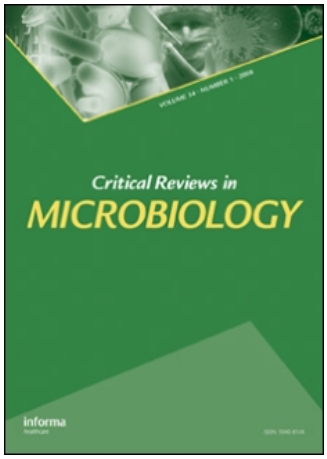

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Microbial Agents Associated with Waterborne Diseases

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I. INTRODUCTION

Four epidemiological categories were initially devised for classifying water-related infectious "diseases" with regard to the engineering technologies required to prevent or control them. The categories defined for water transmission of infectious agents were waterborne (classic and other); water washed (intestinal and body surface); water-based (dependent on intermediate aquatic host(s); and water-related insect vectors (breed in/bite near water).[1,2,3] Waterborne diseases are those transmitted through the ingestion of contaminated water and water acts as the passive carrier of the infectious agent.

The use of operative words such as "waterborne" and "disease" is justified for reports of outbreaks or cases of disease associated with drinking water because traditional epidemiological investigation relies on the occurrence of disease, which for drinking water is primarily waterborne. The use of "waterborne" as a generic term encompassing all infections arising from water use, however, is too simplistic and poses difficulties when attempting a theoretical analysis of water-associated infections, because not all develop from the ingestion of water. Likewise, "disease" should not be used synonymously with "infection", which may be either asymptomatic (without clinical expression) or symptomatic (clinically observable syndrome and therefore a "disease", when the causal agent was identified). It could be argued that if "disease" is not the end result of a water-related infection with a pathogenic agent then the role of water in its epidemiology is unimportant. However, many of the bacteria and viruses that may be spread in low levels by the water route produce asymptomatic infections. This seeding of susceptible individuals could result in an endemic situation with the potential for an epidemic to occur from direct person-to-person, food, or water spread (through contamination with greater levels of pathogenic agents released from infected carriers). Therefore, for the purposes of clarification and general discussion, the use of "infection" instead of "disease" is preferred.

This review attempts to describe present knowledge about waterborne diseases, their epidemiology, and the microbial agents commonly associated.

II. WATERBORNE DISEASES AND ETIOLOGIC AGENTS

A wide variety of bacterial, viral, and protozoan pathogens excreted in feces are

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capable of initiating waterborne infections, although the potential for this, predicted from latency, survival, and infective dose data, which is usually higher for viruses and protozoa, remains unknown in many cases. Waterborne spread of infection by pathogenic agents depends on factors such as pathogen survival in water and the dose required for establishing infection in susceptible individuals. In addition to pathogen survival, latency (the period between pathogen excretion and acquisition of actual infectious power) and pathogen ability for multiplication in the environment are factors influencing the infective dose. The minimum infective dose has been determined only for some bacteria, viruses, or protozoa that are excreted with feces and thus potentially water transmitted.

Therefore, it is important to distinguish different classes of pathogens.

1. Viruses may only remain static in number or die off. They cannot grow in the receiving waters; they cannot, indeed, multiply outside of susceptible living cells. The infective dose of these agents is low, typically in the range of one to ten infectious units.
2. Enteric protozoa such as *Giardia* and *Cryptosporidium* are highly resistant in the aquatic environment and to most disinfectants and antiseptics commonly used in water treatment. Like viruses, they cannot multiply in the receiving waters. The infective dose of *Cryptosporidium* in humans is highly variable in terms of the strain virulence[4] and probably of the host susceptibility.
3. The recognized waterborne bacterial pathogens include enteric and aquatic bacteria. The persistence of enteric bacteria, including *Salmonella* spp., *Shigella* spp., and *Escherichia coli,* in the aquatic environment depends on various parameters, but especially being a function of nutrients present and temperature. Although enteric bacteria are usually assumed to exist under starving conditions, there is evidence that some can grow in fresh water. Other bacterial infectious agents such as *Legionella* spp., *Aeromonas* spp., *Pseudomonas aeruginosa,* and *Mycobacterium avium* are indigenous aquatic organisms that can both survive and proliferate in drinking water. An additional mode of water-related transmission is through the direct inhalation of aerosols, specifically with *Legionella pneumophila*, the etiologic agent of Legionnaires' disease and probably with nontuberculous mycobacteria such as *Mycobacterium avium*. The infectious dose of historic enteric bacteria are in the range of 10^7 to 10^8 cells but much lower with some species, including *Shigella* spp., *Campylobacter* spp., and enterohemorrhagic *E. coli* O157:H7.

This discussion concerns the epidemiology of waterborne diseases, that is to say the rates of infection and characteristics of the diseases principally associated with waterborne transmission in developed countries. The situation is markedly different in developed or developing countries. In many worldwide countries, more illnesses and deaths result from sheer lack of availability of water in quantities sufficient for personal and household hygienic uses than from impurities in drinking water. These deficiencies in quantity or availability, along with malnutrition and lack of medical care, are responsible for the millions of deaths ascribed annually to diarrhea and enteritis, which are water associated, but much less commonly waterborne. In 1997 diarrhea disease ranked first in the WHO report's assessment of causes of morbidity (4.10^9) and sixth in causes of mortality.[5,6] It can be suggested that up to 70% of diarrhea illness could oc-

cur by contaminated food, and therefore 30% could result from polluted water origin. It should also be stated that food responsible for outbreaks could be contaminated by water.

The most complete data on waterborne disease are those reported for the United States by the Center for Disease Control and Prevention (CDC) and the Environmental Protection Agency (EPA). Two or more persons must show the same clinical symptoms before the incident is included in the statistics. Water systems are classified as community water systems, noncommunity systems, and private or individual water systems. National statistics dating back to 1920 on outbreaks associated with drinking water were analyzed by Craun[2,7,8] in an attempt to estimate the effects and adequacy of public health programs, regulations, treatment technologies, and microbial monitoring. Epidemiological course of waterborne disease in the U.S. (Figure 1) is marked by a clear-cut break of bacterial diseases during the period 1920 through 1998 with continuance of shigellosis, emergence of campylobacteriosis and *E. coli* O157:H7 hemorrhagic colitis, and correlatively by extended incidence of protozoan outbreaks from 1970s, including the largest documented incident in Milwaukee in 1993 since record keeping began in 1920. During the last decade, the etiology of acute gastroenteritis (AGI) was not determined in almost half of the outbreaks. However, in many of these outbreaks, a viral etiology (Norwalk agents, human rotaviruses, and adenoviruses) has been suspected and was supported by the detection of Norwalk agents in the US in 1980 to 1990s.[3,9] Several reports and reviews have been published recently in the U.S. on the concern.[6,10,11,12,13,14]

Unfortunately, there is a paucity of data available on the occurrence in Europe of outbreaks of water-related infections. This

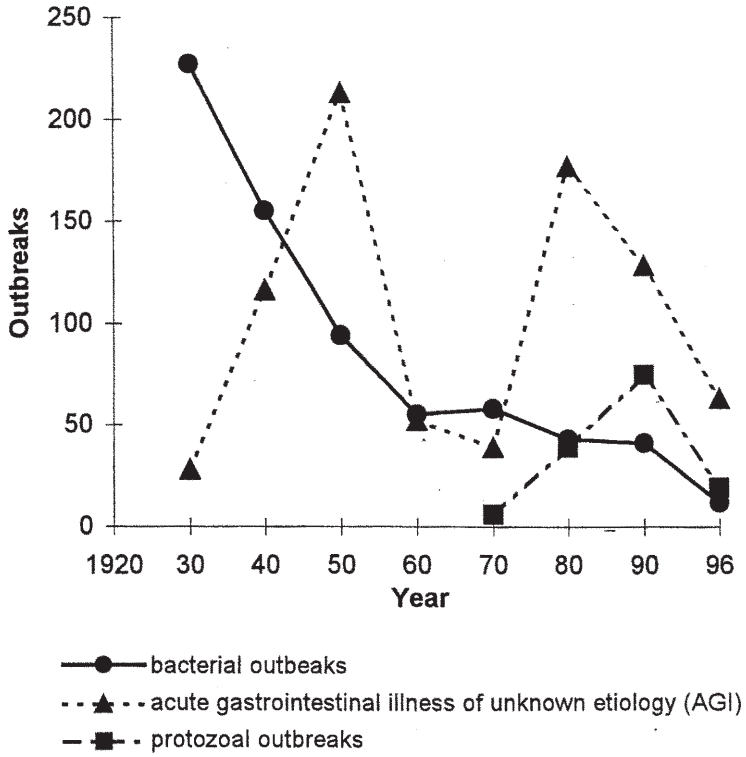

FIGURE 1. Etiology of waterborne outbreaks, United States of America, 1920–1996.

may be because water-related outbreaks are rare in Europe, but it is more likely attributed to the problems associated with epidemiological investigations of such outbreaks. Some reports on European waterborne disease have been published, including the U.K.[15] and Nordic countries,[16] while Hunter[17] conducted the most comprehensive review of waterborne disease world-wide.

