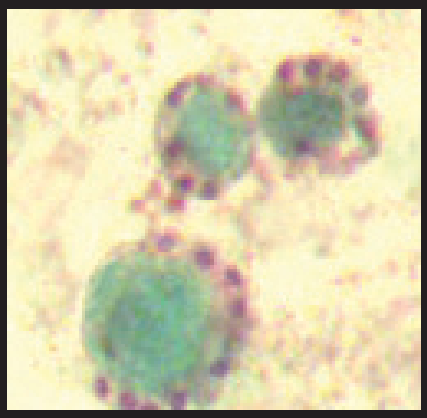
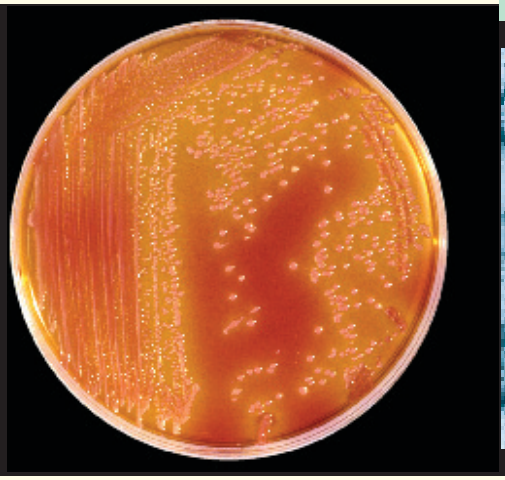
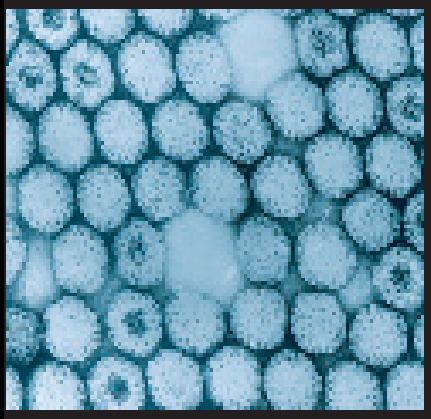
Waterborne Pathogens

MANUAL OF WATER SUPPLY PRACTICES

M48







Second Edition

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Waterborne Pathogens

AWWA MANUAL M48

Second Edition

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MANUAL OF WATER SUPPLY PRACTICES—M48, Second Edition

Waterborne Pathogens

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Preface

Waterborne pathogens can cause life-threatening disease in the immunosuppressed populations of the world and illness in the general population. Reports in print and electronic media of recent waterborne outbreaks and emerging pathogens have expanded public awareness of water quality issues. This awareness places greater demands on government agencies and utilities to effectively inform the public about waterborne agents and related health issues. If outbreaks do occur, having accurate information available is a critical need.

The Organisms in Water Committee of the American Water Works Association (AWWA) prepared this manual for water utility management, water quality, and public relations personnel to use as a reference tool. The manual provides scientific information in a concise, readable format that will be helpful to media, city and state government, and water customers who inquire about waterborne pathogenic organisms. Public health and environmental workers will also find it a convenient reference.

This manual includes chapters on waterborne disease outbreaks; water quality in treatment and distribution systems; water quality monitoring, sampling, and testing; and sections for organisms classified as parasite, bacteria, or virus. Each organism is described in terms of its taxonomic classification and biology, the disease it causes, its mode of transmission, its occurrence, the effectiveness of water treatment methods in controlling it, and monitoring recommendations. Each chapter has a reference section, and a detailed glossary is included at the end of the manual.

Pathogenic organisms reviewed in this manual have been identified in epidemiological investigations of waterborne outbreaks or have the potential to cause such outbreaks. The second edition of the M48 manual expands upon the first with new and revised chapters all reviewed by the section chair. This edition reflects current emerging and re-emerging waterborne pathogens, contemporary procedures, and current literature. The revision also includes improved detection and identification methods and a chapter on molecular detection.

The reader is directed to Appendix A, Additional Resources, at the back of the manual. Appendix A is a list of resources and web links for more information on treatment, security, USEPA resources, and related information.

This is the second edition of the *Waterborne Pathogens* manual. AWWA and the Organisms in Water Committee would appreciate any comments on the manual. Contact Elise Harrington, AWWA Water Quality Engineer, at (303) 794–7711, 6666 W. Quincy Ave., Denver, CO 80235.

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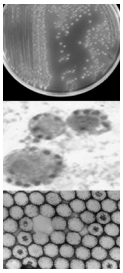


I

Introduction to Water Quality

Waterborne Disease Outbreaks: Their Causes, Problems, and Challenges to Treatment Barriers

Water Quality in Source Water, Treatment, and Distribution Systems

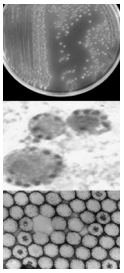
Water Quality Monitoring, Sampling, and Testing

Molecular Detection of Waterborne Microorganisms

Water-related stories are commonly reported on national television news—stories such as toxic algal blooms in coastal waters and watersheds, waterborne disease outbreaks in major cities around the world, and efforts to protect drinking water supplies from feedlot waste or other contaminants. As world economies become increasingly global, waterborne diseases travel independent of national borders. For example, imported agricultural products washed with polluted waters and shipped to the United States have been associated with several waterborne disease outbreaks. These news events have political, economic, and cultural ramifications that either promote or erode water quality and its association with public health.

Utilities must be active and vigilant to reduce the likelihood of outbreaks of waterborne pathogens—regardless of their geographic location. Utilities throughout the world are now focusing more on proactive security measures, which may include protecting against biological, chemical, and radiological contaminants as well as physical threats. The first step to ensure safe water is to assess current levels of contaminants, including chemical, physical, and microbiological, in source waters. Next, a program of watershed protection, education, and continual improvements to reduce and/or contain these contaminants is begun. Then water treatment plant performance must be enhanced by optimizing treatment processes. For systems with groundwater, attention must be focused on data evaluation over time, and they must be alert to potential groundwater contamination that may take decades to become apparent. Water distribution system water quality can degrade once the water has left treatment, and programs should be tailored to meet the utilities' site-specific challenges on a routine basis. Proactive contamination prevention programs for distribution systems include vulnerability assessments, security for storage and distribution systems,

backflow prevention programs, and ongoing education to plumbing and landscape companies. In-house, private, public health, and university laboratories can help implement and maintain proper sampling techniques and monitoring strategies necessary to meet the goal of providing safe drinking water. Chapters 1 through 3 provide an overview of water quality issues related specifically to waterborne pathogens. Chapter 4 provides a discussion of the current information on molecular methods and the role these methods may play in complementing traditional detection of waterborne microorganisms.



Chapter 1

Waterborne Disease Outbreaks: Their Causes, Problems, and Challenges to Treatment Barriers

Gunther F. Craun, Rebecca L. Calderon, and Michael F. Craun

INTRODUCTION

In the United States, state and local public health agencies are responsible for detecting outbreaks, monitoring disease, and performing epidemiological investigations of suspected waterborne outbreaks. When requested, the US Environmental Protection Agency (USEPA) and Centers for Disease Control and Prevention (CDC) help investigate waterborne outbreaks. Each state and territory also has a public health agency with an epidemiological officer whose duties include the investigation of waterborne disease outbreaks. USEPA and CDC also maintain a national waterborne disease outbreak surveillance program, periodically compiling and analyzing statistics for outbreaks that are voluntarily reported.

To be considered a waterborne outbreak, acute illness affecting two or more persons with similar symptoms must be epidemiologically associated with water exposure (Lee et al. 2002). The exception is a single case of chemical poisoning where the water was found to be chemically contaminated (i.e., infantile methemoglobinemia associated with high nitrate concentrations). During an outbreak investigation, water is usually found to be contaminated with coliform bacteria or a chemical; pathogens have been isolated from water samples in some outbreaks.

Most reported outbreaks are associated with water used or intended for drinking or domestic purposes from community, noncommunity, and individual water systems. Outbreaks are also associated with water not intended for consumption (e.g., the use of contaminated springs and creeks by backpackers and campers, and/or accidental ingestion of water while swimming) and water contaminated at its point of use (e.g., a contaminated faucet). The waterborne outbreak surveillance system does not include

outbreaks that occur on cruise ships operating from U.S. ports or cases of endemic waterborne disease.

STATISTICS

Since 1920, 1,836 waterborne outbreaks, 882,592 cases of illness, and 1,152 deaths in the United States have been associated with contaminated drinking and recreational water. Most outbreaks (88 percent) occurred in drinking water systems. Outbreaks caused by the accidental ingestion of water while swimming or during other recreational use have been systematically reported only since 1971, and these events are not included in the statistics presented (Tables 1-1 to 1-3, and Figures 1-1 to 1-6). During 1971 to 1980, only 15 outbreaks were associated with water recreation; during 1981 to 1990, 67 outbreaks were reported; from 1991 to 2000, 160 outbreaks were reported.

The number of waterborne outbreaks reported in drinking water systems shows somewhat cyclical variations (Figure 1-1). From 1931 to 1950 and 1971 to 1990, the highest number of outbreaks, an annual average of 30, were reported. The fewest outbreaks, 12 per year, were reported from 1951 to 1970. An annual average of 17 outbreaks was reported during the most recent 10-year period, 1991 to 2000; this number of outbreaks is slightly higher than that reported during 1961 to 1970 (13 per year) and fewer than that reported during 1981 to 1990 (29 per year).

Usually, more outbreaks were reported in noncommunity systems than in community systems (those serving at least 25 year-round residents). Noncommunity water systems also provide water to the public, but they do not give year-round service. These systems serve primarily transient populations such as institutions, industries, camps,

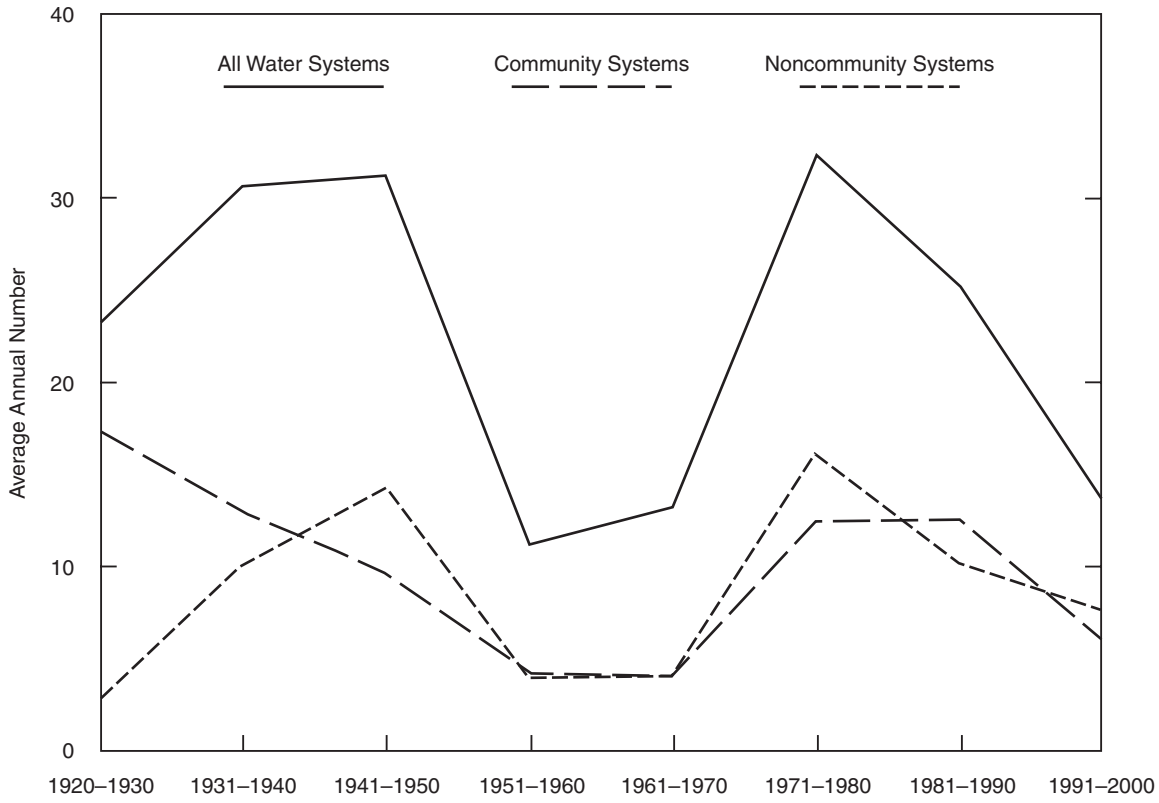


Figure 1-1 Waterborne disease outbreaks in the United States, 1920 to 2000

Table 1-1 Illnesses and deaths associated with drinking water outbreaks in the United States, 1920 to 2000

Time Period	Number of Outbreaks	Cases of Illness	Illnesses per Outbreak*	Deaths
1920–1930	255	102,024	400	669
1931–1940	275	93,306	339	320
1941–1950	313	53,935	172	61
1951–1960	111	12,491	112	10
1961–1970	131	46,399	354	20
1971–1980	324	78,155	241	6
1981–1990	254	63,549	250	6
1991–2000	173	432,733	2,501	60
Totals	1,836	882,592	481	1,152

* Cases of illness are divided by number of outbreaks to calculate illnesses per outbreak.

Table 1-2 Drinking water outbreaks and illness by type of water system in the United States, 1920 to 2000

	Community Systems		Noncommunity Systems	
Time Period	Outbreaks per Year	Illnesses per Outbreak	Outbreaks per Year	Illnesses per Outbreak
1920–1930	17.2	513	2.6	138
1931–1940	12.8	748	8.1	60
1941–1950	9.6	467	14.2	57
1951–1960	4.1	247	3.9	51
1961–1970	3.9	1,023	3.9	111
1971–1980	12.3	483	16.1	113
1981–1990	12.4	289	10.1	268
1991–2000	6.1	6,929	7.7	123

parks, hotels, and service stations that have their own water supplies available for use by employees and the public. Individual water systems are used by residents in areas without community systems. After 1931, a similar reporting trend was observed for outbreaks in both noncommunity and community water systems. An annual average of six outbreaks was reported in community water systems from 1991 to 2000, one-third the number reported from 1920 to 1930. In noncommunity systems, an annual average of eight outbreaks was reported from 1991 to 2000, almost three times the number reported from 1920 to 1930.

Waterborne outbreaks have caused, on average, 481 cases of illness per outbreak. Case numbers vary considerably, however, depending on the type of water system and time period (Tables 1-1 and 1-2). Outbreaks in community systems usually cause more illness than outbreaks in noncommunity systems. During each decade, cases of illness per outbreak ranged from 247 to 6,929 (median = 498) in community water systems and 51 to 268 cases of illness per outbreak (median = 112) in noncommunity systems.

The largest reported waterborne outbreak occurred in the spring of 1993 when contamination of the Milwaukee, Wis., drinking water system with *Cryptosporidium parvum* caused an estimated 403,000 cases of watery diarrhea (MacKenzie et al. 1994; Kaminski 1994). Because of this single outbreak, the average number of illnesses in community water systems during 1991 to 2000 was 6,929 cases per outbreak, more than six times the highest previously reported number of illnesses (Table 1-2). During 1961 to 1970, when two large outbreaks of 16,000 cases each were reported in community systems, an average of 1,023 cases of illness occurred. Excluding the Milwaukee outbreak, 29,733 cases of illness or 173 cases per outbreak occurred in community systems from 1991 to 2000, an illness rate comparable to that observed during 1981 to 1990.

Most deaths associated with waterborne outbreaks occurred before 1940 and were caused by typhoid fever (Craun 1986). Cases reported since 1971 included immunocompromised persons affected during the cryptosporidiosis outbreaks in Milwaukee and in Clark County (including Las Vegas), Nev., in 1993 and 1994. Among the 403,000 cases of illness in the Milwaukee outbreak, an estimated 50 deaths occurred. This estimate is based on a survey of death certificates during the 2-year period before the outbreak and the 2 years inclusive of and following the outbreak. There were 50 more cryptosporidiosis-associated deaths than would have been expected; 46 (85 percent) of these deaths were among persons who had acquired immunodeficiency syndrome (AIDS) as the underlying cause of death (Blair 1994; Hoxie et al. 1996).

In Clark County, most of the reported cases were among immunocompromised persons. Of the 78 persons with laboratory-confirmed *Cryptosporidium* infection during the outbreak, 61 cases were adult human immunodeficiency virus (HIV)-infected adults and 2 cases were HIV-infected children. Goldstein et al. (1996) reported that 32 of the 61 HIV-infected adults had died within 2 months following the outbreak period and at least 20 of the 32 had cryptosporidiosis as a cause of death on their death certificates. Forty of the HIV-infected adults died within the 12 months following the outbreak. However, the 1-year mortality rate for HIV-infected persons in the county was not increased (Kramer et al. 1996). The Clark County deaths are not included in the waterborne outbreak statistics.

Bacterial pathogens caused 15 of the remaining 22 deaths reported since 1971. Seven deaths resulted from community outbreaks of diarrheal illness caused by contamination of water storage tanks with *Salmonella typhimurium*; contamination of water mains with enterohemorrhagic *Escherichia coli* 0157:H7 was responsible for four deaths. Two deaths occurred during an outbreak of mixed etiology (*E. coli* 0157:H7 and *Campylobacter jejuni*) attributed to untreated, contaminated well water at a fair.

Two elderly residents of a nursing home died during an outbreak of shigellosis. One death occurred during an outbreak of undetermined etiology. Six deaths were caused by chemicals. Two persons died after consuming arsenic-contaminated well water, and single deaths were caused by accidental fluoride contamination of a community water system (two outbreaks), ethylene glycol contamination of drinking water used for hemodialysis, and high nitrate in a farm well.

A better perspective of the effects of waterborne disease outbreaks is obtained when the disease burden of the outbreaks and cases is considered. One simple measure of the burden that links the number of cases with their severity is person-days of illness (calculated by multiplying duration of illness by the number of cases). During 1971–2000, information about the median duration of illness data was available for slightly more than 42 percent of the outbreaks of identified or suspected infectious etiology. The reported information was used to estimate duration of illness for those outbreaks in which this measure was not reported, and person-days of illness was calculated by multiplying duration of illness by the number of cases for all infectious disease outbreaks. The estimated burden of enteric illness associated with outbreaks during 1971–2000 was 4,669,480 person-days of illness. The majority of this burden was due to protozoan outbreaks (90 percent), especially the Milwaukee

outbreak. The remaining burden was due to unidentified agents (6 percent), bacterial agents (2 percent), and viral agents (2 percent). The burden as measured by person-days of illness varied from year to year as did the number of reported outbreaks and cases. The burden (person-days ill per year) during the most recent 5 years (mean = 3,478; median = 2,716; lowest = 1,477) was much less than the burden during 1991 to 1995 (mean = 743,567; median = 3,635,960; lowest = 7,102) or 1971–1975 (mean = 16,035; median = 14,854; lowest = 645).

OUTBREAK REPORTING

These statistics do not reflect the actual incidence of waterborne outbreaks or disease. Rather, the observed trends largely reflect surveillance activities of local and state health agencies during various time periods. Many factors influence the degree to which outbreaks are recognized, investigated, and reported in any single year, including interest in the problem and the capabilities for recognition and investigation at the state and local level (Berkelman et al. 1994). The likelihood that individual cases of illness will be detected and epidemiologically associated with water is dependent on many factors including: (a) public awareness of waterborne illnesses, (b) local requirements for reporting cases of particular diseases, (c) the surveillance and investigative activities of state and local public health and environmental agencies, and (d) availability of and extent of laboratory facilities. The recognition of outbreaks largely depends on the effectiveness of surveillance systems, and increased reporting often occurs as the causes and etiologies of waterborne outbreak become better recognized and state surveillance activities and laboratory capabilities increase (Foster 1990; Frost et al. 1995, 1996, 2003). Waterborne disease surveillance is enhanced by educational outreach programs targeting district public health offices, physicians, and the public. Monitoring programs also benefit from designating one individual to be responsible for disease reporting and outbreak investigations; all water-related complaints and health department inquiries are directed to this person. For example, during a 4-year period of intensive waterborne disease surveillance in Colorado from 1980 to 1983, 18 waterborne outbreaks were reported compared to only 6 during the previous 3-year period, when a passive surveillance program was in effect (Hopkins et al. 1985).

While it is generally agreed that reporting of waterborne outbreaks is incomplete, the extent of underreporting is difficult to estimate. Rough estimates suggest that only one half to one third, and even as few as 10 percent, of all waterborne outbreaks are detected, investigated, and reported (Craun 1986; Craun et al. 1992).

Outbreaks in community water systems are more likely to be recognized than noncommunity or individual water systems. Outbreaks affecting small populations are less likely to be recognized than those affecting large populations. Outbreaks characterized by more severe symptoms (bloody or profuse, watery diarrhea; vomiting; fever) are more likely to be recognized than those outbreaks with less-severe symptoms (mild diarrhea or general flu-like symptoms) because persons are more likely to seek medical attention. Outbreaks caused by protozoa and certain bacteria are more likely recognized than viral outbreaks because laboratory analyses are more widely available for clinical specimens.

Not all outbreaks are rigorously investigated. The resources available for an outbreak investigation differ from locality to locality, and the investigation of possible waterborne outbreaks depends on the availability of resources and trained personnel. Improved outbreak investigations can increase our knowledge about important etiologic agents, water system deficiencies, and sources of contamination (Craun et al. 2001).

Several investigators have attempted to estimate the extent to which epidemic waterborne disease is underreported. Hauschild and Bryan (1980) estimated that 1.4 million to 3.4 million cases of both foodborne and waterborne diseases occurred each year in 1974 and 1975. Carlsten (1993) estimated that 350,000 to 875,000 cases of epidemic waterborne disease may occur each year. Considering both epidemic and

endemic waterborne diseases, Morris and Levin (1994) estimated 1.8 million cases and 1,800 deaths annually. Bennett et al. (1987) estimated the annual incidence of waterborne disease to be more than 900,000 cases, accounting for almost 900 deaths. Many believe that Morris and Levin and Bennett et al. have given extreme estimates. Epidemiological studies are currently being conducted by the USEPA and CDC to assess the magnitude of endemic waterborne risks in the United States.

CAUSES OF OUTBREAKS

Contaminated groundwater has caused more waterborne outbreaks than contaminated surface water. In each decade since 1920, contaminated, inadequately treated groundwater caused 44 to 56 percent of all reported outbreaks (Figure 1-2), whereas, inadequately treated surface water has caused 7 to 35 percent of all outbreaks (Figures 1-3 and 1-4). During 1991 to 2000, 46 percent of all outbreaks were attributed to contaminated, inadequately treated groundwater; only 7 percent were attributed to inadequately treated surface water.

Since 1971, the incidence of untreated surface water and groundwater as a cause of outbreaks has declined. Contaminated, untreated groundwater was responsible for 28 to 32 percent of all outbreaks during 1971 to 2000. In previous decades, almost half of all outbreaks were reported in untreated groundwater systems (Figure 1-2). Inadequate or interrupted disinfection of groundwater, however, has increasingly caused outbreaks. During the past quarter-century, 18 to 20 percent of all outbreaks were reported in disinfected groundwater systems. In previous decades, only 2 to 6 percent of all outbreaks occurred in these systems. The observed trends in outbreaks occurring in groundwater systems may be due to an increased emphasis on disinfecting groundwater sources with little or no effort to reduce sources of contamination. Also important may be a lack of effective, continuous disinfection, caused perhaps by a perception among some operators that groundwater needs no disinfection. Although most outbreaks in disinfected groundwater systems since 1971 were the result of inadequate chlorination, several occurred in systems using iodine and ultraviolet (UV) light for disinfection. To reduce waterborne disease risks in groundwater systems, USEPA has proposed the Ground Water Rule (USEPA 2000; 65 FR 91:30194–274) that will specifically state when corrective action including disinfection is required to protect consumers from bacteria and viruses. Other proposed requirements include periodic sanitary surveys to identify deficiencies, hydrogeologic sensitivity assessments for undisinfected systems, source water microbial monitoring for certain systems, and compliance monitoring for systems that disinfect to ensure adequate inactivation or removal of viruses.

From 1920 to 1930, almost 20 percent of all outbreaks were reported in untreated surface water systems (Figure 1-3). Surface water is now rarely used without treatment, and only 8 percent and 4 percent of all outbreaks were reported in untreated surface water systems from 1971 to 1980 and 1981 to 1990, respectively. Only two such outbreaks were reported from 1991 to 2000. Outbreaks in unfiltered, disinfected surface water systems have also decreased, causing only 4 percent of all outbreaks in 1991 to 2000 (Figure 1-3). During the previous two decades, 13 percent and 15 percent of all outbreaks were reported in unfiltered, disinfected surface water systems. This decrease in reported outbreaks in unfiltered surface water systems is likely due to the provisions of the Surface Water Treatment Rule (SWTR) promulgated by USEPA on June 19, 1989 (54 FR 124:27486–541). The SWTR is intended to protect against exposure to *Giardia intestinalis*, viruses, and *Legionella*, as well as selected other pathogens for public systems that use surface water or groundwater under the direct influence of surface water. The SWTR specifies disinfection criteria for community and noncommunity water systems and requires filtration for all but exceptionally high-quality surface water sources. The Interim Enhanced SWTR (USEPA 1998; 63 FR 241:69477–521), Long Term 1 Enhanced SWTR (USEPA 2002; 67 FR 9:1812–44), and Long Term 2 Enhanced SWTR (USEPA 2003; 68 FR 154:47639–795) provide additional

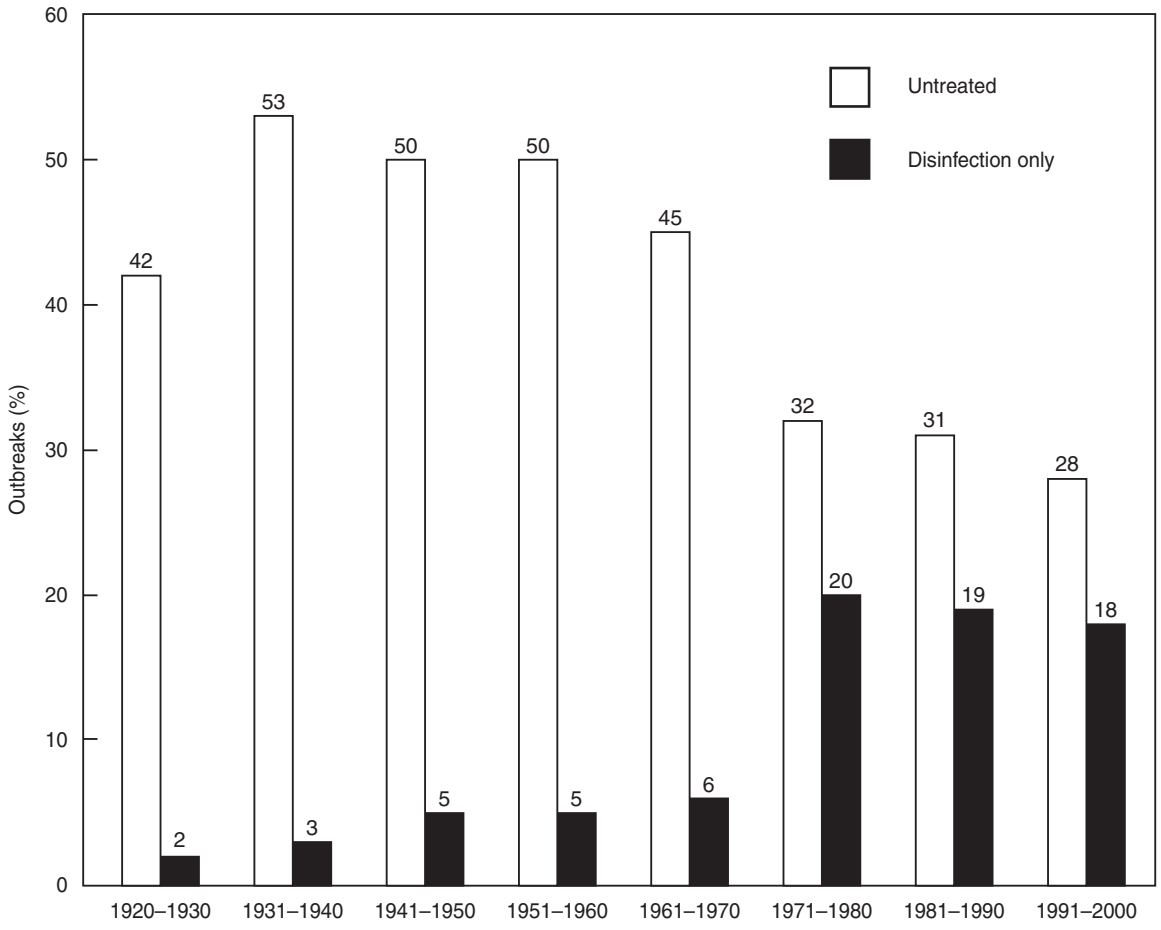


Figure 1-2 Waterborne disease outbreaks in groundwater systems in the United States, 1920 to 2000

protection against *Cryptosporidium* and other waterborne pathogens. Key provisions of the Long Term 2 Enhanced SWTR include source water monitoring for *Cryptosporidium* and additional treatment for filtered systems based on source water concentrations; inactivation of *Cryptosporidium* by all unfiltered systems; disinfection profiling and benchmarking to ensure continued levels of microbial protection while systems take steps to comply with new disinfection by-product (DBP) limits; and covering, treating, or implementing a risk management plan for uncovered finished water storage facilities.

Outbreaks in filtered surface water systems increased from 1981 to 1990, when 7 percent of all reported outbreaks occurred in these systems. This percentage is about the same for outbreaks reported in filtered systems during 1920 to 1930 (Figure 1-4). Inadequate filtration or pretreatment, or both, was responsible for only 3 percent of all outbreaks reported during 1991 to 2000 and only 1 to 3 percent from 1931 to 1980. Whether or not outbreaks in filtered water systems will remain at these low levels depends largely on the success of current efforts to protect source water quality; optimize filtration facilities to remove particles, turbidity, and pathogens; and provide consistent operation of water treatment plants with effective performance monitoring. Programs that identify weaknesses and optimize water treatment have greatly improved performance of surface water treatment plants (Consonery et al. 1997). The

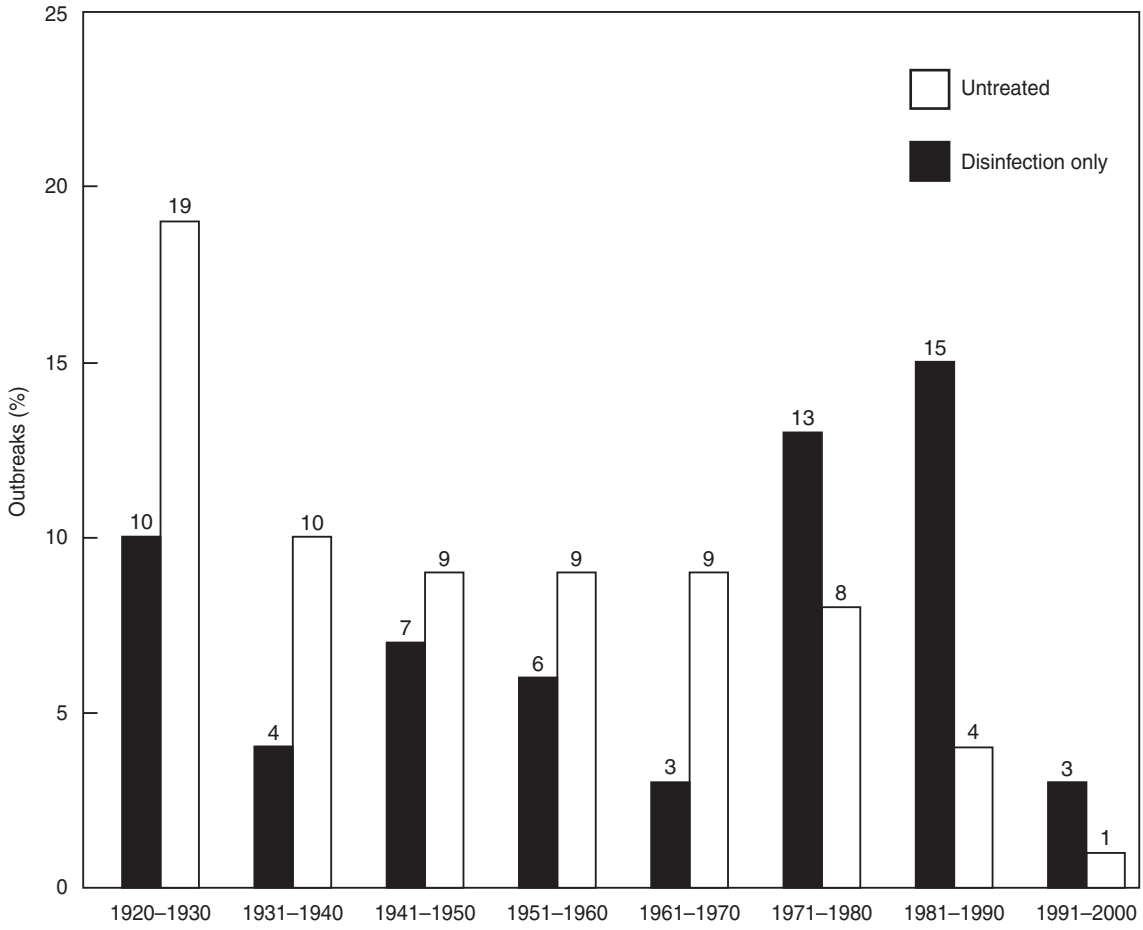


Figure 1-3 Waterborne disease outbreaks in unfiltered surface water systems in the United States, 1920 to 2000

USEPA's surface water rules also include monitoring and compliance regulations to ensure adequate filtration and disinfection. An additional regulation, the Filter Backwash Recycling Rule (USEPA 2001; 66 FR 111:31086–105), requires public systems that utilize direct or conventional filtration and recycle spent filter backwash or other water to return the recycle flows to the water treatment process so that microbial contaminant removal is not compromised.

In addition to protecting water sources and providing adequate treatment, protecting and maintaining the quality of water during distribution and storage is an important priority. Contamination of water in the distribution system from corrosion products, cross-connections and backsiphonage, inadequately protected storage facilities, and repairs to water mains and plumbing were responsible for 21 percent of the waterborne outbreaks reported from 1991 to 2000. From 1920 to 1990, contamination of distribution systems caused 11 to 18 percent of all outbreaks (Figure 1-5). Additional emphasis is needed on identifying and correcting sources of contamination in distribution systems to reduce the number of these outbreaks.

Miscellaneous deficiencies including unknown causes, ingestion of water not intended for drinking, and ingestion of water during recreational activities caused 20 percent of all outbreaks from 1991 to 2000 (Figure 1-6). In previous time periods, miscellaneous deficiencies were responsible for only 4 to 18 percent of outbreaks.

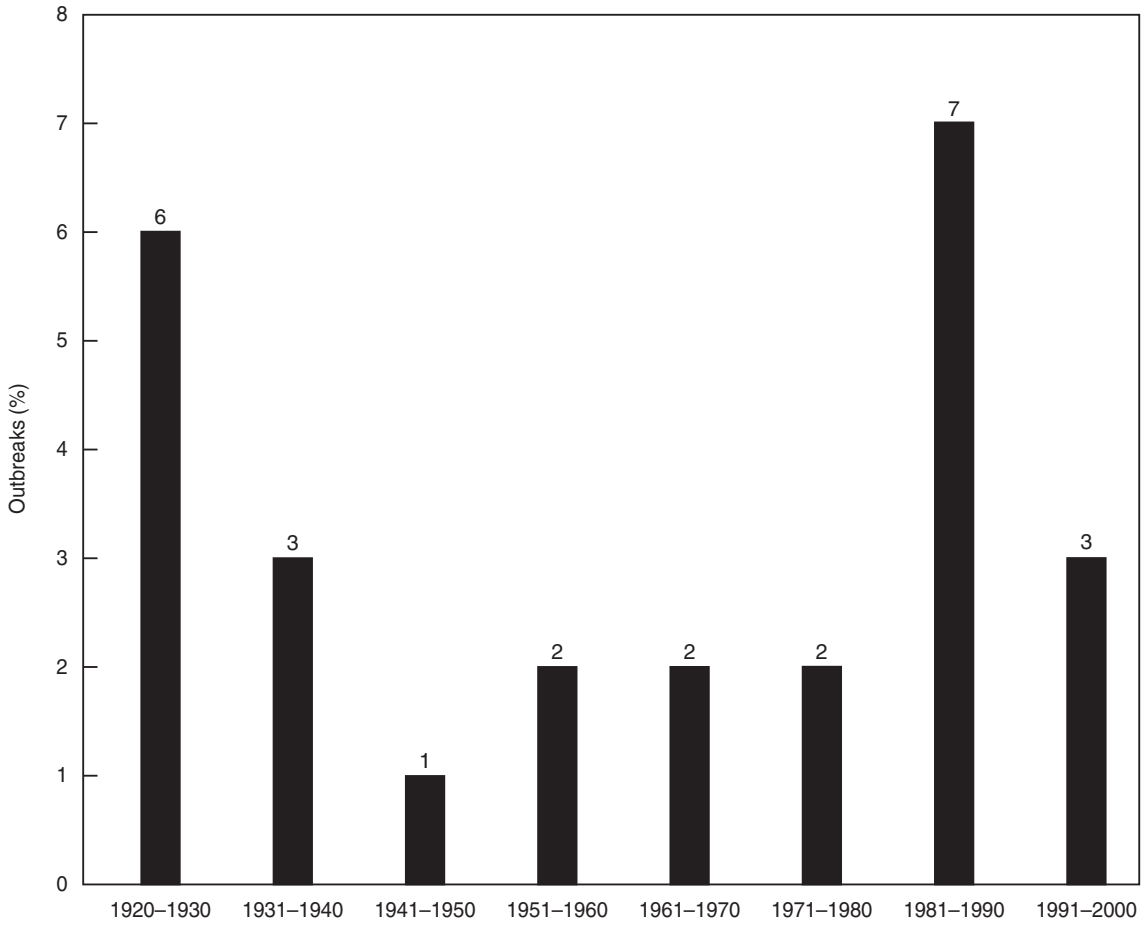


Figure 1-4 Waterborne disease outbreaks in filtered surface water systems in the United States, 1920 to 2000

MICROBIAL WATER QUALITY DURING OUTBREAKS

Of the 751 outbreaks associated with contaminated drinking water during 1971–2000, 665 (89 percent) were of known or suspected infectious etiology. Water samples were collected and examined for total and/or fecal coliforms or *E. coli* during the investigation of 459 (69 percent) of these outbreaks. Coliform bacteria were detected in 359 (78 percent) of these outbreaks. Coliform bacteria were reported less frequently during outbreak investigations in community systems (65 percent) than in noncommunity (84 percent) and individual systems (94 percent). Coliform bacteria were detected in most (91 percent) outbreaks of bacterial etiology; however, they were detected less frequently in outbreaks of viral or undetermined etiology (81–84 percent) and protozoan outbreaks (49 percent). Coliforms have several disadvantages as an indicator for the presence of waterborne viruses and protozoa even when these pathogens may be present in water systems. While most waterborne viruses are easily disinfected, some viruses or an aggregation of viruses may be more resistant than coliforms to water disinfectants. Protozoa are even more resistant to commonly used water disinfectants and can survive longer in water than coliform bacteria.

Water samples were examined for pathogens during the investigation of 122 (18 percent) infectious disease outbreaks during 1971–2000. The etiologic agent was

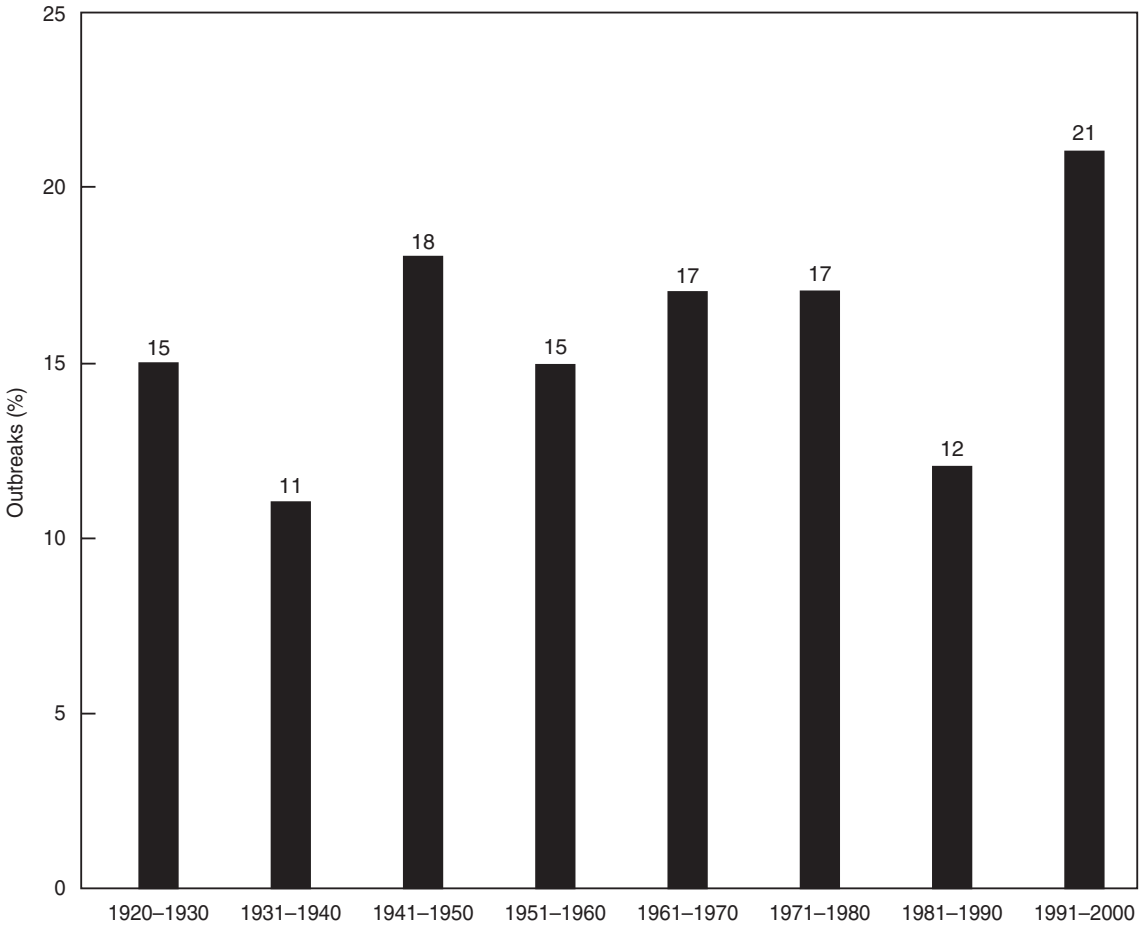


Figure 1-5 Waterborne disease outbreaks from distribution system deficiencies in the United States, 1920 to 2000

identified in water collected from 78 (64 percent) of these outbreaks. *Giardia* cysts or *Cryptosporidium* oocysts were identified in 49 protozoan outbreaks and bacterial pathogens were identified in 19 bacterial outbreaks. *Giardia* and *Cryptosporidium* were frequently detected in water samples when coliforms were not. Waterborne pathogens identified in 10 outbreaks of undetermined etiology included *Giardia*, enteroviruses, *Salmonella*, and toxigenic *E. coli*.

COLIFORM REGULATIONS AND OUTBREAKS

Presence of coliform bacteria may indicate the possibility of fecal contamination, a relationship used to assess the microbiological quality of drinking water. USEPA has established a maximum contaminant level (MCL) and routine monitoring requirements for total coliforms in public water systems. The absence of coliform bacteria in a water supply is usually interpreted as evidence of safe drinking water (i.e., water free of pathogens and having a low risk of waterborne infectious disease). However, waterborne outbreaks have occurred even when the MCL for total coliforms was not exceeded. The USEPA's revised Total Coliform Rule (TCR) requires all public water systems to monitor for total coliforms at a frequency that depends upon the system type (community or noncommunity) and number of persons served (USEPA 1989, 54 FR 124:27544–68; USEPA 1990, 55 FR 118:25064–5).

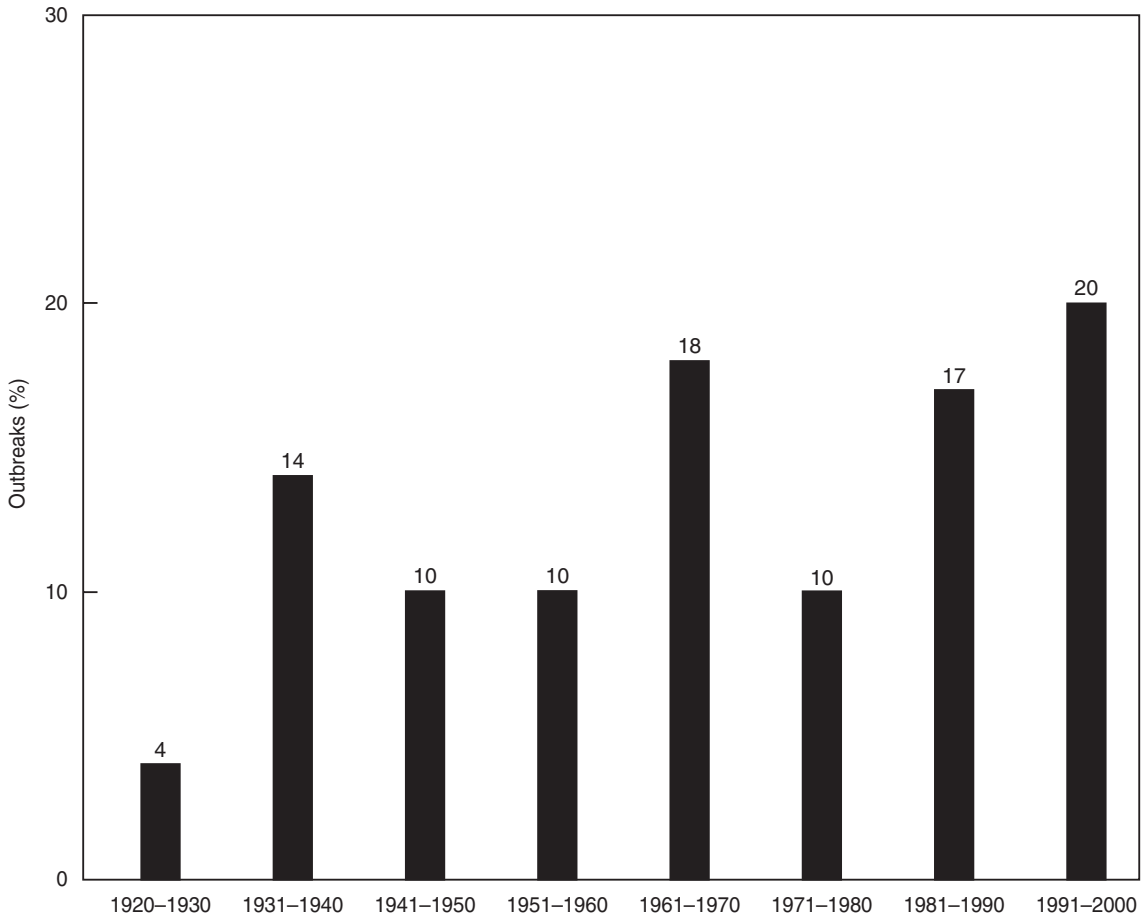


Figure 1-6 Waterborne disease outbreaks from miscellaneous and unknown deficiencies in the United States, 1920 to 2000

For systems that collect fewer than five samples per month, the TCR also requires a periodic sanitary survey. A review of coliform monitoring records during the period 1991–2000 found that the TCR is inadequate to identify public water systems that are vulnerable to an outbreak (Craun 2000; Nwachuku et al. 2002). Similar findings were reported in an earlier study (Craun et al. 1997) where MCL violations were based on the 1975 TCR. In these studies, TCR violations did not differ significantly for public water systems in which an outbreak had been reported and matched, and the comparison systems in which an outbreak had not been reported.

During 1991–2000, only 22 percent of community and 9 percent of noncommunity systems had violated the coliform MCL in the 12-month period before an outbreak, however, when coliform samples were collected during outbreak investigations for the same time period, coliforms were detected in 46 percent of community and 83 percent of noncommunity systems. This may be due to more intensive monitoring during the investigation, insufficient coliform monitoring requirements of the TCR, or both. During an outbreak investigation an intensive effort was often made to determine the water quality, and samples were frequently collected within a few weeks after a contamination event.

ETIOLOGIC AGENTS

Waterborne pathogens in which humans are the sole reservoir of infection, such as *Vibrio cholerae*, *Salmonella typhi*, and hepatitis A, are no longer important causes of outbreaks in the United States (Craun 1990). During the late 1800s and at the turn of the 20th century, when water systems were not filtered or chlorinated, waterborne epidemics of cholera and typhoid fever were common in the United States. Filtration and chlorination of drinking water sources were largely responsible for the dramatic reduction of these diseases and decreased importance of water as a mode of transmission. From 1920 to 1960, typhoid fever continued to be transmitted by contaminated water, but no waterborne outbreaks of cholera were reported (Table 1-3). Since 1971, few waterborne outbreaks of typhoid fever have been reported. Hepatitis A virus caused 22 percent of all waterborne outbreaks from 1961 to 1970, but only 4 percent of outbreaks since 1971.

During 1991–2000, protozoa were the most frequently identified agents in outbreaks; 34 percent of the outbreaks where an agent was identified were either giardiasis (63 percent) or cryptosporidiosis (37 percent). Protozoan outbreaks were responsible for almost 95 percent of all cases of illness reported during this decade. This was primarily due to the outbreak in Milwaukee. In contrast, even though 30 percent of outbreaks in which an agent was identified were bacterial and 27 percent were chemical, these outbreaks contributed to few illnesses. In waterborne outbreaks of bacterial and chemical etiology, 2,981 illnesses and 538 chemical poisonings were reported. The most frequently reported bacterial agents since 1991 include toxigenic *E. coli* (32 percent), *Shigella* (29 percent), and *Campylobacter* (19 percent). The remaining bacterial outbreaks were nontyphoid *Salmonella*, *V. cholerae*, *Pleisomonas shigelloides,* and one outbreak of mixed etiology *E. coli* 0157:H7 and *C. jejuni*. Only 9 percent of the outbreaks reported during 1991 to 2000 were caused by viral agents. Viral outbreaks also contributed little (2,674 cases) to the total case burden during the decade. Most viral outbreaks were caused by norovirus (67 percent); hepatitis A virus caused 22 percent of the viral outbreaks.

Since 1991, *Cryptosporidium*, toxigenic *E. coli*, and *Campylobacter* have become increasingly important as causes of waterborne outbreaks in the United States. During 1991–2000, each of these agents caused from 4 to 8 percent of the reported outbreaks whereas, during 1971–1990, each agent caused less than 1 percent of the reported outbreaks. *Giardiasis* outbreaks decreased dramatically. During 1981–1990, 27 percent of reported outbreaks were caused by *Giardia*; during 1991–2000, only 13 percent of the reported outbreaks were caused by *Giardia*. Hepatitis A outbreaks decreased from 4 to 5 percent of reported outbreaks during 1971–1980 to only 1 percent during 1991–2000. Shigellosis outbreaks also decreased but not as dramatically—from 7 to 5 percent during the same time period. During each decade of the 30-year period, the percentage of norovirus outbreaks remained the same, causing slightly more than 3 percent of all reported outbreaks.

A significant number of drinking water outbreaks were caused by zoonotic agents, *Giardia*, *Campylobacter*, *Cryptosporidium*, nontyphoid *Salmonella*, toxigenic *E. coli*, or *Yersinia*. Zoonotic agents caused 56 percent of the community system outbreaks where an etiology was identified and 41 percent of the noncommunity system outbreaks where an etiology was identified. Outbreaks of zoonotic bacteria were primarily associated with untreated groundwater, inadequately disinfected groundwater, and distribution system contamination through cross-connections, backsiphonage, main breaks, main repairs, inadequately protected storage tanks, or uncovered reservoirs. Few of these outbreaks were associated with inadequately treated surface water. Although inadequate or interrupted treatment of surface water was the most important water system deficiency identified for outbreaks of giardiasis and cryptosporidiosis, the contamination of groundwater and distribution systems was also important. Norovirus and

Table 1-3 Causes of drinking water outbreaks in the United States, 1920 to 2000

Time Period	Disease	Number of Outbreaks	Cases of Illness
1920–1940	Typhoid fever	372	87,675
	Acute gastroenteritis (AGI), unidentified	144	102,814
	Shigellosis	10	3,308
	Amebiasis	2	1,413
	Hepatitis A	1	28
	Chemical poisoning	1	92
1941–1960	AGI, unidentified	265	54,467
	Typhoid fever	94	1,917
	Shigellosis	25	8,951
	Hepatitis A	23	930
	Salmonellosis	5	38
	Chemical poisoning	4	44
	Paratyphoid fever	2	12
	Amebiasis	2	6
	Tularemia	2	36
	Leptospirosis	1	9
	Poliomyelitis	1	16
1961–1970	AGI, unidentified	39	26,556
	Hepatitis A	29	896
	Shigellosis	21	1,735
	Typhoid fever	15	108
	Salmonellosis	7	16,651
	Chemical poisoning	9	45
	AGI, toxigenic *E. coli*	4	188
	Giardiasis	3	176
	Amebiasis	3	39
	Paratyphoid fever	1	5
1971–2000	AGI, unidentified	363	83,397
	Giardiasis	126	28,427
	Chemical poisoning	86	4,477
	Shigellosis	44	9,196
	Hepatitis A	28	827
	AGI, norovirus	27	13,100
	Campylobacteriosis	19	5,604
	Cryptosporidiosis	15	421,473
	Salmonellosis	15	3,203
	AGI, *E. coli* 0157:H7	11	529
	Typhoid fever	5	282
	Yersiniosis	2	102
	Chronic gastroenteritis	2	94
	Cholera	2	28
	AGI, rotavirus	1	1,761
	AGI, *E. coli* 0157:H7 & campylobacterosis	1	781
	AGI, *E. coli* 06:H16	1	1,000
	AGI, *Plesiomonas shigelloides*	1	60
	AGI, *Cyclospora*	1	21
	Amebiasis	1	4

hepatitis A virus are not considered zoonotic, but it is important to recognize that viruses are diverse and complex and have the ability to infect different hosts by genetic changes and expression of different phenotypic properties (Craun et al. 2003).

Important disease-causing agents in the United States now include protozoa and bacteria, with wild and domestic animals and humans serving as reservoirs of infection. Prevention of waterborne transmission of these pathogens requires protection of water sources not only from contamination by human sewage but also from fecal contamination by animals. It is important to reduce concentrations of waterborne pathogens in source waters so that the water treatment that is provided will remove sufficient numbers of waterborne pathogens to prevent infectious dose levels.

The infectious dose for *Giardia* and *Cryptosporidium* is relatively low (Rendtorff 1954; DuPont et al. 1995), and high concentrations of water disinfectants and long contact times are required to inactivate *Giardia* and *Cryptosporidium* (Sterling 1990). Although properly designed and operated granular filters with appropriate pretreatment can effectively reduce the number of cysts and oocysts, granular filters do not provide 100 percent removal. If a water source is heavily contaminated with these parasites, granular filtration may not be able to sufficiently reduce cysts and oocysts below infective dose levels.

Current water filtration and disinfection practices and USEPA regulations have reduced the risk of *Cryptosporidium* outbreaks in surface water systems. However, the role of protective immunity may be important to consider when assessing waterborne cryptosporidiosis risks and future control strategies (Craun et al. 1998; Frost et al. 1997, 1998, 2000). Recent serological-epidemiological evidence suggests that some conventionally-filtered surface water may be a source of low-level exposure and infection (Frost et al. 2002). Serological studies found elevated levels of *Cryptosporidium* infection without an apparent increase in illness in populations where filtered surface water systems meet current water quality standards and regulations. Populations using groundwater sources had lower *Cryptosporidium* infection levels, but when waterborne outbreaks occurred in groundwater systems, these populations had a high incidence of clinically detected cryptosporidiosis.

WATERBORNE PATHOGENS OF EMERGING CONCERN

Better laboratory methods and quicker, more thorough investigation of outbreaks have helped to identify pathogens responsible for waterborne outbreaks (Table 1-3). Outbreaks in which an etiology was not identified declined; 55 percent of all reported outbreaks during 1971 to 1980 were of undetermined etiology but only 46 percent were during 1981 to 1990. However, an etiologic agent was still not identified in 40 percent of all reported waterborne outbreaks during 1991 to 2000. These outbreaks, classified as acute gastroenteritis (AGI) of undetermined causes, may be caused by familiar waterborne pathogens that are not identified because investigations were conducted too late to collect clinical specimens or laboratory analysis was inadequate. Outbreaks have also occurred in which an etiologic agent could not be identified, even after extensive laboratory analysis. Newly recognized waterborne pathogens in recent years include *Cryptosporidium*, *Giardia*, norovirus, rotavirus, *Yersinia*, *Campylobacter*, and *E. coli* 0157:H7. Also recently identified is a waterborne protozoan pathogen similar to *Cryptosporidium* in some morphologic features and is now named *Cyclospora cayetanensis* (Ortega et al. 1993). *Cyclospora* has been identified worldwide in stool specimens from patients with diarrhea and in chlorinated drinking water during an outbreak in Nepal (Rabold et al. 1994). *Cyclospora* may be the organism responsible for a waterborne outbreak in a Chicago hospital in 1990 (Herwaldt et al. 1992).

Other unidentified and suspected pathogens may also be important causes of waterborne illness in the United States. For example, no causative agent has yet been identified for a distinctive chronic diarrheal illness characterized by dramatic, urgent,

watery diarrhea persisting for many months. The first waterborne outbreak of chronic diarrhea was reported in 1987; untreated well water in an Illinois restaurant was implicated as the vehicle of transmission (Parsonnet et al. 1989). Nonbloody diarrhea with a median frequency of 12 stools per day persisted in 87 percent of patients after 6 months. Waterborne transmission is suspected for the atypical mycobacteria (Singh and Yu 1994). Another group of human opportunistic pathogens that might potentially be transmitted by water is the microsporidia (Curry and Canning 1993). A number of other potentially important waterborne pathogens include *Aeromonas* spp., *Helicobacter pylori*, *Toxoplasma*, hepatitis E virus (HEV), enteric adenoviruses, astroviruses, and caliciviruses, which include both the classic calicivirus and small, round, structured viruses, such as norovirus (Benenson et al. 1983; Benenson 1995). Norovirus is an important cause of waterborne outbreaks, and several other pathogens of emerging concern have been associated with the waterborne transmission of disease in other countries. Better assessment of their potential for causing waterborne disease in the United States requires additional information about their occurrence in water sources, their infective doses, and the effectiveness of water treatment processes to remove and inactivate them. Water suppliers should develop their understanding of these waterborne pathogens as more information becomes available in order to assess the vulnerability of their water systems.

SUMMARY

Waterborne disease has largely been controlled in the United States, but outbreaks continue to occur. From 1991 to 2000, 173 waterborne outbreaks and 432,733 cases of illness were reported in public and individual water systems. As evidenced by the cryptosporidiosis outbreak in Milwaukee, large populations can be affected by outbreaks, resulting in a large burden of disease. Although the number of outbreaks and severity of disease associated with drinking water outbreaks in the United States has decreased in recent years, we should not forget that outbreaks of mild or moderate illness, especially if the outbreaks are large, can result in significant economic and personal consequences. Even relatively small outbreaks may be important; depending on the etiologic agent, small outbreaks have been associated with increased mortality. The incidence of waterborne disease in the United States is underreported, and even reported outbreaks may not lead to complete epidemiological investigations or documentation of the full extent of the outbreaks. In addition, endemic waterborne disease risks may be important. Sporadic cases are usually never recognized as outbreaks, and specific epidemiological studies must be conducted to estimate these risks of endemic waterborne disease.

The residual number of waterborne outbreaks that still occur in the United States are preventable, and greater attention should be given to the protection of source water quality for groundwater and surface water; better monitoring of the effectiveness of water treatment; and increased protection of treated water as it is delivered to the tap.

Waterborne disease outbreaks are caused by zoonotic and nonzoonotic pathogens, and each may require different methods of prevention. For example, typhoid fever and cholera were waterborne diseases in the United States at the turn of the century. Chlorination and protection of water sources from sewage contamination were effective in preventing transmission of these diseases by water, because the pathogens are transmitted exclusively among humans, require large infective doses, and are susceptible to disinfection. *Cryptosporidium* and *Giardia* demand much greater emphasis on water treatment processes, but reliance on water treatment alone may not be effective. Wild and domestic animals are sources of the protozoa, and management practices may be needed to limit sources of contamination. With highly contaminated water sources, infective dose levels may be found in tap water even

after filtration. Surface water sources treated only by disinfection may require filtration, and filtration facilities must be properly designed and operated. Outbreaks of cryptosporidiosis and giardiasis have occurred in groundwater systems with sources subject to contamination by surface water or sewage. Chapter 2 of this manual discusses proper source water protection and treatment. Chapter 3 discusses proper monitoring of source and treated water.

Waterborne outbreaks and endemic waterborne disease can be reduced in the United States by effective water treatment, protection of water sources, and increased surveillance programs that include periodic sanitary surveys. Better surveillance to detect possible waterborne outbreaks, more complete investigations of sources of contamination, and improved laboratory capabilities are needed to provide additional information about waterborne agents and reduction of waterborne risks. Epidemiological studies can help assess endemic waterborne disease risks and the effectiveness of water system control measures and regulations.

It is sometimes presumed that coliform-free tap water is unlikely to cause waterborne disease. However, waterborne disease outbreak data suggest this premise is not always correct. Improvements are needed for regulations intended to assess the vulnerability of a water system to an infectious disease outbreak. Additional indicators should be considered, monitoring of source water contamination and water treatment effectiveness should supplement water distribution system monitoring, and the monitoring frequency may need to be increased. The selection of appropriate indicators of fecal contamination and waterborne pathogens should be based on their survivability in water, susceptibility to water disinfectants, and capability to detect increased health risks. Multiple indicators may be needed. *Enterococci*, somatic and male-specific coliphages, and spores of *Clostridium perfringens* have been suggested as possible indicators that should be monitored in addition to coliform bacteria. While water quality monitoring can provide valuable information about contamination events, frequent engineering evaluations such as sanitary surveys are also needed to identify sources of contamination and warn of potential water system deficiencies. Most importantly, water system managers must take steps to correct water system deficiencies when they are identified.

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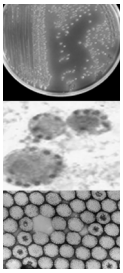

Chapter 2

Water Quality in Source Water, Treatment, and Distribution Systems

Kim R. Fox and Donald J. Reasoner; Security by Kevin R. Gertig

Most drinking water utilities practice the multiple-barrier concept as the guiding principle for providing safe water. This chapter discusses multiple barriers as they relate to the basic criteria for selecting and protecting source waters, including known and potential sources of contamination, water treatment and disinfection processes, security, and educating providers and customers to ensure the highest quality drinking water.

MULTIPLE-BARRIER CONCEPT

The premise behind the multiple-barrier concept is that use of several barriers in a drinking water system is effective in preventing pathogens or other contaminants from reaching the consumer. If one barrier fails, the remaining barriers minimize pathogen presence in the treated water. Although the exact barriers for any single location may differ, essential barriers include

- source water protection
- water treatment plant processes
- disinfection practices
- distribution systems
- security
- education

Source water protection means providing the highest quality source water possible before treatment by controlling use on the watershed, minimizing sewage contamination, protecting the wellhead, and using other strategies that reduce and prevent source water contamination. Finding and developing higher quality source waters also may be necessary or economical.

The water treatment plant barrier encompasses all water treatment processes, including (but not limited to) slow sand filters, enhanced coagulation processes, and membrane systems. The treatment plant must be both designed and operated correctly to maintain a contaminant barrier.

Disinfection processes are normally the final barrier to microorganisms. The disinfection barrier consists of a disinfectant residual throughout the distribution system that limits regrowth of microorganisms and possibly prevents subsequent contamination due to causes like cross-connections.

The distribution system—transmission mains and storage—must be properly maintained to ensure that water quality is not degraded.

Security procedures (such as secured gate access for employees only), physical barriers/items (including fences, cameras, alarms, etc.), and monitoring (on-line/real-time, etc.) protect the water from threats, malevolent acts (causing infrastructure damage), and intentional degradation of water quality, while providing a means to detect and respond to any such incidents.

Lastly, education of water utility personnel and management, public officials, and water consumers is crucial to maintaining safe drinking water.

SOURCE WATER QUALITY

The quality of surface water and groundwater sources used for drinking water supply varies broadly in the United States, ranging from nearly pristine in some areas to extremely poor in others. Ideally, source water should be free of microbial contamination from human and animal activities, free of toxic natural and synthetic chemical contaminants, and of adequate quantity to meet the needs of the population served. Because of increasing population, pollution from domestic and industrial activities, and, in many cases, lack of alternate water supply sources, many communities must use inferior quality source waters for their water supplies. The poorer the source water quality, the greater the need to provide multiple-step treatment to remove microbial and chemical contaminants.

Source water microbial quality may change drastically in a short time period (perhaps just minutes when related to precipitation events) and vary from day to day because of a variety of activities on the watershed. Natural storm events over the watershed create transient changes in water quality by contributing large amounts of both suspended and dissolved materials to the water. Other relatively constant sources of surface water pollutants include agricultural activities, wastewater treatment plants, septic system drainage, and a variety of industrial and manufacturing operations.

Groundwater Protection

Source water protection minimizes the amount of contamination introduced into water before treatment. A groundwater protection program covers three basic areas:

- protecting the aquifer
- locating wells away from potential sources of pollution
- proper well construction

To protect the aquifer, the extent and type of activities in the recharge zone of that aquifer must be controlled based on aquifer characteristics. A confined aquifer may have a protective barrier between surface contamination and the water. On the other hand, an unconfined aquifer may have porous top layers, and contaminated water in the recharge zone surface may percolate quickly into the aquifer. Specific measures to protect a recharge zone include owning and controlling the area or passing zoning ordinances and regulations that prohibit certain land uses within the

recharge area. A formal sanitary inspection can determine how much of a recharge protection program or wellhead protection program is needed.

