., 1981; Feachem et al., 1983; Regli et al., 1991; Sobsey et al., 1993; WHO, 1993; 1996).

Authorities are cautious in defining acceptable risks of infection, but the following proposals serve as valuable guidelines:

- Maximum acceptable risk for drinking-water supplies recommended in the USA (Regli et al., 1991):
  One illness per 10 000 consumers per year.
- Maximum acceptable risk for the recreational use of environmental waters (not swimming pools) recommended in the USA (Environmental Protection Agency, 1986) and Canada (Canadian Guidelines, 1987):
  One illness per 1 000 swimmers.

Based on considerations such as the above, guidelines and standards for water have been formulated by many authorities, and are continually being revised and updated as new information and technology become available (SABS, 1984; Regli et al., 1991; Water Affairs Guidelines, 1993; WHO, 1993; 1996; *Standard Methods*, 1995). Although these guidelines and standards differ in technical details, they generally share certain basic requirements. For instance, they all specify that drinking water should rarely if ever contain total coliform bacteria, and never faecal coliforms or *E. coli*. In addition, guidelines and standards generally specify or

assume that drinking water should be free of pathogens such as viruses and protozoa (European Communities, 1980; Helmer et al., 1991; WHO, 1993; 1996; *Standard Methods*, 1995). However, limits are rarely specified for viruses and protozoa because testing remains complicated and expensive, and beyond the capabilities of most laboratories involved in conventional routine water quality monitoring. Efforts to increase the reliability of monitoring for viruses and protozoa, include the application of additional indicators such as phages (European Communities, 1980; Grabow et al., 1993; *Standard Methods*, 1995) and clostridia (European Communities, 1980; Payment and Franco, 1993), as well as specifications for raw water quality and the efficiency of treatment processes (Regli et al., 1991). However, as progress is being made in the development of more practical techniques, additional analyses are recommended. For instance, tests for viruses have been included in specifications for recreational waters (European Communities, 1975), and examination of drinking water supplies for pathogens including viruses and protozoan parasites has been recommended (European Communities, 1980; Smeets and Amavis, 1981; Regli et al., 1991).

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