It is generally agreed that waterborne outbreak reports are incomplete. A growing number of reports[6,8,18] indicate that waterborne disease is far more prevalent than reported outbreaks. Craun[7,8] suggests that only one-half to one-third, or even one-tenth, of waterborne outbreaks occurring in the U.S. are detected, investigated, and reported. According to Morris and Levin,[18] annual incidence in the U.S. could be 7 to 8 million cases of illness and 1200 deaths attributable to waterborne infectious disease.

III. BACTERIAL ENTEROPATHOGENS

A. Salmonella – Shigella

Waterborne outbreaks of bacterial origin in the U.S. have declined dramatically from 1920 through 1960 to 1970. This applies particularly to typhoid fever, which is seen rarely since 1970 as a result of the widespread application of disinfecting and filtration treatments, introduced between 1890 and 1900. Deaths from waterborne infections were almost entirely attributed to typhoid fever from 1920 to 1945 to 1950.[8] On the other hand, the number of reported outbreaks of salmonellosis has been relatively stable over the years, whereas those of shigellosis have increased (Figure 2). Since 1961, *Shigella sonnei* has become the most prevalent bacterial agent, replacing *S. flexneri* that dominated previously. The change in *Shigella* species causing the majority of cases of illness can probably be explained by a similar change in incidence of *Shigella* serotypes in patients.

B. Campylobacter

Waterborne outbreaks caused by *Campylobacter jejuni* in the U.S. (three outbreaks 1971 to 1980, 10 outbreaks 1981 to 1990, three outbreaks 1991 to 1996)[2,8] demonstrate that *Campylobacter* is now a leading cause of bacterial gastroenteritis in the commu-

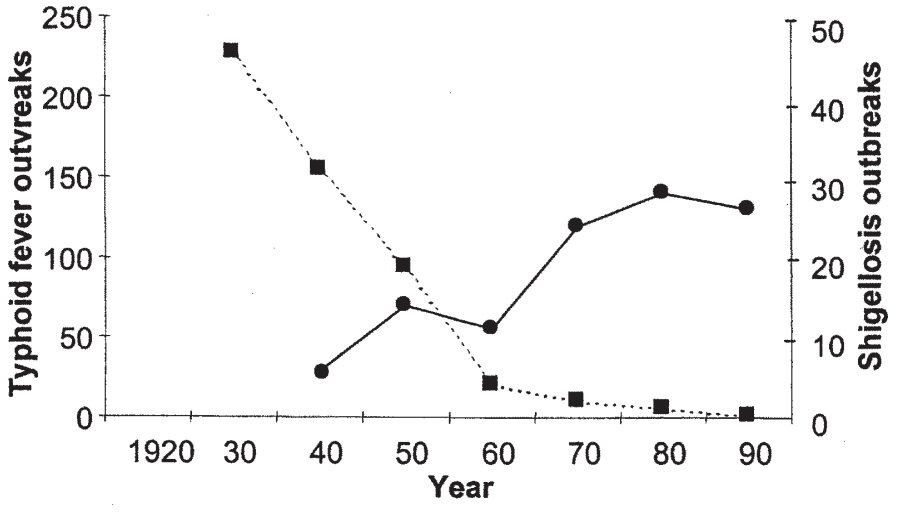

FIGURE 2. Comparison of typhoid fever and shigellosis occurring in the waterborne outbreaks (USA, 1930–1990). ■, typhoid fever outbreaks, ●, shigellosis outbreaks.

nity. It is isolated as commonly as *Salmonella* and *Shigella* from patients with diarrhea; it is the most frequently identified bacterial cause of diarrhea in the U.S. and the U.K. Human campylobacteriosis is a severe disease, often leading to serious sequel and sometimes resulting in death. Following the introduction of an improved surveillance system in England and Wales, the Communicable Disease Surveillance Centre observed 26 outbreaks between 1992 to 1995 in which there was evidence for waterborne transmission of infection.[19] In Nordic–European countries, campylobacters have been known as important human pathogens since the late 1970s. More recently, they were associated with presumed waterborne outbreaks;[20,21] in Finland, a waterborne epidemic in a hospital caused nearly hundred persons to become ill. [22] Other outbreaks have been described in Canada[23,24] and in New Zealand.[25]

Campylobacter species appear to be particularly well adapted to the avian intestinal tract, thus causing poultry to be the primary vehicle of transmission to humans.[26] *Campylobacter enteritis* is a zoonosis and animals used for food production, such as poultry, account for a large reservoir of environmental pollution. Virtually all surface waters contain campylobacters, even in remote areas, where contamination stems from wild birds. Waterborne outbreaks typically follow the consumption of untreated surface water that may be contaminated with bird feces. [27]

The occurrence of thermophilic campylobacters was studied in river and lake waters, some of which was the source of the drinking water. *C. jejuni* was recovered frequently from samples of fresh water.[28,29,30] Campylobacter-type bacteria were found, however, in treated water because they are susceptible to chlorination.[31] The first isolation of *C. jejuni* from groundwater[32] supports the theory that groundwater may be a vehicle for campylobacter transmission. In water microcosm experiments, factors such as temperature, oxygenation, anaerobic conditions, nutrient, and biofilms influence the survival times.[33,34] Survival was enhanced by the presence of autochthonous microflora and nutrient with decreasing temperature and anaerobic conditions. The existence of viable but noncultivable campylobacter, and their power for retaining pathogenicity and virulence[35,36,37] was a debatable point until it appeared elucidated by Cappelier et al.[38] and Talibart et al.[39]

The spread of *Arcobacter* organisms (previously designated as aerotolerant *Campylobacter*) via the drinking water path must be suspected.[40,41] Some cases of acute diarrhea, however, were associated with *Arcobacter butzleri*;[42] little is known about the clinical significance of infections in humans.

C. *Escherichia coli* O157:H7

Escherichia coli O157:H7, first recognized as a pathogen in 1982,[43] is now known as an important cause of bloody diarrhea (hemorrhagic colitis) and renal failure (hemolytic uremic syndrome) in humans. Its pathogenicity has been attributed in part to the production of toxin cytotoxic for Vero cells (a fibroblastic green monkey kidney line cell). The toxin was appropriately named Verotoxin (VT), and the group of *E. coli* that produce VT became known as the Verotoxin-producing *E. coli* (VTEC).[44] Subsequently, other verotoxin-producing *E. coli* strains have been associated with hemorrhagic colitis and hemolytic ureic syndrome.[44] *E. coli* O157:H7, however, is recognized as the most common cause of VTEC-related human illness. VTEC, including *E. coli* O157:H7, are strongly associated with cattle, and they can clearly pass through stomachs of ruminants. The transmission of VTEC O157 is often foodborne, particularly from contaminated ground

beef or raw milk, or person-to-person, and contact with farmed animals has also resulted in human infection. Farmed and wild animals grazing in water catchment areas, however, are a potential source of fecal contamination, and therefore of waterborne VTEC O157 infection. VTEC O157 is particularly adapted for surviving in the aquatic environment, as suggested by outbreaks associated with swimming in lakes (Wang and Doyle, 1998).[45,46] It is capable of surviving for many days, especially at cold temperatures.

The waterborne route of VTEC O157 infection was first clearly demonstrated by an unusually large outbreak by unchlorinated municipal water in the Missouri Community.[47] Since then, waterborne VTEC O157 has been described in sporadic cases and in outbreaks of illness. Chalmers et al.[48] have analyzed published outbreaks of VTEC O157 associated with recreational waters, private and municipal supplies. Despite the potential for large contamination of environment with VTEC O157, however, waterborne infection is relatively rare because VTEC O157 is as susceptible to chlorination as bacterial indicators.[48]

Epidemiological investigations have elucidated the mechanisms by which *E. coli* O157:H7, *Shigella* spp., and campylobacters have become a source of concern recently. The most striking feature is the low inoculum of organisms that may trigger disease. As few as 10 to 100 organisms of the most virulent *S. dysenteriae* type 1 are sufficient to cause clinical dysentery, while the other species may require a 10 to 100 times more elevated dose.[49] The dose required to trigger campylobacteriosis is also low, probably no more than a few hundred bacteria.[50,51] *E. coli* O157:H7, like *Shigella* and *C. jejuni*, appears to have a low infectious dose, approximately some hundred organisms or less.[52]

D. *Yersinia enterocolitica*

Bacteria of the genus *Yersinia* have been isolated commonly from the environment, including water supplies of various types. Environmental strains therefore should be differentiated from serotypes O:3, O:9, O:5, O:27 and O:8 of *Y. enterocolitica*, which are the most frequently ones associated with human infections in Europe, Japan, Canada, and the U.S.[53] These pathotypes are psychrotrophic, and hence can multiply in fresh waters and could constitute a major hazard to drinking water. Epidemiological data concerning waterborne yersiniosis, however, are scarce (Schieman, 1990)[53] and involve only a small number of individuals. A large outbreak of intestinal illness at a Montana ski resort in 1977 was suggested to be waterborne yersiniosis,[54] but the many strains isolated from water sources were later identified as nonpathogenic, leaving the cause of this outbreak uncertain.[17] Three prospective studies from Norway[55,56] and from New Zealand[57] seem to demonstrate that, however, *Y. enterocolitica* could be waterborne in these area.