Wellhead protection prevents surface contamination from entering the well. Installing a well an adequate distance from sources of pollution is a simple means of wellhead protection. Other protective measures include proper design and construction of the well and the wellhead. Decisions must be made before construction concerning the depth of the well, depth of the well casing, depth of the grouting, well pit design, and other factors. Proper design and construction methods are specific to locations and vary with local and state regulations.

Surface Water Protection

When streams, rivers, open ponds, lakes, or open reservoirs must be used as sources of water supply, the danger of contamination is greatly increased. The physical, chemical, and bacteriological contamination of surface water make it necessary to provide greater measures of protection or increased treatment. The three main factors for protecting surface water include

- watershed control
- removing sources of pollution
- properly constructing catchment basins, intakes, and storage facilities

Protecting a surface water source begins with a watershed control program that analyzes watershed hydrology and land ownership, identifies watershed characteristics and activities that could endanger source water quality, and monitors industrial, residential, agricultural, or recreational activities that may adversely impact source water quality. Ownership of the watershed and restricting access is the most stringent control a water utility can have to protect source water quality. When considering operating expenses and capital costs to purchasing costs, it may prove less expensive in the long run to purchase watershed lands. However, control by ownership is less likely as the area of watershed increases. Another way to protect watersheds is to use and enforce zoning restrictions and ordinances. Problems with multiple jurisdictions occur when source watersheds cross political boundaries.

As with groundwater systems, a thorough, periodic sanitary survey to identify specific and potential sources of pollution is necessary to maintain surface source water quality. Ideally, sources of contamination should be identified and corrected before problems occur, but sources can be intermittent and not appear in a cursory sanitary survey. During or after a waterborne disease outbreak, identifying potential sources of the pathogen responsible for the outbreak may prevent future problems or may suggest ways to remove the contaminating source from the watershed or aquifer area. Source waters (both ground and surface) can become contaminated by pathogenic microorganisms from sewage or other waste discharges, septic tank overflows or seepage, urban or agricultural runoff, subsurface movement, recreational usage, or during storage or distribution.

Microbiological Characteristics of Source Water

Source water for a drinking water supply should be the best quality available. The Surface Water Treatment Rule (SWTR) (USEPA 1989c) provides source water quality criteria for water systems wishing to avoid filtration. For such systems, total coliform concentration should be less than 100 per 100 mL, or the fecal coliform concentration less than 20 per 100 mL in 90 percent of samples taken during the previous 6 months. In addition, water turbidity cannot exceed 5 on an ongoing basis, based on grab samples taken every 4 hours (or more frequently) that the system is in operation.

The system must also demonstrate that it maintains disinfection conditions that inactivate 99.9 percent of *Giardia* cysts and 99.99 percent of viruses every day of

operation except any one day each month. With some caveats to these requirements, any supply that cannot meet these microbiological quality criteria must install filtration. Any source water that fails to meet these requirements must be treated to render it safe for human consumption. In the future, developing new regulations or tightening current regulations may further limit the number of unfiltered systems by making the requirements to avoid filtration more stringent.

A source water's microbial quality is extremely variable and depends on many factors, including domestic and feral animal activity on the watershed; human activities on the watershed, including recreational, manufacturing and fabrication, and agricultural activities; municipal pollution inputs from raw sewage to primary and secondary wastewater treatment plant effluents; and storm events over the watershed that wash natural and synthetic contaminants into the surface waters or that percolate into the groundwater aquifers. Microbial contaminants associated with these sources include bacteria, viruses, and protozoa, in both pathogenic and nonpathogenic forms.

Densities of bacteria, viruses, and protozoa vary widely because of wastewater effluents from domestic sewage treatment plant processes (e.g., raw wastewater bypass, primary or secondary treatment, and septic effluents) and the frequency and intensity of storm events that both dilute and degrade the receiving water quality by contributing additional contaminants. Heterotrophic bacterial counts may vary from less than 1 to 10 colony-forming units (cfu)/mL in groundwater to more than 1×10^7 cfu/mL in surface water badly polluted by municipal raw sewage or combined sewage and stormwater overflow effluents. Indicator bacteria (total and fecal coliforms, including *Escherichia coli*) densities in source water can range from less than 1/100 mL in good quality groundwaters to more than 1×10^4/100 mL in surface water badly polluted with municipal raw sewage or combined sewage and stormwater overflow. Few water systems are treating surface water with more than 2×10^4 total coliforms/100 mL or 2×10^3 fecal coliforms/100 mL.

Human viruses found in source waters include enteroviruses (72 types, including polio, Coxsackie A and B, and echovirus), reoviruses (6 types), hepatitis A, hepatitis E, rotavirus, norovirus, adenovirus (types 1 through 49), parvovirus, corona virus, astrovirus and related viruses not yet classified, epidemic non-A and non-B hepatitis viruses, and caliciviruses. All of these organisms present significant health concerns and, if present in source water, must be removed or inactivated by water treatment processes.

While viruses occur more frequently and in greater abundance in surface source waters, they may also be found in groundwater as a result of septic tank effluents, land application of sewage and sewage sludges, and other sources. Viruses are able to travel long distances as water percolates downward and flows through the soil; the distance of travel depends on the type of soil and the specific geology of a given area. Groundwaters can no longer be considered pristine and safe for consumption. If a groundwater source is directly influenced by surface water intrusions, full conventional treatment may be necessary to ensure that the treated water is safe for consumption.

WATER QUALITY IN TREATMENT SYSTEMS

Water treatment processes that serve as protective barriers against microbial pathogens passing into the distribution system must be operated continuously, optimized for the maximum removal capability, and reliably maintained in order to deal with the most extreme water quality changes anticipated. Microorganisms, including various pathogens, are subject to both physical removal by chemical coagulation–flocculation, sedimentation, and filtration and to inactivation by chemical oxidizing agents. The SWTR currently requires overall removal and/or inactivation of 99.9 percent (3 logs)

of *Giardia* cysts and 99.99 percent (4 logs) of viruses; filtered water turbidities are to be less than 0.5 ntu in 95 percent of measurements taken each month.

Physical removal of microorganisms in water treatment is accomplished by sedimentation during source water storage, coagulation–flocculation, sedimentation, and slow or rapid sand filtration or by direct filtration with or without filtration aid. Each step results in a reduction of the microbial density in the water, and the cumulative removal ranges from 90 to 99.9 percent, depending on the actual combination of treatment steps. Adding a chemical disinfection step results in further reduction/inactivation of microorganisms to achieve removals of 99.999 to 99.9999 percent (5 to 6 logs) for bacteria, 99.99 percent (4 logs) for viruses, and 99.9 percent (3 logs) for protozoa. Greater removals may occur when all processes are functioning optimally.

Effective rapid sand filtration removal of bacteria and viruses requires the use of chemical coagulants. Without chemical coagulants, only between 1 and 50 percent of influent viruses may be removed at filter rates of 9.8 to 24 m/hr. Bacteria removal by filtration without chemical coagulants is in the same range as viruses. Virus removals by water treatment have been reported to range from as little as 10 percent following coagulation–flocculation and sedimentation, to greater than 99.9 percent following full conventional treatment, including disinfection with chlorine. Viruses have been found in fully treated water, indicating the process was not fully effective, possibly because of the association of viruses with particulates that escape the filtration process and also protect the viruses from disinfection.

Heterotrophic bacterial removal by conventional filtration, which includes coagulation, ranges from less than 90 to 99.9 percent (1 to 3 logs) through the filtration process. Final disinfection can achieve an additional 99 to 99.99 percent (2 to 4 logs) removal, yielding a total bacterial reduction of 99.999 to 99.99999 percent (5 to 7 logs). For both bacteria and viruses, the effectiveness of filtration and inactivation by disinfection are adversely impacted by cold water temperatures; therefore, both processes must be continuously monitored and optimized.

Microorganisms are usually inactivated (disinfected) by a chemical oxidant. Specific conditions for maximum inactivation effectiveness of any of these oxidants is beyond the scope of this chapter, but the general order of effectiveness of chemical disinfection is as follows: ozone > chlorine dioxide > hypochlorous acid > hypochlorite ion > chloramines. Time, water pH, and water temperature are significant factors that influence a disinfectant's effectiveness to inactivate microorganisms. The order of relative resistance of microorganisms to inactivation by any chemical disinfectant is as follows: protozoan cysts and oocysts >> animal viruses and bacteriophages > bacteria, including coliforms (except for mycobacteria, which may be more resistant than most of the viruses).

The protozoan pathogens *Giardia lamblia* and *Cryptosporidium parvum* have cysts and oocysts, respectively, that are extremely resistant to chemical disinfectants and other adverse environmental effects. Therefore, the primary means of eliminating cysts and oocysts from water during treatment is by chemical coagulation–flocculation and filtration. This combination of processes in rapid sand filtration treatment is effective for removing cysts and oocysts, providing processes are continuously operated, well run, and closely controlled.

Slow sand filtration is also effective for removing parasite cysts and oocysts because of the filtering action of the schmutzdecke. The schmutzdecke is the top layer (a few centimeters in depth) of sand and particulate materials (fine soil particles, plant debris, microorganisms such as algae, etc.) that have been removed from the water as it percolates downward through the sand filter bed. Diatomaceous earth (DE) filtration can be effective for *Giardia* cyst (8 to 18 μm) removal, but may be less effective for removing the smaller sized *Cryptosporidium* oocysts (4 to 6 μm). Recent studies indicate that *Cryptosporidium* oocysts are effectively removed by these treatment processes only when processes are optimized and continuously operated.

Viruses and bacteriophages are typically only slightly more resistant to inactivation by chemical disinfectants than are bacteria. Both viruses and bacteria, when associated with particulate matter, are more difficult to disinfect than freely suspended viruses and bacteria. Turbidity removal must be maximized during treatment to optimize the effectiveness of the disinfection process for any microorganisms that remain in the water following filtration.

WATER QUALITY IN DISTRIBUTION SYSTEMS

The microbiological quality of treated drinking water as it leaves the treatment plant is the highest it will ever be. As water travels through the distribution pipe system to the consumer, its microbiological quality degrades at a variable rate. The degradation process involves several factors, including loss of disinfectant residual; temperature changes; flow velocity changes; biofilm sloughing and stirred-up pipe sediments caused by rapid changes in flow, or even flow reversals; pipe breaks and replacements, and other maintenance activities; intrusions of contaminants into the pipe network from pressure drops and cross-connections; regrowth of bacteria that survived the treatment processes; and growth of bacteria in biofilms on the pipe walls and surfaces in storage reservoirs and tanks.

Some treatment processes, such as preoxidant treatment (ozone or chlorine) of the source water or coagulated (settled) water before filtration, causes an increase in easily assimilable organic carbon (AOC) that can stimulate bacterial growth or regrowth in the distribution system. Heterotrophic bacterial growth or regrowth usually occurs when the free-disinfectant residual drops below 0.2 mg/L, the water temperature is greater than 10°C (40°F), and AOC is greater than 50 µg/L. Biological filtration, if practiced, can reduce AOC in filtered, distributed water. This may reduce, but not necessarily eliminate, bacterial regrowth in the distribution system.

Ultimately, the maintenance of an adequate disinfection residual controls bacterial regrowth in the treated distribution water. The SWTR specifies a minimum disinfectant residual of 0.2 mg/L for chlorine or 0.4 mg/L for chloramine in the distribution system to control microbial growth. These disinfectant concentrations may control bacterial growth in the bulk water but may be inadequate to control biofilm bacterial growth. Thus, the bacterial quality of bulk water leaving the clearwell is usually in the range of less than 1 to 10 cfu/mL measured as heterotrophic plate count (HPC) bacteria. On the other hand, water from other areas of the distribution system (distal areas of the system, dead ends, storage tanks, and reservoirs) may have bacterial densities ranging from 10 cfu/mL to greater than 1×10^5 cfu/mL. Dead-end areas of distribution systems often have high HPC levels along with colored-water problems because of corrosion processes, the long residence time of the water, and loss of disinfectant residual.

Elevated storage tanks often have relatively high HPC levels because of loss of disinfectant residual and, during warm weather, high water temperature. Open storage reservoirs may have high HPC levels due to loss of disinfectant residual and contamination from birds, feral and domestic animals, and the surrounding land.

Practical experience indicates that treated water leaving the clearwell must have a disinfectant residual considerably higher than 0.2 to 0.4 mg/L in order to provide the minimum 0.2 to 0.4 mg/L residual at the ends of the system. Just how much disinfectant residual must be applied may vary widely based on a variety of factors, including the total organic carbon (TOC) present in the finished water that will exert a disinfectant demand; water temperature; age and condition of the distribution system piping; extent of biofilm development; residence time of the water; duration of offline storage in standpipes or reservoirs; type of storage (i.e., covered versus uncovered reservoir); and incidence of posttreatment contamination events that contribute additional disinfectant demand in the form of chemical and microbial contaminants.

Biofilm Effects on Water Quality

Many heterotrophic bacteria that survive or pass through the treatment process are able to adapt to the low nutrient (oligotrophic) conditions of treated drinking water. Once in the distribution system, their ability to associate, temporarily or by colonization (biofilm growth), with surfaces confers survival advantages for the bacteria. Nutrients available to bacteria in the bulk water are usually present at very low levels. This, coupled with the presence of a disinfectant residual, means that bacteria freely suspended in the water are inhibited from growing. However, bacteria associated with surfaces, such as pipe walls and sediments, are exposed to a continuous or even accumulating supply of nutrients from flowing water.

Establishment of a biofilm involves transport of bacteria and nutrients to the pipe wall surface, bacterial adhesion and multiplication, and buildup of microbial growth products. These products include various exopolymeric materials that assist in binding cells to the pipe wall and forming a matrix within which cells are protected from disinfectant. Attachment of bacteria to the pipe wall and other surfaces, or in sediments, provides up to a 3,000-fold increase in disinfection resistance, depending on the surface material. Bacteria adapted to growth under low nutrient conditions have been shown to be more resistant to disinfection than bacteria grown under rich nutrient conditions. Effectively disinfecting biofilms in distribution systems is difficult because of the two factors just discussed. Additionally, the transport of the disinfectant into the biofilm is limited by diffusion kinetics. Reactions of the disinfectant with extracellular matrix material, nutrients from the water that diffuse along with disinfectant, and corrosion products from the pipe itself result in a reduced disinfectant residual available to react with the bacterial cell.

The higher the disinfectant residual present in the bulk phase water, the more likely it is that sufficient disinfectant can diffuse into the biofilm to inactivate bacteria found there. There is evidence that a chloramine residual can exert better control of biofilm bacterial growth than does free chlorine. However, once a bacterial biofilm becomes established in a distribution system, it is virtually impossible to get rid of using chlorine or chloramine. Most distribution biofilms appear to be thin and patchy (discontinuous) rather than uniform and continuous. Maintaining an adequate disinfectant residual undoubtedly limits the extent of development of a biofilm, but the disinfectant residual necessary to do so varies with changes in source water quality and with the performance of treatment processes in removing particulates, nutrients, and microorganisms.

Bacterial growth and regrowth in water distribution systems occurs in biofilms, and biofilm bacteria may enter the bulk phase water by release from the biofilm or by sloughing of biofilm particles from hydraulic shearing. Bacterial densities in the biofilm typically range from about 10^5 to 10^8 cfu/cm^2, measured by culture techniques. Maximum biofilm bacterial densities occur when disinfectant residual is low or nonexistent, whereas lower biofilm densities occur when disinfectant residuals in the bulk water are as high as 1.6 to 1.8 mg/L.

Under some relatively specific conditions, a water utility using a surface water source may experience intermittent or continuous low-level occurrences of coliforms characterized as coliform biofilm, or as growth or regrowth of coliforms in the distribution system. Distribution system age or increased regional temperatures may be part of the problem. Additionally, such coliform biofilm problems have been characterized by

- the inability to maintain a disinfectant residual greater than 0.2 mg/L
- conditioning of the distribution system by exposure to treated water containing relatively high levels of AOC (>100 µg/L) and TOC (>2 mg/L)
- warm water temperatures ($T \geq 15°C$ [59°F]) occurring from late spring to late fall

- a significant percentage of iron pipe in service for more than 75 years
- lack of a regular flushing program for dead-end areas

Although treatment processes may be producing water that meets microbiological standards, low levels of coliform bacteria do pass through treatment and may survive to colonize the biofilm in some areas of the system. When nutrient levels, disinfectant concentration, and temperature conditions are right, these organisms are able to grow, perhaps to levels sufficient that they are released from the biofilm and appear in the bulk water during sample collection.

SECURITY

The tragedy of Sept. 11, 2001, increased awareness of water and wastewater utilities' preparations to deal with possible threats or malevolent acts on water systems. The degree to which each utility responds to threats is widely varied based on their current vulnerability assessment or other input provided by local, state, and federal law enforcement agencies, local and state health departments, homeland security, and the US Environmental Protection Agency (USEPA).

The USEPA has been given the responsibility under Presidential Directive Decision (PDD 63) for the protection of the nation's water and wastewater sector's critical infrastructure. The USEPA was directed to develop methods and procedures that would provide guidance and evaluate protection requirements for its key infrastructures. Readers can stay current on recommendations from the USEPA by frequently checking their Web site on water system security.

Threats

In the May 2001 issue of *Journal AWWA*, Gay Porter Denileon detailed an example of security in the water sector. A threat from a terrorist group indicated that it intended to disrupt water operations in 28 US cities. Water utilities were warned by the FBI of this threat on Jan. 24, 2001, and told to "take precautions and to be on the lookout for anyone or anything out of the ordinary." The threat was later determined not to be credible, but it verified that security in our industry should not be taken for granted. It also alerted us to consider disasters other than natural hazards such as tornadoes or floods for posing risk to infrastructure.

Recent polls indicate that more than half of those polled felt that water would be at least somewhat of a target for terrorist attack. Other places considered to be targets were major businesses, stadium or sports arenas, bridges, and tunnels. Nationwide, security has been increased considerably for some 9,300 "high hazard" dams—whose breach would cause human deaths and major destruction.

Threats can be biological, chemical, radiological, or physical destruction of a system. Having knowledge of where to seek help in your area is critical—the range of possible threats is very broad, although both USEPA and the Centers for Disease Control and Prevention (CDC) have focused on particular contaminants that could potentially be used to purposefully contaminate a water system.

Vulnerability Assessments

The vulnerability assessment (VA) is a systematic process whereby water system vulnerability from the watershed or wellhead throughout the distribution system is ranked by a detailed protocol. It is applicable to any water or wastewater system. It is vital to identify the areas that are "weak links" for a system. During the assessment, each part of the water system is ranked using a numerical matrix method, and once complete, scores delineate which areas pose the most risk.

Utilities were required to complete a vulnerability assessment by the following dates:

Dec. 31, 2002—Utilities serving >100,000 customers

June 30, 2003—Utilities serving between 50,000 and 100,000 customers

Dec. 31, 2003—Utilities serving between 3,300 and 50,000 customers

One of the challenges the water sector faces is how to quickly ascertain water safety for a number of different scenarios. Both USEPA and CDC along with numerous other agencies continue to update possible issues regarding water safety.

Emergency Response Plans

While recent world events have heightened awareness of risk, water utilities deal with risk response and public health protection every day. Utilities are not unfamiliar in dealing with natural disasters and/or emergencies. Waterborne pathogens such as *Cryptosporidium* have increased awareness and development of response plans for emergency situations. Boil-water advisories (chapter 3) are more frequently reported in the news media as utilities are under more strict regulations for the protection of public health. The focus today can also include other concerns such as: microbiological, chemical, and radiological threats, physical security, and detection methods (Figure 2-1).

In addition, cyber security for supervisory control and data acquisition (SCADA) systems may also need to be evaluated for malevolent attack from both insiders and outsiders. Many systems are very reliant on SCADA systems and need to carefully evaluate backup plans in the event the control systems are lost—even for brief time periods.

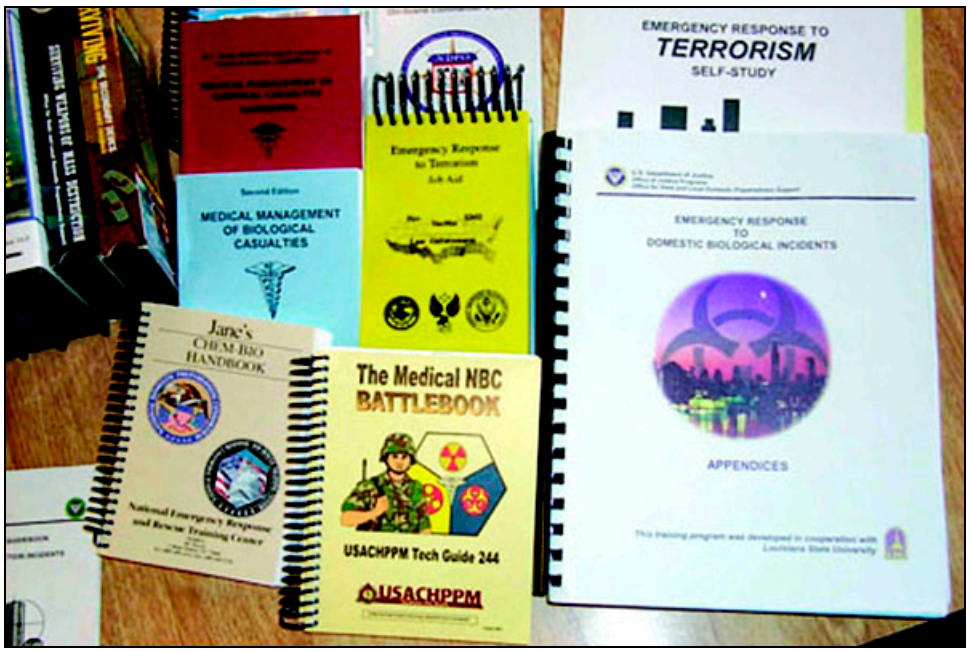

Figure 2-1 Emergency information

Action Plans

One important benefit of conducting a VA is that the utility's operation will be in a better position to react to any emergency—not just a physical or cyber security breach. The Awwa Research Foundation (AwwaRF) describes VAs and procedures that utilities should use to perform a study. Members of AwwaRF can contact their representative for details. Some utilities may choose to hire a consultant to help them update formal assessments.

General Considerations

The following checklist illustrates some basic considerations for utilities. It is not comprehensive and will change over time. All facilities are unique and require unique deliberations. Utilities should contact their local authorities as well as American Water Works Association (AWWA), Water Environment Federation (WEF), USEPA, and CDC Web sites for additional measures and be aware that checklists can change over time because of new threat assessment information.

- Conduct a vulnerability assessment—watershed through distribution systems.
- Write/update emergency operation plans.
- Meet and work closely with local law enforcement agencies.
- Join the FBI Infragard program.
- For utilities using SCADA systems, evaluate computer security and outside connection methods.
- Stay informed about security updates via the AWWA and WEF Web sites at www.awwa.org or www.wef.org.
- Evaluate/update physical protection of facilities.
- Evaluate redundancy for critical equipment.
- Evaluate monitoring frequency and type.
- Evaluate treatment methods and know system contact and detention times.
- Review vents and pressure relief valves for access.
- Test cyber security often.
- Entrances—evaluate access and screening methods.
- Perform background checks on employees.
- Provide training support to employees.
- Know the location of key documents.
- Work with neighbors close to key facilities—they can assist in detection.
- Evaluate disinfection procedures—monitoring and residuals.
- Evaluate storage facilities—site security.
- Consider posting warning signs.
- Evaluate multiple dependencies (gas, electric, communications).
- Evaluate backflow prevention program and modify if warranted.
- Review water quality data with trending.
- Have response systems in place to deal with emergencies.
- Evaluate alternative water sources for emergencies.
- Evaluate mutual aid agreements with surrounding utilities/districts.
- Look for any signs of tampering with fire hydrants and unauthorized or suspicious vehicles making hydrant connections.
- Evaluate vendor quality assurance and delivery for chemicals.

- Evaluate how packages, mail, etc., are handled.
- Consider joining the WaterISAC network.
- Perform a security audit of all buildings, and enhance areas as required.
- Consider installing closed-circuit television/and or alarms.
- Work with your legal staff to evaluate Freedom of Information Act implications for vulnerability assessments.

The Water Information Sharing Analysis Center (WaterISAC, www.waterisac.org) is an excellent resource for utilities to obtain and exchange information in a secure network environment. The WaterISAC is a secure portal by which subscribers can get current updates regarding security issues as well as comprehensive databases for possible contaminants.

Meeting the Challenge

Water treatment professionals will continue to be challenged to do more with limited resources. Planning for the unexpected while maintaining critical services to the public is important. City and county officials need to support their utility's efforts as they consider options for security.

Take time to meet with local emergency response representatives and local emergency planning committees (LEPC). Discussions are beneficial to everyone. Evaluate detection, delay, and response options together—it is usually a good learning experience for both sides. Also, routinely communicate with your local and district health department engineers.

There are many Web resources available for water utility personnel. It should be noted that credible sources are highly recommended for this area because it is so dynamic and can change quickly depending on the homeland security level.

Perhaps the greatest challenge is to avoid becoming complacent after implementation of security improvements and to realize that VAs and security analyses are part of a process that needs to become as integral a part of our periodic planning as budgeting.

Security Resources

American National Red Cross, www.redcross.org/news/terrorism

American Water Works Association (AWWA), www.awwa.org

Association of Metropolitan Sewer Agencies (AMSA), www.amsa-cleanwater.org

Association of Metropolitan Water Agencies (AMWA), www.amwa.net

Centers for Disease Control and Prevention (CDC), www.cdc.gov

Denileon, Gay Porter, "The Who, What, Why, and How of Counterterrorism Issues," *Jour. AWWA*, May 2001

Disaster Resource Center, contact: dera@disasters.org

Federal Emergency Management Agency (FEMA), www.fema.gov

National Infrastructure Protection Center (NIPC), www.nipc.gov

National League of Cities, www.nlc.org/nlc

NSF International, www.nsf.org

Roberson, A., and K. Morley. *Contamination Warning Systems for Water: Providing Actionable Information to Decision-Maker*, American Water Works Association, 2005

Texas Engineering Extension Service—Incident Command System for Public Works–TEEX, Contact: (979) 845-3148

US Environmental Protection Agency (USEPA), Regional USEPA Office or the Task Force, (202) 260-3170, www.epa.gov

USEPA Counterterrorism, www.epa.gov/ebtpages/ecounterterrorism.html

Water Environment Federation (WEF), www.wef.org

Water Infrastructure Security Enhancements Guidance Documents, American Society of Civil Engineers, www.asce.org/static/1/wise.cfm

Water System Security: A Field Guide, American Water Works Association, 2002

Water Information Sharing Analysis Center (WaterISAC), www.waterisac.org

SUMMARY

A water utility's ability to protect, produce, and maintain high quality drinking water is a major challenge. The pressure to produce ever better quality water, driven by both current and proposed regulations, means that water utilities must evaluate their present ability to meet regulatory goals, provide physical security, forecast and plan for treatment technology changes that may become necessary, and balance the costs and benefits of new technologies against the need to increase costs to consumers.

To maintain or improve the microbial quality of drinking water, utilities must address issues such as watershed management and source water protection; maintain good treatment and disinfection practices, including process optimization; develop a thorough understanding of the hydraulics of their distribution system; be aggressive in managing and operating the distribution system, including active programs for cross-connection control and scheduled hydrant and dead-end flushing; develop a proactive microbial monitoring and data collection program (see Figure 2-2); make active use of the data analysis and information stemming from that analysis to implement better treatment and distribution management practices; develop and implement a comprehensive security program; and educate the consumer/public about all of the water quality issues.

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Source: Kevin R. Gertig, Fort Collins Utilities, Fort Collins, Colo.

Figure 2-2 On-line sampling and monitoring equipment

McFeters, G.A. 1990. *Drinking Water Microbiology: Progress and Recent Developments*. McFeters, G.A., ed. Brock/Springer Series in Contemporary Bioscience. New York: Springer-Verlag.

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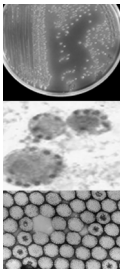


Chapter 3

Water Quality Monitoring, Sampling, and Testing

Jon Bloemaker and Kevin R. Gertig; Heterotrophic by Pierre Payment

Monitoring, sampling, and testing drinking water fulfills two needs—meeting regulatory requirements and identifying areas for water quality improvement so treatment processes can be adjusted for system optimization. The number and types of samples collected and tested to meet regulatory requirements are generally considered the minimum for determining water quality. Increasing the number and types of samples collected and tested provides data more representative of water quality. This data helps operators predict changes in water quality and alter treatment processes or maintenance programs to prevent or reduce the deterioration of finished water quality.

MONITORING

The importance of sample collection timing is frequently overlooked and generally misunderstood. If the timing of sample collection misses the target event, information gained from the program will be misleading, and sampling techniques and laboratory quality-control practices will not improve the final results. The only way to avoid this problem is to thoroughly understand the system being sampled. This requires establishing an adequate history of water quality changes and identifying indicators that will predict future changes.

Surface Waters and Groundwater Under the Influence of Surface Water

Collecting meaningful samples from public water systems using surface water sources or groundwater under the influence of surface water (subject to the same regulations as surface water) requires several considerations.

These monitoring considerations would include clearly defining the intent of the program, implementing a program designed to represent typical, average conditions, and utilizing proper sample timing within the program to ensure test results are representative of true, overall water quality.

A monitoring program should be expanded to include more data than minimum regulatory requirements in order to effectively represent average conditions. For

example, a single, 12-month running average based on samples from four consecutive quarters can be biased from a single atypical result. A more realistic average history requires the monitoring program to be in place for a number of years. Also, increasing the number of sampling events during periods when water usage is high may be more representative. Another approach is collecting samples at widely spaced intervals during times when water quality is relatively constant and collecting more frequent samples when water quality fluctuates. Average water quality may be better represented by a weighted average based either on the amount of water used or the time between sampling events.

A well-designed monitoring program would also account for the changes in activities and conditions within the watershed. Agricultural, recreational, municipal, industrial, and other activities affect downstream water quality. Prior to designing or implementing a monitoring program, a comprehensive and thorough watershed assessment should be performed. The assessment would include an inventory of all potential point and nonpoint sources of contamination. If a monitoring program has been established before such an assessment, the results of the data may be more difficult to interpret.

Point-source discharges are regulated through National Pollution Discharge Elimination System (NPDES) programs in which discharge limits are specified in a permit. Typically, routine point-source discharges from these operations will not cause a sudden change in water quality. Most operations meet their limits during routine operations. The assumption that a waste treatment facility operating within its regulatory discharge limits will not be a major source of microbial contaminants appears sound. However, the atypical event may create a problem, such as discharges from combined sewers during periods of heavy precipitation and hydraulically overloaded wastewater treatment plants. Information about point-source discharges is usually available through local state primacy agencies.

Non-point-source discharges from upstream agricultural facilities may degrade water quality. The locations of feed lots, dairy farms, stables, and commercial agricultural businesses and the waste disposal practices of each operation are factors to consider. Local, county, or state agricultural extension or co-op agents are good starting points for obtaining this information.

The weather may be the most unpredictable influence on water quality. Precipitation, snow melt, wind, drought, and temperature affect water quality. Overland flows of precipitation and snowmelt carry contaminants into receiving streams via nonpoint discharges. In addition, precipitation and snowmelt are responsible for point-source discharges from storm sewers, combined sewers, and treatment plants in urban areas. The direction of the wind may be responsible for the discharge of a stream into a bay or a lake changing course to flow over an intake. Drought conditions result in a reduced dilution factor and may attract wildlife and livestock to the major streams that feed into public water supplies. The influence of discharge from a waste treatment facility is magnified by a reduced dilution factor during droughts. Water temperatures affect the variety and viability of microbiological contaminants, although it may have little bearing on some contaminants' survival in the water environment.

Water providers should be familiar with travel time for the full range of flow conditions in the upstream reaches of their source water. This information may be available for some streams from the US Geological Survey (USGS) or various state agencies. With information on stream flow, a prediction about when an upstream event will affect local water quality can be made and the monitoring schedule and treatment processes adjusted accordingly. Cooperative arrangements with upstream communities and businesses regarding notification of unusual discharges or accidents will aid in short-term changes to monitoring plans.

It may be necessary to develop a monitoring program that includes other potential microbial contaminants besides total coliforms. For source water in the Great Lakes not under the influence of inland streams or shoreline runoff, additional testing beyond total coliforms and heterotrophic bacteria may be unnecessary. At the other

extreme (e.g., an inland river location downstream of urban areas or discharge from an overloaded waste treatment facility), routine tracking of microbial contaminants other than total coliforms is crucial to demonstrate the effectiveness of potable water treatment systems. In the majority of situations between these extremes, a baseline understanding of the types of microbial contaminants impacting the water source should be established. Expansion of a monitoring scheme should include contaminants other than total coliforms, *Escherichia coli*, and fecal coliforms as based on past experiences and overall water quality. Heterotrophic bacteria counts may be useful in clean water conditions. In-house testing programs for *Pseudomonas* sp. or *Klebsiella* sp. may prove useful as indicators of lesser quality source water. Expansion of the monitoring program for *Giardia*, *Cryptosporidium*, or viral testing may yield critical information during nonroutine periods, especially during periods of high runoff.

An example of misleading data is routinely seen in northern US inland streams subject to agricultural runoff. In some such areas, manure is applied to the fields in December and January when the crops are off the fields and the frozen earth supports heavy farm equipment. Immediately following the application, little or no runoff occurs; however, near the end of February or early March, a combination of snowmelt and early spring rains carry various contaminants into receiving streams. A monitoring program performed on the first day of each quarter will likely miss the potential problems. Operators responsible for water systems affected by nonpoint discharges should have the flexibility to change monitoring schedules and collect samples that represent periodic and unusual events. During these events, the number and type of samples should be increased.

Monitoring Frequency

Water treatment professionals must maintain consistent finished water quality to protect public health. The Partnership for Safe Water recommends that conventional water plants strive to meet less than 0.1 ntu in finished water at all times. Water treatment can be most challenging during reservoir turnovers, floods, runoff, aquifer changes, and unidentified upstream discharges. Monitoring plans should be site-specific because of the numerous local variables that make monitoring dynamic. After databases are established, certain parameters can be tested less often, while other parameters may need to be added. An example of a weekly matrix is shown in Table 3-1. Although it is not possible to cover all instances that warrant increased water quality monitoring, the following may be considered for surface waters:

- unusual activity observed in the watershed
- severe weather that affects the source water
- deviations in routine chemical, physical, or biological parameters
- accidents involving hazardous materials spilled in the source water
- high pathogen counts in the source water
- elevated heterotrophic plate counts (HPCs)
- elevated coliform counts
- customer complaints regarding quality
- threats to public water supplies
- problems with chemical feed equipment or loss of process
- washwater that must be returned to the head of a plant without pretreatment or disinfection
- algae blooms
- surface water plants not meeting the requirement of less than 0.1 ntu out of individual filters at all times, or exceeding 0.3 ntu for 10 minutes after the filter is placed on-line

Table 3-1 Example of a weekly sampling matrix

Parameter	Process Location	Goal	Minimum Monitoring Winter*	Frequency Other Times	Sampling Location	Method
Turbidity (ntu)	HT raw	N/A	1 per week	1 per week	Raw water sample station	Nephelometric method using Hach 2100AN turbidimeter; USEPA approved.
	PR raw	N/A	1 per week	1 per week	Raw water sample station	Standard Methods 2130B (Process Control Lab)†
	PR raw	N/A	1 per month	1 per week	Poudre River intake	
	T1 effluent	<1.0	1 per week	1 per week	T1 effluent turbidimeter	
	T2 effluent	<1.0	1 per week	1 per week	T2 effluent turbidimeter	
	T3 effluent	<1.0	1 per week	1 per week	T3 effluent turbidimeter	
	T4 effluent	<1.0	1 per week	1 per week	T4 effluent turbidimeter	
	SS1	<0.1	1 per 12 hours	1 per 12 hours	SS #1 sample tap	
	SS2	<0.1	1 per 12 hours	1 per 12 hours	SS #2 sample tap	
	Filters 1–23 effluent	<0.1	1 per 2 months	1 per 2 months	1–23 filter effluent turbidimeters	
	¾-Mile Michigan Ditch	N/A	N/A	2 per week Apr.–June	Location on upper Poudre River	
	Keal Bridge	N/A	N/A	2 per week Apr.–June	Location on upper Poudre River	
	Narrows Campground	N/A	N/A	2 per week Apr.–June	Location on upper Poudre River	
pH (pH units)	HT raw	N/A	1 per week	1 per week	Raw water sample station	Electrode method using a two-standard calibration; USEPA approved.
	PR raw	N/A	1 per week	1 per 24 hours	Raw water sample station	Standard Methods 4500H+ (Process Control Lab)†
	PR raw	N/A	1 per month	1 per week	Poudre River intake	
	T1 effluent	>6.10	1 per week	1 per week	T1 effluent turbidimeter	
	T2 effluent	>6.10	1 per week	1 per week	T2 effluent turbidimeter	
	T3 effluent	>6.10	1 per week	1 per week	T3 effluent turbidimeter	
	T4 effluent	>6.10	1 per week	1 per week	T4 effluent turbidimeter	
	T4 effluent	>6.10	1 per week	1 per week	T4 floc drywell	
	SS1	7.80–8.00	1 per 12 hours	1 per 12 hours	SS #1 sample tap	
	SS2	7.80–8.00	1 per 12 hours	1 per 12 hours	SS #2 sample tap	
	Wash water return	N/A	1 per week	1 per week	Wash water return overflow	
	¾-Mile Michigan Ditch	N/A	N/A	2 per week Apr.–June	Location on upper Poudre River	
	Keal Bridge	N/A	N/A	2 per week Apr.–June	Location on upper Poudre River	
	Narrows Campground	N/A	N/A	2 per week Apr.–June	Location on upper Poudre River	

Source: Kevin R. Gertig, Fort Collins Utilities, Fort Collins, Colo.

* Winter is defined as October–March.

† American Public Health Association, Water Environment Federation, and American Water Works Association, current edition.

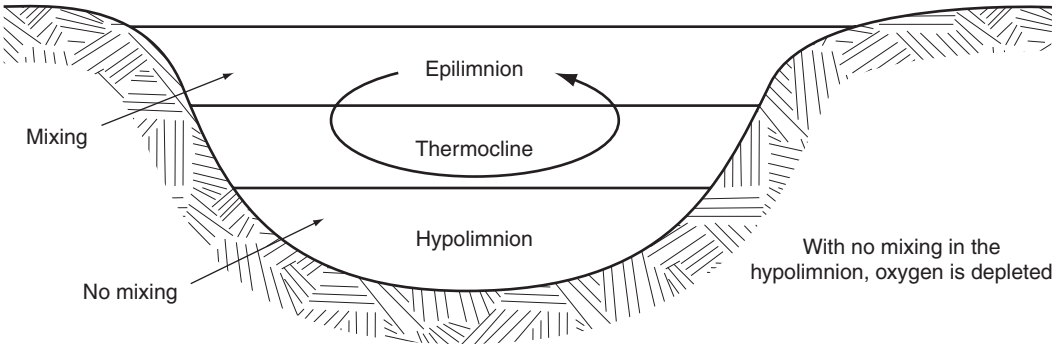


Figure 3-1 Stratification layers of a reservoir

- cross-connections found in the system
- total organic carbon (TOC) levels difficult to reduce in treatment process
- water main breaks or other breaches in the distribution system
- biofilms discovered in the clearwell, storage reservoirs, or distribution system

Reservoir Turnover Monitoring

A surface water condition that may warrant increased monitoring is reservoir turnover. Facilities that take water from surface water impoundments are subject to annual reservoir turnovers. Although site-specific, turnover typically occurs once in the fall and sometimes in the spring. Turnover is caused by the mixing of a formerly temperature-stratified reservoir. Cold, oxygenated water descends to the bottom of the reservoir and replaces warmer water that was anoxic. This turnover can cause quick changes in water quality.

The column of water that exists in most reservoirs has three levels. The upper warmer water is called the epilimnion, the middle level is the metalimnion or thermocline, and the bottom layer is the hypolimnion. Monitoring the water column is site-specific and needs to be studied before designing a finalized monitoring plan. Multiple samples taken at various depths, vertical profiling using multiple sensor datasondes, and continuous on-line raw water quality analyzers all will give a more clear assessment of water quality and, thus, allow the treatment staff to predict water quality changes over time. Depending on the elevation of the intake, the plant may be able to take water at different depths and avert potential problems.

In a stratified reservoir, the three vertical zones (Figure 3-1) of water do not mix and the structure of the water column is determined by the water temperature. Ambient air temperature, influent flow to the reservoir, bacteriological results, algae identification, turbidity, nutrient concentrations, water column dissolved oxygen (DO), and water temperature can all help predict turnover. In some cases, metals such as iron (Fe) and manganese (Mn) may become a problem due to low DO in the hypolimnion layer.

Groundwater Monitoring

Public water supplies using groundwater sources rely on the integrity of the well construction and, more importantly, the natural protection of aquifers. Where well construction is adequate and the aquifer protected, microbiological monitoring programs beyond sampling for total coliforms, *E. coli*, heterotrophic bacteria, and various nuisance environmental bacteria have a limited value. Monitoring programs should be developed to detect changes in water quality that reflect problems with the well itself and natural protective systems. Without adequate well construction or natural protection of the aquifer,

expansion of the monitoring program to include other indicator organisms or pathogens may be warranted.

Water quality in wells is not always consistent; for example, water quality in an aquifer can change. Depending on the aquifer, changes in water quality may become evident with an increase in the rate or total volume of water pumped from the well. Fractured limestone and sandstone formations with little protective overburden appear highly susceptible to microbial and chemical contamination. In one epidemiological investigation, clinical evidence indicated that a hepatitis outbreak was due to contamination of a fractured limestone aquifer from septic tank discharges. The same type of fracturing appears to be responsible for high groundwater velocities.

The key to developing a groundwater system monitoring plan is obtaining information about the aquifer and the wells in use. Performing a source water assessment or developing a wellhead protection program provides most of the information required to develop a monitoring plan. The information gained regarding existing well construction practices is invaluable; a poorly constructed well cannot be expected to produce consistent water quality. Typical problem areas with construction include the wellhead, casing, or grouting. Holes, gaps, or other breaches in the systems allow contaminants access to the well.

To determine well construction problems, sampling may be required within the first few minutes or seconds of operating the well. The sample collector may need to determine water volume by measuring the static water level reading to the bottom of the screen or the open bore hole. The primary samples for examining well integrity usually need to be collected within the first two volumes contained in the well. A delay in collecting the sample may risk diluting the contaminants to below detection limits or losing contaminants by flushing the system with fresh water from the surrounding aquifer. A drop in microbiological counts from the initial samples to after the system has been adequately flushed often indicates construction problems. Total coliform or heterotrophic bacteria counts, or both, can be used as indicators.

Monitoring aquifer-related problems requires detailed knowledge of existing hydrogeological characteristics. For example, it may be necessary to temporarily seal portions of the well with inflatable bladders to isolate one section of the aquifer from another. Samples may have to be collected at specified intervals throughout a pumping test to detect variations in water quality. In general, an increase in selected indicator counts or values indicates aquifer problems. For example, a correlation between an increase in indicator microbial counts with some surface activities, such as precipitation, indicates direct recharge or groundwater under the influence of surface water. An observed increase in coliform counts over time may justify the expansion of the monitoring program to include other possible microbial contaminants.

Other considerations for increasing the monitoring frequency of well systems are

- increased chlorine demand
- sudden decrease in well production
- high levels of metals, such as manganese (Mn) and iron (Fe)
- biofilm indicators, such as slime observed in the distribution system
- redox potential and pH measurements for biofilms
- temperature changes
- coliforms detected at the source
- increased levels of coliforms in unchlorinated well water
- significant change in total dissolved solids

Monitoring Within Treatment Systems

Because the primary purpose of any treatment system is to improve water quality, a change in microbial quality through the treatment process is expected. The purpose of

the monitoring program within treatment systems is to detect those changes. Treatment processes that may change water quality include aeration, coagulation, filtration, softening, and disinfection. Monitoring changes require testing before and after each unit process.

Monitoring changes in groundwater supplies that use simple chemical injection at the discharge piping of a well is straightforward. Sampling is suggested before and following treatment. The untreated or source water tap should be located as close to the wellhead as practical. For wells using vertical turbines, the source water tap should be located far enough from the motor to avoid air currents from the motor. The treated or plant tap should be located downstream from the point of chemical injection at a sufficient distance to permit mixing of the chemical and water. As suggested earlier, the standard indicators of total coliform and heterotrophic bacteria are adequate for most monitoring programs, unless there are problems. Testing for nuisance and fouling bacteria (*Sphaerotilus*, *Leptothrix*, *Crenothrix*, *Gallionella*, and *Clonothrix*) may be performed periodically at the source water tap.

Another way for microbiological contamination to enter the system is a typical iron–manganese removal system with aeration, detention, and filtration. Climatic conditions may assist bacteria in entering the system through the aerator or a poorly sealed detention tank. Inadequate operation and maintenance allows biofilms and slime growths to develop in the various treatment units. Establishing a monitoring program to track coliforms and heterotrophic bacteria in the source, postdetention tank, and filtered water is generally adequate. Monitoring is not as critical in closed or pressure filtered iron–manganese removal systems using potassium permanganate because of the oxidant's disinfection capabilities.

Developing a monitoring program for a standard coagulation, sedimentation, and filtration treatment plant can become cumbersome. Monitoring lime–soda ash and lime softening plants presents the same dilemma. The main issue to consider when developing a program is how the information will be used. Sampling sites can be set up before and after each unit process. However, most information from the monitoring program may not be used to alter treatment processes. Furthermore, the usefulness of information gained may be questionable. One way to avoid this situation is to focus on optimizing unit processes. Limited data from samples collected at the source and before and after the filters may yield information that will be useful. Consideration should be given to sampling from the effluent of each filter rather than at a point of confluence from all filters.

Routine total coliform monitoring at the water tap, pre- and postfiltration, and finished water effluents should be considered the minimum baseline for complete treatment plants. HPCs and postfiltration of the reservoir effluent give a better assessment of the status of microbial removal efficiencies. Monitoring for other contaminants depends on the source or source water makeup. For filtered water, the total coliform count should be zero colony-forming units per 100 milliliters (0 cfu/100 mL). The HPC for filtered water quality should approach 0 cfu/100 mL. Similarly, for any other microbial indicator counts, filtered water quality before disinfection should approach zero.

Because HPC bacteria are not generally associated with waterborne disease and are not described elsewhere in this document, a brief description is included here. HPC bacteria are aerobic and facultative bacteria capable of growing on simple media under specified incubation conditions. Their presence in a drinking water sample is a general indication of water quality deterioration. The occurrence of HPC in a drinking water has never been associated with a disease outbreak, and therefore it is not used as an indicator of a potential health risk. While treated drinking water usually has no detectable HPC organisms, nonchlorinated waters, or high turbidity waters, particularly when the water temperatures rise, can have levels in excess of 10,000 organisms/mL. High HPC levels are also linked with biofilms within distribution systems or associated with point-of-use devices such as carbon filters.

Routine testing for HPC bacteria is not required. It is used primarily as a tool to gain additional insight into the operational integrity of a drinking water system. Changes in HPC levels can often serve as an early warning of system problems or failures. The results are used in conjunction with coliform testing, chlorine residuals, and other operational data.

Microbial monitoring within the pretreatment or coagulation processes may not produce useful information. Floc particles may interfere with reading and interpreting results. Some test methods are based on color change after a specified incubation period, and iron-based coagulants may discolor the test water. Many analytical techniques are based on the theory that bacteria and other contaminants are uniformly dispersed throughout the entire sample aliquot. Coagulation processes concentrate microbial organisms into the bodies of floc particles, producing artificially low or high results, depending on the sampling technique.

Test results of samples from rapid sand or multimedia filter effluent lines may depend on when the filter was last backwashed or restarted. Filters may produce poor quality water immediately following backwash. Also, filters produce lower quality water for a short duration when returned to service after being off-line. Samples collected in the middle of a filter run may produce microbial counts near a "normal" or "average" condition, whereas samples collected at the beginning or end of a filter run are probably biased.

The possibility of concentrating microbial and other contaminants by recycling filter backwash is a major concern. Depending on regulations and the need to reuse backwash water, a monitoring program may be needed to evaluate the safety of this practice. The monitoring program should compare the quality of the backwash and incoming source water.

Primary sampling locations for any treatment facility should include the effluent point of any on-site reservoir and/or the point of entry into the distribution system. Daily sampling for total coliforms and HPCs should be the minimum. Sampling for other microbial contaminants should be considered in the event of a treatment system failure. Treatment failures can include sudden increases in filtered water turbidity or an inability to maintain a turbidity of 0.1 ntu or less in the effluent from any single filter. The ultimate treatment failure would be permitting filters to operate to or beyond the point of breakthrough. Additional monitoring at the filter effluent because of treatment failure should include testing for fecal coliforms or *E. coli*, *Pseudomonas* sp., fecal streptococci, *Giardia*, and *Cryptosporidium*. If the filters responsible for the problem are removed from service before sampling, the alternative sampling site should be the plant tap or the point of entry in the distribution system. Natural events warranting expansion of monitoring include turnover in settling basins, heavy precipitation or runoff, or high winds creating unusual conditions in open treatment basins.

The lack of physical system integrity for finished water storage facilities warrants expansion of the monitoring program beyond a total coliform and HPC program. Integrity issues can include open hatches, improperly designed vents, leaky roofs or covers, and open finished water basins. Depending on the severity of the problem, additional monitoring in the distribution system may be needed.

Biofilms

Biofilms are patches or masses of living and/or dead microorganisms that accumulate inside water storage reservoirs or pipelines (see Figure 3-2). Because treated water is not sterile, some degree of biofilms exists in all systems. Other types of organisms or material can be "sandwiched" between layers in the biofilm, including nematodes, algae, bacteria, pathogens, fungi, and mineral deposits. See chapter 2 of this manual for more information on biofilms.

New research indicates that biodegradable organic carbon (BDOC) and assimilable organic carbon (AOC) may provide nutrients for many biofilm organisms. Systems

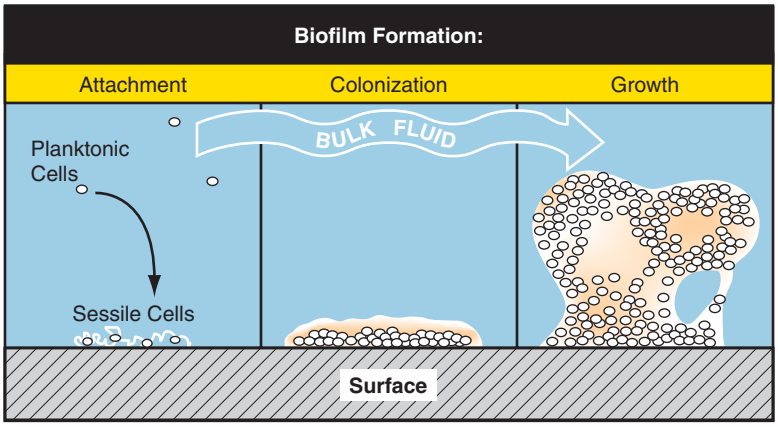


Figure 3-2 Biofilm formation

with more than 100 µg/L BDOC or AOC tend to have more coliform bacteria than those systems with lower levels. Limiting the amount of BDOC and AOC by optimizing water treatment processes is key to reducing this problem. Temperatures greater than 15°C (59°F) may also increase the growth of some bacteria. Plants using ozone and biologically active filters should monitor the BDOC and AOC levels leaving the treatment plant and throughout the distribution system and establish internal operating goals.

Other monitoring actions for biofilms include

- visually inspecting valves and pumps
- using borehole camera inspection
- monitoring and recording temperature trends
- starting and continuing a cross-connection control program
- establishing sampling methods, such as in-well collectors, filters, and grab samples
- observing and sampling during routine systematic flushing
- conducting hydraulic performance tests of the distribution system
- adding specific biofilm investigations with analytical methods or presence–absence tests, and direct microscopic analysis, when applicable
- performing basic inorganic chemical tests
- monitoring organic carbon (AOC/BDOC) levels in source and finished water, where applicable
- optimizing water treatment facilities and strive to meet the Partnership for Safe Water goal of less than 0.1 ntu at all times
- implementing or continuing corrosion control programs
- maintaining disinfectant residuals throughout the system
- monitoring cycling of reservoir levels at various times of the year
- cleaning and disinfecting clearwells and reservoirs on a frequent basis

SAMPLING ISSUES AND TECHNIQUES

The sample collector is responsible for using the correct sample container and sampling technique. If the wrong containers or techniques are used, the validity of the sample results will be questionable. Sampling errors may result in monitoring violations, a loss of laboratory time, and a waste of the public water supply's resources.

The water quality laboratory is usually responsible for providing sample containers for the parameter it intends to analyze. Part of the quality control procedure for any microbiological analytical work requires that the laboratory confirm the sterility of the bottles it uses by testing a portion of the containers it purchases or prepares. For example, the difference between similar sample containers may simply be the preservatives placed in the container before sample collection. Typically, preservatives are dechlorinating agents that must remain in the bottle and not be rinsed out. Laboratories usually do not accept sample containers prepared by or obtained from another laboratory.

Most bacteriological samples have a sample holding time (i.e., the maximum period between the time of sample collection and initiating analysis). The laboratory should supply this information to the sample collector. To meet the holding-time criteria, the sample collector, a courier, or an overnight delivery service may deliver the samples. Whenever practical, samples should be transported iced in a chest or cooler. The laboratory will not accept frozen samples. Samples waiting for delivery should be iced or refrigerated at 4°C. Rapid delivery back to the laboratory is essential to allow sufficient time to perform the analysis.

The sample collector must read and understand the directions for each type of sample. In addition, the sample collector may need to be familiar with field testing for free chlorine or other disinfectant residual monitoring, aseptic sampling procedures, and safe procedures for handling corrosive materials. Either the laboratory or the water supplier should provide this information to the sample collector. Information regarding all sample tap locations and accessibility should be on file.

Example of Directions for Total Coliform or HPC Sampling

1. Do not open the bottle until ready to collect the sample. Do not touch the inside of the cap or bottle. Retain the cap in your hand. (Do not set it down.)
2. Do not rinse the bottle with the sample. The bottle contains sodium thiosulfate to deactivate chlorine. Go to step 3 if taking a sample from a tap. If collecting a sample from a lake or pool, plunge bottle down and move it in a continuous arc down and back up through the water. Discard the top half-inch (12-mm) of sample. Go to step 4.
3. Select a clean faucet and remove attachments, such as aerators, dishwasher connectors, and so forth. Do not use swivel-arm faucets, faucets with one hot/cold water adjustor knob, or faucets at mop sinks. Turn on cold water knob and allow water to run for five minutes (until cold) at full flow from the sampling tap. Immediately before sample collection, reduce flow to avoid splashing, and collect the sample directly into the bottle. Do not use an intermediate container. Do not allow water from the outside surface of the faucet to drip into the bottle. Fill the bottle only to the bottom of the neck.
4. Replace the cap. Record the sample time, date, location, disinfectant residual concentration, water temperature, and sampler's initials. It is critical that paperwork or forms submitted to the laboratory with the sample be complete and legible. The laboratory should provide the sampling chain-of-custody forms, which record information needed by the laboratory to ensure all critical quality control elements have been met and to generate valid analytical results.

Tap Selection for Microbiological Samples

A potential problem with any microbial monitoring program is poor sampling tap selection. Failure to select a proper tap produces misleading results. Swing and mixing faucets and taps with vacuum beakers, aerators, hoses, or filters should not be used. These taps have the potential for bacterial colonies established on surface gaskets and screens

to slough off during sample collection. Other potential problems with some taps include the manner in which the flow leaves the faucet. Flow streams that spray or roll up around the end of the faucet are unacceptable.

For microbiological sampling, simplicity in tap design is key. Manufactured metallic ½-in. (12-mm) faucets with a single shut-off handle and a downturn spout without hose bib threads fulfill this requirement. Sample taps consisting of a petcock or other valve device and approximately 12 in. (300 mm) of ¼-in. (6-mm) tubing formed in a downturned U-shape are ideally suited for sampling. An added advantage of these designs is that the taps can only be used to draw water, reducing the likelihood of contamination by other uses. Unintentional contamination by other parties is a major concern for distribution sites or other locations with unrestricted access to taps. Commercially available sampling boxes directly tapped into the water main are another option for distribution systems where freezing and vandalism are not a problem.

Pathogens

One of the greatest challenges water professionals face is selecting an accurate technique to directly quantify the number of enteric pathogens, including *Cryptosporidium parvum* and *Giardia lamblia*. Monitoring for *Giardia* cysts and *Cryptosporidium* oocysts may be required of certain public water supplies. In some cases, virus monitoring is also necessary, and the search continues for improved techniques for sampling waterborne protozoa (Figure 3-3). Great strides in analytical techniques have been made over the past few years, and research continues for methods that provide quick and accurate results. An overview of the current methods of sampling for pathogens is discussed in following paragraphs.

Field worksheets developed by the laboratory should be filled out completely and checked for errors. They provide key information for the sampler on basic chemistry, physical measurements, and plant statistics at the time of sampling. Plant schematics or diagrams are especially helpful when they indicate the exact location of where samples are taken. Information should always be written in waterproof ink on laboratory forms (Figure 3-4). Chain-of-custody forms may be necessary for transport to the laboratory or compliance monitoring situations.

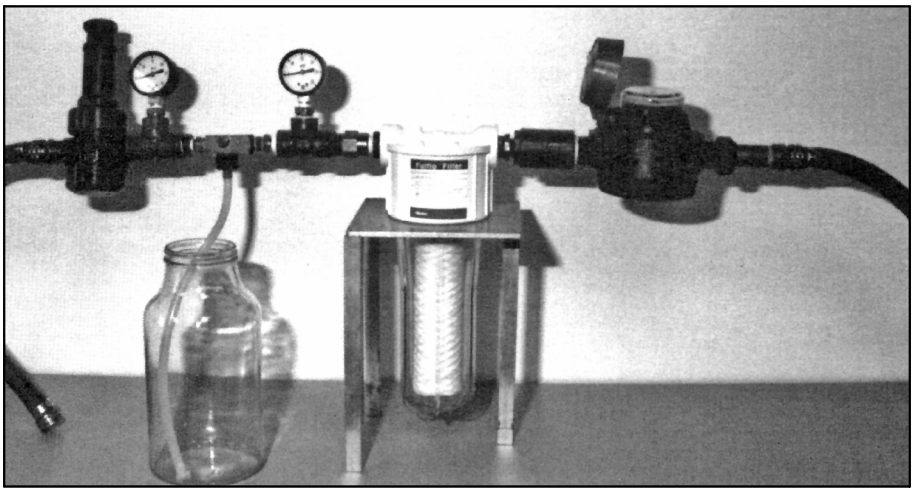


Figure 3-3 Filter housing (1 µm) with appropriate flow-measuring and dechlorinating devices

Analysis Request*

(Please fill out all information)

Client: _____Date: _____ PO#: _____
Person(s) designated to receive results: _____
Address:_____
Phone: _____ Fax:_____
Address for cooler return:_____

Sample Information: (please print clearly)
Sample Location: _____
Sample ID: _____
PWSID#:_____

Start:
Date: _____ Time: _____
Temperature: _____ pH: _____
NTU: _____(required for LT2 clients)
Meter reading: _____

Stop:
Date: _____ Time: _____
NTU: _____
Meter reading: _____

Did filter clog? YES/NO (LT2 clients must filter at least 10 L or two filters)

LT2 clients please initial to approve processing of additional pellets if needed _____

Gallons Sampled: _____ (_____ Liters)
Sample exposed to chlorine? YES/NO
Sample dechlorinated? YES/NO
Sampler's Name: _____

Please fill in where applicable for this sample:
This sample is a:
_____ **Raw** surface water
_____ **Finished** surface water
_____ **Ground** water
Other, please explain _____

Type of Raw Water Source:

Ground Water:	Surface Water:
_____ Spring	_____ Lake/Reservoir
_____ Dug well	_____ Irrigation canal
_____ Horizontal well	_____ Stream/River
_____ Drilled well	
_____ Infiltration gallery	
_____ Artesian well	

Well depth: _____ft
Distance from river/stream/lake: _____ft

Type of Analysis Requested: ***Jan '05–Dec '05***
(Please initial request)

1. **MPA (Microscopic Particulate Analysis) of Surface or Groundwater** _____
2. ***Giardia* and *Cryptosporidium* Analysis**
   –ICR or 1623 with Envirochek™ HV _____
   –Matrix Spike with Envirochek™ HV _____
3. **MPA with *Giardia* and *Cryptosporidium***
   –ICR or 1623 with Envirochek™ HV _____
4. **Photomicrography** _____
5. **Other requests/notes** _____

Laboratory Arrival Information (for use by CHD only)
Courier: UPS☐ FedEx ☐ HD☐ Other☐ _____
Type: Carboy ☐ HV ☐ Poly.☐ Color: _____
Date/Time: _____ **Temp:** _____
Shipping ☐ Cooler Return ☐ None ☐

Type of Disinfection:

_____ Pre-chlorination	_____ Post chloramines
_____ Post chlorination	_____ Ozone

Other _____

Type of Treatment:
_____ None
_____ Disinfection only
_____ Conventional treatment
_____ Direct filtration
_____ Filter to waste cycle
_____ Diatomaceous earth
_____ Slow sand
_____ Pressure filter
_____ Membrane
Other _____
Filter(s) hydraulic loading (gpm/sq ft)_____
Clean filter bed put on line: YES/NO

*For samples taken following protocol established by:
Courtesy—CHDiagnostic & Consulting Service, Inc., 214 SE 19th Street, Loveland, CO 80537
Ph: (970) 667-9789 Fax: (970) 667-9719
customerservice@chdiagnostic.com

Figure 3-4 Example of a laboratory analysis form to be completed in the field (continued on next page)

Method Description

Microscopic Particulate Analysis (MPA) is a survey of the water microbiota that is used to assess water quality. Surface waters (e.g. lakes, reservoirs, and rivers) are analyzed following Microscopic Particulate Analysis (MPA) for Filtration Plant Optimization (EPA 910-R-96-001). Ground waters (e.g. wells, springs, and infiltration galleries) are analyzed following Consensus Method for Determining Groundwaters Under the Direct Influence of Surface Water Using Microscopic Particulate Analysis (MPA) (EPA 910/9-92-029). We modify these methods as follows: sampling with the Pall Envirochek™ HV capsule (Section 11.1.1 of 910-R-96-001), and examination of 3 slides (Section 9.0 of 910/9-92-029).

***Giardia* and *Cryptosporidium* Analysis** is conducted following USEPA Method 1623: *Cryptosporidium* and *Giardia* in water by Filtration/IMS/FA (EPA 821-R-01-025). This method quantifies the number of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts present within a water sample. The analysis can be conducted on raw and treated surface waters as well as ground waters under the influence of surface water. The ICR Method (Microbial Laboratory Manual (EPA/600/R-95/178)) is performed upon request or when wound 1µm nominal porosity polypropylene cartridges are received.

MPA with *Giardia* and *Cryptosporidium* Analysis on the same sample follows the above procedures except particulate elution is modified. The first elution utilizes MPA particle extraction solution followed by a second elution in *Giardia* and *Cryptosporidium* prescribed solution. The first elution suspension is processed separately for MPA analysis, while equivalent volumes from both elution suspensions are combined for *Giardia* and *Cryptosporidium* analysis.

Matrix Spike Analysis is the seeding and recovery of known numbers of *Cryptosporidium parvum* oocysts and *Giardia lamblia* that have been spiked into a water matrix. EPA Method 1623 prescribes a matrix spike on the first sample from each source water and every 20th sample thereafter from that source.

Photomicrography: 35mm slides or prints can be requested. Four photo minimum.

Benthic Macroinvertebrate Analysis follows Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, 2nd edition, EPA 841-B-99-002. Samples are sorted, identified, and enumerated.

OPTION TO INCREASE SAMPLE VOLUME AMOUNT ASSAYED:
Must be requested prior to sample submission

Additional 1623 Pellet Analysis: During processing of the sample, a pellet is generated. The volume of the pellet varies with each sample, however only 0.5 mL of pellet can be analyzed at a time. The analysis can be repeated for each 0.5 mL aliquot of pellet thereby increasing the sample volume analyzed. For LT2 data, examination of 10 L or 2 mL of pellet is required. The client is required to cover the cost of additional analyses.

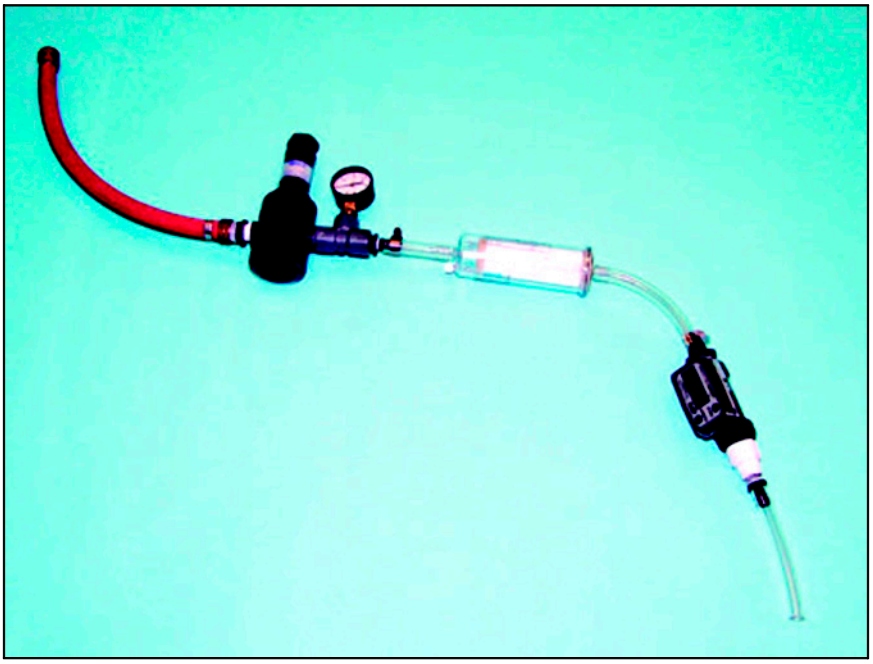
Additional ICR Slide Analysis: Multiple slides can be examined to increase the volume of sample analyzed in many instances. There is a 5 slide maximum per sample. The client will cover the cost of additional slide examinations.

