Detection and Occurrence of Indicator Organisms and Pathogens

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INTRODUCTION

Since 1971, the Centers for Disease Control and Prevention (CDC) and the U.S. Environmental Protection Agency (U.S. EPA) have maintained a surveillance system relating to occurrences and causes of waterborne-disease outbreaks (WBDOs). Levy et al. (1998) summarized data for January 1995 through December 1996 and previously unreported outbreaks in 1994. For the period 1995 to 1996, 13 states reported a total of 22 outbreaks associated with drinking water with a total of 2,567 persons becoming ill. No deaths were reported. The microorganism or chemical that caused the outbreak was identified for 14 of the 22 outbreaks.

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were associated with chemical contamination of the drinking water; the seventh outbreak was attributed to a small round structured virus.

Thirty-seven outbreaks attributed to recreational water exposure from 17 states affected an estimated 9,129 persons, including 8,449 persons in two large outbreaks of cryptosporidiosis. Twenty-two of these 37 were outbreaks of gastroenteritis, 9 were outbreaks of dermatitis, and 6 were single cases of primary amebic meningoencephalitis caused by Naegleria fowleri, all of which were fatal. The etiologic agent was identified for 33 of the 37 outbreaks. Six of the 22 outbreaks of gastroenteritis were caused by Cryptosporidium parvum and 6 by E. coli O157:H7. All of the latter were associated with unchlorinated water (i.e., in lakes) or inadequately chlorinated water (i.e., in a pool). Thirteen of these 22 outbreaks were associated with lake water, eight (36.4%) with swimming or wading pools, and one (4.5%) with a hot spring. Of the nine outbreaks of dermatitis, seven were outbreaks of Pseudomonas dermatis associated with hot tubs, and two were lake-associated outbreaks of swimmer’s itch caused by Schistosoma sp. WB DOs caused by E. coli O157:H7 were reported more frequently than in previous years and were associated primarily with recreational lake water. Tillett et al. (1998) developed a method of categorizing the evidence used to implicate water in disease outbreaks. The scheme takes into account epidemiology, microbiology, and water quality information.

Wastewater treatment workers were found to have a significantly higher prevalence of gastroenteritis, gastrointestinal symptoms (abdominal pain), and headaches compared to college maintenance and operation workers in a retrospective epidemiological study conducted by Khuder et al. (1998).

DETECTION OF MICROORGANISMS

Classically, the detection and enumeration of microorganisms relies on cultural methods. In these methods, the microorganism is grown on either a solid (agar) or liquid (broth) medium, which supplies the nutritional requirements of the organism, or in the case of obligate parasites such as viruses, the organism is grown in a culture of host cells. Once a microorganism has been grown and isolated as a pure culture, identification of the organism is based on biochemical, immunological (serological), and genetic characteristics of the isolate. In many instances, particularly for such well-studied organisms as the coliforms, specific compounds are incorporated into the primary media, which allow for selection and differentiation of the organisms of interest. For example, Endo agar is routinely used for the enumeration of coliforms and other enteric organisms. This agar has sodium sulfite and basic fuchsin to inhibit the growth of Gram-positive bacteria. In addition, lactose is present as a primary substrate for bacterial nutrition. Metabolism of lactose with the formation of acid and gas, a hallmark characteristic of the coliform group, is detected by a color change due to the reaction between acetaldehyde (an intermediate product of lactose fermentation) and the sodium sulfite.

Within the past several decades alternative, noncultural methods for the detection of microorganisms have been developed and are now widely used. These methods are particularly useful for the detection of microorganisms for which no selective media has yet been developed. These methods include the use of specific staining procedures, usually based on serological properties of the organism (immunofluorescence), and molecular methods for the detection and characterization of specific sequences within the DNA or RNA of the organism.

Immunofluorescent detection of microorganisms depends on the ability of antibody molecules to recognize and react with specific three-dimensional regions (epitopes) on the surface of microbial cells. Antibodies can be tagged with fluorescent dyes. Specific microorganisms stained with immunofluorescent dyes can be enumerated by direct counts under an epifluorescent microscope.

Detection of nucleic acid sequences unique to a particular organism involve several distinct procedures. Nucleic acid probes containing nucleotide sequences complementary to a unique sequence of a specific microorganism can be coupled with a variety of reporter molecules (fluorescent dyes and radioisotopes). When mixed with a solution of DNA extracted from an environmental sample, the probe will only bind to those specific target sequences that are complementary to it. Thus, the presence of specific microbial nucleic acid, and presumably then specific microorganisms, can be determined. Frequently, the amount of specific nucleic acids present in environmental samples is too low to be detected directly by gene probes. In this case, there is a need to increase, or amplify, the specific sequences to detectable levels. This is accomplished through the use of the polymerase chain reaction (PCR).

INDICATOR ORGANISMS

Waterborne disease outbreaks can be traced primarily to the contamination of water with fecal matter. Because of the difficulties of monitoring water for all of the known pathogens that can be transmitted by a waterborne route, indicator organisms (e.g., coliforms and thermotolerant coliforms) are used widely as surrogates for the detection of pathogens.

Esham and Sizemore (1998) compared two media, mFC and mTEC, for the enumeration of fecal coliforms in tidal creeks. Counts by mTEC were consistently higher than mFC counts at all salinity ranges. In addition, a significant number of false positives were associated with using mFC in middle and high salinity areas. Lifshitz and Joshi (1998) found the ColiPlate (CF) kit gave estimates of E. coli that were 20% higher than standard membrane filtration when the two methods were compared for testing water samples. The difference increased when samples were spiked with injured cells. They concluded that the CF kit is a more reliable method for use with samples having high levels of injured or weakened cells. m-ColiBlue24 (m-CB) was compared to m-Endo medium and an International Organization for Standardization (ISO) standard coliform medium, lactose agar with Tergitol 7, for the analysis of indicator organisms in bottled water (Grant, 1998). Coliform analysis was conducted on 104 brands of bottled water from 10 countries. Presumptive coliform colonies were found in 5.8% of the samples with m-CB, 1.9% with m-Endo, and 11.5% with lactose agar with Tergitol 7. None of the presumptive coliforms from any of the three media were verified as true coliforms in subsequent analysis.

Defined substrate methods (e.g., Colilert) for the detection of coliforms and Escherichia coli have been widely accepted over the past decade. Eckner (1998) reported on a comparison of the Colilert system to the Swedish standard methods using multiple-tube fermentation or membrane filtration on 338 water samples. The Colilert system was found to be more sensitive than Swedish standard methods for detecting coliform bacteria and of equal sensitivity for detecting Escherichia coli. Landre et al. (1998) reported that Aeromonas sp. at low concentrations can yield a false positive reaction using Colilert reagent 4 weeks short of shelf-life expiration. They caution against the use of aged Colilert for

Literature Review 1999

531
monitoring water quality because it could lead to an overestimation of coliforms. \(\beta\)-Galactosidase activity of \textit{E. coli} after chlorination was less reduced than CFUs, indicating that detection and enumeration methods based on this enzyme may be more sensitive to injured cells (Tryland et al., 1998). Because bacteria other than coliforms can produce \(\beta\)-galactosidase and \(\beta\)-glucuronidase, the possibility exists that defined substrate tests may give false positive results. Tryland and Fiksdal (1998) examined this possibility by measuring the activity of these two enzymes in noncoliform water isolates. In all cases, enzyme activity in the noncoliforms was significantly less than in the coliforms, leading the authors to conclude that noncoliform bacteria must be present in significantly higher concentrations than coliforms to interfere with the enzymatic detection of these organisms.

Alfonso et al. (1998) compared a new chromogenic medium, Chromocult Coliform (CC) agar, to mFC medium. There were no statistically significant differences between the two media. Specificity of CC agar was found to be related to incubation temperature and the authors recommended lowering the incubation temperature from 44.5 to 41 °C to improve enumeration of metabolically injured fecal coliforms.

Entry into the viable but nonculturable (VNC) state is a survival mechanism that bacteria can adopt in an adverse environment. When in this state, bacteria are still viable but are unable to form colonies on growth medium. Lleo et al. (1998) examined the possibility of Gram-positive species entering the VNC state using exponential-phase cultures of \textit{Enterococcus faecalis} inoculated in filtered, sterilized water from Lake Garada, Italy. During the 60-day study, the number of total cells stained with a fluorescent dye or counted with a Coulter Counter remained constant, while the number of cells capable of forming colonies on Tryptic Soy Agar (TSA) declined rapidly. On day 14, no colonies could be observed when 50 mL of inoculated lake water were plated; however, the cells showed active uptake of amino acids and \textit{E. faecalis} specific DNA was detected by PCR throughout the experiment. The data obtained in this study lend further support to recent research that examines mechanisms that bacteria can adopt in an adverse environment.

Identification of enterococci species by DNA hybridization with recently designed oligonucleotide probes was superior to results obtained with a common biochemical test panel (Frahm et al., 1998). Using these findings, a procedure for the detection of enterococci in water samples was developed, consisting of a short enrichment followed by an amplification step and a hybridization reaction in microtiter plate format. The detection limit was 1 CFU/mL, and results were available within 26 hours. \textit{Enterococcus faecalis} was shown to survive for prolonged periods under conditions of complete starvation established by incubation in tap water (Hartke et al., 1998). During incubation in the oligotrophic environment, cells from the exponential-growth and early-stationary phases became progressively more resistant to environmental conditions and to sodium hypochlorite.

Bahirathan et al. (1998) evaluated the antibiotic susceptibility of 54 isolates of yellow-pigmented enterococci to determine if antibiotic resistance patterns could be used as a specific indicator of human and nonhuman sources of fecal contamination of water. Their results suggested that vancomycin-susceptibility testing may have value in the identification of sources of pollution because there was a significantly higher resistance to vancomycin in isolates from wild sources compared with that from other sources. The ability of \textit{Enterococcus faecalis} to transfer various genetic elements under natural conditions was tested in two municipal sewage water treatment plants (Márzinek et al., 1998). The transfer rate between different strains of \textit{E. faecalis} under natural conditions was, compared to that under laboratory conditions, significantly lower, indicating that gene transfer should not take place under natural conditions.

Sartory et al. (1998a) evaluated two media (mCP medium and Tryptose Sulphite Cycloserine [TSC] agar) for recovery of \textit{Clostridium perfringens} in environmental and partially treated drinking water. TSC recovered significantly greater numbers of \textit{C. perfringens} than mCP. There was a significant number of false presumptive positive and negative isolates on mCP. Therefore, the authors concluded that TSC is a more suitable medium for the routine monitoring of water supplies for the presence of \textit{C. perfringens}. Edwards et al. (1998) examined the distribution of \textit{C. perfringens} in sediment cores near an untreated wastewater outfall at McMurdo Station, Antarctica. \textit{Clostridium perfringens} decreased with depth in the sediment and with distance from the outfall.

Efstratiou et al. (1998) evaluated the usefulness of total coliforms, fecal coliforms, and fecal streptococci in predicting the presence of \textit{Salmonella} spp., \textit{Staphylococcus aureus}, and \textit{Candida albicans} in sewage polluted coastal water. Total coliforms gave a better correlation with the presence of \textit{Salmonella} spp. and \textit{Staph. aureus} than either of the two fecal groups of organisms. Fecal coliforms were better predictors of the presence of \textit{Candida albicans} in moderately polluted areas. The authors concluded that monitoring of total coliforms, without the measurement of fecal coliforms or fecal streptococci is adequate in moderately contaminated coastal waters. Nola et al. (1998) conducted a microbiological survey of 5 spring water points and 10 wells located in various residential districts and selected according to geographical location and number of users in Yauande, Cameroon. \textit{Pseudomonas aeruginosa}, \textit{Aeromonas hydrophila}, and indicators of fecal contamination were frequently found in the water samples. The density of fecal bacteria was strongly correlated with \textit{P. aeruginosa} in spring water and with \textit{A. hydrophila} in well water.