Y. enterocolitica became the repository for a large number of "atypical" or *Y. enterocolitica*-like strains. Usually isolated from terrestrial and fresh water ecosystems, they are often referred to environmental strains, including nonpathogenic serotypes of *Y. enterocolitica* and the related species *Y. aldovae*, *Y. bercovieri*, *Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. mollaretii,* and *Y. rohdei*.[58] These aquatic environmental strains were, however, relatively common in the examined water supplies.[59,60,61]

E. *Vibrio cholerae*

Cholera caused by toxigenic *Vibrio cholerae* is a major public health problem concerning developing countries, where *out-*

breaks occur in a regular seasonal pattern and are associated with poverty and poor sanitation. Among the 193 currently recognized O serogroups *of V. cholerae*, only serogroups O1 and O139 have been associated with epidemics of cholera. The other serogroups usually referred to as non-O1, non O-139, can cause sporadic diarrhea.[62] This sharp distinction between serogroups is related to virulence associated genes. The strains belonging to serogroups O1 and O139 (more than 95%) produce cholera toxin (CT) and colonization factor known as toxin-coregulated pilus (TCP) that is coordinately regulated with CT production. In contrast, the strains of non-O1 non-O-139 (more than 95%) serogroups lack these two virulence genes.

From 1817 to 1994, seven distinct pandemics of cholera occurred; all these were caused by *V. cholerae* O1. During the first six pandemics, cholera remained principally confined to South and Southeast Asia, whereas the seventh pandemic reached West and East Africa and South America. In late 1992, *V. cholerae* belonging to a non-O1 serogroup (now referred as O139 Bengal) caused explosive epidemics of cholera through India, Bangladesh, and neighboring countries.

Systematic surveillance of cholera in these countries from 1992 to 2000 revealed temporal alternation or coexistence of *V. cholerae* O1 and O139 in different regions.[63,64,65] A clone of serogroup O37 demonstrated epidemic potential in the 1960s.[66] The close evolutionary relationships among O1, O139 and O37 epidemic clones indicates that new cholera clones were likely arisen from a lineage that was already epidemic or closely related to such a clone.[63,66,67,68] An endemic focus of cholera was reported in the U.S. in 1970 to 1990s. Most cases were associated with the consumption of undercooked crabs or shrimp, or raw oysters from the Gulf of Mexico.[69] In Europe, several countries were affected (concerned) by cholera in the 1990s.[70,71,72,73]

V. cholerae is now recognized as an autochtonous member of the microflora in many aquatic environments such as in riverine and estuarine areas. The importance of water ecology is suggested by the close association of *V. cholerae* with surface water and population interacting with the water-food also plays an essential role, although in many instances water is the source of contamination of foods.[74] Many ecological aspects remain unknown to explain seasonal appearance epidemic *V. cholerae* strains and outbreaks of cholera. Recent data suggest that *Vibrio* features can be involved:

1. The state of an aquatic reservoir of *V. cholerae* O1 or O139 being capable not only to survive in water but also to form a complete component of the ecosystem.[75] It has been postulated that under stress conditions the vibrios can be converted to a viable but nonculturable form (VNC)[76,77,78] that can be reverted back to live infectious bacteria.
2. The major pathogenic gene in toxinogenic *V. cholerae* are clustered in several chromosomic regions (CTX genetic element and TCP pathogenicity island) that are capable of being propagated horizontally to non-O1 and non-O139 strains by lysogenic conversion.[63] More recently, Chakraborty et al.[62] have demonstrated the occurrence and expression of critical virulence genes in environmental strains of *V. cholerae* that appear to constitute an environmental reservoir of virulence genes. These new data on the ecology of *V. cholerae* appear to be of great significance. Likewise, that non O1 and non O139 strains are more commonly detected than O1 and O139 strains, in fresh water and estuarine areas, is also relevant. [63,79,80]

In outbreaks with a bacterial etiology, the CDC and the EPA make efforts to relate waterborne-disease surveillance with processing deficiencies. Thus, it has been established that untreated surface or ground water, inadequate disinfecting procedures or distribution system were the major causes of waterborne outbreaks; control of such deficiencies is usually within reach. These conclusions substantiate the basic concepts of management of enteric diseases by multiple barriers and monitoring by using indicators.[6]

IV. PUTATIVE BACTERIAL PATHOGENS THAT GROW IN WATER SUPPLIES

Like fecal pathogens that may have access to the aquatic environment and survive or remain viable but nonculturable, other bacterial pathogens are able to multiply in water even with low levels of organic nutrients. They are opportunistic pathogens in humans, but concern about their occurrence in drinking water is disputed. The most important organisms to consider are *Pseudomonas aeruginosa, Aeromonas, Legionella,* and *Mycobacterium avium* complex.

A. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an ubiquitous microorganism, inhabitant of fresh waters, soil, and plants. This bacterium has been isolated from numerous vegetables, such as tomatoes, radishes, cucumbers, onions, and lettuces[81] at rates capable of reaching 10^3/g. Its presence is constant and abundant in waste waters[82,83] and consequently in surface waters that receive polluted effluents. Its growth in water is not directly linked to the organic matter content, because it can develop abundantly in the purest of waters. *P. aeruginosa* is a species of considerable versatility and a significant pathogen that can cause infection in a variety of plants, insects, and warm-blooded animals. In man it is an opportunist pathogen, well known in the hospital environment; it seems likely to be the cause of 10 to 20% of nosocomial infections. Its extreme resistance to antibiotics explains why this ubiquitous bacteria has been selected to colonize the skin and mucous membranes of patients.

As some *P. aeruginosa* strains are capable of producing enterotoxins, the enteropathogenicity of this species was sometimes surmized. Since 1894, many later publications have recognized this bacterium as an enteric pathogen and causative agent of diarrhea in infants and children.[84,85,86,87] Enteric disease associated with septicaemia was described earlier by Dold in 1918[88] as "Shangai fever", a prolonged febrile illness that affected both children and adults. As expounded by Hardalo and Edberg[89] the colonization of children by *P. aeruginosa* quickly ceases once the environmental sanitary conditions are corrected. Moreover, each of these "infections" was diagnosed before there were adequate means of precluding a viral or protozoan etiology. Community acquired *P. aeruginosa* gastrointestinal disease with sepsis rarely occurs in healthy infants, that is, those who lack identified underlying immunological or haematological problems.[90] On the other hand, *P. aeruginosa* can be a colonizer of the gastrointestinal tract in immunocompromised and hospitalized adults and children. There have been no significant outbreaks reported in recent decades, possibly as result of better hygienic control measures and diagnostic techniques.[90] Moreover, a 1969 study[91] demonstrated that the ingestion of up to 10^6 viable *P. aeruginosa* did not lead to infection or colonization, but only to a brief period of recovery of the organism from the stool.

In the past, Hoadley[92] considered the presence of *P. aeruginosa* in drinking water as a public health risk. However, *P. aeruginosa* is predominantly an environ-

mental organism and fresh surface water an ideal reservoir. As a consequence of contemporary lifestyles, *P. aeruginosa* reaches relatively high numbers in food and on moist surfaces. Daily, substantial numbers of the species are ingested with our food, particularly with raw vegetables, while our body surfaces also are in continuous contact with the organism. The risk to the general population originating from *P. aeruginosa* in drinking water is insignificant, and Hardalo and Edberg[89] think that attempts at guideline for *P. aeruginosa* in drinking water would not yield public health protection benefits. On the other hand, this bacterium is primarily a nosocomial pathogen.[93] There is abundant evidence that specific hosts are at risk for an infection with *P. aeruginosa*, including patients with deep neutropenia, cystic fibrosis, severe burns, and those subject to foreign device installation.[94,95,96] For these highly vulnerable hosts water supplies in hospitals with *P. aeruginosa* should be avoided.

B. *Aeromonas*

Many experimental, clinical, and epidemiological data tend to lend credence to the assertion that *Aeromonas* may be etiologically involved in diarrhea illness.[97,98,99] Some authors are more cautious and consider that only some strains are likely to be pathogenic, a situation similar to that with *E. coli* and *Y. enterocolitica*.[100] Beyond any doubt, *Aeromonas* may be isolated as many times from the feces of patients with diarrhea as persons without diarrhea, suggesting that *Aeromonas* would as a rule be a nonpathogenic "fellow traveler". The most striking argument against the role of *Aeromonas* in human diarrhea emerged from studies of Morgan et al.[101] with human volunteers; despite that high challenge doses were used, this investigation failed to establish *Aeromonas* spp. as an enteropathogen. However, the pathogenicity of aeromonads may be strain or pathovar related.