Source: CHDiagnostic & Consulting Service, Inc., Loveland, Colo.

Figure 3-4 Example of a laboratory analysis form to be completed in the field (continued)

Preparation of the sampling devices as well as carefully planning where and how to take the samples is essential. When using cartridge filter sampling units, recommendations on specific detergents and cleaning techniques should be discussed with the laboratory. Field personnel or staff should be trained on assembly, disassembly, cleaning, and care of cartridge filter units and housing units that can become a problem because of improper setup or poor seals at the top of the housing. Flushing the sample taps, hoses, and housing assembly thoroughly before use is essential. Latex gloves need to be worn when inserting the polypropylene 1-µm filter to limit contamination.

Sampling methods for water containing chlorine residuals require a special procedure involving injecting a flow-proportional dechlorination solution during sample collection. The main purpose of the proportional dechlorination system is to eliminate the chlorine residual that may subsequently interfere with identification procedures. Specific attention to flow rate and feed rate of the dechlorination agent is critical. Flowmeters for the 1-µm filter units should be calibrated each year by a meter shop and checked more often with a graduated cylinder to ensure accuracy.



Source: Kevin R. Gertig, Fort Collins Utilities, Fort Collins, Colo.

Figure 3-5 Filter device

Filter devices such as the one shown in Figure 3-5 have been developed and are in routine use. This type of unit is self-contained and requires similar sampling protocols to those previously discussed. After sample collection, the inlet and outlet of the unit are simply sealed and delivered to the laboratory for analysis.

Transport and storage of the sample to the laboratory should be expedited by using overnight services. Communication should be already established between the sampler and the laboratory. Coolant materials, such as Blue Ice, should never be in direct contact with the filters. Coolers need to be cleaned regularly and the coolants sealed to prevent leakage. If care is not taken in sealing the filter before delivery to the laboratory, cross-contamination can occur during transit.

In a critical situation, samples may need to be collected over a short time period, and sampling supplies may be difficult to secure late at night, on weekends, or on holidays. An ample supply of filters and appropriate sampling equipment should always be available for systems known to have the possibility of enteric pathogens in the source or finished water. For smaller systems, the names of several neighboring larger water plants, and after-hours phone numbers for vendors and laboratories should be available. Equipment expense can be overwhelming for the small system, and sharing resources may be the key link in protecting public health.

Figure 3-6 shows a pathogen sample kit with various types of hoses, connectors, and dechlorination equipment stored and ready for use. Sampling kits for *Giardia* and *Cryptosporidium* analysis should contain the following items:

- inlet hose with adapters for various sample ports
- backflow preventer
- pressure regulator with pressure gauge
- fluid-proportioning injector with pressure gauge

Source: Kevin R. Gertig, Fort Collins Utilities, Fort Collins, Colo.

Figure 3-6 Pathogen sample kit. Various types of hoses, connectors, and dechlorination equipment can be stored and ready for use.

- calibration column for proportioning solution, when applicable
- 1-µm nominal porosity filter and holder
- water meter
- flow control valve–limiting control orifice
- plastic sampling bags
- ice packs with plastic sealed covers
- sample labels
- stopwatch for calculating flow rates
- calculator to determine dechlorination rates, if applicable
- latex gloves
- sodium thiosulfate and reagent-grade water (may be secured through your laboratory)
- graduated cylinders for calibrating sodium thiosulfate additions
- equipment for shipping 10-L samples to the laboratory, if applicable

The following factors should be taken into account when sampling for pathogenic organisms:

Surface water source

- Confirm the sample point does not have any chemical pretreatment.
- Collect samples after small reservoirs or presedimentation basins, if possible.
- Review the plant process with the laboratory using a plant schematic to describe the sample location. This can help the analyst understand the sample and situation.
- Sample as described by the laboratory using the most recent USEPA-approved method.

Groundwater

- Sample at the discharge of the pump.
- Flush all lines for a minimum of five minutes before sampling.
- Discuss with the laboratory all required specific monitoring data.
- Provide a schematic of the system to the laboratory.

Finished water

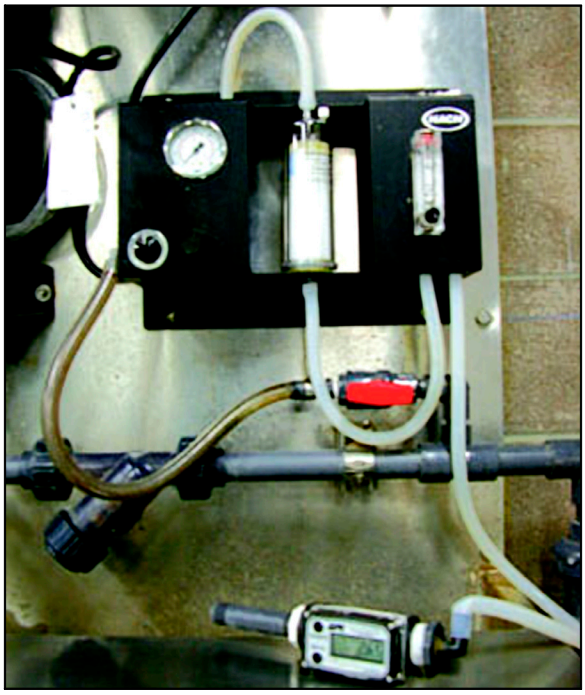
- Sample before chlorination, if possible.
- Dechlorination is required if a sample cannot be taken before chlorination. This procedure requires adding sodium thiosulfate while taking care to maintain proper disinfectant feed rates to the system. Discuss with the laboratory.
- Use the plant schematic to indicate the point of sample for the laboratory.

Sampling Stations or Points

Representative sampling is critical to building a valid database of water quality information. Such a database is valuable because a utility can see seasonal trends, quality improvements, and action plans. Although water quality databases are usually in a computer program, small water suppliers may simply use organized files with specific locations referenced.

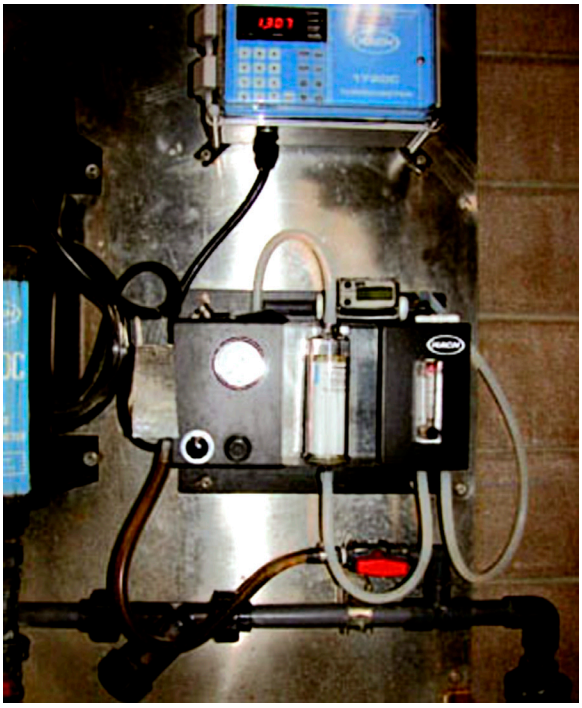
Larger systems may prefer establishing sampling station points throughout a water treatment facility and in the water distribution system. One type of sample station is shown in Figure 3-7. If desired, grab samples can be taken at the polyvinyl chloride (PVC) line or pathogen sampling taken at the ¾-in. (19-mm) hose bibs for each filter shown in Figure 3-8. On-line analyzers can be used to monitor filtered water quality. Information from on-line analyzers is then routed through programmable logic controllers and logged onto a computer database for storage. The information can then be archived in database format for retrieval, trending, and establishing control limits for out-of-target results. Because on-line instruments may have different ranges of accuracy, each parameter needs to be evaluated individually. Quality assurance (QA) and quality control (QC) programs to ensure instrument calibration or standardization are essential for on-line equipment. One of the major benefits of the sample station concept is that it makes available both real-time data and information on grab samples.

On-line units or running sample taps around the clock may not be justified or practical for smaller systems. Small systems rely on the experience and knowledge of the system operator. Maintaining consistency in the sampling technique and using best judgment to obtain the most representative samples will yield accurate results. The system operator should keep judicious records and ascertain the integrity of the system by sampling the source water and the storage and distribution systems.



Source: Kevin R. Gertig, Fort Collins Utilities, Fort Collins, Colo.

Figure 3-7 Sample station



Source: Kevin R. Gertig, Fort Collins Utilities, Fort Collins, Colo.

Figure 3-8 On-line turbidimeter sample station with ¾-in. (19-mm) sample taps for MPA and pathogen monitoring

Proactive sampling by water systems can be a helpful troubleshooting tool. For example, if a plant or system operates under unusual conditions (e.g., loss of process), sampling with 1-µm units for microscopic particulate analysis (MPA) could provide invaluable information if the units are placed on-line during the event. Analysis of the sample filters can provide important information to the water system if used as a proactive tool rather than a reactive test method. Too often, facilities try to piece the puzzle of events together later and not when a problem is taking place.

LABORATORY SELECTION

A certified laboratory must be used to provide analytical data intended to meet the compliance monitoring requirements of the Safe Drinking Water Act (SDWA). However, certification is not a guarantee that the laboratory will always produce valid results. Certification means that the laboratory has successfully met the requirements of the primacy jurisdiction and that certain QC practices are in place. Most primacy jurisdictions do not require public water supplies to use certified laboratories for testing beyond the monitoring requirements of the SDWA. Also, most primacy agencies do not offer laboratory certification for contaminants for which monitoring has not been specified by the SDWA.

The use of a certified laboratory for optional, nonregulatory testing assures the client that the laboratory is at least aware of certain QC practices that are necessary to produce valid results. One of the certification requirements specifies that laboratories have and maintain an updated QA manual. Depending on contractual arrangements, the laboratory's QA program may be reviewed by the client. A good correlation between the manual and any audit report of the laboratory is an indication of the quality expected from the facility. A review of the QA manual will give the client an idea of the laboratory's capabilities. A failure to fully understand these capabilities and corresponding limitations can become a problem between environmental laboratories and their clients. The capabilities a client should understand include the inherent variabilities in producing microbial data, time required for analysis and reporting of final results, and cost.

Clients of environmental laboratories should understand that different analytical methods may produce different results. Also, there should be an understanding that there is variability in results produced using a single method. Each analytical method has its own recovery rate for a given sample matrix. One method may be capable of recovering 92 percent of the colonies or organisms present, while a different analytical method may have a recovery rate of 87 percent. The difference of 5 percent indicates final results from the two techniques may vary. Microbial techniques report a fixed number of colonies or organisms per a fixed volume or simply confirm their presence or absence. If the analytical technique was developed to determine the number of colonies per 100 mL and the density is 1 cfu/200 mL, a sample volume of 100 mL will not always yield positive results.

The time needed for an environmental laboratory to return analytical results to its clients depends on a variety of factors, including workload, staffing, equipment, and analytical methods. For bacteriological tests, there may be different incubation periods for two different analytical techniques. In addition, one technique may require subsequent confirmatory testing while another technique may be self-confirmatory. A comparison of two *Standard Methods* procedures, 9221 and 9223, is an excellent example for demonstrating the differences in time required to produce final results (APHA, WEF, and AWWA 2005). Method 9221B, the multiple-tube fermentation technique, requires up to 48 hours for the presumptive stage and possibly an additional 48 hours (for a possible total of 96 hours) for the confirmatory stage of the test. Confirmed and final results are available from Method 9223B, Colilert, within 24 to 28 hours.

Another factor for differences in turnaround times involves how laboratories process sample results. After bench data are produced, time is required to read, interpret, and record the information. Laboratories need to review the QC associated with the sample before the results are released. Lack of staff on weekends or holidays may further slow down reporting time. In some instances, the laboratory may issue verbal or electronic reports as soon as the results are approved as meeting the QC requirements. Under no circumstances should a verbal report be the sole means of reporting the results to the client. Most primacy jurisdictions require either a hard copy or an easily retrievable electronic database report. Forwarding a hard copy of the results requires additional time. Reliance on a mailed hard copy as the only means for reporting results to a client may add potentially unacceptable delays in taking action to correct water quality problems.

USE OF RESULTS

After results are received from the laboratory, the public water utility has legal obligations and several decisions to make regarding the release of the information. The utility must report all monitoring results for contaminants covered by the SDWA to the primacy jurisdiction. Reporting a limited subset of results could be considered an attempt to conceal problems. The public water supply may be required to periodically release information to its consumers in a summarized report. Releasing information provides an opportunity to build support for system improvements and associated costs. In addition, information can prove systems in place are providing high quality water to customers. A utility should avoid releasing information too often, which can result in the message being ignored. Releasing too little or possibly inaccurate information also should be avoided.

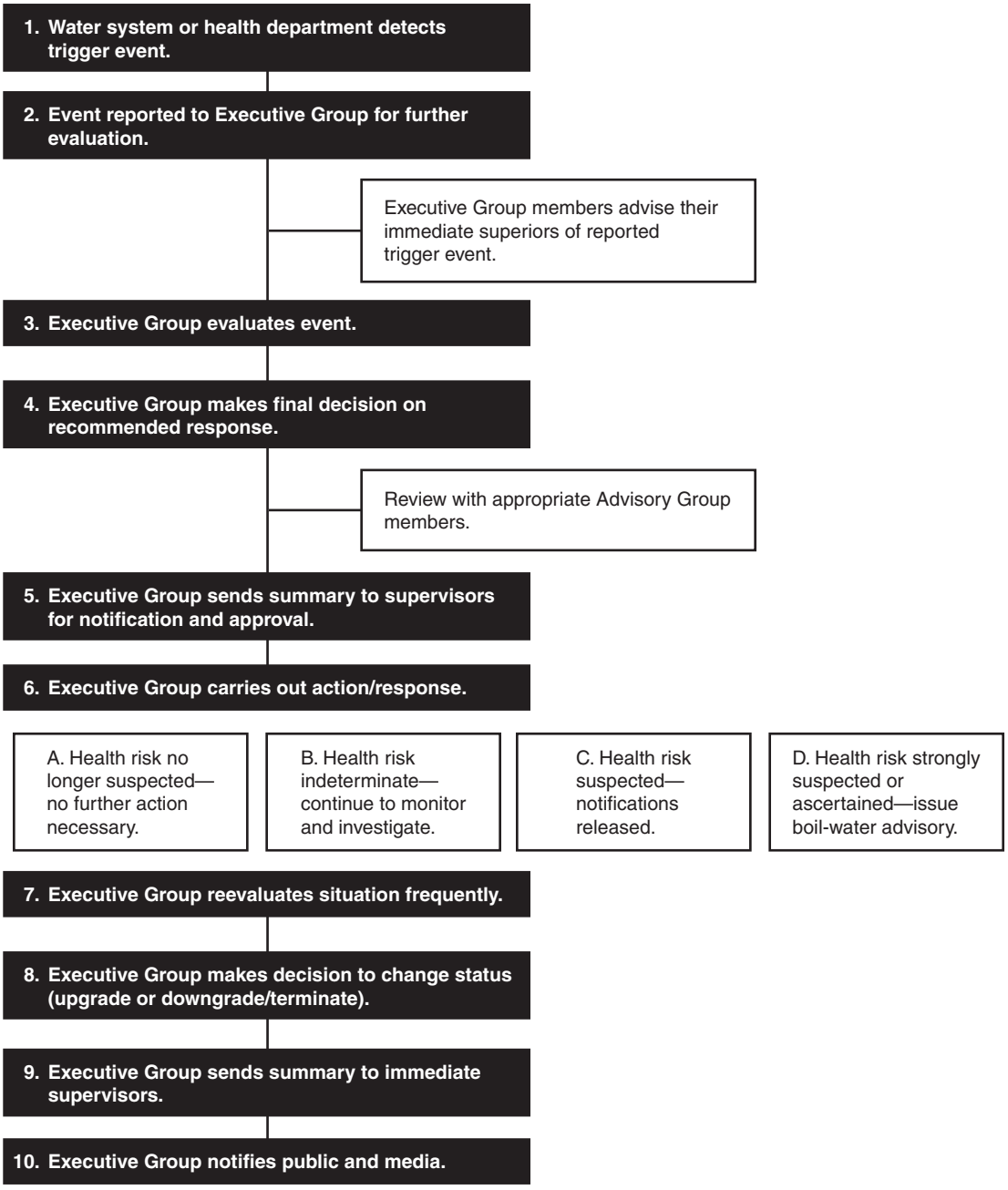
Action Plans

Although every water treatment operator strives to produce high quality water, there is a possibility of pathogens passing treatment barriers. Using current methods, simply monitoring for *Giardia* or *Cryptosporidium* cannot assure the water supplier that water is free of pathogens at all times. Therefore, water professionals must develop boil-water advisory (BWA) criteria and action plans before there is a problem, not during a crisis.

A course of action should be in place if a test result indicates positive fecal coliform, *Giardia*, or *Cryptosporidium* in finished water. BWAs require a considerable amount of thought if they are to be carried out in a timely fashion. One of the most important aspects of BWAs is determining what circumstances will trigger the event, which may involve violation of the total coliform rule, breakdown in treatment, pathogen test result, or an increase in diarrheal disease in the community.

Action plans should be developed by task teams including the operator, local and state health departments, hospitals, nursing homes, physicians, pharmacies, and so forth. A sample flowchart is shown in Figure 3-9. There is no single perfect action plan for all utilities; each must be tailored to the specific area and system. It is essential that plant staff focus on the challenge of fixing the problem that prompted the BWA thus protecting public health. Text of the public notification regulation can be found in the SDWA Reauthorization. Smaller systems might be able to share resources with larger utilities or to network using electronic mail or phone.

Water treatment professionals must regard public health protection as the highest priority. If a test result or condition exists that may breach the public health water quality standard, BWAs must be considered. BWAs should be issued only after carefully evaluating the circumstances. Once the criteria to issue a BWA have been met, prompt action is necessary. Customer confidence may be eroded or elevated depending on the timeliness and accuracy of the information they receive.



Source: Centers for Disease Control and Prevention 1997.

Figure 3-9 Sample flowchart for action/response plan

Considerations Leading to Boil-Water Advisories

The use of analytical results in deciding whether to issue a BWA requires considerable subjective judgment. Professionals at both the public water utility and the primacy jurisdiction making the decision must consider the consequences of issuing a BWA, including its ultimate costs, or delaying its issuance while waiting for additional information.

BWAs erode public confidence if they are not issued in time or issued too often. Financial costs to the public supply can be substantial, but the final costs for not issuing an advisory when needed may be devastating. Failure to issue a timely advisory could lead to serious public health, financial, and public relations consequences.

Results from a single sample or set of samples are typically not cause for issuing a BWA. Issuance usually requires additional confirmation, including subsequent sample results, other water quality data, and reports on treatment system operations failure or a breach in the system. In certain circumstances, delays in taking action while waiting for additional microbial results may not be prudent.

Public water systems using surface water or groundwater under the influence of surface water are more vulnerable to treatment system breakdowns and may justify immediate issuance of an advisory. Loss of disinfection residuals at the point of entry for these supplies is one reason to take immediate action. Loss of disinfection residuals at the point of entry for protected groundwater supplies in most cases would not be considered cause for the immediate issuance of a BWA without other mitigating circumstances. High filter effluent turbidities should be considered cause for immediate action. If the point-of-entry turbidity levels exceed the regulatory treatment standards or if there are sudden increases in turbidity levels from a single filter, then the water supplier and the primacy agency should determine whether or not an advisory is warranted. Other indicators of treatment system failures needing joint review include sudden changes in water quality or increases in finished water particle counts. Concurrent detection of microbial indicators of fecal contamination and a treatment system failure increases the possible need to issue an advisory.

Professional judgment and discretion are necessary in making decisions on the issuance of an advisory for distribution system failures. Failures include loss of pressure, documented cross-connection backflow, major water main breaks, and breaches in the integrity of water storage facilities. Microbial results supporting the decision to issue a BWA in the event of distribution system problems are not always readily available because of delays associated with analysis. In situations where microbial results are available and system failures are documented, the decision to issue an advisory should be straightforward.

Detection or sudden increase of any microbial indicators in a single sample or set of samples is not sufficient grounds to issue a BWA. However, the change is grounds for other actions such as collecting additional samples. Public water supplies have a regulatory-mandated responsibility to collect additional samples in the event of total coliform detection within the distribution system. Similar, but voluntary, actions regarding sample collection should take place when monitoring for other microbial indicators or pathogens reveals unexpected results. In addition, it may be advisable to consider collecting samples for other indicator organisms in the event of a positive sample, depending on the system and its conditions.

Before the onset of any problems, it is advisable for representatives of a public water supplier to meet with the local primacy jurisdiction agency to discuss the criteria for issuing public notices or BWAs. These discussions should include the actual wording and conditions for issuing the advisory as well as the comfort zones for all parties.

General issues to consider when issuing a BWA include

- reviewing data that may indicate a public health problem and evaluating the integrity of the samples and related quality assurance and control
- repeating the sampling immediately
- involving local and state health departments as soon as possible
- starting with the source water and following analytical results through to the finished water
- trying to identify cause and effect

- determining the identity of the pathogen or organism of concern, and identifying and evaluating possible courses of action
- evaluating plant or system performance before, during, and after the sample was taken
- reviewing the last sanitary survey and performing portions again, if warranted
- looking for changes or unusual conditions to see if there is a correlation to sampling results
- reviewing HPC and coliform occurrence data before, during, and after the event
- evaluating the system's capability for contact time ($C \times T$) and whether or not the organism can be deactivated

Factors to consider involving the distribution system include

- reviewing water main flushing procedures
- implementing a systematic flushing plan as soon as practical (site-specific or systemwide)
- communicating with restaurants, hospitals, and health-care and kidney dialysis centers
- contacting local hospitals, nursing homes, day-care centers, and physicians to ascertain recent changes in the public health
- evaluating the area for possible cross-connections
- determining the availability of resources to deal with all of the issues
- establishing additional water quality monitoring sites to determine where other samples will be taken and their frequency
- determining if system line breaks or other unusual situations in the distribution system occurred before the discovery of positive fecal coliform

Considerations When Issuing Boil-Water Advisories

Once the decision has been made to issue a BWA, the utility needs to do the following:

- Identify the specific person responsible for communicating with the media.
- Establish one site that will be the command center during the BWA.
- Meet with the task team and identify factors that trigger issuance of a BWA.
- Evaluate criteria for the BWA (e.g., Centers for Disease Control and Prevention 1997).
- Evaluate the conditions that need to be met to rescind a BWA.
- Include the laboratory in all briefings.
- Increase watershed or well system monitoring and surveillance.
- Acquire additional sterile sample bottles, filters, chlorine-residual test equipment, and other critical supplies.
- Increase monitoring programs for source, treated, and finished water.
- Assign a task team to solve the source water or treatment problem.
- Ask for guidance from local or state health departments, if necessary.
- Maintain close communication with officials at local and state health departments.
- Use technology such as fax machines and electronic mail preprogrammed with contacts for situation updates.

- Identify possible sources of bottled water or mobile tanks for water distribution to customers.
- Have public education materials ready to hand out to all water users.
- Check the contact time at the time of the alert and keep time-series logs.
- Sample as frequently as needed, but focus on public health protection.
- Keep one phone line open for internal communications.
- Maintain status boards posted with assignments and updates to ensure communication between staff and town or city leaders.
- Obtain references on action plans from the American Water Works Association (AWWA), the Centers for Disease Control and Prevention (CDC), and USEPA. Correlate these references with what is happening during an event.
- Prepare daily briefing reports understandable to the public. Use care in the spoken and written word; it is easy to say or write something that can be misinterpreted.
- Tailor BWAs to be site-specific for each situation.
- Keep a time-sequenced log of events.

Rescinding the Boil-Water Advisory

The following steps should be taken before the BWA is rescinded:

- Determine if treatment is adequate and take immediate action if it is not.
- Check source water and determine if it is of acceptable quality.
- Analyze the data that prompted the BWA and determine whether or not it could continue to be an issue due to the sampling as discussed previously.
- Focus on the cause and effect of the BWA.
- Develop a strategy for completion if temporary repairs or remedies are taken.
- Create a document recording all information leading up to, during, and after the incident.
- Work with health departments and the medical community to review epidemiological data.

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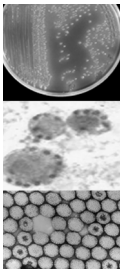


Chapter 4

Molecular Detection of Waterborne Microorganisms

Paul A. Rochelle and Kellogg J. Schwab

Environmental waters that are used as sources of drinking water may be contaminated with a wide range of pathogenic microorganisms intermixed with a dominant background of naturally occurring nonpathogenic microbial populations. While correctly operating water treatment facilities will remove the vast majority of microbial contaminants, one of the best protective measures against waterborne disease outbreaks is to protect watersheds from contamination in the first place. An important aspect of such protection is the ability to detect pathogens with specific and sensitive assays. Over the past 15 years, the polymerase chain reaction (PCR) and other molecular biology–based techniques have begun to revolutionize the detection of pathogenic bacteria, viruses, and protozoa in clinical and environmental samples. Traditionally, drinking water treatment plants monitor fecal coliforms and other indicator organisms to provide an approximate measure of potential fecal contamination and evaluate efficacy of removal or inactivation of pathogenic microorganisms (chapter 3). Conventional cell culture or animal assays for specific microbial pathogens are not routinely conducted because these methods are expensive, labor intensive, and time-consuming. Molecular methods enable rapid detection of pathogens in water by providing levels of sensitivity and specificity difficult to achieve with traditional culture-based assays, which often take days to perform. Molecular methods have revolutionized our understanding of the composition, phylogeny, physiology, and function of microbial communities in the environment. Published applications of molecular techniques to drinking water issues include direct detection of pathogens in water, fecal source tracking of either indicator microorganisms or specific pathogens, and as detection methods for in-vitro infectivity and disinfection assays (Abbaszadegan et al. 1999; Foulds et al. 2002; Huang et al. 2000; LeChevallier et al. 2003; Sails et al. 2002; and Wang et al. 2004).

However, although molecular methods were first applied to the detection of potential waterborne pathogens in the 1980s, they have not yet been adopted on a routine basis by the water industry, as they have been in the clinical diagnostic and, to a lesser extent, the food industries. This is due to the lack of standardization, the relatively few researchers and utilities using molecular tools to address water-related microbial issues, and the unique challenge presented by attempting to detect very low concentrations of target organisms in relatively large volumes of water (10–1,000 L).

The extensive literature describing the application of a wide variety of molecular techniques to the study of environmental samples would seem to indicate that that the procedures are routine and the results unquestionable. However, a certain amount of evaluation and optimization must be conducted to ensure that results obtained using these techniques are reliable and consistent. Without a thorough understanding of the limitations of these methods, as well as their advantages, end users may be disappointed by molecular results. Molecular methods should not be considered as an alternative to conventional microbiological techniques. Rather, they provide an additional suite of tools that can be used to complement traditional methods. Nevertheless, the potential benefits of molecular technologies and the valuable information and insights gained through their application in recent years demonstrate that there is a strong role for these methods in the future.

An illustrative example of the utility of molecular methods is provided by the increased knowledge that has been gained about waterborne *Cryptosporidium* in recent years. For many years it was assumed that cows were responsible for much of the *Cryptosporidium* contamination detected in surface waters. However, because of the application of a variety of molecular methods, it is now known that there are two species of *Cryptosporidium* (*C. parvum* and *C. hominis*) that are responsible for most cases of human cryptosporidiosis and that *C. hominis* is almost exclusively a human pathogen. Thus, human contamination is a significant source of oocysts in environmental waters. Also, there appear to be many animal host-specific strains of *Cryptosporidium* that are detected in water and whose significance to human health is not yet known. Molecular methods have also been used to detect a broad range of protozoal, enteric virus and bacterial pathogens in water (Table 4-1) in addition to nonpathogenic but problematic microorganisms in water, such as nitrifying bacteria. They have also been used to measure the infectivity of protozoa and viruses recovered from water.

SAMPLE RECOVERY, CONCENTRATION, AND EXTRACTION

A major drawback to the implementation of many of molecular methods is the extensive sample processing and purification required before the techniques can be applied. Typically, relatively large volumes of water (1–1,000 L) need to be reduced to volumes that are compatible with molecular detection technologies (<10 mL). In many cases, the sample volume analyzed in an individual molecular reaction is 1–10 µL. Water samples are typically concentrated by filtration using a variety of filter formats depending on the sample volume and type of microorganism being targeted (Figure 4-1). All of the considerations of sample collection and transport that are described in chapter 3 apply whether traditional microbiological techniques or molecular methods will be applied. Sample filtration and concentration can follow the initial stages of approved methods such as membrane filtration for bacteria, US Environmental Protection Agency (USEPA) Method 1623 (LeChevallier et al. 2003) for protozoa, and the Information Collection Rule (ICR) method for viruses or can use more research-oriented techniques such as hollow-fiber ultrafiltration, vortex flow filtration, and continuous centrifugation. Following a combination of elution and centrifugation procedures, nucleic acids are extracted from the resulting concentrate. Alternatively, when relatively small (47-mm) polycarbonate membranes are used to filter microorganisms, nucleic acids can be extracted directly without further elution or centrifugation. For readily cultivable organisms such as many bacteria, an enrichment step in appropriate growth media (either selective or nonselective) can greatly increase detection sensitivity.

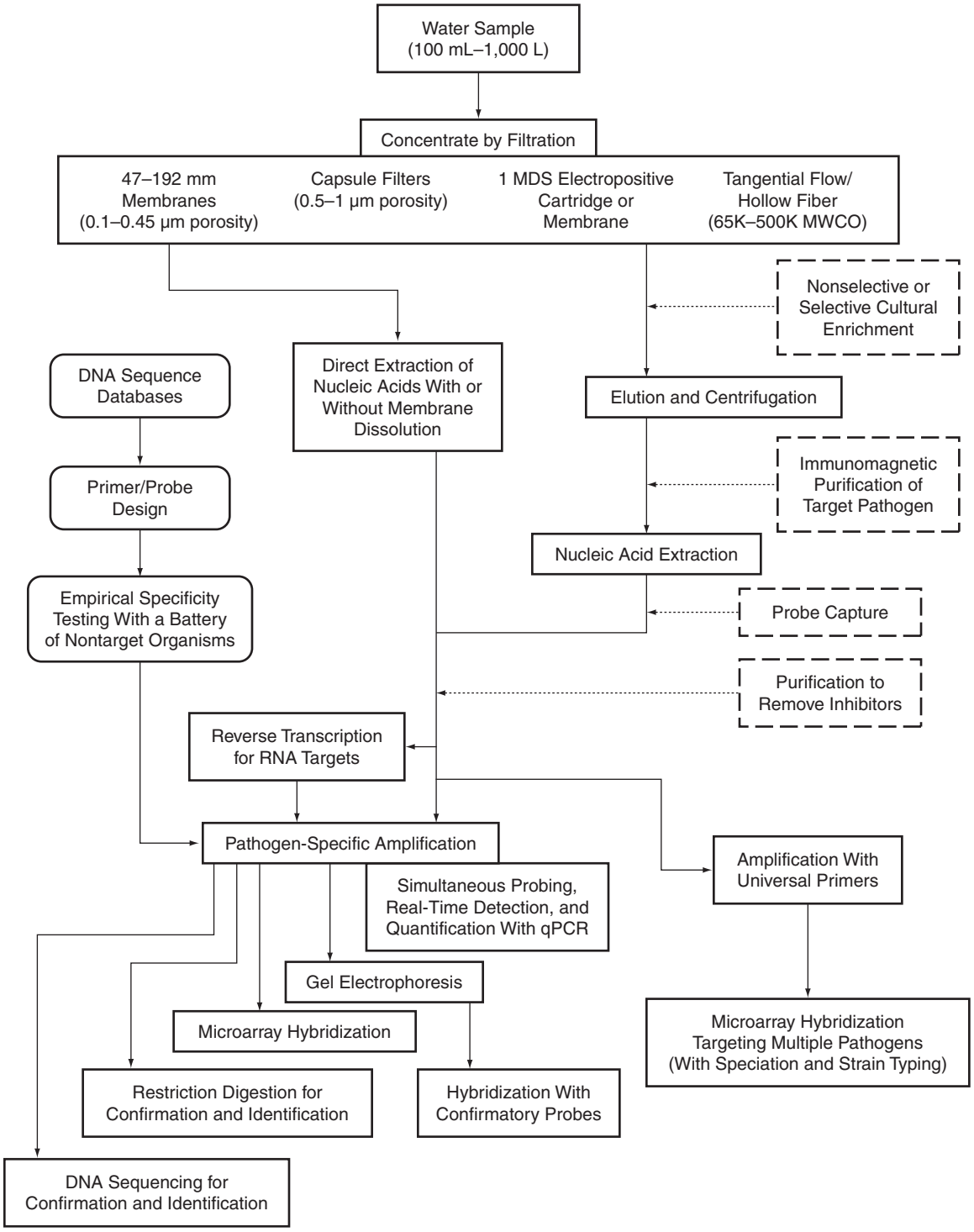
Sample inhibition resulting in false negatives or partial inhibition leading to inaccurate estimates of the number of microorganisms can be especially problematic when analyzing nucleic acids extracted from environmental concentrates. Current studies are attempting to identify and evaluate sample preparation techniques that are high-throughput and cost-effective, efficient at reducing inhibition, require

Table 4-1 Examples of molecular assays for detection of pathogens and indicators in aquatic sample

Organism	Assay Method	Matrix	Reference
Adenoviruses	PCR	River water and urban sewage	Pina et al. 1998
Bacteroidetes	PCR and qPCR	Coastal water	Bernhard and Field 2000
Caliciviruses	RT-PCR	Source and treated drinking water	Huang et al. 2000
Campylobacter spp.	PCR-ELISA*	River water and sewage	Sails et al. 2002
	PCR and FISH	Surface waters	Moreno et al. 2003
Candida spp.	qPCR	Spiked tap water	Brinkman et al. 2003
Cryptosporidium spp.	Cell culture/PCR	Surface water	LeChevallier et al. 2003
	PCR and fingerprinting	Storm water	Xiao et al. 2000
Cyanobacteria	qPCR	Surface water	Foulds et al. 2002
Cyclospora cayetanensis	PCR-RFLP	Spiked surface water concentrate	Shields and Olson 2003
Escherichia coli O157:H7	qPCR	Artificial wetlands	Ibekwe et al. 2002
Giardia lamblia	PCR	Wastewater	Mayer and Palmer 1996
Hepatitis A virus	NASBA	Spiked wastewater	Jean et al. 2001
	RT-PCR/molecular beacon	Spiked groundwater	Abd El Galil et al. 2004
	RT-PCR	Groundwater	Abbaszadegan et al. 1999
Legionella pneumophila	qPCR	Hospital water systems	Wellinghausen et al. 2001
Microsporidia	PCR/sequencing	Surface and groundwater	Dowd et al. 1998
Mycobacterium avium	NASBA/molecular beacon	Spiked drinking water	Rodriguez-Lazaro et al. 2004
Naegleria fowleri	Nested-PCR/sequencing	Drinking water	Marciano-Cabral et al. 2003
Noroviruses	RT-PCR	River water	Lodder and de Roda Husman 2005
Pseudomonas aeruginosa	PCR/probe capture	Drinking water	Frahm et al. 2001
Reoviruses	Cell culture/RT-PCR	Surface waters	Spinner and Di Giovanni 2001
Rotavirus	RT-PCR	Groundwater	Abbaszadegan et al. 1999
Salmonella spp.	Enrichment-PCR	Surface waters	Yanko et al. 2004
Toxoplasma gondii	PCR	Surface waters	Villena et al. 2004
Vibrio spp.	Multiplex PCR-microarray	Shellfish	Panicker et al. 2004
Vibrio parahemolyticus	PCR	Spiked coastal water	Myers et al. 2003
Yersinia enterocolitica	Nested PCR	Spiked environmental waters	Waage et al. 1999

*Enzyme-linked immunosorbent assay

minimum training for use, and are reproducible from user to user. The ideal extraction method should yield relatively intact deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), with very little loss of material from a wide range of environmental water types, and minimize or eliminate coextraction of assay inhibitors. In addition, extraction should be rapid to allow pathogen detection assays to move towards real-time monitoring and should preferably avoid the use of toxic solvents. Specific purification of target organisms by immunomagnetic separation (also referred to as immunocapture) from environmental concentrates prior to DNA extraction has been demonstrated to circumvent inhibition and allow sensitive detection by PCR. Recirculating



Source: Paul A. Rochelle, Metropolitan Water District of Southern California.

Figure 4-1 A generalized approach for the application of PCR and other molecular assays to detect pathogens in water. Selection of sample concentration method, the level of sample enhancement or purification (dashed lines), and particular detection assay depend on the required sensitivity, specificity, throughput, laboratory capabilities, time, and cost constraints.

immunocapture systems have also been evaluated as an alternative to filtration and centrifugation. Postextraction purification of nucleic acids to remove inhibitors using one of the many commercially available kits or in-house methods may also be necessary for successful application of molecular assays. One of the greatest challenges in the application of highly sensitive techniques such as PCR to environmental samples is maintaining the integrity and pristine nature of the sample prior to, and during, nucleic acid extraction. Consequently, although dedicated separate facilities are not required for the application of molecular assays to environmental samples, a high degree of laboratory organization, coordination of workflow, spatial and temporal separation of functions within the laboratory, and rigorous quality assurance and control (QA/QC) procedures are necessary to minimize the risks of contamination. Reference to the USEPA guidance manual on QA/QC procedures for the application of PCR to environmental samples is recommended (USEPA 2004).

DETECTION METHODS

Once extracted, specific pathogen nucleic acids can be detected using a myriad of molecular assays (Figure 4-1). Numerous molecular methods have been developed for detecting microorganisms in water. These include PCR and its derivatives reverse transcriptase (RT)-PCR and quantitative qPCR, nucleic acid hybridization in membrane and microarray formats, and fluorescent in-situ hybridization (FISH). Additional techniques such as nucleic acid sequencing, repetitive (rep)-PCR, and restriction enzyme digestion are used for confirmatory identification, speciation, and subtyping following detection. While most techniques are designed to target specific pathogens or indicator organisms, others such as universally primed PCR and denaturing gradient gel electrophoresis can be used to identify unknown organisms in environmental samples. General advantages and limitations of molecular assays are summarized in Table 4-2.

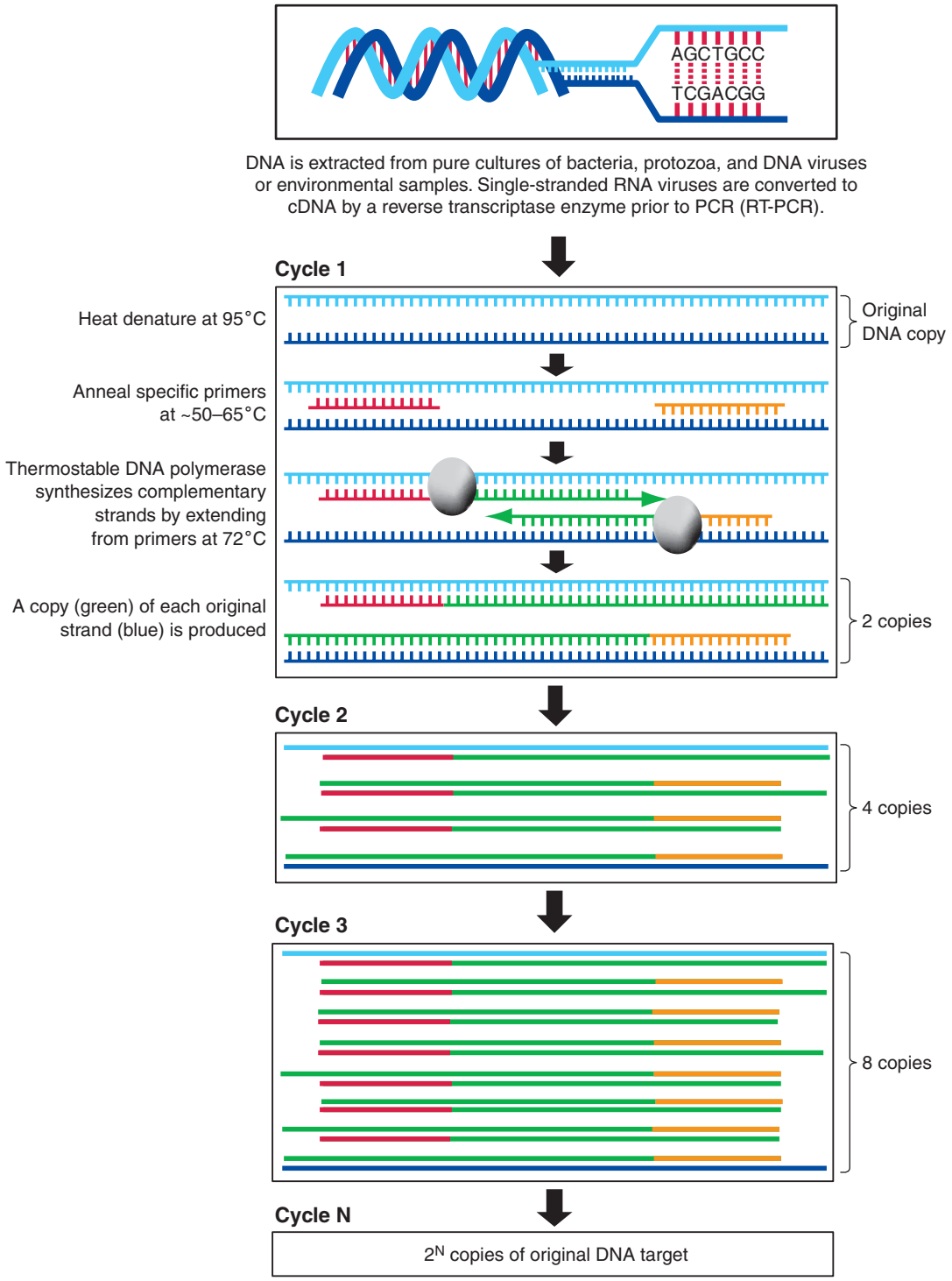
In-vitro nucleic acid amplification techniques offer the most promise for pathogen detection in water because of their potential for extreme sensitivity and specificity. Of the amplification techniques, PCR is by far the most predominant technique in use. Other amplification methods that have been applied in clinical diagnostics include strand displacement amplification (SDA), nucleic acid sequence–based amplification (NASBA), loop-mediated isothermal amplification (LAMP), ligation-mediated amplification (LMA), and rolling circle amplification. Conventional PCR assays utilize three cyclic temperature steps to amplify specific sequences of DNA: (1) denaturation of double-stranded DNA into single-stranded DNA, at an elevated temperature (typically 94°C); (2) annealing of pathogen-specific primers to the single DNA strands at a temperature dictated by the sequence of the primer-target hybrids (usually 50–65°C); and (3) template-directed extension of the single-stranded primers by a thermostable DNA polymerase at 72°C to yield double-stranded DNA (Figure 4-2). Two-step protocols have also been developed that combine annealing and extension into a single process at 68–72°C. Isothermal amplification methods such as NASBA operate at a single temperature. Because each round of temperature cycling during PCR doubles the amount of DNA, billions of copies of the specified sequence can theoretically be made from a single target and, therefore, be easily detected. Acquiring a thermal cycler (microprocessor-controlled automated heating block) is typically the largest expense when introducing PCR into a traditional microbiology laboratory (Figure 4-3). The capacity of commercially available thermal cyclers ranges from 24 to 384 simultaneous reactions, which can be further increased with slave units operating from a single controller.

With appropriate selection of PCR primers and optimization of PCR conditions, the amplified DNA sequence can be unique to the level of genus, species, or strain of organism. Therefore, PCR can have very high specificity and sensitivity for nucleic

Table 4-2 Characteristics of molecular methods

Criteria	Strengths	Limitations
Sensitivity	Potentially extremely sensitive. Under ideal conditions a single cell or virus particle can be detected by amplification methods.	Sample matrix effects, presence of inhibitors, inefficient nucleic acid extraction methods can reduce sensitivity by orders of magnitude.
Specificity	Because most methods rely on nucleic acid sequences as primers and/or probes, assays can be designed to be highly specific, depending on the availability of nucleic acid sequence information.	Availability of sequence information may indicate unjustified specificity. Often difficult to empirically test an appropriately broad suite of nontarget organisms to evaluate specificity.
Multiplexing	Multiple genes can be targeted for a specific pathogen, increasing the confidence in positive results. Also, multiple pathogens can be targeted in a single assay, thus reducing processing time and costs.	The presence of many primers/probes in complex assay mixtures can lead to interference, nonspecific amplification, and amplification artifacts.
Throughput	Many samples can be processed simultaneously. Instrumentation is available for automated extraction of multiple samples, and thermal cyclers can process up to 384 samples at one time.	Processing many samples at one time increases the potential of cross-sample contamination.
Analysis speed	Because many samples can be processed simultaneously, the analysis time per sample is greatly reduced. Also, rapid cycle technologies reduce cycling times from hours to minutes.	Molecular detection technologies can be very rapid (<15 minutes), but sample concentration and processing and assay setup can require substantially more time.
Enumeration	Most methods are amenable to some form of quantification.	Direct enumeration is not possible. Although relative quantification is well established, absolute quantification methods are somewhat complex and still need to be validated.
Cost	Instrumentation is generally no more expensive than many of the pieces of equipment in water quality laboratories.	Compared to traditional microbiological techniques (e.g., agar plates), there is a considerable initial cost in setting up a facility, and some essential reagents are expensive.

acid detection. Publicly accessible nucleic acid sequence databases and readily available primer design software allow in-house primer and probe design, but many-pathogen-specific primers are commercially available. However, rigorous evaluation of the specificity and sensitivity of all primers is necessary if end users are going to be confident of their results. Multiplex PCR assays have been developed that can detect up to nine different pathogens in a single reaction tube. A wide variety of thermostable DNA polymerase enzymes are available, and selection of the most suitable enzymes depends on the purpose of the assay, the size of the amplified fragment, and the assay conditions. Some enzymes have proofreading ability that translates into a low error rate while others have greater efficiency in amplification of long (>1 kb) DNA fragments. Enzymes have also been developed to allow automated hot start reactions that minimize the likelihood of contamination.



Source: Paul A. Rochelle, Metropolitan Water District of Southern California.

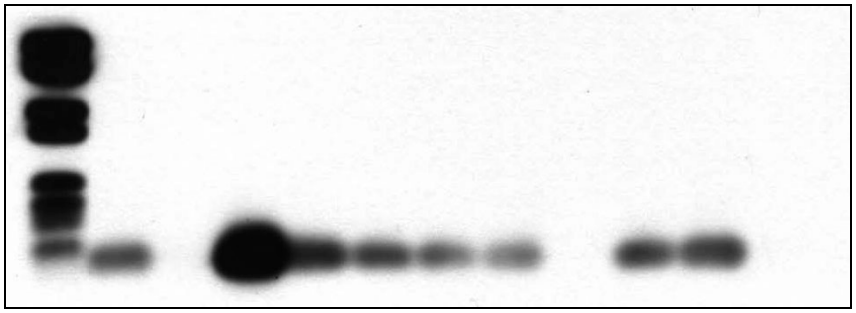
Figure 4-2 Amplification of DNA by PCR. Reactions are typically performed for 30–50 cycles, and each cycle takes 0.5–5 minutes, depending on the type of thermal cycler and size of amplification target.

Source: Paul A. Rochelle, Metropolitan Water District of Southern California.

Figure 4-3 Thermal cyclers used to automate the temperature cycling steps of polymerase chain reactions

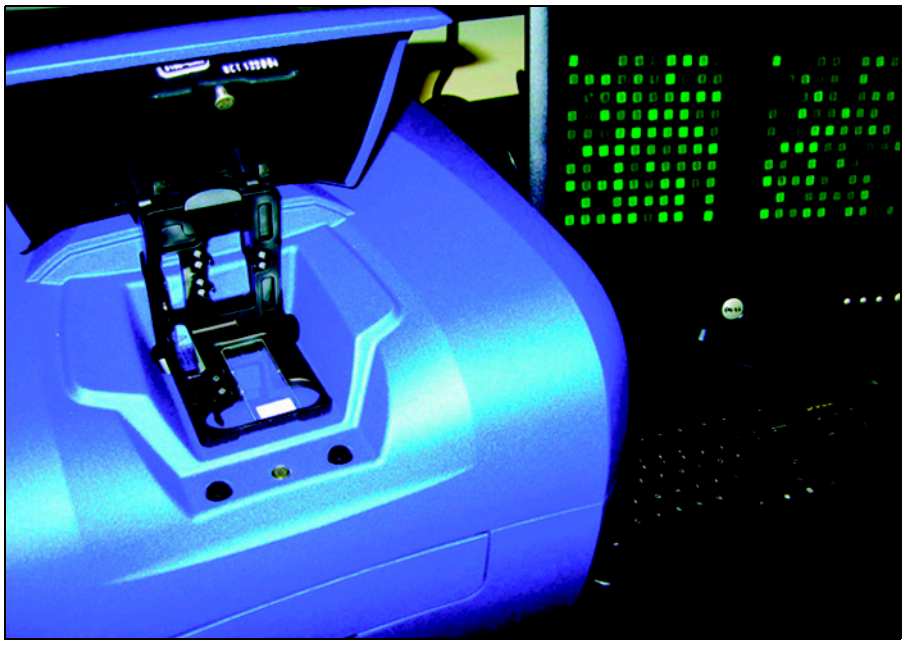
Although PCR can greatly improve specificity and sensitivity of nucleic acid detection, samples and reactions must be handled with care to prevent contamination or inhibition. In addition to strict QC procedures, many researchers have incorporated the use of uracil-N-glycosylase (UNG), internal standard DNA, and hot start methods to address these issues. UNG is an enzyme that degrades uracil-containing PCR products prior to amplification. Contaminating PCR products from previous laboratory experiments, during which deoxythymidine triphosphate (dTTP) was replaced with deoxyuridine triphosphate (dUTP) during amplification, are thereby degraded and are no longer potential sources of contamination. Hot start PCR methods reduce background and increase specific yield by preventing nonspecific primer annealing/extension during PCR setup. Inhibitory compounds such as humic acids may completely prevent amplification or change the reaction kinetics or efficiency. The addition of a known amount of internal standard DNA to the PCR master mix allows identification of decreased PCR signal due to the presence of inhibitors. Thus, the probability of reporting false-negative samples due to complete or partial inhibition of the PCR is greatly reduced.

Following amplification, reaction products are analyzed by gel electrophoresis followed by probe hybridization (Figure 4-4) or they can be applied to microarrays. Microarrays are small glass slides containing many oligonucleotide probes; low-density arrays typically contain <200 probes, whereas high-density arrays may contain >10,000 probes (Figure 4-5). Such arrays may carry probes for multiple pathogens, multiple gene targets for each pathogen (to increase detection confidence), polymorphism-specific probes for speciation and genotyping, or virulence-related genes to determine the disease-causing potential of detected pathogens. While their application to the detection of pathogens in environmental samples is still in the early stages of development (Wang et al. 2004), they have the potential to greatly increase the interrogative capabilities of molecular detection assays.



Source: Paul A. Rochelle, Metropolitan Water District of Southern California.

Figure 4-4 Results of amplification reactions are usually visualized by agarose gel electrophoresis followed by hybridization with specific oligonucleotide probes

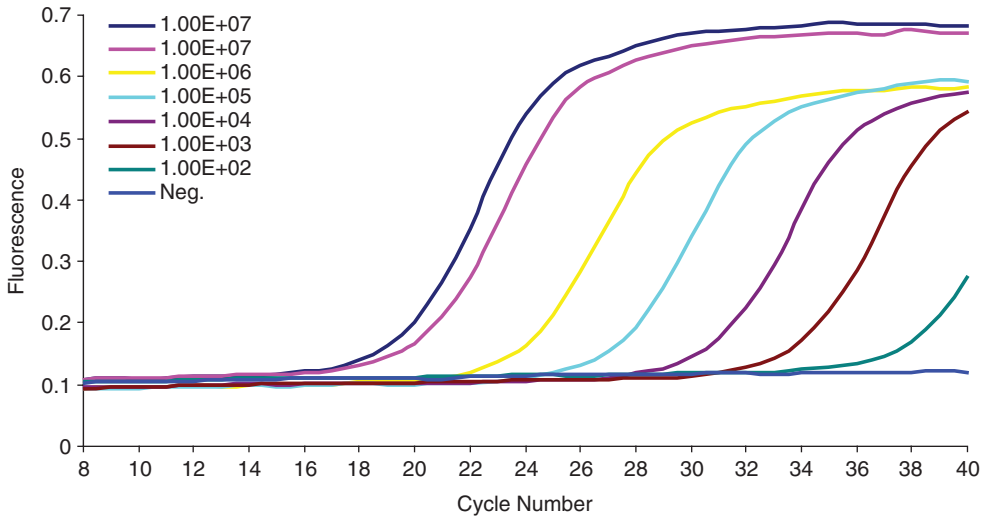


Source: Paul A. Rochelle, Metropolitan Water District of Southern California.

Figure 4-5 Microarray readers record the results of slide-bound hybridization assays. Microarrays contain hundreds to many thousands of individual probes, and the results are displayed as an array of fluorescence signals.

QUANTIFICATION

One of the limitations of the application of molecular techniques to environmental samples is that the results obtained are frequently qualitative rather than quantitative. While it is useful to know what is in a sample, it is far more informative if the relative abundance of different organisms can be determined and also if the absolute density of an organism in a particular sample can be calculated. One of the reasons that PCR has not progressed further as a diagnostic tool for pathogen detection in environmental samples is that the basic approach is not quantitative. For example,



Source: Paul A. Rochelle, Metropolitan Water District of Southern California.

Figure 4-6 Quantitative PCR formats allow the generation of amplification products to be monitored in real time

the conventional microbial detection methods that are often compared to PCR (e.g., plate counts and microscopy), while having many other limitations, are quantitative; they provide the analyst with a number of cells, or colony-forming units, or plaque-forming units. Therefore, developing PCR into a more quantitative technique is an important goal for many researchers.

Recent advances in PCR technology, such as TaqMan methods that use fluorogenic probes and specialized detectors, allow for quantitative analysis. In qPCR the formation of the PCR amplification curve is monitored after each amplification cycle and the amount of amplicon present is determined during the early exponential phase of DNA amplification when reaction components are not rate-limiting and variability is low, providing a reproducible and quantitative assay of nucleic acid. Quantitative PCR techniques include TaqMan methods, Scorpion probes, fluorescence resonance energy transfer (FRET) techniques, and molecular beacons, all of which involve incorporation of fluorogenic probes into PCR assays and allow real-time monitoring of amplification results (Figure 4-6). These qPCR methods do not allow real-time monitoring of microorganisms in water samples since sample processing (concentration and nucleic acid extraction) is still required prior to the detection assay. Apart from the quantification aspects, many of the qPCR methods incorporate probes into the assay, which increases the overall specificity of the reaction.

VIABILITY AND INFECTIVITY

Another potential limitation of molecular detection methods is that they generally produce presence–absence results but do not provide information on the viability of detected microorganisms. Thus interpreting the significance to human health of a molecular assay positive result is difficult. Most molecular techniques detect a small fragment of nucleic acid, a single protein, or identify a specific receptor. A positive signal does not provide any information on the infectious nature of the identified microorganism. Detection of specific DNA sequences does not indicate whether or not an organism is viable or infectious because DNA does not degrade rapidly once a cell

dies. Also, free DNA, released upon cell lysis, can be readily detected in aquatic samples. The detection of RNA, particularly messenger RNA (mRNA), by either RT-PCR or FISH has been proposed as a measure of viability (e.g., for *Cryptosporidium* in water), based on the assumption that only active cells will contain RNA; RNA, and mRNA in particular, is far more labile than DNA and degrades rapidly once a cell dies. However, there are substantial variations in the decay rates of different RNA molecules and of RNA from different organisms. Also, extraction of amplification-quality RNA from environmental samples is more difficult than for DNA. Consequently, these methods have not been widely adopted for direct assessment of viability. However, molecular methods have been coupled with in-vitro cell culture to provide sensitive and specific detection of infectious pathogens. These applications allow an assessment of the infectivity of pathogens in water, provide methods for evaluating the efficacy of disinfectants and water treatment strategies, and have demonstrated equivalency with animal infectivity models for some pathogens.

ALTERNATIVE METHODS

Another approach to molecular detection of waterborne pathogens is based on the use of ligand-binding assays to recover and detect target microbes. Many microbes possess specific surface receptor molecules or epitopes that bind to specific molecular targets and have various functions, such as cell attachment, transport of molecules for nutrition, or molecular processing for immune response or other biological activities. As these molecules and their corresponding targets are elucidated, the molecules to which these epitopes bind can be used for microbe capture and detection—analogous to the use of antibodies for such purposes. Because some microbe surface receptors are used for initiation of infection in host cells, the ability of the microbes to bind to their specific target ligand can be used to detect intact, chemically functional, and potentially infectious or viable microorganisms. Such assays for viability based on the ability to bind to specific receptors are now under development for waterborne microorganisms and are likely to be developed further.

Other chemical and biochemical constituent analysis also can be used to ascertain the presence of viable organisms (e.g., adenosine triphosphate [ATP] detection with luminescence detection), the presence of specific toxins, or the organism's protein profile. An important new area for pathogen detection is mass spectrometry. In this approach, samples, including whole viruses, protozoa, or bacteria, are introduced into the mass spectrometer and ionized, and the fragmentation pattern is detected. The ability to detect macromolecules is enabled by developments in sample introduction through ionization including matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI).

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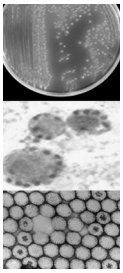


II

Introduction to Bacterial Pathogenic Agents

Acinetobacter
Aeromonas
Campylobacter
Cyanobacteria
Enterohemorrhagic *Escherichia coli*
Escherichia coli
Flavobacterium
Helicobacter pylori
Klebsiella
Legionella
Mycobacterium avium complex
Pseudomonas
Salmonella
Serratia
Shigella
Staphylococcus
Vibrio cholerae
Yersinia

Bacteria are unicellular microorganisms that are simpler and smaller than parasitic pathogens such as *Giardia* and *Cryptosporidium* but larger and more complex than viruses. The US Environmental Protection Agency compiles a list of bacterial agents that are known or anticipated to contaminate public water systems. Although the factors in establishing this list are numerous, considerations include that the organism can invade the human body and produce a toxic reaction in tissues or organs. The candidate may be the cause of waterborne outbreaks, defined as two or more individuals sickened by a common agent using water as the vector. Two general classifications of bacterial pathogenic agents include Traditional and New or Emerging.

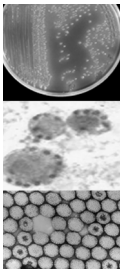
TRADITIONAL BACTERIAL PATHOGENS

Traditional bacterial pathogens are those responsible for persistent waterborne outbreaks. Some traditional pathogens such as *Vibrio* or *Salmonella* are particularly virulent and responsible for catastrophic losses of life during historical outbreaks of cholera or typhoid fever, respectively. Other traditional pathogens are less virulent and characterized as "opportunistic." This group of bacteria should not pose a risk to healthy adult humans, but individuals with weakened immune systems (i.e., infants, elderly, AIDS victims) are susceptible. *Pseudomonas aeruginosa*, generally recognized

as a classic opportunistic pathogen, is not known to infect healthy tissue. However, any type of human tissue (intestinal, respiratory, joint, dermal, etc.) compromised by illness and/or injury, is vulnerable to *Pseudomonas* infection.

NEW OR EMERGING PATHOGENS

Emerging pathogenic bacteria are those relatively recently identified as new species. Recent identification may be the result of improved detection methodologies or the discovery of truly new strains that appear because of environmental pressures or spontaneous mutations. For example, prior to the early 1980s, gastroenteritis was attributed to stress and/or spicy cuisine and treated using psychotherapy or diet modification. Since *Helicobacter pylori* was identified as the causative agent for gastroenteritis, this affliction is effectively treated with antibiotics. Similarly, in 1885 *E. coli* was established as a generally harmless strain of human enteric bacteria. However, since the emergence of *E. coli* subspecies 0157:H7 in the late 1970s, serious outbreaks worldwide are now attributed to this genus. It is probable that new pathogenic strains will continue to emerge as detection methodologies become more sophisticated and/or existing bacterial species mutate to adapt to new environmental conditions.



Chapter 5

Acinetobacter

Mic Stewart
Revised by Paul A. Rochelle

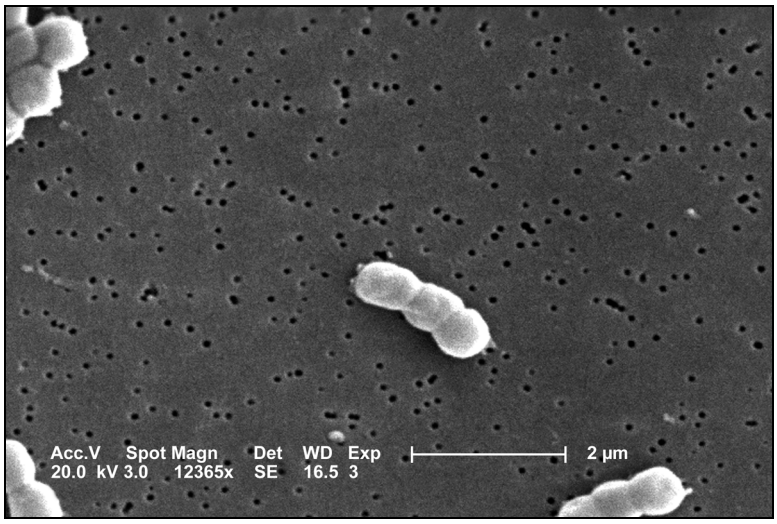
DESCRIPTION OF THE AGENT

Bacteria of the genus *Acinetobacter* have undergone many taxonomic classifications under at least 15 different generic names. Members of this genus are classified in the family Moraxellaceae, which also includes the genera *Moraxella*, *Psychrobacter*, and other related genera. The genus comprises at least 23 genomic species based on DNA-DNA hybridization patterns, 17 of which have been given species names. The most important clinical species are included in the *A. calcoaceticus*, *A. baumannii* complex (Figures 5-1 and 5-2). Other species include *A. haemolyticus, A. johnsonii, A. junii, A. lwoffii,* and *A. radioresistens*.

Acinetobacters are rod-shaped, 0.9 to 1.6 µm in diameter and 1.5 to 2.5 µm in length but become more spherical in shape during stationary growth. These organisms are aerobic, do not form spores, and are gram-negative but may be gram-variable in pure culture. Cells exhibit "twitching motility" due to the presence of polar fimbriae. Many strains are encapsulated and can demonstrate considerable aggregation depending on growth conditions. Strains grow between 20° and 37°C with a temperature optima of 33° to 35°C. Acinetobacters are catalase positive, oxidase negative, and nonfermentative. Most strains grow in defined media containing a single carbon and energy source.

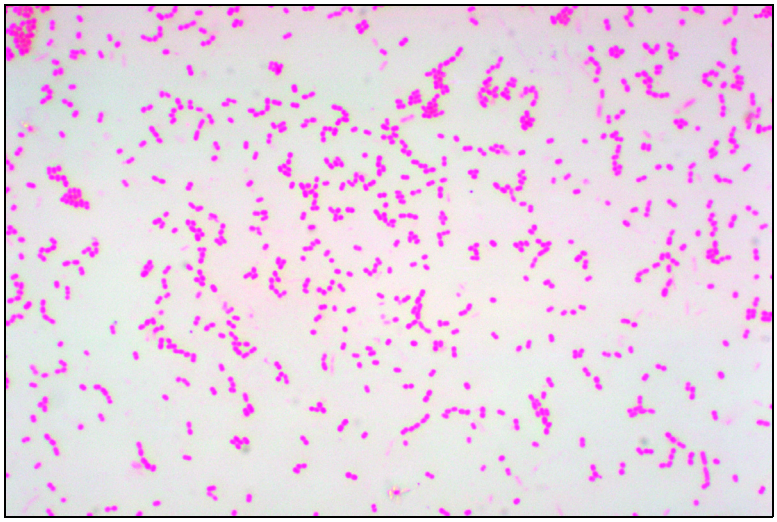
DESCRIPTION OF THE DISEASE

Acinetobacters, generally considered to be of low virulence, have been increasingly associated with nosocomial (hospital-acquired) infections, including septicemia, meningitis, endocarditis, brain abscesses, lung abscesses, pneumonia, empyema, urinary tract infections, eye infections, and skin and wound infections. Factors enhancing the virulence of acinetobacters include (1) presence of a capsule, (2) adhesion to human epithelial cells due to fimbriae, capsular polysaccharide, or both, (3) production of enzymes that may damage tissue lipids, and (4) production of an endotoxin. Antibiotic treatment for *Acinetobacter* infections includes amikacin, carbapenem, imipenem, ceftazidime, pefloxacin, ciprofloxacin or colistin, although resistant strains of *A. baumannii* in particular are becoming increasingly problematic to treat. Some clinical isolates are resistant to all of the commonly available antibiotics.



Source: M.J. Ardvino, J. Carr, J. Swenson, Centers for Disease Control and Prevention.

Figure 5-1 Scanning electron micrograph of gram-negative, nonmotile *Acinetobacter baumannii* bacterium



Source: Dr. W.A. Clark, Centers for Disease Control and Prevention.