Pianetti et al. (1998) evaluated the usefulness of traditional indicator organisms in predicting the presence of \textit{Salmonella}, \textit{Campylobacter}, \textit{Aeromonas}, and \textit{Yersinia} spp. in river water. In 168 water samples analyzed, 22.6% were positive for \textit{Salmonella}, 30.9% were positive for \textit{Campylobacter}, 23.2% were positive for
Aeromonas, and 4.7% were positive for Yersinia. No correlation between indicator organisms and these potential pathogens were found, indicating that routine monitoring of river water for indicator organisms may not be protective of public health.

Pruss (1998) reviewed studies on uncontrolled waters, such as seas, lakes, and rivers, to evaluate the health risks caused by poor microbiological quality of recreational water. Most studies reported a dose-related increase of health risk in swimmers with an increase in the indicator bacteria count in recreational waters. The indicator microorganisms that correlated best with health outcomes were enterococci/fecal streptococci for both marine and freshwater and E. coli for freshwater. European Union (E.U.) bathing water standards set a guide level for thermotolerant coliforms of 100/100 mL and an imperative level of 2 000/100 mL. Van Asperen et al. evaluated these standards in a study of the risk of gastroenteritis among triathletes. Swimming in freshwaters that failed the guide level but still met the imperative level was associated with a significant risk of gastroenteritis, indicating that the imperative level may not be protective of public health. Fleisher et al. (1998) found that bathers were at increased risk of gastroenteritis, acute respiratory illness, and eye infections compared to nonbathers even at beaches meeting both the U.S. EPA and E.U. criteria for recreational waters. Elliott (1998) proposed a method for combining the temporal concentration frequency distribution of indicator organisms in recreational water with a concentration response relation to obtain the averaged illness rate.

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Sibille et al. (1998) examined the microbial community composition (especially bacteria and protozoa) to obtain direct and indirect proof of protozoan feeding on bacteria in two distribution networks, GAC water (i.e., water filtered on granular activated carbon) and nanofiltered water. Protozoan ingestion of bacteria was indirectly shown by adding E. coli to the experimental distribution systems. Unexpectedly, E. coli was lost from the GAC water-supplied network more rapidly than from the nanofiltered water-supplied network. The authors concluded that the presence of protozoa in drinking water distribution systems may regulate the autochthonous and allochthonous bacterial populations.

Perrot et al. (1998) examined the development of coliform transport in the Quabbin Reservoir. The main contributor of coliforms to the lake water was E. coli. In their data, they calculated a log removal time for E. coli of 10 meters below the surface, indicating that light was the most important factor in die-off. No correlations between coliform numbers and temperature, oxygen, pH, conductivity, or transparency were found in a study of the bacteriological quality of a shallow lake in the lower Salado River Basin, Argentina (Emilian and Gonzalez de Paiva, 1998). The main contributor of coliforms to the lake water was shown to be floating vegetation and aquatic macrophytes, particularly during high water periods. Calvo et al. (1998) determined aerobic heterotrophic bacteria, fecal and total coliforms, fecal streptococci, and coliphages in five protected lakes in the Antequera area of Spain. Most of the lakes contained fecal streptococci. The authors concluded that coliform bacteria were not adequate indicators of fecal pollution in these lakes.

Momba et al. (1998) found that biofilm formation occurred even in the presence of residual disinfectant concentrations (16.5 mg/L H2O2; 1 mg/L monochloramine; 0.2 mg/L free chlorine) within the first day after disinfection. Barbeau et al. (1998) reviewed the role of biofilms on medical and dental devices in relation to nosocomial infections. They noted, in particular, that colonization of dental lines by Legionella pneumophila is an important public health concern.

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Personne et al. (1998) examined the impact on a karst aquifer of a river that receives effluents from a sewage treatment plant just upstream from the point of recharge. The rate of infiltration, of subsurface transport, and of dissemination of contaminants into a zone usually protected from contamination was recorded. The results confirmed the vulnerability of fissured aquifers to contamination. Pavelic et al. (1998) modified and improved the design of diffusion chambers allowing the measurement of the survival of Enterobacter faecalis in the presence and absence of porous material (aquiifer solids) in wells. Die-off of the organisms was found to fit a broken-stick model and not a log-linear function. Based on their data, they calculated a log removal time for E. faecalis of 17 days. Buckley et al. (1998) found significantly higher concentra-
The presence of *Salmonella* and its relationship with indicators of faecal pollution was investigated in aquatic habitats (Polo et al., 1998). The highest frequency of isolation of *Salmonella* was in rivers followed by freshwater reservoirs and seawater. The sporadic presence of *Salmonella* on beaches with low concentrations of faecal streptococci may represent a potential risk for bathers. Absence of *Salmonella* was observed only on beaches with very low densities of indicator organisms. Shellfish (mussels and clams) and shellfish-growing waters were examined for indicator bacteria according to the EC regulations, *Salmonella* spp., coliphages and anti-*Salmonella* phages (Legnani et al., 1998a). Samples from both natural-growing areas along the coast and from authorized shellfish-harvesting beds were contaminated by coliphages at a significantly higher level than the corresponding bacterial indicators for fecal contamination. Coliphage concentrations were significantly more correlated with faecal indicators in marine waters and sediments, but no correlation was found in shellfish, thus showing their low specificity as indicators of fecal pollution of human origin in shellfish of economic importance.

Fjendbo Jorgensen et al. (1998) described a device for direct heating of water by solar radiation in a flowthrough system of copper pipes. The authors showed that it is possible to eliminate coliform and thermotolerant coliform bacteria from naturally contaminated river water by heating to temperatures of 65 °C or above. Artificial additions of *Salmonella typhimurium*, *Streptococcus faecalis*, and *Escherichia coli* to contaminated river water were also inactivated after heating to 65 °C and above. The total viable count could be reduced by a factor of 1000. To provide a good safety margin the authors recommended that an outlet water temperature of 75 °C be used. At that temperature the daily production was about 50 L of decontaminated water per square meter of solar panel.

Disinfectant concentration was found to be the sole predictive variable in a study of the microbiological quality of indoor swimming pools (Ibarluzea et al., 1998). In pools disinfected with chlorination, 2.6 mg/L of free chlorine was required to ensure that there was a 90% probability of microbiologically safe bathing water. In pools disinfected with electrolytically generated copper and silver ions, 3.4 mg/L copper was required for the same level of safety.

Rawat et al. (1998) found that a gamma radiation dose of 2 kGy could effectively reduce the coliform load in raw sewage to a level in which it could be safely employed in agricultural practice. The performance of extruded, polymeric, tubular, microfiltration membranes for the disinfection of sewage effluent was evaluated by Till et al. (1998). A smaller membrane (0.45 µm) was significantly more efficient at removing fecal coliforms from effluent than a larger membrane (1.2 µm) with a rejection rate comparable to existing systems. The larger membrane was significantly better, however, for treating influents.

**BACTERIAL PATHOGENS AND EMERGING PATHOGENS**

*E. coli* O157:H7. *E. coli* O157:H7 is an enterohaemorrhagic strain of *E. coli*. The organism produces a toxin known as vero-cytotoxin which is similar to the toxin produced by *Shigella*. Infection with this organism is associated with haemorrhagic colitis. In a small proportion of the cases, particularly in children, the infection can progress to haemolytic uremic syndrome, a life-threatening disease. Risk factors associated with *E. coli* O157:H7 infection were identified in a study at 10 medical centers throughout the U.S. (Slutsker et al., 1998). Univariate analysis indicated that drinking unchlorinated well water or swimming in a pond, in addition to the typical food-associated factors (e.g., ingestion of undercooked hamburger) were significantly associated with infection. In an epidemiological study of sporadic *E. coli* O157:H7 infections in Scotland, Coia et al. (1998) found that a high proportion of confirmed cases were associated with exposure to farm animals or their byproducts, gardening, or suspected or confirmed household water supply problems before the onset of illness. Samples from cattle, other domestic and wild animals, flies, feeds, and water troughs were collected from 12 cattle farms and tested for *E. coli* O157:H7 (Hancock et al., 1998). *E. coli* O157:H7 was isolated from 10 of 320 water trough sample sets (biofilm and water). The bovine and water trough isolates from two farms showed a high degree of genetic similarity, indicating that water troughs may serve as a reservoir for this organism. A 14-month longitudinal study was conducted on four dairy farms in Wisconsin to ascertain the source(s) and dissemination of *E. coli* O157:H7 (Shere et al., 1998). *E. coli* O157:H7 was isolated from cattle, feed, flies, a pigeon, and water associated with heifers on two farms. When present in animal drinking water, *E. coli* O157:H7 was disseminated through the cattle that used the water source. *E. coli* O157:H7 was found in water at <1 to 23 CFU/mL. Genetic analysis demonstrated that a single strain comprised a majority of the positive samples on the farms.

Wang and Doyle (1998) studied the survival of *E. coli* O157:H7 in filtered and autoclaved municipal water, in reservoir water, and in water from two recreational lakes for a period of 91 days at 8, 15, or 25 °C. Greatest survival was in filtered autoclaved municipal water and least in lake water. Regardless of the water source, survival was greatest at 8 and least at 25 °C. These studies indicate that *E. coli* O157:H7 is a hardy pathogen that can survive for long periods of time in water, especially at cold temperatures. In addition, the results suggested that *E. coli* O157:H7 can enter a VNC state in water. Warburn et al. (1998) found that *E. coli* O157:H7 could survive for up to 300 days when inoculated into bottled water. Scanning electron microscopy of the bottles indicated that the cells attached to and multiplied on the container walls.

The efficiency of selective enrichment broths for the recovery of acid/salt stressed *E. coli* O157:H7 was determined (Stephens and Joyntson, 1998). Significantly fewer stressed cells were recovered by all the selective enrichment broths containing bile salts or VCC antibiotics compared to the nonselective controls. The use of such enrichments to recover low numbers of stressed *E. coli* O157:H7 may result in failure to detect the organism. Jackson et al. (1998) reported on a 16-month-old girl living on an Ontario dairy farm who developed an *E. coli* O157:H7 infection. Initial testing of the well water on the farm was negative for *E. coli* by standard methods; however, culture of selected total coliform isolates on sorbitol-MacConkey agar resulted in the isolation of *E. coli* O157:H7.

*Salmonella*. A case-control study was carried out to investigate an outbreak of acute gastroenteritis among a military detachment stationed in a rural area of Castellon, Espana (Pac et al., 1998). *Salmonella richmond* was isolated in 5 of the 14 stool cultures performed. An association was also discovered between the illness and consumption of water from an aqueduct that flowed near the
The risk of suffering from the illness rose with the amount of water consumed. Chemical and bacteriological analyses of the aqueduct water indicated the presence of fecal contamination. An outbreak of gastroenteritis due to *S. ohio* whose origin was the consumption of water from a drinking fountain was described for the first time by Molinero et al. (1998). This fountain had no chlorination system. *S. ohio* was isolated from the water and from 2 of the 13 stool specimens analysed. A molecular epidemiology study of *Salmonella* serotype Enteritidis was carried out by ribotyping and randomly amplified polymorphic DNA (RAPD) typing of 38 food and 25 water strains, which were epidemiologically unrelated and collected in Spain from 1985 to 1996 (Landers et al., 1998). Their results supported the fact that organisms representing at least 40 genomic groups are currently circulating in Spain but that only the organisms of five groups predominate and these fall into a single subcluster or lineages. Organisms of four infrequent groups were only collected from sewage or environmental waters.

