

# Microbiological Safety of Drinking Water: United States and Global Perspectives

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Waterborne disease statistics only begin to estimate the global burden of infectious diseases from contaminated drinking water. Diarrheal disease is dramatically underreported and etiologies seldom diagnosed. This review examines available data on waterborne disease incidence both in the United States and globally together with its limitations. The waterborne route of transmission is examined for bacterial, protozoal, and viral pathogens that either are frequently associated with drinking water (e.g., *Shigella* spp.), or for which there is strong evidence implicating the waterborne route of transmission (e.g., *Leptospira* spp.). In addition, crucial areas of research are discussed, including risks from selection of treatment-resistant pathogens, importance of environmental reservoirs, and new methodologies for pathogen-specific monitoring. To accurately assess risks from waterborne disease, it is necessary to understand pathogen distribution and survival strategies within water distribution systems and to apply methodologies that can detect not only the presence, but also the viability and infectivity of the pathogen. — *Environ Health Perspect* 107(Suppl 1):191–206 (1999). <http://ehpnet1.niehs.nih.gov/docs/1999/Suppl-1/191-206ford/abstract.html>

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In many countries, microbiologically safe drinking water is considered a fundamental human right, but the definition of microbiologically safe is unclear. What appears to be harmless to the healthy individual may potentially be fatal to immunocompromised and elderly populations.

The dramatic decline in incidence of waterborne disease in the early 1900s after introduction of water treatment and disinfection has been documented in detail by numerous authors (1,2). However, there is reason to be concerned for the future microbiological safety of drinking water, in both developing and developed countries (3). This is because *a*) source waters continue to receive agricultural, industrial, and municipal wastes; *b*) water treatment and distribution systems age and deteriorate; *c*) water supplies are overwhelmed by excessive demand; and *d*) there appears to be an increase in diseases, or at least an increased recognition of disease, caused by pathogens with varying degrees of resistance to treatment and disinfection (3,4).

Critical issues facing developed countries relate to undervaluation of water and the

public misconception in many areas of a limitless resource. Technology is generally adequate to provide multiple barriers to drinking water contamination through protection of water sources, appropriate treatment and disinfection, and programs to upgrade deteriorating distribution networks. However, resources generally are not sufficient to adequately achieve this multibarrier approach. Protection of water sources often infringes on rights of upstream communities and levies high economic costs for provision of adequate wastewater treatment. Many U.S. cities are faced with deteriorating distribution systems with inadequate programs for replacement or upgrade. In addition, toxicity of disinfection byproducts (DBPs) is a potentially important issue that is affecting water management decisions on appropriate levels of disinfection to protect the microbiological quality of water (5).

In developing countries, issues discussed for the United States apply but in a more extreme sense. Many diseases, such as cholera, shigellosis, typhoid, and hepatitis A, are endemic and may regularly reach

epidemic proportions. Population susceptibilities can be extremely high through exposure to many other infectious diseases, chemical pollutants in both air and water, and from malnutrition (3,6). Water scarcity becomes a major issue (7). This is often through inadequate and highly contaminated sources, but also can occur through limited capacity of treatment plants built for populations considerably smaller than they currently supply. This can often mean supply is limited to 1 to 2 hr/day, resulting in stagnant conditions in already deteriorated pipelines (8). Problems in developing countries can often be linked to either limited or a complete absence of wastewater treatment.

## Definition of Waterborne Disease

It is appropriate to discuss briefly how we define a waterborne disease. In principle, almost all enteric pathogens and opportunistic pathogens that are transmissible by the fecal–oral route can be transmitted through water. However, the rate of inactivation in the water environment and infectious dose are the critical characteristics of an organism that defines the risk of a waterborne outbreak of disease. *Vibrio cholerae*, *Shigella* spp., *Campylobacter jejuni*, *Giardia lamblia*, and *Cryptosporidium parvum* can clearly be considered waterborne diseases (other routes of infection are food, soil, person to person, etc.); however, they are all enteric pathogens that may survive but cannot proliferate in treated drinking water (4,9). There are also environmental pathogens that can both survive and proliferate in drinking water, including *Legionella pneumophila*, the *Mycobacteria*, and a large number of other opportunistic pathogens. As the potential number of pathogens and opportunistic pathogens is vast, this review will focus on infectious agents that either have been directly linked to the drinking water supply or for which there is strong evidence that transmission can occur by the waterborne route. A summary of important waterborne pathogens, their infectious doses, and estimated incidence where available is presented in Table 1. However, it is important to note that Table 1 can only reflect characterized pathogens, potentially underestimating total numbers by orders of magnitude.

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Abbreviations used: AGI, acute gastrointestinal disease (of unknown etiology); AOC, assimilable organic carbon compounds; CDC, Centers for Disease Control and Prevention; DBPs, disinfection byproducts; U.S. EPA, U.S. Environmental Protection Agency; GI, gastrointestinal; HAV, hepatitis A virus; HEV, hepatitis E virus; IC, intracellular survival and/or growth; MAC, *Mycobacterium avium* complex; MMWR, *Morbidity and Mortality Weekly Reports*; ORT, oral rehydration therapy; PCR, polymerase chain reaction; SRSV, small round-structured virus; VNC, viable but nonculturable; WBDOs, waterborne disease outbreaks; WHO, World Health Organization.

**Table 1.** Pathogens in drinking water: infectious dose, estimated incidence through consumption of drinking water in the United States, survival in drinking water, and potential survival strategies.<sup>a</sup>

	Infectious dose <sup>b</sup>	Estimated incidence <sup>c</sup>	Survival in drinking water, days	Survival strategies <sup>d</sup>
<b>Bacteria</b>				
<i>Vibrio cholera</i>	10 <sup>8</sup>	(very few) <sup>d</sup>	30	VNC, IC
<i>Salmonella</i> spp.	10 <sup>6-7</sup>	59,000	60-90	VNC, IC
<i>Shigella</i> spp.	10 <sup>2</sup>	35,000	30	VNC, IC
Toxigenic <i>Escherichia coli</i>	10 <sup>2-9</sup>	150,000	90	VNC, IC
<i>Campylobacter</i> spp.	10 <sup>6</sup>	320,000	7	VNC, IC
<i>Leptospira</i> spp.	3	?	?	?
<i>Francisella tularensis</i>	10	?	?	?
<i>Yersinia enterocolitica</i>	10 <sup>9</sup>	?	90	?
<i>Aeromonas</i> spp.	10 <sup>8</sup>	?	90	?
<i>Helicobacter pylori</i>	?	High	?	?
<i>Legionella pneumophila</i>	>10	13,000 <sup>e</sup>	Long	VNC, IC
<i>Mycobacterium avium</i>	?	?	Long	IC
<b>Protozoa</b>				
<i>Giardia lamblia</i>	1-10	260,000	25	Cyst
<i>Cryptosporidium parvum</i>	1-30	420,000	?	Oocyst
<i>Naegleria fowleri</i>	?	?	?	Cyst
<i>Acanthamoeba</i> spp.	?	?	?	Cyst
<i>Entamoeba histolytica</i>	10-100	?	25	Cyst
<i>Cyclospora cayentanensis</i>	?	?	?	Oocyst
<i>Isospora belli</i>	?	?	?	Oocyst
The microsporidia	?	?	?	Spore, IC <sup>f</sup>
<i>Ballantidium coli</i>	25-100	?	20	Cyst
<i>Toxoplasma gondii</i>	?	?	?	Oocyst
<b>Viruses<sup>g</sup></b>				
Total estimates	1-10	6,500,000	5-27 <sup>h</sup>	Adsorption/absorption

Abbreviations: ?, unknown; IC, intracellular survival and/or growth; VNC, viable but not culturable. <sup>a</sup>Except where noted, data are compiled from Morris and Levin (2), WHO (10), Hazen and Toranzo (11), and Geldreich (12). <sup>b</sup>Infectious dose is number of infectious agents that produce symptoms in 50% of tested volunteers. Volunteers are not usually susceptible individuals, and therefore these numbers are not useful for risk estimates. <sup>c</sup>U.S. point estimates. <sup>d</sup>Very few outbreaks of cholera occur in the United States, and these are usually attributable to imported foods (14). <sup>e</sup>Data from Breiman and Butler (14). <sup>f</sup>Possible IC with microsporidialike organisms (15). <sup>g</sup>Includes Norwalk virus, poliovirus, coxsackievirus, echovirus, reovirus, adenovirus, HAV, HEV, rotavirus, SRSV, astrovirus, coronavirus, calicivirus, and unknown viruses. <sup>h</sup>Estimated for HAV, Norwalk virus, and rotavirus (13).