Figure 5-2 Gram-negative bacterium *Acinetobacter calcoaceticus* using gram-stain technique

RESERVOIRS FOR THE AGENT

Acinetobacters can be isolated from soil, seawater, fresh water, estuaries, sewage, contaminated food, and mucosal and outer surfaces of animals (including fish) and humans. Acinetobacters may be part of the normal bacterial flora in more than 25 percent of healthy humans and have been isolated from moist areas of the body, including the axillae, groin, oral cavity, respiratory tract, between the toes, and, to a lesser extent, the digestive tract. *Acinetobacter* spp. have been found in 27 percent of hospital sink traps, 20 percent of hospital floor swab cultures, and in 12 percent of air samples

in wards containing patients infected by this organism. Studies documenting the prevalence of *Acinetobacter* in soil, surface water, estuaries, thermal springs, and groundwater found 93, 96, 50, 42, and 38 percent, respectively, of the samples tested from these environments contained this organism.

MODE OF TRANSMISSION

The ubiquitous nature and environmental persistence of acinetobacters aid in the transmission of these organisms in hospitals. High colonization rates of the skin, throat, respiratory system, and digestive tract of hospital patients together with the occurrence of acinetobacters as part of the normal bacterial flora in humans provide a significant source of this organism. Consequently, transmission of this organism through person-to-person contact or contact with medical instrumentation, equipment, or surfaces throughout the hospital ensures the continual persistence of acinetobacters. Studies documenting acinetobacters in air samples collected in the vicinity of infected patients indicate that aerosolization may also be an important transmission method. Water supply may be another important mode of transmission because these organisms are often part of the profile of heterotrophic bacteria found on granular activated carbon (GAC) and sand filters, in distribution system pipe biofilm, and point-of-use devices. Water baths used to warm up dialysis fluids have been implicated as the source of *Acinetobacter* infection in hospital patients.

METHODS FOR DETECTING THE AGENT

Acinetobacters can be isolated and cultivated on ordinary laboratory media. Organic Medium 79, Mineral Medium with Crude Oil, Peptone Yeast Extract Medium, Trypticase Soy Agar with Glycerol, and Trypticase Phytone Medium may all be used to cultivate the organism. Selective and differential media, such as Sellers Agar, Herella Agar, and MacConkey Agar, may also be used. Eosin-Methylene Blue Agar and mAC agar may be used to differentiate *Acinetobacter* species isolated from potable water environments. Optimum growth temperatures for most strains is 33° to 35°C; however, growth at lower temperatures also occurs. Acinetobacters from soil and water can be enriched by inoculation of 20 mL of an acetate–mineral medium with 5 mL of a water sample or a filtered 10 percent soil suspension followed by vigorous aeration at 30°C or at room temperature. Acinetobacters favor a slightly acidic (pH 5.5 to 6.0) growth environment. Biotyping, serotyping, phage typing, bacteriocin typing, protein profiles, multilocus enzyme electrophoretic typing, plasmid profiles, pulsed-field gel electrophoresis, ribotyping, and other molecular-based methods help discriminate among acinetobacters.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

The occurrence of *Acinetobacter* spp. in contaminated food, as commensal flora of humans and animals and in the aquatic environments provides evidence of the ubiquitous and adaptive nature of these organisms. In studies involving groundwater, *Acinetobacter* was directly detected in 38 percent of samples, and comprised 54 percent of the heterotrophic bacteria population in another. In a study of a chlorination distribution system, *Acinetobacter* was found to be the most commonly isolated organism, comprising 5.5 percent of the total organisms identified. During a chlorine failure of the same system, *Acinetobacter* comprised the second highest portion of heterotrophic bacteria. Studies have indicated that 3 to 24 percent of pneumonia patients using mechanical ventilation devices were infected with at least one *Acinetobacter* sp., which suggests this organism is an emerging pathogen under these conditions.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Although studies on the survival of *Acinetobacter* in water are scarce, there exists data involving survivability in other environments. For example, an *Acinetobacter* spp. was shown to survive for up to 6 days after inoculation on dry filter paper, which is a harsh environment for any bacterial strain. Such survivability is comparable to *Staphylococcus aureus*, but considerably longer than *Escherichia coli* and *Pseudomonas* spp. Survival on hospital floors and washcloths has also been documented. Studies of *Acinetobacter*-infected patients indicate that this organism could be recovered from articles contained in the room for up to 13 days after discharge of the patient.

DOCUMENTED WATERBORNE OUTBREAKS

Acinetobacters have been associated with outbreaks in hospital settings but not in treated public drinking water supplies.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Conventional treatment by coagulation and filtration removes between 76 and 99 percent (0.6 to 2 logs) and 50 and 99.5 percent (0.3 to 2.3 logs), respectively, of bacteria. Disinfection results during the treatment process can vary appreciably depending on the disinfectant used and the relative resistance of the organism; however, typical removals range from 99 to 99.99 percent (2 to 4 logs). The use of a disinfectant residual in the distribution system provides additional microbial inactivation. Naturally occurring *Acinetobacter* spp. were observed to have inactivation rates similar to other heterotrophic bacteria, such as *Moraxella*, *Aeromonas*, *Pseudomonas*, and *Alcaligenes*, when exposed to chloramines. Other studies, however, have indicated that acinetobacters can develop increased resistance to chlorine, chloramines, and chlorine dioxide when grown under conditions favoring cell aggregation.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

No regulatory guidelines for acinetobacters in drinking water have been established in the United States. Treatment of drinking water and effective distribution maintenance programs are important in controlling acinetobacters in drinking water. Control of antibiotic usage to minimize development of resistant strains and good housekeeping practices, including equipment decontamination, strict attention to hand washing, and effective isolation procedures of infected patients, are important control factors for acinetobacters in hospitals.

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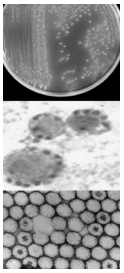


Chapter 6

Aeromonas

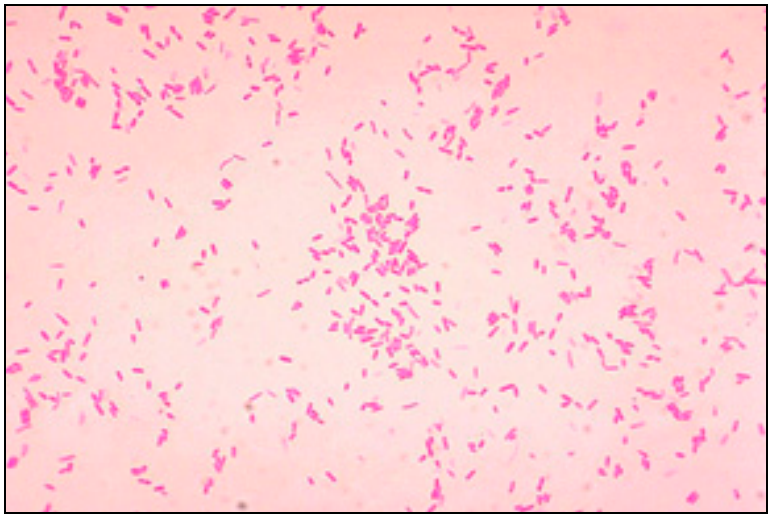
Nelson P. Moyer
Revised by Jon Standridge

DESCRIPTION OF THE AGENT

The genus *Aeromonas* of the family Aeromonadaceae consists of 19 recognized DNA hybridization groups, comprising 17 genospecies and 14 phenospecies (3 genospecies remain unnamed). Aeromonads are facultatively anaerobic, chemoorganotrophic, nonsporulating, oxidase-positive, gram-negative rods or coccobacilli, 0.3 to 1.0 µm in diameter and 1.0 to 3.5 µm in length, motile by a monotrichous polar flagellum, and appearing singly or in short chains. The psychrophilic aeromonads pathogenic for fish are nonmotile. The mesophilic aeromonads important to environmental microbiologists and the water industry are *A. hydrophila*, *A. sobria*, and *A. caviae*. These grow in a temperature range between 15 and 38°C on a variety of eugonic media (Figure 6-1). When grown at 35°C for 18 hours on sheep blood agar plates, colonies are round, convex, measuring 2 to 3 mm in diameter, creamy to mucoid in consistency, translucent gray in appearance, with or without hemolysin production. They are strongly hydrolytic, producing a number of proteolytic, saccharolytic, hemolytic, and lipolytic extracellular enzymes. They are differentiated from similar oxidase-positive bacteria, such as *Plesiomonas*, by gelatin hydrolysis and failure to ferment inositol, and from the *Vibrio* by their resistance to O/129 (150 µg) and inability to grow in the presence of 6 percent sodium chloride.

DESCRIPTION OF THE DISEASE

Aeromonads are pathogenic for fish and aquatic animals, and they represent an economic threat to the aquaculture industry. Human disease is characterized by a broad range of pathology, from self-limiting gastrointestinal illness to septicemia and death. Soft-tissue infections occur within hours following penetrating injuries in aquatic environments or wound contamination with surface water. Local suppurative infections are characterized by pain, swelling, erythema, and edema. Fever accompanies septicemia and disseminated infections. Complications of soft-tissue infections include cellulitis, necrotizing fasciitis, myositis, sepsis, meningitis, pneumonia, peritonitis, osteomyelitis, and endocarditis, among others. Gastrointestinal disease develops within a few days following ingestion of contaminated food or water and may be an acute, cholera-like illness characterized by profuse watery diarrhea or a less-severe diarrhea, with fever and abdominal cramping.



Source: Dr. W.A. Clark, Centers for Disease Control and Prevention.

Figure 6-1 *Aeromonas hydrophila* gram stain

A chronic form of the disease with multiple soft to loose stools per day, but without marked dehydration, is characteristically associated with children under the age of 5 years, who have a history of therapy with antibiotics to which aeromonads are resistant. Repeated or prolonged antibiotic therapy disrupts normal intestinal flora and provides a competitive environment for aeromonads in which they can opportunistically colonize. Untreated water from wells that contain aeromonads places infants and children at risk of chronic *Aeromonas* gastroenteritis. Adults diagnosed with "irritable bowel" syndrome may be suffering from undiagnosed chronic *Aeromonas* gastroenteritis. Rapid diagnosis by bacterial culture and performance of antimicrobial susceptibility tests is necessary for successful treatment of extraintestinal *Aeromonas* infections. Shock-chlorinating wells and distribution systems may be necessary to suppress aeromonads.

RESERVOIRS FOR THE AGENT

Aeromonads are found in fresh water, estuarine and marine environments, sediments, sludge, and sewage. They have been recovered from chlorinated and nonchlorinated drinking water supplies worldwide, where they contribute to regrowth and biofilm problems in distribution systems. They are readily isolated from surface waters, flocs, and sediments in nature, and from the surfaces and intestinal contents of aquatic animals. Aeromonads have been cultured from domestic and hospital water systems, bottled drinking water, renal dialysis units, and home aquaria. Their presence in groundwater indicates surface water or aquatic animal contamination. Aeromonads have been isolated from produce, meats, and shellfish. Water may be the major source of contamination of foods.

MODE OF TRANSMISSION

Soft-tissue infections occur following traumatic exposure to contaminated water, as do wound and eye infections. Persons with underlying disease or immunosuppression are at increased risk of dissemination following infection. Gastrointestinal infections result from ingestion of contaminated food or water. Aeromonads have been reported

as a cause of travelers' diarrhea. Person-to-person transmission occurs through the fecal–oral route, typically from substandard personal hygiene or diapering infected infants. Aeromonads enter water supplies from incomplete treatment, new construction, and maintenance and repair activities.

METHODS FOR DETECTING THE AGENT

Direct plating methods are recommended for culturing aeromonads from polymicrobic sources, such as surface water, sewage, sediments, and sludge, when they are present in sufficient number (≥1 colony-forming unit [cfu]/mL). Spread plates or pour plates of diluted samples are required to isolate aeromonads present in high numbers. Membrane filtration and multiple-tube fermentation (MTF) techniques have been used to enumerate aeromonads with differential and selective media. Recovery of aeromonads from groundwater or treated drinking water may require resuscitation or enrichment when present in low numbers (≤10 cfu/100 mL). Alkaline peptone water, pH 8.6, may be used directly, with Moore swabs or Spira bottles, or with membrane filtration for enrichment of aeromonads at very low levels or when aeromonads are heavily outnumbered in a sample by competitive bacteria. Aeromonads are presumptively identified by their colony morphology and hemolytic reaction on blood agar plates, the oxidase test, and growth characteristics in either TSI/LIA/MIO (see glossary) or Kaper's multi-test medium. Complete identification of phenospecies depends on biochemical tests. Molecular methods including a rapid multiplex polymerase chain reaction (m-PCR) method that allows for the rapid detection of *Aeromonas* have been developed, but are not extensively used.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Aeromonads grow to high numbers in surface waters containing organic material (total organic carbon [TOC], assimilable organic carbon [AOC], biochemical oxygen demand [BOD], or biodegradable organic matter [BDOM]), and they have been proposed as indicators of water pollution and the trophic state of water. Populations of aeromonads exhibit marked seasonal variation, with the highest counts occurring in the summer. The incidence of gastroenteritis cases in humans parallels the seasonal peak in natural *Aeromonas* populations. *A. caviae* is the predominant species in fecally contaminated waters. While aeromonads have been isolated from up to 27 percent of chlorinated drinking water supplies, their numbers do not correlate with the presence of coliform bacteria, which suggests regrowth or biofilm colonization in the distribution system. The percentage of humans found to have aeromonads in their intestines and show no symptoms of disease ranges from a low of 0.1 percent in developed countries to a high of 30 to 50 percent in countries without effective water and sewage sanitation. Swine, sheep, cattle, and poultry harbor aeromonads in their intestinal tracts at low levels without evidence of disease.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Aeromonads survive in the environment at temperatures between 2 and 42°C, and association with sediments and aquatic animals ensures their survival at ambient temperature extremes. They tolerate a range between pH 5.2 and 9.8. Population densities in sewage sludge (>10^8 cfu/mL), wastewater (10^2–10^7 cfu/mL), rivers (10–10^4 cfu/mL), and lakes and reservoirs (1–10^2 cfu/mL) illustrate the ubiquitous nature of aeromonads in the environment. In one study, groundwater isolates of *Aeromonas* from wells have been shown to be genetically unrelated to *Aeromonas* isolated from patients with gastrointestinal illness.

DOCUMENTED WATERBORNE OUTBREAKS

Recently, aeromonads have been recognized as the cause of waterborne and foodborne outbreaks of disease; however, no outbreaks of *Aeromonas* gastroenteritis have been documented in association with treated public drinking water supplies. Counts of aeromonads in contaminated well water associated with cases of gastroenteritis range between 0.7 and 460 cfu/mL. The potential for outbreaks is likely to occur only in susceptible individuals (e.g., infants, children on ampicillin, persons with underlying disease, or the immunosuppressed).

Because significant contamination is unlikely in a municipal system, the probability of a large identifiable outbreak is remote. Recognition of increased numbers of sporadic cases in a community is equally unlikely, because sporadic cases are below the detection threshold of current epidemiological surveillance systems. *Aeromonas* gastroenteritis is not a reportable disease in most states, and clinical laboratories rarely include a search for aeromonads in their routine stool culture procedures, except by physician request. Colonization of treatment plants and distribution systems has been documented, and anecdotal information suggests that susceptible persons may be at risk of developing *Aeromonas* gastroenteritis as the result of continuous exposure to low levels of aeromonads in treated drinking water.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Chlorination is sufficient to control aeromonads in public drinking water supplies with properly maintained distribution systems, provided the free chlorine residual remains above 0.5 mg/L at the distal ends of the system. Free chlorine residuals below 0.5 mg/L are associated with an increased number of positive water samples, and growth in filter beds, softeners, and flocculation and sedimentation basins. Studies also suggest that the breakthrough of *A. hydrophila* in filtration beds may occur before turbidity from the filtration beds begins to rise. Aeromonads colonize pipes and plumbing fixtures at the distal ends of distribution systems in biofilms. Granular activated carbon (GAC) sequesters nutrients that promote growth of aeromonads. Regular removal of sludge from sedimentation basins, and proper chlorination, filter maintenance, backwashing procedures, and hydrant flushing are required to control *Aeromonas* colonization of public water supplies.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

While no regulatory standards for the permissible number of aeromonads in drinking water have been established in the United States, operators can assume that their systems are functioning properly when the heterotrophic plate counts (HPCs) remain below 10 cfu/100 mL in distribution water. The European Community has established a drinking water standard of no more than 20 cfu/100 mL for aeromonads in water leaving the treatment plant, and no more than 200 cfu/100 mL in distribution system water. Use of biologically activated filters, and aging distribution systems with established biofilm, make it difficult to completely eradicate aeromonads from drinking water supplies. Aeromonads have been reported as a cause of heterotrophic interference in coliform tests. Using the MTF method, lightly turbid lauryl tryptose broth (LTB) tubes that are clear at 44.5°C usually contain aeromonads. Canada recently established an *Aeromonas* maximum contaminant level (MCL) of 0 cfu/100 mL for bottled drinking water.

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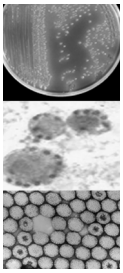


Chapter 7

Campylobacter

Colin R. Fricker

DESCRIPTION OF THE AGENT

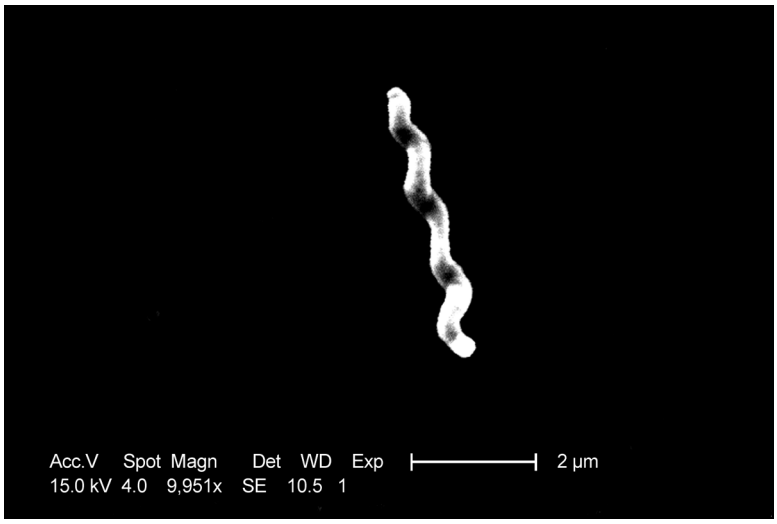
The genus *Campylobacter* belongs to the family Vibrionaeceae and contains at least 14 species. Campylobacters are microaerophilic, oxidase-positive, gram-negative, slender, curved rods that are motile by means of a single polar flagellum. Healthy cells are spiral or curved, 0.2 to 0.5 µm wide and 0.5 to 5.0 µm long. Older or damaged cells may form coccal bodies. The campylobacters of most concern to the water industry are the "thermophilic" group. Of greatest concern to human infection are *C. jejuni*, *C. coli*, and *C. upsaliensis* (Figure 7-1).

DESCRIPTION OF THE DISEASE

The campylobacters cause a variety of diseases in a wide range of animals and in humans. In humans, the principal symptom is acute diarrhea that clinically cannot be distinguished from most other acute bacterial infections of the gut. While the incubation period may range from 1 to 8 days, 2 to 3 days is more common. The onset of diarrhea is usually sudden and may be preceded by a prodromal flu-like illness, acute abdominal pain, or both, which may mimic the symptoms of appendicitis. The diarrhea may be profuse and watery, probably due to the production of a cholera-like enterotoxin, or may be dysenteric and contain blood and mucus. The disease is usually self-limiting and patients' stools are normally culture negative after 3 weeks, although occasionally excretion persists in excess of 3 months. Complications of *Campylobacter* enteritis are relatively uncommon; 1 to 2 percent of patients may suffer from a reactive arthritis, and infection has also been linked with GuillainBarré syndrome. In human volunteer studies, the infectious dose has been shown to vary considerably, although infection has been caused by ingestion of a few hundred organisms.

RESERVOIRS FOR THE AGENT

Campylobacter enteritis is principally a zoonotic disease and the organisms are harbored in the intestines of a wide variety of domestic and wild animals, particularly birds. Campylobacters have been found in almost all bird species that have been examined. They are particularly prevalent in poultry, likely a major source of human infection. Water probably has a major role in disseminating infection in flocks of poultry although in experiments, chlorination of the drinking water supply had no effect



Source: Dr. P. Fields, Dr. C. Fitzgerald, Centers for Disease Control and Prevention.

Figure 7-1 Scanning electron micrograph of *Campylobacter jejuni* bacteria; magnified 9,951×

on the rate of colonization of birds. Campylobacters can be found in most natural fresh and marine waters, even in remote areas, and their occurrence tends to be higher during autumn and winter. They are also found in high numbers in domestic sewage and undisinfected treated sewage effluents.

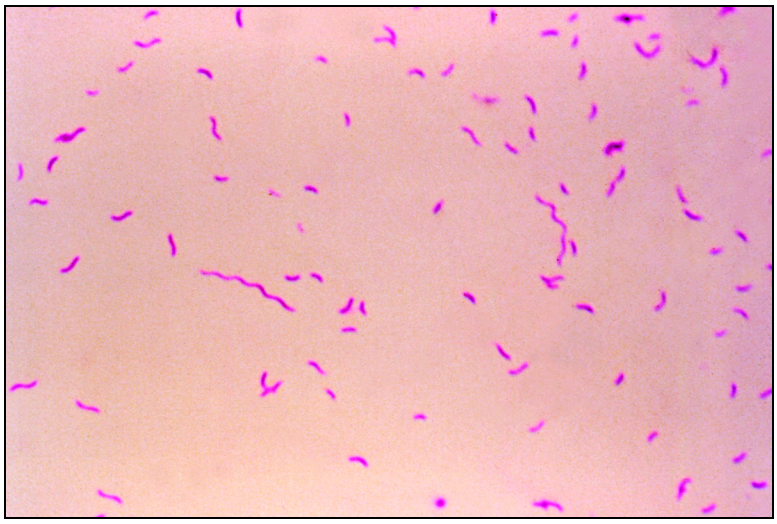
MODE OF TRANSMISSION

Campylobacters are transmitted by the fecal–oral route. Person-to-person transmission is relatively uncommon and is usually restricted to young children. Direct transmission from animals to humans is relatively common, either through occupational exposure to infected animals or from household pets. Indirect transmission, through consumption of contaminated food or water, is by far the most common route of infection, despite the fact that campylobacters are easily killed by normal cooking temperatures. Poorly prepared or undercooked chicken is probably the major cause of human infection. Outbreaks associated with consumption of unpasteurized milk or contaminated water have frequently been reported.

METHODS FOR DETECTING THE AGENT

Most strains of campylobacters are sensitive to oxygen and require CO_2 for adequate growth. Both *C. jejuni* and *C. coli* can use hydrogen as a major energy source. While most strains will grow on relatively simple media, either complex or defined, they prefer the addition of blood, serum, or powdered carbon (Figure 7-2). *C. jejuni* and *C. coli* grow optimally at 42 to 43°C but will not grow below 30°C. Biochemically, the campylobacters are relatively inactive and do not use sugars. Several biotyping schemes have been developed based on tests such as H_2S production, hippurate hydrolysis, and DNA hydrolysis.

Since the first successful isolation of campylobacters from human feces in the early 1970s, a wide spectrum of media have been formulated, although most have been designed with detection from food or feces rather than water. In the 1980s most work on the detection of campylobacters in water and the environment used Preston broth (a nutrient broth base containing lysed blood, trimethoprim, rifampicin, polymyxin B, and amphotericin B) together with a supplement known as FBP (containing



Source: Centers for Disease Control and Prevention.

Figure 7-2 Gram-stained image of *Campylobacter fetus* with 7% addition of rabbit blood agar plate

ferrous sulfate, sodium metabisulfite, and sodium pyruvate) followed by plating on Preston agar. Further work, aimed at eliminating the need for inclusion of blood, demonstrated that the use of ceferaperazone in a charcoal-containing medium worked well. For optimal recovery of campylobacters from water, a preenrichment culture should be used lasting for 2 to 4 hours, followed by enrichment and plating onto selective media. However, procedures have not been optimized, and some degree of experimentation may be required.

As a general procedure for the detection of *C. jejuni* and *C. coli*, water samples should be concentrated by filtration through a 0.22-μm filter. Then the filter is placed in nonselective broth containing FBP supplement, incubated at 42°C for 4 hours, after which antibiotics are added. Incubation is continued for 24 hours, then the broth plated onto Preston agar and incubated for 48 hours in a microaerobic environment before examination. Isolates should then be examined by gram stain and oxidase and catalase reactions and then further identified where required using the limited number of biochemical tests available. Serotyping schemes are available based on either heat labile or heat stable antigens; the procedure based on heat stable (lipopolysaccharide) antigens is preferred for routine use. Several procedures based on a combination of enrichment culture and the polymerase chain reaction (PCR) have been described, but there are few situations, if any, where their use can be justified.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

The campylobacters that cause infection in humans are unable to grow in water, but they may still be present in relatively high numbers. Marked seasonal variation occurs in naturally contaminated waters, with higher numbers being found during the cooler months of the year. *C. jejuni* appears to be the predominant species in aquatic environments, the source of which is often sewage or wildlife, particularly birds. Experiments have suggested that *C. jejuni* may survive better in water than *C. coli* or *C. lari*, although this has not been extensively researched. The number of individuals without

symptoms carrying campylobacters is low in developed countries, but may be significant in developing areas where poor standards of hygiene exist. Campylobacters are rarely found in chlorinated drinking water and when present are usually the result of post-treatment contamination. Some workers believe that campylobacters may frequently be present in municipal drinking water in a viable but nonculturable form. This has yet to be substantiated, and infection from ingestion of contaminated poultry products appears to be the main cause of human infection.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Campylobacters survive well in aquatic environments, particularly at temperatures below 5°C. Their survival appears to be enhanced by the presence of organic material and low dissolved oxygen. The numbers present in surface waters are usually low (<100/mL), although in wastewater numbers as high as 10^4/mL have occasionally been detected. Groundwater contains no campylobacters unless contaminated with surface water. Fully treated water subject to adequate disinfection will be free from *Campylobacter*.

DOCUMENTED WATERBORNE OUTBREAKS

A large number of waterborne outbreaks of *Campylobacter* have been reported in the literature, often affecting hundreds or even thousands of individuals. Despite these large outbreaks, the organism causing the outbreaks has seldom been isolated from the drinking water supply. The lack of isolation may be due to sporadic occurrence, poor detection techniques, or the presence of viable but nonculturable organisms. Sources of these waterborne outbreaks have included surface water, unchlorinated water storage tanks contaminated with bird feces, groundwater contaminated by surface runoff, and mains contaminated by cross-connection. While drinking water may be a major risk factor, there is no evidence that campylobacters can colonize or even survive in water distribution systems, thus, consumption of properly treated water is unlikely to result in infection.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Normal disinfection procedures using chlorine are sufficient to kill campylobacters in drinking water supplies, and the organisms appear to be somewhat more susceptible to chlorine than *Escherichia coli*. In laboratory experiments, *E. coli* survived better than campylobacters and is likely a good indicator for the potential presence of campylobacters. Campylobacters are not found in water in the absence of *E. coli*. Water systems that are maintained free of *E. coli* will be free of campylobacters.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

While there are no specific standards for the presence of campylobacters in drinking water, their potential presence is covered by the *E. coli* standard.

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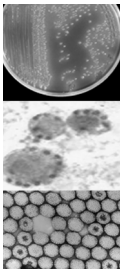


Chapter 8

Cyanobacteria

David W. Fredericksen and Edwin E. Geldreich
Revised by Dawn A. Karner

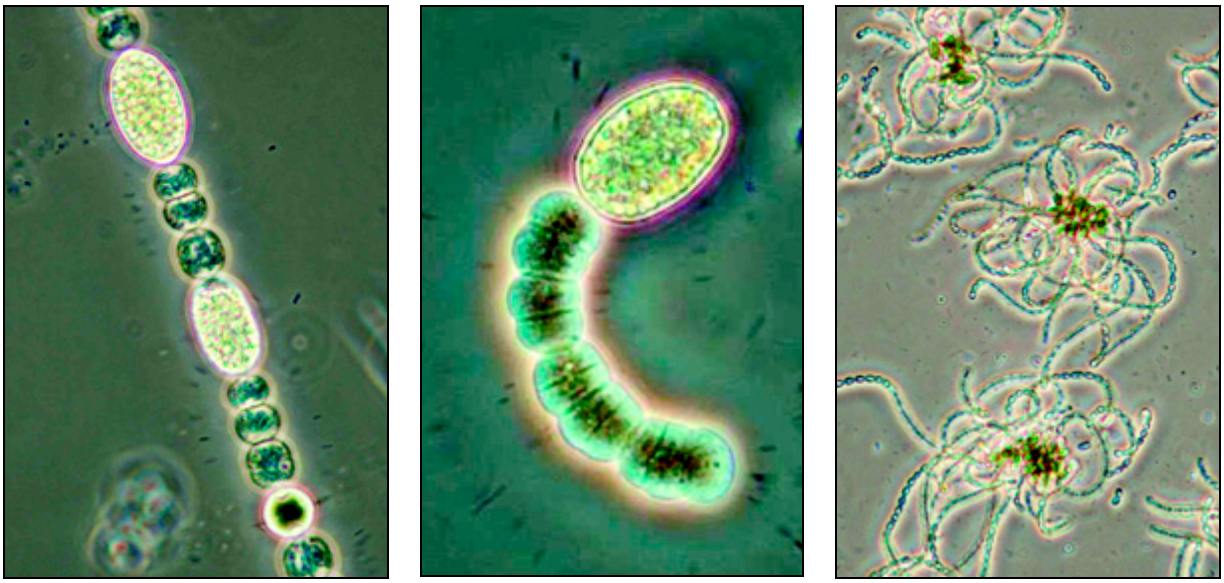
DESCRIPTION OF THE AGENT

Cyanobacteria (blue-green algae) are best known for the unsightly scum they produce during summer blooms in eutrophic freshwater lakes, reservoirs, and in the sluggish backwaters of rivers. However, they are becoming more recognized for the toxins that they can produce. These organisms are classified as a type of bacteria (prokaryotic) rather than as a type of algae, which are eukaryotic. Worldwide, at least seven genera, 13 species, and several strains have been identified. In North America, at least six genera have been identified as toxin producers, including two strains of *Anabaena flos-aquae*, *Aphanizomenon flos-aquae, Cylindrospermopsis raciborskii, Microcystis aeruginosa*, and *Nodularia* sp. Illustrations of these organisms may be found in an appendix to the latest edition of *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association, American Water Works Association, and Water Environment Federation, current edition).

In size, cyanobacteria range from 1 µm for unicellular types to over 30 µm for multicellular species. The group is gram-negative and some possess a metabolic pathway that can fix nitrogen in the dark. They also carry out oxygenic photosynthesis, like higher plants, by splitting water to free oxygen in aerobic sunlight environments. Some produce gas vacuoles that allow them to float, and others have a gliding mechanism that is poorly understood. There are also two different types of differentiated cells in their structure—heterocysts and akinetes (spores) (Figures 8-1 and 8-2).

DESCRIPTION OF THE DISEASE

Some cyanobacteria can produce toxins that, if ingested in sufficient concentration, may cause gastrointestinal upsets and/or affect the central nervous systems in mammals. Body contact exposure through water recreation may induce skin irritations that lead to a rash. The most convincing case history reported is that of a physician who fell into a lake containing a heavy bloom of cyanobacteria and swallowed an estimated 250 mL of water. A few hours later, he suffered stomach pains, nausea, vomiting, painful diarrhea, fever, headache, and pains in limb muscles and joints that lasted for 2 days. Samples of slimy green stool from the patient were subjected to laboratory analysis for pathogens (including virus) and revealed no *Salmonella* or *Entamoeba*, but many cells of *Anabaena* and *Microcystis*.



Source: Grant Williams-Jones, Chemist, Fort Collins Utilities, Fort Collins, Colo.

Figure 8-1 *Anabaena* sp.

Two categories of toxins may be produced by cyanobacteria—neurotoxins and hepatotoxins. Observations of poisoning of domestic animals show that when mucous membranes are exposed to cyanobacterial neurotoxins, symptoms develop in 4 to 10 minutes and death within 30 minutes. The effects of hepatotoxins can cause symptoms in exposed animals within 30 minutes and death within 24 hours. Using cobra venom as a point of reference, the alkaloid neurotoxins produced by species of *Anabaena flos-aquae* and *Nodularia spumigena* are equally potent with 200 µg/kg body weight as the lethal dose (LD_{50} of 200 µg/kg). The hepatotoxins produced by *M. aeruginosa* and some strains of *Aphanizomenon flos-aquae* are more than twice as potent as cobra venom with 9 µg/kg being the LD_{50}.

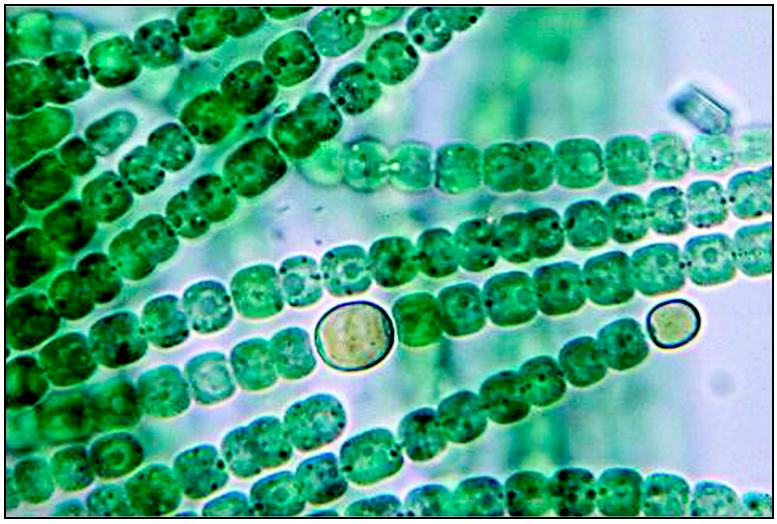
Some of these toxins have been found to be tumor promoters and oncogenic in laboratory animals. One of the toxins isolated from *Anabaena* is an alkaloid that can cause neuromuscular blocking activity. Another toxin, isolated from *M. aeruginosa*, affects the cardiovascular systems and produces lesions on the livers of a variety of laboratory animals when administered orally or by intraperitoneal injection. Fortunately, when cyanobacterial toxins are present in a drinking water source, conventional drinking water treatment processes, when operating correctly, have been shown to reduce toxin concentrations to levels that are safe for human consumption.

RESERVOIRS FOR THE AGENT

Stagnant water, sediments, and soil appear to be significant reservoirs for these organisms. While high densities of cyanobacteria may appear in the feces of infected animals, there is no evidence to support their common presence in the normal intestinal flora of healthy, warm-blooded individuals.

MODE OF TRANSMISSION

The most frequent mode of transmission is by water ingestion, however, body contact water sports in areas of blooms of cyanobacteria are also of serious concern.



Source: Grant Williams-Jones, Chemist, Fort Collins Utilities, Fort Collins, Colo.

Figure 8-2 *Anabaena* sp.

METHODS FOR DETECTING THE AGENT

To detect cyanobacteria, microscopic examination using simple wet-mount staining techniques with India ink or methylene blue are used to identify and examine morphological characteristics. Another method is to culture cyanobacteria. Water samples are first blended with glass beads or treated by ultrasound to break filamentous forms prior to streaking on agar plates. There are a variety of selected mineral media available (D-medium, ASM-1, BG-11, and WC) with incubation at 25°C under cool white fluorescent light. Some recalcitrant cyanobacteria may not be freed easily of contaminants, thus, physical and chemical separation schemes may be necessary. Success in achieving pure cultures depends on persistence and patience. Laboratory analyses to detect the toxins produced by cyanobacteria are becoming more available. Analytical techniques such as liquid chromatography (LC), gas chromatography (GC), and enzyme-linked immunosorbent assay (ELISA) can identify and quantify the toxins in a sample.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Because cyanobacteria are not dependent on a fixed source of carbon, they are widely distributed throughout aquatic environments. Cyanobacteria are to be found in the early stages of soil formation, whether it is converting bare rock or decomposing debris. They are also naturally occurring in stream sediments, overlying surfaces of slow-moving streams, receiving waters for a variety of waste discharges and treated effluents, rural storm runoff, marshes, drainage canals, rice fields, and marine waters. Massive growths of these bacteria often occur during the summer in surface waters at densities of 500 cells or more per milliliter.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Surface waters with slow flow and high nutrients under summertime conditions often support persisting populations of cyanobacteria. These organisms, like many algae,

are stimulated by water temperature and high concentrations of inorganic nitrogen (N) and phosphorus (P). Most of these growths occur when nitrogen and phosphorus concentrations reach 0.8 and 0.4 mg/L, respectively. Their capability to be a source of biological nitrogen fixation in soils and water is also a significant contributor to long-term survival and an important role in the ecological succession of microorganisms in the environment. Some cyanobacteria also possess specialized spore cells called akinetes, which enable them to get through harsh conditions such as overwintering.

DOCUMENTED WATERBORNE OUTBREAKS

There have been numerous reports of poisonings of livestock, pets, and wildlife by waters laden with cyanobacteria. Outbreaks of human gastroenteritis from ingestion of toxic cyanobacteria in a public water supply occurred in Charleston, W. Va., and the area served by the Anacostia Reservoir near Washington, D.C., during the drought years of 1930 and 1931. In 1981, an outbreak of human poisoning occurred in northeastern Pennsylvania where 12 children and one adult were affected by an *Anabaena* sp. bloom. Then in 1990, a localized outbreak of diarrhea occurred among residents of a Chicago apartment building. This incidence was traced to cyanobacteria toxins in the open water supply storage tank. Apparently, the cover to the tank had been inadvertently left ajar so that light and airborne cyanobacteria in dust particles gained access, grew, and in time, released toxic by-products throughout the plumbing system. More recently in 1996, an outbreak of illness occurred in 131 patients at a clinic in Brazil. It was established that these patients were exposed intravenously to the hepatotoxin microcystin while undergoing routine renal dialysis treatment. Consequently, 100 patients developed acute liver failure with 52 dying by the end of 1996. Further investigation revealed that the water used in the dialysis treatment was not properly purified prior to use.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

For utilities using surface water supplies, cyanobacteria are well known for their association with taste-and-odor problems, often regarded as a matter of aesthetics. Many utilities with seasonal taste-and-odor occurrences spend millions of dollars on powdered activated carbon (PAC) to remove the offending compounds. Removal of intact cyanobacteria cells by utilizing separation techniques such as coagulation is an important first step in treating water with toxin producing cyanobacteria. In light of recent information on cyanobacteria, PAC, granulated activated carbon (GAC), and ozone treatments may be very important to achieve toxin removal. Furthermore, for those water systems using disinfection as the only surface water treatment, there is always the threat of a seasonal passage of cyanobacteria and deposition of their dead cells in the distribution pipe network. Such an occurrence provides a source of assimilable organic carbon (AOC), which is a potential nutrient for bacterial regrowth.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

Neither cyanobacteria nor their obnoxious metabolites are regulated by the US Environmental Protection Agency (USEPA) or by states, except under guidelines stating that drinking water must be potable. As defined, potable water must not only be safe, but clear and free from any objectionable tastes and odors, regardless of origin. However, the World Health Organization (WHO) has set a drinking water quality guideline of 1.0 μg/L for microcystin-LR, one toxin that can be produced by certain cyanobacteria.

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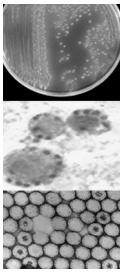

Chapter 9

Enterohemorrhagic *Escherichia coli* (O157:H7 and Others)

Alan J. Degnan and Jon Standridge

DESCRIPTION OF THE AGENT

Enterohemorrhagic *Escherichia coli* (EHEC), a subgroup of the species *E. coli*, is considered separately in this chapter due to its significance to the water industry. The basic microbiology of the organism is presented in chapter 10 of this manual, along with a discussion of other pathogenic and nonpathogenic *E. coli*. EHEC, and more specifically *E. coli* O157:H7, has been identified as the causative agent in several recently documented waterborne disease outbreaks. EHEC are of particular concern because of their unique ability to produce shiga toxin (also known as verotoxin), which results in serious, sometimes life threatening disease. EHEC is also referred to as shiga toxin-producing *E. coli* (STEC) in the more recent literature. The EHEC group is comprised of more than 100 serotypes including O157:H7, O111:H8, O145:NM, O45:H2, O118:H2, O104:H21, O113:H2, O121:H19, and O26:H11. O157 is the most significant serogroup in the United States and accounts for approximately one half of the EHEC outbreaks, producing an estimated 73,000 illnesses and 60 deaths per year.

DESCRIPTION OF THE DISEASE

The illnesses caused by EHEC and related *E. coli* serotypes may involve initial symptoms of diarrhea, followed by severe abdominal cramping, headache, watery and then bloody diarrhea. The average incubation period is 3 to 4 days. The duration of the illness is generally about 7 days, but may persist for longer periods. The diarrhea and cramping results from enterotoxins attacking the intestinal lining and/or invading the cell wall, causing intestinal hemorrhaging (bloody diarrhea) in advanced cases. In some cases, the disease may progress to kidney failure, an extreme condition called hemolytic uremic syndrome (HUS). HUS may begin as the patient (generally children and debilitated adults) becomes irritable and appears pale due to anemia. The patient will cease urinating and appear bloated because of fluid retention resulting from

toxin-damaged kidneys. Hospitalization with intravenous hydration therapy and sometimes kidney dialysis is often necessary. The kidney failure is often permanent, requiring continued dialysis or a kidney transplant.

RESERVOIRS FOR THE AGENT

The predominant source for EHEC, as with nonpathogenic *E. coli*, is the mammalian intestinal tract, especially that of cattle. Outbreaks caused by EHEC can most often be traced to cattle and/or products thereof, such as ground beef, nonpasteurized milk, or exterior surfaces (hides, pens, etc.). The origin of EHEC in nonmammalian sources such as soil, fruits, vegetables, and water (drinking and surface) may often be traced back to livestock and/or their by-products.

MODE OF TRANSMISSION

EHEC is spread by the fecal–oral route. Beef products that have been fecally contaminated during slaughter and served undercooked are the primary means of transmission. Fecally contaminated recreational water and drinking water are also well recognized routes of transmission, making up 12 percent of all documented outbreaks. Additionally, the disease can be passed directly from person to person or animal to person. EHEC are generally considered to have a low infective dose. Children under the age of five, the elderly, and people whose health is weakened (i.e., people who have long-term illnesses such as cancer or acquired immunodeficiency syndrome [AIDS]) are at greater risk of severe illness.

METHODS FOR DETECTING THE AGENT

Routine water quality testing based on the presence–absence of total coliform bacteria and nonpathogenic *E. coli* are excellent surrogate tests for fecally contaminated water that might contain EHEC. However, it is important to note that these standard methods will not actually detect the pathogenic EHEC. The EHEC group, specifically *E. coli* O157:H7, grows poorly at 44.5°C and is often negative for beta-glucuronidase. Initial screening tests for EHEC in water involve filtration and/or immunocapture concentration followed by plating on selective agar media. EHEC strains isolated on the selective media can be further confirmed by testing for antigenic characteristics that produce reactions with specific antisera. Molecular-based techniques that focus on detecting the genes that support pathogenicity traits such as toxin-production, attachment to the intestinal wall, and/or cell invasion may also be employed for final confirmation.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

EHEC has been detected in both drinking water and recreational waters. It is widely accepted that EHEC originates primarily from agricultural mammals, and many scientific surveys focus on dissemination of the agent from such sources. In one such survey, 21 of 29 lots of cattle produced at least one fecal specimen containing *E. coli* O157, while 38 percent of the hides were similarly positive. EHEC were isolated from about 2 percent of the swine sampled in slaughterhouses in the United States, Norway, and Japan.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

E. coli O157 can survive at a range of temperatures, is extremely acid tolerant, and can survive and grow in both aerobic and anaerobic environments. It can persist for more than 3 weeks in fruit and salad vegetables, and up to 4 months in turfgrass.

Farm studies show that calves inoculated with *E. coli* O157 shed the bacteria for up to 24 days. Also, the agent has been shown to spread amongst herds via common drinking water sources. *E. coli* O157 has been reported to survive up to 90 days in river water, depending on water source and temperature, and more than 300 days in bottled water. Prolonged contamination of groundwater supplies by animals or their feces has been implicated in outbreaks of *E. coli* O157 worldwide.

DOCUMENTED WATERBORNE OUTBREAKS

EHEC has recently emerged as an important waterborne disease pathogen. It has been implicated in several documented waterborne disease outbreaks. The first well-publicized outbreak happened in Cabool, Mo., where *E. coli* O157:H7 was epidemiologically linked to line breaks in the distribution system of an undisinfected water supply. Several other small waterborne outbreaks have also been documented. A 1997 Washington state outbreak sickened four with one hospitalization. A Wyoming outbreak had an estimated 157 illnesses. Illinois and Texas outbreaks sickened 3 and 22 persons, respectively. In 1999, an outbreak of O157:H7 was linked to a seasonal water supply at rural fairgrounds in Washington County, New York. The epidemiological study identified 921 infections (116 laboratory confirmed as O157:H7), 65 hospitalizations, 11 cases of hemolytic uremic syndrome, and two deaths. In 2000, California reported an outbreak with 5 cases; Idaho, 4 cases; and Ohio, 29 cases. Also in 2000, Walkerton, Ontario, Canada, experienced a severe outbreak that resulted in 600 illnesses and seven deaths.

EFECTIVENESS OF WATER TREATMENT PROCESSES

The multiple-barrier approach to water treatment is recommended for the elimination of EHEC from drinking water. There is conflicting information regarding the efficacy of coagulation, sedimentation, and sand filtration on the removal of the organisms. Since the infective dose is fairly low, disinfection becomes the primary means of eliminating EHEC from finished water. Standard engineering practice indicates that a chlorine residual of at least 0.2 mg/L must be maintained at all points in the distribution system.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

The US Environmental Protection Agency (USEPA) has no specific regulations regarding EHEC. The multiple-barrier approach to water treatment, including disinfection coupled with monitoring for total coliform and indicator *E. coli*, is assumed to effectively control bacterial contaminants including EHEC. The World Health Organization (WHO) recognizes waterborne disease and, more specifically, waterborne disease from emerging pathogens such as EHEC to be a significant global health issue. WHO also recommends a multiple-barrier approach to control of bacterial pathogens in water where disinfection is the most important barrier.

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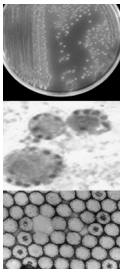


Chapter 10

Escherichia coli

Eugene W. Rice
Revised by Alan J. Degnan

DESCRIPTION OF THE AGENT

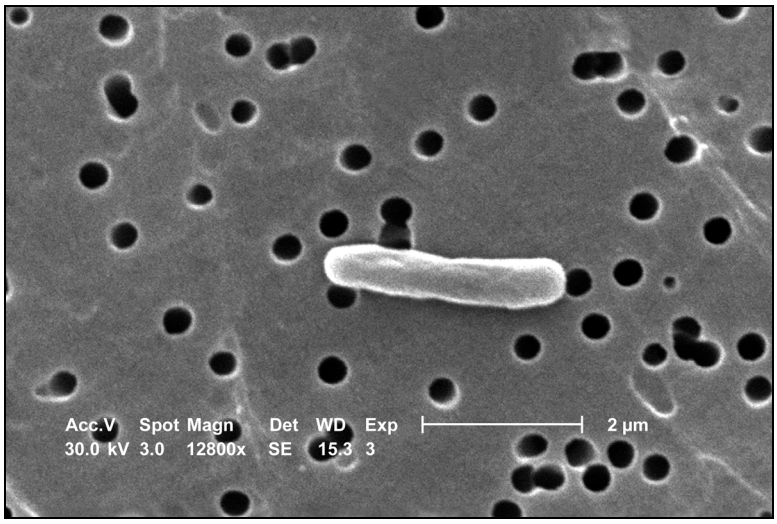
Escherichia coli (E. coli) is a bacterial species that is a major constituent of the normal intestinal flora of humans and warm-blooded animals. *E. coli* is the predominant species comprising the fecal coliform group of bacteria. The organism is a gram-negative, facultatively anaerobic, rod-shaped bacterium about 0.5 to 2.0 µm in size (Figure 10-1). While most members of this species are considered harmless commensal organisms, some strains are responsible for gastrointestinal illness. Pathogenic *E. coli* causes diarrheal illness, especially among children in developing countries and in travelers to these locales. Pathogenic strains have been categorized into seven groups, based on serological and virulence characteristics. These groups include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), diffuse adherent *E. coli* (DAEC), and enterohemorrhagic *E. coli* (EHEC), also known as shiga toxin–producing *E. coli* (STEC). Because of the emergence of the EHEC/STEC group, (including the 0157:H7 serotype) in several recent waterborne disease outbreaks, it is discussed separately in chapter 9.

DESCRIPTION OF THE DISEASE

Symptoms and pathology vary among the groups of pathogenic *E. coli*. Infectious dose studies have shown that 10^8 to 10^{10} organisms are required to produce infection. The infectious dose may be about 10^6 organisms when stomach acids are neutralized before ingestion.

Infections caused by EPEC have most commonly been associated with diarrhea in infants, with some reports of high mortality rates. Acquisition of immunity is thought to be the primary reason for the low occurrence rate in adults. Typical symptoms include watery diarrhea, fever, and dehydration. The incubation period in infants is not known. The duration of illness is generally 1 to 3 days, however, persistent diarrhea for more than 14 days has been reported. Adherence to intestinal mucosa with eventual destruction of microvilli is the primary mode of pathogenesis. Adhesion has been linked to a specific plasmid encoded adherence factor.

ETEC is a common cause of traveler's diarrhea and pediatric diarrhea in developing countries. Symptoms include watery diarrhea, abdominal pain, and vomiting. Fever, if present, is generally low grade. The incubation time is approximately 1 to 2 days. The



Source: E. Sowers, J. Carr, Centers for Disease Control and Prevention.

Figure 10-1 Scanning electron micrograph of rod-shaped *Escherichia coli* bacterium; magnification 12,800×

illness may last from 3 days to several weeks. ETEC strains produce enterotoxins, which may be either heat labile, heat stable, or both. The heat-stable toxin is very similar to the toxin produced by *Vibrio cholerae*.

Strains of EIEC produce a disease similar to that caused by *Shigella*. Watery diarrhea accompanied by abdominal cramps, fever, and muscle pain characterize this illness. In most cases, blood is not present in the stool. The incubation period is relatively short, with symptoms usually occurring in less than 24 hours after exposure. The ability to invade and multiply within epithelial cells is associated with a plasmid carried by these bacteria.

RESERVOIRS FOR THE AGENT

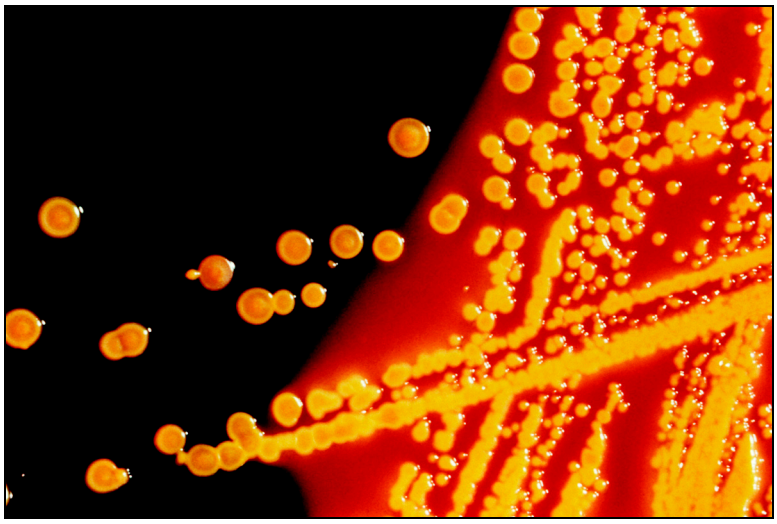
Humans serve as the principal reservoir for all pathogenic *E. coli* groups, with the exception of EHEC. Nonpathogenic *E. coli* are derived from all warm-blooded animals and may represent 95 to 99 percent of all coliforms found in feces of these animals.

MODE OF TRANSMISSION

Pathogenic *E. coli* groups are spread by the fecal–oral route. Fecally contaminated food or water as well as direct contact are primary modes of transmission. Water contaminated with sewage has been implicated in both recreational and drinking water disease outbreaks.

METHODS FOR DETECTING THE AGENT

Typical biochemical reactions used for identification of *E. coli* include fermentation of lactose, production of indole from tryptophane, a positive methyl red test, a negative Voges-Proskauer test, and inability to use citrate as a sole carbon source (Figure 10-2). The presence of enzymes β-glucuronidase and β-galactosidase can be detected simultaneously in colorimetric assay methods which confirm the presence of *E. coli.* In most instances, with the possible exception of suspected outbreak situations, standard methods for detecting fecal coliforms or *E. coli* should be sufficient for determining the



Source: Centers for Disease Control and Prevention.

Figure 10-2 *Escherichia coli* bacteria grown on a Hektoen enteric agar plate medium

sanitary quality of water. During outbreaks, standard procedures for *E. coli* may be used to analyze for the presence of EPEC and ETEC groups.

When analyzing for specific pathogens, it is always advisable to assay large volumes of water (1 L or more). Samples may be concentrated by membrane filtration or centrifugation and assayed using appropriate media. The use of enrichment broths facilitates the recovery of stressed organisms. Definitive identifications must be based on both biochemical and serological characterizations.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

All of the major *E. coli* groups have been associated with water. Human sewage is the major source for EPEC, EIEC, and ETEC in contaminated water. In any fecal contamination event, nonpathogenic *E. coli* should always occur in numbers exceeding pathogenic strains. Thus, wild type *E. coli* may be used as an indicator for the presence of these pathogens.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Numerous reports have shown that *E. coli* is capable of surviving in the aquatic environment. Factors such as water type (i.e., surface or ground), water temperature, and nutrient levels directly affect survival mechanisms. Research studies have usually found that survival times for pathogenic *E. coli* do not vary widely from survival times for normal wild type *E. coli* strains under similar conditions in the aquatic environment.

DOCUMENTED WATERBORNE OUTBREAKS

Waterborne transmission is an important factor in the epidemiology of diarrheogenic *E. coli*. EPEC infections, while being most common in children, can also occur in adults. A major EPEC outbreak affecting more than 100 individuals attending a conference center occurred in 1971 near Washington, D.C. The pathogen (serotype 0111)

was isolated from drinking water and fecal specimens. An unchlorinated groundwater source contaminated by sewage was the source of the outbreak. A similar waterborne outbreak of EPEC had been previously reported from Sweden in 1965.

One of the largest waterborne pathogenic *E. coli* outbreaks in the United States occurred in 1975 at Crater Lake National Park in southwestern Oregon. More than 2,000 individuals experienced gastrointestinal disease. The causative organism was an ETEC strain (serotype 06) that produced both heat-labile and heat-stable toxins. This serotype was found in both the water and fecal samples. A shallow spring supplying the park's drinking water supply was found to be contaminated by sewage. Although chlorination was used, areas of the distribution system were found to contain no detectable chlorine residual. Two ETEC (serotype 0159) outbreaks reported from Japan were also linked to contaminated drinking water.

A waterborne outbreak caused by EIEC (serotype 0124) was reported from a children's camp in Hungary. A leaking sewer was implicated in contaminating the drinking water. The causative organism was isolated from patients' stools and a reservoir supplied by a spring water source.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Proper disinfection of drinking water should control most strains of *E. coli*. Various studies have shown that *E. coli* is readily inactivated by chlorination, with $C \times T_{99}$ values reported at 0.2 mg-min/L or less. In the United States, current disinfection guidelines targeting protozoan and viral pathogens in surface water supplies are sufficient for controlling *E. coli*. An adequate disinfectant residual must be maintained in all areas of the water distribution system.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

Adherence to national standards for coliform bacteria in drinking water provides the best protection from waterborne disease outbreaks caused by pathogenic *E. coli*. Suspected outbreaks should be reported to local and national public health authorities. International measures should be instituted if an epidemic involves the populations of more than one country. The World Health Organization (WHO) established International Collaborating Centres to assist in disease management on the global level. Diarrheal illness caused by *E. coli* is one of the diseases covered by these centers.

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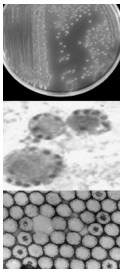


Chapter 11

Flavobacterium

Edwin E. Geldreich
Revised by Alan J. Degnan

DESCRIPTION OF THE AGENT

The genus *Flavobacterium* contains aerobic, gram-negative, asporogenous bacilli that are nonmotile, oxidase-positive, nonfermentative, nonglucose oxidizers. They produce pigmented colonies (when grown at incubation temperature below 35°C) that may be yellow, orange, and red to brown. These pigments are nonsoluble in media. The exact taxonomy of some of these organisms is still in debate because they usually do not demonstrate typical reactions (or any at all unless incubated for an extended time) in most biochemical identification media used to speciate gram-negative bacteria. As a consequence, estimates of the number of species vary from 30 to 70.

DESCRIPTION OF THE DISEASE

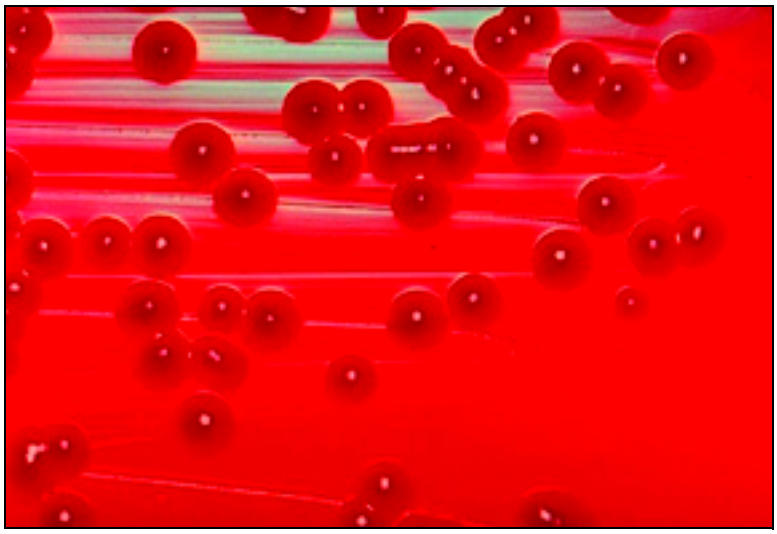
Clinically, the most important species of *Flavobacterium* are *F. meningosepticum*, *F. breve*, and *F. odoratum*. *F. meningosepticum* is the species most frequently involved as an opportunistic pathogen in nosocomial infections, including meningitis (particularly in infants), pneumonia, endocarditis, and septicemia (Figure 11-1).

RESERVOIRS FOR THE AGENT

Soil and water appear to be significant reservoirs for *Flavobacterium*. Detecting these chlorine-resistant organisms to some degree in any water supply is not unusual. Stagnation of building plumbing systems can provide opportunities for *Flavobacterium* colonization, and they can be found in hospitals. *Flavobacterium* may also be introduced through microbial growth on devices connected to the water supply.

MODE OF TRANSMISSION

The route of exposure may be by ingestion, body contact with water supply, or by person-to-person contact in hospitals.



Source: Dr. W.A. Clark, Centers for Disease Control and Prevention.

Figure 11-1 Blood agar plate culture of *Flavobacterium meningosepticum*

METHODS FOR DETECTING THE AGENT

The most successful recovery of *Flavobacterium* in water supply is with R2A spread plates or as microfiltrated medium with incubation at 28°C for 5 to 7 days. Pigmented colonies that develop need to be purified and tested for reactions to various biochemicals. While some success may be achieved using commercially prepared multitest kits, more definitive results may be achieved by placing the biochemical agent in a diluted R2A formulation and incubating for an extended time at 28°C. As with other waterborne pathogen genera, efforts to improve sensitivity and rapidity of detection methods currently involve investigation of polymerase chain reaction (PCR), DNA fingerprinting (i.e., pulsed-field gel electrophoresis), genotyping, and biotyping.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Normally, flavobacteria are not part of the resident microflora of humans. In the aquatic environment, flavobacteria are ubiquitous, being found in soil, water, sewage, vegetation, and dairy products.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Little is known about the environmental conditions in the distribution system that favor the colonization of these heterotrophic bacteria, but the available organic concentration needed may be less than 1 µg of carbon per liter. Current evidence suggests that conditions contributing to *Flavobacterium* regrowth include absence of free chlorine residual, water temperatures above 15°C, accumulations of bacterial nutrients (assimilable organic carbon [AOC] and specific inorganics) in pipe sediments, and static water conditions.

DOCUMENTED WATERBORNE OUTBREAKS

Waterborne outbreaks are thought to be caused by *Flavobacterium* colonization in stagnant plumbing systems. In one case, six office workers complained about intense abdominal cramping 6 to 8 hours after drinking water from fountains demonstrated to contain high counts of *Flavobacterium*. A sufficient quantity of *Flavobacterium* was likely ingested through water consumption to cause endotoxin gastroenteritis. Further investigation of the plumbing pipe network suggested that the bacterial colonization occurred in ½-in. copper lines leading to drinking fountains. *Flavobacterium* was not isolated from the tubing or reservoirs within the refrigerated fountains. No stagnation was evident in the public water supply lines entering the modular office buildings. To date, no substantial waterborne outbreaks specifically attributed to *Flavobacterium* have been reported.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Flavobacterium species appear to be chlorine-resistant. One strain was shown to survive a 10-minute exposure to 10 mg/L of chlorine. During a 5-month study of a New England metropolitan water supply, *Flavobacterium* species were detected in the disinfected water from five of nine finished water reservoirs. In another study of the seasonal occurrence of various pigmented bacteria (including *Flavobacterium*) in a treated municipal water system in the Midwest, these organisms were detected not only in the raw water supply but thought to enter from soil in line breaks and subsequent repairs to the distribution system. The predominant pigmented bacteria at most locations in the treatment basins or from distribution sites were yellow and orange strains. A small number of pink organisms also occurred in water collected at selected distribution sites.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

There are no national or international guidelines for this opportunistic pathogen in water supply, recreational waters, and sewage effluents.

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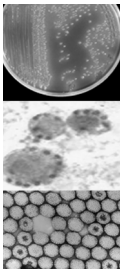

Chapter 12

Helicobacter pylori

Katherine H. Baker and Alan J. Degnan

DESCRIPTION OF THE AGENT

The genus *Helicobacter* was first recognized as a phylogenetic and taxonomic unit in 1989, though the species infecting humans had been isolated several years earlier. Originally classified as *Campylobacter pyloridis*, this organism is now designated *Helicobacter pylori.*

Members of the genus *Helicobacter* belong to the epsilon (e) subgroup of the proteobacteria. Based on 16S rRNA sequence data, there are more than 25 species within this genus as well as a number of proposed species awaiting formal recognition. All *Helicobacter* spp. colonize the digestive system of a vertebrate host with each species showing a very narrow host range. Furthermore, within the host a particular species will colonize either the gastric or enteric portion of the digestive system, but not both. In human hosts, *H. pylori* colonizes the gastric (stomach) environment.

H. pylori is a gram-negative, curved to spiral-shaped organism approximately 0.5 × 3 µm in size. It is highly motile with four to six sheathed unipolar flagella. Each flagellum is tipped with a distinctive bulb visible under the electron microscope. *H. pylori* is oxidase, catalase, and urease positive. In standard microbiological tests, it does not ferment or oxidize carbohydrates.

H. pylori grows best at a near neutral (6–7) pH under microaerobic or CO_2-enriched (10 percent CO_2) atmospheres. It does not grow under anaerobic conditions. The organism can tolerate very low pHs only in the presence of urea.

In aged cultures or under conditions of environmental stress, *H. pylori* undergoes a morphological transformation into a coccoid form. It is unclear if the coccoid form is an adaptation to stress, a dormant form of the organism, a viable-but-nonculturable (VBNC) cell, or an indication of cell death. Cultures with 50 percent or greater of the cells in the coccoid form usually do not produce colonies on solid media.

DESCRIPTION OF THE DISEASE

The majority of individuals colonized with *H. pylori* never develop a clinically recognized disease. In a minority of those infected, *H. pylori* is associated with a variety of gastric disorders including chronic gastritis, peptic and duodenal ulcer disease, mucosal-associated lymphoid tissue (MALT) lymphoma of the digestive tract, and adenocarcinoma of the stomach. Between 60 and 95 percent of all peptic ulcers are caused by *H. pylori* infection. Eradication of the microorganism using antibiotic treatment

leads to the cure of these ulcers. Colonization with *H. pylori* is known to increase the risk of an individual developing atrophic gastritis which often leads to gastric cancer. In fact, *H. pylori* is classified as a Class I carcinogen by the International Association of Cancer Registries (IACR). Gastric cancer is the second leading cause of cancer deaths worldwide.

RESERVOIRS FOR THE AGENT

The only known reservoir for *H. pylori* is the human stomach. There are several proposed possible reservoirs for the organism, including biofilms within water distribution systems and intracellular association with protozoa; none of these have been definitely proven.

MODE OF TRANSMISSION

The mode of transmission of *H. pylori* is through fecal–oral routes. In addition, several studies have presented data supporting an oral–oral route.

The only environment from which *H. pylori* can be routinely cultured is the human stomach. Epidemiological and indirect detection methods have supported transmission of *H. pylori* through contaminated food and contaminated water. The significance of these routes of transmission is still under investigation.