Typhoid fever, a severe systemic illness transmitted through food or water, is caused by the bacterium *Salmonella* serotype *typhi*. Luby et al. (1998) evaluated risk factors for developing typhoid fever in a setting where the disease is endemic (Karachi, Pakistan). One-hundred cases with blood culture-confirmed *Salmonella* *typhi* between July and October 1994 and 200 age-matched neighborhood controls were compared. Eating ice cream, eating food from a roadside cabin during the summer months, taking antimicrobials in the 2 weeks preceding the onset of symptoms, and drinking water at the worksite were all independently associated with typhoid fever.

*Shigella*. Faruque et al. (1998a) reviewed the clinical and epidemiological features of 390 children under 5 years of age infected with either *Shigella dysenteriae* type I or *Shigella flexneri* attending a diarrhoea treatment center from 1993 to 1995 in Dhaka, Bangladesh. Use of antibiotics at home, use of water from tubewells or pipe-water for drinking, and lack of sanitary facilities were the behavioral and environmental factors strongly associated with *S. dysenteriae* type I infection. Tshimanga et al. (1997) investigated a July 1994 outbreak of *Shigella dysenteriae* type I at a textile factory in Bulawayo, Zimbabwe. Thirty seven of 58 workers who drank borehole water were ill compared to 1 of the 17 who did not. Water samples from the two boreholes yielded numerous fecal coliforms.

*Vibrio*. Cholera, caused by certain strains of *Vibrio cholerae*, is the first disease for which a waterborne route of transmission was shown. Paneth et al. (1998) reviewed the history of John Snow's 1854 investigation proving a waterborne route of transmission for cholera. In a cautionary note that is applicable today, they noted that England's Board of Health did not accept Snow's hypothesis for a waterborne route of infection but instead clung to their preconceived notion that cholera was spread by bad air.

Epidemic cholera is caused by toxigenic strains of *V. cholerae*, of which strains O1 and O139 are associated most often, but not exclusively, with epidemic outbreaks. Faruque et al. (1998) reviewed the epidemiology, genetics, and ecology of toxigenic *V. cholerae*. They emphasized the close association among *V. cholera*, surface water, and the population interacting with the water. They also noted that molecular epidemiological studies have revealed significant clonal diversity among toxigenic strains and continual emergence of new epidemic clones. Borroto (1998) noted an inverse relationship between toxigenic *V. cholerae* O1's ability to survive and the altitude above sea level of freshwater lakes where the organism is introduced. He suggested that this geographic pattern could be used as a basis for the design of a sampling and monitoring program for the organism in Latin America, where cholera is becoming endemic. Uchiyama (1998a) assessed the persistence of *V. cholerae* non-O1 in water during the cold season. The results suggested that *V. cholerae* non-O1 would be able to exist in river sediment during the cold season. Colaco et al. (1998) isolated *V. cholera* O1 strain El Tor biotype in 86% of 2,585 water and sewage samples analyzed indicating that fecal contamination of water is the most common source for rapid spread of the organism. In contrast, only 2.1% of 91 food samples examined by the same researchers were positive for the organism. The presence of *V. cholerae* non-O1 in water supplies for human consumption in the city of Campeche and rural locality of Becal was investigated by Isaac-Marquez et al. (1998). *V. cholerae* non-O1 was detected in 5.9% of the samples obtained in deep pools of Campeche. Studies conducted in Becal and the neighborhood of Morelos in Campeche indicated that collected samples harbored *V. cholerae* non-O1 in 31.5 and 8.7%, respectively.

Clark et al. (1998a) reviewed the microbiology and epidemiology of the 1994 to 1995 cholera outbreak in the Ukraine. During this epidemic, 1,370 cases of cholera occurred with 32 fatalities. Isolates of *V. cholerae* O1, serotype Ogawa, biotype El Tor were isolated from sewage, sea and surface water, freshwater, and marine fish. Because all but one of the environmental isolates were very similar to isolates from infected individuals, the authors concluded that environmental transmission was a significant factor in the epidemic. Molecular analysis of 37 *V. cholerae* and 4 non-cholera *Vibrio* isolates indicates that all Ukrainian toxigenic *V. cholerae* were closely related to each other and to an isolate from a patient from Pakistan. In addition, these strains were closely related to the seventh pandemic strains from Asia supporting the hypothesis that the Ukrainian epidemic of 1994 to 1995 was caused by toxigenic environmental strains surviving since the 1991 Ukrainian epidemic (Clark et al., 1998b).

Seventy clinical strains of *Vibrio cholerae* O1, isolated from 1982 to 1996 in Samutsakorn, a port city 30 km southwest of Bangkok, where cholera occurs at low levels with regular seasonality, were characterized to investigate if there were any differences among the O1 strains isolated before, during, and after an epidemic outbreak (Dalsgaard et al., 1998). Genetic analysis of O1 strains isolated during and after the appearance of O139 suggested that the *V. cholerae* O1 strain may reemerge from an environmental source. A total of 127 strains of *Vibrio cholerae* (117 *V. cholerae* O1 and 10 nonagglutinating strains), isolated from a recent cholera outbreak in Senegal and 4 strains isolated in Guinea-Bissau (during the survey of a cholera epidemic that occurred 10 months before the Senegalese one), were analyzed by Aidara et al. (1998). Strains were characterized by conventional methods (biochemical and serologic identification and susceptibility to antimicrobial agents) and PCR for several specific genes. Conventional methods showed that all strains of *V. cholerae* O1 belonged to serotype Ogawa, biotype El Tor. There was no difference between environmental O1 strains isolated from water and strains isolated from patients with cholera, suggesting that fecally contaminated water is an important reservoir for infection.

*V. cholerae* non-O1 strains were isolated from river water more frequently than from seawater in a study by Uchiyama (1998b). Although the number of *V. cholera* isolated from river water did not correlate with environmental parameters, the variation in numbers suggested that the organism adhered to floating matter in the water. Thomson et al. (1998) tested drinking water from 12 sites in different geographic areas of Vellore, South India, where cholera had been reported. Non-O1, Non-O139 strains of *V. cholerae* were
detected in 41% of the drinking water samples tested. In addition, 100% of the water, sediment, and plankton samples from by nearby test lakes and 87% of the open sewers sampled contained viable non-O1, non-O139 V. cholerae. Son et al. (1998) found significant genetic diversity between isolates of V. cholerae O139 from surface water in Malaysia. In addition, all four strains isolated showed multiple resistance towards antibiotics. Goncalves et al. (1998) examined the prevalence of V. cholerae O1 infection in 1,196 individuals living in Manacapuru, Amazonas State. There was no significant statistical difference between infected and noninfected individuals when analyzed against housing patterns, sanitary facilities, source and treatment of water, destination of domestic waste, sex, or profession. Household location, number of occupants per household, age, and schooling showed significant statistical differences in infection prevalence.

The occurrence of Aeromonas spp., V. cholerae, and Plesiomonas shigelloides in freshwater from various sources in Araraquara, State of Sao Paulo, Brazil, was determined by Pasetto Falcao et al. (1998). Samples from 10 distinct irrigation systems used in vegetable cultivation, from 5 distinct streams, from 2 reservoirs, from 1 artificial lake, and from 3 distinct springs were analyzed. No P. shigelloides was found. V. cholerae non-O1 was found in five irrigation water samples and in three stream samples. Aeromonas sp. were isolated in two samples of irrigation water, in three streams, and in one reservoir.

Vibrio vulnificus infections are highly lethal infections associated with consumption of raw shellfish and exposure of wounds to contaminated seawater. Shapiro et al. (1998) summarized V. vulnificus infections reported to the Centers for Disease Control and Prevention from 23 states. Between 1988 and 1996, 422 infections were reported; 45% were wound infections, 43% primary septicemia, 5% gastroenteritis, and 7% from undetermined exposure. A total of 96% of the patients with primary septicemia had consumed raw oysters; 61% with primary septicemia died. An improved selective medium, cellobiose-colistin (CC) agar was described by Hoi et al. (1998a). CC agar gave a higher plating efficiency of V. vulnificus cells than did cellobiose-polymyxin B-colistin (CPC) agar, mCPC agar, or thiosulfate-citrate-bile salts-sucrose (TCBS) agar. Arias et al. (1998a) compared the effectiveness of culture-based methods and a DNA-based method for the detection of V. vulnificus from seawater and three types of shellfish collected from the coastal waters of Valencia, Spain. Of 32 seawater samples, only 1 yielded positive results by direct detection by PCR, whereas 5 were positive by culture methods. Except for one sampling, positive results by direct detection did not correlate with confirmed strains obtained from culture media. The authors suggest that the best strategy for detection of this organism consists of a combination of culture-based methods and DNA-based procedures.

Genetic relationships among 132 strains of Vibrio vulnificus (clinical, environmental, and disease-ee isolates from different geographic origins, as well as seawater and shellfish isolates from the western Mediterranean coast, including reference strains) were analyzed by random amplified polymorphic DNA (RAPD) PCR (Arias et al., 1998b). There was an overall agreement regarding the high level of homogeneity of disease-ee isolates in contrast to the genetic heterogeneity of Mediterranean isolates suggesting the existence of autochthonous clones present in Mediterranean coastal waters. Hoi et al. (1998b) found that V. vulnificus is ubiquitous in warm marine climates and concluded that seawater can serve as a reservoir for the organism facilitating the spread of V. vulnificus to marine animals and subsequently to humans.

Aeromonas. Aeromonas spp., gram negative, nonspore forming, facultatively anaerobic bacilli belonging to the family Vibrionaceae are associated with a variety of infections in humans. Usually causing a mild, self-limiting diarrhea, which in some cases can become protracted and develop into chronic colitis, some members of the genus are also responsible for soft tissue infection and bacteremia. Fioretini et al. (1998) examined two estuaries, one of which received wastewater treatment system effluent, on the Italian coast of the Adriatic Sea between September 1994 and August 1995 for the presence of Aeromonas spp. There was no correlation between water temperature and numbers of aeromonads isolated. Isolates identified as Aeromonas caviae were the most prevalent in polluted water; while A. hydrophila was most often isolated from cleaner water. Borrell et al. (1998) identified 938 Aeromonas isolates from environmental and clinical sources. Most pathogenic species were prevalent in environmental samples, with A. veronii biotype sobria being the most common in lakes and reservoirs and in treated drinking water and with A. caviae being the most common in seawater and milk products. Aeromonas hydrophila was the second most prevalent species isolated in untreated drinking water. A major drinking water distribution system in northeast Scotland was monitored over a 12-month period to determine the prevalence of mesophilic Aeromonas (Gavriel et al., 1998). Aeromonas were isolated from 21 of the 31 reservoirs. The probability of isolation typically decreased with increasing levels of chlorination. A seasonal pattern in the incidence of Aeromonas emerged with infrequent isolation during the winter period increasing to a peak during the summer. An association was demonstrated between the pattern of Aeromonas isolations and that of rainfall. No relationship was apparent between incidence of Aeromonas and total heterotrophic plate counts. Legnani et al. (1998b) studied the occurrence of Aeromonas spp. in drinking water supplies in a mountain area in northeast Italy (the Dolomites). This water is exposed to a low level of pollution and systematic chemical disinfection is not used. Out of 7,395 water samples analysed over a 3-year period, 1,623 were found to be positive for Aeromonas. Seventy-two of the strains were identified as A. hydrophila, 7% as A. caviae and 9% as A. sobria. The percentage of recovery from surface water (approximately 40%) was found to be higher than that of groundwater. Aeromonas spp. were isolated from 7% of samples from the distribution network and showed no significant variations compared with water from reservoirs. No correlation was found between the concentration of Aeromonas spp. and fecal indicator organisms.