## Organization

This review attempts to describe current knowledge about waterborne disease and the relative safety of drinking water in both developed and developing countries. It necessarily reflects a rapidly changing field. Continued development of molecular techniques has resulted in an increasing ability to detect pathogens in drinking water, and it is likely that many new pathogens will be identified in the future. These techniques will find wide-ranging applications for pathogen monitoring in source and finished waters in both developed and developing countries. They also have the potential to redefine our ability to assess health risks from drinking water from indicator-based risk (coliforms, etc.) to pathogen-specific monitoring (3).

The review addresses the following topics: *a*) the burden of waterborne disease, both globally and in the United States, with discussion of the dramatic underreporting

of disease; *b*) etiology of waterborne disease, e.g., direct contamination of water by enteric pathogens and growth of opportunistic pathogens within distribution system biofilms; *c*) characteristics of waterborne pathogens; *d*) water treatment and pathogen survival strategies; *e*) advances and limitations in methodology; and *f*) critical needs for future research.

## The Burden of Waterborne Disease

### Global Estimates

Mortality and morbidity from waterborne disease can be very high. The World Health Organization's (WHO) World Health Report for 1996 (16) estimates total mortality from diarrheal disease at over 3 million cases for 1995, with more than 80% among children under age 5. Total morbidity was estimated at over 4 billion; however, the authors suggest that

up to 70% of diarrheal episodes may be caused by contaminated food, suggesting that roughly 1.2 billion episodes of infectious disease occur annually from contaminated water. Foodborne outbreaks of infectious disease can of course originate through food preparation with contaminated water. Low levels of pathogens in drinking water may rapidly multiply to infectious doses when associated with food. In addition, a susceptible host can become infected from drinking water and subsequently spread disease to others through person to person contact (10). Provision of microbiologically safe drinking water therefore has dramatic impacts not only on incidence of waterborne disease but also on secondary transmission pathways.

In 1995 diarrheal disease ranked first in the WHO report's assessment of causes of morbidity and fourth in causes of mortality. The 1998 World Health report (17) provides similar estimates for morbidity from diarrheal disease in 1997 (4 billion) but reduced estimates for mortality (2.5 million), which is consistent with the 1997 report (18). Although remaining first in WHO's morbidity ranking, it dropped to the sixth cause of mortality (Table 2), overtaken by both tuberculosis and chronic obstructive pulmonary disease. Amoebiasis and giardiasis show no changes in either morbidity or mortality estimates between 1993 and 1997 (Table 2). However, cholera morbidity decreased from 380,000 in 1993 to 145,000 in 1997 (officially reported cases), presumably reflecting a decline in the South American epidemic. The major success in reduction of waterborne disease is in the number of cases of dracunculiasis, from 3.2 million in 1986 to 70,000 in 1997 (17); this is currently one of WHO's most successful elimination programs.

Lower mortality figures may reflect changes in reporting or improved diagnosis of diarrheal diseases rather than an actual decline. However, they may also reflect an improvement in intervention measures over 1995 and preceding years (19). The WHO 1996 report discusses agreements with a number of agencies and organizations to provide technical assistance in epidemic diarrhea control and preparedness. For example, this included support for a number of African countries in areas of policy formulation, developing surveillance systems, and strengthening laboratory services. In addition, countries faced with outbreaks of cholera or dysentery received technical assistance and emergency

**Table 2.** Global rates of morbidity and mortality for selected infectious diseases with significant transmission through contaminated water<sup>a</sup>.

Disease	1993		1995		1996		1997	
	Morbidity, × 1,000	Mortality, × 1,000	Morbidity, × 1,000	Mortality, × 1,000	Morbidity, × 1,000	Mortality, × 1,000	Morbidity, × 1,000	Mortality, × 1,000
Diarrhea	1,821,000 (1)	3,010 (4)	4,002,000	3,115	4,002,000 (1)	2,473 (6)	4,000,000 (1)	2,455 (6)
Amoebiasis	48,000	70	—	—	48,000	70	48,000 (11)	70
Typhoid	—	—	16,000	> 600	—	—	—	—
Cholera								
Officially reported	380	6.8	384	11	120	6	145	10
Estimated <sup>b</sup>	500	—	6,000	120	—	—	—	—
Giardiasis	500	—	—	—	50	—	500	—
			200,000 <sup>c</sup>	—	—	—	—	—
Dracunculiasis	2,000	—	122	—	130	—	70	—

<sup>a</sup>Except where noted, data are compiled from WHO World Health Reports (16–19). Numbers in parentheses refer to WHO's ranking for selected causes of morbidity and mortality. <sup>b</sup>Officially reported figures for cholera are in dramatic contrast to these estimated numbers of 6 million cases per year, and 120,000 deaths reported in Sack (20).

<sup>c</sup>Numbers of individuals with symptoms of giardiasis in Asia, Africa, and Latin America (16).

supplies. Of course, political conditions frequently prevented these supplies from reaching the populations at risk.

Oral rehydration therapy (ORT) has been extremely successful in reducing mortality from diseases such as cholera (21). A study in Mexico examined the impact of selected public health interventions on the reduction of mortality from diarrheal diseases in children under 5 years of age (22). The authors confirmed the importance of ORT, together with literacy campaigns for women, in reducing diarrheal disease. However, improvements in sanitation and a massive immunization program against measles resulted in greater reduction. Presumably, the immunization program had the indirect consequence of reducing children's susceptibility to diarrheal disease.

### Waterborne Disease in the United States

The primary source of information on waterborne disease in the United States is the Centers for Disease Control and Prevention (CDC), which, together with the U.S. Environmental Protection Agency (U.S. EPA), have maintained a passive surveillance system since 1971. CDC surveillance for waterborne disease reports is published in the *Morbidity and Mortality Weekly Reports (MMWR)* approximately every 2 years. The most recent report for the period 1993 to 1994 estimated that 405,366 persons became ill in the United States from consuming contaminated drinking water, including the cryptosporidiosis outbreak in Milwaukee that is thought to have infected 403,000 persons (23). Table 3 presents the total number of outbreaks and associated cases reported to the CDC during this time period. Of a total of 30 outbreaks, 11 were attributed to untreated groundwater,

7 to treatment deficiencies, 8 to distribution system deficiencies, and 4 were of unknown cause. It is clear from Table 3 that these data are dramatically skewed by the Milwaukee outbreak. In fact, if that outbreak were excluded, the total number of cases is only 2,366. This gives an entirely false impression of the scale of the problem due to underreporting of waterborne disease in the United States (discussed below), and is an argument for far better surveillance systems (2,3).

### The Problem of Underreporting and the Epidemiologic Approach

*MMWR* waterborne disease reports are useful for partially characterizing the epidemiology of waterborne disease outbreaks (WBDOs), identifying etiologic agents, and determining causes of WBDOs (all stated goals of the CDC and U.S. EPA surveillance system). There are necessarily many deficiencies, but the primary problem is dramatic underreporting of waterborne disease and large numbers of acute gastrointestinal diseases of unknown etiology (AGI). The problem of AGI is probably underestimated by the most recent *MMWR*. Previous reports on waterborne disease (Figure 1) have generally identified 50% or less of the causative agents. Underreporting of waterborne infectious disease in the United States, as well as globally, makes the problem of assessing rates very difficult. Accurate estimates of waterborne disease are virtually impossible. This is primarily due to the large number of asymptomatic cases, symptomatic cases for which no treatment is sought, cases for which treatment is sought but no specific diagnosis given and/or no information obtained on routes of exposure, and cases for which diagnosis is obtained but not reported (2). A recent analysis of infectious