METHODS FOR DETECTING THE AGENT

Direct isolation of *H. pylori* from water and other environmental samples has met with limited success. In environmental samples, overgrowth by other microorganisms and the fastidious nature of the organism usually precludes the isolation of *H. pylori*. *H. pylori* typically grows slowly (5 to 8 days incubation) on complex media (e.g., blood agar) with added supplements. Supplements frequently incorporated into the media include whole blood, serum, heme, charcoal, egg yolk emulsion, or cornstarch. In addition, antibiotic mixtures (e.g., polymyxin B, amphotericin B, vancomycin, trimethoprim, and cefsulodin) often are incorporated into the medium to inhibit other organisms. Growth is best in a modified atmosphere supplemented with carbon dioxide. The recent development of selective and differential media for *H. pylori* and of specific concentration methods (e.g., immunomagnetic separation) should make isolation from environmental samples more likely.

The majority of the research concerning the presence of *H. pylori* in environmental samples, therefore, has relied on indirect molecular and immunological methods for detection of the organism. These include polymerase chain reaction (PCR) amplification and subsequent detection of a suitable nucleic acid sequence, fluorescent in-situ hybridization, and fluorescent antibody staining.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

The gastrointestinal tract of mammals is the established domain of *Helicobacter pylori*, although research suggests existence in the natural environment, including surface and drinking waters. Estimates of the occurrence of *H. pylori* in humans ranges from 10 to 90 percent, depending upon social parameters such as population densities, socioeconomic status, ethnicity, allergies, food-handling practices, sanitation practices, and/or age. Nonhuman mammalian species associated with *H. pylori* include ferrets, raccoons, swine, sheep, rodents, and primates. Domestic dogs and cats are said to carry a unique species of *Helicobacter*, although it may be transmissible to humans. Associations between *H. pylori* and the natural environment have been demonstrated with

soil, feces, and vegetables, with perhaps the strongest connection being surface and drinking waters.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

In independent laboratory experiments, *H. pylori* in water was cultured up to 4 days, but survived up to 20 days in a VBNC state. Factors affecting survival in water include pH, temperature, salinity (ionic strength), and biofilm availability.

DOCUMENTED WATERBORNE OUTBREAKS

Although adult human carriers of *H. pylori* in the United States are estimated as high as 50 percent, outbreak sources have not been identified to date.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Research conducted by the U.S. Environmental Protection Agency (USEPA) and others indicates that *H. pylori* is killed by chlorine at levels commonly used by municipalities for water treatment. Residual free chlorine at 0.5 mg/L was shown to inactivate about 1,000 *H. pylori* per milliliter within 80 seconds. Compared to *Escherichia coli*, *H. pylori* was shown to be more susceptible to monochloramine but more resistant to chlorine and ozone.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

No national or international guidelines exist in regard to *Helicobacter pylori* in waters.

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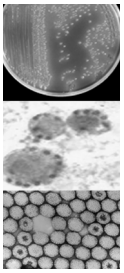


Chapter 13

Klebsiella

Edwin E. Geldreich
Revised by Jon Standridge

DESCRIPTION OF THE AGENT

The genus *Klebsiella* includes at least seven currently recognized species and 72 serotypes. Most of these organisms are of environmental origin without significance to human health, while other strains of the same genus originate in the intestinal tract of warm-blooded animals. They are found as human pathogens, commensals in humans, in soil and water, and as plant pathogens. Five of these (*K. pneumoniae*, *K. oxytoca*, *K. rhinoscleromatis*, *K. planticola*, and *K. ozaenae*) are known to be clinically significant. Like other coliform bacteria, *Klebsiella* are lactose fermenting, nonmotile, gram-negative bacilli (Figure 13-1). Under certain conditions they form a gelatinous encapsulation.

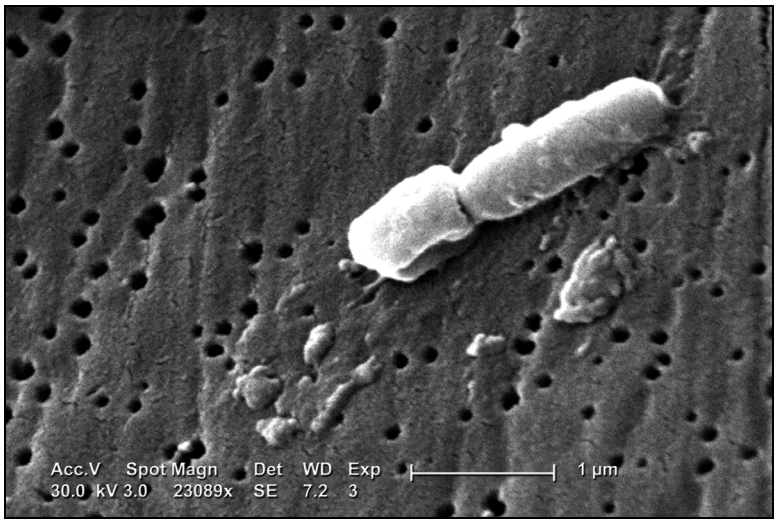
Klebsiella typically use citrate and give a negative methyl red and positive Voges-Proskauer reaction (Figure 13-2), although some strains have atypical biochemical reactions, such as fermenting glucose at 5°C or lactose fermentation at 44.5°C (fecal *Klebsiella*). An estimated 60 to 85 percent of all *Klebsiella* isolated from feces and clinical specimens are positive in the fecal coliform test and identify as *K. pneumoniae*. As a consequence, classification of these bacteria into a clear-cut scheme has been difficult.

DESCRIPTION OF THE DISEASE

K. pneumoniae, particularly antibiotic-resistant serotypes, can cause human infections of the respiratory system, genitourinary tract, nose, and throat, and occasionally meningitis and septicemia. *Klebsiella*-caused infection is sometimes a primary etiology agent but more often is found in mixed infection or as an opportunistic invader. Virulence does not appear to depend on capsule formation. In a study of 94 hospitals, the infection rate (16.7 infections per 100 patients) for pathogenic *K. pneumoniae* was the cause of 1.1 percent of all nosocomial deaths. Infections of the urinary system, lower respiratory tract, and surgical wounds were the most frequent cause of *Klebsiella*-associated illness or deaths.

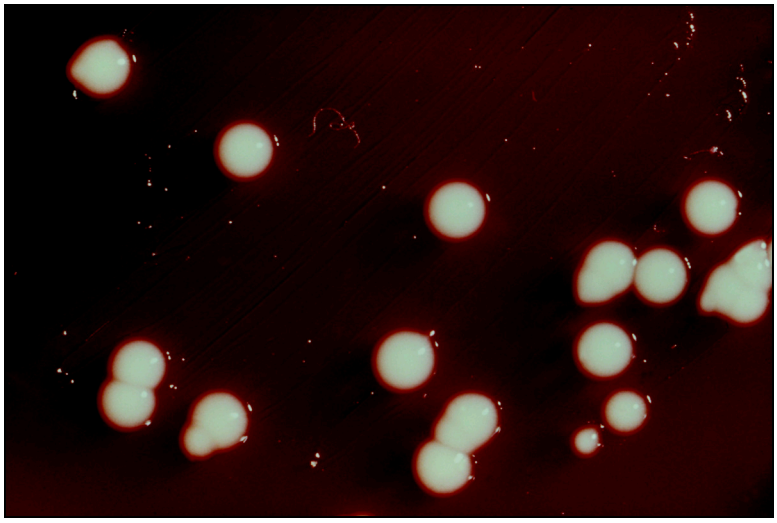
RESERVOIRS FOR THE AGENT

Approximately 30 to 40 percent of all warm-blooded animals, humans included, have *Klebsiella* in their intestinal tract, with individual densities ranging up to 10^8 *Klebsiella*



Source: J. Carr, Centers for Disease Control and Prevention.

Figure 13-1 Scanning electron micrograph of *Klebsiella pneumoniae* bacterium



Source: Centers for Disease Control and Prevention.

Figure 13-2 Blood agar plate of gram-negative, small rod-shaped *Klebsiella pneumoniae* bacteria

per gram of feces. *K. planticola* and *K. terrigena* have their origins in the environment, being found on fruit and vegetables, dairy products, seed embryos, internal and external tree tissues, hay, and cotton. Wood pulp, paper mills, textile finishing plants, and sugar cane processing operations may release 10^4 to 10^6 *Klebsiella* per milliliter of effluent and account for about 50 to 90 percent of the total coliform population of such effluents. Water storage tanks constructed from fresh redwood lumber have been shown to be a source of *Klebsiella* in the distribution network.

MODE OF TRANSMISSION

Transmission is often from body contact with water supply during bathing, ingestion, or by person-to-person contact through poor hand-washing habits in hospitals or senior citizen–care institutions. Inhalation of moisture from vaporizers using drinking water contaminated with *Klebsiella* should also be considered a risk to some individuals.

METHODS FOR DETECTING THE AGENT

The public health significance of *Klebsiella* can be very difficult to interpret unless the available information is supplemented by data on key growth responses and antibiotic resistance characteristics. Fortunately, the biochemical characteristics of commercial multitest systems provide speciation for most isolates purified from coliform colonies on M-Endo agar cultures. A more desirable approach is the use of the differential medium M-Kleb agar in connection with the membrane filter (MF) procedure. This approach provides quantitative information on total *Klebsiella* density in the sample and, in conjunction with a biochemical multitesting of positive (dark blue or dark gray) colonies, yields identification of species. Pulsed-field gel electrophoresis (PFGE) is considered the "gold standard" for tracking *Klebsiella* outbreaks in hospital environments. Because PFGE is time consuming and expensive, PCR-based fingerprinting techniques have been developed that are both expeditious and cost-effective.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Surface water and unprotected groundwater receive *Klebsiella* from both environmental and fecal sources. Environmental strains are introduced to the source of raw water intake from urban and rural runoff and by discharges of wood, paper, and textile processing wastes. Fecal *Klebsiella* enter the water in point-source discharges from municipal sewage and meat processing works and in non-point-source discharges from farm animal waste runoff.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Densities of *Klebsiella* in surface waters are influenced by available nutrients and seasonal water temperatures. For example, *Klebsiella* may survive for 20 days in laboratory "pure water" as a consequence of trace organics introduced to the supply as contaminates from the air. Those strains of *Klebsiella* that persisted in this environment developed into a mucoid mutant that dominated over time.

DOCUMENTED WATERBORNE OUTBREAKS

While numerous outbreaks of *Klebsiella* infections have been associated with water in medical facility environments, no community waterborne outbreaks have been reported to be caused by *Klebsiella* in public water supplies. The lack of evidence of increased illness in a community during coliform biofilm events involving *Klebsiella* may relate to difficulties in gathering reports of water-related illness cases among susceptible people at home, in the work environment, and the hospital setting. Furthermore, most of the *Klebsiella* waterborne occurrences do not involve fecal strains. In those infrequent situations in which the laboratory analyses reveal fecal *Klebsiella* in the distribution system, the colonization sites must be destroyed to avoid more frequent releases of this opportunistic pathogen at higher densities into the water supply. Infective dose (ID_{50}) values for environmental and clinical isolates of *Klebsiella* have been reported to be between 3.5×10^1 and 7.9×10^5 cells/mL. Therefore, ingestion of 100 mL of drinking water (approximately one glass of water) containing 3.5×10^1 *Klebsiella* per milliliter could present a risk to susceptible individuals.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Many of the water systems reporting coliform occurrences in their distribution networks have noted that the predominate organism was a member of the *Klebsiella* genus. Although klebsiellae can be controlled effectively by adequate disinfection in a clean pipe environment, these organisms can be protected by particulate material, porous pipe sediments, biological debris, macroinvertebrates, and disinfection demand products. Furthermore, *Klebsiella* can encapsulate, which provides some protection from disinfectants. As the organisms become established in this type of environment, growth beyond meager subsistence can result in the periodic sloughing of cells into water flowing past the sites. This condition can persist until elevated disinfectant residuals penetrate the protective habitat and inactivate the microbes. Systematic flushing of the entire system, sanitizing of all new line extensions, cross-connection control, cleaning of storage reservoirs and standpipes, and corrosion control in iron pipes are often effective in suppressing the seasonal reoccurrence of these biofilm events.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

Klebsiella organisms are coliform bacteria and as such are included in the national and international guidelines for total coliform occurrences. Generally these standards limit total coliforms to less than one organism or their absence in 100 mL of treated drinking water. World Health Organization (WHO) guidelines have established a limit of less than 10 total coliforms per 100 mL of untreated groundwater, provided no fecal coliforms are detected in the sample.

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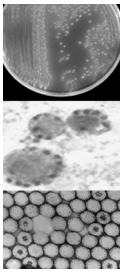


Chapter 14

Legionella

Nancy H. Hall

DESCRIPTION OF THE AGENT

Legionella are bacteria of the family Legionellaceae. They are facultative, gram-negative, non–spore-forming, small bacilli (from 0.3 to 0.9 µm wide by 2 to 20 µm or more in length) that do not grow on ordinary laboratory media (Figure 14-1). The genus *Legionella* is composed of at least 46 different species and 70 sets of serogroups. *Legionella* bacteria are ubiquitous in the aquatic environment and survive in water system biofilms. They are able to colonize hot-water tanks, cooling towers, distribution systems, and other water sources. Probably all *Legionella* species can cause illness, but only about half of the known species have been implicated in human disease; the remainder have been isolated from environmental sources, usually water. The species *Legionella pneumophila* causes most *Legionella* infections (Figure 14-2).

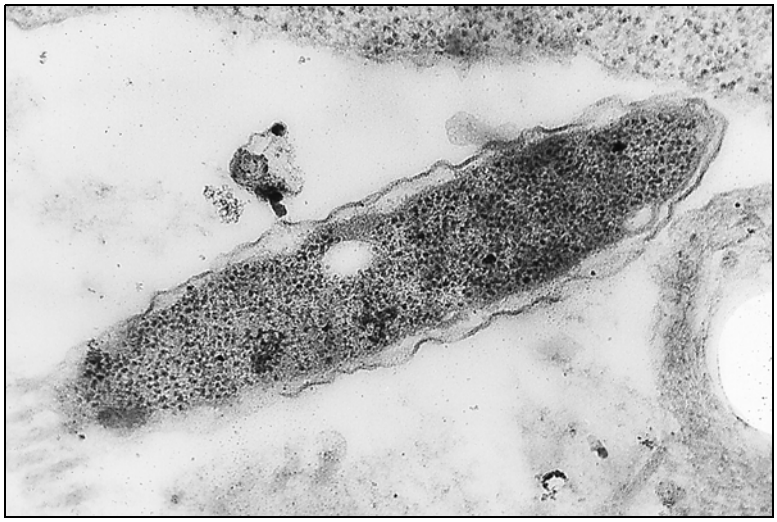
DESCRIPTION OF THE DISEASE

The disease caused by *Legionella* bacteria is called legionellosis. The two forms of legionellosis are Legionnaires' disease and Pontiac fever. In the United States, the frequency of Legionnaires' disease is estimated to be 8,000 to 18,000 cases a year, although public health officials suspect this is a low estimate. The occurrence of Pontiac fever is estimated to be 2 to 100 times more frequent than Legionnaires' disease.

Legionnaires' disease is a severe respiratory illness characterized by pneumonia. The incubation period for Legionnaires' disease is 2 to 10 days, with an attack rate of 1 to 6 percent. The risk of contracting Legionnaires' disease is greater for individuals with underlying disease and immunosuppression. Additional risk factors include being a male over 50 years of age, cigarette smoking, excessive use of alcohol, and surgery.

Pontiac fever is a self-limiting, nonpneumonic, influenza-like illness. No known cases of pneumonia or deaths with Pontiac fever are documented. The incubation period of Pontiac fever usually ranges between 20 and 48 hours, with a mean of 36 hours and an attack rate of nearly 100 percent.

Five methods of diagnosis for legionellosis are culture isolation from respiratory secretions or tissues, direct fluorescent antibody test on respiratory secretions or tissues, indirect fluorescent antibody test performed on acute and convalescent sera, nucleic acid hybridization for detection of *Legionella* rRNA, and urinary antigen detection by radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), or agglutination assay.



Source: Centers for Disease Control and Prevention.

Figure 14-1 *Legionella pneumophila*

RESERVOIRS FOR THE AGENT

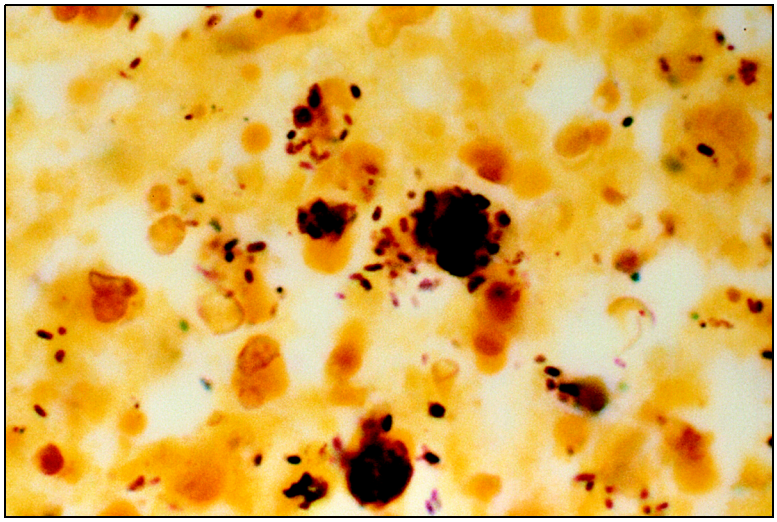
Legionella bacteria are ubiquitous in aquatic environments, both natural and artificial, and some species have been recovered from soil. These organisms have been found in lakes, rivers, and streams, and have colonized artificial environments such as cooling towers, evaporative condensers, and hot-water tanks, whirlpool spas, decorative fountains, potable water distribution systems, with and without association of disease. These artificial environments are thought to act as either amplifiers or disseminators of legionellae. The temperature of the water appears to be a major determinant of *Legionella* colonization in artificial settings. Legionellae are most often isolated from environmental water sources between 35 and 45°C and killed at temperatures above 60°C.

MODE OF TRANSMISSION

The mode of transmission is by inhalation of moist aerosols contaminated with *Legionella* bacteria. *Legionella* in the environment is amplified in a water system, cooling tower, whirlpool spa, and other sources. The bacteria are transmitted through water vapor (aerosolization) to a susceptible host. In immunosuppressed individuals, the bacteria can cause legionellosis.

METHODS FOR DETECTING THE AGENT

The three primary methods used to detect legionellae in environmental samples are culture, direct fluorescent antibody test, and polymerase chain reaction (PCR). Culture has been the method of choice for detection of legionellae from environmental samples but is sometimes difficult because of interference or overgrowth by other organisms. Selective media and pretreatment techniques (acid and heat treatment) are usually necessary to enhance recovery and reduce overgrowth. Sample concentration by either filtration or centrifugation before culture may be necessary to detect low numbers of legionellae in relatively clean samples. Environmental samples may also contain legionellae that are viable but nonculturable; any culture method will typically underestimate the total population density.



Source: Centers for Disease Control and Prevention.

Figure 14-2 Silver-stained micrograph of lung tissue specimen revealing *Legionella pneumophila* bacteria

The direct fluorescent antibody test has been used to see legionellae in its natural habitat and is a very useful diagnostic tool. This technique does not provide absolute specificity and does not allow viable and nonviable cells to be differentiated unless secondary staining with tetrazolium dyes is performed.

A PCR test kit (EnviroAmp® *Legionella* kit*) has been used successfully to detect *Legionella* in environmental water samples but is no longer commercially available. Primer sequences of the macrophage infectivity potentiator (*mip*) gene of *L. pneumophila* and 16S and 5S rRNA genes have been used in PCR assays. PCR assays may be helpful when viable, nonculturable legionellae are thought to be present. The major limitations of this method are cost, presence of inhibitory substances, and necessity of a dedicated PCR laboratory.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Legionella are ubiquitous in the environment and are found in water, both natural and treated, as well as in water distribution and storage systems.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Legionella bacteria thrive in the freshwater environment under a wide range of temperatures (about 5 to 63°C) and pH (5.5 to 9.2). Legionellae become attached to surfaces in an aquatic environment forming a biofilm and protective barrier. Several studies have shown a unique association between legionellae and protozoa in the environment. *Legionella* bacteria have the capacity to survive inside free-living protozoa (e.g., amoebas), which may explain how these bacteria evade water purification systems. Legionellae are more readily isolated from warm than from ambient sites, as demonstrated by their frequent occurrence in domestic hot-water tanks and hot-water

* Perkin Elmer, Branchburg, N.J.

Table 14-1 Notable outbreaks of Legionellosis

Location	Setting	Date	Cases/Deaths	Source
Pontiac, Mich.	Health department	August 1968	144/0	Evaporative condenser
Philadelphia, Pa.	Hotel	August 1976	221/34	Unknown
Los Angeles, Calif.	Hospital	1977–1980	175+/28	Potable water
Pittsburgh, Pa.	Hospital	1979–1981	100+/?	Potable water
Seattle, Wash.	Hospital	1984–1988	21/9	Nebulizer
Staffordshire, England	Hospital	1985	163/39	Cooling system
South Australia	Statewide	1987–1989	30/0	Potting soil mixes
Bogalusa, La.	Grocery store	November 1989	34/2	Produce mister
Bermuda	Cruise ship	1994	50/0	Whirlpool spa
Bovenkarspel, Netherlands	Flower show	February 1999	188/21	Whirlpool spa display
Murcia, Spain	City	July 2001	449/6	Cooling towers

plumbing systems. Other factors that encourage *Legionella* growth include pH, stagnation, sediment formation, presence of nutrients in natural-rubber sealing washers and gaskets, and presence of protozoa.

DOCUMENTED WATERBORNE OUTBREAKS

Some of the more notable outbreaks caused by *Legionella* bacteria are listed in Table 14-1. The largest US outbreak of Legionnaires' disease occurred among persons attending the American Legion convention held in Philadelphia, Pa., in July 1976, in which more than 220 cases and 34 deaths were reported. The world's largest outbreak of Legionnaires' disease occurred in Murcia, Spain, in July 2001 with more than 800 suspected cases (449 confirmed). Epidemiologic investigation implicated the cooling towers of a hospital located in the northeastern part of the city. The majority of nosocomial legionellosis cases have been linked to contaminated hospital water distribution systems. The reservoirs that have been associated with community-acquired legionellosis have been cooling tower or evaporative condensers, and more recently, whirlpool spas. Most community-acquired cases occur in the summer months, whereas nosocomial legionellosis occurs year-round. Although *Legionella* bacteria have been implicated in numerous outbreaks, the recovery of legionellae from environmental sources does not constitute proof of the source of the infecting agent. Four criteria must be met to confirm the source of the *Legionella* outbreak: (1) association between exposure to a potential source, (2) identification of the mechanism of aerosol production, (3) occurrence of disease established, and (4) isolation of similar *Legionella* subtypes from patient and suspected environmental site.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Most strategies for prevention of legionellosis in the absence of disease are based on outbreak investigations and control. Although routine maintenance procedures for water systems may not prevent legionellosis, the following measures have been shown to reduce the prevalence of legionellae in water systems:

- Maintain hot water at 50°C or higher in the return and cold water below 20°C.
- Limit thermal stratification in central hot-water storage equipment.

- Periodically clean and remove sediment in central hot-water storage tanks and cooling towers.
- Maintain hot-water tank temperature between 71 and 77°C.
- Remove obstructions to flow or conditions of stagnation.
- Consistently provide adequate maintenance and disinfection control procedures, especially for whirlpools and cooling towers.

Additional control measures used after outbreaks include the following: cleaning of scale or sediment accumulation or replacement of faucets and showerheads, and supplemental chlorination of the heated water to achieve 1 to 2 mg/L of free residual chlorine. If outbreaks of legionellosis are to be prevented, control methods must focus not just on legionellae but the whole microbial community, particularly biofilm. The accumulation of microbial slime and sludge that supports and protects *Legionella* bacteria in these systems must be reduced.

Studies indicate that the use of monochloramine as a biocide in municipal water systems is more effective than chlorine at killing *Legionella* in biofilms. The protective effect of monochloramine has been reported in the literature. Case-control and retrospective studies have shown that hospitals using chlorine as a disinfectant were more likely to experience a problem with nosocomial Legionnaires' disease than those using monochloramine. Municipal water disinfection with monochloramine may provide an effective means of reducing the incidence of Legionnaires' disease.

Another treatment strategy involving copper–silver ionization is a relatively new approach to controlling *Legionella* in hot-water distributions systems. Some hospitals have used these systems successfully and some have not.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

The Surface Water Treatment Rule (SWTR) requires a treatment technique (filtration and disinfection for surface water supplies) rather than a maximum contaminant level (MCL) for *Legionella* to significantly reduce the transport and potential for colonization in the distribution system. Following the outbreak linked to a grocery store mist machine, the US Food and Drug Administration (FDA) issued cleaning and maintenance guidelines for the use of ultrasonic vegetable misters. The Centers for Disease Control and Prevention (CDC) *Guidelines for Prevention of Nosocomial Pneumonia* state that only sterile fluids should be used in nebulizers or humidifiers in the hospital setting (CDC 1997). The American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) developed Guideline 12-2000 to provide information and guidance to minimize *Legionella* contamination in building water systems. Technical standards W551 and W552 were established by the German Association of Gas and Water Branches to minimize the number of systems with low hot-water temperatures and to determine whether a system is contaminated. The CDC Recommendation for Cruise Ship Prevention of Spa-Associated Legionnaires' Disease states that existing health guidelines should be followed for whirlpool maintenance and vigorous filter cleaning and maintenance should be performed frequently (Jernigan et al. 1996). Guidelines for the control and prevention of legionellosis for cooling towers have been established in many countries (e.g., Australia, New Zealand, and the United Kingdom). The Australian standards are the most restrictive, requiring tower registration and submission of building plans, site surveys, maintenance procedures, and other documentation to regulatory authorities.

The value of routine monitoring for legionellae of water sources not implicated in disease remains questionable, since legionellae are ubiquitous and long-term decontamination is difficult. Most public health investigators recommend environmental

sampling for legionellae only after two or more epidemiologically linked cases have been identified. Currently, environmental sampling is not recommended after individual community-acquired cases because of the considerable time and expense required to collect samples from the many potential sources in the environment.

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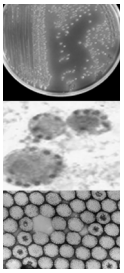


Chapter 15

Mycobacterium avium Complex

Mark W. LeChevallier

DESCRIPTION OF THE AGENT

Members of the *Mycobacterium avium* complex (i.e., *M. avium* and *intracellulare*) are acid-fast, rod-shaped bacteria, 0.2 to 0.6 by 1.0 to 10.0 µm in size. Mycobacteria are aerobic, nonmotile bacilli that do not form spores. The cell walls of the bacteria contain high levels of lipid (waxy) material and are not affected by staining procedures that use acid alcohol. Therefore, they are described as acid fast.

DESCRIPTION OF THE DISEASE

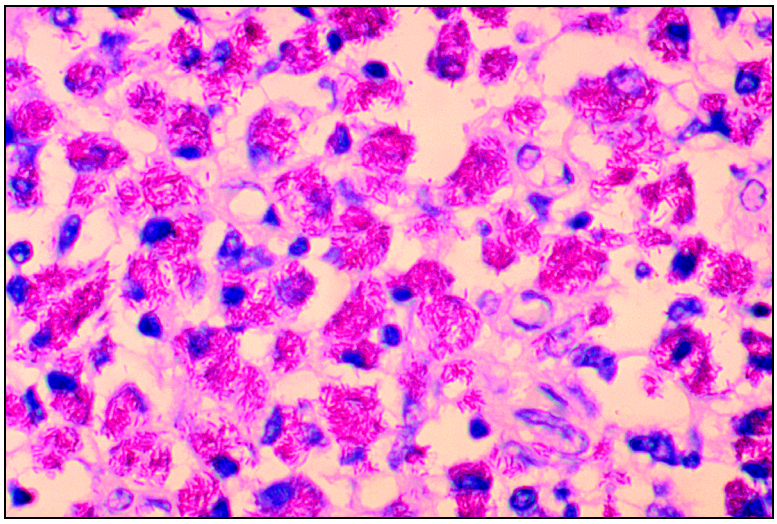
Members of the *M. avium* complex (MAC) are opportunistic human pathogens and can infect the lungs, producing cough, fatigue, weight loss, low-grade fever, and night sweats similar to *M. tuberculosis*. From the lungs, the organism can be disseminated throughout the body. The greatest increase in MAC infections is seen in acquired immunodeficiency syndrome (AIDS) patients. Approximately 25 to 50 percent of these patients suffer debilitating and life-threatening infection (Figure 15-1).

RESERVOIRS FOR THE AGENT

M. avium complex are ubiquitous in the environment and have been found in soil, house dust, water (wastewater, surface and groundwaters, and drinking water), animals, and poultry. *M. avium* levels can be hundreds or thousands times higher in soils than in treated drinking water.

MODE OF TRANSMISSION

M. avium complex can be transmitted through inhalation or ingestion of contaminated water, soil, or other materials. Evidence for environmental transmission, especially in immunocompromised individuals include (1) the frequency of gastrointestinal colonization increases as the stage of human immunodeficiency virus (HIV) advances, (2) higher frequency of isolation of MAC from the gut than from the respiratory tract, and (3) gastrointestinal symptoms (e.g., nausea, vomiting, and diarrhea).



Source: Centers for Disease Control and Prevention.

Figure 15-1 *Mycobacterium avium-intracellulare* infection of lumph node in patient with AIDS

METHODS FOR DETECTING THE AGENT

Isolation of MAC organisms from water or biofilm samples is difficult because of their very slow growth (i.e., greater than 7 days for colony formation). Optimum growth temperature is 37°C. Disinfection is required to kill nonmycobacteria that can overgrow the mycobacterial colonies. As an alternative, mycobacterial selective media can be used. These media permit direct isolation and enumeration of MAC without decontamination.

Identification of MAC isolates is achieved through application of chemical or molecular techniques. Characteristic mycolic acids and other cell wall lipids of *M. avium* can be identified using capillary gas chromatography (GC) or high-performance liquid chromatography (HPLC) methods. Deoxyribonucleic acid (DNA) probes are commercially available for identification of MAC, as well as multiplex polymerase chain reaction (PCR) techniques.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Members of the MAC (i.e., *M. avium* and *intracellulare*) are found in natural waters and drinking water distribution systems throughout the United States. Table 15-1 summarizes the number of MAC colony-forming units (cfu) per 100 mL of drinking water samples reported by different authors.

The differences in the numbers may reflect different pretreatment steps used in the processing of environmental samples, different isolation media, and variations in water quality.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Members of the MAC are resistant to chlorination and are able to grow in water samples to which no additional nutrient substrate had been added. Further, they grow over a wide range of temperatures (e.g., 15 to 45°C) and salinities (e.g., 0 to 2 percent NaCl).

Table 15-1 Summary of reports on *Mycobacterium avium* complex in water

Study	Sample Types	*M. avium* cfu/100 mL
Haas et al. (1983)	Distribution system	0.08–0.14
Carson et al. (1988a)	Hospitals	14.5–195
duMoulin et al. (1988)	Hospitals	141
Fischeder et al. (1991)	Distribution system	10–45,000
von Reyn et al. (1993)	Distribution system and hospitals	20–10,000
Glover et al. (1994)	Distribution system and dwelling	0.20–2

M. avium complex organisms are found in highest numbers in waters of low dissolved oxygen, of high organic matter, and of high zinc concentrations. Recent evidence suggests that the level of biodegradable organic material can influence the growth of mycobacteria in drinking water. DNA fingerprinting studies have shown that single, unique strains can persist as long as 41 months in a water distribution system.

Because of their lipid cell walls, MAC organisms are hydrophobic and readily colonize surfaces. The reported resistance of mycobacteria to zinc and copper may aid the development of biofilms of these organisms on plastic, copper, or galvanized (i.e., zinc-coated) pipes.

DOCUMENTED WATERBORNE OUTBREAKS

M. avium strains have been detected in nebulizer reservoirs, ice machines, hot- and cold-water faucets, toilets, sinks, and other water sources in patient care sites. The strains were serologically similar to clinical isolates. *M. avium* strains from infected AIDS patients were shown to be genetically related to isolates recovered from water to which the patients were exposed through drinking or bathing. An epidemiological study of 290 homes of HIV patients found MAC in 0.76 percent (4 of 528) water samples, 1 of 397 (0.25 percent) food samples, but in 55 percent of 157 soil samples taken from potted plants. Some of the soil isolates were similar by serotyping and multilocus enzyme electrophoresis analysis. Other environmental mycobacteria may be of health concern. *M. xenopi* was detected in drinking water faucets and showers from 5 of 11 apartments of patients infected with the organism.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Physical Removal by Coagulation and Filtration

The hydrophobic nature of the mycobacteria cell wall promotes attachment of the organism to surfaces, and most mycobacteria in untreated raw water will be attached to suspended particulate material. Therefore, treatment processes that reduce particle counts and turbidity will be effective for removal of mycobacteria. Coagulation conditions (dose, pH, temperature, alkalinity, turbidity, and the level and type of natural organic matter) can impact the efficiency of microbial removal, with slightly better overall reductions under pH conditions (5 to 6.5) optimum for removal of total organic carbon (TOC).

The presence of biodegradable organic matter in water has been associated with the growth of mycobacteria. Mycobacteria can grow within the treatment process, and growth on filter media has been observed to when ozone-treated water was filtered through granular activated carbon (GAC) filters. GAC used in point-of-use treatment

Table 15-2 Comparison of disinfection conditions ($CT_{99.9\%}$ in mg min/L) for *Giardia* cysts and *M. avium*

Disinfectant	*Giardia* Cysts	*M. avium*
Chlorine	46	130
Monochloramine	700	580
Chlorine dioxide	11	7
Ozone	0.48	0.13

NOTE: Data are for pH 7.0, 23–25°C.

devices can accumulate bacterial nutrients and neutralized disinfectant residuals, and growth of *M. avium* in point-of-use filters has been observed even in the presence of 1,000 mg/mL of silver. *Mycobacterium* species attached to cellulose acetate membrane filters 25 times more effectively than did *Escherichia coli* cells.

Disinfection

Mycobacteria are very resistant to disinfection, and free chlorine $CT_{99.9\%}$ values for the *M. avium* strains are 700 to 3,000 times greater than that for *E. coli.* Similarly, the $CT_{99.9\%}$ values of the *M. avium* strains for chlorine dioxide and ozone are at least 100- and 50-fold greater (respectively) than the *E. coli* strain. Isolates grown in low-nutrient tap water show greater resistance (between 4 and 15 times) to free chlorine disinfection than isolates grown in rich media. Disinfection requirements for inactivation of *Giardia* cysts may not be sufficient for treatment of mycobacteria. *M. avium* are more resistant than *Giardia* to free chlorine disinfection, but equal or more sensitive than *Giardia* to monochloramine, chlorine dioxide, and ozone. However, both organisms are more sensitive to disinfection than are *Cryptosporidium* oocysts (see Table 15-2).

Inactivation data for mycobacteria by ultraviolet (UV) light vary among *Mycobacterium* species with values for 1 log reduction ranging between 2.4 and 24 mJ/cm^2. Mycobacteria are capable of photoreactivation, with an increase of 40 to 56 percent following exposure to visible light for 1 hour.

Temperature

The resistance of mycobacteria to heat and freezing may influence its survival in water systems and in treatment residuals. Certain thermophilic mycobacteria species survive at temperatures above 55°C; whereas, under the same conditions others are quickly destroyed. It has been speculated that differences in sensitivity to heat could account for some of the seasonal occurrence between different isolates of mycobacteria (see Table 15-3).

Freezing is one mechanism for dewatering water treatment sludges through a freeze/thaw process. Mycobacteria can survive freezing conditions in contaminated ice machines for prolonged periods with counts actually increasing after freezing (–75°C in nutrient broth), presumably due to disaggregation of bacterial clumps.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

There are no national or international guidelines for mycobacteria in water. *M. avium* and *M. intracellulare* have been included in a list of candidate contaminants for possible

Table 15-3 Decimal reduction times (D) of *Mycobacteria* at 50, 55, 60, and 70°C

Species	Strain	D (sec) at Temperature of			
		50°C	55°C	60°C	70°C
M. avium	DSM* 43216	60,750	3,210	240	2.3
M. chelonae	DSM 43283	10,130	1,360	260	5
M. fortuitum	DSM 43271	6,330	1,520	220	2
M. intracellulare	DSM43224	32,950	1,470	91	4.5
M. kansasii	Water isolate	3,970	560	59	<10
M. kansasii	Water isolate	4,700	350	27	<10
M. marinum	ATCC† 927	4510	750	60	<10
M. phlei	DSM 750	NR	4,120	420	2.8
M. scrofulaceum	NCTC‡ 10803	56,030	3,650	320	5.6
M. zenopi	NCTC 10042	NR**	20,730	1,980	22.5

* DSM: Deutsche Sammlung von Mikroorganismen and Zellkulturen.
† ATCC: American Type Culture Collection.
‡ NCTC: National Collection of Type Cultures.
** NR: No reduction in colony-forming units over a period of 48 hours.

regulation by the US Environmental Protection Agency (USEPA). The World Health Organization (WHO) declined to consider mycobacteria in their 1997 revisions of the WHO guidelines for drinking water quality.

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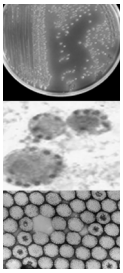


Chapter 16

Pseudomonas

Edwin E. Geldreich
Revised by Alan J. Degnan

DESCRIPTION OF THE AGENT

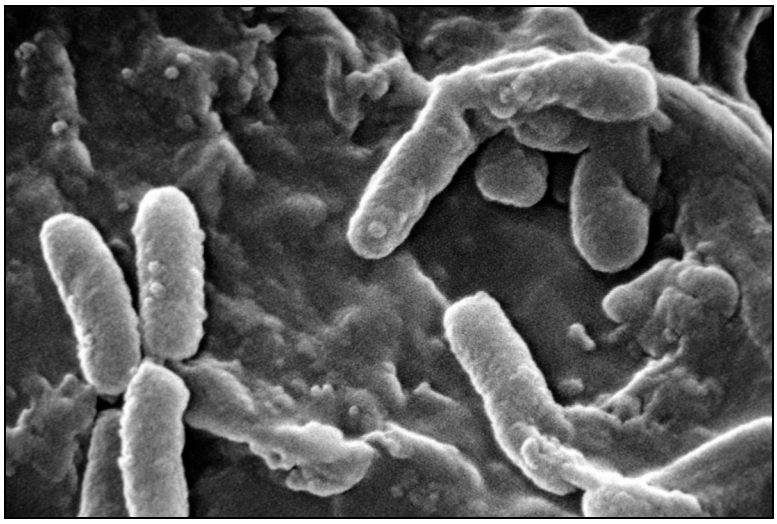
The genus *Pseudomonas* includes more than 29 species. Pseudomonads are gram-negative rods that may be motile because of polar flagella. Highly mucoid strains may lack motility. No spores are produced and metabolism is rapid in a variety of aerobic environments at ambient temperatures. These organisms are often glucose-nonfermenting, but tests for acid production from various other carbohydrates are not clear-cut, leading to ambiguities in species interpretation. Most fluorescent pseudomonads, including *P. aeruginosa*, *P. fluorescens*, and *P. putida* (Figure 16-1), produce water-soluble pigments (yellow, green, and red), while other strains (e.g., some *P. aeruginosa*) form a nonfluorescent water-soluble blue pigment. Development of these pigments (which may be an erratic trait of some strains) are influenced by nutritional factors, choice of media for cultivation, and incubation temperatures between 20 and 42°C.

DESCRIPTION OF THE DISEASE

The ability of *P. aeruginosa* to rapidly colonize a variety of environments, including the susceptible human, makes it a major opportunistic pathogen, particularly *P. aeruginosa* serogroup 11 and possibly serogroup 9. Infection with *P. aeruginosa* is usually associated with hospitalized patients as a secondary infection (nosocomial infections). Also susceptible are infants, individuals with suppressed immune systems from chemical therapy, and older people with reduced natural barriers to infection. Bacteremia attributable to *Pseudomonas* has become a major concern in the management of trauma as well as in the management of susceptible patients recovering from burns, intensive surgery, and others exposed to cancer therapy. *P. aeruginosa* is a cause of severe epidemic diarrhea of infants, ocular infections, cystic fibrosis, spa- and whirlpool-associated folliculitis, osteomyelitis, and malignant external otitis.

RESERVOIRS FOR THE AGENT

Pseudomonas species are ubiquitous bacteria able to flourish in a wide variety of habitats (e.g., surface water, aquifers, bottled water, distilled water, seawater, soils, and vegetation). *P. aeruginosa* has been isolated from cold waters only following storms, suggesting soil and vegetation may be reservoirs of these bacteria found in surface waters.



Source: Centers for Disease Control and Prevention.

Figure 16-1 Scanning electromicrograph of *Pseudomonas aeruginosa*

MODE OF TRANSMISSION

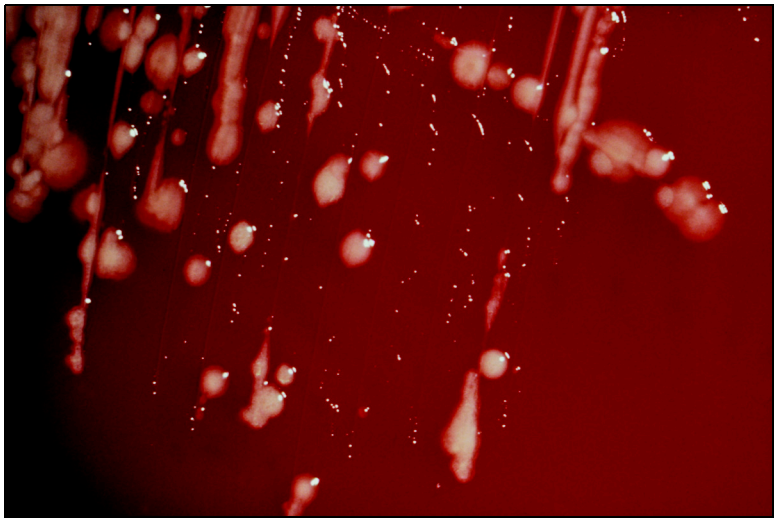
The route of exposure to *Pseudomonas* may be ingestion of food or water, body contact with water supply during bathing, or person-to-person contact in hospitals.

METHODS FOR DETECTING THE AGENT

Most species can grow in mineral base medium without growth factors. Growth is often optimal at 15 to 30°C, although *P. aeruginosa* grows well at 41.5°C. Membrane filtration and multiple tube fermentation (MTF) techniques are available for the enumeration of *P. aeruginosa* in water samples. M-PA agar is used in the membrane filter procedure with incubation at 41.5°C for 72 hours (Figure 16-2). Typical colonies are flat in appearance with light outer rims and brownish to green–black centers. Confirmation involves milk agar incubated at 35°C for 24 hours. *P. aeruginosa* hydrolyzes casein and produces a yellowish to green diffusible pigment. Asparagine broth is used in the MTF test with incubation at 35°C for 24 hours. Production of a greenish fluorescent pigment constitutes a positive presumptive test. Confirm positive tubes in acetamide broth for a purple color within 36 hours at 35°C incubation. Efforts to improve sensitivity and rapidity of detection methods for *P. aeruginosa* include molecular-based polymerase chain reaction (PCR), DNA fingerprinting, pulsed-field gel electrophoresis (PFGE), genotyping, and biotyping.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

The infrequent occurrence (3 to 19 percent) of *Pseudomonas* in the human intestinal tract suggests that colonization of the gastrointestinal system rarely occurs in healthy adults, possibly due to potent host-defense mechanisms against this group of bacteria. Because municipal sewage contains a mixture of domestic wastes, industrial discharges, and intermittent stormwater runoff, *P. aeruginosa* will be found in 90 percent of sewage samples. While *P. aeruginosa* is the most significant species in some drinking



Source: Centers for Disease Control and Prevention.

Figure 16-2 *Pseudomonas aeruginosa* growing on a blood plate agar

water supplies, others found in the distribution system include *P. fluorescens*, *P. mallei*, and *P. putida*. *P. stutzeri* has been found in bottled waters. Bacteria previously identified as pseudomonads that have been found in distribution systems and bottled water include *Burkholderia cepacia* (formerly *P. cepacia*), *Stenotrophomonas maltophilia*, (formerly *P. maltophilia*), *Brevundimonas diminuta* (formerly *P. diminuta*), *Delftia acidovorans* (formerly *P. acidovorans*), and *Comamonas testosteroni* (formerly *P. testosteroni*).

Some pseudomonads are among the prominent denitrifiers. Others grow prodigiously in sand or carbon filtration beds and on tertiary treatment devices, such as reverse osmosis (RO) and electrodialysis membranes. Biofilm development in distribution system sediments and home water treatment devices attached to public water supplies are also important contributors. Water supply attachments used with dental equipment and poorly maintained whirlpools are also potential sources of these organisms.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Densities of *P. aeruginosa* in surface waters receiving waste and stormwater discharges may range from 1×10^1 to 1×10^4 cells per 100 mL and are influenced by available nutrients and seasonal water temperatures. Low-density subsistence of pseudomonads in bottled water and distilled water may continue for months because of the organism's ability to slow down metabolism and survive on trace amounts of carbon and nitrogen sources in the product water.

DOCUMENTED WATERBORNE OUTBREAKS

P. aeruginosa found in a contaminated water supply has been linked to one waterborne outbreak that occurred in a newborn nursery. The groundwater supply had become contaminated by seepage of sewage and infiltration of contaminated surface water. However, recently reported waterborne disease outbreaks related to *P. aeruginosa* are associated solely with swimming pools and/or hot tubs.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Pseudomonas organisms may grow prodigiously in the water–air interfaces of process basins, sand filters, granular activated carbon (GAC) beds, and distribution system sediments unless proper measures are taken to control their colonization. Frequent removal of scum, careful control of backwashing procedures, and sediment removal in the pipe network are essential. This implies that distribution systems must be adequately flushed to maintain a disinfectant residual above 0.5 mg/L at the distal ends of the system. Home water treatment devices using carbon filters or RO and electrodialysis membranes can also be amplifiers of these organisms unless attention is given to careful maintenance of these units.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

Because *P. aeruginosa* is the most prevalent *Pseudomonas* in human disease, its occurrence has been limited to less than one organism in 250 mL of bottled drinking water by the European Community. There are no federal regulations on restricting *Pseudomonas* in drinking water supplies.

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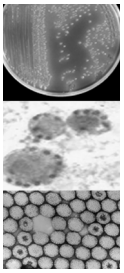


Chapter 17

Salmonella

Terry C. Covert and Mark C. Meckes

DESCRIPTION OF THE AGENT

The genus *Salmonella* is included in the family Enterobacteriaceae, which is a large and diverse group of bacteria found in soil, water, wastes, plants, and the normal flora of animals. Over 2,000 *Salmonella* serotypes are listed by the Kauffmann-White antigenic scheme. Salmonellae are facultatively anaerobic, non–spore-forming, gram-negative bacilli, 2 to 5 µm long and 0.8 to 1.5 µm wide, usually motile by peritrichous flagella, except *S. pullorum* and *S. gallinarum*. Gas is usually produced from glucose, nitrates are reduced to nitrites, citrate is used, and most *Salmonella* produce hydrogen sulfide. Salmonellae are positive for lysine decarboxylase and ornithine decarboxylase, with the important exceptions of *S. paratyphi A* and *S. typhi*. Lactose is usually not fermented, with the exception of *S. arizonae*. Salmonellae yield negative Voges-Proskauer and positive methyl red tests and do not produce cytochrome oxidase.

DESCRIPTION OF THE DISEASE

The genus *Salmonella* includes a wide variety of serotypes pathogenic for humans or animals, and usually for both. Three clinically distinguishable forms of salmonellosis occur in humans, including gastroenteritis, enteric fever, and septicemia. Gastroenteritis is an infection of the colon usually occurring 18 to 48 hours after ingestion of the organism. It is characterized by diarrhea, fever, and abdominal pain. The infection is usually self-limiting, lasting 2 to 5 days. Enteric fever is most often caused by *S. typhi* (typhoid fever) and the paratyphoid bacilli, *S. paratyphi A*, *B*, and *C*. Enteric fever from *S. typhi* is more prolonged and has a higher mortality rate than paratyphoid fever. Symptoms include sustained fever, diarrhea, abdominal pain, and may involve fatal liver, spleen, respiratory, and neurological damage. Typhoid fever symptoms persist for 2 to 3 weeks. Enteric fevers from other than *S. typhi* have a shorter incubation period, 1 to 10 days, compared to 7 to 14 days for typhoid fever, and the symptoms are less severe. *Salmonella* septicemia is characterized by chills, high remittent fever, anorexia, and bacteremia. Organisms may localize in any organ of the body and produce focal lesions resulting in meningitis, endocarditis, pneumonia, or osteomyelitis.

RESERVOIRS FOR THE AGENT

Reservoirs of *Salmonella* are domestic and wild animals, including poultry, swine, cattle, birds, rodents, and pets such as tortoises, turtles, chicks, dogs, and cats. Humans also serve as a reservoir (e.g., convalescent carriers and those with asymptomatic infections). The occurrence of chronic carriers is rare in humans but is common in birds and animals.

MODE OF TRANSMISSION

Infection from *Salmonella* occurs through ingestion of food, milk, or water contaminated with feces from infected hosts or by ingestion of the infected meat products. *S. typhi* and *S. paratyphi* only colonize humans; therefore, infection with these organisms indicates exposure to human feces. Most often this involves ingestion of the organisms from food or water contaminated with human excreta. Unlike *S. typhi* and *S. paratyphi*, nontyphoidal *Salmonella* spp. are widely distributed in nature and closely associated with animals. Consumption of contaminated poultry, meat products, and eggs are the most common sources of transmission. Meat products can become contaminated with fecal matter during slaughter. Improper storage or undercooking allows the organisms to proliferate. *Salmonella* infection is also widespread in rodents. When the bacteria are excreted in the feces, contamination of food and water permits transmission of the infection to humans. Person-to-person, fecal–oral transmission does occur and has been a problem in health care facilities traced to inadequate hand washing.

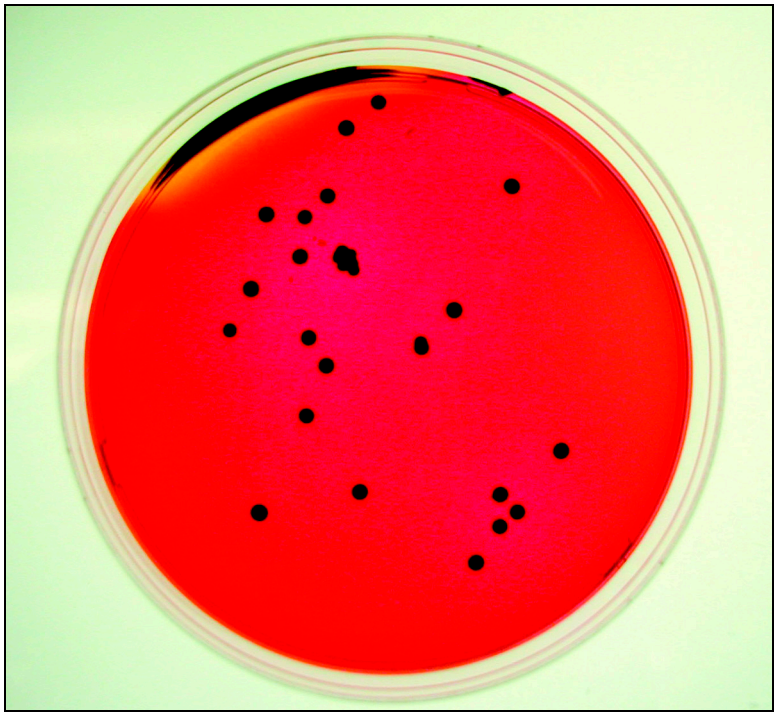
METHODS FOR DETECTING THE AGENT

Selective enrichment is needed for isolation of salmonellae from samples containing mixed bacterial flora (i.e., feces, food, and environmental). Selective enrichment increases the ratio of *Salmonella* to other bacterial cells. Three selective enrichment media commonly used are tetrathionate broth, selenite broth, and Rappoport-Vassiliadis (RV) medium. The semisolid modification of Rappaport-Vassiliadis medium (MSRV) has been shown to work well with food and environmental samples. Numerous selective plating media have been used to isolate *Salmonella*. These media are differential and vary from highly selective to low selectivity. Laboratories should include a highly selective medium along with low and or moderately selective media when isolating salmonellae. Salmonellae grow readily on routine selective, differential media forming 2- to 3-mm diameter colonies in 24 to 48 hours (Figure 17-1). Colonies may be circular with a smooth surface and an even edge, or flat with an uneven surface and serrated edge. The temperature range of growth is from 10 to 43°C, with an optimum temperature of 37°C (Figure 17-2). Elevated incubation temperatures, including 40, 41.5, and 43°C, help suppress background growth and may improve detection of salmonellae but also suppress growth of some serotypes, including *S. typhi*.

After primary isolation on selective media, presumptive *Salmonella* isolates can be tested with commercial identification systems or screened with triple sugar iron agar, urea broth, and lysine iron agar. Isolates with *Salmonella* biochemical profiles should be tested with commercial polyvalent O group, H, and Vi antisera. With environmental samples, a large sample volume usually should be examined (1 L or more). Concentration of the organisms can be accomplished using Moore swabs, membrane filtration, diatomaceous earth, or large-volume samplers.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Salmonellosis represents a major communicable worldwide disease problem. The annual occurrence of typhoid fever is estimated at 17 million cases, with 600,000



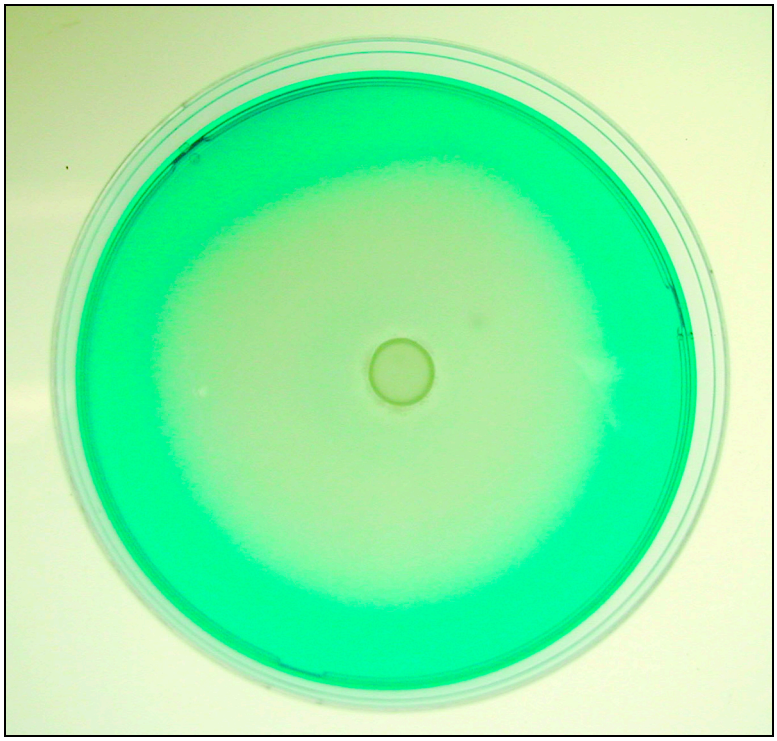
Source: T.C. Covert and M.C. Meckes, USEPA.

Figure 17-1 *Salmonella typhimurium* colonies on XLD agar

deaths. In contrast to typhoid fever, with humans being the sole source of the organism, animals and animal products are major sources of other *Salmonella*. Animal husbandry studies have shown approximately 50 percent of US chickens are culture positive for *Salmonella*. Cold-blooded animals such as turtles are also carriers and sources of infection. Large numbers of *Salmonella* may be present in contaminated surface water and waste treatment plant influents and effluents. Studies have shown that 80 percent of activated sludge treatment plant effluents and 58 percent of contaminated surface waters may contain *Salmonella*.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Bacterial survival in aquatic ecosystems is affected by numerous factors, including protozoa, antibiosis, organic matter, algal toxins, dissolved nutrients, ultraviolet (UV) light, heavy metals, and temperature. Recent studies of survival of enteric pathogens in aquatic environments have shown these organisms may enter a viable but nonculturable physiological state. Because the vast majority of detection and enumeration techniques require culturing using selective media, the detection of viable organisms may be compromised. Salmonellae are frequently isolated in polluted waters and can persist in high-nutrient waters. Wastewater treatment reduces but does not eliminate indicator bacteria and *Salmonella*. Reported *Salmonella* levels range from 1 to 1,100/100 mL from nondisinfected wastewater effluents. *Salmonella* discharged in the effluents from municipal wastewater treatment plants were reported to survive for an extended time in the nutrient-rich water of the Red River of North Dakota during the sugar beet processing season.



Source: T.C. Covert and M.C. Meckes, USEPA.

Figure 17-2 Growth of migrated *Salmonella typhimurium* cells on Rappaport-Vassiliadis (MSRV) medium, semisolid modification

DOCUMENTED WATERBORNE OUTBREAKS

The vast majority of waterborne outbreaks of salmonellosis are classified as acute gastrointestinal illness of unknown etiology. Waterborne outbreaks in the United States usually involve poor-quality source water, inadequate treatment, or contamination of the distribution system (e.g., cross-connections). Large outbreaks of waterborne salmonellosis have not been reported in the United States, with the exception of the Riverside, Calif., *S. typhimurium* gastroenteritis outbreak in 1965, which affected 18,000 people. The water supply was implicated, but the source of contamination was not determined. During 1976 to 1980, 223 cases of waterborne salmonellosis were reported from small water suppliers and private wells. In 1993 a waterborne outbreak of *S. typhimurium* in Gideon, Mo., affected more than 650 persons and resulted in 7 deaths. Contamination was traced to a water storage tower with defective roof vent covers that allowed free access by birds.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Disinfection is an important barrier in prevention of human exposure to pathogens in drinking water. Chlorination is effective for inactivating *Salmonella* in properly maintained distribution systems using conventional treatment (coagulation, sedimentation, filtration, and disinfection). Chlorine residuals need to be at least 0.2 mg/L throughout the distribution system including the distal ends of the distribution system. Early studies have shown that *S. typhi* is as susceptible to chlorine disinfection as *Escherichia coli*.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

There are no permissible levels of *Salmonella* in drinking water. Because many waterborne pathogens are difficult to detect or enumerate in water and the methods are often impractical to use for routine monitoring, the US Environmental Protection Agency (USEPA) and World Health Organization (WHO) have adopted total coliform, fecal coliform, and *E. coli* microbiological standards for drinking water. These organisms are used as indicators of water treatment efficacy, distribution system integrity, and recent fecal contamination. The coliform group of organisms are present in the normal intestinal flora of humans and warm-blooded animals and are eliminated in large numbers in fecal wastes. The presence of fecal coliform or *E. coli* in a drinking water supply usually is interpreted as evidence of possible presence of enteric bacterial pathogens. Implicitly, routine coliform monitoring is to provide an alert against treatment barrier failure.

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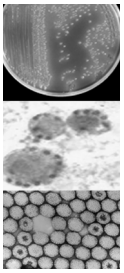


Chapter 18

Serratia

Edwin E. Geldreich
Revised by Jon Standridge

DESCRIPTION OF THE AGENT

The *Serratia* genus is composed of small, gram-negative, motile coccobacilli that characteristically give positive reactions for citrate, motility, Voges-Proskauer, ONPG (see glossary), and fermentation of mannitol and trehalose. Lactose is fermented slowly or not at all. *Serratia marcescens* is the type species and produces a nondiffusible red pigment that is more pronounced when incubated at temperatures between 25 and 30°C (Figure 18-1). Most pathogenic *Serratia* strains isolated in clinical laboratories are nonpigmented. Other strains are plant pathogens found in the environment.

DESCRIPTION OF THE DISEASE

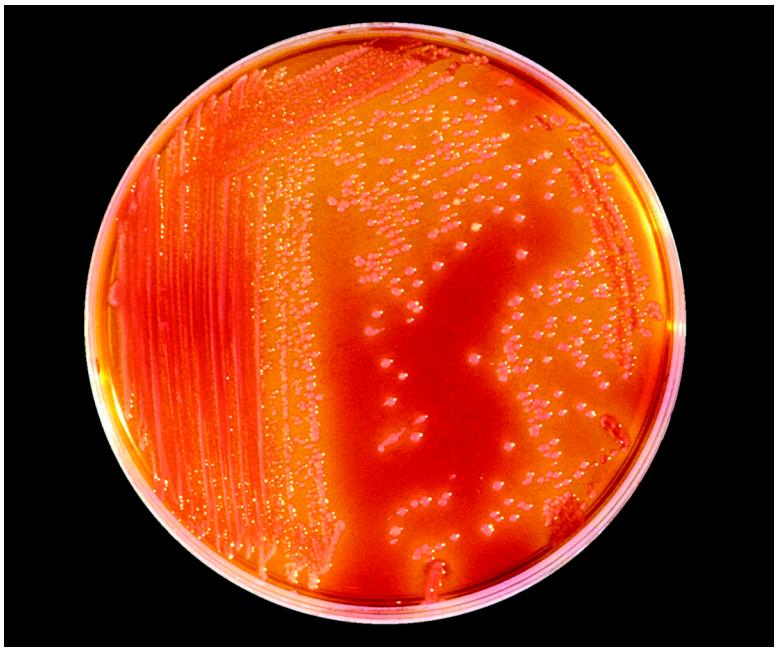
S. marcescens, *S. odorifera*, *S. rubidaea*, and the subgroup *S. liquefaciens* are recognized as potential opportunistic pathogens that may spread in epidemic proportions causing nosocomial infections in hospital patients. *S. marcescens* is a frequent cause of infections ranging from cystitis to life-threatening bloodstream and central nervous system infections. In one case, *Serratia* was isolated from the feces of a 2-month-old infant who had developed diarrhea. The antibody titer to this organism in the feces increased eightfold while the antibody titers against five "O" groups of *Escherichia coli* remained unchanged, indicating *Serratia* was the causative agent. Because many hospitals harbor multiple antibiotic-resistant strains of *S. marcescens*, resistance may be transferred to various pathogens in the clinical environment.

RESERVOIRS FOR THE AGENT

Serratia strains are considered to be ubiquitous in the environment. They may be found in surface and groundwater, soil, decaying vegetation, insects, decaying meat, and spoiled milk.

MODE OF TRANSMISSION

Serratia strains are spread by person-to-person contact and by contaminated water from sumps in hospital equipment. *S. marcescens* infections have been transmitted via medical solutions and peritoneal-dialysis effluents. Two studies on the contribution of



Source: Centers for Disease Control and Prevention.

Figure 18-1 An agar culture plate cultivated gram-negative rod-shoped, and anaerobic *Serratia marcescens* bacteria

water-supply-associated bacteria and patient or consumer illness suggest that water-supply-derived organisms are part of the problem but not necessarily the major source of nosocomial infections in the hospital environment or in the drinking water at the home tap.

METHODS FOR DETECTING THE AGENT

Heterotrophic plate count (HPC) media will often reveal the presence of *Serratia* in water samples when incubated on a nondifferential medium (i.e., R2A medium) in the range of 20 to 30°C using either the membrane filtration (MF) procedure or a spread plate technique. There are no differential media specifically for detecting *Serratia*. Serological typing, biotyping, bacteriocin typing, phage typing, plasmid analysis, polymerase chain reaction amplification of enterobacterial repetitive intergenic consensus (ERIC-PCR) sequences, RAPD-PCR, and ribotyping have all been used as epidemiological tools in medical facility outbreak investigations.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Serratia and other pigmented bacteria appear to occur seasonally in high-quality waters (such as private wells, distribution systems, finished reservoir supplies, and bottled water). Colonization may occur in a variety of attachment devices, including drinking water fountains, ice machines, point-of-use treated water, laboratory high-quality water systems, humidifying units, and hemodialysis equipment. *Serratia* have also been found in the heterotrophic bacterial population on media substrate in granular

activated carbon (GAC) filters. Densities in water are variable, most often being less than 100 organisms/mL unless predominate colonization occurs in a biofilm.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Decaying vegetation is often a niche for the survival of *Serratia* in the watershed. Cannibalism of *Serratia* cells may continue for months, with rises and falls of the population. Persistence in tap water is about 100 days, and much longer in contaminated well water. In distilled water, Serratia may survive for 48 days at room temperature.

DOCUMENTED WATERBORNE OUTBREAKS

While more than 50 hospital outbreaks of infection have been due to *S. marcescens*, no waterborne outbreaks have been documented in the literature.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Serratia frequently can be isolated from treated water because these organisms are more chlorine-resistant than many nonpigmented aerobic, heterotrophic bacteria. They may also enter the distribution system via soil contamination during line breaks and their repairs. Because this organism passes through conventional treatment barriers, control is best achieved by reducing sediment accumulations in pipe networks and water storage reservoirs. Removing scum around process basins will suppress habitat sites for *Serratia* and many other organisms.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

No national or international guidelines limit the occurrence of *Serratia* in the water supply.

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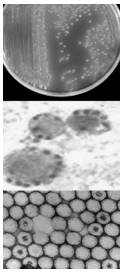


Chapter 19

Shigella

Nelson P. Moyer
Revised by Alan J. Degnan

DESCRIPTION OF THE AGENT

The genus *Shigella* of the family Enterobacteriaceae consists of the four species (serogroups) *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C), and *S. sonnei* (serogroup D). Serogroups A, B, and C are subdivided into 12, 13, and 18 serotypes, respectively.

Shigella species have been taxonomically related to *Escherichia coli* by DNA–DNA hybridization studies. Shigellae are facultatively anaerobic, chemoorganotrophic, nonsporulating, nonmotile, oxidase-negative, gram-negative rods, 0.3 to 1 µm in diameter and 1 to 6 µm in length, appearing singly, in pairs, and in short chains. The optimum temperature for growth is 37°C. When grown on blood agar, colonies are translucent, circular, convex, and measure 1 to 2 mm in diameter.