The ability of Aeromonas hydrophila to attach to various water distribution pipe surfaces (stainless steel, copper, and polybutylene) after different contact times at ambient and storage temperatures was examined by Assanta et al. (1998). Aeromonas cells could easily attach to all surface types after exposures as short as 1 or 4 hours at 4 and 20°C. Polybutylene, a low-energy surface, followed by stainless steel, was most colonized by Aeromonas cells, whereas few cells were observed on copper, which has a high surface energy. Pettibone (1998) found a strong correlation between numbers of Aeromonas spp. and both indicator bacteria and total suspended solids in samples from the Buffalo River throughout the year. Samples from four sites on upstream tributaries of the Buffalo River showed a correlation between Aeromonas spp. and both indicator organisms and total suspended solids during the summer months only indicating that different conditions influencing the occurrence and survival of these organisms exist in different water bodies. Despite the lack of correlation between Aeromonas spp. and indicator organisms in certain water bodies during the winter,
he suggested that standard fecal coliform analyses may adequately assess public health risks from Aeromonas spp. until a media capable of quantitative recovery of this organism is developed. Multiple antibiotic and heavy metal resistant strains of Aeromonas were isolated from raw drinking water supplies, irrigation waters, and runoff waters receiving sewage in Chilli (Miranda and Castillo, 1998). Moderately polluted waters showed lower antibiotic resistance and metal susceptibility than either unpolluted or highly polluted ones. Antibiotic resistance could not be related to the level of fecal pollution. All Aeromonas spp. examined in a study by Sisti et al. (1998) were more susceptible to chlorine than Escherichia coli. The effect of the chlorine compound was markedly influenced by water temperature. At a summer water temperature (20 °C), the efficacy of the chlorine concentrations tested was found to be two to three times lower compared to that found at a winter temperature (3 °C).

Campylobacter. Campylobacter spp. are now recognized as a major cause of gastroenteritis associated with the ingestion of contaminated food and water. Furtado et al. (1998) reported that Campylobacter was associated with the majority of waterborne disease outbreaks from private water supplies in England and Wales between 1992 and 1995. Stanley et al. (1998) isolated C. jejuni from a spring impacted by a dairy farm situated within the hydrological catchment in the Arnside area of Cumbria, U.K. Several of the strains isolated from the water had identical biotypes to strains isolated from the dairy herd. C. jejuni was not isolated in the absence of fecal coliforms. Thermotolerant Campylobacter were isolated in 70% of the water samples examined from rivers and lakes in the Warsaw region of Poland with the highest contamination associated with the presence of municipal sewage (Popowski et al., 1997). Interestingly, Campylobacter spp. were also isolated, but to a lesser extent, from areas impacted by the droppings of wild animals.

Examination of 390 sewage samples from three sewage treatment plants in Rio de Janeiro, Brazil, resulted in the isolation of 169 thermophilic strains of Campylobacter dominated by C. jejuni biotype I, C. coli biotype I, and C. jejuni biotype II (Lauria-Filgueiras and Hofer, 1998). Both human and animal pathogenic biotypes were isolated from activated sludge during the initial processing steps. Isolation of Campylobacter decreased significantly after the aeration tank allowing the authors to conclude that the aeration tank could be a barrier to Campylobacter survival.

Thomas et al. (1998) used a combined enrichment and conductivity protocol for the detection of C. jejuni from river water at a concentration of 1 CFU per mL. Campylobacter could be detected in under 39 hours demonstrating an improvement over a conventional membrane filtration technique.

In water microcosm experiments, Buswell et al. (1998) examined the survival times of Campylobacter isolates. Survival times were much longer at 4 and 10 °C than at 22 and 37 °C. The survival times of cultures were considerably longer in the presence of autochthonous water microflora than in the sterile microcosms. Persistence times within biofilms were much longer when they were determined by detection methods not involving culturing. Immunofluorescent-antibody staining demonstrated that the pathogen persisted up to the termination of the experiments after 28 and 42 days of incubation at 30 and 4 °C, respectively. Hazeleger et al. (1998) examined the physiological activities of Campylobacter at several environmental temperatures. Cellular activity, and hence persistence, could be measured at temperatures as low as 4 °C. In addition, the organism was capable of chemotaxis and aerotaxis at all temperatures and thus may be able to move to more favorable environments. Both osmolality and temperature were found to be significant factors in the persistence of Campylobacter spp. (Reezal et al., 1998). None of the Campylobacter examined (C. jejuni, C. lari, and E. coli) grew in media with an osmolality of 130 mosmol and a temperature below 42 °C. In media with low osmolalities, the number of viable cells declined rapidly at any of the temperatures examined. At nongrowth temperatures, inoculation into higher-osmolality media (175 mosmol and above) resulted in the organism persisting up to 96 hours. Thus, the presence of dissolved salts in water environments may enhance the persistence and environmental transport of this organism.

Acrobacter. A total of 147 Campylobacter-like strains were isolated from six drinking water treatment plants in a 2-year investigation conducted by Jacob et al. (1998). The strains were characterized by bio- and serotyping according to the scheme for Arcobacter butzleri. The result was that 100 strains were typed as Arcobacter butzleri, 17 strains were typed as Arcobacter butzleri-like and 6 strains as Campylobacter jejuni/coli, and 24 strains were typed as Arcobacter spp. The strains isolated from the treatment plants showed the same serotypes as described for human isolates. Therefore, the spread of Arcobacter via the drinking water path must be suspected.

Arcobacter enrichment medium (AM), newly developed by Oxoid, was compared with two Campylobacter enrichment media (Preston broth [Oxoid] and LabM broth) and with Arcobacter basal medium (ABM) as a control by Aabay and Corry (1998). Twenty strains of Arcobacter and Campylobacter spp. were tested for growth, with target inocula of less than 4 CFU/mL. Medium none of the Campylobacter spp. grew in the complete AM, and only one grew (very poorly) in the ABM. However, AM supported good growth of all three species of Arcobacter (A. butzleri, A. skirrowii, and A. cryaerophilus), which have been associated with human and animal disease.

Helicobacter. Helicobacter pylori is now recognized as the causative agent of peptic ulcer disease and certain types of stomach cancer. Zhou et al. (1997) evaluated Helicobacter pylori infection among 1,084 healthy people among the Yi and Han nationalities at Yuxian County of Yunnan Province. The overall infection rate was 51.1%. Higher rates were detected among those who drank river water or unboiled-water than among those who drank tap water, well water, or boiled water. Lindkvist et al. (1998) also found the type of water consumed to be a risk factor associated with H. pylori infection in a study involving 242 children aged 2 to 4 years old in rural Ethiopia. On the other hand, Elitssur et al. (1998) evaluated the prevalence rate of Helicobacter pylori in children from urban and rural areas of West Virginia. Helicobacter pylori infection prevalence correlated with increasing age, family crowding, and community location (urban or rural) but not with gender, water source used (city or well), or socioeconomic status. The prevalence rate in the children of West Virginia was higher than any data previously reported from the U.S. Souto et al. (1998) likewise found no correlation between H. pylori infection and drinking water consumption in a study of 204 individuals from a rural area of the State of Mato Grasso, Brazil. Beggue et al. (1998) found a correlation between consumption of food from street vendors associated with increased risk of H. pylori infection in a study of 104 children (0 to 17 years of age) in Lima, Peru, but no correlation with drinking water source. Thus, the possible role of drinking water in the transmission of H. pylori remains unclear.

The survival of Helicobacter pylori in artificially contaminated milk and tap water was investigated by Fan et al. (1998). Helicobacter pylori could survive for up to 10 days in milk at 4 °C.
storage but only 4 days in tap water with a steady decrease of colony-forming units. However, electron microscopy clearly showed that the nonculturable coccoid form was present in tap water that had been kept at 4 °C for 7 days. Hulten et al. (1998) analyzed municipal water, treated wastewater, and well water from Sweden for the presence of Helicobacter spp. using PCR analysis for DNA specific to this genus. They found 9 of the 24 wells, 3 of the 25 wastewater samples, and 3 of the 25 municipal tap water samples assayed were positive for Helicobacter indicating that this organism may be transmitted via water. In light of this possibility, it is interesting to note that Friis et al. (1998) found that wastewater treatment plant workers reported a higher incidence of peptic ulcer disease than other workers. Hegarty and Baker (1998) developed a combined fluorescent-antibody vital staining procedure for the detection and enumeration of H. pylori in water samples. Using this technique, they surveyed surface and groundwater samples in central Pennsylvania, U.S. H. pylori was detected in 77% of the samples analyzed. In 72% of these samples, no E. coli was detected. Johnson, Rice, and Reasoner (1997) studied three strains of Helicobacter pylori to determine their resistance to chlorination. The organisms were readily inactivated by free chlorine and therefore should be controlled by disinfection practices normally employed in the treatment of drinking water.

Legionella. Legionella spp. were first recognized as pathogenic organisms as a result of the 1976 outbreak of pneumonia associated with the 58th annual convention of the American Legion in Philadelphia. One hundred and eighty-two cases of pneumonia and 29 deaths were associated with this outbreak. Since its initial isolation, Legionella has been shown to be transmitted by direct inhalation of aerosols. Brieman and Butler (1998) reviewed the ecology of Legionella emphasizing the capacity of the organism to multiply within amoebae in warm water and the use, during the 20th century, of devices that maintain water at warm temperatures and produce aerosols. They noted that the disease is much more common than previously appreciated with at least 13,000 cases estimated to occur per year in the U.S.

Saint (1998) described modifications to the EnviroAmp Legionella detection system that permit the rapid analysis of bacterial colonies taken from Legionella selective media. The modified technique provides a convenient and cost-effective alternative to standard confirmation procedures. L. pneumophila was detected in 21 water samples from 11 sites from hot spring baths in Kanagawa, Japan (Kuroki et al., 1998a). Naegleria, Platyamoeba, Acanthamoeba, and two other genera of free-living amoebae, which may harbor Legionella, were detected in 22 samples from the sites. In addition, the authors noted that 13 water samples contained N. lovaniensis. Although N. lovaniensis is nonpathogenic, it is considered an indicator organism for places that are suitable for the growth of N. fowleri, the causative agent of primary amebic meningoencephalitis in man. These same authors (Kuroki et al., 1998b) surveyed the occurrence of both Legionella species and free-living amoebae in whirlpool baths installed in 11 private houses, 8 public baths, and 13 spas. Free-living amoebae that are known to be the hosts of Legionella were isolated from 24 out of 32 water samples. Further studies were conducted for 10 consecutive weeks to monitor the occurrence of both free-living amoebae and Legionella in the whirlpool baths of four private houses. Free-living amoebae, such as Hartmannella and Vexillifer a, and L. pneumophila were consistently isolated from all the water samples throughout the monitoring periods. Management practices such as frequent washing of filter elements or frequent addition of tap water to bath basins was recommended to reduce microbial contaminants.

Luck et al. (1998) used DNA restriction patterns to compare Legionella pneumonia isolated from a 44-year-old woman who developed pneumonia after cerebral surgery with isolates from water samples. When colonies from the water specimens were analyzed, a serogroup 12 strain complementary to that found in the clinical specimen was identified. In addition, the same serogroup one strain was isolated from the patient and the water system. Environmental cultures of hot water tanks, faucets, and showerheads were performed in six health care facilities in Allegheny County, Pennsylvania, according to health department guidelines (Goetz et al., 1998). Legionella was isolated from the water distribution system in 83% of the facilities. Fiore et al. (1998) investigated a pneumonia outbreak in a Pennsylvania town using epidemiological and molecular microbiological studies to determine the outbreak source and interrupt transmission of disease. Case-patients were more likely than controls to have been within 1000 feet of the hospital during the 2 weeks prior to illness. Legionella pneumophila serogroup 1 (Lp-1) was isolated from hospital cooling towers (CTs) and rooftop air samples but not from hospital potable water or community CTs. Hospital CT and air Lp-1 isolates matched all five patient isolates.