Aeromonas spp. are widely associated with environmental waters; most available strains were not from human fecal origin, but represented the natural habitat of the organism. The concentration of mesophilic aeromonads range from 10^6 to 10^8/ml in crude sewage to 10 to 10^3/ml in clean river water like in superficial ground water.[102,103,104,105] Aeromonads readily multiply in domestic or industrial wastewater and are also found in siphons, sinks, and drainage systems.[103] Temperature is an important factor for enhancing the colonization by aeromonads in wastewater. That is probably the reason why *Aeromonas* spp., which are psychrotrophic organisms, outgrow coliforms in sewage.[103,106,107] Hence, aeromonads are natural residents in wastewater and fresh surface water, their rate is closely related to organic load and temperature. On the other hand, it appears that aeromonads survive poorly in nutrient-poor waters in comparison with other autochtonous oligotrophic bacteria.[103,108] Since 1962, we have demonstrated that 30% of drinking water samples found positive for "fecal-coliforms" indeed contained strains of *Aeromonas* that would have falsely indicated that the sample was positive in the fecal coliform test.[109] These observations have since been confirmed by many teams.[103,110,111]

The frequent presence of *Aeromonas* in drinking water raised the question of its role as an enteric pathogen, because production of enterotoxins and/or adhesins had been demonstrated.[74,112,113] Burke[110] observed that *Aeromonas* spp.-associated gastroenteritis was closely correlated with mean number of *Aeromonas* spp. in water samples within the distribution systems. The epidemiological relationship between aeromonads isolated from humans and those isolated from distribution system has been studied by typing.[114]

These investigations demonstrated conclusively that the aeromonads isolated from the public water supply were unrelated to those isolated from patients with gastroenteritis. With regard to the epidemiological relationship with drinking water, in contrast to other waterborne pathogens, no clearly defined outbreaks of diarrhea illness due to *Aeromonas* have ever been reported, although this bacterium is frequently isolated from water.[115]

C. *Legionella*

The genus *Legionella* had at least 42 named species,[116] among which *L. pneumophila* serogroup 1 is most frequently related to human disease. *Legionella* infections can lead to two forms of disease, namely, legionellosis or Legionnaires' disease, an acute purulent pneumonia and Pontiac fever, a self-limiting nonpneumonic disease consisting of fever and mild constitutional symptoms. Infection is the result of inhalation of contaminated aerosols.

Legionella is a common inhabitant, usually in low numbers, of natural aquatic habitats and of water supplies that meet drinking water standards.[117] A number of abiotic factors, of which temperature is the most important one, significantly influence *Legionella* survival and growth.[118] Therefore, hot water tanks, cooling systems, and towers,[119] because of their heat-exchanging function, serve as bacterial "amplifiers". Then, *Legionella* spp. are able to colonize all parts of internal distribution systems of hospitals, hotels, or buildings.[120,121,122] Thus, these bacteria can potentially infect susceptible persons through aerosols created in showers bubble baths, sinks, etc.

Evidence also indicates that *amoebae* and other protozoa may be natural hosts and "amplifiers" for legionellae in the environment. Invasion and subsequent intracellular replication of *L. pneumophila* within protozoa in the environment should play a major role in the transmission of Legionnaires' disease.[117,123,124,125,126] Recently, it has been shown that viability and infectivity of environmental noncultivable *L. pneumophila* can be restored by intracellular replication within protozoa.[127] Intracellular growth even enhances the infectious power of *L. pneumophila* to human-derived cells.[128] At some stage after the ingestion of bacteria, amoebae produce vesicles that contain high numbers of legionellae, which may reach the alveoli of the lungs and there constitute an infectious dose.[129]

It appears that it is impossible to prevent the contamination of water supply systems and reservoirs with legionellae during extended periods of time by thermal eradication or hyperchlorination.[124] The risk of infection following exposure to *Legionella* cannot be assessed and remains open to speculation.[130] Therefore, risk management strategies should be introduced to control *Legionella* at locations where a health risk is recognized, that is, in water supply systems of hospitals, in public spas, in swimming pools, and whirlpools. Quantifying relative risks for exposed robust vs. debilitated persons is yet impossible. However, special measures of surveillance and intervention should be introduced for people with reduced resistance against respiratory disease, such as elderly people, diabetics, etc.

D. Nontuberculosis Mycobacteria

The *Mycobacterium tuberculosis* complex (*M. tuberculosis*, *M. bovis*, and *M. africanum*) is composed of species pathogenic for man and animal, and "nontuberculosis mycobacteria" (NTMs) or "mycobacteria other than *M.* tuberculosis", include the formerly called "atypical" mycobacteria. In a benchmark review[131] the evidence was summarized that some nontuberculosis mycobacteria were able to cause disease. The most common among these include the *Mycobacterium avium* complex

(MAC) composed of *M. avium* and *M. intracellulare* clearly different species based on DNA-DNA homology and 16S rRNA sequences.[132] On the other hand, on the basis of DNA homology and phenetic relatedness, it has been proposed that *M. avium*, *M. paratuberculosis,* and the wood pigeon bacillus be placed in one species with the designations *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, *M. avium* subsp. *silvaticum,* respectively.[133] It should be noted that a "MAIS" complex, including *M. avium*, *M. intracellulare*, and *M. scrofulaceum* has been occasionally described. However, *M. scrofulaceum* is genetically and phenetically distinct from *M. avium* and *M. intracellulare* and is not a member of the *M. avium* complex.[134] Several other reviews[135,136,137,138] have contributed to an increase in evidence that the environment and more particularly water may be the vehicle by which these organisms infect or colonize man.

The concern about nontuberculosis mycobacterial disease has been changed radically by the emergence of the AIDS epidemic throughout the world. Before the AIDS epidemic and still today in immunocompetent people, nontuberculous mycobacterial disease was primarily pulmonary and the major pathogens were *M. kansasii*, *M. avium,* and *M. intracellulare*. In the absence of evidence of person-to-person transmission, it was suggested that man is infected from environmental sources via aerosols.[131] *M. scrofulaceum* was considered the causative agent of cervical lymphadenitis in children, and *M. marinum* was associated with skin infections originating from aquaria or swimming pools. However, with the advent of the AIDS epidemic in the U.S. and Europe, in AIDS patients and other immunodeficient individuals, nontuberculous mycobacterial disease is usually systemic with acid-fast organisms being isolated more commonly from either blood or stool and caused principally by *M. avium*. Therefore, infections possibly occur via the lungs or gastrointestinal tract. Thus, a wider range of sources and routes of exposure has had to be investigated in AIDS patients. In Africa and other areas of the developing world, where the incidence of tuberculosis is high, the rate of nontuberculous mycobacterial disease in AIDS people is low.[139] In all probability AIDS patients die of other infections before they reach a stage at which slow onset *M. avium* disease develops.

E. Occurrence of Nontuberculous Mycobacteria and Transmission in Water

In contrast to tuberculous bacteria that live and grow in human tissue, nontuberculous bacteria are free-living saprophytes that are widely distributed in the environment: water, soil, dust, and aerosols.[138,140,141] They have been recovered from many piped and treated drinking waters throughout the world.[135,136,142,143,144,145,146] NTMs are not contaminants picked up from another source, but residents able to survive and grow in water.

The physiological characteristics of nontuberculous mycobacteria have provided an understanding of their ecological distribution;[138] (1) they grow best at low pH values, that is, between 5 and 5.5, and (2) microaerobically;[138] (3) *M. avium* strains grow at 45°C, unlike *M. intracellulare* that can grow to only 42°C; (4) they grow equally well in water with a high level of salt and are found in large numbers in brakish swamps and estuaries; (5) they are hydrophobic and collect largely at air-water interfaces; (6) they are relatively resistant to heavy metals and may be isolated from water by highly polluted metals; (7) it has been shown that they may be responsible for degradation of a wide variety of xenobiotic organic coumpound; they are capable of transforming compounds not readily biodegradable such as humic and fulvic acids;[147] (8) their high resistance to disinfection

by chlorine undoubtedly contributes to their persistence in drinking water systems.[138] Several experimental data suggest a role for protozoa present in water environments as host for pathogenic mycobacteria, such as *M. avium*.[148,149] These attributes favor their survival and even occasional growth in natural waters such as fresh water, salt water, and estuaries, in piped and treated waters such as drinking and domestic waters, swimming pools and aquaria, public baths, and whirlpools.[135,136,138,141,146,148] Increases in the immunodeficient population and the prevalence of nontuberculous mycobacteria in water systems contribute to an emerging problem of waterborne mycobacterial infections.