DESCRIPTION OF THE DISEASE

Shigella species cause acute gastroenteritis and dysentery in humans by invading the intestinal mucosa. Shigellosis is characterized by diarrhea, fever, nausea, vomiting, and cramps. Illness ranges from mild, self-limiting diarrhea to toxic megacolon and hemolytic uremic syndrome. Most cases in the United States are caused by *S. sonnei*. The incubation period for shigellosis is usually 1 to 3 days. While mild cases of shigellosis are self-limiting, antibiotic treatment is necessary in severe cases. Antimicrobial susceptibility tests must be performed before therapy, because multiple drug-resistant strains are becoming prevalent in the United States. Organisms are detectable in stool for up to 1 week after symptoms resolve. Immunity from natural infection is short-lived and no effective vaccine is available.

RESERVOIRS FOR THE AGENT

Infected humans are the only significant reservoir.

MODE OF TRANSMISSION

Transmission occurs primarily through direct or indirect fecal–oral contact with patients or carriers. The infective dose is low, between 10 to 100 bacteria. Fecally

contaminated water, food, and milk also transmit disease. Flies can transfer bacteria from feces to foods.

METHODS FOR DETECTING THE AGENT

Methods for the reliable recovery of shigellae from the environment are not yet available. Membrane filtration (MF) and centrifugation have been used to concentrate environmental samples, with and without subsequent broth enrichment cultures. GN broth enrichment (see glossary) has been used to recover *Shigella* from stools and other samples where they may be present in low numbers. Recently, polymerase chain reaction (PCR) has shown promise for detection of shigellae in environmental samples. Diagnosis of shigellosis is made by recovery of the shigellae from stool or rectal swabs on differential and selective culture media, such as MacConkey agar or xylose lysine deoxycholate (XLD) agar. Clear colonies from MacConkey agar and colorless to pinkish colonies from XLD agar plates are inoculated into triple sugar iron (TSI) and lysine iron agar (LIA) slants that are incubated for 18 hours at 35°C. Presumptive *Shigella* cultures appear as nonmotile, gas negative, hydrogen negative isolates producing alkaline slants and acid butts in TSI and LIA. A small amount of growth from the LIA slant is suspended in normal saline and used to conduct slide agglutination tests with homologous antisera for serogroups A, B, C, and D. Serotyping is usually restricted to outbreak investigations. *Shigella* species are biochemically differentiated from *E. coli* by the lysine and motility tests. Novel methods currently under evaluation for detection of *Shigella* species include antimicrobial susceptibility testing, pulsed-field gel electrophoresis (PFGE), and phage typing, among others.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

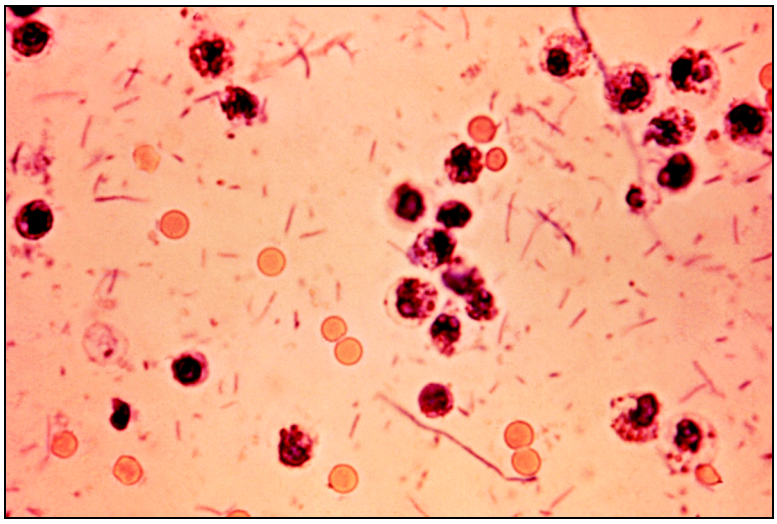
Shigellosis occurs worldwide and is most common in areas where sewage treatment and personal hygiene are poor to nonexistent (Figures 19-1 and 19-2). Most cases in the United States occur in children, and secondary transmission to household contacts is common. Outbreaks of shigellosis occur in institutional settings, such as day-care centers, mental hospitals, nursing homes, and refugee camps, from contamination of food and water, and by person-to-person contact. Shigellosis is associated with travel to developing countries where sanitation is lacking. Shigellosis outbreaks may also occur in hogans (Native American dwellings) and colonies (Latin America border communities) where flowing water supply is not available.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Shigellae do not survive well at acid pH, so buffered transport media at pH 7.4 to 7.6 are required if clinical specimens are not processed immediately after they are collected. Shigellae are sensitive to chlorination at normal levels, and they do not compete favorably with other organisms in the environment. They persist up to 4 days in river water. Viable but nonculturable forms have been reported in estuarine waters, but the infectivity of nonculturable shigellae is unknown.

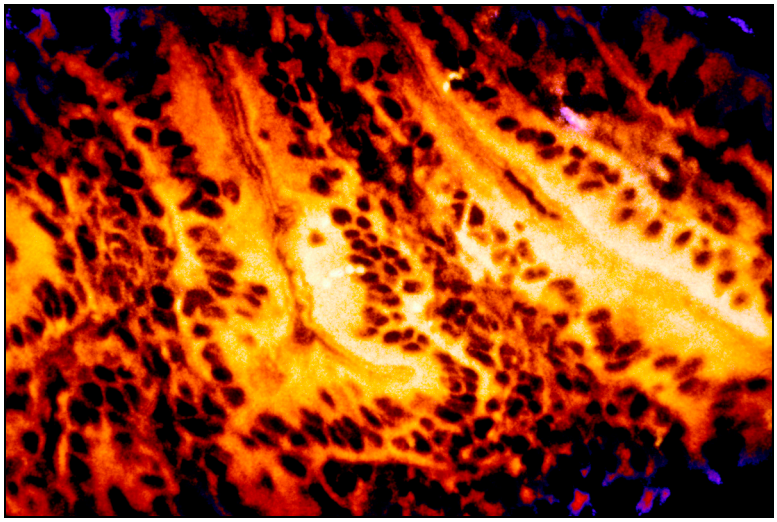
DOCUMENTED WATERBORNE OUTBREAKS

Waterborne outbreaks of shigellosis most commonly result from fecal contamination of nonchlorinated private and noncommunity water supplies. Treatment deficiencies, septic tank contamination of wells, or cross-connections between wastewater and potable water lines are most commonly implicated in drinking water outbreaks. The availability of running water in the home is key to reducing the spread of cases through



Source: Centers for Disease Control and Prevention.

Figure 19-1 Stool excudates in a patient with shigellosis; also known as *Shigella* dysentery



Source: Dr. S. Formal, Walter Reed Army Institute of Research and Centers for Disease Control and Prevention.

Figure 19-2 *Shigella* sp. bacteria penetrating the intestinal mucosa

poor personal hygiene habits. Outbreaks are also associated with recreational exposure to fecally contaminated swimming and wading pools, and polluted surface water such as lakes and ponds. Sewage contamination of shellfish beds has caused outbreaks of foodborne disease associated with oysters. Shigellae caused 17 of 21 (81 percent) of drinking water associated outbreaks, and 12 of 71 (17 percent) of recreational water associated outbreaks reported to the Centers for Disease Control and Prevention (CDC) between 1985 and 1994. Sixteen states reported waterborne shigellosis outbreaks during this 10-year period. Drinking water outbreaks occur year-round, while recreational water outbreaks typically occur in summer months between May and

September. *S. sonnei* was implicated in 11 of 12 drinking water outbreaks and in 16 of 17 recreational water outbreaks. One outbreak each in drinking and recreational water was caused by *S. flexneri*, and *S. boydii* was recovered from victims of one of the recreational water outbreaks (Mississippi River near Dubuque, Iowa) caused by *S. sonnei*. Although waterborne shigellosis outbreaks persist globally (i.e., Israel, Japan, and Greece), outbreaks have not occurred in recent years in the United States.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

Shigella species are enteric pathogens of humans and should be absent from the environment. No occurrence of this pathogenic agent in food or water is considered safe. Isolation of cases, stool precautions, hand washing, and proper diaper and sewage disposal are the primary control mechanisms for prevention of shigellosis. Water and wastewater treatment processes incorporating disinfection are sufficient for inactivation of shigellae.

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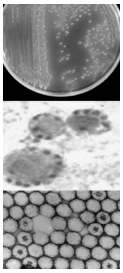


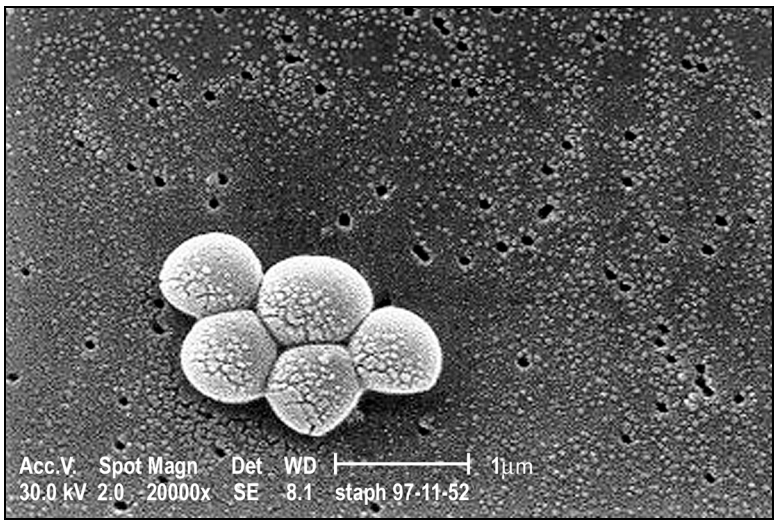
Chapter 20

Staphylococcus

Edwin E. Geldreich
Revised by Jon Standridge

DESCRIPTION OF THE AGENT

Staphylococci are gram-positive cocci that may occur as single cells, in pairs, packet clusters, or as short chains of several individual cells (Figure 20-1). Species in this genus are usually nonmotile, catalase positive, and ferment glucose. Some strains encapsulate or form a slime layer. While most species are facultative anaerobes, some strains of *Staphylococcus aureus* grow more favorably in aerobic environments. All strains of this species are potential pathogens. *S. aureus* strains are coagulase positive and often form a yellow to orange pigmentation on some media after 2 to 3 days of incubation, although certain antibiotic-resistant strains may vary in the intensity of color development. Of the coagulase negative species, *S. epidermidis* and *S. saprophyticus* have been associated with human infections.



Source: J. Biddle, Centers for Disease Control and Prevention.

Figure 20-1 Scanning electron micrograph of methicillin resistant *Staphylococcus aureus* bacteria

DESCRIPTION OF THE DISEASE

S. aureus, *S. epidermis*, and *S. saprophyticus* may be the opportunistic pathogens associated with infections of the skin (cellulitis, pustules, boils, carbuncles, and impetigo), bacteremia, peritonitis associated with dialysis, genitourinary infections, and postoperative wound infections. *S. aureus* may also cause meningitis, osteomyelitis, and violent diarrhea and vomiting from ingestion of the enterotoxin caused by the organism growing in food. *S. aureus* concentrations in drinking water can be a health concern for individuals in contact with water for extended periods, such as from dishwashing, whirlpool therapy, and dental hygiene. Densities of 200 to 400 cocci/mL have been shown to set up a carrier state in the nose of 50 percent of newborn infants, and an *S. aureus* density of a few hundred cells/mL in a water contact may induce infection in traumatized skin.

RESERVOIRS FOR THE AGENT

Major reservoirs of staphylococci include warm-blooded animals (in the skin, nose, ear, and mucous membranes), sewage, and stormwater runoff. *S. aureus* and other species of this genus are a major component of the normal human flora of the skin and to a lesser extent in fecal wastes.

MODE OF TRANSMISSION

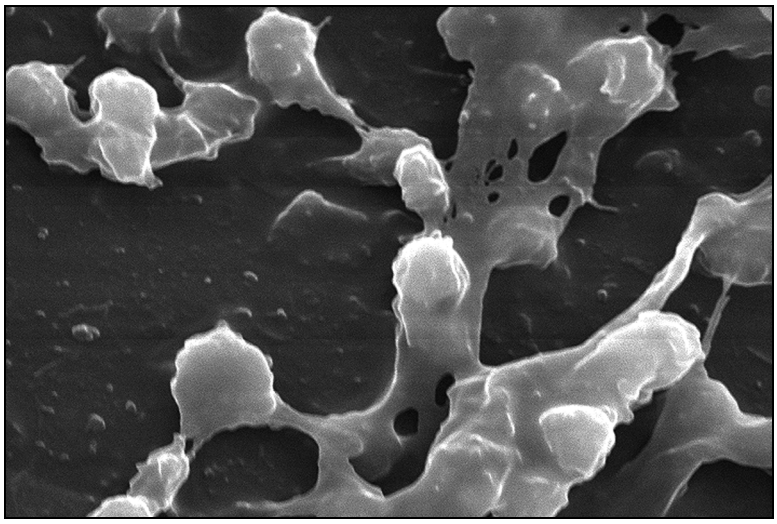
The major concern with *S. aureus* in water transmission is contact with cuts and scratches on the skin, infecting the ears or the eyes during bathing, or in water used to prepare uncooked foods. Ingestion is the pathway for gastrointestinal infections from contaminated foods, or for individuals on intensive antibiotic therapy.

METHODS FOR DETECTING THE AGENT

Pigmented *Staphylococcus* colonies can be seen on heterotrophic plate count (HPC) cultures done on samples taken from the water distribution system. A better characterization of the extent of these organisms in drinking water will require the use of more selective media and procedures such as described in *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association, Water Environment Federation, and American Water Works Assocation, current edition). The recovery of *S. aureus*, *S. epidermis*, and *S. saprophyticus* should be emphasized rather than total staphylococci in the heterotrophic bacteria population. Unfortunately, no specific media are available for these three opportunistic pathogens. Some success has been reported for the use of M-staphylococcus broth in a modified multiple-tube procedure or Baird-Parker agar in the membrane filter (MF) procedure. Presumptive results are verified by placing pure cultures from the MF into a commercial multitest system for biochemical reactions. Any turbid tubes in the multiple-tube test are confirmed in Lipoviettin-salt-mannitol agar streak plates. Conventional polymerase chain reaction (PCR) and real-time PCR protocols have been used extensively in medical facility staphylococci outbreak investigations. These same methods could theoretically be applied to any waterborne outbreak event.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

The density of coagulase positive *Staphylococcus* (i.e., *S. aureus*) ranges from 10^2 to 10^4/g in the normal human fecal flora, but occurrence is quite variable (10 to 93 percent) (Figure 20-2). Coagulase negative strains of *Staphylococcus* may be found in



Source: R.M. Donlan, PhD; J. Carr; and Centers for Disease Control and Prevention.

Figure 20-2 Highly magnified electron micrograph of *Staphylococcus aureus* bacteria found in an indwelling catheter

31 to 59 percent of feces from healthy people. Because of their presence in feces and on human skin, staphylococci are not surprisingly the most numerous bacteria shed by swimmers in natural bathing waters and chlorinated swimming pools. In one study, approximately two thirds of the staphylococci in bathing waters were *S. aureus*. Densities of *S. aureus* in both studies ranged from a few to several hundred cells per 100 mL. *S. aureus* was found to be one of the two most concentrated opportunistic pathogens in urban stormwater, ranging from 10 to 1,000 organisms/mL. In a study of private water supplies in Oregon, *S. aureus* was isolated from more than 6 percent of 320 rural water supplies. Inspection of HPC data on these samples suggested a 63 percent *S. aureus* occurrence for HPC above 300 organisms/mL. Colonization sites of these and other opportunistic pathogens in public and private water supplies are most often aerator screens on faucets. In public and private water systems, persistence or growth may be localized in slow-flow pipe sections and dead ends where sediments accumulate.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

In drinking water, *Staphylococcus* may persist at 20°C for 20 to 30 days, provided trace amounts of organic nutrients are available. Growth in water is slow at temperatures below 20°C, and merely at subsistence rate below 10°C.

DOCUMENTED WATERBORNE OUTBREAKS

S. aureus and several other staphylococci species are opportunistic pathogens in the hospital environment, and water supply may play a subordinate role in disease transmission. However, no waterborne outbreaks caused by staphylococci, or *S. aureus* in particular, have been documented. The health risk of staphylococci associated with exposure to poor-quality small water systems and private water supplies is undocumented.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Numerous reports suggest the staphylococci are more resistant to ultraviolet (UV) light radiation at ambient temperature than *Salmonella* and *Escherichia coli*, and require 45 minutes or more contact time. Filtration of surface waters may entrap significant numbers of these heterotrophic bacteria, but some will pass through water treatment barriers. Systematic flushing of distribution systems to reduce the nutrient source for these and other opportunistic bacteria is important. Hospital maintenance crews must periodically flush the building's water supply lines and clean all water supply attachment devices on a scheduled basis to suppress colonization of staphylococci and other opportunistic bacteria.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

No national or international guidelines limit this opportunistic pathogen in water supply.

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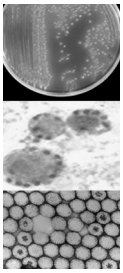


Chapter 21

Vibrio cholerae

Gary A. Toranzos and Alan Toro
Revised by Alan J. Degnan

DESCRIPTION OF THE AGENT

Vibrio cholerae are gram negative, slightly curved or twisted rods, 1.5 to 3 µm in length and about 0.5 µm in width (Figure 21-1). They are motile by a single flagellum, and may occur singly or in chains which appear as short spirals. *Vibrio* are alkaline tolerant, and therefore media used to select for *Vibrio* in mixed cultures might be as high as pH 9.6. Colonies on alkaline peptone agar may be distinguished from those of *Escherichia coli* by their relatively thin, translucent appearance at 24 hours. Cells maintained long term on agar usually lose the curve and appear as straight rods, under microscopic examination.

More than 130 *V. cholerae* serogroups have been reported, generally divided into the categories 01 and non-01. Until 1992, only the toxigenic 01 had been associated with cholera epidemics and pandemics.

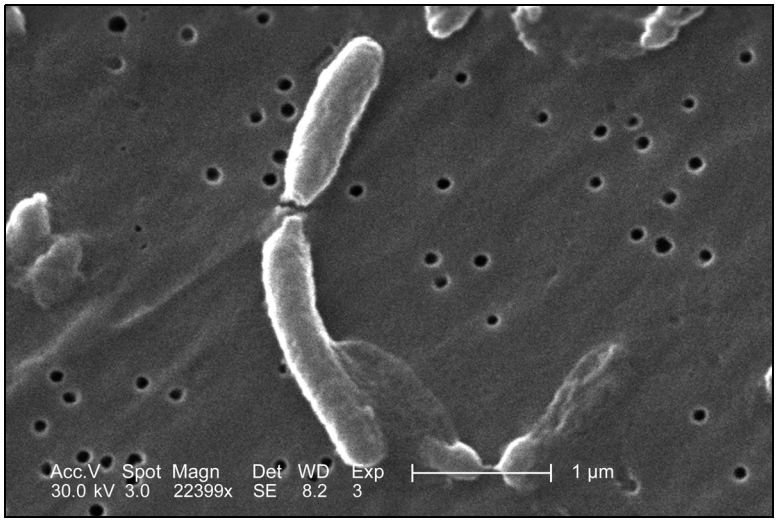
However, O139 has also been isolated in Argentina (Faruque et al. 1997) and Mexico (Parveen et al. 2003). In both of these instances, however, although the isolates were serologically O139, they were negative for the presence of the cholera toxin. The public health risk as a result of the presence of these microorganisms and their role in the environment remain to be seen. Nonetheless, public health workers should be aware of the possible presence of this serotype and thus clinical *Vibrio* spp. isolates should be tested against the appropriate sera.

DESCRIPTION OF THE DISEASE

V. cholerae causes infections ranging from asymptomatic to profuse watery diarrhea, known as "rice water stools," and vomiting, which lead to rapid and severe dehydration and possibly death within 1 to 5 days. Under extreme circumstances, death may occur within 2 to 24 hours. The rapid rates of fluid loss and electrolyte imbalance make imperative the necessity for rehydration therapy.

RESERVOIRS FOR THE AGENT

V. cholerae has been shown to survive for extended periods in environmental waters, especially where feces from infected individuals may be introduced (i.e., discharge of ship ballast waters). In nature, *V. cholerae* may be associated with zooplankton,



Source: J. Carr and Centers for Disease Control and Prevention.

Figure 21-1 Scanning electron micrograph of two separating *Vibrio cholerae* bacteria

sediments, and shellfish that are grown in polluted waters. Toxigenic 01 strains were shown to survive in aquatic environments for years in the United States and Australia, even in total absence of fecal contamination. Studies have shown that US Gulf Coast marshes may be an environmental reservoir for *V. cholerae* 01. But the most important reservoir may still be symptomatic and asymptomatic human carriers who shed the microorganisms in their feces.

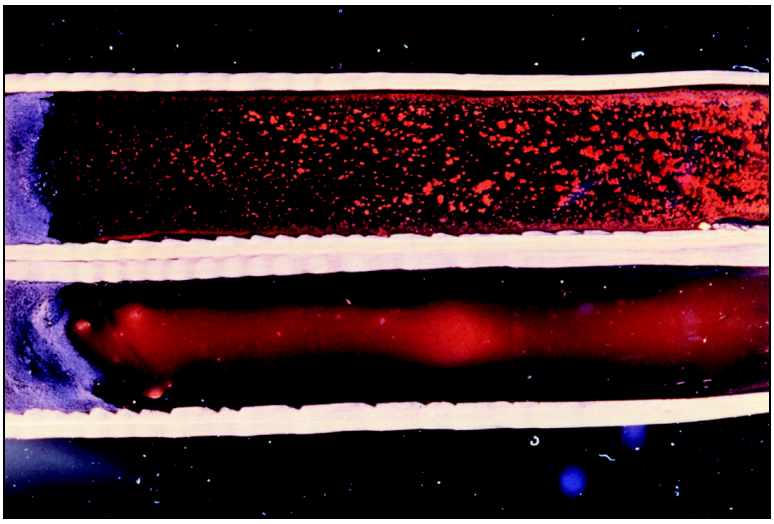
MODE OF TRANSMISSION

Contaminated food seems to be the most prevalent mode of transmission for *V. cholerae*, but contaminated water used to wash foodstuffs can serve as the starting point of an epidemic. Most cases of cholera in the United States have been associated with the consumption of raw shellfish and undercooked seafood, especially oysters. No person-to-person transmission has been documented, possibly as a result of the high infectious dose required for *V. cholerae*. However, the microorganism may grow to infectious concentrations in low-acid foods (i.e., rice) if introduced after cooking by contaminated food handlers.

Drinking water may become a vector for *V. cholerae* whenever conditions of long-term storage, lack of disinfectants, and exposure to human contact occur.

METHODS FOR DETECTING THE AGENT

The most commonly used medium for the isolation of *V. cholerae* is alkaline peptone water (APW) at a pH of 8.4. Modifications to APW to increase selectivity include addition of 1 to 3 percent NaCl, plus 0.05M Tris buffer (pH 8.4) to maintain alkaline pH range. *V. cholerae* are typically present in low concentrations in environmental waters, and thus large volumes (Š1 L) are used for sampling. In low-turbidity waters, membrane filtration is used, while in turbid waters, a gauze pad (Moore swab) is placed in the stream for approximately 48 hours. Filters or pads are then placed into APW and incubated at 35°C for up to 24 hours. At increments of 6, 15, and 18 hours, aliquots from the upper portion of the APW are streaked onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar. Typical *V. cholerae* appear as yellow colonies; however, further



Source: Centers for Disease Control and Prevention.

Figure 21-2 A chick RBC test on a slide used to diagnose cholera

confirmation via biochemical assay or polyvalent sera is necessary for confirmation (Figure 21-2). Molecular techniques for toxigenic strains of *V. cholera* are currently being used more for strain characterization than for detection.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Contaminated human feces remain the predominant reservoir for *V. cholerae*, while such factors as low pH and temperature in the environmental probably cause populations to remain low. However, concentrations up to 10^5 and 10^7 cells/mL were observed in raw sewage in Peru and in association with a hospital treating cholera victims, respectively. Low-acid foods contaminated after cooking (i.e., rice) have been shown to be the source of several cholera outbreaks.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Conditions such as pH and moisture strongly influence the survival of *V. cholerae* in the environment. Studies have shown that 90 percent of microorganisms seeded into freshwater were inactivated after 18 hours, while in seawater, such inactivation occurred at 95 hours, a fivefold increase in survival time.

DOCUMENTED WATERBORNE OUTBREAKS

Cholera is typically a disease of poor sanitation and/or high population densities. Waterborne outbreaks account for all the large epidemics that have occurred in the last century. Table 21-1 lists selected, relatively recent, documented waterborne outbreaks.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Chlorine disinfection at levels used in conventional drinking water treatment is very effective for *V. cholerae* inactivation, as are other types of disinfection. Strains

Table 21-1 Selected recent cholera outbreaks worldwide

Year	Location	Vehicle	Cases	Deaths
2005	Burundi, Africa	Heavy rainfall	105	5
2004	Niger, Africa	Heavy rainfall	137	5
2002	Malawi, Africa	Groundwater	22,023	609
2001	Afghanistan	N/A	4,499	114
1998	Peru	Heavy rainfall	2,863	16
1988	Delhi, India	Groundwater	N/A	1,500
1975	Portugal	Bottled water	2,467	48

producing a "rugose" colony morphology seem to be more resilient to chlorine disinfection. *V. cholerae* has not been shown to survive for long under the conditions of treated effluents, thus proper treatment of waste is important.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

According to the International Health Regulations, every member country must immediately notify the World Health Organization (WHO) of any cholera cases. Active surveillance of clinical cases should be ongoing and information on cases or probable cases of cholera should be exchanged at the local, national, and international levels.

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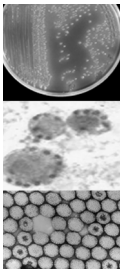


Chapter 22

Yersinia

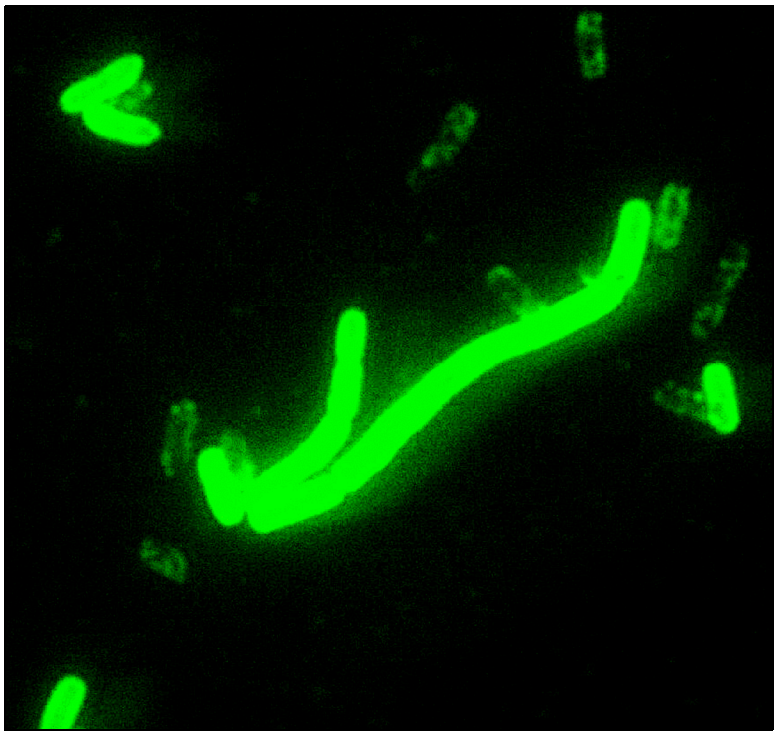
Colin R. Fricker

DESCRIPTION OF THE AGENT

The genus *Yersinia*, family Enterobacteriaceae, was named after the French bacteriologist Yersin. It is composed of at least 11 species at present, the type species of which is *Y. pestis* (Figure 22-1). However, the taxonomy is confused and further work is required to establish the exact taxonomic position of several members of the genus. As with other members of the Enterobacteriaceae, the yersinias are facultatively anaerobic, gram-negative, nonsporulating, oxidase-negative rods that ferment glucose with production of acid. The cells are 0.5 to 0.8 µm in diameter and 1 to 3 µm in length, in some ways resemble *Pasteurelleae*, and can be pleomorphic depending on the age of culture and the medium on which it was grown. All species are nonmotile when grown at 37°C, but motile by means of peritrichous flagella when grown below 30°C, except for *Y. pestis* which is never motile. The yersinias will grow on simple media and tolerate bile salts.

DESCRIPTION OF THE DISEASE

The yersinias are responsible for a plethora of diseases in man and animals. In humans the disease may occasionally be present as enteritis or enterocolitis, but more frequent symptoms are a septicemia and generalized disease or more commonly acute inflammation of the mesenteric lymph glands (referred to as pseudoappendicitis). *Y. enterocolitica* was first isolated from a human skin ulcer in 1939 and is now recognized as an important cause of gastrointestinal disease in human beings. The spectrum of disease in animals is similar to that encountered with *Y. pseudotuberculosis*, and the organism has been associated with abortion in sheep. *Y. enterocolitica* can be responsible for acute septicemias not unlike those caused by *Y. pseudotuberculosis* but is far more commonly associated with gastroenteritis. The disease is usually found in children under the age of seven, tends to occur mainly in autumn and winter, and is characterized by fever, diarrhea, abdominal cramps, and sometimes vomiting. The symptoms often last for about 2 weeks, but the disease is usually self-limiting, although secondary immunological complications occur fairly frequently. In older children and adults, *Y. enterocolitica* usually causes a terminal ileitis and the pseudoappendicitis syndrome.



Source: L. Stauffer, Oregon State Public Health Laboratory and Centers for Disease Control and Prevention.

Figure 22-1 Direct fluorescent antibody stain of *Yersinia pestis*; magnification 200×

RESERVOIRS FOR THE AGENT

While *Y. pestis* and *Y. pseudotuberculosis* are specific pathogens of man and animals, *Y. enterocolitica* and some other species are normally saprophytic and can be found in animal feces where they occur as part of the normal flora and in soil and water. In addition, many foods may be contaminated with *Y. enterocolitica;* pork and unpasteurized milk in particular have been implicated as causes of outbreaks of disease. The yersinias certainly occur as saprophytes in freshwater systems, and they have been isolated from slow sand filter beds where they appear to be capable of growth. Essentially, the yersinias can be found where one might expect to encounter coliform organisms.

MODE OF TRANSMISSION

Y. enterocolitica is transmitted by the fecal–oral route, and various foodstuffs, milk, and water have been implicated as vehicles of disease. In addition, increasing evidence shows that gastrointestinal disease due to these organisms may be a zoonosis. Sick puppies were implicated as the source of an outbreak in the United States.

METHODS FOR DETECTING THE AGENT

Most yersinias will grow on laboratory culture media designed for the growth of coliform bacteria but cannot easily be differentiated from other coliforms. They ferment carbohydrates with the production of acid (but seldom gas) and are oxidase negative and catalase positive. For example, some strains are positive for ONPG (see glossary) and will grow well in media designed to detect coliforms. A further alkali

treatment prior to plating has been used by some workers to reduce competing flora. There are many variations on the time and temperature of incubation, particularly of enrichment broths, but no comprehensive study has been undertaken to determine optimum conditions for recovery of yersinias from water. Identification of isolates growing in Colilert from treated water samples in the United Kingdom has shown that the incidence of yersinias in such waters is negligible.

The preferred medium for detecting yersinias from water is by membrane filtration (MF) followed by incubation on CIN agar at 32°C for 24 hours. Typical colonies of *Yersinia* will develop as a dark-red bull's-eye surrounded by a zone of transparency. Some strains of *Yersinia* fail to grow and some coliforms may also give this bull's-eye appearance. Considerable care should be taken in identifying any colonies that form. An alternative approach is to use an enrichment culture. Test portions can first be incubated in a preenrichment medium at pH 8.3 at low temperature, either 15°C for 2 days or 4°C for 7 days, followed by enrichment in bile-oxalate-sorbose broth, which should be incubated at 25°C for 3 to 5 days. Methods for the detection of yersinias using polymerase chain reaction (PCR) have also been developed, but these have not been widely used and are of doubtful benefit.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Yersinia have been isolated from a wide variety of different water sources, and no particular seasonal difference is seen in the frequency of their isolation. Furthermore, in the limited number of studies that have looked at other water parameters, such as pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and salt concentration, no correlation was seen. Additionally, yersinias were present in the absence of "coliform" organisms, although some strains of *Yersinia* are regarded as coliforms (e.g., UK definition). Yersinias have also been found fairly frequently in chlorinated drinking water supplies, particularly in Europe, where in most cases they were present together with fecal coliforms. From the limited serotyping data available, strains most often found in water are not those commonly associated with human disease.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Many strains of *Yersinia* are able to grow at low temperatures (e.g., 4°C) and in laboratory experiments they were shown to survive periods in excess of 1 year at 4°C. They will also survive and in some cases grow in waters up to 25°C, reaching levels as high as 10^3 colony-forming units (cfu)/mL. Their presence has been demonstrated in a wide variety of water types with different levels of eutrophication and salinity and in sewage. They are often found in samples of sewage sludge and, although the major pathogenic strains are not frequently encountered, some strains survive for periods greater than 1 year.

DOCUMENTED WATERBORNE OUTBREAKS

Yersinias are recognized mainly as foodborne pathogens but may often be found in water. The low occurrence of yersinias in treated (chlorinated) drinking water has meant that disease associated with consumption of fully treated drinking water has not been reported. Apparent outbreaks of *Yersinia*-related disease have occasionally been associated with consumption of untreated water, the largest of which occurred in Montana in 1977. In this outbreak, no clinical evidence of *Yersinia* infection was demonstrated and no yersinias were isolated from patient stools. The tentative diagnosis of *Yersinia* infection was based solely on the finding of various strains of yersinias in

water that had been consumed by those affected. The likelihood of detecting small outbreaks of *Yersinia*-related disease is low because most laboratories do not specifically look for yersinias in patient stools. If a low incidence of waterborne yersiniosis occurs, it is likely to be unrecognized. Larger waterborne outbreaks associated with treated water are unlikely because the organisms are mostly removed by water treatment. If water were contaminated after treatment (e.g., by cross-connection), other etiological agents would be far more likely to be recognized than yersinias.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Yersinias appear to have a similar sensitivity to chlorine as *Escherichia coli*, thus routine chlorination should be adequate for control of the presence of yersinias in treated drinking water and distribution systems. Strain-to-strain variation in chlorine sensitivity has been seen, but this has not been shown to present a significant risk. As with other bacteria, there is a relationship between chlorine concentration, water temperature, and rate of inactivation, with higher temperatures resulting in more rapid cell death. Suboptimal chlorine treatment has been shown to yield cells that will grow in liquid media but not on agar substrates. However, the chlorine susceptibility of these organisms suggests that routine water treatment, including an adequate level of chlorination, is likely to prevent the risk of *Yersinia* infection.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

While no regulatory standards have been established specifically for yersinias in the United States or elsewhere, depending on the definition used, some yersinias may be detected as coliforms. Because the procedures for detecting yersinias specifically are so poor, a regulation covering yersinias will not likely be implemented in the foreseeable future.

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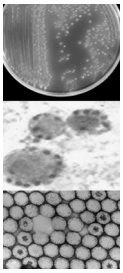


III

Introduction to Parasitic Pathogenic Agents

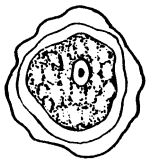
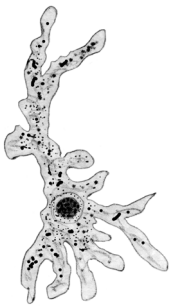
Acanthamoeba spp.
Ascaris lumbricoides
Balamuthia mandrillaris
Balantidium coli
Blastocystis hominis
Cryptosporidium parvum and *Crytosporidium hominis*
Cyclospora cayetanensis
Entamoeba histolytica
Giardia lamblia
Isospora belli
Microsporidia
Naegleria fowleri
Schistosomatidae
Toxoplasma gondii
Trichuris trichiura

Waterborne parasites, including various kinds of worms and protozoa, are acknowledged to be the leading identifiable agents of diseases acquired from drinking water. Worldwide, waterborne parasites have played a major role in shaping history, and they continue to make their mark on human endeavor. Many cause acute effects such as diarrhea and bloody urine, but some infections can result in chronic conditions including dementia, underdevelopment in children, cancer, and death. Close relatives of the flat worms that cause swimmer's itch are responsible for serious human disease in Africa, Asia, and Central and South America. Schistosomiasis is endemic in 74 developing countries and ranks second only to malaria in terms of socioeconomic and public health importance in tropical and subtropical countries. Wide-scale urinary tract infection with *Schistosoma haemotobium* among French troops was probably one of the factors in Napoleon's failed Egyptian campaign in 1799. The protozoa comprise a large group of extremely diverse unicellular organisms, and while there are many pathogenic species, only a few are currently recognized as potential problems for the water industry. Antonie van Leeuwenhoek first observed *Giardia lamblia* in 1681 from his stools, using the recently invented microscope, but it was not until the 1960s that *Giardia* was recognized as a significant cause of human disease. *Cryptosporidium parvum* was first described by Ernest Tyzzer in 1912, but was not acknowledged as a significant threat to human health until the 1980s. In the last 20 years, these two protozoa have caused waterborne disease outbreaks cumulatively affecting hundreds

of thousands of people; up to 60 percent of all *Giardia* infections are caused by drinking contaminated water. Other pathogenic protozoa may represent potential waterborne health threats but there have either been no documented outbreaks or small outbreaks, and isolated cases have occurred sporadically.

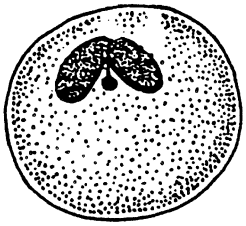
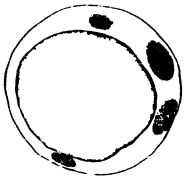
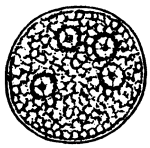
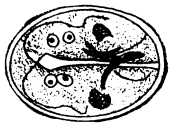
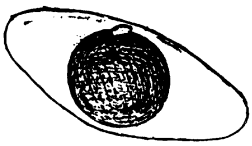
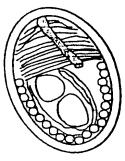
Some of these organisms are common natural inhabitants of aquatic environments while others enter bodies of water as a result of fecal contamination. A few are opportunistic pathogens, but most are frank pathogens infecting individuals regardless of their immune status, although the ultimate disease outcomes may be different. The incidence of carriage in the general population may be relatively high for some of these parasites, even in developed nations. Importantly, many of the parasitic protozoa form cyst or oocyst stages that allow them to survive in the environment and make them resistant to some drinking water treatment practices. With the exception of *Cryptosporidium* and *Giardia*, there are no approved methods for detecting protozoa in water. Consequently, waterborne parasites will continue to play a major role in human life. The water industry must always aspire to mitigate against parasites such as those described in this section as well as those that will be recognized as agents of waterborne disease in the future.

Table III-1 Parasitic pathogenic agents discussed in section III

Organism	Diagnostic or Infective Stage	Physical Appearance	Occurrence
Acanthamoeba spp.		15–20-µm star-shaped, hexagonal, polygonal, or spherical cyst	Ubiquitous; worldwide; Keratitis syndrome per recreational water activities and contact lens wearers
Ascaris lumbricoides		Ellipsoid 35–50 × 50–70-µm fertilized egg with knobby albuminous coat	Worldwide; most common in warm countries and where sanitation is poor
Balamuthia		15–30 µg, spherical cyst characterized by three layers in its wall; an outer ectocyst, a middle mesocyst, and an inner endocyst	Worldwide

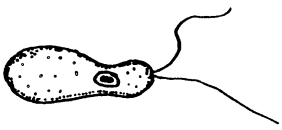
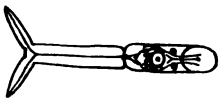
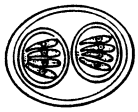
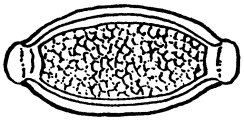
(Table continued on next page)

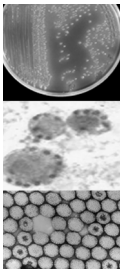
Table III-1 Parasitic pathogenic agents discussed in section III (continued)

Organism	Diagnostic or Infective Stage	Physical Appearance	Occurrence
Balantidium coli		Spherical or oval 50–70-µm cyst. Macronucleus and micronucleus can be obscured. The largest intestinal protozoan of humans	Worldwide; incidence of infection in human host is low
Blastocystis hominis		6–40-µm spherical cyst, characterized by a large, central body surrounded by small, multiple nuclei	Worldwide
Cryptosporidium parvum		Spherical, thick-walled, 4–6-µm oocyst containing 4 sporozoites	Worldwide
Cyclospora cayetanensis		Spherical 8–10-µm oocysts unsporulated and sporulated. The sporulated oocyst is the infectious stage	Worldwide
Entamoeba histolytica		Spheroid 10–20-µm cyst with a refractive hyaline cell wall containing 4 nuclei; may contain chromatoid body	Worldwide
Giardia lamblia		Round to oval 8–18-µm long × 5–16-µm wide cyst containing 2 to 4 nuclei and distinctive axoneme	Worldwide
Isospora belli		Ellipsoidal unsporulated and sporulated oocyst 20–30-µm long × 10–19-µm wide	Worldwide; tropical regions of the Western Hemisphere
Microsporidia *Enterocytozoon bieneusi* *Encephalitozoon (Septata) intestinalis*		Spore size ranging from 1.5 to 2.5 µm	Ubiquitous; worldwide

(Table continued on next page)

Table III-1 Parasitic pathogenic agents discussed in section III (continued)

Organism	Diagnostic or Infective Stage	Physical Appearance	Occurrence
Naegleria fowleri		10–35-µm limax-like amoeba trophozoite	Worldwide; associated with aquatic activities
Family Schistosomatidae		Cercaria breaks out of snails into water	Cosmopolitan; United States, Canada, Europe, Mexico, Central America, Japan, Malaya, and Australia
Toxoplasma gondii		10-µm × 12-µm mature oocysts with 2 sporocysts containing 4 sporozoites each	Worldwide
Trichuris trichiura		22–25-µm × 50–55-µm ovoid fertilized egg; at each tip is a translucent plug.	Worldwide



Chapter 23

Acanthamoeba spp.

Govinda S. Visvesvara and Hercules Moura

DESCRIPTION OF THE AGENT

Small, free-living amoebae belonging to the genus *Acanthamoeba* are mitochondria-bearing eukaryotic protozoa that normally live in soil, fresh water, brackish water, and even in sewage and sludge. They feed on bacteria and multiply in their environmental niche as free-living organisms. They cause infections of the human brain, lungs, skin, and eye and feed on human tissue (Figure 23-1). Because of their ability to live as free-living organisms in nature and as pathogens living within their hosts, they are also called amphizoic amoebas. Because they cause a fatal disease of the central nervous system (CNS) called granulomatous amoebic encephalitis (GAE) in humans, especially in immunocompromised patients, they are often referred to as opportunistic pathogens. Additionally, *Acanthamoeba* spp. have also been known to harbor/support many different pathogens such as *Legionella,* mycobacteria, *Francisella tularensis, Chlamydia*, etc., and certain viruses, which accentuate the public health importance of these organisms.

The genus *Acanthamoeba* consists of as many as 20 species that are placed in three groups based on their morphology. Unlike *Naegleria fowleri*—the only species of *Naegleria* that causes human disease—several species of *Acanthamoeba* (e.g., *A. castellanii*, *A. culbertsoni*, *A. divionensis*, *A. healyi*, *A. rhysodes*, *A. hatchetti*, *A. polyphaga*, etc.) are known to cause infection in humans.

Acanthamoeba has two stages in its life cycle—the trophozoite and the cyst. *Acanthamoeba* trophozoites measure 15 to 45 µm and are characterized by the presence of fine, tapering, spine-like projections, called *acanthapodia*, from the surface of the body, which are periodically protruded and retracted. The trophozoites usually have one nucleus with a large, dense nucleolus. *Acanthamoeba* divide by conventional mitosis, in which the nucleolus and the nuclear membrane disappear during cell division. Numerous mitochondria, ribosomes, and vacuoles are present within the cytoplasm. *Acanthamoeba* does not have a flagellated stage but differentiates into a cyst during adverse conditions. The cysts are uninucleate and double-walled with a wrinkled, proteinaceous outer ectocyst and an inner cellulose-containing endocyst that may be stellate, polygonal, oval, triangular, or round. Pores or ostioles are present at the point of contact between the ecto- and the endocyst, and the pores are usually covered by an operculum.

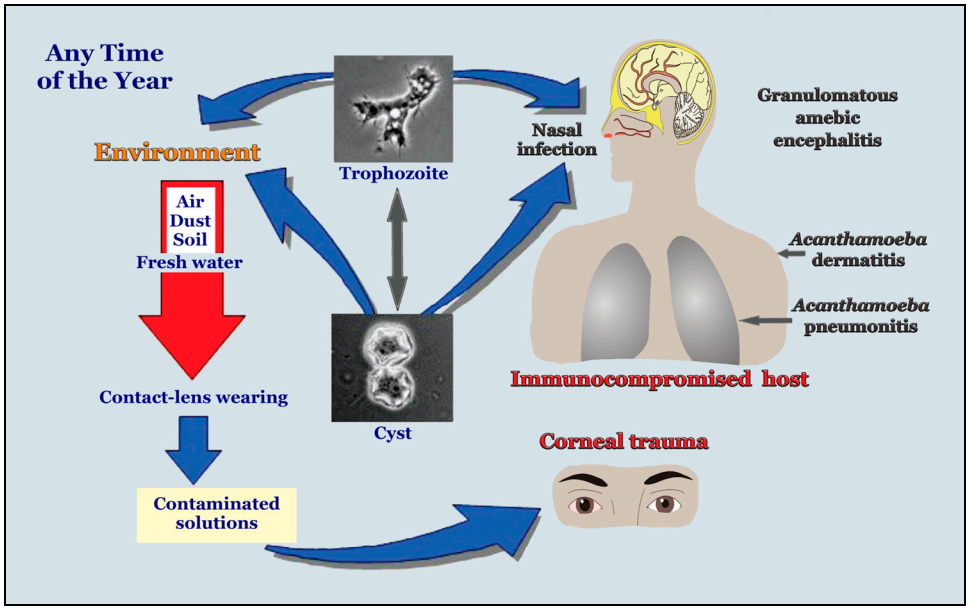


Figure 23-1 Infections due to *Acanthamoeba* spp.

DESCRIPTION OF THE DISEASE

Several species of *Acanthamoeba* (*A. castellanii*, *A. culbertsoni*, *A. rhysodes*, *A. polyphaga*, *A. divionensis*, and *A. healyi*) cause a chronic and usually fatal GAE and skin lesions that may last for several weeks to even months. GAE is a slowly progressive CNS disease with no clear-cut incubation period. It has an insidious onset, lasting from a few days to several weeks or months. The most common clinical symptoms include headache, irritability, confusion, seizures, dizziness, drowsiness, and sometimes diplopia, lethargy, and hemiparesis. Some patients, especially those with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), develop skin abscesses, lesions, or erythematic nodules. Microscopically, the lesions consist of hemorrhagic necrosis of CNS parenchyma, with variable amounts of subacute and chronic inflammatory reaction, with amoebic trophozoites and cysts around and within the walls of blood vessels. Because of a granulomatous reaction, the disease caused by *Acanthamoeba* is called GAE. Trophozoites and cysts may be found in liver, lungs, kidneys, prostate, lymph nodes, skin, and other organs, suggesting hematogenous dissemination.

More than 150 cases (>100 in the United States) of granulomatous disease caused by *Acanthamoeba* with and without the involvement of skin lesions have been reported from around the world. Though many of these patients have been treated with a number of antiviral, antibacterial, antimycotic, and antiprotozoal therapeutic agents, except in three documented cases, all have died.

Acanthamoeba spp. also cause a painful vision-threatening disease of the cornea, *Acanthamoeba* keratitis (AK). If the infection is not treated promptly, it may lead to a chronic ulceration of the cornea, loss of visual acuity, and eventual blindness and enucleation. More than 3,000 cases of AK have been reported worldwide. The principal risk factor for AK is the use of soft contact lenses and nonsterile homemade saline in developed countries and ocular trauma in developing countries.

AK is characterized by severe ocular pain, a 360° or partial paracentral stromal ring infiltrate, recurrent corneal breakdown of the epithelia, and a corneal lesion refractory to commonly used ophthalmic antibacterial medication. Although *Acanthamoeba* is

resistant to commonly used chemotherapeutic agents, especially in the cyst stage, even at very high concentrations some patients with AK have been treated successfully with topical application of a combination of drugs, including propamidine isethionate (Brolene), clotrimazole, neosporin suspensions, chlorhexidine gluconate, and polyhexamethylene biguanide (PHMB). Currently, the recommended method of treatment is PHMB or chlorhexidine with or without Brolene.

RESERVOIRS FOR THE AGENT

Although infection with *Acanthamoeba* has been documented in a number of animals such as gorillas, monkeys, bovines, ovines, dogs, horses, kangaroos, and even in invertebrates, no human or animal reservoir for *Acanthamoeba* spp. has been identified, possibly because no survey has been conducted to identify sources of such reservoir hosts. Both invertebrates, such as aquatic insects and their larvae, mollusks, and crustaceans, and all classes of vertebrates should be surveyed for their ability to act as reservoirs for these amoebas.

MODE OF TRANSMISSION

Unlike *N. fowleri*, no clear-cut and direct evidence shows that *Acanthamoeba* GAE can be directly acquired from water, though this is quite likely. The portal of entry in the case of GAE is probably through the lower respiratory tract, ulceration of the skin or mucosa, or any open wounds. CNS disease is probably acquired by hematogenous spread from a primary site, such as the respiratory tract or skin lesion. Recent cases of GAE in AIDS patients who first developed skin abscesses and later developed CNS symptoms strongly suggest secondary dissemination to the CNS from a primary location, such as skin.

AK, however, has been directly traced to the use of contaminated saline solution made by dissolving salt tablets with commercially available unsterile bottled water. There are also indications that AK has been acquired from recreational water (e.g., hot tubs, swimming pools, and lakes).

METHODS FOR DETECTING THE AGENT

Acanthamoeba spp. can be easily detected and isolated from the suspected source, such as soil, water, sewage, cerebrospinal fluid, and small pieces of tissue (e.g., brain, lungs, skin, and cornea). Samples should be collected aseptically and can be stored for short periods at room temperature, but never frozen. Personnel handling specimens should take appropriate precautions, such as wearing gloves, surgical masks, and working in a biological safety cabinet. Water samples should be sent to a qualified reference laboratory for testing.

Acanthamoeba can be easily isolated from suspected samples by inoculating them into nonnutrient agar plates coated with *Escherichia coli* or *Enterobacter aerogenes*. *Acanthamoeba* can be identified by the presence of acanthapodia in the trophozoites or its characteristic double-walled cysts. An immunofluorescence test is also available to detect *Acanthamoeba* in the tissue samples. A polymerase chain reaction (PCR) test is also available for its detection in contact lens paraphernalia and tissue specimens.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

GAE due to *Acanthamoeba* is a chronic illness that occurs primarily in immunosuppressed, chronically ill, or otherwise debilitated persons with no history of exposure to fresh water. A few cases, however, have occurred in healthy persons with no known

immunodeficiency or risk factors for HIV. *Acanthamoeba* is also known to cause CNS and other organ infections in canines, ovines, bovines, monkeys, kangaroos, rabbits, and even in fish and reptiles.

Members of the genus *Acanthamoeba* are ubiquitous and occur worldwide. They have been isolated from soil; freshwater lakes and ponds; sewage and sludge; dust in air; thermal effluents of power plants; swimming pools; hot springs; brackish water and seawater; ocean sediments; frozen water; tap water; bottled water; heating, ventilating, and air conditioning units; bacterial, mycotic, and mammalian cell cultures; vegetables; and mushrooms.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Acanthamoeba can survive indefinitely in the environment as cysts that are resistant forms and are able to withstand adverse conditions. For example, *Acanthamoeba* has been isolated from bottled water, ocean sediments, antarctic soil, and frozen swimming areas in Norway.

DOCUMENTED WATERBORNE OUTBREAKS

No definite outbreaks of *Acanthamoeba* encephalitis have been described; however, as agents of keratitis, *Acanthamoeba* has been identified in hot-tub water, bottled water, and contact lens cleaning and holding solutions, particularly homemade saline solution, indicating that these sources have the potential for transmission of *Acanthamoeba*.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Several factors, including pH, temperature, and biological and biochemical demands that influence the growth of amoebas, also influence the amoebicidal effects of chlorine and other disinfectants. Scattered reports based on experimental laboratory data suggest that *Acanthamoeba* spp. are more resistant to chlorine than *Naegleria* spp. Isolated reports of resistance of *A. culbertsoni* to high levels (40 mg/L) of chlorine exist; however, other reports indicate that chlorine levels of 1.25 mg/L chlorine is enough to kill *Acanthamoeba*, including *A. culbertsoni*. Of the four disinfectants used, deciquam 222 (a quarternary ammonium compound) was found to be the most effective amoebicide, followed by chlorine, chlorine dioxide, and ozone. Another compound, Baquacil (a 20 percent solution of PHMB), used in the disinfection of swimming pools in New Zealand, has been found to be a good amoebicidal agent. Purified PHMB is now being used therapeutically in AK cases.

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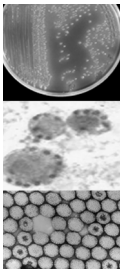

Chapter 24

Ascaris lumbricoides

Huw V. Smith, Anthony M. Grimason, and Celia Holland

DESCRIPTION OF THE AGENT

Ascaris lumbricoides, superfamily Ascaridoidea, is the large roundworm of humans. The parasite exists in three distinct forms, namely, the egg or ovum, the larva, which migrates through various organs of the body, and the adult, which resides in the intestinal tract. The sexes are separate. Similar species parasitize the pig, horse, dog, and cat. Parasitic nematodes have five consecutive developmental stages in their life cycle. The fertile egg develops into the first-stage larva, which molts to become the second-stage larva. The second-stage larva molts a further two times, into the third- and fourth-stage larva before it becomes adult. Both infertile (Figure 24-1) and fertile (Figure 24-2) eggs can be found in feces and the environment.

Adult females are fertilized in the intestine by adult males and have a massive propensity for egg laying. One adult female can lay in excess of 200,000 ova daily and can contain more than 25 million eggs at any one time. Fertilized eggs are excreted unembryonated in feces. Embryonation, whereby the fertilized, unsegmented embryo develops into a larva, occurs in the environment. A moist microclimate, ambient temperature, and protection from ultraviolet (UV) light encourage embryonation. Embryonation to the infectious egg (containing the second-stage [L2] larva within the eggshell) takes between 10 and 14 days, under ideal conditions. Following ingestion, the L2 in the infective egg is stimulated to hatch in the intestine. Hatched L2 penetrate the wall of the jejunum and, within a few days, migrate to the liver, via the vasculature draining the intestine, or the lymphatics. Migration, via the heart, to the pulmonary capillaries, ensues where larvae remain resident for a period of about 2 weeks and molt into third-stage (L3) larvae. Third-stage larvae migrate into the alveolar spaces, up the respiratory tree, and are swallowed. Acid-resistant L3 larvae pass through the stomach, undertaking the final molt into the L4 in the intestine, then grow rapidly. After about 8 weeks, adult female worms commence egg laying. When an adult female worm has mated with a mature male, fertile eggs are laid. The cycle from infection to egg laying adult normally takes 10 to 16 weeks.

The transmissive stage is the fertilized egg, and the infective stage is the fully embryonated egg which contains the L2 larva. Fertilized eggs, which do not contain a fully developed L2 larva, are not infective. The two commonly identified stages are (a) fertilized or unfertilized eggs and (b) adult male and female worms. Fertilized ova differ in morphology from unfertilized eggs. Fertilized eggs are excreted unembryonated and embryonate to infectivity (containing a fully developed L2) in the environment.

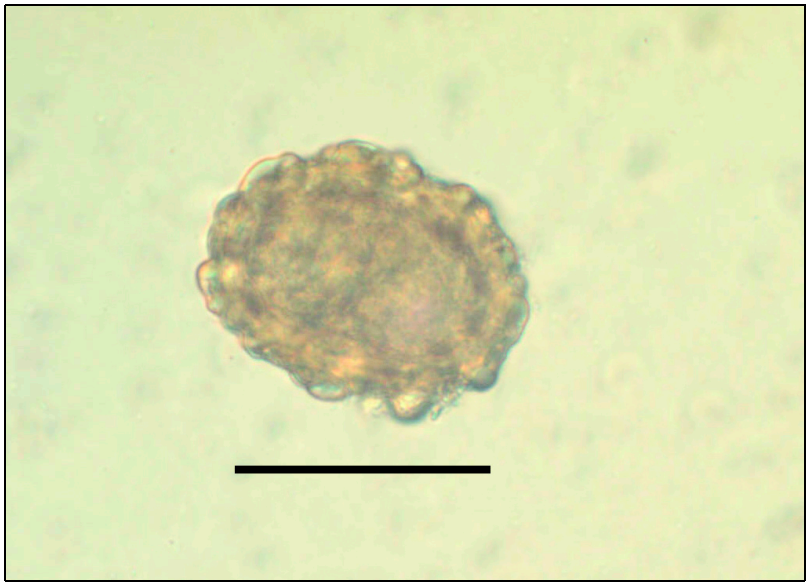

Figure 24-1 *Ascaris lumbricoides* infertile egg (bar represents 60 µm)

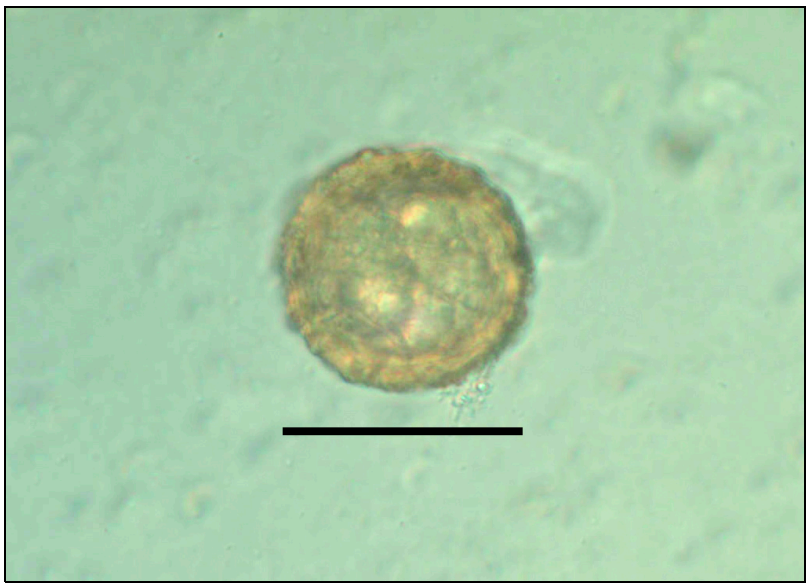

Figure 24-2 *Ascaris lumbricoides* fertile egg (bar represents 60 µm)

Eggs are oval to spherical in shape. Voided ova are generally a yellowish brown in color, ellipsoid (ovoid) to circular, measuring 35 to 50 µm by 50 to 70 µm. The fertile ovum is encased in a knobbly (mammilated), bile-stained outer albuminous coat, which protects the developing larva in the environment and, also being sticky, adheres to a variety of surfaces. Unfertilized eggs are elongated and larger than fertilized eggs, measuring 43 to 47 µm by 85 to 95 µm, have thin shells with a grossly irregular mammilated layer.

DESCRIPTION OF THE DISEASE

The prepatent period (2 to 3 months) is the interval of time between ingesting infectious eggs and the first appearance of ova in the stool. The incubation period is the interval of time between ingesting infectious eggs and the development of recognized symptoms or signs of illness. This can vary from a few days to several months depending upon the number of infectious ova ingested and the susceptibility of the individual. In light infections, individuals may remain asymptomatic.

Symptoms can be due to either migrating larvae, adult worms, or both and are more severe when the larval and/or adult worm burden is high. Pulmonary and intestinal infection, allergy to *Ascaris* allergens, and other complications can all give rise to symptoms. With high infectious doses (>2,000 ova), pneumonitis and liver enlargement can occur. Larval *A. lumbricoides* allergens stimulate local and peripheral eosinophilia and can produce facial edema. Adult worms may produce mild abdominal pain; however, individuals infected with low doses often only become aware of infection when ova are found in the stool or when an adult worm is passed either in the stool or migrates out of the mouth or nostrils. As the cycle from infection to egg laying adult female normally takes between 10 and 16 weeks, in these instances, the period between infection and recognition of symptoms can be greater than 16 weeks. Occlusion of other orifices associated with the alimentary tract, including the eustachian tubes, trachea, and bile and pancreatic ducts are also associated with adult *A. lumbricoides* infections. Intestinal obstruction can occur due to the presence of large numbers of intestinal adult worms, *A. lumbricoides* being responsible for 35 percent of intestinal obstruction in endemic areas. Chronic ascariasis is widely recognized as a contributory factor to impaired nutritional status, and infected children who receive anthelmintic treatment show increases in weight-for-age and height-for-age after treatment.

RESERVOIRS FOR THE AGENT

Humans are the reservoir for *A. lumbricoides*. Coprophagous animals such as pigs, dogs, cats, and chicken which feed on human feces, act as transport hosts by dispersing or redistributing fertile ova to other sites associated with human activities. Filth flies can also act as transport hosts because of their association with feces and their ability to travel over large distances. Filth flies ingest 1 to 3 mg feces over 2 to 3 hours. *Ascaris* ova have been found in surface water, groundwater, and seawater but not in treated drinking water. They have also been detected in night soil, sewage (Tables 24-1 and 24-2), sludge (Table 24-3), soil, particularly in the vicinity of houses, and on crops, utensils, and beaches.

Densities of *Ascaris* sp. ova can vary greatly in raw wastewaters and are indicators of endemic disease within the human and nonhuman population. Other factors include the size and socioeconomic status of the population, the percentage of the population sewered, shock loadings from the discharge of pit latrine tankers, the provision of separate or combined sewers, and seasonal and sampling factors. *Ascaris* ova occur more frequently and in significantly larger numbers than *Trichuris* ova.

Large numbers of *Ascaris* ova (up to 291,600 ova/L) have been reported in raw sewage, but the methods used currently underestimate their true occurrence and concentration. The use of different methods for sampling, concentration, and analyses make the data from different studies difficult to compare; however, lower concentrations of ova in raw and treated effluents are reported from developed countries rather than from developing countries.

MODE OF TRANSMISSION

Transmission can occur by any route where infective ova are ingested by a susceptible human host. This necessitates the external maturation of ova to infectivity in the environment, thus contact with recently voided feces is not a risk. In endemic areas, major routes of transmission include the following:

- defecation by infected individuals, especially children, in backyards or compounds, which leads to the contamination of soil, fingers, hands, and cooking utensils
- use of untreated human feces as fertilizer (night soil), especially for crops that receive minimal heating prior to consumption
- defecation by agricultural workers in or near to the fields in which they work (defecating in or near flowing water is often seen as being more sanitary than defecating into latrines where often no water supply is available for hand washing)
- use of untreated sewage effluent for irrigating crops and untreated and treated sludge for fertilizing crops that receive minimal heating prior to consumption
- consumption of contaminated unfiltered drinking water or communal drinking water
- redistribution of fertile ova to other uncontaminated sites associated with human activities by coprophagous animals such as pigs, dogs, cats, and chickens that feed on human feces.

METHODS FOR DETECTING THE AGENT

Detection of *Ascaris* ova, either in feces or in the environment, is by the morphological and morphometric examination of suspect organisms by bright field microscopy. The microscopic examination of a fecal smear or concentrate (e.g., formalin-ether or salt flotation) can reveal the presence of either fertile or unfertilized ova. A concentration technique is recommended for diagnosing light infections. Single-sex infection of adult male worms, or immature or old female adult worms, cannot be diagnosed microscopically by fecal examination because no ova are produced.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

A. lumbricoides appears to be specific to humans; however, mature worms can occur in nonhuman primates, and mammals can be infected experimentally. Human infections with *Ascaris suum* (from pigs) cannot be ruled out in endemic areas where pigs and humans live closely together as recent evidence from Central and North America indicates that cross-infection could occur even though the two parasite populations appear to be reproductively isolated.

The most recent estimates for the worldwide prevalence of ascariasis are 1,273 million infections (24 percent prevalence). Infections are generally acquired during the first or second year, peak in children aged 5 to 15 years, and then stabilize across the adult age classes. Prevalence can be influenced by climate, season, and socioeconomic environment. Climate can influence egg survival and embryonation; the prevalence of ascariasis is lower in arid African countries than in those with higher rainfall. The prevalence of ascariasis is intimately linked to the socioeconomic conditions; the poorer the conditions, the higher the prevalence of ascariasis. Factors such as housing type, water source, and availability of latrines show a significant relationship with *Ascaris* prevalence. In contrast to age-prevalence curves, age-intensity curves indicate

that intensity peaks in the 5-to-15-year-olds and declines markedly in adults. Adults that continue to be infected but their worm burdens are significantly lower than those of children. The number of worms within a host population are not distributed normally but follow an aggregated or overdispersed frequency distribution. Thus, the majority of hosts harbor few or no worms and the minority of hosts harbor heavy worm burdens. Clearly, the most heavily parasitized individuals are more at risk from morbidity and mortality, and contribute the most to environmental contamination and the transmission of infection.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Ascaris ova can occur commonly in the environment, densities being dependent upon the level of infection in the indigenous population. *Ascaris* ova can be found in surface water, groundwater, and seawater but not in treated drinking water. They can also be present in feces, night soil, sewage (Tables 24-1 and 24-2), sludge, soil, and on crops and beaches. The viability of *Ascaris* spp. ova exposed to a variety of environments is summarized in Table 24-3.