The effects of various concentrations of sodium chloride solutions and different temperatures on survival of Legionella pneumophila were investigated by Holler et al. (1998). It was found that at temperatures between 4 and 20 °C, Legionella organisms survived in salt solutions up to 3% NaCl. Only the combination of high temperatures, i.e., 30 and 37 °C, with NaCl concentrations greater than 1.5%, reduced cell numbers significantly. It was interesting to note that the addition of small amounts of NaCl (0.1 to 0.5%) enhanced survival of L. pneumophila, suggesting a protective effect of NaCl. To obtain information about conditions encountered in the environment, the survival experiments were repeated in sterile seawater from the Baltic Sea and the North Sea. The marked bacterial die-off, especially at higher temperatures, was not observed in natural seawater. All these results indicate that L. pneumophila can survive in the marine environment.

Lin et al. (1998a) reviewed the factors promoting the colonization of water distribution systems with Legionella and methods to control the growth of this organism. Conditions within water systems that promote colonization included water temperature, configuration and age of the hot water tank, physicochemical constituents of the water, plumbing materials, and commensal microflora, while control measures included superheat-and-flush, copper–silver ionization, ultraviolet light, instantaneous heating systems, and hyperchlorination. They noted that although each of the disinfection methods has been proven to be effective in the short term, long-term disinfection has been difficult.

Kool et al. (1998) found that hospitals supplied with drinking water containing free chlorine as a disinfectant were more likely to have a reported outbreak of Legionnaires’ disease than those that used water with monochloramine as a disinfectant. On the basis of these observations, they suggested that 90% of the outbreaks associated with drinking water might not have occurred if monochloramine had been used for residual disinfection.

Stout et al. (1998) compared the efficacy of metal ions versus the superheat-and-flush method of disinfection for the control of L. pneumophila. The reduction in Legionella colonization after copper–silver ionization was significant compared to the superheat-and-flush. The authors concluded that a properly maintained and monitored copper–silver ionization system was more effective.
than the superheat-and-flush method for reducing the recovery of Legionella from the hospital water distribution system. A copper-silver ionization system was evaluated for the control of L. pneumophila in hot-water recirculation lines (Liu et al., 1998). Four weeks after activation of the system, no distal sites were positive for Legionella. Legionella recolonization did not occur for 6 to 12 weeks. A significantly higher copper concentration was found in the biofilm than in the bulk water. This is likely to be the reason that the copper-silver ionization system had the residual effect of preventing early recolonization.

Steinert et al. (1998a) examined the factors involved in the occurrence of Legionellaeaeae in a hospital water system and the recontamination by Legionella pneumophila after a thermal disinfection procedure. Three months after the heat treatment (70 °C), the regrowth of the two prevalent Legionella strains reached the original level of cell numbers. Genetic analysis revealed the strains to be survivors of the decontamination.

Mycobacterium avium complex. Atypical mycobacteria are responsible for a variety of diseases, particularly in immunocompromised individuals. Mycobacterium avium complex (MAC) organisms have been isolated from water and soil. It is now generally accepted that environmental sources, especially natural waters, are the reservoirs for most human infections caused by MAC. Wallace et al. (1998) reviewed nosocomial outbreaks and pseudo-outbreaks caused by the nontuberculous mycobacteria (NTM). These outbreaks have been recognized for more than 20 years and continue to be a problem. Most of these outbreaks have involved the rapidly growing mycobacterial species Mycobacterium fortuitum and M. abscessus. The reservoir for these outbreaks is generally municipal and (often separate) hospital water supplies. They noted that these mycobacterial species and others are incredibly hardy, able to grow in municipal and distilled water, thrive at temperatures of 45 °C or greater (M. xenopi and M. avium complex), and resist the activity of organomercurials, chlorine, 2% concentrations of form-aldehyde and alkaline glutaraldehyde, and other commonly used disinfectants.

Lin et al. (1998) found that Mycobacterium avium was significantly more resistant to disinfection with copper–silver ions than was Legionella pneumophila. Water, both in the city water supply and hospital environment, was found to be the major source of transmission of Mycobacterium xenopi, an opportunistic pathogen that causes pulmonary infections (Badalk et al., 1998).

Steinert et al. (1998b) compared the growth of Mycobacterium avium in coculture with the free-living amoeba Acanthamoeba polyphaga with the growth of M. avium when it was separated from amoebae. Although viable mycobacteria were observed within amoe-bal vacuoles, there was no significant difference between bacterial growth in coculture and bacterial growth separately. In contrast, Legionella pneumophila multiplied only in coculture.

Stenotrophomonas (Pseudomonas). Stenotrophomonas (Pseudo-monas) maltophilia is an emerging nosocomial pathogen in patients following therapy for malignancy. The identification of sources and routes of transmission of this bacterium is of importance in the development of strategies to prevent nosocomial infections. Wilkinson and Kerr (1998) determined the prevalence of S. maltophilia in both carbonated and noncarbonated commercially available bottled water. Whereas carbonated water did not yield bacterial growth, 22 samples of noncarbonated products had viable bacterial. Three samples yielded S. maltophilia and a further eight grew Pseudomonas species. Isolates of S. maltophilia of both clinical and environmental origin were able to survive or grow in noncarbonated mineral water over a range of pH and temperature values, including refrigeration temperatures.

Tropheryma. Whipple’s disease is a systemic disorder in which a gram-positive rod-shaped bacterium is constantly present in infected tissues. After numerous unsuccessful attempts to culture this bacterium, it was eventually characterized by 16S rRNA gene analysis to be a member of the actinomycetes. The name Tropheryma whippelli was proposed. Until now, the bacterium has only been found in infected human tissues. There is no evidence for human-to-human transmission. Maiwald et al. (1998) reported the detection of DNA specific for the Whipple’s disease bacterium in 25 of 38 wastewater samples from 5 different sewage treatment plants in the area of Heidelberg, Germany. Their data argues for an environmental source for infection with the Whipple’s disease bacterium.

Bacterial endotoxin. Two sequential outbreaks of respiratory disease among lifeguards at an indoor swimming pool with water spray features were investigated by Rose et al. (1998). Pool air and water were sampled for fungi, bacteria, amoebae, endotoxin, and respirable particulates. Analyses indicated increased levels of endotoxin in pool air and water (relative to control pools) and gram-negative bacterial colonization of water sprays. Use of water spray features generated a 5.2-fold increase in the number of respirable particles and up to an 8-fold increase in air endotoxin levels. Lifeguards in this indoor swimming pool developed granulomatous lung disease associated with endotoxin-containing respirable bioaerosols from water spray features, which ventilation system improvements did not prevent.

**VIRUSES**

Viruses are a diverse group of acellular, obligately parasitic, organisms. They are incapable of metabolism, growth, and replication outside of a host cell but can persist in the form of infectious virions within environmental media.

Because viruses cannot be grown outside of a host organism, detection of viruses depends on either cell infection assays or molecular techniques for the detection of viral genetic material. In addition, because most viruses have an extremely low infectious dose detection of viruses in water necessitates concentration of virions from large (100 L or more) of water.

**Bacteriophages.** Bacteriophages are viruses that infect bacterial cells. Typically they are specific for a particular genus or even species of bacteria. Although bacteriophages are not directly associated with human diseases, they have been widely proposed as indicator and model organisms for the detection of fecal pollution and for modeling viral transport in the environment.

Approximately 1 000 fecal samples from a variety of animals and 64 sewerage samples were assayed for male specific bacteriophage by Calci et al. (1998). All samples tested harbored these viruses. Over a period of 10 months, Deborde et al. (1998) assayed coliphage levels in the effluent from a multiuser septic tank and in the groundwater below the down-gradient edge of the drainfield. Their results indicated that the levels of coliphage present in the effluent were high enough to allow them to be used as indicators of fecal contamination in groundwater.

Dowd et al. (1998a) investigated the influence of viral isoelectric point on viral adsorption onto aquifer sediment material using five different spherical bacteriophages having differing isoelectric points in laboratory transport studies. The authors developed a model of virus migration in the soil columns using a one-dimen-sional transport model in which kinetic sorption was included. The data suggested that the isoelectric point of a virus is the predeter-
mining factor controlling viral adsorption within aquifers; however, when virus particles are more than 60 nm in diameter, viral dimensions become the overriding factor.

Virus elimination as a result of the wastewater treatment processes of natural lagooning and activated sludge was studied by Benyahya et al. (1998) using <i>QX-174</i> and MS2 phages as model viruses. The two treatments tested removed both phages with equal efficiency and both were eliminated more rapidly than rhodamine, the inorganic tracer used for comparison. Chendorain et al. (1998) studied MS2 transport as a model for human enteric viruses in constructed wetlands. They found removal to be 97 ± 3% with most viral removal observed within the first 3 months. Iranpour (1998) seeded a secondary effluent that had been previously filtered through a trimedia filter with MS2 bacteriophage. The effluent was then processed using microfiltration (MF) and reverse osmosis (RO) units. Use of the RO unit resulted in removal of viruses to undetectable levels with the MF method reducing levels by less than one order of magnitude. The use of oxidized coal to remove viruses from water was investigated by Cioe et al. (1998). The oxicoal product was found to be able to remove not only coliphages but also various pathogenic human viruses from seeded water sources. Removal was dependent on the type of virus, the period of exposure, and the concentration of oxidized coal.

Miller et al. (1998) evaluated a male-specific bacteriophage plaque assay for process control verification for a wastewater treatment plant processing pork slaughterhouse wastes. Numbers of plaque plaque-forming units per gram or milliliter showed greater variation and were usually lower than standard indicators, including total coliform or <i>Escherichia coli</i> counts.

Gantzer et al. (1998) attempted to determine if somatic coliphages, <i>Bacteroides fragilis</i> phages, or the enterovirus genome were appropriate indicators for infectious enterovirus in water. RT-PCR was used to detect the enterovirus, genome, cell culture was used for the infectious enteroviruses and plaque formation for the bacteriophages. They observed a significant correlation between the plaque concentration and enterovirus (infectious form and genome). Maitland et al. (1998) investigated the viricidal activity and the mechanism of action of sodium hypochlorite using the <i>Pseudomonas aeruginosa</i> PA01 phage FI16 as a model virus. The bacteriophage was inactivated with a low concentration of the oxidant, and the final effluent indicated a decline in the number of enterovirus comparable with standard indicators, including total coliform or <i>Escherichia coli</i> counts.

Hepatitis virus. Viral hepatitis is an inflammation of the liver caused by one of several very different viruses. Currently, five hepatitis viruses, denoted by a letter of the alphabet (A through E) are recognized as causing human disease with hepatitis A (HAV), hepatitis B, and possibly some other forms of non-A, non-B hepatitis virus being most commonly associated with waterborne transmission. Exposure to infectious agents in the workplace continues to be a concern. Issues that are currently being addressed include whether wastewater treatment workers should be vaccinated against hepatitis A (Warlen and Hoff, 1998). Using the salivary assay for IgG anti-HAV Brugha et al. (1998) studied 241 employees of a water and sewerage company who may have had occupational exposure to sewage. They determined that exposure to raw wastewater was a significant risk factor for hepatitis A and that this was independent of other known risk factors. In a cross-sectional study the employees of a wastewater treatment plant were tested for hepatitis B virus (HBV) markers—HBsAg, anti-HBs, anti-HBc—to determine the prevalence of HBV infection and assess the risk of exposed wastewater workers becoming infected to evaluate the necessity for appropriate vaccination (Arvanitidou et al., 1998). The overall prevalence of HBV markers was 43.9% with 6.6% of the employees being HBsAg carriers. Logistic regression analysis confirmed that only exposure to wastewater was independently associated with positivity for HBV infection. The authors concluded that workers exposed to wastewater should be vaccinated against hepatitis B virus. Salamo and Copello (1998), on the other hand, found no evidence of an increased prevalence of positive hepatitis A markers in a study on occupational risks of a group of wastewater workers of the city of Genoa versus a control group of nonexposed subjects.