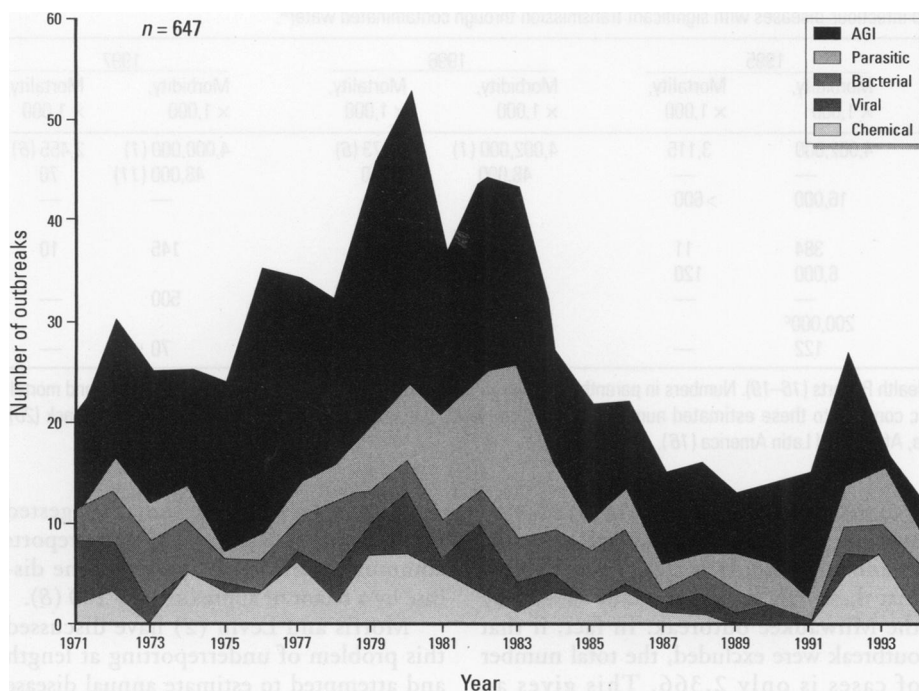
diseases in Hyderabad, India, suggested that hospital incidence data underreports community incidence of waterborne disease by a factor of approximately 200 (8).

Morris and Levin (2) have discussed this problem of underreporting at length and attempted to estimate annual disease incidence in the United States for *Salmonella* spp., *Shigella* spp., *Escherichia coli*, *Campylobacter* spp., *G. lamblia*, *C. parvum*, and viruses. Their estimates were based on low and high estimated incidences obtained from the literature. There is a considerable range in estimates, as different methodologies were followed that ranged from follow-up investigations of outbreaks to models of likely infection rates based on distribution of pathogens in surface waters. As a result, these estimates must be viewed cautiously. Morris and Levin (2) reported point estimates of annual incidence of 7.1 million cases of mild-to-moderate infections, 560,000 moderate-to-severe cases, and 1,200 deaths attributable to waterborne infectious disease. However, the authors emphasize the inadequacy of the data available for accurately estimating these incidence rates.

**Table 3.** Waterborne disease outbreaks associated with drinking water, by etiologic agent, United States, 1993–1994.<sup>a</sup>

Etiologic agent	Outbreaks	Cases
<i>Cryptosporidium parvum</i>	5	403,271
AGI	5	495
<i>Giardia lamblia</i>	5	385
<i>Campylobacter jejuni</i>	3	223
<i>Salmonella</i> serotype		
Typhimurium	1	625
<i>Shigella sonnei</i>	1	230
<i>Shigella flexneri</i>	1	33
Non-O1 <i>Vibrio cholerae</i>	1	11
Chemical exposures	8	93

<sup>a</sup>Adapted from Kramer et al. (23).



**Figure 1.** Number of waterborne disease outbreaks associated with drinking water, by year and etiologic agent—United States, 1971–1994. Reproduced from Kramer et al. (23).

A critical question for the United States is whether incidence of waterborne disease is increasing. Kramer et al. (23) report that numbers of outbreaks reported for 1993 and 1994 are comparable with those reported for the previous few years except for 1992 when higher numbers were reported. Since 1971, the highest number of waterborne diseases were reported between 1970 and 1983 (Figure 1). The authors note, however, that changes in reported numbers of waterborne disease may in part reflect changes in surveillance activities and not increased or decreased risks from water supply systems. Morris and Levin (2) conclude that although the incidence of waterborne disease declined dramatically through the early 1900s, over the past 30 or 40 years this decline in cases has reversed and, in particular, the number of cases per outbreak has increased.

A growing body of literature indicates that waterborne disease is far more prevalent than reported outbreaks indicate. An intervention study (24) published in 1991 resulted in a reassessment of the quality of drinking water and the potential importance of undiagnosed gastrointestinal (GI) disease in North America. The researchers conducted a randomized trial to evaluate quality of drinking water meeting current microbiological standards. By using a control group of study participants supplied with reverse

osmosis units, they were able to estimate that 35% of reported GI illnesses were water related. Subsequent epidemiologic studies have attempted to link turbidity records with GI disease (25,26). These studies do appear to present weak relationships between utility-measured turbidity and hospital-reported GI disease. Unfortunately, because of the prospective nature of the studies, it is not possible to link disease to a causative pathogen in the drinking water. However, the increasing ability to archive samples for future pathogen analysis could provide these linkages if utilities could be persuaded to archive samples during periods of elevated turbidity, heavy rainfall, etc.

It is difficult to compare U.S. statistics with waterborne disease on a global scale. Many other countries have surveillance systems for waterborne disease, e.g., the U.K. Communicable Disease Surveillance Centre. However, maintenance of infectious disease databases varies tremendously between countries, and many developing countries are not even aware of diseases such as cryptosporidiosis. One interesting statistic is derived from an analysis of WBDOs in Israel between 1976 and 1985 (27). According to these authors, Israel experienced 18.7 times the number of community WBDOs per capita as reported for the United States. In general, lack of

comparable data and access to disease statistics and water quality data make global assessments of the burden of waterborne disease almost impossible. These issues are complicated by the poor quality of epidemiologic data linking disease with consumption of drinking water. Epidemiologic studies may be easier to conduct in developing countries where hospital incidence data may be more readily available and populations can be surveyed by administration of health related questionnaires. Using this approach, a recent epidemiologic study in Hyderabad, India, linked distribution system condition, lack of residual chlorine, and sewage status around the home to rates of GI disease. This information has subsequently provided impetus for programs to upgrade water delivery and wastewater collection systems (8).

## Etiology of Waterborne Diseases

Rather than focus further on the inadequacy of existing data linking GI disease to contaminated water, it is probably instructive to examine the etiologies of waterborne infectious agents where known. If the water supply becomes contaminated with *Shigella* spp., *Salmonella* spp., pathogenic *E. coli*, *Campylobacter* spp., *G. lamblia*, or pathogenic viruses, morbidity, and in some cases, mortality, may occur. These organisms enter the drinking water distribution system through fecal contamination of untreated ground or surface water, treatment failure, or distribution system failure [interconnections with contaminated surface waters or waste water collection systems (4)]. However, we are also seeing disease associated with well-maintained water delivery systems. These are the diseases that result from environmental pathogens such as *Legionella* or *Mycobacteria* spp., or from protozoa that are highly resistant to chlorination through formation of cysts or oocysts (e.g., *C. parvum*). Additionally, a current concern for the public health community are the opportunistic pathogens that may survive and grow within distribution system biofilms. Biofilms develop on the inner surfaces of distribution system pipes because of colonization by microorganisms. They are essentially an accumulation of microbial cells, extracellular products, and inorganic and organic debris (4). These films can easily develop to > 100  $\mu$ m thick and provide a protective environment for the species mentioned above and for species of *Pseudomonas*, *Aeromonas*,

*Klebsiella*, *Acinetobacter*, *Xanthomonas*, *Moraxella*, and the many others whose etiology is ill defined. The water distribution system provides unique conditions for the biofilm community to develop. The biofilm in turn provides a nutrient-rich, disinfection-protected environment for pathogen survival. It is also a potential site for transfer of virulence and antibiotic resistance factors. The biofilm is also a source of disinfection demand and a site of accelerated corrosion. Continuous sloughing of the film contributes to "dirty water" and is a source of infectious boli that provide an inoculum for new connections or "cleaned"/repaired pipeline and can also be disseminated to the consumer's tap (4).

The following section reviews the state of knowledge of specific waterborne pathogens. Although the focus is on bacteria, protozoa, and viruses, there is also the potential, though poorly characterized, for pathogenic fungi and helminths to enter drinking water supplies (28). In addition, other invertebrates (e.g., crustacea, insect larvae) may pass into distribution systems, particularly from unfiltered supplies, protecting ingested pathogens from disinfection (29,30).

## Bacterial Pathogens

### The Case of Cholera

Much has been written about cholera and, indeed, as with the other major infectious diseases (e.g., smallpox, the plague), epidemic cholera has played an important role in the development of societies worldwide (31,32). Now well into the seventh pandemic since 1817, cholera continues to affect population demographics, particularly in countries with inadequate sanitation (33). The situation is dramatically exacerbated in countries subjected to periodic flooding, as in Bangladesh (34).