Von Reyn et al.[144] were among the first to document a relationship between infections in AIDS patients, and water as the source of the *M. avium* complex, examination of isolates from patients and from waters by PFGE showing identical patterns. The study from Aronson et al.[150] also supports the possibility that potable water is a source of the nosocomial spread of *M. avium* infections in hospitals. Recirculating hot water systems, used in many institutions such as hospitals, hotels, apartments, and office buildings, may allow thermotrophic and chlorine-resistant mycobacteria to persist and colonize once they have been introduced from municipal systems.[136,142] Infection with *M. avium* complex is thought to occur from colonization of the gastrointestinal tract,[151] although respiratory access has also been documented.[152] Therefore, hot water showers may be the source of infection, but it cannot be excluded that drinking water acts as a possible source, because a common tap may deliver both hot and cold water.[153]

Before the emergence of the AIDS epidemic, *M. kansasii* infection were generally more common than *M. avium* complex infections. The AIDS epidemic has had, in most countries, a striking effect on the incidence of disease caused by the *M. avium* complex but not *M. kansasii*. Indeed, between 1984 to 1992 there has been a 10-fold increase in the number of *M. avium* complex infections compared with *M. kansasii* infections in England.[154] *M. kansasii* disease in immunocompetent patients (e.g., those with AIDS) can be disseminated or exclusively Pulmonary. There have been a number of reports relating the occurrence of *M. kansasii* in drinking water and shower heads.[135,138,155,156] Therefore, like with the *M. avium* complex infections, the water exposure route may be possible.

M. paratuberculosis is the agent of Johne's disease in cattle. Several data have suggested that *M. paratuberculosis* may be also implicated with Crohn's disease in humans, which is a chronic, invalidating, inflammatory disease of the gastrointestinal tract.[138] Epidemiologic studies of incidence and geographic distribution of disease have led Herman Taylor et al.[157] to propose that it is due to the transmission of *M. paratuberculosis* via water contaminated by cattle feces. If it is later proved that *M. paratuberculosis* or wood pigeon strains of *M. avium*[158] are the responsible bacteria of disease, human infection would also occur through exposure to contaminated water (e.g., drinking or aerosols).

V. *HELICOBACTER PYLORI*

The assumption that *Helicobacter pylori* is waterborne needs to be substantiated. Half of the world's population is infected with *H. pylori*, making it a pathogen of potentially great significance. Although in the majority of cases, infection is harmless, many infected people develop chronic gastritis, peptic ulcer disease, or gastric cancer.[159] Although all evidence shows that *H. pylori* is well suited to attach to the gastric mucus and the gastric epithelium, it is difficult, however, to establish whether this ecological niche is the only one. Studies suggest four transmission ways:[160,161,162,163]

1. By the fecal-oral route, the bacterium, excreted by feces, might colonize water sources, becoming available to be transmitted to man;
2. By the oral-oral route, *H. pylori*, which colonizes dental plaque and saliva, may be transmitted by saliva to other individuals (person to person transmission);
3. By the gastric-oral way, the typical modality of transmission in childhood, by contaminated vomit;
4. Finally, by the gastric-gastric route, the bacterium might be transmitted by endoscopic procedures.

The natural route of transmission is by gastric juice, specifically as a result of epidemic vomiting in childhood.[161,162] The different modalities of transmission may be contemporaneously involved, however, because no one *per se* is able to explain the widespread occurrence of *H. pylori* infection. Many studies have examined the possibility that *H. pylori* is waterborne. *H. pylori*-specific DNA was detected in sewage,[164] surface water,[165] and water supplies,[166,167,168] although the organisms should be readily inactivated by free chlorine.[169] Actively respiring bacteria were found by monoclonal antibody in the majority of surface and shallow groundwater samples tested in the U.S.[170] The survival capacity of *H. pylori* is related to the noncultivable coccoid form that may persist up to 20 to 30 days in water[165,170,171] and also in food.[172]

Studies of the prevalence or seroprevalence suggested that drinking water might play some role in infection with *H. pylori*,[168] which is unconfirmed by other works.[173,174] More and more data show that *H. pylori* DNA can be detected by PCR from feces samples of infected individuals or patients with peptic ulcers,[175,176,177,178] strongly suggesting fecal-to-oral transmission. The detection of *H. pylori* antigen in stools (Hp SA) brings new promise for diagnosing *H. pylori* infection.[179] The results obtained from *H. pylori* infection in an experimental murine model also seem to support an oral-fecal route as the mode of transmission.[180]

However, many characteristics make *H. pylori* a special bacterium in the world of human pathogens. A long way remains for the epidemiology of transmission, while the environmental occurrences of this pathogen are better defined.

VI. PATHOGENIC PROTOZOA

The most prevalent enteric protozoa associated with waterborne disease include *Giardia lamblia* and *Cryptosporidium parvum*.[127,181,182,183,184,185] The last species, *Cyclospora cayetanensis, Isospora belli,* and many Microsporidian species appear like emerging pathogen protists.[186,187,188,189]

A. *Giardia, Cryptosporidium*

As a result of proliferation and evolution in the intestinal tract, cysts (*Giardia*), oocysts (*Cryptosporidium*, *Cyclospora*, *Isospora*) or infective spore (Microsporidia) are produced and excreted in feces in a fully infective form (*Giardia, Cryptosporidium*, Microsporidia), or like immature stages (*Cyclospora, Isospora*), which will shortly complete their development in the environment acquiring their infectious power. Giardiasis has become the most common cause of human waterborne disease in the U.S. over the last 30 years. The first outbreak was documented in 1965 at Aspen, Colorado.[2] From then until 1996, 122 outbreaks involving 27,000 patients have been reported (Table 1).

The Apicomplexan *Cryptosporidium* has only more recently been recognized as a cause of waterborne outbreaks. The first occurred in 1985 in Texas, with sewage-

TABLE 1
Enteric Protozoa and Waterborne-Disease Outbreaks

Enteric protozoa	Waterborne-disease outbreaks	High risk for AIDS
Prevalent pathogens		
Giardia lamblia	from 1961 : 122*	
Cryptosporidium parvum	from 1985 : 10*	+++
Emerging pathogens		
Cyclospora	1994 : 1*	?
Microsporidia	no related	+++
Isospora	no related	+++

* in U.S.A.

contaminated ground water as the source of the infection.[190] The outbreak in Milwaukee was the largest documented waterborne incident ever in the U.S. since 1920. An estimated 403,246 cases of diarrhea were recorded over a span of 2 months, 4400 persons were hospitalized and 69 deaths were recensed among immunocompromised patients.[191,192] From 1985 to 1996, 10 outbreaks occurred in the U.S. that resulted in 419,914 cases of illness.[184] Many outbreaks have also been reported in the U.K.[17] The size and severity of waterborne cryptosporidiosis have prompted investigations in depth about their etiology and transmission. A striking result was that many outbreaks of giardiasis and cryptosporidiosis were found associated with potable water, even when processing systems had been operated in accordance with conventional standards of water treatment, and while current microbiological standards were met.[183,181,193] Relying on *Cryptosporidium* as a model protozoan pathogen, investigations by Casemore,[181] Rose,[194] Current and Garcia,[182] and Meinhart et al.[183] attempted to clarify the transmission patterns for these diseases, and the problems to be overcome in their management and monitoring for compliance. These are briefly reviewed below.

1. A broad variety of animal reservoirs, including farm livestock, pets, and wildlife spread oocysts to man and the water environment, particularly through agricultural and human effluents, compounded by an abundant rainfall. In addition, zoonotic and person-to-person transmission occurs frequently. Oocysts excreted by man and animals in large numbers and in fully infective form constitute probably the main risk factor for the spread of *Cryptosporidium* in aquatic environment.[183,194,195] The contamination of water sources may lead to penetration of water treatment plants and drinking water supplies.[17,183,196]
2. *Cryptosporidium* oocysts possess a substantially elevated resistance against disinfectants used in water decontamination, with the exception of ozone and chlorine dioxide.[197,198] The physical removal of the parasite by coagulation and filtration should, in theory, overcome the risk. All outbreaks of waterborne cryptosporidiosis that occurred in the U.S. between 1985 and 1993, however, hit communities where surface water supplies had been filtered and where processing equipment met federal and state standards. According

to Gale,[199] available evidence suggests that many pathogenic microorganisms, including cryptosporidia, are clustered to some degree, even within small volumes, exposing some drinking water consumers to much higher doses than others. By assuming that enteric pathogens are randomly dispersed, current models underestimate the risk of contracting infectious diseases.[200] Modeling pathogen densities in drinking water obtained from source water, and performance criteria for treatments, for example, removal of efficiencies calls for more information on the degree to which treatment process (e.g., filtration and coagulation) affect pathogen clustering. Also, the practice of recycling filter backwash water can lead to breakthrough of oocysts into finished water.[201] Finally, oocysts of *Cryptosporidium* can attach to and persit in biofilms, then be released by sloughing into the water system. By such mechanisms, some persons may be exposed to infectious clusters, whereas the majority of consumers are not.