Arnal et al. (1998) used RT-PCR to detect the hepatitis A virus (HAV) genome in HAV-contaminated artificial seawater and compared it to HAV detection in cell culture. In the former, the genome was detectable for 232 days compared with 35 days for cell culture. These results indicated that ability to detect the genome may not be a valid indicator of viral survival in cell culture and suggested the need for additional studies on the effect of environmental factors in seawater on genome stability and maintenance of viral infectivity. Divizia et al. (1998) used an antigen capture PCR (AC-PCR) technique followed by membrane hybridization to detect hepatitis A viruses in wastewater. Assays of 10 samples each of raw wastewater, wastewater after oxidation, and the final effluent indicated a decline in the number of hepatitis A positive samples. However, finding hepatitis A viruses in the effluent of the treatment plant indicates it may be a source of environmental contamination. Jothikumar et al. (1998) used immunomagnetic capture (IC) and polymerase chain reaction (PCR) techniques to concentrate hepatitis A (HAV) virus from environmental samples. They determined this technique was able to detect less than one plaque-forming unit (PFU) of HAV in water and wastewater and was specific for HAV.

Clayson et al. (1998) studied a hepatitis outbreak that occurred from January 29, 1995, to March 15, 1995, in a military training camp in Nepal. Sera from patients and soldiers not exhibiting symptoms were tested for hepatitis A, B, C, and E using commercially available ELISA kits. PCR was also used to detect hepatitis E virus, which was determined to be the cause of the outbreak. The source was thought to be fecal contamination drinking water. Of the 488 soldiers not exhibiting symptoms, 83 were found to be recently infected without experiencing symptoms of the disease. It was suggested that the apparent infection detected may be a typical response in areas endemic for the disease.

A hepatitis outbreak that occurred in 1993 in the indigenous population of Djibouti, Republic of Djibouti (East Africa), and in local French soldiers and their families was reported by Coursaget et al. (1998). They indicated that this is the first report of a waterborne outbreak caused simultaneously by hepatitis A and E viruses. Commercially obtained ELISA tests were used to detect hepatitis A, B, C, and E viruses. They noted that hepatitis E was found almost exclusively in the indigenous population and that anti-hepatitis E antibodies were present in 19% of the indigenous control group. Hepatitis A was found primarily in French individuals. Singh et al. (1998) reported on an outbreak of hepatitis E that occurred in the city of Saharanpur (Uttar Pradesh, India) between April 1992 and December 1992. There were 3,682 cases of the disease. A contaminated water supply resulting from treated water leaking from pipes and passing through sewerage holes was determined to be the cause of the outbreak.
Using RT-PCR followed by nested PCR, Pina et al. (1998a) analyzed 37 samples of raw wastewater for hepatitis E virus from an area in Barcelona, Spain, where the virus is not endemic. The virus found in the single positive sample was inoculated into rhesus monkeys, isolated, and characterized. It was found to have 98% identity with a Madras, India, isolate. Tomar (1998) reported on information obtained from examination of 10,500 cases of hepatitis E virus in pediatric populations. The vast majority of cases were associated with fecal contamination of drinking water, and the author suggested the use of a residual chlorine concentration of at least 0.5 mg/L for a minimum of 30 minutes to prevent outbreaks of this disease.

Viral et al. (1998) studied two populations from low socioeconomic areas in Rio de Janeiro, Brazil, for their age-specific prevalence of anti-hepatitis A antibodies (anti-HAV). Sera from the two populations was collected in 1978 (population 1, neonates to age 6) and 1995 (population 2, ages 1 to 23). They found a difference in the levels of antibodies in the two groups with population 1 having a higher incidence of anti-HAV (65.5%) compared with 32.1% for population 2. They also noted a lower exposure of young children in population 2 compared with that of population 1. These results may have reflected the better living conditions of the children in population 2 who had access to safer water and better sanitation.

Adenovirus. Adenoviruses are DNA viruses most commonly associated with respiratory infections and conjunctivitis in humans. In addition, there is some evidence from animal studies that adenoviruses are associated with the development of tumors.

Paparopoulou and Vantarakis (1998) investigated a July 1995 outbreak of pharyngocconjunctivitis caused by adenoviruses occurred among athletes participating in a swimming contest in a town in southern Greece (Peloponese). At least 80 persons displayed symptoms of the illness. Poor chlorination was probably the cause of the outbreak (residual chlorine less than 0.2 mg/L) because after hyperchlorination the spread of adenoviruses stopped. They noted that rapid detection of adenoviruses in the municipal swimming pool water by nested polymerase chain reaction (PCR) amplification allowed quick control of the outbreak. Castignolles et al. (1998) detected adenovirus in nucleic-acid extracts from the Seine River estuary by a two-step amplification of a 220-bp segment of the conserved coding region of type 2 adenovirus hexon protein L3. The primers used in this study detected the most prevalent adenovirus serotypes in human disease in France but not other virus strains or bacteria.

Pina et al. (1998b) used PCR to detect human viruses in wastewater (urban and slaughterhouse), shellfish, and river and seawater. Their results indicated that both the adenoviruses and the hepatitis A viruses detected in the environment came primarily from humans, with the former being the most frequently detected virus over the course of a year. All enterovirus and HAV positive samples were also positive for human adenoviruses suggesting the use of PCR to detect adenoviruses as an indicator of human viruses in environmental samples.

Enteroviruses. Enteroviruses are a genus of viruses belonging to the family Picornaviridae. Traditionally, these viruses have been divided into four subgroups—polioviruses, coxsackievirus A, coxsackievirus B, and echoviruses—based on differences in host range, growth in cell lines, and pathogenicity. Enteroviruses are widespread in aquatic environments and are fairly easy to cultivate; therefore, they have frequently been used as indicator viruses in a manner analogous to the use of fecal coliforms as indicator bacteria.

PCR has been considered by a variety of workers for detection of viruses in environmental samples. Protocols for using this procedure to detect enteroviruses are provided by Wyn-Jones and Sellwood (1998). Caillou et al. (1998) obtained sludge samples from a plant that treats wastewater from Tucuman, Argentina. Samples were shaken, centrifuged and treated with antibiotics before inoculation into cell culture. Using this method enteroviruses were detected in 5 of the 12 samples assayed. Using cell culture and RT-PCR, Reynolds et al. (1998) attempted to detected enterovirus in marine samples obtained from recreational waters in Mamala Bay, Hawaii. The results indicated that recreational waters may present a health hazard to the public. They found cell culture methods to be more effective than RT-PCR.

Tanaka et al. (1998) attempted to determine the possible risks associated with use of reclaimed wastewater. They considered a variety of applications using data from California on enteric virus concentrations in unchlorinated secondary effluents. They used the reliability criterion of meeting less than 10⁻⁴ annual risk of infection at least 95% of the time. They indicated the need for a larger enteric database and the use of standardized protocols. Vantarakis and Paparopoulou (1998) compared the use of nested PCR and cell culture to detect enteroviruses from 120 coastal water samples from the Greek Achaia coastline. They found nested PCR to be an effective method for the detection of both enteroviruses and adenoviruses. They also reported that the presence of these viruses did not correlate with the presence of bacterial fecal indicators in the waters tested. Li et al. (1998) reported on the use of an electroscopic filter media particle to adsorb enteric viruses. They used this method of viral concentration with a variety of enteric viruses and report high levels of recovery.

Poliiovirus. Adu et al. (1998) determined that the majority of 22 wastewater samples collected from the southwestern part of Nigeria were positive for type 1 wild polio virus. Because this virus is from human contamination, finding it suggests that the Nigerian polio immunization program has been unsuccessful. Sobsey et al. (1998) examined polio virus type 1 (PV1) and MS2 coliphage to determine their persistence in water and wastewater and their inactivation by free chlorine, chlorine dioxide, and UV radiation. Their results indicated that RT-PCR detects inactivated viruses resulting in false positive results. Tsai and Parker (1998) developed a RT-PCR to quantitate polio virus in environmental samples. Quantitation was facilitated by the use of an internal standard that coamplified with the poliovirus.

Using poliovirus-1, Quignon et al. (1998) showed a highly significant linear relationship between virus inactivation rate and water conductivity for sterilized water samples. Using sterile saline solutions, however, they demonstrated that this apparent relationship was false, i.e., virus inactivation rate was not affected by water conductivity alone. With the hypothesis that salts may serve to potentiate the antiviral activities of certain microbiological substances that are found in the water samples, they explained the apparent relationship observed. They noted that effective water treatment could be responsible for the removal of both viruses and some virus-inactivating factors. Alternatively, virus-inactivating capability of a given water resource could be enhanced along with its hardness or its degree of mineralization.

Viral gastroenteritis. In addition to the specific viruses discussed above, there are a variety of viruses associated with viral gastroenteritis. Diarrheal diseases associated with these organisms, including rotavirus, Norwalk virus, Calicivirus, and Coronavirus, affect between 3 billion and 5 billion individuals per year with up
to 10 million deaths. Although the viruses can be transmitted by a variety of fecal-oral routes, outbreaks of viral gastroenteritis are frequently associated with contaminated water.

The adsorption of Norwalk viruses (NV) to a variety of soil types was studied by Meschke and Sobsey (1998). The results obtained were compared to that of two other enteric viruses—poliovirus 1 (PV), which absorbs strongly, and MS2, which absorbs weakly. NV adsorption was intermediate between that of PV and MS2.

Shin and Sobsey (1998) studied the effect of 2 mg/L of preformed monochloramine on Norwalk virus (NV), as assayed by quantitative RT-PCR. NV results were compared to those obtained for poliovirus 1 (PV1) and coliphage MS2, which were assayed by infectivity and RT-PCR. A comparison of the detection techniques indicated that RT-PCR underestimated the effect of monochloramine. It was also determined that preformed monochloramine was not an effective disinfectant for any of the viruses tested.

A review article by Smith et al. (1998) provides information on animal caliciviruses, which are present in ocean reservoirs. These viruses can be a source of human infection but, unlike the human caliciviruses, can be cultivated in vitro. Their potential as indicators for the uncultivable animal caliciviruses is discussed in this review.

**PROTOZOA**

Twenty-six outbreaks associated with waterborne transmission of disease were reported to the Public Health Laboratory Service Communicable Disease Surveillance Center between January 1, 1992, and December 31, 1995 (Furtado et al., 1998). The majority of the outbreaks were associated with drinking from public (10) or private (9) water supplies, and 4 outbreaks were associated with swimming pool water. *Cryptosporidium* was thought to be the causative agent of the public water supply and swimming pool water outbreaks, indicating a continuing risk of *Cryptosporidium* transmission. Kramer et al. (1998) reported the first *cryptosporidiosis* outbreak associated with the use of recreational water. The outbreak lasted for approximately 1 month and affected more than 2,000 people. Exposure to lake water was found to be strongly associated with disease. It is thought that runoff and infected swimmers were the source of the organism. Willocks et al. (1998) reported the largest *cryptosporidium* outbreak due to groundwater. They suggested that the 340 cases reported in North Thames in the spring of 1997 resulted from ingestion of tap water that was obtained from a deep chalk borehole. It was not known how the oocysts gained entrance to the borehole.