Inadequate sanitation and hygiene practices can rapidly result in outbreaks of cholera. This was clearly seen during the 1991 South American epidemic that continues sporadically to this day and has caused an estimated 1 million or more cases and 10,000 deaths through 1994 (35). Where infrastructure breaks down and wastewater mixes with water supplies, cholera and other disease epidemics emerge. It is important to note that a waterborne disease epidemic is likely to increase population susceptibility to other diseases.

There are multiple routes of transmission of a waterborne disease such as cholera,

many of which can be traced to cultural behavior, particularly in developing countries. For example, it is now well documented that traditional water supplies in many communities also serve as washing and play areas as well as receptacles for human and animal wastes (3). Funeral practices have been implicated in transmission of cholera, in particular, food handling for large community meals following preparation of a body (36,37).

**New Areas for Cholera Research.** There are currently 155 'O' serogroups of *V. cholerae* (38), of which only O1 and, more recently, O139 presently appear capable of causing epidemic cholera (through the presence of the virulence genes for cholera toxin). However, horizontal gene transfer can occur between O1 and non-O1 serogroups of *V. cholerae*, and existing toxigenic strains are thought to have originally evolved from nontoxigenic environmental strains (39). Horizontal gene transfer has also been demonstrated between O1 and non-O1 strains (40), and there is strong evidence that bacteriophages may be involved in transfer of virulence factors (41,42). In addition, a number of non-O1 and non-O139 serotypes can cause clinical disease despite the absence of the typical virulence (CT) genes (43). The significance of clinical disease caused by these non-O1 and non-O139 serotypes is currently unclear. However, the emergence in early 1993 of serotype O139 with epidemic potential suggests that other serotypes could also develop this potential. Serotype O139 apparently has enhanced environmental and antimicrobial resistance (44,45), a potentially alarming trend in the long history of cholera.

Environmental reservoirs for *V. cholerae* make eradication of this disease almost impossible. The bacterium has been associated with blue crabs, shellfish, copepods, and aquatic vegetation [reviewed by Colwell (33)]. There is compelling evidence that *V. cholerae* may proliferate in copepod egg sacks, which can then be ingested in untreated drinking water (46,47). These associations mean that spread of these aquatic organisms, as in a copepod bloom, could potentially cause spread of the disease. Researchers have implicated seasonal outbreaks of cholera in Bangladesh with blooms of aquatic organisms (48) and plankton blooms may also have accelerated spread of cholera in Peru in 1991, although direct evidence is lacking. The association of pathogens such as cholera with plankton has important implications for increased risk of

disease through stimulation of plankton blooms. It has been suggested that nutrient enrichment through anthropogenic activity and even climate change (in particular, warming trends) may be important factors in the spread of waterborne disease (49).

### Other Bacterial Diseases

Other major bacterial diseases frequently linked to consumption of drinking water are caused by *Shigella* spp., *Salmonella* spp., pathogenic *E. coli*, and *Campylobacter* spp. All have multiple routes of infection and may be transmitted through water, food, soil, or person-to-person contact. However, in each case, major outbreaks of these diseases have been linked to consumption of contaminated water. Morris and Levin (2) have estimated that within the United States, contaminated water is responsible for 35,000 cases of shigellosis, 59,000 cases of salmonellosis, 150,000 cases of infection with pathogenic *E. coli*, and 320,000 cases of campylobacteriosis (Table 1). Most *Shigella* spp. are human enteric pathogens that cause diarrheal illness worldwide and are usually directly linked to sewage contamination (50,51). Major shigellosis epidemics have occurred after flooding in Africa (52). Typhoid, caused by *Salmonella typhi*, is now rare in developed countries but remains endemic in a number of developing countries (53) and can reach epidemic proportions given poor sanitary conditions. This occurred in Chile during the 1980s and was partially linked to irrigation of vegetables with wastewater coupled with increased rainfall, poor water treatment, and deteriorating economic conditions (54). An analysis of strain diversity of *S. typhi* from Chile during endemic and epidemic periods suggested that the epidemic was probably attributable to multiple sources including environmental reservoirs (sewage and river water) (55,56).

Non-*typhi* species of *Salmonella* cause large numbers of GI disease in both the United States and worldwide, with reported increases in infection rates for some countries (57). For example, an outbreak of *Salmonella typhimurium* directly attributable to water occurred in the United States in Missouri in 1993, sickening more than 650 persons and causing 7 deaths (58). As with many *Campylobacter* spp. and pathogenic *E. coli*, most *Salmonella* spp. are zoonotic and may contaminate surface and groundwaters through agricultural runoff or directly through wildlife or domestic animal feces. Outbreaks of these diseases therefore can

occur in the absence of direct contamination from human sewage. Pathogenic *E. coli* are a major cause of diarrheal disease worldwide. In fact, recent epidemiologic studies have implicated *E. coli*, including enteropathogenic, enterotoxigenic, and enterohemorrhagic strains, as the major identified cause of diarrheal disease (59–61). Few studies, however, have distinguished between different routes of infection for this pathogen, although almost all pathogenic strains, including strains with multiantibiotic resistance (62), can be isolated from contaminated water (63,64). Drinking water has been thought to be contaminated during repairs to distribution system pipelines (65) and through cattle manure-contaminated surface runoff into well water (66).

*Campylobacter* is reported to be the most frequently isolated enteric pathogen from humans in the United Kingdom since 1981 (67) and one of the commonest causes of diarrhea in developed countries (68). Outbreaks are often linked to water (67,69–71). Morris and Levin (2) report the highest numbers of waterborne bacterial disease in the United States for campylobacteriosis. However, as these authors discuss, *Campylobacter* infections are not routinely reported to the CDC and therefore their true incidence is very difficult to determine. In other countries the relative frequency of this diarrheal disease appears different from that in the United States (Table 4). This presumably reflects differences in environmental conditions, host susceptibilities, routes of exposure, and limitations in diagnostic approaches.

**Bacterial Diseases Less Clearly Associated with Water.** There are a number of bacterial diseases that are less frequently associated with water but for which there is compelling evidence for a waterborne route of infection. These include the zoonotic diseases, leptospirosis, tularaemia, and yersiniosis. Recently, leptospirosis was the cause of epidemic disease in Ecuador after the breakdown of infrastructure caused by flooding (74). There are many animal hosts for the different *Leptospira* species (75), and the disease is most commonly thought to be spread through contamination with the animal's fecal matter or urine. Although leptospirosis outbreaks are frequently linked with contaminated water (75–80), there is remarkably little information on the pathogen's survival strategies outside of the animal host. Tularemia also has been epidemiologically linked to consumption of contaminated water (81,82). The disease is

caused by *Francisella tularensis* and two subspecies have been reported. The primary reservoir of the milder type B appears to be aquatic rodents, providing ample opportunity for infection of humans by the waterborne route, whereas the highly virulent type A is carried primarily by cottontail rabbits and ticks (83).

Thirty-five percent of non-*pestis* *Yersinia* infections were attributed to the waterborne route in Bennett et al.'s 1987 review on infectious and parasitic diseases (84), yet little direct evidence of this route of infection is presented in the literature. Major outbreaks have occurred in the United States, but evidence suggests that transmission was through food (85). In a recent review of this pathogen, it was suggested that other than identified foodborne outbreaks, sources are speculative (85). Further work clearly is required to establish whether water can be a significant route of exposure to this pathogen. Epidemiologic evidence has linked *Yersinia* infections to water (86) and *Yersinia* spp. are readily isolated from drinking water. However, based on serologic and biochemical characteristics, one study has suggested that *Yersinia* isolates from drinking water plants are not of public health significance (87).

Aeromonads also are frequently isolated from drinking water (88,89), and although they are known to be opportunistic pathogens and causative agents of non-GI infections [e.g., wound infections from exposure to water (90)], direct evidence that GI disease may occur from ingestion of waterborne aeromonads, in particular, *Aeromonas hydrophila*, is also limited (91). *Plesiomonas shigelloides* is another pathogen that has been implicated in waterborne GI

disease (92), but as with *A. hydrophila*, direct evidence that this is a major route of infection is also limited. A number of other opportunistic pathogens can be readily isolated from drinking water supplies, often within biofilms (4); these include *Pseudomonas* spp., *Flavobacterium* spp., *Klebsiella* spp., *Acinetobacter* spp., *Xanthomonas* spp., and *Moraxella* spp., among others (11). A recent risk estimate of opportunistic bacterial pathogens in drinking water suggests that levels of risk are generally less than 1/10,000 for a single exposure (93). However, as the authors point out, seasonal trends in concentrations are not examined, dose-response studies are inadequate, particularly for susceptible populations, and there is no assessment of risks from multiple exposures.