3. The infective dose of *Cryptosporidium* in humans is not exactly known. Experiments in human volunteers[202,203] obtained dose-response curves indicating a median infective dose (ID_{50}) of 132 oocysts and a minimum infective dose of less than 30. A more recent study[4] reported that the minimal infective dose for immunocompetent volunteers can vary between 10 and 1000 oocysts in terms of the *Cryptosporidium* strain. Studies using animal models showed that as few as one to ten oocysts may initiate an infection.[183,204] However, IDs vary strongly with the viability of oocysts that depends on incurred environmental stress, and on their virulence. Nevertheless, the model of risk assessment developed by Perz et al.[185] tends to demonstrate that transmission of oocysts may occur at low levels of the pathogen in drinking water.[205] With respect to the Milwaukee epidemic, mathematical modeling combined with epidemiological data suggest that persons might indeed become infected after exposition to only one oocyst.[192] Waterborne cases have been related, however, in the absence of detectable oocyst levels as in the Las Vegas outbreak.[206]
4. Recent molecular studies on *Cryptosporidium* genetic diversity are changing basically the usual views about the epidemiological pattern of cryptosporidiosis. Until recently, *C. parvum* was the species considered like responsible for cryptosporidiosis both animals and humans. Consequently, human cryptosporidiosis was usually considered a typical zoonosis. In fact, on the basis of isoenzymatic and molecular studies, two genotypes (1 and 2) were revealed in the species *C. parvum*. Genotype 1 was found only in humans, whereas the genotype 2 was found in both infected humans and livestock. Moreover, cross-infection studies have shown that the bovine genotype infects readily mice and cattle, while the human genotype does not.[207,208,209] Multilocus analysis indicated that the two genotypes are genetically isolated.[210,211] These results suggest the existence of two *C. parvum* separate transmission cycles: one that may occur in humans or animals, and other that exclusively passes through humans. However, subsequent studies are revealing a situation that is still more complex. Thus, in addition to the two major bovine and human genotypes, six other genotypes were identified in *C. parvum* isolates from domestic or wild mammals (dog, mouse, pig, ferret, marsupial, and monkey). Furthermore,

the species *C. meleagridis* (from birds), *C. felis,* and *C. muris* have been identified in human patients from the New or the Old World.[212] Indeed, as only molecular methods allow species or genotype identification of human, animal, or environmental *Cryptosporidium* isolates,[213] new *Cryptosporidium* taxonomy and cryptosporidiosis transmission patterns are being defined on molecular bases.

5. In immunocompromised persons, particularly AIDS patients, symptoms of illness are of a greater severity than in immunocompetent people, which may result in death of the former.[185,183] Most waterborne cryptosporidiosis outbreaks have substantiated the more fulminant reaction in AIDS patients. Among the 69 deaths in the Milwaukee outbreak the majority of victims were AIDS patients.[191]

B. *Cyclospora, Isospora*, Microsporidia

In addition to *Giardia* and *Cryptosporidium*, Apicomplexa protozoa like *Cyclospora*, *Isospora,* and many Microsporidian species are emerging as opportunistic pathogens and may have waterborne routes of transmission. Overall, recently species have received attention because of their high infection rates in AIDS patients.[186,188,214,215]

Before 1995, the parasite *C. cayetanensis* was primarily described in gastroenteritis among children living in poor sanitary conditions and in travelers who had visited developing countries. Several outbreaks of *Cyclospora* infection (or isolated cases) have linked to waterborne transmission[216,217,218] but in only one case[216] were *Cyclospora* oocysts demonstrated in drinking water. In 1996, the largest ever reported outbreak of cyclosporiasis, affecting more than 1400 persons in North America[219] was associated with eating fresh raspberries from Guatemala. Most likely, fecal-polluted water, used for spraying biocides on fruit, was the indirect source. Notwithstanding technologic limitations, *Cyclospora* have been recovered in limited numbers from water sources and vegetables.[188,191]

Unlike *Cryptosporidium,* which has many known animal hosts,[220] *Cyclospora*-like organisms have been recovered from ducks, chickens, dogs, and primates,[187] perhaps having been passed through these hosts. The Apicomplexa *Isospora belli* is among the protozoa that most commonly causes gastroenteritis in immunocompromised hosts, such as patients with AIDS.[221] The waterborne transmission of this parasite is possible and some epidemiological data suggest this route.[6]

Microsporidia protists are obligate, intracellular, spore-forming protozoan parasites. Their host range is extensive and includes most invertebrates and all classes of vertebrates. Five microsporidian genera have been associated with human disease primarily in immunocompromised persons. They are the following *Enterocytozoon*, *Encephalitozoon*, *Trachipleistophora*, *Vittaformae,* and *Nosema*. *Enterocytozoon bienensi,* and *E. intestinalis* are the most prevalent Microsporidian parasite that cause gastrointestinal infection in AIDS patients;[222,223] Microsporidia could also be a possible cause of traveler's diarrhea.[224] Microsporidia may disseminate to cause systemic infection as well as ocular infection affecting cornea, conjonctiva, liver, and biliary tract infection. They have been recognized as a group of pathogens that have potential for waterborne transmission,[14,225] and recently an evaluation of methodologies was developed for the detection of human pathogenic Microsporidia in water.[226] However, the study by Fournier et al.[227] shows a low rate of water contamination by *E. bienensi*, suggesting that the

risk of waterborne transmission to humans is limited.

VIII. VIRAL PATHOGENS

The modern era in environmental virology is characterized by the recognition of hepatitis E virus (HEV) and the more recently identified enteric virus capable of producing waterborne outbreaks.[228,229,230,231,232] More than 15 different groups of viruses, encompassing more than 140 distinct types, can be found in the human gut. They are excreted by patients and find their way into sewage.

These viruses can be divided into three categories with respect to their epidemiological significance (Table 2).

1. Some are either nonenteropathogenic, or cause illness unrelated with the gut epithelium; these include poliovirus, coxsackieviruses A and B, echovirus, hepatitis A and E viruses, and some other human enteroviruses.
2. A relatively small group of viruses has been incriminated as the causes of acute gastroenteritis in humans, and fewer still have been proven to be true etiologic agents; they include Norwalk virus and other caliciviruses, rotaviruses, astroviruses, and some enteric adenoviruses.
3. A third category encompasses putative enteropathogens, such as coronavirus, enterovirus, torovirus, parvovirus, and reovirus, for which a causal relationship is still unproven.[228]

TABLE 2
Human Viral Pathogens or Putative Pathogens That Can Be Transmitted by Fecally Polluted Water

Virus	drinking water	recreational water	shellfish
Pathogen viruses			
Enteroviruses :			
Polioviruses	+/-	-	-
Coxsackie A viruses	-	-	-
Coxsackie B viruses	-	-	-
Echoviruses	-	-	-
Enteroviruses 68-71	-	-	-
Hepatitis A viruses	+	+	+
Hepatitis E-like viruses	+	-	+
Gastroenteritis virus (enteropathogenicity proven) :			
Human calivirus			
Norwalk-like viruses	+	+	+
Sapporo-like viruses	+	+	+
Astroviruses	+	-	+
Rotaviruses	+	-	-
Enteric adenoviruses 40, 41	-	-	-
Putative enteropathogens			
Coronaviruses	Toroviruses		
Enteroviruses	Parvoviruses		
Picobirnaviruses	Pestiviruses		

adapted from Metcalf, Melnick and Estes (1995)

The occurrence of a virus in a fecal specimen from a patient with gastroenteritis does not prove that the virus is indeed the causative agent of diarrhea. The first step in an attempted confirmation procedure relies on establishing the identity of the virus by molecular biology (PCR), immunological assessment, or after it has grown in culture. The second step in demonstrating causality is based largely on guidelines developed from epidemiological knowledge over the years.[233]

A. Hepatitis

Hepatitis A virus (HAV) and Hepatitis E virus (HEV) are associated with epidemics and sporadic cases of hepatitis; they are transmitted by the fecal-oral route, and therefore sewage polluted water may constitute a source.[17]

The most established virus group associated with waterborne outbreaks is hepatitis A virus. It results in widespread epidemics and constitutes an important cause of asymptomatic infections in young children. Mosley[234] has summarized reports of 50 outbreaks from 1895 to 1964. Twenty-nine of them show features supporting transmission by water. The highest incidence of waterborne infectious hepatitis in the U.S. has been in 1950 to 1960s and 1961 to 1970s with, respectively, 18 outbreaks (757 cases of illness) and 29 outbreaks (896 cases of illness). It has declined since 1971 with 16 outbreaks in 1971 to 1980 and 11 outbreaks in 1981 to 1990.[2,8] Since 1991, only two outbreaks caused by hepatitis virus A were reported.[235,236]

Hepatitis E is much less widespread and mostly confined to tropical and subtropical areas. Infection can be more severe than hepatitis A, with a high incidence of cholestasis and increased mortality in pregnant women (20%). In 1991, the largest waterborne outbreak of viral hepatitis E yet reported occurred in Kasspur, India, with an estimated total of over 79,000 cases of disease.[237] Another waterborne outbreak occurred in Delhi in 1955 to 1956 affected approximately 29,300 persons.[238] Such large-scale hepatitis epidemics are invariably waterborne and caused by HEV.[239,240] Recent evidence indicates that HEV might also be prevalent at a low level in Europe.[241]

B. Viral Gastroenteritis

The recognized viral agents of gastroenteritis include rotavirus, calicivirus, astrovirus, and some enteric adenovirus. Rotaviruses are classified by antigenic groups A, B, and C, subgroup, and serotype. Group A rotaviruses are the most common cause of diarrhea disease in infants and young children. Group C rotaviruses, like group A strains, principally cause diarrhea in young children, age 4 months to 4 years. In contrast to other human rotaviruses, group B rotaviruses are responsible predominantly for adult diarrheal disease, therefore designated as adult diarrhea rotavirus or ADVR.