Table 24-1 Occurrence of *Ascaris* sp. ova in raw sewage

Country	*Ascaris* sp. ova and / or occurrence (% removal)
Argentina	0–1.66 ova/L
Bangladesh	59,800–291,600 ova/L, mean 211,122 ova/L
Brazil	192–1,757 ova/L
Canada	Mean 150 ova/L
Cayman Islands	32 ova/L; (5.5%)
China	0–79 ova/L; median 3 ova/L (82.3%)
Colombia	Mean 183 ova/L
Egypt	Mean 9.6 ova/L
France	Detected, numbers not stated*
Germany	38 ova/L
India	(Study 1) mean 3,770 ova/gal
India	(Study 2) $3.1 \times 10^5 - 8.4 \times 10^9$ ova/0.5 mgd
Iran	1,000–13,000 ova/L, mean 6,500 ova/L (100%)
Japan	10–80 ova/L
Kenya	61.5–133.5 ova/L
Mexico	Detected, numbers not stated*
Morocco	2,200 ova/L
Pakistan	50–68 ova/L, mean 60 ova/L
Puerto Rico	651 ova/17 L
Russia	60 ova/L
Syria	100–800 ova/100 mL
United States	5–110; mean 30 ova/L (100%)

* Data on the occurrence and removal of *Ascaris* sp. ova are normally presented as occurrence and removal of helminth ova. Some publications refer to *Ascaris* spp., others to *Trichuris* spp., and yet others to mixtures of both or mixtures of various intestinal helminth ova.

Table 24-2 Occurrence of *Ascaris* sp. ova in sewage effluents

Country	Treatment	*Ascaris* sp. ova and/or (% removal)
Bangladesh	OXFAM emergency double-vault septic tank	
	Tank 1 effluent	Mean 1,739 ova/L
	Tank 2 effluent	Mean 35 ova/L
	OXFAM permanent double-vault septic tank and mini-pond (n = 2) system	
	Double-vault effluent	9,120–25,000 ova/L; mean 22,084 ova/L (89.5%)
	Pond 2 effluent	2200–8,200 ova/L; mean 5,417 ova/L (97.5%)
China	Septic tank (3 compartments)	100%
	Sewage treatment	>85%
Brazil	Waste stabilization pond (pilot system)	
	Anaerobic pond effluent	0–50 ova/L
	Facultative pond effluent	0–15 ova/L
	Maturation pond 1 effluent	0–10 ova/L
	Maturation pond 2 effluent	0–33 ova/L
	Maturation pond 3 effluent	0–13 ova/L
	Upward anaerobic sludge blanket reactor effluent	Mean 11.76 ova/L
	Facultative pond effluent	Detected, numbers not stated*
Egypt	Trickling filter effluent	Mean 1.59 ova/L (79%)
	Trickling filter and gravel bed hydroponic	0 ova/L
India	Sedimentation	Mean 1,260 ova/gal (67%)
	Activated sludge	Mean 58.6 ova/L (93%)
	Trickling filter	95.7%
	Septic tank	Mean 3 ova/gal (99.4%)
	Septic tank and trickling filter	Mean 8 ova/L (83%)
	Septic tank, trickling filter, and chlorinator	Mean 1.1 ova/L (98%)
	Biological disc	79.2%
	Aerated lagoon	0–20 ova/L (70%)
	Oxidation ditch (pilot scale)	0–4 ova/L (84%)
	Waste stabilization pond 1 effluent	$0–2.6 \times 10^8$/0.5 mgd
	Waste stabilization pond 2 effluent	$0–1.1 \times 10^5$/0.5 mgd
Jordan	Stabilization pond system	5 ova/L
Kenya	Waste stabilization pond system	100%
	Sewage irrigation	85% (not stated)
Nigeria	Conventional plant (sedimentation, trickle filtration, activated sludge, secondary sedimentation)	100%

(Table continued next page)

Table 24-2 Occurrence of *Ascaris* sp. ova in sewage effluents (continued)

Country	Treatment	*Ascaris* sp. ova and/or (% removal)
Puerto Rico	Primary sedimentation	2.8 ova/L (93%)
	Sedimentation	35–74%
	Activated sludge and secondary sedimentation	<1 ova/L (97–100%)
	Trickling filter (pilot scale)	<1 ova/L (94.7–99.8%)
Russia	Sedimentation	20 ova/L
	Trickling filtration	13 ova/L
	Trickling filtration and secondary sedimentation	2 ova/L
	Stabilization pond system	0.5 ova/L
South Africa	Sedimentation (settled sewage)	19 ova/L
	Trickling filter effluent	1 ova/L
	Trickling filtration and secondary sedimentation	1 ova/L
United Kingdom	Primary sedimentation	76%
United States	Aeration and sedimentation	2–30 ova/L; mean 15 ova/L (50%)
	Final chlorination	0–20 ova/L; mean 6 ova/L (80%)

* Data on the occurrence and removal of *Ascaris* sp. and *Trichuris* sp. ova in sewage are normally presented as occurrence and removal of helminth ova. Some publications refer to *Ascaris* spp. others to *Trichuris* spp. and yet others to mixtures of both or mixtures of various intestinal helminth ova.

Ascaris ova are among the most environmentally resistant of intestinal pathogens. Because the embryo develops into the infective larva outside the body of the host, *Ascaris* ova are well suited to prolonged survival in the environment. The development of the embryo into the infective larva and its survival in the environment depend upon aerobic metabolism, but these ova can also survive in anaerobic conditions. *Ascaris* ova remain viable for months without oxygen, although their development is interrupted. Under conditions of ambient temperature (22 to 35°C), moisture, shade from UV light, and oxygen, embryonation to infectivity takes 12 to 14 days for *Ascaris* ova. At lower temperatures, embryonation takes longer. Infective ova of *Ascaris* can remain viable for several years. In temperate climates, *Ascaris* ova can remain viable for 7 years in moist, loose soils with moderate shade. Ova survive longer when buried in soil and clay soils, which retain moisture and enhance survival. *Ascaris* ova do not embryonate below 18°C, can remain viable for 43 days at –23°C, and can continue development when the temperature is elevated. In arctic tundra, *Ascaris* ova may remain viable for up to 100 years. *Ascaris* ova are resistant to cold desiccation and chemical insults such as strong salt solutions and moderate concentrations of formalin and hypochlorite. However, exposure for short periods of time (hours) to temperatures above 37°C will kill developing embryos.

There is a need to address the concentrations of viable ova remaining in conventionally treated sludge samples, which can be used as fertilizer for domestic and agricultural purposes. In Europe, there is a move towards some form of heat treatment of sludge as a minimum before sludge application to agricultural land, used to grow food crops, is allowed. In the United Kingdom, currently there is a voluntary agreement on a ban upon the use of untreated sludge on land used for growing food crops.

Table 24-3 Viability of *Ascaris* sp. ova in sewage, sludge, and water

Country	Sample Type	Percentage of Viable *Ascaris* sp. Ova Detected
Brazil	Primary facultative pond sludge—near outlet	Viable ova detected
	Biosolids	56.78%
	Untreated sludge	58.8%
	Anaerobic treated sludge	44%
	Untreated sludge plant C	50%
Czech Republic	Dried sludge	Viable ova detected
France	Sludge + milk of lime (5 min at 55°C; lab-scale)	<1 ova / 10 g TS
	Sludge + milk of lime (2 min at 60°C; lab-scale)	<1 ova / 10 g TS
	Sludge + slaked lime (40%) (4 min at 60°C; lab-scale)	<1 ova / 10 g TS
	Sludge + quick lime (22%) (>60 min at 51°C; full-scale)	<1 ova / 10 g TS
	Sludge + quick lime (24%) (75 min at 55°C; full-scale)	<1 ova / 10 g TS
	Sludge + quick lime (26%) (5 min at 58°C; full-scale)	<1 ova / 10 g TS
Mexico	Flocculated prim. sludge and thickened (15 days)	90%
	Flocculated primary sludge and sedimentation	Mean 93 viable ova/g TS
	Flocculated primary sludge and sedimentation + 15% CO at 21.3°C/2 hr	Viable ova detected*
	Flocculated primary sludge and sedimentation + 15% CO at 23.8°C/2 hr	Viable ova detected*
	Waste stabilization pond (primary facultative) inlet	~300 viable ova/g FS
	Waste stabilization pond (primary facultative) outlet	~10 viable ova/g FS
	Waste stabilization pond (primary facultative) inlet	~300 viable ova/g FS
	Waste stabilization pond (primary facultative) outlet	<100 viable ova/g FS
	Waste stabilization pond (anaerobic pond):	
	Waste stabilization pond (anaerobic pond) inlet	~50 viable ova/g FS
	Waste stabilization pond (anaerobic pond) outlet	~90 viable ova/g FS
Slovak Republic	Aerobic digestion (33.3°C)	37.2%
	Aerobic digestion (38.7°C)	33.4%
	Thermophilic digestion (48.5°C)	0%
South Africa	Raw sludge	74%
	Seawater for 2 days (laboratory scale)	3%
United Kingdom	Anaerobic mesophilic digestion (34°C/26 days)	No effect
	Aerobic thermophilic digestion (55°C/28 days) after 1 hr	~50%
	Aerobic thermophilic digestion (55°C/28 days) after 2 hr	<0.5%

(Table continued next page)

Table 24-3 Viability of *Ascaris* sp. ova in sewage, sludge, and water (continued)

Country	Sample Type	Percentage of Viable *Ascaris* sp. Ova Detected
	Aerobic thermophilic digestion (55°C/28 days) after 4 hr	0%
	Thermophilic digestion (53°C) for 1 hr	0%
	Thermophilic digestion (45°C) for 3 hr	No effect
	Anaerobic mesophilic digestion (35°C/13.3 days)	No effect
United States	Raw sewage	87%
	Aeration and sedimentation	85%
	Chlorinated effluent	88%
	River (below effluent discharge)	81%
	Irrigation (abstracted surface) water	86%
	Irrigation (abstracted surface) water	83%
General information		
Ensiled swine feces—7 days		95%
Ensiled swine feces—14 days		57%
Ensiled swine feces—28 days		40%
Ensiled swine feces—56 days		70%
Ensiled clover/grass mixture—42 days		67%

TS = total solids; FS = fecal solids.

* Data on the occurrence and removal of *Ascaris* sp. ova are normally presented as occurrence and removal of helminth ova. Some publications refer to *Ascaris* spp., others to *Trichuris* spp., and yet others to mixtures of both or mixtures of various intestinal helminth ova.

DOCUMENTED WATERBORNE OUTBREAKS

Although associated with foodborne outbreaks of disease, there is no documented waterborne outbreak of ascariasis in association with treated public drinking water supplies.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Effective coagulation and filtration processes remove ova. Chlorine and chloramine disinfectants are ineffective against *Ascaris* ova. Exposure to UV light early on in embryonation will destroy the developing embryo. Conventional sewage treatment processes are not effective in destroying ova. Ova need to be retained for 10 to 12 months in septic tanks, cesspools, etc., before they are all destroyed.

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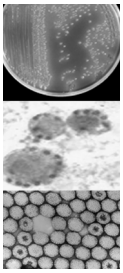

Chapter 25

Balamuthia mandrillaris

Govinda S. Visvesvara and Hercules Moura

DESCRIPTION OF THE AGENT

Balamuthia mandrillaris, a small, free-living amoeba that has been recently found in soil, has been identified to cause a fatal disease of the central nervous system (CNS) in humans and other animals.

The trophozoite of *B. mandrillaris* is slightly larger than *Acanthamoeba* and *Naegleria fowleri*, measures 15 to 60 µm in size, and exhibits, especially in tissue cultures, spider-like movement by producing finger-like pseudopodia (Figure 25-1a). Like *Acanthamoeba* it has only two stages, trophozoite and cyst, in its life cycle. The cyst is usually spherical, measures 15 to 30 µm, and is characterized by three layers in its wall: an outer ectocyst, a middle mesocyst, and an inner endocyst (Figure 25-1b). Both trophozoite and cyst stages of the organism have a single vesicular nucleus with a large centrally placed nucleolus. The trophozoite may also have two or three nucleoli. Occasionally, binucleate forms are seen in the trophic stage.

DESCRIPTION OF THE DISEASE

Like *Acanthamoeba, B. mandrillaris* causes a chronic, slowly progressive, fatal CNS disease—granulomatous amoebic encephalitis (GAE), with an insidious onset and no clear-cut incubation period. Some patients, especially those with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), develop skin lesions, abscesses, or erythematic nodules. Common clinical symptoms include headache, irritability, confusion, seizures, dizziness, drowsiness, and sometimes diplopia, lethargy, and hemiparesis like those found in infections with *Acanthamoeba*. Microscopically, the lesions consist of hemorrhagic necrosis of CNS parenchyma, with variable amounts of subacute and chronic inflammatory reaction, with amoebic trophozoites and cysts around and within the walls of blood vessels. Trophozoites and cysts may be found in lungs, kidneys, lymph nodes, and skin, suggesting hematogenous dissemination.

More than 100 cases (>60 in the United States) of granulomatous disease caused by *B. mandrillaris*, with and without the involvement of skin lesions, have been reported from around the world.

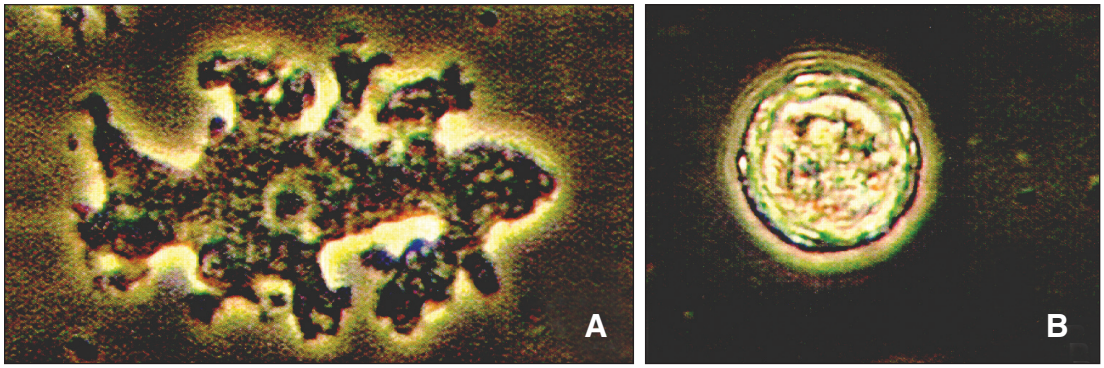

Figure 25-1 *Balamuthia mandrillaris* (a) trophozoite and (b) cyst

RESERVOIRS FOR THE AGENT

Although, infection with *B. mandrillaris* has been documented in gorillas, chimpanzees, orangutans, monkeys, dogs, horses, and sheep, no human or animal reservoir for *B. mandrillaris* has been identified so far.

MODE OF TRANSMISSION

Like *Acanthamoeba* GAE, there is no evidence that *B. mandrillaris* can be directly acquired from water, though this is quite likely. Like *Acanthamoeba*, the portal of entry in the case of GAE is probably through the lower respiratory tract, ulceration of the skin or mucosa, or any open wounds. CNS disease is probably acquired by hematogenous spread from a primary site, such as the respiratory tract or skin lesion. Recent cases of GAE in AIDS patients who first developed skin abscesses and later developed CNS symptoms, strongly suggest secondary dissemination to the CNS from a primary location, such as skin.

METHODS FOR DETECTING THE AGENT

B. mandrillaris can be detected and isolated from the suspected human tissue such as brain, lungs, and skin. Samples should be collected aseptically and can be stored for short periods at room temperature and usually not frozen although occasionally it is possible to isolate the organism if cysts are present in the tissue. Personnel handling specimens should take appropriate precautions, such as wearing gloves, surgical masks, and working in a biological safety cabinet.

Fresh tissue specimens should be macerated and inoculated into monolayers of tissue cultures (e.g., monkey kidney cells) and incubated at 37°C. Gentamicin (100 µg/mL) and amphotericin B (1 µg/mL) should be added to prevent bacterial and fungal contamination. Viable *B. mandrillaris*, if present in the sample, would feed on the monkey kidney cells, multiply, and differentiate into cysts when all the monkey kidney cells are gone. An immunofluorescence test is available for the detection of *B. mandrillaris* in Formalin-fixed and paraffin-embedded tissue sections. A polymerase chain reaction (PCR) test has been developed recently to identify *B. mandrillaris* in fresh or frozen tissue.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

B. mandrillaris infection has occurred in both immunocompromised and immunocompetent people. Also, infections with *B. mandrillaris* have been identified in a number of animals including gorillas, chimpanzees, orangutans, monkeys, dogs, horses, and sheep. Only recently, *B. mandrillaris* has been isolated from soil although its nutritional requirements are not known. It is believed that it feeds on other small free-living amoebas present in the environment.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Nothing is known about the survival of *B. mandrillaris* in the environment. However, it is believed that cysts can survive adverse conditions in the environment.

DOCUMENTED WATERBORNE OUTBREAKS

B. mandrillaris has not been isolated from water, and no definite waterborne outbreaks of *B. mandrillaris* encephalitis have been described.

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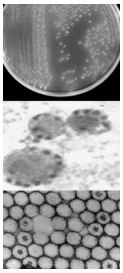


Chapter 26

Balantidium coli

Lynne S. Garcia

DESCRIPTION OF THE AGENT

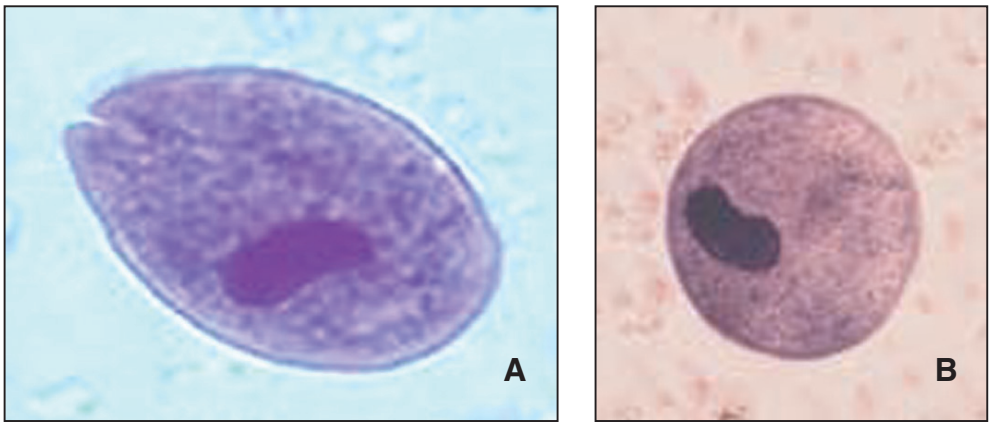
While many of the organisms in the phylum Ciliophora are free-living, members of the genus *Balantidium* are parasites found in the intestinal tract of invertebrate and vertebrate hosts. *B. coli* is the only pathogenic ciliate of humans and is the largest of the protozoa that are parasites in humans. Both the trophozoite and cyst forms are found (Figure 26-1 and Table 26-1). The trophozoite is quite large, approximately 50 to 100 µm in length, oval in shape, and covered with short cilia. The anterior end is somewhat pointed and is provided with a cytostome. The posterior end is broadly rounded. The cytoplasm has many vacuoles containing bacteria and debris. The trophozoite has two nuclei, one very large bean-shaped macronucleus and a small round micronucleus. The organisms normally live in the large intestine where the trophozoites feed on cell debris, starch grains, bacteria, and mucus. The cilia beat in a sequence, producing waves along the surface of the trophozoite. The organisms move in a spiral motion and can move backward by reversing the cilia.

The cyst is formed as the trophozoite moves down the intestine. Nuclear division does not occur in the cyst; therefore, only two nuclei are present—the macronucleus and the micronucleus. The cyst measures from 50 to 70 µm and is the infective form for humans. The parent multiplies asexually by transverse binary fusion; conjugation has also been documented, but is rare.

DESCRIPTION OF THE DISEASE

Some individuals with *B. coli* infections are asymptomatic, others develop symptoms of severe dysentery similar to those caused by amoebiasis. Symptoms usually include diarrhea or dysentery, tenesmus, nausea, vomiting, anorexia, and headache. Insomnia, muscular weakness, and weight loss have also been reported. The diarrhea may persist for weeks to months before dysentery develops. Tremendous fluid loss may occur, characteristic of a type of diarrhea similar to that seen in cholera or in some coccidial infections. In healthy individuals, the infection may resolve spontaneously or may become latent.

B. coli has the potential to invade intestinal tissue by mechanical and enzymatic action on the mucosal surface. *B. coli* may penetrate the mucosa with cellular infiltration in the area of the developing ulcer. Some of the abscess formations may extend to the muscular layer. The ulcers may vary in shape, and the ulcer bed may be full of pus



Source: Lynne S. Garcia.

Figure 26-1 (a) *Balantidium* trophozoite and (b) *Balantidium coli* cyst

Table 26-1 Characteristics of *Balantidium coli*

Species	Shape and Size	Motility	Number of Nuclei	Other Features
Balantidium coli trophozoite	Ovoid with tapering anterior end; 50–100 µm in length; 40–70 µm in width; usual range: 40–50 µm	Ciliates: rotary, boring; may be rapid	One large kidney-shaped macronucleus, one small round micronucleus, which is difficult to see even in the stained smear; macronucleus may be visible in unstained preparation	Body covered with cilia, which tend to be longer near cytostome; cytoplasm may be vacuolated
Cyst	Spherical or oval, 50–70 µm; usual range: 50–55 µm		One large macronucleus visible in unstained preparation; micro-nucleus difficult to see	Macronucleus and contractile vacuole are visible in young cysts; in older cysts, internal structure appears granular; cilia difficult to see within the cyst wall

and necrotic debris. Although dissemination to the lungs in infections with *B. coli* is very rare, it has been reported in at least two immunocompromised patients and should be considered in the examination of bronchial secretions.

The organism can be identified in routine stool examinations, particularly wet preparation examinations of fresh and concentrated material, using low-power (10×) or high-power (40×) objectives. The organism can easily be seen in a wet preparation on low power. Organism recognition and identification on a permanent stained smear may be very difficult. The protozoa are so large that stains obscure any internal morphology. *B. coli* organisms may even be confused with helminth eggs because of their size, particularly when the cilia are not visible.

Tetracycline is the current drug of choice for treatment of *B. coli*, although it is considered investigational for this infection. The dosage is 500 mg, four times daily for 10 days. Iodoquinol or metronidazole may be used as alternatives.

RESERVOIRS FOR THE AGENT

The natural habitat for *B. coli* is the large intestine of humans, monkeys, and pigs. *B. coli* is widely distributed in hogs, particularly in warm and temperate climates, and in monkeys in the tropics. Human infection is found in warmer climates, sporadically in cooler areas, and in institutionalized groups with low levels of personal hygiene.

MODE OF TRANSMISSION

Infections with *B. coli* are transmitted through infective cysts by the fecal–oral contamination of food or water. A patient who is symptomatic with diarrhea is passing the trophozoite (noninfectious form). However, an individual who is an asymptomatic carrier without diarrhea will be passing the infectious cyst form. The usual source of infection for humans is the pig.

METHODS FOR DETECTING THE AGENT

The same direct microscopic examination approach used for other protozoan parasites (amoebas and flagellates) can be used for *B. coli*; however, the same potential problems exist. These problems include lack of specific monoclonal-based reagents for high specificity and recovery problems related to low organism numbers. Many species of ciliates live in water and can be found in fecal specimens contaminated by water containing these organisms. They usually belong to the genera *Chilodon* or *Cyclidium* and can be confused with *B. coli* when compared morphologically.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Although *B. coli* has been reported from many different simian hosts, domestic hogs probably serve as the most important reservoir host for human infection. In areas where pigs are the main domestic animal, the incidence of human infection can be quite high. Particularly susceptible to infection are persons working as pig farmers or in slaughterhouses (e.g., 28 percent infection in New Guinea). Human infection is fairly rare in temperate areas, although once the infection is established, it can develop into an epidemic, particularly where poor environmental sanitation and personal hygiene are found. This situation has been seen in mental hospitals in the United States. Because the mode of transmission is ingestion of infective cysts through contaminated food or water, preventive measures involve increased attention to personal hygiene and sanitation.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Cysts in feces remain viable in stool for 1 to 2 days at room temperature and disappear in approximately 1 week. Like most intestinal protozoa, the cysts are relatively resistant to environmental conditions, but will rapidly die when exposed to drying or direct sunlight.

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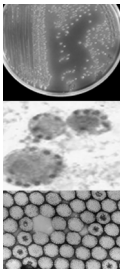


Chapter 27

Blastocystis hominis

Lynne S. Garcia

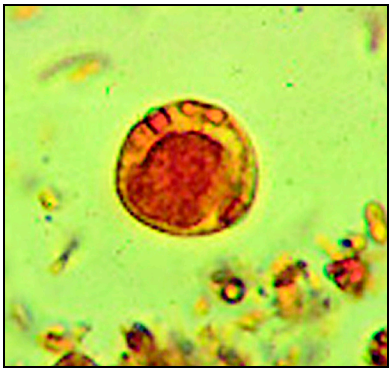
DESCRIPTION OF THE AGENT

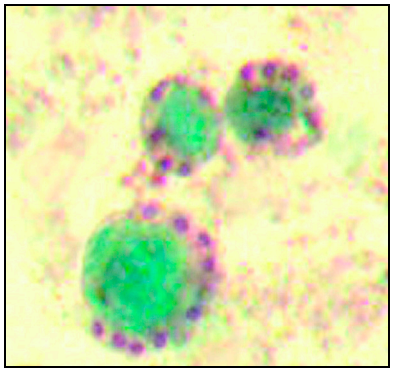
Blastocystis hominis was classified as an organism related to *Blastomyces* spp., a cyst form of a flagellate, or a yeast of the genus *Schizosaccharomyces*. However, this organism has been reclassified as a protozoan. Analysis of 10 stocks of *B. hominis* isolated from human stools revealed two distinct groups of organisms. Other studies on 61 isolates suggest that there may be four separate serologic groups. Further studies will be needed to determine whether or not these groups should be classified as separate species and whether or not they have epidemiologic significance. More recent biochemical studies have revealed at least two variant strains.

B. hominis is capable of pseudopod extension and retraction, reproduces by binary fission or sporulation, and has a membrane-bound central body that takes up 90 percent of the cell (Table 27-1). The organism is strictly anaerobic, normally requires bacteria for growth, and ingests bacteria and other debris. The classic form usually seen in the human stool specimen varies in size from 6 to 40 µm and is characterized by the large central body that may be involved with carbohydrate and lipid storage. The more amoebic form can occasionally be seen in diarrheal fluid but may be extremely difficult to recognize. *B. hominis* is usually identified by the more typical round form with the central body (Figures 27-1 and 27-2).

Table 27-1 Morphologic criteria used to identify *Blastocystis hominis*

Species	Shape and Size	Other Features
Blastocystis hominis	Organisms are generally round, measure approximately 6 to 40 µm, and are usually characterized by a large, central body (looks like a large vacuole) surrounded by small, multiple nuclei; central body area can stain various colors (trichrome) or remain clear	The more amoebic form can be seen in diarrheal fluid, but will be difficult to identify; due to variation in size, may be confused with various yeast cells





Source: Lynne S. Garcia.

Figure 27-1 *Blastocystis hominis* iodine stain

Figure 27-2 *Blastocystis hominis* trichrome stain

DESCRIPTION OF THE DISEASE

When *B. hominis* is present in large numbers in the absence of other parasites, bacteria, or viruses, it may cause diarrhea, cramps, nausea, fever, vomiting, and abdominal pain and require therapy. In patients with other underlying conditions, the symptoms may be more pronounced. Incidence of this organism appears to be higher than suspected in stools submitted for parasite examination. *B. hominis* should certainly be considered the possible pathogen in patients with symptoms, but in whom no other etiologic agent has been identified. Other recent studies suggest that when a symptomatic *B. hominis* infection responds to therapy, the improvement probably represents elimination of some other undetected pathogenic organism, such as *Entamoeba histolytica, Giardia lamblia,* and *Dientamoeba fragilis*. Data from other geographic areas indicate that *B. hominis* is commonly seen in stool samples, although likely nonpathogenic. Consequently, the true role of this organism in terms of colonization or disease is still somewhat controversial. Molecular typing of *B. hominis* reveals extensive genetic diversity in morphologically identical strains, and detection by microscopy alone may not be sufficient to confirm the role of this organism as an etiologic agent in human disease.

In a recent study from Germany, the prevalence and clinical significance of *B. hominis* in a large group of human immunodeficiency virus (HIV) patients was investigated. The data from this study of 262 patients suggest that the isolation of *B. hominis* does not justify treatment, even in symptomatic, severely immunocompromised patients. Symptoms will go away without therapy in most patients, or other infectious or noninfectious etiologies will be identified. Therapy should be limited to patients with persistent, unexplained symptoms after a thorough evaluation, screening, and examination of multiple stools.

RESERVOIRS FOR THE AGENT

B. hominis has been confirmed in humans, monkeys, apes, pigs, and possibly guinea pigs, and multiple species are likely involved. *B. hominis* is also widespread in avian hosts. In Australia, the organism has been found in dogs, cattle, sheep, horses, pigs, chickens, geese, ducks, ostriches, alpacas, llamas, koalas, and wombats. Molecular and phylogenetic analysis of *Blastocystis* isolates from various hosts indicates that many of the isolates found in animals have zoonotic potential or have cross-transmissibility among heterogeneous hosts, including humans. Zoonotic genotypes of *Blastocystis hominis* detected in cattle and pigs by polymerase chain reaction (PCR) with diagnostic

primers and restriction fragment length polymorphism analysis of the small subunit ribosomal RNA gene appear to be potential sources of human infection.

MODE OF TRANSMISSION

B. hominis infections are transmitted through infective cysts (central body forms) found in fecal–oral contamination of food or water. Stages other than the cyst form will be destroyed on contact with water. The presence of thin- and thick-walled cysts have been reported. Thin-walled cysts are thought to be autoinfectious, leading to multiplication of the organism in the intestinal tract. The thick-walled cysts are responsible for external transmission via the fecal–oral route. This life cycle might explain the high percentage of positive carriers in many studies where the percentage of patients with *B. hominis* is often much higher than those infected with other intestinal protozoa.

METHODS FOR DETECTING THE AGENT

Methods used to detect other protozoan parasites (amoebas and flagellates) can be used. However, the lack of specific monoclonal-based reagents for high specificity and recovery of low organism numbers and small organism size are problems of which to be aware. Morphologically, these organisms can resemble large yeast forms, or even artifacts.

Routine stool examinations are very effective in recovering and identifying *B. hominis*, although the permanent stained smear is the procedure of choice. Examination of wet preparations may not easily reveal the organism. If the fresh stool is rinsed in water before fixation (for the concentration method), *B. hominis* organisms, other than the cysts, will be destroyed, thus possibly yielding a false-negative report. In those laboratories performing wet preparation examinations only, studies indicate that fecal culture has a higher positive yield for *B. hominis* than stool microscopy.

Both enzyme-linked immunosorbent assay (ELISA) and fluorescent antibody (FA) tests have been developed for detection of serum antibody to *B. hominis* infections. Demonstration of a strong antibody response is consistent with the ability of this organism to cause symptoms. In a second study using FA, serum antibody production both during and following *B. hominis* symptomatic disease is immunological evidence for the pathogenic role for this protozoan.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

B. hominis is likely transmitted via the fecal–oral route through contaminated food or water. Although other possible modes of transmission are not defined, the incidence and apparent worldwide distribution indicate the traditional route of infection. Prevention would probably involve improved personal hygiene and sanitary conditions.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Because prevalence numbers of *B. hominis* in human stool samples are often reported to be higher than other protozoans, infective cyst forms have been shown to remain viable at least as long as the other protozoa. Like most intestinal protozoa, the cysts are relatively resistant to environmental conditions but will die when dried or exposed to direct sunlight.

DOCUMENTED WATERBORNE OUTBREAKS

In a study of 904 army personnel in Thailand in 2001, increased risk of being infected with *B. hominis* was associated with being a private, working in a specific unit, and consuming unboiled drinking water. Although other modes of transmission could not be ruled out, waterborne transmission of *B. hominis* was indicated at this army base.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

No information on effective water treatment processes is available.

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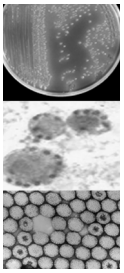


Chapter 28

Cryptosporidium parvum and *Cryptosporidium hominis*

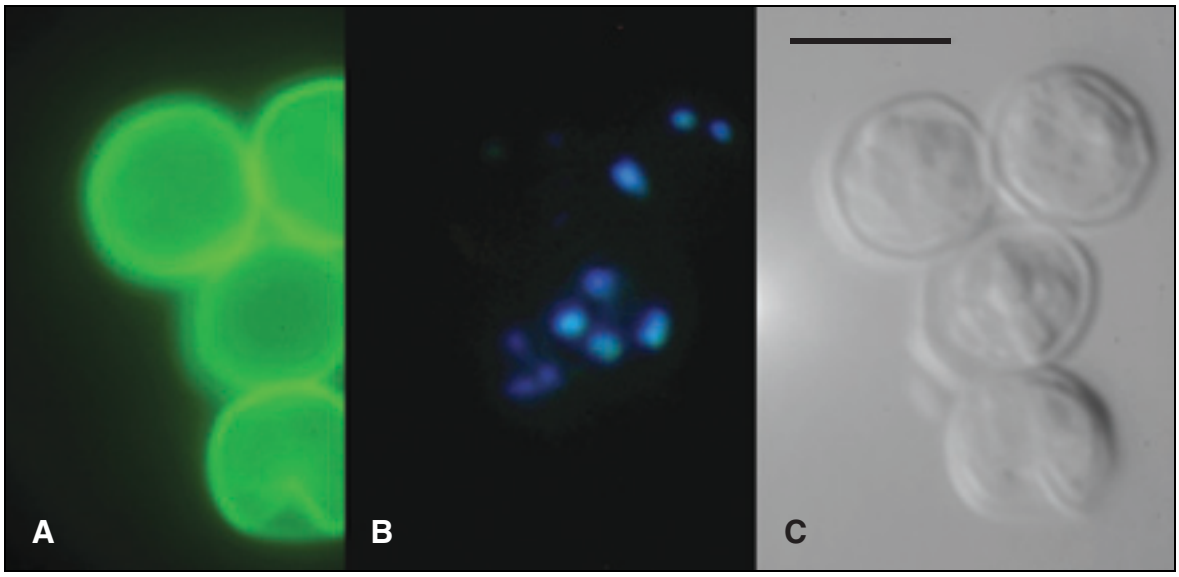
Charles R. Sterling and Marilyn M. Marshall

DESCRIPTION OF THE AGENT

Tyzzer described and named the genus *Cryptosporidium* in 1907 and 1910. In 1912 he detailed the morphology and life cycle of *Cryptosporidium parvum*. As a species, *Cryptosporidium* was first linked to morbidity and mortality with the description of *C. meleagridis* in turkeys in 1955. In 1971 *Cryptosporidium* was associated with bovine diarrhea, thus sparking veterinary interest. In 1976 two independent groups reported the first cases of human cryptosporidiosis. In 1982 cases of severe diarrhea involving *Cryptosporidium* and associated with the acquired immunodeficiency syndrome (AIDS) in humans was reported to the Centers for Disease Control and Prevention (CDC). Since 1984, when the first waterborne disease outbreak associated with *Cryptosporidium* was reported, to the massive Milwaukee, Wis., outbreak in 1993, medical, public health, and water treatment personnel have become increasingly aware of this parasite and its potential for causing disease. Until quite recently, most human infection was attributed to *C. parvum*. Recent advances in genotyping have added several species of *Cryptosporidium* to the list that can infect humans and potentially cause disease. These include the recently described *C. hominis, C. felis,* and *C. canis*, and *C. meleagridis*. The two species of *Cryptosporidium* of most interest to the water industry are *C. parvum*, which can infect both humans and animals, and *C. hominis*, which infects humans only (Figure 28-1).

Cryptosporidium is classified in the suborder Eimeriorina and family Cryptosporidiidae. The suborder Eimeriorina also contains the medically important coccidian parasites *Isospora belli* and *Toxoplasma gondii*. The transmissible stage of *Cryptosporidium* is a mature, thick-walled oocyst, 4 to 6 µm in size. When oocysts are ingested along with food or water, the oocyst cell wall opens (excystation) in the small intestine releasing sporozoites that attach to and invade epithelial cells of the gastrointestinal tract. Excystation usually requires reducing conditions, pancreatic enzymes, and bile salts; however, it may occur in warm aqueous solutions without any special stimuli.

Sporozoites that have penetrated enterocytes develop into trophozoites. The trophozoite stage divides by asexually forming two generations of meront stages containing merozoites. Merozoites from the first generation can infect neighboring cells and continue to recycle or produce a second generation of meronts which will go on to



Source: CHDiagnostic & Consulting Service, Inc., Loveland, Colo.

Figure 28-1 Photographs of *Cryptosporidium parvum* oocysts at 1,000× magnification. A: Immunofluorescence (fluorescein) using a monoclonal antibody-based assay; B: DAPI stain highlighting sporozoite nuclei in two of the four oocysts; C: Differential interference contrast (DIC) microscopy depicting intact oocysts. Bar represents 5 μm.

initiate the sexual cycle where zygotes are formed after fertilization. Zygotes either develop into environmentally resistant, thick-walled oocysts (80 percent) that are released in feces and can transmit infection from one host to another or into thin-walled oocysts (20 percent), autoinfective life cycle forms that can maintain the parasite life cycle in the host. This stage and the type I meronts of the asexual cycle are believed to be largely responsible for the continuation of life-threatening disease in immunodeficient persons who do not have repeated exposure to environmentally resistant oocysts.

DESCRIPTION OF THE DISEASE

Profuse and watery diarrhea is the most common symptom of cryptosporidiosis. Other symptoms include abdominal pain, nausea, fever, and fatigue. The incubation period is from 5 to 28 days, with a mean of 7.2 days. The duration of the symptoms vary according to the immune status of the host. In patients with compromised immune systems, the illness may be life threatening. Most other hosts with intact immune systems are able to clear the infection. Patients with late-stage AIDS (i.e., those with CD4 lymphocyte counts below 200/mm^3), regardless of age, are highly susceptible to cryptosporidioisis and, after exposure, develop prolonged, severe, life-threatening diarrhea. Controlled infection studies conducted in immunocompetent individuals have demonstrated that an infectious dose may be as low as 30 oocysts.

RESERVOIRS FOR THE AGENT

As previously mentioned, several species of *Cryptosporidium* have recently been shown to infect humans. This implies that zoonotic transmission can and does occur. Studies have demonstrated that most zoonotic transmission likely involves *C. parvum*. The

young of most animal species are particularly prone to infection and diarrhea caused by *C. parvum*, and this likely contributes to environmental contamination and even direct fecal–oral zoonotic transmission.

MODE OF TRANSMISSION

Because oocysts of *Cryptosporidium* are fully sporulated when excreted by an infected host, close personal contact with infected individuals or animals can result in parasite transmission. This most likely occurs with person-to-person contact between family and household members, sexual partners, health-care personnel, and day-care employees and attendees. Another route is animal-to-person (zoonosis), involving pets, farms animals, and laboratory animals. Water is the largest potential common source of transmission and has been associated with numerous disease outbreaks.

METHODS FOR DETECTING THE AGENT

Detection methods for *Cryptosporidium*, such as those specified in *USEPA Method 1622:* Cryptosporidium *in Water by Filtration/IMS/FA* and *Method 1623:* Giardia *and* Cryptosporidium *in Water by Filtration/IMS/FA*, require that large volumes of water be passed through sampling filters and concentrated by centrifugation (USEPA 2001a, b). The pelleted material is then subjected to immunomagnetic separation (IMS) and placed on well slides for sample preparation and microscopy. Microscope slides are stained with 4',6-diamidino-2-phenylindole (DAPI) and a *Cryptosporidium*-specific monoclonal antibody (MAb). Slides are then read at 1,000× using a microscope equipped with epifluorescence, and Nomarski differential interference optics. Oocysts are confirmed by size, shape, and internal morphological characteristics. Variation in sample collection, turbidity, and weather conditions may influence results, as explained in chapter 3. Potential molecular-based detection methods as polymerase chain reaction (PCR), real-time PCR, microsatellite and microarray technology are in the experimental testing stage in some laboratories. Each technique has strengths and weaknesses that must be evaluated before these techniques become approved for standardized water quality methods. A review of molecular techniques that may have utility in detecting *Cryptosporidium* is provided in chapter 4.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT,

Cryptosporidium oocysts are widely distributed in water and have been reported in 87 percent of source water samples and 27 percent of drinking water samples. In the massive Milwaukee outbreak, investigators found that the severity of illness in Milwaukee residents, as defined by more frequent abdominal cramping, nausea, vomiting, and fever, was greater than in previously described sporadic cases. In addition, the severity of illness was even greater in visitors to Milwaukee than its residents. Of both visitors and residents with laboratory-confirmed cryptosporidiosis, 39 percent had recurrent bouts of watery diarrhea that lasted an average of 2 days after having had normal stools for 2 to 14 days. Numerous outbreaks of cryptosporidiosis associated with swimming pools have also been reported. Cryptosporidiosis has been on the *Morbidity and Mortality Weekly Report* (MMWR, published by the CDC) Summary of Notifiable Diseases, United States, since 1999. This is a collection of the official statistics for the reported occurrence of nationally notifiable diseases in the United States. These statistics are collected and compiled from reports to the National Notifiable Diseases Surveillance System (NNDSS). Many physicians, however, are still unfamiliar with the parasite, and many persons do not seek medical attention for diarrheal illness. The

mean prevalence rate for *Cryptosporidium* infection is between 1 and 3 percent in Europe and North America, but is considerably higher in underdeveloped countries, where levels of infection range from 5 percent in Asia to approximately 10 percent in Africa. Children of underdeveloped countries are particularly prone to infection with infection rates reaching 100 percent in some areas. As previously noted, *Cryptosporidium* is also a zoonotic parasite. Young farm animals, and particularly calves, have high rates of infection worldwide.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

As long as the thick, two-layered oocyst wall remains intact, *Cryptosporidium* remains quite environmentally stable. Oocysts can survive for months in cold, moist environments, such as lakes and streams. Oocysts in water can retain viability and infectivity even after freezing at –15°C for 8 to 24 hours; however, oocysts can be rendered noninfectious when water temperatures of 64.2°C or higher are held for two minutes or longer. Oocysts are also susceptible to drying at 18–28°C after 4 hours.

DOCUMENTED WATERBORNE OUTBREAKS

Outbreaks associated with *Cryptosporidium* have been documented in the United States since 1984. These outbreaks have occurred in water systems using well and spring water treated solely by chlorination and in filtered surface water systems. *Cryptosporidium* is the etiological agent that has caused the greatest number of reported individuals to become ill from waterborne disease in the United States in recent times. Outbreaks caused by this organism are often unrecognized. In the Milwaukee outbreak that occurred in 1993, the organism was first identified from stool samples of ill patients. This information, along with the treatment plant records and other epidemiological data, pointed to the water supply as a likely source of infection. Oocysts were only later recovered in ice prepared using water from Milwaukee that had been collected and stored during the outbreak dates. This underscores the difficulty frequently associated with isolating the agent at the time of the outbreak.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Utilities should consider all surface water to be contaminated with *Giardia* or *Cryptosporidium.* Watersheds should be managed to limit the introduction of *Cryptosporidium* into the drinking water supplies. Continuous optimization of plant performance for turbidity and particle removal are key monitoring parameters that should be used by utilities to signal possible problems. Treatment conditions (e.g., sedimentation, coagulation, and filtration) should be optimized to produce a filter effluent turbidity of 0.1 ntu or less. When filter effluent turbidity ranged between 0.1 and 0.3 ntu, *Cryptosporidium* presence was as much as 90 percent (1 log) greater than when filter effluent turbidity was 0.1 ntu or less. The effectiveness of chemical disinfection is dependent upon the disinfectant, pH, temperature, disinfectant demand in the water, and the organism being inactivated. *Cryptosporidium* is resistant to chlorine-based disinfectants, so that oocysts escaping filtration could remain viable after chlorine treatment. The $C \times T$ for ozone inactivation of *Cryptosporidium* is approximately 25 to 35 times higher than that required for ozone inactivation of *Giardia.* Temperature plays an important role in ozone inactivation, and thus water systems in colder climates cannot achieve the required levels of *Cryptosporidium* inactivation with ozone. Ultraviolet (UV) light is the latest and most effective method for control of *Cryptosporidium.*

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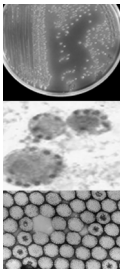

Chapter 29

Cyclospora cayetanensis

Ynés Ortega

DESCRIPTION OF THE AGENT

Cyclospora belongs to the family Eimeriidae. The species name *cayetanensis* was derived from the university where it was initially studied, Cayetan (Universidad Peruana Cayetano Heredia), and -ensis (Latin, belonging to). In 1979 *Cyclospora*-like organisms were first observed in Papua New Guinea. The organisms were thought to represent a new species of *Isospora* because of the structure of its sporocysts. From 1985 on, organisms 8 to 10 µm in size, staining red with modified acid-fast stains, and autofluorescing under ultraviolet (UV) light were reported with increasing frequency in humans worldwide. They were described as CLBs (coccidian-like bodies, cyanobacter-like bodies), or blue-green algae, and held responsible for diarrheal illness. Researchers eventually showed that CLBs belonged to the coccidian genus *Cyclospora*. *Cyclospora* was linked to previous reports of CLB infections based on the organism's biological characteristics and patients' symptoms and response to treatment.

Noninfectious *Cyclospora* oocysts are passed in the feces of infected individuals. These unsporulated oocysts require about 7 to 15 days to sporulate and become infectious (Figure 29-1). Unsporulated and sporulated oocysts measure 8 to 10 µm in diameter. Intracellular stages of the parasite have been observed in the cytoplasm of jejunal enterocytes of infected individuals. Asexual stages have been observed by electron and light microscopy. An animal model and an in vitro cultivation system for *Cyclospora* will help confirm the life cycle and identify possible mechanisms of pathogenesis.

Three other *Cyclospora* species that are morphologically similar to *C. cayetanensis* have since been described in nonhuman primates.

DESCRIPTION OF THE DISEASE

Onset of illness caused by *Cyclospora* has been reported as abrupt in 68 percent and gradual in 32 percent of adult patients. Infections typically last an average of 7 weeks, up to 4 months in acquired immunodeficiency syndrome (AIDS) patients. Symptoms mimic those caused by cryptosporidiosis, including mild nausea, anorexia, abdominal cramping, and watery diarrhea. Diarrhea alternating with constipation is commonly reported. Most adult patients reported weight losses of up to 5 to 10 percent. Some patients have had flatulence, joint pain, and night sweats. Infection in children is less severe and is usually asymptomatic. *Cyclospora* was detected in 12 percent of Nepalese children between 18 months and 5 years of age.

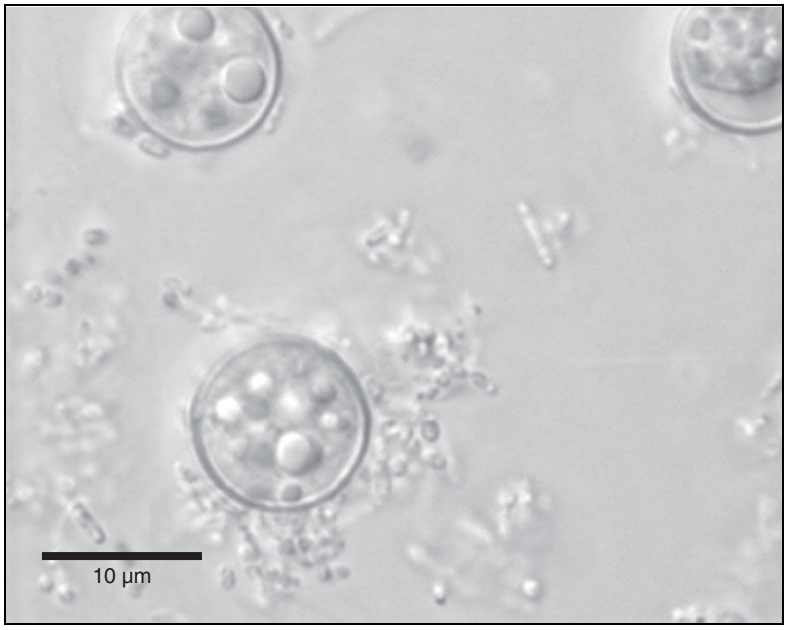

Figure 29-1 *Cyclospora cayetanensis* unsporulated oocyst. Bar represents 10 µm.

RESERVOIRS FOR THE AGENT

Humans are the only natural host, although organisms similar to CLBs have been observed in chimpanzees and baboons.

MODE OF TRANSMISSION

Epidemiological evidence strongly suggests water can transmit *Cyclospora*, especially because of the 1- to 2-week sporulation time. Most reports of infection have come from predominantly coastal cities or regions. Consumption of untreated water or reconstituted milk with this water has led to infection. Indigenous *Cyclospora* infections in many countries including the United States have been described in patients with no travel history.

Accidental ingestion of contaminated water while siphoning water from a tank or cleaning a flooded basement have been reported as possible sources of infection. Epidemiological evidence suggested that water from a rooftop reservoir was responsible for an outbreak involving 20 individuals, most of whom were physician residents in a Chicago hospital. In another instance, 12 of 14 British soldiers and dependents stationed in Nepal developed diarrhea. Examination of the chlorinated drinking water, consisting of river and municipal water, at the camp demonstrated the presence of *Cyclospora* oocysts.

Infection associated with the consumption of contaminated produce has also been reported. During May and June of 1996, approximately 850 cases of cyclosporiasis were confirmed in the United States and Canada, nearly all in areas east of the Rocky Mountains. Epidemiological investigation suggested the infections were due to eating contaminated strawberries and raspberries. During 1997 *Cyclospora* outbreaks were reported in Virginia, Maryland, the District of Columbia, and Canada. These outbreaks were associated with the consumption of raspberries and basil. In 1998 importation of Guatemalan raspberries was not permitted in the United States, and

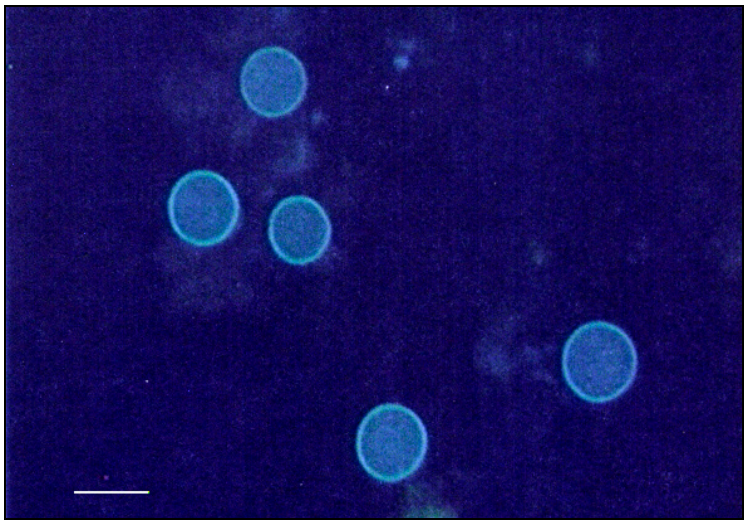

Figure 29-2 *Cyclospora cayetanensis* oocyst autofluorescence when observed using an epifluorescence microscope with UV excitation filter set (330–365 nm). Bar represents 10 µm.

no cases of cyclosporiasis were reported during that year. However, Canada continued to import raspberries from Guatemala and reported some cases of *Cyclospora* that were associated with these raspberries. From 1998 to 2004, sporadic cases of cyclosporiasis have been reported every year in the United States. Basil, berries, and lettuce continue to be implicated in these outbreaks.

METHODS FOR DETECTING THE AGENT

Light microscopy of wet mounts, modified acid-fast staining, and autofluorescence are currently used for identifying *Cyclospora* (Figure 29-2). The nested polymerase chain reaction (PCR) may provide specificity and sensitivity to some laboratories and will be useful in population and environmental studies. Restriction fragment length polymorphism (RFLP) analysis of the PCR-amplified products (18S ribosomal RNA) does differentiate *Cyclospora* from *Eimeria* strains. This is particularly important when examining environmental samples and food matrices. *Cyclospora* oocysts stain variably using a modified acid-fast technique and measure 8 to 10 µm in diameter. They stain best using the modified carbolfuchsin technique. Immunomagnetic separation has been described for *Cyclospora* identification. Magnetic beads tagged with wheat germ agglutinin (WGA) have been evaluated to capture oocysts nonspecifically. Based on the use of monoclonal antibodies or other probe-specific technologies, specific detection tests are being developed to better define the epidemiology of this organism and determine the extent to which water and other routes can transmit the organism.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Organisms similar in appearance to *Cyclospora* have now been described from patients with protracted diarrheal illness in North, Central, and South America; the Caribbean; Africa; Southeast Asia; Australia; England; and Eastern Europe. *Cyclospora* and *Cryptosporidium* oocysts have been detected in vegetables purchased from some markets in Lima, Peru. Other sources of infection include travelers or individuals infected

with *Cyclospora*, contaminated food, and possibly insects as transport hosts. Epidemiological studies will be invaluable in helping to sort the mechanisms of transmission and to define control against infection with *Cyclospora*.

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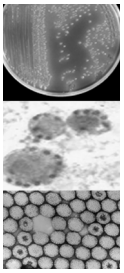


Chapter 30

Entamoeba histolytica

William E. Keene

DESCRIPTION OF THE AGENT

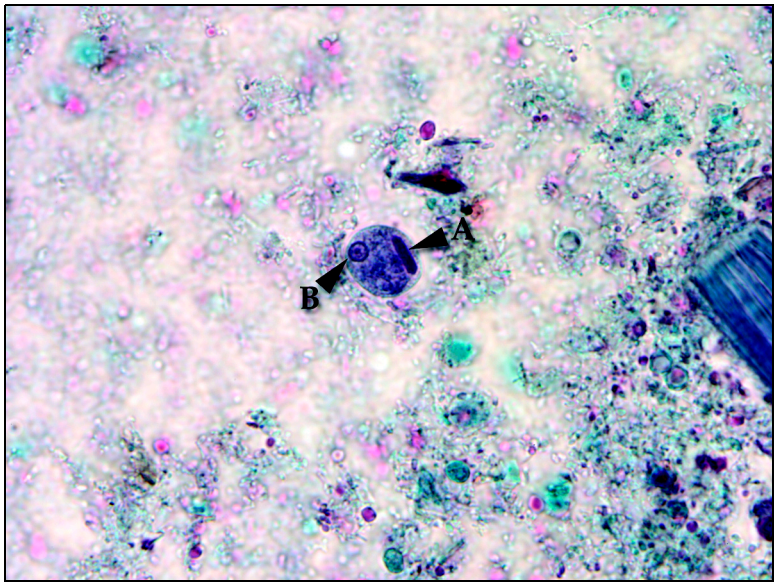
Entamoeba histolytica is a protozoan parasite of humans with a long—albeit infrequently documented—history of waterborne transmission. Morphologically indistinguishable amoebae classified as "*E. histolytica*" since 1903 have been recognized as two species since the 1990s. The pathogenic species retains the name *E. histolytica*, while its apparent twin, considered to be nonpathogenic, is now called *E. dispar*. Despite their identical appearance, genetic data suggest that these species diverged millions of years ago. Unless associated with clinical disease, or phagocytosed red blood cells are seen within the parasites in fresh stool specimens, *E. histolytica* and *E. dispar* can only be distinguished by using serology (most people with invasive *E. histolytica* infections develop antibodies) or recently developed, species-specific polymerase chain reaction (PCR) and antigen-detection assays. Even today, most diagnostic screening is limited to microscopic examination of fecal specimen morphology ("O & P exam").

This distinction between species, based originally on isoenzyme patterns ("zymodemes") and later on DNA analysis, must be borne in mind when reviewing the older medical literature, where everything was called *E. histolytica*. The relative prevalence of *E. dispar* is much higher than that of *E. histolytica,* as much as 10 times by some estimates, but no doubt varies around the world. Invasive amebiasis is now rarely identified in the US-born population absent a history of travel in the developing world.

E. histolytica has a direct life cycle (i.e., no intermediate hosts) with two stages—trophozoite and cyst. The trophozoite is a "ameboid" blob of changing outline and dimension (Figure 30-1). Cysts are spheroid (10 to 20 µm in diameter). Humans become infected by swallowing cysts. Trophozoites develop following excystation in the small intestine and are the form that can cause symptoms. Trophozoites reproduce by fission and under some conditions develop into cysts. Trophozoites, cysts, or both are shed in feces. Cysts are immediately infective, while trophozoites die rapidly outside the human host, and, because they cannot survive transit through the acidic stomach environment, are not infective.

Although morphologically distinguishable, *E. histolytica* is sometimes confused with other harmless human amoebae, including *E. hartmanni*, (which is smaller) and *E. coli*[*] (which has up to eight nuclei per cyst). Even human leukocytes found in stool

**Entamoeba coli*, not to be confused with the bacterium *Escherichia coli*.



Source: Melanie Moser, Centers for Disease Control and Prevention.

Figure 30-1 A trichrome stain of single-celled parasite *Entamoeba histolytica*; (A) chromatoid body, (B) nuclei

can be misidentified as amoebas. Pseudo-epidemics resulting from poor laboratory work have been documented.

DESCRIPTION OF THE DISEASE

While most infections with *E. histolytica* are asymptomatic, amebiasis can be a serious and even life-threatening disease. The prevalence of infection worldwide is high enough that—at least in the pre-AIDS (acquired immunodeficiency syndrome) era—amebiasis ranks as the third leading killer among parasitic diseases, causing an estimated 40,000 deaths annually (far behind malaria and schistosomiasis).

Infections begin with parasite excystation in the intestinal lumen, but in some persons, trophozoites burrow into and even penetrate the intestinal wall. If that happens, parasites can spread to adjacent tissues or via the bloodstream to almost any part of the body. The clinical manifestations depend on where the amoebas are localized. Infections are usually categorized as intestinal, extraintestinal, or both.

With symptomatic infections, recurrent diarrhea of varying degrees is common, often with mucus and visible or occult blood, with or without fever, abdominal pain, and tenesmus. In fulminant amebiasis, the onset is abrupt with profuse bloody stools, high fever, and severe abdominal pain. Chronic colitis develops in some patients, with bloody stools and abdominal pain that may persist for months or years without treatment. Liver abscess is the most common form of extraintestinal amebiasis, but the infection can spread to the lungs, brain, perineum, or other sites.

Amebiasis is usually diagnosed by a stool exam for ova and parasites. Trophozoites can be visualized on wet mount of fresh stool or in trichrome-stained material preserved in polyvinyl alcohol (PVA). Cysts can be identified in concentrated and stained stool collected in formalin. Shedding can be intermittent, and three or more samples may be necessary to identify more than 90 percent of cases. PCR and antigen detection tests

that distinguish *E. histolytica* from *E. dispar* in stool specimens are now available, but they are not yet widely used.

Antibodies are usually detectable within 7 to 10 days of invasive infections, and are diagnostic for infection with pathogenic *E. histolytica*. Although titers typically fall with successful treatment, they can remain elevated for years, making serologic results sometimes difficult to interpret.

The incubation period is variable and obviously undefined for persons with asymptomatic infections. In one well-studied outbreak of more than 300 cases, incubation periods ranged from several to 120 days, including 25 percent by 11 days, 50 percent by 20 days, and 75 percent by 36 days.

Medical therapy is usually successful for most infections, albeit complicated by side effects of antimicrobials, and, in some areas, drug availability. Noninvasive (intralumenal) infections can be treated with diloxanide furoate, iodoquinol, or paromomycin. Invasive disease (colitis/dysentery, liver abscess, or other extraintestinal infections) is usually treated with metronidazole or tinidazole, followed by a regimen aimed at lumenal parasites.

RESERVOIRS FOR THE AGENT

Infected humans, particularly asymptomatic carriers, are the only reservoirs of significance. Persons with frank diarrhea or dysentery tend to shed predominantly or exclusively trophozoites and hence are relatively unimportant transmitters of infection. Conversely, asymptomatically infected persons shed most or almost exclusively cysts, up to 15 million a day, according to one estimate. Shedding may be intermittent and can persist for years in untreated individuals.

MODE OF TRANSMISSION

Transmission is by the fecal–oral route, reflecting direct person-to-person spread or exposure to contaminated food, water, or fomites. Cysts can also be dispersed by flies. A number of waterborne outbreaks have been identified over the years. In contrast, foodborne outbreaks of amebiasis have not been documented. The infectious dose is low; inocula of 2,000 to 4,000 cysts produced infections in 100 percent of 42 human volunteers.

METHODS FOR DETECTING THE AGENT

Water samples can be concentrated by filtration and examined microscopically or cultured, but routine testing is not indicated.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Before *E. histolytica* and *E. dispar* were distinguished, it was estimated that as much as 10 percent of the world's population had amebiasis. In some parts of the world, pathogenic *E. histolytica* are commonly found. In the United States, *E. dispar* is probably much more prevalent than *E. histolytica*, particularly among native-born residents. Amebiasis is most commonly reported among recent immigrants, travelers, homosexual men, and institutionalized persons, but many of these may be harmless *E. dispar* infections.

Amebiasis was common among homosexual men in the pre-AIDS era, but prevalence has probably declined in recent years. The vast majority of amoebic infections in homosexual men in the United States are probably *E. dispar*. In contrast to cryptosporidiosis, amebiasis does not pose an increased risk to immunocompromised individuals.

While still reportable in most states, amebiasis was dropped from the list of nationally notifiable diseases in 1994. Raw surveillance tallies are difficult to interpret, as *E. histolytica* and *E. dispar* infections are rarely distinguished by clinical laboratories, and asymptomatic infections are rarely diagnosed. Sporadic reported cases may get little followup by public health agencies. Absent high attack rates, even large, communitywide outbreaks could escape detection.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Unlike the fragile trophozoites, amoebic cysts are resistant to environmental degradation. Survival is primarily a function of temperature. At temperatures above freezing, *E. histolytica* cysts rarely survive longer than 3 months. Cysts are rapidly killed by modest heat, with survival times of less than two minutes at 50°C. Cysts are also killed by freezing. *E. histolytica* does not multiply outside the human host.

DOCUMENTED WATERBORNE OUTBREAKS

Waterborne outbreaks of amebiasis are rarely documented because (1) when they occur, they are hard to detect, and (2) they probably do not occur very often. In every case where adequate information is available, local disruptions in distribution systems or consumption of untreated water have been the cause.

According to US Environmental Protection Agency (USEPA) records, only eight waterborne outbreaks have been reported in the United States—the most recent in 1984. These are typically very small clusters (median number of cases, seven). None have involved large community systems.

The largest is the infamous 1933 Chicago World's Fair outbreak, with more than 1,400 cases documented, including almost 100 fatalities. This outbreak was traced to cross-connections and contamination of drinking water reservoirs at two hotels with sewage from corroded and leaking waste lines (Figure 30-2). Chicago was the site of another large waterborne outbreak in 1934 after firefighters and bystanders at a huge stockyard fire drank from watering trough taps, not realizing that they connected to a nonpotable source. The only other sizable US outbreak occurred in 1953 at a large (1,500 employees) factory in South Bend, Ind. That outbreak was traced to a leak in a potable water suction line that ran through a pool of sewage.

Only a handful of outbreaks have been reported in Europe. A British outbreak was attributed to a broken sewer line contaminating a well; a cluster in Sweden was caused by a sewage backup that flooded a drinking water reservoir.

The largest outbreak of amebiasis ever documented was recently reported from Tbilisi, Republic of Georgia, amply illustrating the continuing potential of this parasite to cause widespread disease whenever water treatment or distribution systems are inadequate. Serosurvey results in the capital city suggested that between 84,000 and 225,000 persons may have been infected over several months in 1998. Secondary transmission via foodborne and person-to-person routes likely contributed to the outbreak.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

Amebiasis is a potential problem when unfiltered drinking water is contaminated with human feces. Outbreaks have occurred when chlorinated water supplies became contaminated with sewage. There are no recommendations for specifically monitoring or testing source or effluent drinking water for *E. histolytica*. In the event of a suspected waterborne outbreak, ad hoc protocols would be developed by public health agencies working with water utility engineers.

Source: William E. Keene, Oregon Public Health Services.

Figure 30-2 Site of 1933 World's Fair hotel *E. histolytica* outbreak

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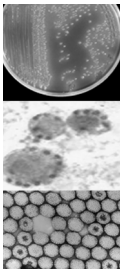


Chapter 31

Giardia lamblia

Frank W. Schaefer III

DESCRIPTION OF THE AGENT

Giardia lamblia is an obligate protozoan parasite that infects numerous mammals including humans, dogs, cats, beavers, and muskrats. Protozoa are unicellular animals which, unlike bacteria and viruses, possess membrane-bound genetic material or nuclei and other assorted cellular organelles. They exhibit various forms of locomotion and reproduction that have been used to categorize them into broad groups (phyla); however, the taxonomy of the protozoa is a dynamic evolving process about which there is little agreement (Lee et al. 1985). Protozoa fall into two broad nontaxonomic groupings depending upon whether they are parasitic or free-living.

Parasitic forms live in or on another host organism, and this host–parasite interaction is always detrimental in some fashion to the host. In the case of *Giardia,* the parasite is found within the lumen of the small and large intestine making it an enteric parasite. Most enteric protozoans have two stages in their life cycles (see Figure 31-1). Because *Giardia* is an obligate parasite, it must have a host to complete its life cycle. In the environment, *Giardia* has a cyst that is round to oval in shape with dimensions ranging from 8 to 18 µm long by 5 to 15 µm wide. The *G. lamblia* cyst has up to four nuclei, a claw hammer–shaped median body (clusters of microtubules), and axonemes (basal portions of flagella within cytoplasm) that can be seen giving the cyst the appearance of a face. Once inside the host, the cyst is stimulated to excyst (Schaefer 1990) and release an undifferentiated trophozoite in the duodenum. The trophozoite is an actively feeding, growing, and reproducing stage with two nuclei, a claw hammer–shaped median body, and four pairs of bilaterally symmetrical flagella. The trophozoite is shaped like a pear cut in half lengthwise with dimensions ranging from 12 to 15 µm long by 6 to 8 µm wide. Stimuli in the host's intestinal tract induce *Giardia* to produce a resistant, dormant transmission form, which is referred to as a cyst.