*Ameoba*. Mathers et al. (1998), analyzing data for the period from January 1993 to December 1996, determined that the highest incidence of amoebo-like keratitis in Iowa occurred in June and November. They compared this data with reports on the incidence of amoeba in groundwater from Tulsa, Oklahoma, a region with a similar climate. They indicated a yearly increase in the incidence of the amoebo-like keratitis and concluded that the numbers of amoeba in surface water may have an effect on the incidence of the infection.

Meier et al. (1998) determined the risk factors associated with an outbreak of presumed *Acanthamoeba* keratitis using 31 patients diagnosed between July 1993 and December 1994. The major risk factor was found to be the use of contact lenses. Regional flooding was also suggested as a risk factor. It is not clear how important a role the flooding played, if at all, because the diagnostic techniques used were changed at the same time as the outbreak occurred.

*Naeegleria fowleri* is a free-living amoebalagellate that normally lives in warm water. The organism causes primary amoebic meningoencephalitis (PAM). Although infections with this organism are rare, the high fatality rate associated with PAM makes this an important waterborne pathogen. Permin et al. (1998) studied the use of filtration and centrifugation to concentrate *Naeegleria fowleri* vegetative cells and cysts that had been mixed with *N. lovaniensis* and *N. australiensis*. Ten replicates of 100 and 10 mL were counted using the most probable number method. A higher percentage of cysts than trophozoites were recovered by both concentration techniques. The authors also noted that because neither method of concentration provided consistent recovery rates, they would not provide accurate information on environmental samples containing low levels of organisms. Gupta et al. (1998) studied the effect of irrigating hardwood with secondary treated wastewater effluent by determining the numbers and species of soil protozoa and nematodes. Use of the wastewater stimulated protozoan growth and resulted in a change in the composition of both protozoan and nematode populations. Species of both *Naeegleria* and *Acanthamoeba* were detected in the irrigated plots.

Sampling for amoebae was done in six hospital hot-water systems and moist sanitary areas previously determined to be contaminated with *Legionella* (Rohr et al., 1998). Swabs were used to sample the moist areas of the system. Approximately 30% were found to be positive for amoebae. *Acanthamoeba* and *Naeegleria* were two of the five genera of amoebae detected in the swabs. Szenasi et al. (1998) indicated that although molecular methods used to differentiate human pathogenic and nonpathogenic strains of *Naeegleria* and *Acanthamoeba* are becoming available, there continues to be a need for rapid, specific tests. They reported on a survey carried out in Hungary on the isolation and identification of free-living amoebae and potentially pathogenic strains of these organisms.

*Cryptosporidium* and *Giardia*. *Cryptosporidium* and *Giardia* are the two most widely recognized protozoan pathogens for which a waterborne route of transmission has been demonstrated. Both cause chronic diarrheal disease, particularly in immunosuppressed individuals. The organisms undergo a complex life cycle during which cysts or oocysts are produced. These structures are highly resistant to disinfection but still remain infectious. Infectious dose for both organisms is extremely low with as few as 10 to 30 cysts or oocysts capable of causing clinical infection. Cryptosporidium is now recognized as a common cause of diarrhea throughout the world. Although more than 20 species of *Cryptosporidium* are now recognized, only one, *C. parvum*, is considered to be a human pathogen. Giardiasis is caused by *Giardia lamblia*, recently renamed *G. duodenalis*.

A review by Fricker and Crabb (1998) indicates areas where knowledge of Cryptosporidium is limited as well as new methods for monitoring water. It also suggested approaches that can be taken to lessen the danger of this parasite to our water supply.

Moore et al. (1998) tested the ability of four commercial antibodies to detect *Cryptosporidium* after treatment with sodium hypochlorite and sodium metaperiodate. They observed damage to the *C. parvum* epitopes recognized by the commercial antibodies. This damage did not reduce viability of the oocysts but did result in decreased detection. Vesey et al. (1998) developed a fluorescence in situ hybridization (FISH) technique for the detection of *Cryptosporidium* oocysts in water. The oligonucleotide probe targets a unique sequence in the 18S ribosomal RNA of *C. parvum* and was determined to be species specific. Positive results (fluorescence) was observed only with oocysts capable of excys-
tation. The authors suggested coupling this technique with immunofluorescence staining for detection of viable oocysts in water samples.

In an attempt to subtype Cryptosporidium parvum Tyzzer, 1912, McAulchin et al. (1998) evaluated the use of SDS-PAGE Western-blotting of an oocyst wall antigen. Using this technique allows for the differentiation of multiple types of the parasite. Brasseur et al. (1998) indicated that excystation alone is not a valid indication of oocyst viability. They suggest that infectivity for enterocytic Caco2 tissue cells provides the necessary specific information on the viability of C. parvum oocysts. Deng and Cliver (1998) used genomic DNA isolated from Cryptosporidium parvum oocysts by a specific immunomagnetic separation—in vitro excystation procedure and subjected to randomly amplified polymorphic DNA analysis using sequence-independent primers to differentiate strains of C. parvum isolated from different sources. Using this technique, an estuary C. parvum isolate was easily differentiated from several bovine isolates, while five bovine isolates of the same origin were indistinguishable from each other.

Bukhari et al. (1998) compared immunomagnetic separation (IMS) kits for the separation and concentration of C. parvum oocysts in waters with turbidity levels that ranged from 50 to 500 nephelometric turbidity units. The effect of oocyst age on recovery was also studied using one of the commercial kits. The results obtained were variable depending on the kit used and the level of turbidity present. Sartory et al. (1998) studied the recovery of Cryptosporidium oocysts from water using a novel filter system. The ability of this system to recover oocysts from various volumes of tap and river water inoculated with a range of organisms was in the 90% range. After using a potassium citrate flotation concentrate to further purify the cysts, recovery levels were 60%. The authors indicated this is superior to conventional methods of oocyst recovery that use wound cartridge and membrane filters. A RT-PCR method for simultaneous detection of Cryptosporidium and Giardia (oo)cysts that can be used for the direct analysis of primary water concentrates was developed by Kaucner and Stinear (1998). The technique was compared with an immunofluorescence assay using concentrated environmental samples. The RT-PCR technique resulted in an increase in the detection of viable Giardia cysts, but fewer positive samples of viable Cryptosporidium were detected, suggesting that species other than parvum were present in the water. Matheson et al. (1998) report on the Gelman Environchek capsule, which is used to concentrate Cryptosporidium and Giardia (oo)cysts from water. They indicate that multiple samples can be treated in 1 hour and after concentration any purification technique can be used. Studies indicate that recovery for Giardia (80%) is higher than that for Cryptosporidium (70%).

Tirfiat et al. (1998) determined that Giardia cysts could be quantified using direct immunofluorescence and their viability confirmed using the fluorogenic dyes (4',6-diamidino-2-phenylindole) DAPI and propidium iodide (PI) along with differential interference microscopy (DIC). Using Giardia cysts obtained from feces and wastewater sludge, they indicated that they obtained significantly better results than when standard counting methods were employed.

A case control study of a cryptosporidiosis outbreak (52 diagnosed cases) in the Wirral peninsula indicated no significant association between the outbreak and use of water from the single water plant, which uses the river for source water (Hunter and Quigley, 1998). However the epidemiological studies coupled with the detection of oocysts in treated water during the investigation fit the Public Health Laboratory Service criteria for associating use of the water with the outbreak. The authors suggested that case control studies will be of lesser value in situations in which treated surface waters are used. The population of individuals drinking these waters may obtain immunological protection from exposure to low levels of cysts in the water. A Cryptosporidium outbreak occurred on a U.S. Coast Guard cutter that had been supplied with water from Milwaukee in March 1993 (Moss et al., 1998). The authors found that individuals with confirmed cases had consumed significantly more water while on the boat than individuals not considered as confirmed cases.

A total of 18% of 222 children with diarrhea in the study area of 4 Zambian townships were determined to have cryptosporidiosis (Nchito et al., 1998). Specific areas of the study area were characterized as being high or low risk based on the levels of oocysts in the water supply. Results suggested that waterborne contamination may be a major factor in spreading infection and that the incidence of the disease may not be associated other factors such as contact with animals, nutritional status, and parental education. Sareidi and Bava (1998) studied the incidence of Cryptosporidium in hospitalized pediatric patients. They found the frequency of this illness to be greater in younger children (less than 18 months) who have diarrhea, who are immunodeficient, and who live in areas that do not provide the appropriate sanitary conditions or safe sources of drinking water. The incidence of Cryptosporidium infection in asymptomatic children in four Aymara communities in the Bolivian Altiplano was approximately 30% (Esteban et al., 1998). Single stool samples were taken from 377 children aged 5 to 19. There were no significant differences when age and sex were considered. The high incidence of asymptomatic infection was thought to be associated with the poor living conditions of the children. The authors suggested that continuous exposure to the parasite may confer protection resulting in the high levels of asymptomatic children.

Asymptomatic HIV-positive injecting drug users in Manipur State in Northeast India were studied by Anand et al. (1998) to determine if they were infected with a variety of protozoa. They found a high incidence of Cryptosporidium in these individuals, although they did not exhibit the characteristic disease symptoms associated with these organisms such as diarrhea. It was suggested that these individuals may function as carriers potentially introducing the pathogens into water. Perez et al. (1998) used a risk analysis approach to determine the role of tap water in the spread of Cryptosporidium using adults and children with and without AIDS as four population subgroups. Applying their model to the New York City population, they calculated the numbers of tap water-related cases per year in the AIDS subgroups to be 34 and in the non-AIDS subgroups to be 6.

Eisenberg et al. (1998) analyzed data from the 1993 Milwaukee Cryptosporidium outbreak (March 23) to develop a model of the epidemic. They determined that an earlier outbreak occurred on March 1 and suggested that if this one had been detected, 85% of the subsequent cases might have been prevented. Their data also suggested a 3 to 7 day incubation period, increased levels of oocysts in the water, and a decrease in the treatment efficiency of the water. Morris et al. (1998) carried out a retrospective study to determine if there were Cryptosporidium outbreaks in Milwaukee that preceded the massive April 1993 outbreak. They examined hospital data as well as turbidity data from the Milwaukee water works and determined that for more than a year before the 1993 outbreak there was an association between cases of gastroenteritis and turbidity. They noted the lag period observed between increased turbidity and cases of gastroenteritis was similar to that
observed in the 1993 outbreak. They concluded that drinking water associated cases of cryptosporidiosis occurred before the 1993 outbreak. Griffin et al. (1998) carried out a telephone survey questioning individuals in Milwaukee, the site of the massive 1993 Cryptosporidium outbreak. A total of 610 people participated in the study. The results indicated that the media served as the main source of information for many individuals, strongly influencing their concerns about the possibility of future infections by Cryptosporidium. Proctor et al. (1998) studied data that were available during the Milwaukee Cryptosporidium outbreak. They suggested that the use of surrogate measurements of morbidity, which are available prior to laboratory results, can help lower the magnitude of outbreaks. Various methods that may be applied in these situations were discussed.

After observing long term survival of Cryptosporidium in artificial seawater, Fayer et al. (1998a) studied the ability of oysters to take up (oo) cysts. Oysters from Chesapeake Bay were obtained and examined for the presence of both Giardia and Cryptosporidium using immunofluorescence and bioassays. Giardia was not found in any of the samples. Cryptosporidium was found indicating that oysters in natural waters can be a source of infection. The ability of oysters to take up infectious oocysts from seawater was tested by Xiao et al. (1998) who used strain-specific diagnostic tools based on 18S ribosomal RNA sequence variations to detect organisms in a variety of samples. They found Cryptosporidium genotype 1 associated with human diseases and genotype 2 in the majority of oysters that they obtained from Chesapeake Bay, suggesting that oysters may be used as indicators of the quality of estuarine waters. In a study by Graczyk et al. (1998a), Asian freshwater clams (Corbicula fluminea) were exposed to Cryptosporidium parvum oocysts for 24 hours. By this time, oocysts were no longer detectable in the water. Acid fast staining and immunofluorescent antibody (IFA) techniques were used to assay the clams for oocysts with the highest levels of recovery obtained when IFA was used on tissues from the gills and GI tract. Oocysts released from the clams were consistently found associated with fecal matter.