*Helicobacter pylori* is another pathogen that has recently received considerable attention because of its potential association with gastric ulcers and stomach cancer (94). Compelling evidence from epidemiologic and laboratory-based studies suggests there could be a waterborne route of infection for this pathogen (95–97). Although a recent study suggests that *H. pylori* is rapidly inactivated by chlorine (98), there is a need for further examination of its prevalence in unchlorinated drinking water and for potential disinfection-resistant strategies.

**Legionella and the Mycobacteria.** *Legionella* and *Mycobacteria* spp. are worth considering separately because they are truly environmental pathogens that appear to have found a unique ecologic niche in our drinking and hot water supplies. Although *Legionella* is a newly recognized pathogen (99), there is evidence that it may

**Table 4.** Examples of relative frequency of diarrheal diseases from three different studies.

	Diarrheal cases, %	Reference
Lao, n = 880		
<i>Campylobacter</i> spp.	4.4	Yamashiro et al. (59)
<i>Shigella</i> and <i>E. coli</i> spp.	45.0	
<i>Salmonella</i> spp.	0.6	
Rotavirus	6.1	
Crete, n = 3,600		
<i>Salmonella</i> spp.	13.6	Samonis et al. (72)
<i>Campylobacter</i> spp.	4.7	
Enteropathogenic <i>E. coli</i>	3.9	
<i>Shigella</i> spp.	0.7	
<i>Yersinia enterocolitica</i>	0.7	
<i>Aeromonas hydrophila</i>	0.05	
Italy, n = 618		
Rotavirus	23.6	Caprioli et al. (73)
<i>Salmonella</i> spp.	19.2	
<i>Campylobacter</i> spp.	7.9	

Other pathogens observed in only a limited number of cases.

be a major cause of bacterial pneumonia, at least in the United States, with estimates of at least 13,000 cases per year [Table 1 (14)]. The evidence is strong for a water-related mode of transmission for *Legionella*, which appears to be ubiquitous in the environment (100). Numerous reports have linked Legionnaires' disease, caused by *L. pneumophila*, to residential and hospital water supplies (101–104). Stout and colleagues (105) showed that hot water storage tanks provide conditions that support *L. pneumophila* concentrations in excess of  $10^4$  colony-forming units/ml. They argue that the organism tends to multiply in areas of sediment accumulation where essential nutrients are readily available to support its fastidious growth requirements.

Far less research has been conducted on the risk of transmission of the nontuberculous mycobacteria from drinking water, although they have been shown to colonize potable water systems (106,107) and appear to be as environmentally ubiquitous as *Legionella* spp. (108). There is reason to be concerned about the routes of exposure to these mycobacteria. *Mycobacterium avium* complex (MAC) causes disseminated infections in as many as 40% of AIDS patients (109), and infections may be increasingly prevalent in populations with no apparent predisposing factors (110). As with *L. pneumophila*, they have been isolated repeatedly from recirculating hot water systems (107), and can survive in coculture with protozoa. (Survival strategies of these and other pathogens are discussed in greater detail in "Water Treatment and Pathogen Survival Strategies.")

## Protozoan Diseases

### *G. lamblia* and *C. parvum*

Smith and Lloyd (111) have recently reported that these two protozoa are responsible for more than 600 million infections worldwide, of which a significant proportion are waterborne. For example, 60% of *Giardia* cases are estimated to be waterborne in the United States (84), with a point estimate of the annual incidence of giardiasis of 260,000 cases (2). Until recently, giardiasis was the most frequently reported waterborne disease (112,113). Cryptosporidiosis has now overtaken giardiasis, with a point estimate of 420,000 annual waterborne cases in the United States (2). Table 5 compares surveys of potable water supplies for *C. parvum* and *G. lamblia*.

Over the past 10 years, there has been considerable emphasis on *C. parvum* research. This is partly because of the scale of waterborne outbreaks seen in 1989 (116) (estimated 13,000 cases) and again in 1993 (117) (estimated >400,000 cases) but also because the outbreaks were associated with filtered water supplies apparently meeting all appropriate standards for that time. Although *C. parvum* oocysts are small (4–6  $\mu$ m) relative to *G. lamblia* cysts (10–12  $\mu$ m) (113), well-maintained filtration units should remove most oocysts. In fact, outbreaks in filtered water systems have generally been traceable to poor operational procedures. Once *C. parvum* oocysts have entered the distribution system, however, residual chlorine concentrations that inactivate other pathogens are inadequate.

Cryptosporidiosis in Milwaukee in 1993 was the largest documented waterborne disease outbreak to have ever occurred in the United States (117). A telling statistic is taken from Bennett et al.'s (84) assessment of infectious disease rates in the United States, with estimates of only 50 cases of cryptosporidiosis per year and none associated with water. In fact, outbreaks are reported with increasing frequency not only in the United States (116–118) but also globally in both developed (119,120) and developing countries (121,122). It is probable that reporting of cryptosporidiosis infections will increase dramatically as awareness of this disease increases.

Seroprevalence studies indicate that exposure to *C. parvum* is widespread and that asymptomatic infection occurs frequently. In a study of 803 children in Oklahoma, 13% of children under 5 years of age, 38% of children 5 to 13 years of age, and 58% of adolescents 14 to 21 years of age were seropositive (123). In three villages in rural Anhui, eastern China, up to 57.5% of children less than 16 years old were seropositive. In the same study,

almost 100% of randomly selected serum samples from children less than 4 years of age in Fortaleza, Brazil, were seropositive (122). In these and the many other studies of seroprevalence, exposure to *C. parvum* occurs far more frequently than records of the disease would suggest. For example, in Germany *C. parvum* is reported in approximately 2% of diarrheal patients; however, antibodies are detected in 15.4% of all samples tested (124).

Considerable research has been directed toward understanding the etiology of cryptosporidiosis, in particular, its transmission from animal host through the water supply to the human host. As with *Giardia* and many of the other pathogens described in this review, *C. parvum* has a broad host range, making elimination of this pathogen from the watershed virtually impossible. Both *Giardia* cysts and infectious *C. parvum* oocysts recently have been shown to be disseminated by waterfowl (125). The environmental ecology and public health implications of *C. parvum* have been reviewed by Rose (126).

### Other Pathogenic Protozoa

Although much attention has been given to *G. lamblia* and *C. parvum*, many other pathogenic protozoa may be transmitted by the water route. In addition to the above, Marshall et al. (113) reviewed the association with water of *Naegleria fowleri*, *Acanthamoeba* spp., *Entamoeba histolytica*, *Cyclospora cayentanensis*, *Isospora belli*, and the microsporidia. Additional protozoan pathogens that may be transmitted by the waterborne route include *Ballantidium coli* and *Toxoplasma gondii*.

*N. fowleri* is the causative agent of primary amoebic meningoencephalitis, which is rapidly fatal. The pathogen has been isolated from surface waters (127), and a 1973 report implicated a domestic water supply as the source of transmission (128). Although relatively few cases of the

**Table 5.** Surveys of potable water supplies for *C. parvum* and *G. lamblia*; percentage of samples containing oocysts or cysts.

Region	Prevalence, %	Reference
17 U.S. states, 1991		Rose et al. (112)
<i>Cryptosporidium</i> oocysts	17	
<i>Giardia</i> cysts	0	
14 U.S. states and 1 Canadian province, 1991		LeChevallier et al. (114)
<i>Cryptosporidium</i> oocysts	27 (13.3) <sup>a</sup>	
<i>Giardia</i> cysts	17 (<9) <sup>a</sup>	
72 Canadian municipalities, 1996		Wallis et al. (115)
<i>Giardia</i> cysts	18.2 (26.6) <sup>b</sup>	

<sup>a</sup>Estimates of viability based on cyst or oocyst morphology. <sup>b</sup>Estimate of viability, assessed by dye exclusion.

disease occur, recent information on seroprevalence suggests that exposure to *Naegleria* spp. may be relatively common (113). There is also some evidence that *N. fowleri* may play a role in distribution of *L. pneumophila*, as this bacterium has been shown to increase in number after ingestion by viable *N. fowleri* cells (129). In contrast to *N. fowleri*, *Acanthamoeba* spp. have often been found in tap water and even bottled mineral water. Although *Acanthamoeba* spp. is the causative agent in a number of diseases, including a form of encephalitis, ingestion of the organism has not been directly linked with disease. It could, however, account in part for a widespread seroprevalence (113). Ingestion of *Acanthamoeba* spp. is also a potentially serious public health threat because of their role in transmission of bacterial pathogens (130,131). *E. histolytica* is a common contaminant of untreated drinking water, particularly in developing countries and, according to Marshall's review (113), has been suggested to infect 12% of the world's population with high associated rates of mortality.