The most notorious of caliciviruses that have been identified as a cause of gastroenteritis was the agent termed Norwalk virus. This originated from an outbreak of epidemic gastroenteritis in an elementary school in Norwalk, Ohio, in 1968. Many other related viruses were later classified by IEM as small round structured viruses (SRSV). These induded the Hawaii, Snow Mountain, Montgomery Country, Taunton, Otofuke, Sapporo, and the Osaka agents. Most of these have been identified as calicivirus, like over 50 other SRSVs. The family *Caliciviridae* includes the following four genera: vesivirus, lagovirus, Norwalk-like viruses (NLV), and Sapporo-like viruses (SLV).[242] Viruses in the NLV and SLV genera have been found only in humans, but recent data suggest that calves and pigs may be reservoir hosts of NLVs.[232] Genetically, the human

caliciviruses (Hu CVs) contains at least three genetic clusters or genogroups (GC), that is, GGI, GGII NLV, and GGIII SLV.[243] However, recently accumulated sequences of HuCV strains indicate these genogroups can be further divided.[244,245,246,247,248]

Some SRSVs from infant stools with diarrhea were named astrovirus for the characteristic five- or six-pointed star configuration that was evident on the surface of the viral particles. To date, seven serotypes of human astroviruses (HAstV) have been identified by enzyme immuno-assays immunoelectron microscopy or reverse transcriptase-polymerase chain reaction (RT-PCR).[249,250] Phylogenetic analysis identified two genogroups A and B correlated with serotypes.[251]

There are currently 47 serotypes of human adenoviruses, subdivided into six subgroups A through F. Many types of adenoviruses have been isolated from stools, but only types 40 and 41 have been consistently associated with gastroenteritis.[252] The two serotypes from the F subgroup are colloquially referred to enteric adenoviruses (EAd).

Rotaviruses are one of the most important causes of infantile gastroenteritis in the world. If untreated, rotavirosis can result in severe dehydration and death, especially in developing countries. Calicivirus and astrovirus gastroenteritis are generally mild, typically explosive but self-limited, with symptoms lasting from 1 to 4 days after a mean incubation time of 2 days. The clinical picture of adenovirus gastroenteritis is similar to that of rotavirosis, but the incubation period lasts 8 to 10 days.

In American statistics viral enteritis outbreaks of drinking water occur infrequently: 10 between 1971 and 1980, 15 between 1981 and 1990, 1 from 1991 to 1996. Because of their low incidence they have not been included in Figure 2. However, it is more than likely that a marked fraction of gastroenteritis of unidentified origin (AGI) is indeed associated with a viral aetiology (Section II). In recent years, the predominant part of SRSVs, especially NLVs, was revealed in several waterborne outbreak reports.[253,254,255,256] Epidemiological studies of seroprevalence confirm that a large number of infections owing to Norwalk viruses occur throughout the year.[257] Data from Lodder et al.[229] suggest that NLVs may exceed rotaviruses in importance as a cause of illness in the Netherlands. The sensitive new molecular techniques based on the reverse transcriptase-polymerase chain reaction (RT-PCR) available now have made it possible to detect low levels of a wide variety of enteric viruses. The results obtained[154,231,258] also support epidemiological data that suggests low-level transmission of viruses by drinking water supplies.

C. Other Candidate Viruses

A relatively small group of viruses has been incriminated as the causes of acute gastroenteritis in humans, but while most of them could be identified in fecal specimens from patients with diarrhea, not all were necessarily the etiologic agent. Coronaviruses have been associated to humans gastroenteritis[259] to neonatal necrotising entero-colitis[260] and were found regularly in the feces of patients with nonbacterial gastroenteritis.[261] Despite 2 decades of research to assess their causality, the results so far have been inconclusive. Other viruses such as picobirnaviruses, toroviruses, enteroviruses, reoviruses, and parvoviruses were also identified in fecal specimens from children and adults with gastroenteritis, and HIV-infected patients.[233,249,261,262] However, their role as an etiologic agent of diarrhea in the human population remains to be determined because many are also found in healthy controls. More recently, immune response has been intro-

duced as a parameter in such investigations.[233]

D. Factors Affecting Viral Infections

Certain characteristics of enteric viruses point to their potentially important role in waterborne outbreaks.

1. More than 140 types of human or animal enteric viruses can be found in sewage (Table 2). Some of them have proven enteropathogenicity, other just being putative enteropathogens in humans.[233] Most of the novel viral agents (caliciviruses, astroviruses) found in human diarrhea specimens are known to cause gastroenteritis in different animal species.
2. Viruses, found in the feces of symptomatic or asymptomatic hosts attain numbers up to 10^{10} infectious doses per gram, excreted for 1 to 4 weeks. The number of viruses currently detectable in raw sewage can reach 10^2 to 10^3 infectious units per liter.
3. The wide distribution of enteric viruses is demonstrated by sero-epidemiological surveys of North American populations: most of the adults testing positive.[257]
4. Many methods have been applied for eliminating viruses from water; all marred by the small size (20 to 100 nm), multiplicity of the virions, their resistance being much higher than that of bacteria, and various analytical shortcomings, such as the variation in microbial load of raw water, seasonal effects, and no random dispersion of pathogens within large volumes. After drinking water treatments, viruses are still occasionally detectable.[263] The main limitation of water treatments is that they may promote further clustering of pathogens, exposing some water-drinking consumers to much higher doses than others.
5. The number of viruses required to initiate infection can be estimated in animal models or human volunteers. Under appropriate conditions, as little as one infectious particle of rotavirus can trigger disease in animal[264] or human models.[265] From studies in volunteers with Norwalk and related viruses,[266,267] astroviruses,[268] enteroviruses,[265] it appears that the number of virions needed to infect man or animals is low in terms of infectious units: Payment[269] rightly stated, "The cell culture infectious unit is only a fraction of the real number of infectious viruses". For rotaviruses, the proportion of culturable among electron microscopically detectable viruses can be as high as 1:1000 or more.[270] In the most enteropathogenic bacteria the ID50 are generally high, on the order of 10^6 to 10^8 except some species (cf. Section II).
6. In some waterborne outbreaks of gastroenteritis, viruses were isolated from water that met current bacteriological standards and contained an adequate chlorine level.[2,269,271,272,273] The isolation of viruses from samples of "bacteriologically safe" potable water indicates that bacteriological indicators are inadequate to monitor the occurrence of viruses in water.

IX. GASTROENTERITIS OF UNDETERMINED ETIOLOGY

In the majority of outbreaks of acute gastroenteritis related in the U.S., the etiology could not be determined. Between 1940 to 1950, 213 outbreaks were recorded and 177 in 1970 to 1980. Because waterborne outbreak reporting system is voluntary, the

differences observed in the frequency of their occurrence might be largely attributed to poor recording by State and Local Health Departments.[2] Although, in principle, many incidents could have a bacterial or protozoan etiology, many of these outbreaks could be of viral origin.

A bacterial cause is relatively easy to identify, while filtration and disinfecting procedures effectively eliminate these agents. Also, some outbreaks were clinically and epidemiologically consistent with Norwalk-like virus infection.[9] Finally, the detection of enteric viral pathogens was impossible at that time. Even in 1970 to 1980 *Giardia* and *Cryptosporidium* were only rarely recognized as etiologic agents.