Fayer et al. (1998b) tested the ability of oocysts obtained from calves and held at various temperatures and times to infect neonatal BALB/c mice. Cyst infectivity was determined by microscopic observation of developmental stages in sections of mouse gut or a positive PCR for C. parvum DNA in samples of mouse ileum. Oocysts held from 0 to 20 °C were found to be infectious even after 24 weeks, with levels of infectivity varying depending on the time and temperature of storage in deionized water.

Youssef et al. (1998) used direct fluorescent monoclonal antibodies and Ziel-Neelsen staining to detect Cryptosporidium parvum in various Alexanderia, Egypt, water sources. They found C. parvum in uncovered water tanks, associated with the shore area near two canals and in one swimming pool. Although they found the direct fluorescence technique to be more sensitive in C. parvum detection, the alternative method allowed for the detection of other pathogen protozoans.

Hirata and Hashimoto (1998) determined that microfiltration (MF) and ultrafiltration (UF) were appropriate methods for drinking water treatment. Bench-scale experiments were run using MF (nominal pore size of 0.25 μm) and UF (nominal molecular weight cutoff of 13,000 daltons) modules in a cross-flow mode. Results from studies in which 10^6 oocysts/L were tested with both modules and higher levels tested with MF indicate that this technology can be used to remove high levels of oocysts from contaminated influent waters. The use of membrane filtration for the removal of Cryptosporidium and Giardia (oo) cysts was studied by Falk et al. (1998). After filtration, the (oo) cysts were observed using phase contrast microscopy and counted using a haemocytometer. The method was found to be more effective for the removal of Giardia cysts. Studies by Hoffmann et al. (1998) indicate that chlorine dioxide may be a more appropriate disinfectant than chlorine in treating waters with Cryptosporidium contamination.

Bertolucci et al. (1998) suggested the use of pretreatment to improve raw water quality. They investigated a natural lagoon (an abandoned gravel quarry) used for short-term storage of raw water. Decreased levels of Cryptosporidium and Giardia (oo) cysts were detected in the effluent from this reservoir. Ho and Tam (1998) monitored two rivers in Hong Kong for E. coli, Giardia cysts and Cryptosporidium oocysts. Although bacterial levels in the two rivers were similar, the levels of Giardia varied, reflecting the type of wastewater treatment used along the river. Low levels of Cryptosporidium oocysts were reported. These results indicated a lack of correlation between bacterial indicators of fecal contamination and the presence of protozoan parasites.

Atherholt et al. (1998) attempted to determine if a relationship existed between the presence of Cryptosporidium and Giardia (oo) cysts and other biological or environmental factors that were more easily monitored. Rainfall was considered to be a major factor resulting in the increase of (oo) cysts as well as other microorganisms.

Ground and surface water supplies of San Pedro Sula, Honduras, were assayed by Solo-Gabriele et al. (1998) for the presence of Cryptosporidium and Giardia (oo) cysts in June 1996 using the immunofluorescent antibody technique. Giardia cysts were present in higher numbers in surface waters, with the reverse situation observed in groundwaters. The Cryptosporidium levels detected were similar to those reported for other surface water supplies in North America, but Giardia levels were higher than expected.

From July, 1993, to December, 1995, Karanis et al. (1998) assayed samples from six water treatment plants for the presence of Giardia and Cryptosporidium. Cysts and oocysts were found in the raw and treated waters tested. The results indicate the need for optimization of water treatment practices. The authors determined that combining slow sand filtration, infiltration, disinfection, sand, and activated carbon filtration resulted in the most effective treatment regime.

Feces obtained from calves with diarrhea and healthy calves were analyzed by de Verdier Klingenberg and Svensson (1998) for rotavirus, Cryptosporidium and Escherichia coli K99+. A statistically significant correlation was found between the presence of group A rotavirus and diarrhea. Although Cryptosporidium oocysts were observed in smears from 19% of the fecal samples, there was no statistically significant correlation between diarrhea and the presence of oocysts. E. coli K99+ was not detected in any of the fecal samples.

The sedimentation kinetics of Cryptosporidium oocysts and Giardia cysts in environmental settings was studied by Medema et al. (1998). The attachment of (oo) cysts to particulate matter in settled secondary effluent was determined to occur rapidly with 30% of (oo) cysts attaching within minutes of exposure to the effluent and approximately 75% attachment after one day. Once attached, the sedimentation velocity of the (oo) cysts was determined by the size of the associated particle. The authors suggested that (oo) cyst attachment to particles plays a major role in the subsequent fate of the organisms.

544

Water Environment Research, Volume 71, Number 5
Emerging Protozoan Pathogens: Cyclosporidia, Microsporidia, and Toxoplasma. With the increased number of immunosuppressed individuals in the population in recent decades, several new protozoan pathogens have been recognized. *Cyclosporidia cayetanensis* is a recently described protozoon associated with prolonged self-limiting and relapsing diarrhea. It has a worldwide distribution with a higher prevalence in tropical and subtropical countries. The first known human cases of illness caused by *Cyclospora* infection (i.e., cyclosporiasis) were reported in 1979. Cases began being reported more often in the mid-1980s. *Microsporidia* are obligately parasitic protozoa that lack mitochondria. They are becoming increasingly well known in the medical arena through pathologies they cause in humans with impaired immune systems. Several species of microsporidia infect HIV-positive patients. Most frequent is *Enteroctoctoziun bieneusi. Encephalitozoon hellem* and *cuniculi*, and a new species designated *Septata*, have also been reported to cause human disease. *Toxoplasma gondii* is an ubiquitous organism that can cause several clinical syndromes in animals and humans, including one of the most common central nervous system infections in patients with AIDS.

Graczyk et al. (1998b) introduced Asian freshwater clams (*Corbicula fluminea*) into waters contaminated with *Cyclospora cayetanensis* oocysts. After 24 hours oocysts were not observed in the water. Analysis of clam hemolymph and gill smears indicated the clams had taken up the *Cyclospora*, although by day 18 post-exposure oocysts were not recoverable from the clam tissue or feces. Additional experiments indicated that the number of oocysts recovered decreased as a function of clam size. The results obtained in this study suggest that *C. fluminea* can be used as a biological indicator for contamination with *C. cayetanensis* oocysts. Sturbaum et al. (1998) attempted to detect *Cyclospora* oocysts in wastewater using current techniques. The detection of *Cyclospora cayetanensis* indicate that contaminated wastewater may be a source of the pathogen.

Dowd et al. (1998b) extracted and screened DNA from 14 samples of wastewater effluent, surface water, and groundwater using PCR amplification and PCR sequencing. Database homology comparison indicated that pathogenic microsporidia were found in half of the 14 samples tested, indicating that water may transmit these pathogens.

The world's largest outbreak of waterborne toxoplasmosis occurred in a municipality in the western Canadian province of British Columbia (Isaac-Renton et al., 1998). When drinking water emerged as a possible source of infection during the outbreak investigation, a laboratory method was needed to attempt detection of the parasite *Toxoplasma gondii*. The method developed was based on the current U.S. EPA method for detection of *Cryptosporidium* oocysts. Collection of large-volume drinking water samples and cartridge filter processing were unchanged, although identification of *Toxoplasma* oocysts in the filter retentate was carried out by using a previously described rodent model. Validation of the method developed was tested by using oocysts from a well-characterized *Toxoplasma* strain.

HELMINTHS AND OTHER PARASITES

Bouhoum and Schwartzbrod (1998) compared the stools of "wastewater farming" children exposed to raw wastewater (El Azzouzia area) and those of a control group. Of the exposed children, 73% were infected with one or more helminths versus 30% of the control group. The main parasites were *Ascaris* and Trichuris. The El Azzouzia children were more heavily infected. Thus, "wastewater farming" children are exposed to detectable risk from the parasitic nematodes in raw wastewater. Kosoff et al. (1998) surveyed the prevalence of *Ascaris lumbricoides, Trichuris trichiura,* and *Hymenolepis nana* in two adjacent, but socioeconomically distinct, urban Costa Rican communities: a squatter settlement and a community with access to modern wastewater treatment facilities. The prevalence of these infections was significantly higher in the former. Although squatter children (1 to 14 years old) were more heavily infected with *A. lumbricoides* and *H. nana* than squatter adults, the same pattern was not observed for *T. trichiura.*

Buitrón and Galván (1998) compared a 21-day in vitro incubation technique with a vital staining technique for the detection of viable helmint eggs in wastewater. They reported a mean recovery efficiency of 50.3 ± 2.8% of the eggs added to spiked samples. Although the 21-day incubation technique, which depends on the microscopic detection of developing helmint larvae within the ova, gave adequate results, the addition of the vital stain did not significantly improve detection of viable ova. They concluded that the vital staining technique may be better suited to a rapid detection method. Johnson et al. (1998) developed a simple procedure using *Ascaris suum* as a model for *Ascaris lumbricoides* in testing the ability of sludge treatment processes to kill the eggs of parasitic roundworms. After 1 week in a mesophilic anaerobic digester, 95% of *A. suum* eggs produced two-cell larvae into vitro, with 86% progressing to motile larvae. After 5 weeks in the digester 51% progressed to motile larvae. Between 42 and 49% of eggs stored in a sludge lagoon for 29 weeks were viable and able to develop motile larvae.

Cercarial dermatitis (swimmer's itch) is an inflammatory response to penetration of skin by schistosome parasites while swimming or wading in lakes. Lindblade (1998) investigated risk factors for cercarial dermatitis to determine whether limnological characteristics of the lake were associated with development of the condition. Factors associated with the development of cercarial dermatitis identified in the study included age, the time of day at which exposure to lake water occurred, and the month in which exposure to lake water took place. In addition, development of cercarial dermatitis was significantly associated with exposure to lake water in the area of the lake with the highest algae content and shallowest depth. Chamot et al. (1998) investigated the occurrence of cercarial dermatitis in Geneva, Switzerland. Overall, 153 bathers out of 555 reported probable cercarial dermatitis. Of the cases, 11.1% noticed more than 30 skin lesions, 19.6% described severe itching, 50.3% used a drug treatment, 3.9% visited a doctor, and 15% claimed they would reduce their bathing activities. History of cercarial dermatitis, time spent in the water, hour of the day, barometric pressure, and maximum daily atmospheric temperature predicted disease occurrence in multivariate analysis. Although a benign disease, cercarial dermatitis may have a negative effect on the local water recreation industry.

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**Geographic Information Systems**

**Michael W. Sweeney**

Geographic information systems (GIS) provide powerful tools for spatial analysis. However, their inherent capabilities for complex and dynamic analysis remain limited. Simulation models, on the other hand, are powerful tools for complex and dynamic situations. But they often lack the intuitive visualization and spatial-analysis functions that GIS offers. The integration of GIS and simulation models, within a common and interactive graphical user interface, produce more powerful, easy-to-use, and easy-to-understand planning and analysis information systems.

**GROUNDWATER MODELING AND MAPPING**

Fuest et al. (1998) compiled and stored long-term groundwater monitoring data, spatial databases of land-use patterns, livestock figures, and soil and meteorological data in a GIS and overlaid with regionalized aquifer contamination to facilitate the hazard mapping of top aquifer contamination for Osnabrueck, Germany. An aquifer vulnerability study in the southwest Trans-Danubian Central Range resulted in plotting an atlas to be a useful tool in the hands of land-users and waste-disposal managers, helping them to...