In addition to *Cryptosporidium*, the family Coccidia includes the genera *Cyclospora* and *Isospora*, of which *C. cayatensis* and *I. belli* may have waterborne routes of transmission. As with many of the other waterborne pathogens, increasing numbers of immunocompromised individuals both in the United States and globally contribute to increased reporting of disease from these organisms. *Cyclospora* spp. is a recently recognized pathogen that has been linked directly to water in only one case (132), although an association with water has been suggested in a limited number of other outbreaks (133). Contaminated fruits and vegetables are thought to be a major route of transmission (134,135), but it is likely that the water route is underestimated because of the lack of epidemiologic evidence (113). A recent study shows that the pathogen can be detected in wastewater (136); therefore, contamination of drinking water would seem to be a potential risk. Little direct information is available on the waterborne route of transmission for *I. belli* and *B. coli*, although epidemiologic evidence suggests this may be the case (137,138). *B. coli* has been isolated from stored water used for drinking and cooking in Hyderabad, India (139).

Canadian researchers have recently provided strong epidemiologic evidence that an outbreak of toxoplasmosis caused by *T. gondii* in British Columbia, Canada,

was associated with the municipal water supply (140).

The microsporidia are an interesting group of protozoa that have recently received attention because of high infection rates in AIDS patients (141). Drinking water would seem to be a potential route of infection, as certain species can survive for extended periods of time and maintain infectivity (up to 1 year in 4°C water), although it is not known if species that infect humans have this capability (113). Potentially pathogenic microsporidia, however, have been isolated from surface waters (142).

## Viral Pathogens

In many ways, viruses are the most poorly understood area of research on waterborne disease. A number of authors have suggested that Norwalk virus and Norwalk-like viruses are the major causes of both food- and waterborne illnesses worldwide (143). In fact, in Bennett et al.'s (84) analysis of infectious and parasitic diseases, Norwalk virus was the only viral agent included as transmissible by water. Payment et al. (144) confirmed that exposure to Norwalk virus is common by examining seroprevalence of the virus in tap water drinkers participating in a prospective epidemiologic study. In fact, seroprevalence reached 100% in older study participants (those ≥ 60 years of age). Norwalk virus was found to be the major cause of a 1994 outbreak of waterborne viral gastroenteritis in Finland, affecting 1,500 to 3,000 people. However, adenovirus, small round-structured viruses (SRSV) and rotaviruses were also confirmed as causative agents (145). A recent article (146) has also epidemiologically linked SRSV to an outbreak of gastroenteritis from sewage-contaminated well water in the United States. This is an important study, as it illustrated the importance of molecular techniques in linking viruses in drinking water with clinical samples.

There is also strong epidemiologic evidence that hepatitis A (HAV) and rotaviruses are frequent causes of waterborne disease (147). HAV is reported to be the first virus definitively shown to be transmitted by water, and numerous outbreaks have been documented [reviewed by Rose and Gerba (148)]. These authors feel that on the basis of its epidemiology, HAV is one of the most prevalent waterborne viral pathogens. Rotaviruses are also frequently reported in outbreaks (Table 4) and, together with enteroviruses, have been isolated from chlorinated drinking water systems

(149,150). These viruses are a primary cause of traveler's diarrhea. They are also a major cause of infantile gastroenteritis and have been reported to be responsible for 50% of hospitalized cases of diarrheal illness in temperate climates (148,151).

Bennett et al.'s analysis (84) suggested that 300,000 cases of waterborne infection were caused annually by Norwalk virus in the United States. Rose and Gerba's 1986 review of viruses in treated drinking water (148) listed more than 110 types of enteric viruses capable of environmental transmission. These included types of poliovirus, coxsachievirus, echovirus, reovirus, adenovirus, HAV, rotavirus, and Norwalk virus. SRSV, astrovirus, coronavirus, calicivirus, and, at least for non-U.S. water systems, hepatitis E (HEV) (152) can now be added to the list of waterborne viruses. Bennett et al.'s estimates based on Norwalk virus alone must therefore underestimate waterborne viral disease to a considerable extent. Morris and Levin (2) provide a point estimate of 6.5 million annual cases of waterborne viral disease in the United States, with an estimated mortality of 0.005%. As previously discussed, many assumptions necessarily made in these estimates may dramatically underestimate or overestimate burden of disease.

HEV is an interesting example of a waterborne virus whose etiology is poorly understood. Apparently endemic in a number of countries, HEV does not always cause disease (153). Outbreaks associated with water have occurred in Kanpur (153), Somalia (154), Vietnam (first recognized outbreak) (155), and Nepal (156). Mortality can be high, particularly among pregnant women, i.e., estimated to be 13.8% in the Somalia outbreak. According to the U.S. EPA (152), outbreaks of 100,000 cases of HEV have occurred in China, with mortality rates approaching 20% for pregnant women. This suggests regionally based differences in exposure pathways, infection rates, and virulence of HEV, but currently these differences are unclear (3). A recent analysis of a waterborne epidemic of acute hepatitis in Djibouti (157) suggested that both HAV and HEV were responsible but not equally distributed between French expatriates and Djibouti residents. HEV was almost exclusively found in the Djibouti residents, raising important questions about population immunity.

As previously discussed, reported numbers of GI disease are only a fraction of the actual incidence (2,8). It is probable that a high percentage of unreported cases

as well as AGI of unknown etiology could be caused by viruses, as these infections are often minor and hard to characterize. Further application of molecular techniques will undoubtedly provide stronger evidence linking waterborne viruses with GI disease (146), see also "Advances and Limitations in Methodology").

## Water Treatment and Pathogen Survival Strategies

It is beyond the scope of this review to discuss water treatment in detail; however, many excellent texts address this topic (28,158). Filtration (with precoagulation/flocculation) remains one of the most effective means to minimize pathogen loading to the distribution system. However, disinfection is still necessary to protect public health. Modern treatment plants often apply disinfection prior to filtration (primary disinfection) and to the finished water to maintain a residual in the distribution system (secondary disinfection). Choice of disinfectant may be important in the patterns of pathogen survival. Ozonation is becoming increasingly popular as a primary disinfectant, as it requires a much shorter C·T (concentration × contact time) for deactivation of the cysts and oocysts of *Giardia* and *Cryptosporidium* compared to chlorine. Ozonation has additional advantages in that it oxidizes nuisance organics that convey odor, taste, and color problems to finished water. However, ozonation byproducts are poorly characterized (160). In addition, the strong oxidizing capability of ozone may also result in production of readily assimilable organic carbon compounds (AOC) that have been shown to stimulate biofilms and coliform regrowth in distribution systems (159). Approaches to reduction in AOC involve removal through biologic filters or adsorption to granular-activated carbon (28), technologies that rapidly increase the cost of water treatment beyond the scope of many utilities.

The reactivity of ozone also results in minimal residual disinfection capacity in the distribution system. To maintain residual disinfection, either chlorine or chloramine is applied to drinking water. This is generally the case for both filtered and unfiltered water supplies. Although chloramine is a less effective biocide than free chlorine, it maintains a residual concentration for a longer period of time in the distribution system. It also appears to permeate biofilms more effectively than free chlorine and to control their development.

Other advantages of chloramination over chlorination include reduced production of chlorinated byproducts. However, there are reported problems with increased numbers of nonpathogenic bacteria and associated taste and odor (28). The solution appears to be an occasional return to free chlorine and frequent flushing of the system.