X. ENDEMIC GASTROENTERITIS

A few studies carried out in Canada and later in France seem to point at endemic infections associated with drinking water complying with bacteriological standards. Payment et al.[274,275] in Montreal, followed a randomized trial design in order to quantify the health hazard, which remains after a standard, well-conducted treatment of bacteriologically contaminated surface water has been completed. This study showed that municipal tap water drinkers had a significantly higher rate of acute gastrointestinal illnesses (35%) than those provided with a domestic water filter, despite that the treatment used in the community water system included predisinfecting, flocculation, sand filtration, ozonation, and final chlorine or chlorine dioxide disinfecting procedures meeting the North American microbiological and physicochemical water quality standards. Payment et al.[276] suggest that heterotrophic, potentially pathogenic agents, for example, *Bacillus* spp., could be responsible for these incidents. This assumption has never been confirmed, however, while this disproportionately high percentage of *Bacillus* in the studied water supplies has not been found by other authors.[277]

In order to assess the residual health risk after mere disinfecting of piped water processed from ground water, an epidemiological study was conducted in France by Zmirou et al.[278] The crude incidence rate of diarrhea was 1.4 times more frequent among children drinking treated water than among controls. The morbidity observed in this study comprises small sporadic outbreaks, along with an endemic occurrence of acute gastrointestinal infection. Even when days with 'epidemics' were excluded from the analysis, the routine occurrence of acute gastroenteritis events is still greater in relying on water, suggesting that microbiological contamination is continuous.

In Vermont, where giardiasis was the most common disease with annual incidence rates higher than other states, waterborne transmission was suggested to be an important cause of non-outbreak related cases; indeed, rates of infection were highest in persons that drank nonfiltered city water.[279] These observations have been confirmed by Fraser and Cook[280] in New Zealand and by Dennis et al.[281] in New Hampshire, where drinking untreated surface water and recreational water exposure were strongly associated with endemic giardiasis.

Cryptosporidium spp. probably present enhanced endemic risks compared with *Giardia* spp., because of their more frequent occurrence in surface water and higher resistance to water purification technology, including filtration. Perz et al.[185] used a risk assessment model to examine the potential role of tap water in endemic infections with *Cryptosporidium* spp. In comparing the model output with surveillance data for cryptosporidiosis, the analysis showed that low-level transmission via tap water can represent an important exposure route for endemic *Cryptosporidium* infection.

Endemic transmission of protozoan infections results from the low infectious dose of these organisms for man, and their heterogeneous distribution in clusters in treated waters. Similarly, enteric viruses might be involved in outbreaks of endemic waterborne gastroenteritis. On the other hand, a similar role for bacterial enteropathogens seems less likely because their infectious dose is usually higher and their resistance to water purification treatments lower.

These data demonstrate that management of endemic transmission of waterborne infections call for many supplemental epidemiological and analytical data. Such information has to be provided by prospective studies on populations that have been exposed to different levels of pathogens, including control subjects. The outcome of such investigations may well call for improvements in customarily applied water purification treatments aiming at an unconditionally safe drinking water supply.

XI. TAKING INTO ACCOUNT EXPOSURE OF IMMUNOCOMPROMISED SUBJECTS

In all waterborne cryptosporidiosis outbreaks it has been observed that immunocompromised persons, such as AIDS patients, are at greater risk than immunocompetent persons for developing severe illness, and for a potential lethal outcome. In the Milwaukee epidemic,[192] at least 69 fatalities were recorded amongst immunocompromised patients. In the outbreak that occurred in 1994 in Clark County (Nevada),[193,282] 63 of the 78 laboratory confirmed cases were in HIV-infected adults, among whom 32 died. Thus, current data indicate that during waterborne outbreaks immunocompromised persons, such as AIDS patients, can acquire cryptosporidiosis more likely than immunocompetent persons, while illness is more severe and life threatening.

Not surprisingly, more recently reported cases of cyclosporiasis, and diarrhea with *Isospora* or Microsporidia in stools include overrepresentation of immunocompromised patients with AIDS.[184] This follows the pattern of substantially enhanced risks for the classic group of debilitated subjects: the very young, old, pregnant, and immunocompromised (YOPI) category.[200] It is striking that YOPIs seem to incur more risks, particularly from exposure to protozoa, more specifically *Cryptosporidium* spp. than from other pathogens. This prompted Public Health Authorities to launch an information campaign targeted at YOPIs recommending to take specific measures aiming at reducing the risk for waterborne cryptosporidiosis, including boiling water before use, using water filters, or bottled water and avoiding swimming in lakes, rivers, or public swimming pools. This does not exempt these authorities, however, from the obligation to manage health risks associated with low-level oocyst contamination of fully treated drinking water. Such efforts should be guided by surveillance systems and by epidemiologic studies designed for assessing the public health significance of low levels of *Cryptosporidium* oocysts.

SUMMARY

Many classes of pathogens excreted in feces are able to initiate waterborne infections. There are bacterial pathogens, including enteric and aquatic bacteria, enteric viruses, and enteric protozoa, which are strongly resistant in the water environment and to most disinfectants. The infection dose of viral and protozoan agents is lower than bacteria, in the range of one to ten infectious units or oocysts.

Waterborne outbreaks of bacterial origin (particularly typhoid fever) in the developing countries have declined dramatically from 1900s. Therefore, some early bacterial agents

such as *Shigella sonnei* remains prevalent and new pathogens of fecal origin such as zoonotic *C. jejuni* and *E. coli* O157:H7 may contaminate pristine waters through wildlife or domestic animal feces. The common feature of these bacteria is the low inoculum (a few hundred cells) that may trigger disease. The emergence in early 1992 of serotype O139 of *V. cholerae* with epidemic potential in Southeast Asia suggests that other serotypes than *V. cholerae* O1 could also getting on epidemic.

Some new pathogens include environmental bacteria that are capable of surviving and proliferating in water distribution systems. Other than specific hosts at risk, the general population is refractory to infection with ingested *P. aeruginosa*. The significance of *Aeromonas* spp. in drinking water to the occurrence of acute gastroenteritis remains a debatable point and has to be evaluated in further epidemiological studies. *Legionella* and *Mycobacterium avium* complex (MAC) are environmental pathogens that have found an ecologic niche in drinking and hot water supplies. Numerous studies have reported Legionnaires' disease caused by *L. pneumophila* occurring in residential and hospital water supplies. *M. avium* complex frequently causes disseminated infections in AIDS patients and drinking water has been suggested as a source of infection; in some cases the relationship has been proven.

More and more numerous reports show that *Helicobacter pylori* DNA can be amplified from feces samples of infected patients, which strongly suggests fecal-to-oral transmission. Therefore, it is possible that *H. pylori* infection is waterborne, but these assumptions need to be substantiated.

Giardiasis has become the most common cause of human waterborne disease in the U.S. over the last 30 years. However, as a result of the massive outbreak of waterborne cryptosporidiosis in Milwaukee, Wisconsin, affecting an estimated 403,000 persons, there is increasing interest in the epidemiology and prevention of new infection disease caused by *Cryptosporidium* spp. as well as monitoring water quality. The transmission of *Cryptosporidium* and *Giardia* through treated water supplies that meet water quality standards demonstrates that water treatment technologies have become inadequate, and that a negative coliform no longer guarantees that water is free from all pathogens, especially from protozoan agents. Substantial concern persists that low levels of pathogen occurrence may be responsible for the endemic transmission of enteric disease. In addition to *Giardia* and *Cryptosporidium*, some species of genera *Cyclospora*, *Isospora,* and of family Microsporidia are emerging as opportunistic pathogens and may have waterborne routes of transmission.

More than 15 different groups of viruses, encompassing more than 140 distinct types can be found in the human gut. Some cause illness unrelated with the gut epithelium, such as Hepatitis A virus (HAV) and Hepatitis E virus (HEV). Numerous large outbreaks have been documented in the U.S. between 1950 and 1970, and the incidence rate has strongly declined in developing countries since the 1970s. Hepatitis E is mostly confined to tropical and subtropical areas, but recent reports indicate that it can occur at a low level in Europe. A relatively small group of viruses have been incriminated as causes of acute gastroenteritis in humans and fewer have proven to be true etiologic agents, including rotavirus, calicivirus, astrovirus, and some enteric adenovirus. These enteric viruses have infrequently been identified as the etiologic agents of waterborne disease outbreaks, because of inadequate diagnostic technology, but many outbreaks of unknown etiology currently reported are likely due to viral agents. Actually, Norwalk virus and Norwalk-like viruses are recognized as the major causes of waterborne illnesses world-wide.

The global burden of infectious waterborne disease is considerable. Reported numbers highly underestimate the real incidence of waterborne diseases. The most striking concern is that enteric viruses such as caliciviruses and some protozoan agents, such as *Cryptosporidium,* are the best candidates to reach the highest levels of endemic transmission, because they are ubiquitous in water intended for drinking, being highly resistant to relevant environmental factors, including chemical disinfecting procedures. Other concluding concerns are the enhanced risks for the classic group of debilitated subjects (very young, old, pregnant, and immunocompromised individuals) and the basic requirement of to take specific measures aimed at reducing the risk of waterborne infection diseases in this growing, weaker population.

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