Whichever mode of disinfection is employed, one that reduces biofilm formation or one that more effectively eliminates suspended bacteria, pathogens will not be entirely eliminated from the distribution system. It appears that water treatment and, in particular, disinfection provide selection pressures on pathogens that promote a wide range of survival strategies. Almost all bacteria tested in the laboratory appear to be capable of taking advantage of some form of resistance mechanism. Although contested by one report (161), the viable but nonculturable (VNC) form has now been documented for a large number of bacteria including pathogens such as enterotoxigenic *E. coli*, *V. cholerae*, *Salmonella* spp., *Shigella* spp., *C. jejuni*, and *L. pneumophila* (162–165). Exposure of enteric bacteria to stress may result in the VNC state, in which survival is enhanced by a dormant or injured condition that prevents culturing on selective media. Although these bacteria are no longer detectable by standard microbiologic methods, they retain their pathogenicity and may still cause disease if ingested by the human host (166).

Many different bacteria have been shown to survive and in many cases grow within protozoan hosts. Studies have shown that this survival mechanism protects the pathogen from disinfection and may also be important in initiation of virulence and transmission of disease (99,167). King and co-workers (131) were able to show that a number of different bacteria, including pathogens, could be ingested by the protozoa *Acanthamoeba castellanii* and *Tetrahymena pyriformis*. These protozoa were then able to survive and grow in concentrations of free chlorine that killed free-living bacteria (10 and 4 ppm, respectively). The tested bacterial strains, including the pathogens *S. typhimurium*, *Yersinia enterocolitica*, *Shigella sonnei*, *Legionella gormanii* and *C. jejuni*, could be subsequently cultured from the treated protozoans. Considerable attention has been focused on the intracellular replication of *L. pneumophila* in protozoan hosts (168,169). The protozoan *A. castellanii* has recently been shown to resuscitate VNC

*L. pneumophila* to a culturable and infective state (170). Berk and colleagues (167) have shown that two *Acanthamoeba* spp. expelled vesicles containing viable *L. pneumophila* and suggest that these vesicles could be important agents for transmission of disease.

A recent study (130) reports that *Mycobacterium avium* resides within outer walls of *Acanthamoeba polyphaga* cysts and can grow saprozoically on products secreted by the organism. This is in contrast to *L. pneumophila*, which is found within the cysts, and suggests that there are separate reservoirs for these two opportunistic pathogens during unfavorable conditions. It has been hypothesized that the mode of exposure to these organisms can effect disease outcomes. For example, ingestion of amoebae containing *L. pneumophila* results in exposure to high numbers of infective organisms. These organisms are not only adapted to parasitize amoebae but also are ideally suited to parasitize alveolar macrophages. The result is Legionnaires' disease (14). Ingestion of biofilm-associated or free-living *L. pneumophila* may result in exposure to lower numbers of infective organisms with disease outcomes such as Pontiac fever or minor infections (99). These hypotheses have yet to be rigorously tested but may also be important in assessing disease outcomes from exposure to other pathogens.

Considering the increasing literature on biofilms, it is surprising that comparatively little information is published on the patterns of pathogen survival (including viruses and protozoa) in drinking water biofilms. The biofilm provides a nutrient-rich, protective environment that should promote survival of enteric pathogens (4). Redox dyes have been used to differentiate respiring and nonrespiring cells in mixed *K. pneumoniae* and *Pseudomonas aeruginosa* biofilms (171). Greater respiratory activity was measured in cells deep in the biofilm after application of biocide, consistent with the biofilm's protective role. Also, experiments to test substratum topography on susceptibility of *Salmonella enteritidis* biofilms to trisodium phosphate indicated that the bacterium survives in greater numbers in thicker biofilms associated with artificial crevices (172). Microcosm experiments have also provided evidence that *Campylobacter* spp. persist for extended periods (several weeks) within biofilms (173). There is also limited information on persistence of *L. pneumophila* in biofilms (174), focused on types of plumbing

materials colonized by this pathogen. Recent unpublished research has shown that a clinical isolate of MAC can survive for several months within a model *P. aeruginosa* biofilm (175). More research on the role of biofilms in pathogen survival is needed to begin to estimate risks of disease from biofilm sloughing within the distribution network.

Far less is known about protozoan and viral survival mechanisms. Many protozoa form cysts or oocysts that are extremely resistant to disinfection and, as already discussed, many of the bacterial pathogens take advantage of these cysts for their own survival strategies. A recent report identified microsporidialike parasites infecting amoebae (*Vanella* spp.) isolated from a domestic potable warm-water system (15). If the parasites are confirmed as microsporidia, it will be interesting to see if the intracellular survival state is common for these organisms, including species that infect humans.

There is no information in the literature on survival of pathogenic viruses in protozoa, although this survival strategy should not be ruled out. Adsorption to particles or even colloidal organic material has long been suspected as a mechanism to convey disinfection resistance to viruses. Certainly, the rotaviruses and many of the enteroviruses appear to survive in chlorinated water (147). There is also some evidence for reactivation of previously iodine-disinfected bacteriophages (MS2) when proteinaceous material is present in the water (176). The limited literature on drinking water biofilms and viruses has focused primarily on the importance of bacteriophages in gene transfer in biofilms (177). As with many of the bacterial and protozoan pathogens, the role of the biofilm is probably important in enteric virus survival, but considerable research is needed to obtain a clearer understanding of mechanisms involved.

### Advances and Limitations in Methodology

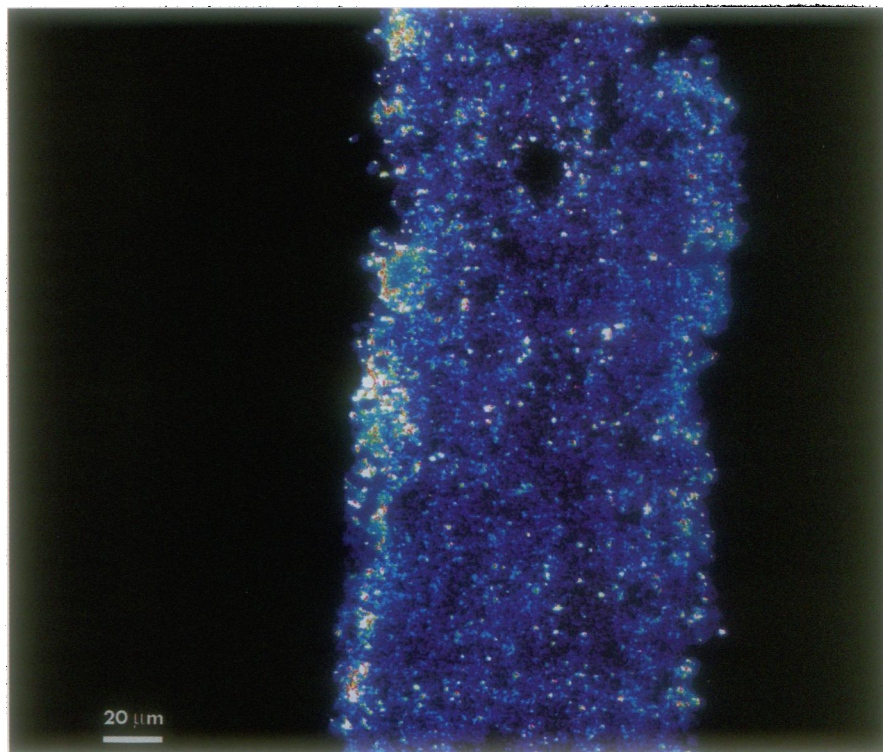
The advent of microscopic techniques to image biofilms and to prepare thin sections using, for example, cryosectioning and confocal microscopy (171), allows direct observation of pathogens within biofilms by using specific antibody staining (175,178). This is beginning to help in investigations on the protective role of biofilms. For example, Figure 2 shows a clinical isolate of *M. avium* that has both survived and proliferated under a biofilm

of *P. aeruginosa* in a circulating model tap water system (175).

New methodologies are changing the approach to monitoring pathogens in drinking water and will therefore affect our ability in the future to assess risk. At present it is difficult to establish concentrations of specific, viable, and infective pathogens in drinking water. The increasingly widespread use and continuing development of flow cytometry, *in situ* polymerase chain reaction (PCR) to the single-cell level, magnetic separation techniques, and reverse transcription PCR (179–183) is dramatically improving detection of specific pathogens and pathogen viability in drinking water. Accelerated development of these techniques has been stimulated partly by the U.S. EPA Information Collection Rule—the requirement to monitor source waters for protozoa and viruses (184). Considerable effort has gone into developing routine techniques for *Cryptosporidium* monitoring. However, immunocapture PCR that is sufficiently sensitive and specific to monitor extremely low numbers of viruses in drinking water samples is also

at an advanced stage of development (179,185). The critical challenge is how to make these new methodologies cost effective for pathogen monitoring, particularly in developing countries where resources are extremely limited.

Risk assessment methodologies also need further development. Any calculation of risk depends on considerable speculation concerning exposure pathways to the drinking water supply, infectious dose, and population susceptibility. Although attempts have been made to assess risks from drinking water pathogens, and in some cases models do appear to approximately predict incidence of disease (186), uncertainties are enormous. Far better risk assessment methodologies are needed that take into account the uneven distribution of pathogens in drinking waters (187), include better estimations of infectious dose, and can more accurately predict the infectivity of an organism under environmental conditions (188,189). In addition, the inclusion of interactions among microbes and between microbes and chemicals in models that attempt to define



**Figure 2.** Confocal micrograph of a mixed biofilm of *Pseudomonas aeruginosa* and *Mycobacterium avium*. Biofilms were incubated with rabbit polyclonal antibody to Erdman lipoarabinomannan, a *Mycobacterium* cell-wall lipopolysaccharide, followed by goat anti-rabbit antibody conjugated to rhodamine. *M. avium* (brightly stained) appears to be clustered close to the biofilm substratum interface (left side) (micrograph courtesy of R. Rogers (Biomedical Imaging Laboratory, Harvard School of Public Health, Cambridge, MA). Bar = 20  $\mu$ m.

exposure risk, as is currently being attempted for chemicals alone (190), will eventually be necessary if accurate assessments are to be made.

## Concluding Issues

The global burden of infectious waterborne disease is enormous. Reported numbers dramatically underestimate incidence of waterborne disease, particularly the low-level endemic diseases that are widespread in both developed and developing countries. Pathogen survival strategies ensure that no treatment approach will be entirely successful in eliminating all pathogens from the drinking water supply. However, multiple barriers and optimization of treatment design can help to minimize the risks. Table 6 illustrates a typical approach.

There are, of course, effective alternatives to the scheme in Table 6 and approaches to water treatment and disinfection are an active area of research. For example, there is considerable interest in large-scale applications of membrane technologies for removal of pathogens and high molecular weight organic compounds. To achieve a multibarrier approach requires considerable resources and most water supply utilities are unable to meet these costs. At least for developed countries, a better understanding of the economic and health consequences of waterborne disease, attainable only through better monitoring and surveillance systems, may

help both the public and policy makers understand the value of microbiologically (and chemically) safe drinking water (3).

In developing countries where resources may be grossly inadequate, particularly in rural or transient communities, much can still be achieved by basic hygiene and sanitation programs. Population susceptibility may be reduced by immunization programs for other endemic diseases (22) and low-cost intervention programs can be introduced (191,192). With any intervention program, care must be taken to avoid creating new problems while resolving existing ones. For example, widespread arsenic poisoning has resulted in Bangladesh and West Bengal from contaminated groundwater, the result of programs to reduce epidemic diarrheal disease from use of surface waters (193,194).

## New Waterborne Diseases

A wide range of factors promote waterborne disease epidemics. When hygienic conditions are compromised, waterborne disease outbreaks appear inevitable. Irrigation with wastewater, floods and other natural disasters, poor source water quality, and inadequate or aging water treatment facilities or failing distribution system networks are all contributory factors. This has always been the case; however, alarming trends are becoming evident in the emergence and resurgence of waterborne diseases. There has been a

resurgence of older diseases in certain parts of the world, e.g., cholera in South America. However, it is more difficult to define the emergence of a new disease (195). New routes of exposure to previously uncharacterized pathogens may result in emergence of disease. Increasing numbers of susceptible individuals (very young, elderly, pregnant women and immunocompromised) (196) could provide an extensive human reservoir for opportunistic pathogens and promote changes in virulence patterns, even in developed countries. In addition, increased adaptation to the human host could increase infection rates in populations with no underlying susceptibilities. Clearly, these are areas in which far more research is necessary if future risks from waterborne disease are to be accurately evaluated.

Infectious agents categorized as emerging diseases and not recognized until recently, or at least not in association with water, include *L. pneumophila*, *C. parvum*, *E. coli* O157, *V. cholerae* O139, hepatitis E, and *H. pylori*. Perhaps we should also add to this list every waterborne pathogen that has developed resistance to antibiotics or changed apparent virulence as they emerge as higher mortality risks. Multiple antibiotic resistance has been shown to be widespread in waterborne bacterial pathogens, and, as for nonwaterborne pathogens, is well-documented and represents one of the greatest threats to public health (197–200). Examples exist for almost all waterborne bacterial pathogens and represent what seems to be an inevitable consequence of extensive use of antibiotics, not only in the human population but also in agriculture and aquaculture (201). Transfer of antibiotic and virulence factors in drinking water biofilms is a poorly understood area of research, but in principle they provide an ideal environment for horizontal gene transfer (177,202,203). Biofilms could, therefore, represent an important risk factor in dissemination of antibiotic and virulence genes. In addition, genes for polysaccharide synthesis, conveying increased resistance to chlorine and preference for biofilm formation, could also be transferred in drinking water biofilms.

## Critical Needs

In summary, needs for the future microbiological safety of water include:

- More realistic valuation of water. This requires better education on the value and limitations of the resource for both the public and policy makers.

**Table 6.** A multibarrier approach to maximize microbiological quality of water.<sup>a</sup>

Watershed protection that minimizes anthropogenic and wildlife impacts on source water, including programs to reduce the impact of waterfowl, particularly near water intake sites.

A treatment system with sufficient capacity to maintain adequate pressure throughout the distribution system for 24 hr/day, and that minimizes opportunities for microbial colonization in the distribution system. This could include

- Coagulation-flocculation and sedimentation<sup>b</sup> to remove colloids, associated microorganisms, debris, and macroorganisms
- Preozonation<sup>c</sup> to effectively kill microorganisms in source waters, reduce odor, taste and color, precursors for DBPs, and reduce the amount of chlorine/chloramine necessary to maintain a system residual
- Filtration to further remove particulates and microorganisms, including granular or biologic activated carbon<sup>d</sup> to remove AOC
- Chloramination<sup>e</sup> to minimize biofilm formation and reduce DBPs, with intermittent chlorination<sup>f</sup> and system flushing

A rigorous program to upgrade distribution system networks and prevent interconnections through leakage, backflushing, improper hydrant use, etc.

Information taken primarily from Geldreich (28). <sup>a</sup>To be protective of public health, a rigorous monitoring program for microbial and chemical contaminants in source and finished waters needs to be maintained. This should be easily cross-referenced with a surveillance system for GI disease. <sup>b</sup>Coagulation-flocculation and sedimentation is recommended by WHO prior to primary disinfection to reduce DBP formation (10). If ozone is used as a primary disinfectant, then this should also reduce formation of AOC. <sup>c</sup>If high concentrations of bromine are suspected in the source water, ozonation may cause formation of bromates and alternatives should be considered (160), e.g., chlorine, chlorine dioxide or chloramines. <sup>d</sup>It is important to note that these filters may themselves become a site for regrowth of coliforms or opportunistic pathogens, without careful control of bacterial growth. <sup>e</sup>Chloramination may be particularly appropriate in deteriorating distribution systems as it is more effective than free chlorine at the higher pHs used for corrosion control. <sup>f</sup>Alternating disinfection may reduce the ability of pathogens to adapt to the drinking water environment.

- Improved surveillance systems. The burden of waterborne disease is constantly underreported and surveillance systems are inadequate. Intervention studies (24) and population surveys are necessary to provide a clearer understanding of disease burden from contaminated water in both developed and developing countries.
- Improved water treatment. Water treatment approaches are needed that minimize selection for treatment-resistant pathogens, biofilm formation, and production of disinfection byproducts.
- Improved monitoring. Cost-effective, pathogen-specific monitoring is needed to begin to evaluate risk in both developed and developing countries.
- New disease. Improved techniques, including predictive models, are needed to recognize conditions that result in resurgent or emerging disease.
- Risk assessment. Improved risk assessment methodologies are necessary to better model exposure scenarios and provide realistic estimates of pathogen infectivity.
- Population susceptibility. A better understanding is needed of the role of increasingly susceptible populations in transmission and perpetuation of waterborne disease.
- Global issues. Reduction in the burden of waterborne disease and the risks of new disease emergence requires an aggressive surveillance system on a global scale [e.g., using the online ProMED system (204)]. The international community must be prepared to provide rapid assistance, without regard for political boundaries when epidemic or new disease is suspected.

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