Trophozoites generally do not survive outside the host unless they are in a specialized culture medium. While trophozoites are not thought to be a major mode of transmission, they are capable of initiating infections. Cysts are a different story, as they are known to survive for long periods outside the host especially in cool water (Bingham et al. 1979).

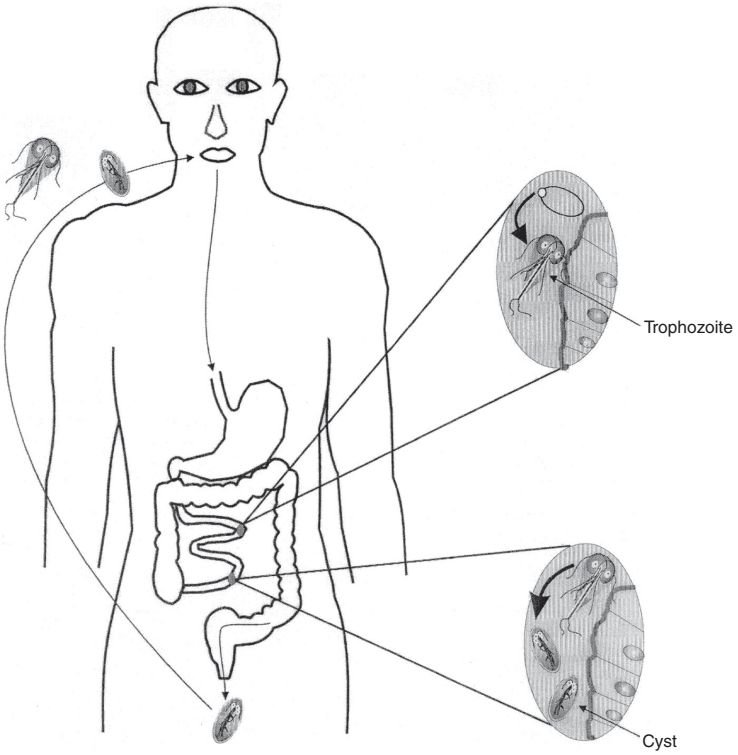


Figure 31-1 *Giardia lamblia* life cycle

DESCRIPTION OF THE DISEASE

While *Giardia lamblia* is known to produce gastrointestinal distress including diarrhea, weight loss, flatulence, cramps, belching, distention, anorexia, vomiting, fatigue, mucus in the stool, bloody stool, and/or foul smelling stool, to name a few (Wolfe 1990), it must be borne in mind that the parasite can produce a continuum of pathologies ranging from no symptoms to extremes of illness requiring hospitalization. A complete understanding of how pathogenesis is produced is not available; however, it is known that the strain of parasite, along with the immune competency and health status of the host, are important factors. According to Faubert and Belosevic (1990), the attachment and detachment of trophozoites to the microvilli of the small intestine appear to cause mechanical damage resulting in a reduction of the villus to crypt ratio. This in part may explain why some infected individuals exhibit alterations in lactase activity and have a decreased ability to absorb nutrients like fat, glucose, xylose, carotene, folic acid, and vitamin B_{12}.

The time from infection until the demonstration of parasites is called the prepatent period and is dependent upon the number of parasites in the dose, and the health and susceptibility of the host. Rendtorff (1954), in a study of human volunteers receiving 10 cysts, reported the prepatent period to be 9.1 days on average. The mean prepatent period of American travelers to the Soviet Union was 12 to 15 days, with a range of 1 to 75 days (Walzer et al. 1971; Brodsky et al. 1974). In light of information on giardiasis in Leningrad at the time of these studies, it is now felt that the prepatent period for human giardiasis is in the range of 12 to 19 days (Jokipii et al. 1985). Individuals, especially asymptomatics, who receive no treatment, may remain infected, pass cysts, and be a source of infection for years.

Diagnosis is based upon either finding trophozoites and/or cysts in fecal material or by demonstrating trophozoites from a duodenojejunal aspirate or biopsy. Because *G. lamblia* produces cysts intermittently in some individuals, demonstration of cysts in fecal material may be difficult. Furthermore, the cyst densities vary depending on how many are being produced by the parasite. For these reasons, either collecting three consecutive stools or collecting stools on alternate days for a week has been shown to increase test reliability. The use of antibiotics, antacids, kaolin products, paregoric, oily laxatives, and most enema preparations are contraindicated when trying to demonstrate cysts and trophozoites, for these medications may cause masking or the disappearance of the parasites. In addition to examining microscopically for cysts and trophozoites directly in a wet mount of stool, cysts may be concentrated from a stool using either formal-ethyl acetate sedimentation or sucrose flotation. When numerous stool examinations are negative but *Giardia* is still suspected, examination of intestinal fluid is done. A duodenal tube, endoscopy, or an Enterotest (HEDECO, Mountain View, Calif.) are the usual options used for the examination of intestinal fluid. Duodenojejunal aspiration or biopsy may also be negative if the infection is not widespread in the area examined.

Cysts have been classically detected and confirmed using bright field microscopy and Lugol's iodine which allows for demonstration of median bodies, axonemes, and nuclei. Once detected the cysts are measured using a calibrated ocular micrometer to ensure that they fall within the correct size range. Recently the use of immunofluorescent antibody stains and epifluorescent microscopy has gained acceptance. In wet mounts, trophozoites have a characteristic falling or turning leaf way of swimming which helps the trained eye identify them in duodenojejunal aspirates. Highly skilled microscopists are also known to use both phase contrast and differential interference contrast microcopy to identify this parasite.

Giardia lamblia infections are susceptible to treatment with quinacrine (Atabrine), metronidazole (Flagyl), furizolidone (Furoxone), and paramomycin (Humatin) (Davidson 1990). The drug of choice depends upon the condition of the patient and the experience of the physician. Although the Centers for Disease Control and Prevention (CDC) recommends quinacrine and metronidazole, these two drugs are not approved for this purpose by the Food and Drug Administration. Furazolidone, which is marketed as a liquid, is used to treat children, because they are more likely to comply with this treatment regimen. Each drug has side effects and contraindications. Metronidazole has been shown by Legator et al. (1975) to produce mutagenic effects in the Ames *Salmonella* test at drug levels found in the urine and serum of patients taking therapeutic doses. For this reason it is never given to pregnant women. Instead paromomycin, a minimally absorbed amino glycoside in humans, can be used to treat pregnant women; however, the most conservative course of action is to avoid treatment until after delivery.

MODE OF TRANSMISSION

These parasitic protozoans have simple, direct life cycles, and all are transmitted as fecal contaminants of food and/or water (Owen 1984). Furthermore, person-to-person transmission is known and may take many forms. For example, institutional environments in which hygienic conditions are not good, like poorly run day-care centers and nursing homes, are well-documented sources of transmission. Venereal transmission has also been widely reported as a result of sexual activity of certain patient groups. Also of concern is transmission of the parasite from pets to humans.

METHODS FOR DETECTING THE AGENT

The method used to detect *Giardia* cysts in water is an immunofluorescence detection procedure. To detect low levels of cysts in water, large volumes of water are sampled.

Currently, *Giardia* cysts are concentrated from water by retention on either a Pall Gelman Envirochek filter, a Pall Gelman Envirochek HV filter, or an IDEXX Filta-Max foam filter. Retained particulates and cysts are eluted from the filter and reconcentrated by centrifugation. The pelleted *Giardia* cysts are affinity purified from the other debris using Dynal's immunomagnetic separation Dynabead GC-Combo kit. The concentrated and purified cysts are stained on a teflon well slide with a direct fluorescent antibody, counterstained with 4',6-diamidino-2-phenylindole (DAPI), and are examined using epifluorescent microscopy. Figure 31-2 illustrates representative results obtained using these fluorescence and differential contrast microscopic techniques. Cysts are classified according to specific criteria including immunofluorescence, size, shape, DAPI-stained nuclei, and internal morphological characteristics, and the results are reported in terms of empty cysts, amorphous cysts, cysts with one internal structure, and cysts with more than one internal structure per 10 L. All these cyst categories are added together to calculate the total number of cyst-like objects detected per 10 L. The internal morphological characteristics that can be detected in some cysts include nuclei, axonemes, and median bodies. The fluorescent DAPI counterstained nuclei, in conjunction with demonstration of internal morphological characteristics using differential interference contrast optics, enables the confirmation of more internal structures.

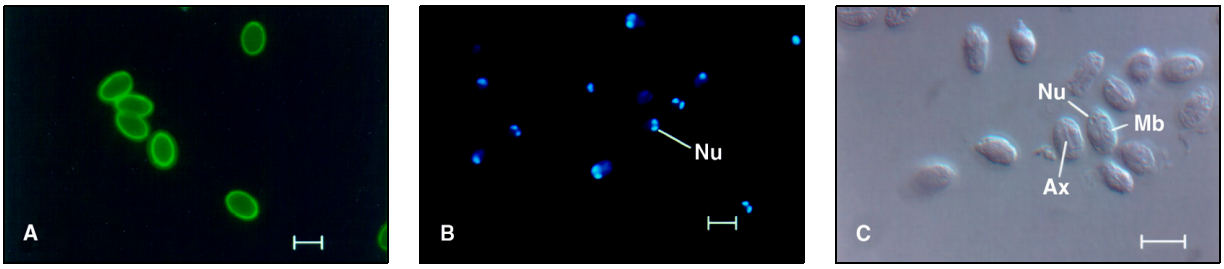
OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND ENVIRONMENT

Giardia lamblia is a ubiquitous parasite, as it occurs all over the world. Besides temperate and tropical locations, it is known to also occur in arctic locations. Thirteen percent of adults and up to 50 percent of children may be infected, but asymptomatic. *Giardia* is the most frequently identified protozoan parasite in the United States and Britain. Occurrence of clinical symptoms in these countries ranges between 2 percent and 20 percent depending upon the socioeconomic status and age of the group. According to the CDC, giardiasis is the most commonly diagnosed intestinal parasite in public health laboratories in the United States (Furness et al. 2000).

Giardia has many mammalian reservoir hosts that can carry the infection in addition to humans. However, the degree to which *Giardia* infections in animals contribute to illness in humans remains unclear. This is due to the fact that few adequately controlled cross-species transmission studies have been reported. *Giardia lamblia*-like cysts have been observed in numerous mammals and birds. However, it is now known that isolates from the great blue heron are *G. ardeae* and not *G. lamblia* (Erlandsen et al. 1990). Similarly, *G. psittaci* cysts from budgerigars resemble *Giardia* cysts from humans but lack the trophozoite ventrolateral flange morphology required for *G. lamblia* (Erlandsen and Bemrick 1987). Efforts by Erlandsen et al. (1991) to infect laboratory mammals with *G. ardeae* and *G. psittaci* have been unsuccessful. Consequently, *Giardia* from birds is not likely to be a threat to humans. *Giardia* cysts isolated from humans have been transmitted to rats, gerbils, guinea pigs, beavers, dogs, cats, raccoons, bighorn sheep, mouflon sheep, and pronghorn sheep (Davies and Hibler 1979). Moreover, beavers can be infected with human *Giardia* cysts at doses ranging between 50 and 500 cysts (Erlandsen et al. 1988).

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Temperature is known to have a pronounced effect on the viability of *Giardia* cysts. Human source cysts suspended in tap water and stored at –13, 8, 21, 37, and 100°C were evaluated for viability using in vitro excystation (Bingham et al. 1979). As might be expected, cysts exposed to freezing and thawing, as well as boiling, did not survive. Cysts stored at 8, 21, and 37°C remained viable for up to 77, 24, and 4 days,



Source: Michael W. Ware, US Environmental Protection Agency.

Figure 31-2 *Giardia lamblia* cysts stained with an immunofluorescent (A) antibody, (B) DAPI, and also viewed by (C) Nomarski DIC microscopy: Nu = nucleus; Mb = median body; Ax = Axonemes. Bars represent 10 μm.

respectively. In a study by deRegnier et al. (1989), storage conditions of *G. muris* cysts were examined. Cysts were suspended in lake water at 15 and 30 ft and in river water. Cysts suspended in tap water and reagent water were stored in a laboratory refrigerator. In the lake water, cysts remained viable for 28 days at 15 ft and 56 days at 30 ft. The cysts exposed to river water remained viable for up to 28 days. Cysts stored in tap water were no longer viable after 14 days. The control cysts which were stored in reagent water in the laboratory refrigerator remained viable up to 84 days. This helps explain why US waterborne outbreaks of giardiasis have been found clustered in mountainous states, where water temperatures are low but not freezing.

DOCUMENTED WATERBORNE OUTBREAKS

According to Craun (1990), *Giardia* is the most commonly identified pathogen in waterborne outbreaks in the United States. During the period 1971–1985, it was reported that 92 (18 percent) of the waterborne disease outbreaks were due to giardiasis, resulting in 24,124 cases of illness. By comparison, less than 2 percent of all reported cases of typhoid fever, salmonellosis, shigellosis, or infectious hepatitis were associated with waterborne outbreaks in the United States since 1951. Some of the more notable giardiasis outbreaks have occurred in Aspen, Colo., in 1965–1966; Rome, N.Y., in 1974–1975; Camas, Wash., in 1976; Berlin, N.H., in 1977; Vail and Estes Park, Colo., in 1978 and 1979; Bradford, Pa., in 1979; and Pittsfield, Mass., in 1986. For the period 1989–1990 (Herwaldt et al. 1991), *G. lamblia* was identified as the etiological agent for 7 of 12 outbreaks of gastroenteritis for which an agent was identified. A great many of these waterborne giardiasis outbreaks involved surface water systems receiving minimal treatment only involving disinfection. Other outbreaks have occurred in systems using groundwater which were found to be fecally contaminated. Most of these outbreaks have occurred in systems classified as small community systems which have a minimum of 15 or more connections serving customers year round. Perhaps less publicized are the outbreaks that have occurred in noncommunity systems like those in camps and parks that serve a transient, seasonal population.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Logsdon (1988) reported that filtration processes, including diatomaceous earth filtration, slow sand filtration, and coagulation-filtration, when applied appropriately, can remove *Giardia* cysts at levels of 99 percent or more. Chemical disinfectants like free chlorine, chloramine, chlorine dioxide, and ozone are known to inactivate *Giardia*

cysts (Jarroll 1988) when appropriate conditions of pH, temperature, disinfectant concentration, and contact time are used. Disinfectant $C \times T$s (disinfectant concentration multiplied by contact time) required to inactivate 99 percent of *Giardia* cysts range from 9 to 342 mg·min/L. Also, ultraviolet light at a dose of 2 mJ/cm^2 can inactivate greater than 4 $\log_{10}$ *Giardia* cysts in water (Shin et al. 2000). Because all surface water is subject to contamination by *Giardia* cysts, a multibarrier approach to water treatment is the most effective approach. In addition to good water treatment practices, like filtration and disinfection, this multibarrier system also includes watershed protection and distribution system maintenance.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

At the present time in the United States, there are no numerical standards (maximum contaminant level, MCL) for *Giardia* cysts in water due to the limitations in monitoring methods. US regulations for drinking water are based on the Safe Drinking Water Act (SDWA) of 1974 which was amended in 1986. The US Environmental Protection Agency (USEPA) proposed filtration of all surface water supplies; however, the US Congress in the SDWA amendment of 1986 required the USEPA to establish criteria to be used by the states to decide which water systems must filter. The final regulation was published by the USEPA in 1989 and addressed treatment for *Giardia*, viruses, and *Legionella.* In brief, all water systems are required to provide treatment capable of removing or inactivating 99.9 percent of *Giardia* cysts whether filtration is part of the treatment or not. As part of this rule, $C \times T$ tables for various disinfectants are supplied as well as requirements for effective filter operation.

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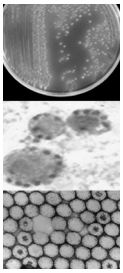


Chapter 32

Isospora belli

Lynne S. Garcia

DESCRIPTION OF THE AGENT

Isospora belli was first described by Virchow in 1860, and was officially named by Wenyon in 1923. This coccidian is included in the family Eimeriidae and produces oocysts, long and oval, 20 to 33 µm in length by 10 to 19 µm in width (Table 32-1). *I. belli* is the only member of the genus that is parasitic to humans, but many species of *Isospora* are found in wild and domestic animals.

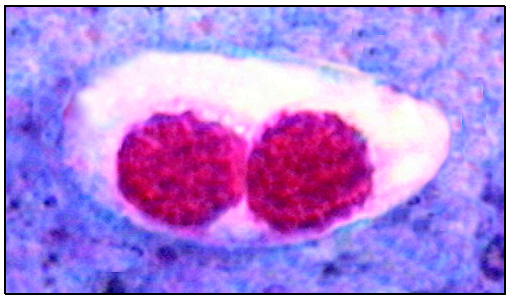
Schizogonic and sporogonic stages in the life cycle of *I. belli* have been described from human intestinal mucosal biopsies. Development in the intestine usually occurs within the epithelial cells of the distal duodenum and proximal jejunum, and eventually oocysts are passed with the stool. Usually the oocyst contains only one immature sporont (Figure 32-1); however, two may also be present. Development continues outside the host where it matures into twin sporocysts, each containing four sporozoites (Figure 32-2). The sporulated oocyst is the infective stage that excysts in the small intestine, releasing the sporozoites that penetrate the mucosal cells and initiate the life cycle. The life-cycle stages are schizonts, merozoites, gametocytes, gametes, and oocysts, and are structurally similar to other coccidia.

DESCRIPTION OF THE DISEASE

Clinical symptoms include foul smelling, foaming diarrhea, which may last for months to years, causing weight loss, abdominal colic, and fever. Patients who are immunosuppressed, particularly those with acquired immunodeficiency syndrome (AIDS), often experience profuse diarrhea associated with weakness, anorexia, and

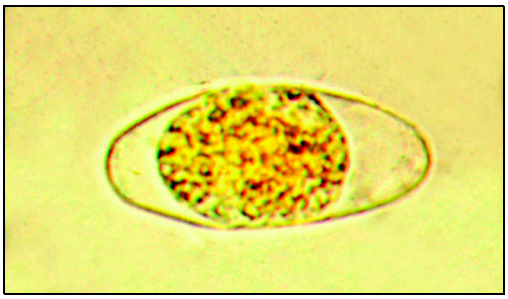
Table 32-1 Characteristics of *Isospora belli*

Species	Shape and Size	Other Features
Isospora belli	Ellipsoidal oocyst; usual range 20–33 µm long, 10–19 µm wide; sporocysts rarely seen broken out of oocysts, but measure 9–11 µm.	Mature oocyst contains two sporocysts with four sporozoites each; usual diagnostic state in feces is immature oocyst containing spherical mass of protoplasm (diarrhea) (intestinal tract)



Source: Lynne S. Garcia.

Figure 32-1 *Isospora belli* immature oocysts (iodine wet mount)



Source: Lynne S. Garcia.

Figure 32-2 *Isospora belli* mature oocyst (modified acid-fast stain)

weight loss. The diarrheal and other symptoms may be persistent under immunosuppressive therapy. This infection has been reported in homosexual men, all of whom were immunocompromised and had diarrhea for several months. In patients with AIDS, eosinophilia has been strongly associated with isosporiasis, particularly in those patients without weight loss, but with low CD4+ cell counts (<100–200 cells/mm^3). The finding of unizoic cysts of *I. belli* in lymphoid tissue of a patient with AIDS may be responsible for drug resistance and/or relapses. Also in those patients with severe malabsorption, failure to reach therapeutic levels of nitazoxanide in plasma and bile may be responsible for treatment failure.

Examination of a fecal specimen for the oocysts is recommended. However, wet preparation examination of fresh material either as the direct smear or as concentrated material is preferable to the permanent stained smear. The oocysts are very pale and transparent and can easily be overlooked. They can also be very difficult to see if the concentration sediment is performed from polyvinyl alcohol (PVA)-preserved stool. The light level should be reduced and additional contrast obtained with the microscope for optimal examination. Oocysts may not be recovered even with a positive biopsy specimen if small numbers of organisms are present. In a patient with diarrhea, the oocyst usually contains only a single sporoblast. These organisms are acid-fast and can also be demonstrated by using auramine-rhodamine stains. Organisms tentatively identified as *I. belli* with auramine-rhodamine stains should be confirmed by wet smear examination or acid-fast stains, particularly if the stool contains other cells or excess artifact material as in a stool with more normal consistency. Charcot-Leyden crystals derived from eosinophils are often seen in fecal specimens from infected individuals.

RESERVOIRS FOR THE AGENT

I. belli is thought to be the only species of *Isospora* that infects humans; however, many different species of *Isospora* infect a wide range of carnivores and passeriform birds, as well as amphibians, reptiles, rodents, swine, and primates other than humans.

MODE OF TRANSMISSION

I. belli is transmitted through ingestion of water or food contaminated with mature, sporulated oocysts. The parasite may be transmitted sexually by direct oral contact with the anus or perineum, although this mode of transmission is probably much less

common. The oocysts are very resistant to environmental conditions and may remain viable for months if kept cool and moist. Diagnostic methods for laboratory examinations may tend to miss the organisms when they are present. Because transmission is via the infective oocysts, prevention centers on improved personal hygiene measures and sanitary conditions to eliminate possible fecal–oral transmission from contaminated food, water, and environmental surfaces.

METHODS FOR DETECTING THE AGENT

No specific environmental detection methods are available. The same methods for identifying other protozoan parasites (amoebas and flagellates) can be used. Potential problems are the lack of specific monoclonal-based reagents for high specificity and recovery of low organism numbers. The oocysts will stain with auramine-rhodamine and modified acid-fast stains, both of which could be used on environmental isolates. However, oocysts from *I. belli* would have to be differentiated from those found in other animal hosts. In a comparison of the Modified Ziehl-Neelsen stain (MZN) with the newer method, the acid-fast-trichrome (AFT), the authors concluded that the sensitivity and specificity of the MZN was superior to that of the AFT. However, with minor changes, the AFT method would be appropriate for use in the diagnosis of intestinal coccidia, as well as the possible detection of microsporidia.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Although *I. belli* is cosmopolitan, certain tropical areas in the western hemisphere have well-defined locations of endemic infections. These organisms can infect both adults and children, and intestinal involvement and symptoms are generally transient unless the patient is immunocompromised. *I. belli* has also been implicated in traveler's diarrhea.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

The oocysts of coccidia can withstand exposure to various chemical and physical agents that harm most organisms. The oocyst wall is remarkable in its ability to protect the oocyst contents. Studies indicate that sunlight for as short a time as 4 hours and freezing below about –7°C were lethal for a relative, *Eimeria zuernii*, and survival was directly proportional to relative humidity. Environmental infectivity studies using oocysts from chickens have demonstrated that *E. acervulina* could be recovered from soil plots as long as 86 weeks. *E. tenella* and *E. maxima* and oocysts of *I. belli* disappeared from all experimental soil plots in less than a year's time. No severe infection with any of the three species was produced after 34 weeks.

DOCUMENTED WATERBORNE OUTBREAKS

There are no documented waterborne outbreaks of *I. belli*.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Disinfection studies have not been carried out, but effective coagulation and filtration or slow sand filtration should be effective.

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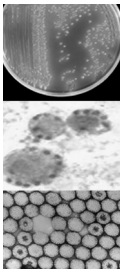

Chapter 33

Microsporidia

Ann Cali

DESCRIPTION OF THE AGENT

Microsporidia is the common name for a group of spore-forming, intracellular, obligate (requiring a host), protistan parasites of the phylum Microspora. The source of the name for this group is the small, resistant spore, which contains a single, long, coiled polar filament. This filament everts when the spore is ingested and the reproductive portion of its life cycle is intracellular; spores are very resistant to environmental factors and serve as the main source of transmission to other hosts.

Within the order Microsporidia are approximately 20 families, a hundred genera, and over a thousand species. The Microsporidia are ubiquitous, infecting every major group of animals and man. Insects, fish, and mammals are the host groups containing the majority of the known Microsporidia. Consequently, their spores are found throughout the environment. In humans, Microsporidia are considered opportunistic, occurring in immunocompromised individuals or immunoprivileged locations, such as the eye. About a dozen different types of Microsporidia are known to infect man.

The microsporidian life cycle is divided into three phases—infective, proliferative, and sporogonic. The infective phase contains the spore stage, from its completed development in a host cell to the stimulus that triggers the eversion of the long polar filament, thus becoming a tubule, through which the spore contents is injected into a proper host cell. This event initiates a new infection and may occur within an infected host with autoinfective spores. This event may also occur in the external environment, when spores are passed out of the body in feces, urine, or other body fluids that may contaminate other individuals or their food. Autoinfective and environmental spores must be properly stimulated by pH or specific ions in order to extrude their polar tubule. The forceful eversion of the polar tubule enables it to pierce a host cell.

The sporoplasm then emerges through the tubule and passes into a proper host cell, and the proliferative phase begins. Parasite growth and division produce many organisms within the host cell cytoplasm. The sporogonic phase of spore formation is usually indicated by secretions deposited on the parasite plasmalemma, commonly called a *thickened membrane*. These cells (sporonts) may undergo from one to many cell divisions before they become sporoblasts. After the last cell division, the resulting sporoblast cells undergo a metamorphosis into spores.

Because some spores are autoinfective, they evert their polar tubules within the same host in which they were formed, which provides additional sites of infection. The number of spores produced in sporogony, how they are produced, and the host–parasite

interface all contribute to genus determination. Three types of host-parasite interfaces are (1) direct contact with the host cells' cytoplasm, (2) indirect contact with the host cells' cytoplasm through an envelope secreted by the parasite called a *sporophorous vesicle* or *pansporoblast*, and (3) indirect contact by production of a parasite-and-host-produced vacuole-like envelope called a *parasitophorous vacuole*.

The transmissible stage of the Microsporidia is the spore. Among the Microsporidia that infect humans, spore sizes range from 1 μm × 0.7 μm to 5 μm × 3 μm. The more common species are in the 1 to 2 μm range. The most distinguishing feature of the spores is the tubule inside the spore. The tubule can sometimes be forced to evert on a microscope slide. The spore's highly refractile coat is visible by phase contrast microscopy of water-containing spores or infected cells.

DESCRIPTION OF THE DISEASE

Approximately a dozen microsporidian species infect humans. The disease and associated symptoms vary considerably, and depend on the organism species causing the infection. The most commonly reported species are those that infect the intestinal tract (with diarrhea and wasting as the presenting symptoms) and ocular infections causing a keratoconjunctivitis. Among the organisms producing these two clinical syndromes are disseminating species that can cause a myriad of other clinical symptoms. The incubation period is not known. Diagnosis is by microscopic observation of the spore stage collected in fecal samples, biopsies, scrapings, or body fluids, especially urine and sputum. Scattered data on prevalence averages around 25 percent for chronic diarrhea patients.

RESERVOIRS FOR THE AGENT

Encephalitozoon cuniculi, originally described in rabbits and other laboratory animals, is now known to infect two types of birds and over 30 types of mammals, including dogs, cats, rodents, and humans. Many organisms described in humans have also been reported in animals. As Microsporidia are so newly described and so ubiquitous in the environment, other reservoirs are continuously identified.

MODE OF TRANSMISSION

Microsporidia produce a resistant spore stage that survives in the environment and is responsible for infection in new hosts. Those that pass from the body in feces, urine, or other body fluids may contaminate hands, food, clothing, ground, and water. Infection by ingestion is the most common route of infection, but ocular infections could occur by touching the eyes with contaminated fingers. In both instances, water could be a source of infection if the spores are present. Microsporidia are probably as ubiquitous in humans as they are in other organisms, but they may be self-limiting and thus undiagnosed in the immunocompetent population. Undiagnosed hosts could, however, be responsible for producing great numbers of spores in the environment.

METHODS FOR DETECTING THE AGENT

Detecting environmental spores first requires collection, which may be accomplished in a number of ways. For large quantities of water, a series of progressively smaller sieves should be used to remove other organisms and debris. Microscopic contents can then be concentrated by continuous flow centrifugation at 10,000 rpm into 50-mL tubes. Sediment from these tubes or any other samples may be examined live by phase contrast microscopy. Fresh, unfixed material may be placed on the slide in a small amount of water, cover slipped, and examined on high power. Microsporidian

Table 33-1 Microsporidia reported in humans

Microsporidian Family	Genus, Species, and Spore Size	Tissues Infected	Manifestations
Enterocytozoonidae	*Enterocytozoon bieneusi*, 1–1.6 × 0.7–1 µm	Intestinal enterocytes Hepatobiliary tract	Diarrhea Hepatitis
Encephalitozoonidae	*Encephalitozoon cuniculi*, 2–2.5 × 1–1.5 µm	Hepatobiliary tract	Hepatitis Peritonitis
	Encephalitozoon-like or sp.,* 2–2.5 × 1–1.5 µm	Ocular conjunctiva	Keratoconjunctivitis
	Encephalitozoon hellem† (disseminating), 2–2.5 × 1–1.5 µm	Ocular conjunctiva Sinuses, lung, urinary	Keratoconjunctivitis Sinusitis
	Septata intestinalis‡ (disseminating), 2.0 × 1–1.2 µm	Male genitourinary tract Enterocytes, lamina propria Biliary tree Urinary tract Ocular conjunctiva Sinusitis and respiratory tract	Renal failure Diarrhea Infected gall bladder Renal failure Keratoconjunctivitis
Pleistophoridae	*Pleistophora* sp., 4 × 2 µm	Skeletal muscle	Myositis
	Pleistophora ronneafiei, 4 × 2 µm	Skeletal muscle	Myositis
	Trachipleistophora hominis, 4 × 2.4 µm	Skeletal muscle	Myositis Encephalitis
	Trachipleistophora anthropophthera (disseminating)†, I = 3.7 × 2 µm, II = 2.4 oval µm	Heart, brain, kidneys	
Nosematidae	*Brachiola (Nosema) connori,* 4–4.5 × 2–2.5 µm	Intestinal/disseminated	Diarrhea
	Nosema-like,** 3 × 2 µm	Skeletal muscle	Myositis
	Vittaforma corneae (*Nosema corneum*), 3.7 × 1 µm	Corneal stroma	Blindness
	Nosema ocularum, 5 × 3 µm	Corneal stroma	Blindness
	Brachiola vesicularum, 2.9 × 2 µm	Skeletal muscle	Myositis
	Brachiola (Nosema) algerae	Skeletal muscle	Keratoconjunctivitis
Unknown affiliation	*Microsporidium africanum*, 4.5–5 × 2.5–3 µm	Corneal stroma	Blindness
	Microsporidium ceylonensis, 3.5 × 1.5 µm	Corneal stroma	Blindness

* These reports were published before *E. hellem* was described and are probably that organism.
† Parasite has been reported to infect many tissues; only primary ones are listed here.
‡ Parasite has been reported to infect enterocyte, macrophage, fibroblastic, and endothelial cells, resulting in reports of infection in many tissues. Only some are listed here. Now called *Encephalitozoon intestinalis*.
** This report was published before *Brachiola vesicularum* was described. It is the same organism.

spores are very refractile and many light microscopes equipped for birefringence can be used. Both fresh and fixed spores may be seen in this way.

Other followup procedures should be used on suspected microsporidian spores. Forcing the spore to extrude its polar tubule is positive proof of live material. Seeing the polar tubule in the spore with an electron microscope is the best evidence, although many light microscopic stains can demonstrate the spore's presence. PAS stain (see glossary) reveals a small granule at the anterior end of the spore, which is also diagnostic. For fixed material, Webers' modified trichrome, Giemsa, Heidenhain

iron hematoxylin, Warthin-Starry silver stain, or Gomori methenamine silver (GMS) gram stains are the best light microscopy stains for demonstrating spores. General fluorescent stains such as calcofluor that combine with the chitinized spore coat or specific fluorescent antibody stains to particular Microsporidia can also be used for identification.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

The more than 1,000 species of Microsporidia are ubiquitous in nature and infect a wide range of vertebrate and invertebrate hosts. Table 33-1 indicates the occurrence of Microsporidia reported in humans. The detection of human-pathogenic Microsporidia has been reported in rivers, groundwaters, and crop irrigation water using molecular methods for identification (Dowd et al. 1998, 2003; Sparfell et al. 1997; Thurston-Enriquez et al. 2002).

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

The survival of Microsporidia varies from species to species. Most research has focused on the animal Microsporidia. In insects, some survive a very short time while others survive for years. Some specific studies include the effects of ultraviolet (UV) light on various densities of living and dead microsporidian spores. Waller found the viability and infectivity of *Encephalitozoon cuniculi* spores lasted up to 4 months in the environment and reported their sensitivity to various temperatures, disinfectants, and drugs (Waller 1979).

EFFECTIVENESS OF WATER TREATMENT

The physical removal of spores in source water using pilot-scale conventional treatment with alum coagulation, flocculation, sedimentation, and filtration has been shown ineffective, removing only 1 to 1.5 log of the spores seeded (Harrington et al. 2003). However, inactivation studies using chlorine (Wolk et al. 2000; Johnson et al. 2003) and UV (Huffman et al. 2002; Marshall et al. 2003; Johnson et al. 2003) show that disinfection may be accomplished but is variable depending on the species in question. Preliminary data have also been produced for other chemical disinfectants like chloramines, ozone, and chlorine dioxide (Jacangelo et al. 2002).

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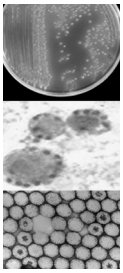

Chapter 34

Naegleria fowleri

Govinda S. Visvesvara and Hercules Moura

DESCRIPTION OF THE AGENT

Small, free-living amoebas belonging to the genus *Naegleria* are eukaryotic protozoa commonly found in soil, fresh water, sewage, and sludge. *Naegleria* normally feed on bacteria and multiply in their environmental niche as free-living organisms. Only one species of *Naegleria*, *N. fowleri*, can cause a fatal disease of the central nervous system (CNS) in humans and feed on human tissue.

N. fowleri and other members of the genus *Naegleria* are also called *ameboflagellates* because of a flagellate stage in addition to the trophozoite and the cyst stages in their life cycle (Figures 34-1). The trophozoite of *N. fowleri* is a small limax-like amoeba that measures 10 to 35 µm in size and moves with smooth hemispherical bulges from its body. The uroid or the posterior end is sticky and often has several trailing filaments. The cytoplasm contains a number of dumbbell-shaped mitochondria and abundant ribosomes. All stages of the organism have a single nucleus, although binucleate forms are occasionally seen in the trophic stage. The nucleus has a large, dense, centrally placed nucleolus. The amoebas reproduce by binary division. During the nuclear division, the nucleolus and the nuclear membrane persist and the nucleolus divides to form polar bodies. This type of nuclear division is called *promitosis*.

The pear-shaped biflagellate stage is a transient nonfeeding and nondividing phase formed because of altered environmental conditions. The flagellate usually reverts back to the trophic stage. The cysts measure 7 to 15 µm in size, are smooth-walled, and may have one or two pores flush on the surface of the cyst wall.

DESCRIPTION OF THE DISEASE

N. fowleri causes an acute fulminating (rapidly occurring) disease of the CNS called primary amebic meningoencephalitis (PAM). The disease has an abrupt onset and occurs in previously healthy children and young adults who have had contact with fresh water 3 to 10 days before the onset of symptoms (Figure 34-2). PAM is characterized by severe headache, spiking fever, stiff neck, photophobia, and coma leading to death within 3 to 10 days after the onset of symptoms. The trophozoites of *N. fowleri* enter the nasal passages during water sport activities, make their way into the CNS, and cause an acute hemorrhagic necrosis leading to the destruction of the olfactory bulbs and the cerebral cortex. Patient survival depends on prompt diagnosis and aggressive treatment.

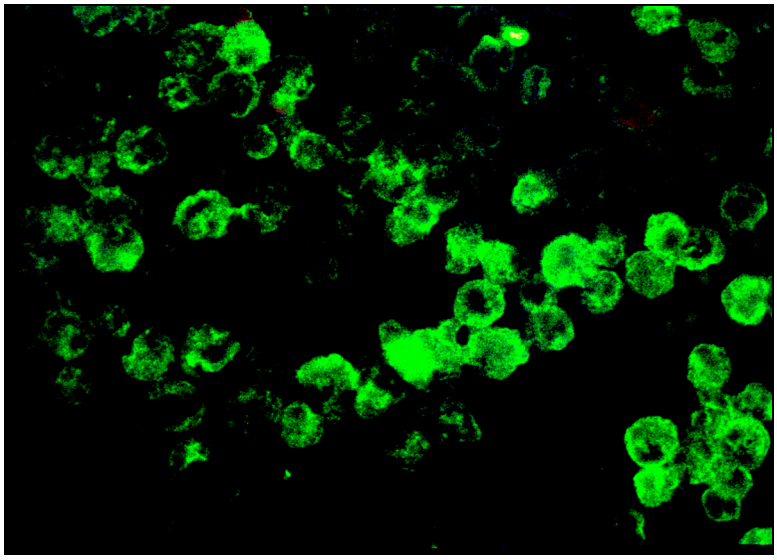

Figure 34-1 Photomicrograph of amebic meningoencephalitis caused by *Naegleria fowleri*

RESERVOIRS FOR THE AGENT

Although, infection with *N. fowleri* has been documented in a South American tapir and cattle, no human or animal reservoir for *N. fowleri* has been identified so far. However, birds such as ducks and geese that have a fairly high body temperature may act as reservoirs for the thermophilic *N. fowleri*. Aquatic mammals (beavers, otters, and muskrats) also may prove to be a source for *N. fowleri*. A comprehensive survey of animals that inhabit aquatic habitats and reservoirs needs to be conducted.

MODE OF TRANSMISSION

N. fowleri is transmitted through the nasal passages. The trophozoites and possibly the flagellates enter the nostrils during swimming, diving, splashing while wading, or waterskiing in lakes and other bodies of fresh water. The trophozoites make their way into the olfactory lobes through the cribriform plate and cause an acute hemorrhagic necrosis that destroys the olfactory bulbs and the cerebral cortex.

METHODS FOR DETECTING THE AGENT

Materials suspected of containing *N. fowleri*, such as cerebrospinal fluid (CSF), brain tissue, soil and water, must be collected aseptically in order to detect and isolate the agent. The specimens may be kept at room temperature (24 to 28°C), or at 4°C for short periods of time (but never for more than 24 hours), and should never be frozen. Personnel handling the specimens must take appropriate precautions, such as wearing surgical masks and gloves and working in a biological safety cabinet.

The best method to identify *N. fowleri* in the CSF is to centrifuge the sample, remove the supernatant, and prepare several microscope slides by placing one drop of the sediment on each slide. One of the slides is coverslipped and examined under a microscope. *N. fowleri* can be identified by its rapid and directional movement. Other slides should be stained, preferably with Giemsa or trichrome, and examined under a microscope. *N. fowleri*, if present, can easily be identified by its nucleus with a centrally placed large nucleolus. An immunofluorescence test is also available to detect

Figure 34-2 Transmission of primary amebic meningoencephalitis due to *N. fowleri*

N. fowleri in the CSF sample as well as in formalin-fixed paraffin-embedded brain tissue sections. The CSF and the macerated brain tissue may be processed for culture on an agar plate coated with *Escherichia coli* or *Enterobacter aerogenes*. A polymerase chain reaction (PCR) test is also available for the detection of *N. fowleri*.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

More than 200 cases of PAM caused by *N. fowleri* have been reported worldwide, including >100 cases in the United States. *N. fowleri* is found worldwide and has been isolated from fresh water, including tap water, thermal discharges of power plants, heated swimming pools, hydrotherapy and remedial pools, aquaria, sewage, and even from nasal passages and throats of healthy individuals. *N. fowleri* is thermophilic and therefore proliferates when the ambient temperature is raised. PAM cases occur typically in the hot summer months and coincide with the increase in the number of people engaging in aquatic activities in freshwater bodies, such as lakes and ponds, as well as improperly chlorinated swimming pools that may harbor the amoebas. It is estimated that one case of PAM occurs in approximately 2.5 million swimmers.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

N. fowleri, especially the cyst forms, can likely survive for long periods in moist soil and water. *N. fowleri* cysts held at 4°C for up to 8 months were able to produce PAM in mice. The cysts become nonviable if dried, frozen, or heated above 51°C.

DOCUMENTED WATERBORNE OUTBREAKS

Most cases of PAM have occurred in children and young adults who have had a history of swimming, diving, wading, and waterskiing in swimming pools, lakes, ponds, rivers,

thermally polluted water, and hot springs. A series of PAM cases involving 16 young persons in the former Czechoslovakia between August 1962 and September 1965 was traced to a swimming pool that received heated river water treated with chlorine. It was later found that *N. fowleri* amoebas were present in an area with inadequate chlorine levels. Several cases of PAM in the Richmond, Va., area were all traced to one of three lakes in that area. *N. fowleri* has also been isolated from the drinking water in Australia, and several cases of PAM that occurred in summer months in South Australia have been attributed to children splashing in small home-made pools that used a household water supply. It was suggested that the household water was warmed to 35–45°C by the sun because it was carried in pipes aboveground for long periods. Recently, a child in Georgia, United States, died of PAM due to *N. fowleri* because of swimming and playing in a small river where the water temperature was 33°C. Further, two children in Arizona died of PAM, one after playing in a jacuzzi and the other after swimming in a backyard swimming pool, both filled with household unchlorinated water tapped from a deep aquifer.

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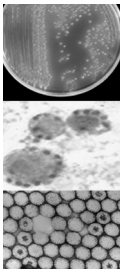


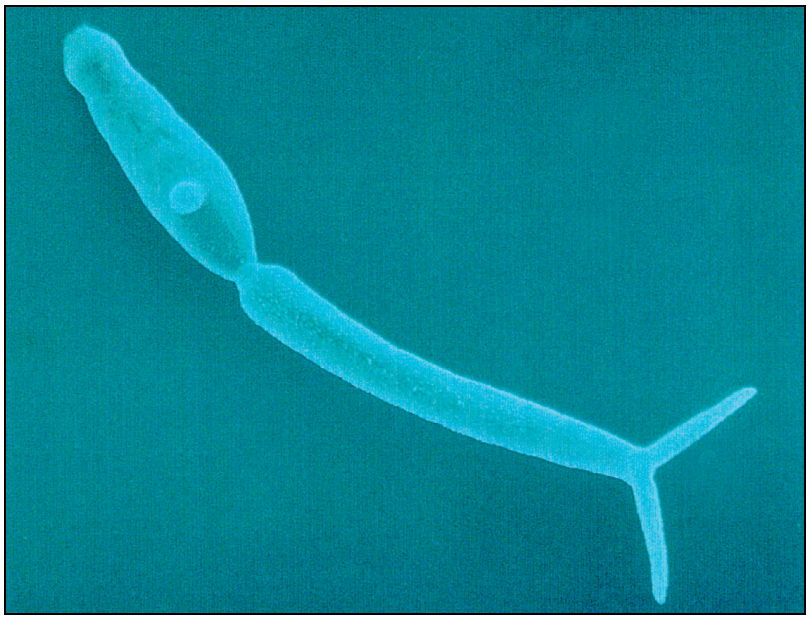
Chapter 35

Schistosomatidae

Harvey Blankespoor

DESCRIPTION OF THE AGENT

The parasites that cause swimmer's itch (schistosome cercarial dermatitis) belong to a group of flatworms known as digenetic trematodes of the family Schistosomatidae (Figure 35-1). Although schistosomes found in humans (*Schistosoma*) may be responsible for this condition, the disease is usually associated with nonhuman species, particularly those that use birds as final or definitive hosts. Some common genera that cause swimmer's itch include *Gigantobilharzia, Ornithobilharzia, Schistosomatium,* and *Trichobilharzia*.



Source: Harvey Blankespoor, Hope College, Holland, Mich.

Figure 35-1 Scanning electron micrograph of cercaria causative agent for *Schistosoma*

The life cycle of nonhuman schistosomes involves an aquatic snail and a warm-blooded vertebrate, usually an aquatic bird. Eggs of the parasite are voided with the host's feces. On contact with water, a free-living, nonfeeding stage (miracidium) hatches from the egg and actively swims for up to a day. If the miracidium contacts an appropriate snail species, it penetrates the tegument or, with some species, is ingested by the snail. It then migrates to a specific region and elongates to form a complex of germinating sacs called mother sporocysts. Usually a second generation (daughter sporocysts) are asexually produced and they produce another life-cycle stage known as cercariae. These nonfeeding, free-swimming larvae emerge from the snail and live approximately 24 hours. If they contact a suitable bird species, they enter the host and migrate to the lungs and liver before entering the mesenteric veins to develop into egg-producing adults. If they enter a human, the cercariae die in the skin; sensitive individuals will develop swimmer's itch.

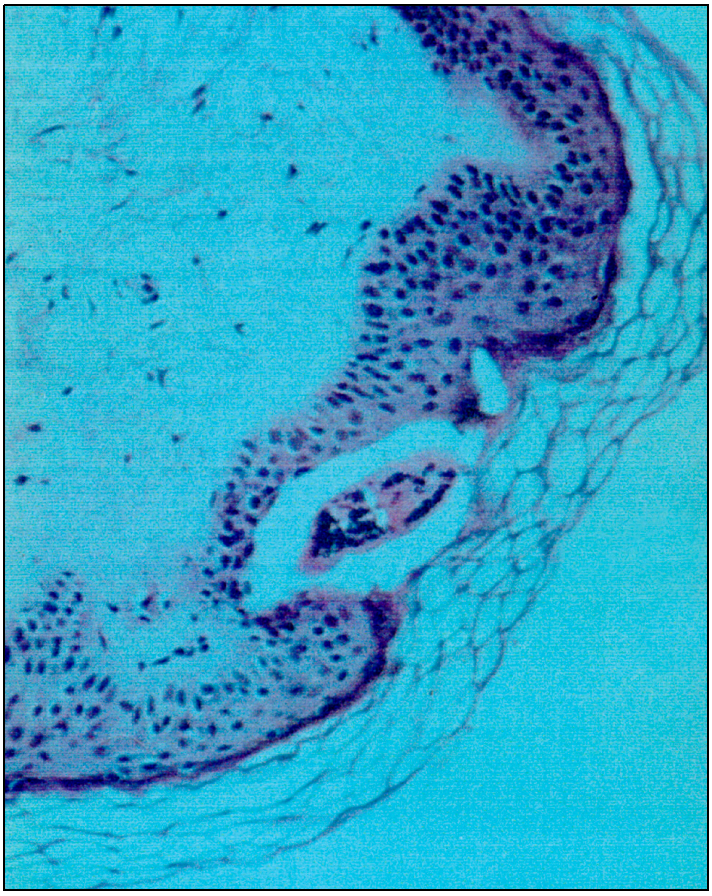
Cercariae are fork-tailed (furcocercous), range in length from 200 to 300 µm, and have a body and tail. The body is elongated and contains four pairs of penetration glands, a ventral sucker, an anterior holdfast organ, and two eye spots. The outer covering, or tegument, is covered with minute spines that make the cercaria difficult to withdraw from vertebrate skin once penetration has begun. The tail is 20 to 40 µm in diameter with a length of 100 to 200 µm. It has a main stem and two distal flaps called furcae; finfolds may or may not be present. Distinguishing characteristics of the cercaria include the absence of a pharynx, the presence of a ventral sucker (protrusible acetabulum), two flame cells near the proximal end of the tail, and four pairs of penetration glands.

DESCRIPTION OF THE DISEASE

Swimmer's itch is a delayed hypersensitivity response to the cercarial stage of nonhuman schistosomes. Cercariae that are unable to penetrate the dermis layer of the skin of nonsuitable hosts, such as humans, die, and within a half hour a small red area may appear at the site of penetration. This spot may continue to increase in size for about 24 to 30 hours. Once it becomes reddened and raised, it is called a *papule* and forms where each cercaria enters the skin (Figure 35-2). Itching continues for several days. The symptoms vary depending on the age and sensitivity of the individual, type or lack of medication used, amount of irritation from scratching, and the species of schistosome that causes the dermatitis. Diagnosis is based on finding papules only on body areas exposed to water within the past few days (Figure 35-3). Treatment includes prescription drugs containing antihistamines to minimize itching and topical steroid (cortisone) creams to reduce the swelling. A nonprescription drug, Ken-Tox, provides relief for this condition. Outbreaks of swimmer's itch in Michigan usually occur from the end of May to early September, but latitude and seasonal fluctuations may affect this timing. Although swimmer's itch has the potential to occur on all lakes, it seems most common on larger recreational lakes with sandy or rocky shorelines. Onshore winds appear to concentrate the cercariae close to the water's edge.

RESERVOIRS FOR THE AGENT

In the Midwest, as many as 20 species of nonhuman schistosomes can cause swimmer's itch. Most of the snail intermediate hosts belong to families Lymnaeidae or Physidae. A few species of family Planorbidae can harbor dermatitis-causing nonhuman schistosomes. Vertebrates that serve as final or definitive hosts for the dermatitis-producing schistosomes include ducks; geese; swans; gulls; some perching birds, such as blackbirds and starlings; and rodents, such as rats, muskrats, and voles.



Source: Harvey Blankespoor, Hope College, Holland, Mich.

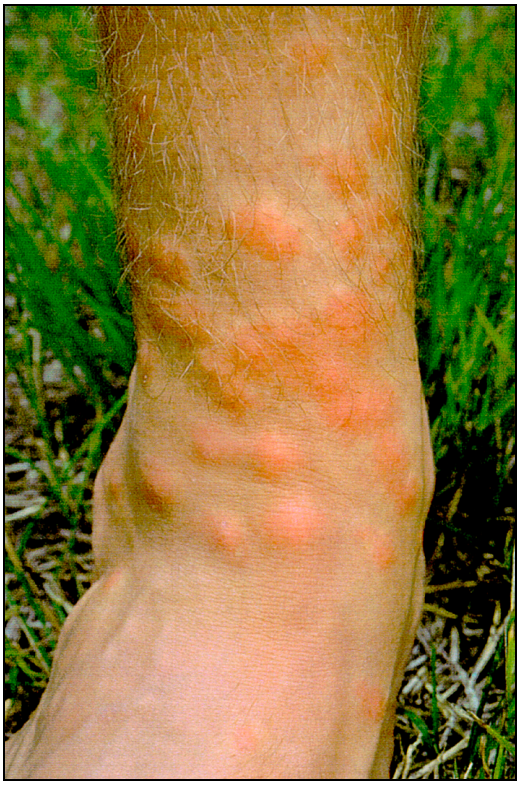
Figure 35-2 Section of human skin with the parasite isolated

MODE OF TRANSMISSION

Free-living larval stages of nonhuman schistosomes are only transmitted in water. Hatched, ciliated miracidium must contact the snail intermediate host in the water. Furthermore, cercariae leaving the snail must swim in water to come in contact with the vertebrate host. Transmission is not possible from snail to snail or from bird to bird.

METHODS FOR DETECTING THE AGENT

Cercariae are difficult to detect in natural bodies of water for several reasons. First, the larvae are small and nearly transparent. Second, it is nearly impossible to predict where they will occur. Factors affecting their presence and abundance include the density and location of snail hosts, wave action, water currents, and time of day. If cercariae are subjected to harsh handling techniques, the body and tail separate, leaving the larval flatworm immobile. Finally, they are difficult to distinguish morphologically from cercariae of some other digenetic trematodes (e.g., Strigeidae) that do not cause swimmer's itch. Filtering large volumes of lake or pond water, then staining the intact cercariae is not a reliable way to isolate and identify schistosome cercariae.



Source: Harvey Blankespoor, Hope College, Holland, Mich.

Figure 35-3 Ankle affected by *Schistosoma*

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Cercariae of avian schistosomes typically live for 1 day, depending on the water temperature. The larvae live longer at lower temperatures and die more rapidly at warmer ones. Depending on the species, cercariae emerge at a specific time each day, usually with the onset of light. This emergence coincides with the habits of the vertebrate host involved in the cycle for the maximum contact opportunity.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Two methods are used to control swimmer's itch. The traditional method is to apply a molluscicide in mid-June to break the life cycle by destroying the snail intermediate host. This type of treatment has met with mixed results, and the long-term effects of this toxin on aquatic ecosystems is a concern. A new method of control focuses on capturing and treating the birds with an anthelmintic drug, Praziquantel. Seed-eating birds can be fed treated corn or bread.

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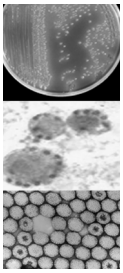

Chapter 36

Toxoplasma gondii

J.P. Dubey

DESCRIPTION OF THE AGENT

Toxoplasma gondii is an obligate (requiring hosts), intracellular, coccidian parasite usually transmitted by ingestion of infective tissues from animals through the placenta, or ingestion of food and water contaminated with infected cat feces. Cats excrete a resistant form of *T. gondii* (oocysts) that are noninfective in their feces. After defecation, the development of infective sporozoites inside the oocyst requires 1 day or more, depending on environmental conditions. Oocysts are 10 × 12 µm in size, can survive up to 18 months in unfavorable environmental conditions, and are remarkably resistant to most disinfectants.

After a warm-blooded animal ingests fecal-contaminated food or water, the oocyst ruptures in the intestine and releases eight sporozoites. Sporozoites multiply in the intestinal cells and in associated lymph nodes, and tachyzoites (rapidly multiplying forms) are formed. Tachyzoites are banana-shaped and measure 6 × 2 µm. Tachyzoites disperse to the rest of the body through blood and lymph, eventually encysting in the brain, skeletal and cardiac muscles, and liver. Encysted *T. gondii* organisms are called bradyzoites (slowly multiplying forms). Tissue cysts are microscopic (less than 70 µm in diameter) and survive in tissues for the duration of the life of the host. Tachyzoites are found during the acute phase, whereas encysted bradyzoites (tissue cysts) are found predominantly in the latent, chronic phase. Bradyzoites are about 7 × 2 µm in size and a tissue cyst may enclose over 1,000 bradyzoites.

After infected tissues are ingested, enzymes dissolve the wall of the tissue cyst, releasing the bradyzoites that infect intestinal cells of the host. After entering host cells, bradyzoites transform into tachyzoites. These intracellular tachyzoites may undergo repeated intracellular divisions. Ultimately, they are dispersed throughout the body and encyst in tissues. The life cycle of *T. gondii* is completed when tissue cysts are ingested by cats and oocysts are then formed.

DESCRIPTION OF THE DISEASE

T. gondii causes mental retardation, loss of vision, hearing impairment, and mortality in congenitally infected children. Although toxoplasmosis is mostly an asymptomatic infection in adults, it causes serious disease and death in immunosuppressed persons, especially those with acquired immunodeficiency syndrome (AIDS). In AIDS patients, latent *T. gondii* infection can be reactivated resulting in an overwhelming disease.

Encephalitis is the predominant feature of toxoplasmosis in AIDS patients. *T. gondii* is also a major cause of abortion in sheep and goats and can cause mortality in virtually all warm-blooded animals. It is very pathogenic for marsupials and New World monkeys, especially in zoos.

RESERVOIRS FOR THE AGENT

Cats, including wild Felidae, are the only definitive hosts for this parasite.

MODE OF TRANSMISSION

Human beings and other animals become infected primarily by ingesting food or water contaminated with oocysts or by ingesting infective animal tissues that contain tissue cysts of *T. gondii*. During pregnancy of infected hosts, *T. gondii* can multiply in the placenta and spread to fetal tissues. Although such transplacental infection can occur during any stage of gestation, the fetus is affected most severely when the pregnant female becomes infected during the first half of gestation.

Soil contaminated by feral cats is probably a greater risk than soil contaminated by cats housed indoors. *T. gondii* infection is higher in feral cats than in domestic cats because they hunt birds and small mammals in which a sylvatic cycle of *T. gondii* is found.

METHODS FOR DETECTING THE AGENT

T. gondii oocysts are 10 to 12 μm in diameter and can be seen with a microscope. A bioassay should be performed because other coccidians have similar oocyst morphology. Methods used to concentrate *Cryptosporidium* and *Giardia* oocysts from water by filters can be applied to examine water reservoirs for *T. gondii* oocysts. The final sediment from filters should be inoculated orally into laboratory mice and be examined for *T. gondii* infection.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

T. gondii infects virtually all warm-blooded hosts. It is one of the most widespread infections of human beings.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

T. gondii oocysts are highly resistant to environmental influences and survive freezing and drying. They are also resistant to ammonia, chlorine, and commonly used disinfectants, including formalin. They are killed by heat (70°C) but will survive for 30 minutes at 56°C. They are killed by 0.30 KgY gamma irradiation. Irradiation may be a practical way of decontaminating fruits and vegetables contaminated with oocysts.

DOCUMENTED WATERBORNE OUTBREAKS

An outbreak of toxoplasmosis involving many patients was epidemiologically linked to a water reservoir in British Columbia. Another small outbreak occurred in U.S. Army soldiers who drank water from a pond during jungle exercises in Panama. In both of these episodes, persons had persistent symptoms of acquired toxoplasmosis, including chorioretinitis.

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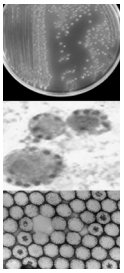


Chapter 37

Trichuris trichiura

Huw V. Smith, Anthony M. Grimason, and Celia Holland

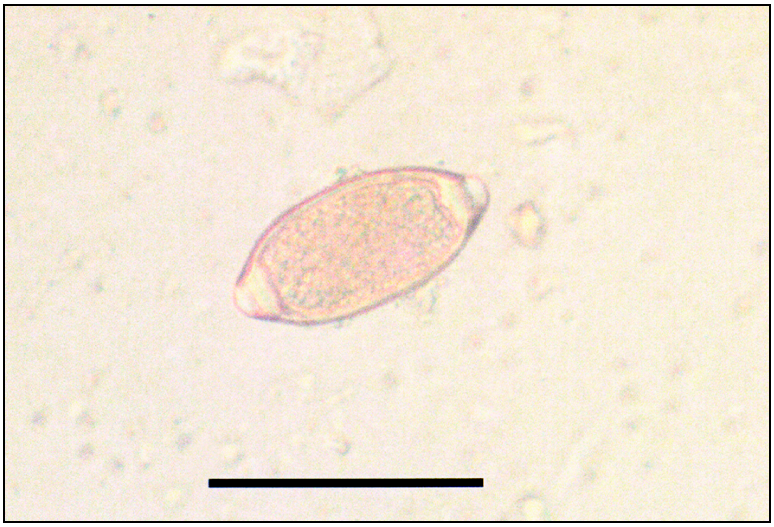
DESCRIPTION OF THE AGENT

Trichuris trichiura, superfamily Trichuroidea, is a nematode parasite of man, commonly known as whipworm because of the whip-like appearance of the adult stage. The parasite exists in three distinct forms, namely, the egg or ovum, the larva, which migrates through various organs of the body, and the adult, which resides in the large intestine of human beings. The sexes are separate. Similar species parasitize other primates, rodents, camels, and some domesticated animals. The genus is also referred to by its former name *Trichocephalus*, especially in the Spanish and French medical literature. *Trichuris* is translated as "hairtail," whereas *Trichocephalus* is translated as "hairhead."

Adult males and females reside in the cecum, with their anterior ends anchored in a tunnel within the epithelial cells lining the cecum, and with their posterior ends lying free in the lumen. Adult females, fertilized by adult males, can lay up to 20,000 ova daily, and fertilized eggs are excreted unembryonated in feces (Figure 37-1). Embryonation occurs in the environment. A moist microclimate and ambient temperature encourage embryonation. Development from fertile egg to the first-stage larva (L1) within the eggshell takes between 14 and 28 days, under ideal conditions. At this stage the larva within the egg is infectious to other susceptible human beings. Following ingestion, the L1 in the infective egg is stimulated to hatch in the intestine. Hatched L1 pass from the small intestine into the large intestine where they penetrate the epithelial lining of the crypts, predominantly those of the cecum. Larvae develop and molt through the second, third, and fourth larval stages and become adult worms at this site. The cycle from infection to egg laying adult normally takes about 8 weeks.

The transmissive stage of *T. trichiura* infection is the fertilized egg, and the infective stage is the fully embryonated egg which contains the L1 larva. Fertilized eggs, which do not contain a fully developed L1 larva, are not infective.

The most commonly identified stage is the fertilized egg. Fertilized eggs are excreted unembryonated and embryonate to infectivity (containing a fully developed L1) in the environment. Eggs are ovoid in shape (lemon-shaped) and are generally brown in color due to the uptake of bile pigments present in the intestine. Ova measure 22 to 24 µm by 50 to 55 µm and have a brown, smooth eggshell. At each tip of the lemon-shaped ovum is a translucent plug. *T. trichiura* are readily distinguished from the ova of other intestinal parasites of humans. The adult female worm measures



Source: H.V. Smith, Scottish Parasite Diagnostic Laboratory.

Figure 37-1 *Trichuris trichiura* egg (bar represents 60 μm)

30 to 50 mm in length and the adult male 30 to 45 mm with a tightly coiled posterior end with a single spicule. At the anterior end of adults of both sexes is the mouth, without lips, but with a stylet which is used to penetrate the mucosal lining of the gut. The posterior end of the female tapers to a point whereas the posterior end of the male is curved or hook-shaped. Both sexes have a narrower anterior portion and a wider posterior portion, the wider posterior portion representing the handle of a whip.

DESCRIPTION OF THE DISEASE

The prepatent period (about 2 months) is the interval of time between ingesting infectious eggs and the first appearance of ova in the stool. The incubation period is the interval of time between ingesting infectious eggs and the development of recognized symptoms or signs of illness and is dependent upon the number of infectious ova ingested and the susceptibility of the individual. In light infections, individuals are asymptomatic, and there has been much reluctance in accepting that heavy infections give rise to disease. Symptoms occur when adult worms number 50 to 150. When several hundred or thousand adult worms are resident, a pancolitis with chronic illness, known as *Trichuris* dysentery syndrome (TDS) can occur. The principal features of TDS are chronic diarrhea, anemia, and growth retardation. Symptoms include anorexia, pica, lower abdominal pain, mucoid diarrhea, dysentery, and rectal prolapse (especially in intense trichuriasis). As the cycle from infection to egg laying adult female normally takes about 8 weeks, the period between infection and recognition of symptoms, especially systemic symptoms, is often greater.

Trichuris is frequently found concurrently with *Ascaris*, hookworm, and *Entamoeba histolytica*. Significant positive correlations between egg counts for *Ascaris* and *Trichuris* have been demonstrated in individual children.

RESERVOIRS FOR THE AGENT

Humans are the reservoir of *T. trichiura*. Coprophagous animals such as pigs, dogs, cats, and chicken which feed on human feces can act as transport hosts by dispersing

Table 37-1 Occurrence of *Trichuris* sp. ova in raw sewage

Country	*Trichuris* sp. Ova and/or Occurrence (% removal)
Argentina	0–3.03 ova/L
Bangladesh	13,800–52,800 ova/L, mean 39,044 ova/L
Brazil	0–57 ova/L
Cayman Islands	273 ova/L; (5.5%)
Colombia	Mean 63 ova/L
Egypt	0.5 ova/L
France	Detected, numbers not stated*
Germany	Detected, numbers not stated*
India	(Study 1) mean 33 ova/gal
India	(Study 2) 1.2–2.4 × 10^5 ova/0.5 mgd
Iran	500–1,500 ova/L, mean 750 ova/L
Japan	10–20 ova/L
Mexico	Detected, numbers not stated*
Morocco	7 ova/L
Pakistan	Detected, numbers not stated*
Puerto Rico	689 ova/17 L
Syria	0–100 ova/100 mL

* Data on the occurrence and removal of *Trichuris* sp. ova in sewage are normally presented as occurrence and removal of helminth ova. Some publications refer to *Ascaris* spp., others to *Trichuris* spp., and yet others to mixtures of both or mixtures of various intestinal helminth ova.

or redistributing fertile ova to other sites associated with human activities. Pigs, lemurs, and monkeys may act as reservoir hosts. Filth flies can also act as transport hosts because of their association with feces and their ability to travel over large distances. Filth flies ingest 1 to 3 mg of feces over 2 to 3 hours. *Trichuris* ova have been found in surface water, groundwater, and seawater but not in treated drinking water. They have also been detected in night soil, sewage (Tables 37-1 and 37-2), sludge (Table 37-3), soil, and on crops and beaches.

Densities of *Trichuris* sp. ova can vary greatly in raw wastewaters and are indicators of endemic disease within the human and nonhuman population. Other factors include the size and socioeconomic status of the population, the percentage of the population sewered, shock loadings from the discharge of pit latrine tankers, the provision of separate or combined sewers, and seasonal and sampling factors. *Ascaris* ova occur more frequently and in significantly larger numbers than *Trichuris* ova.

Large numbers of *Trichuris* ova (up to 52,800 ova/L) have been reported in raw sewage, but the methods used currently underestimate their true occurrence and concentration. The use of different methods for sampling, concentration, and analyses make the data from different studies difficult to compare; however, lower concentrations of ova in raw and treated effluents are reported from developed countries rather than from developing countries.

Table 37-2 Occurrence of *Trichuris* sp. ova in sewage effluents

Country	Treatment	*Trichuris* sp. Ova and/or (% removal)
Bangladesh	OXFAM emergency double-vault septic tank 1 effluent	Mean 40 ova/L (99.95%) not detected (100%)
	OXFAM emergency double-vault septic tank 2 effluent	
	OXFAM permanent double vault septic tank and mini-pond (n = 2) system	
	Double-vault effluent	1,920–10,800 ova/L; mean 5,942 ova/L (84.8%)
	Pond 2 effluent	640–2,200 ova/L; mean 1,480 ova/L (96.2%)
Brazil	Stabilization pond pilot system	
	Anaerobic pond 1 Effluent	0–2 ova/L
	Anaerobic pond 2 Effluent	0–3 ova/L
	Anaerobic pond 3 Effluent	0 ova/L
	Upward anaerobic sludge blanket reactor effluent	Mean 0.1 ova/L
	Upward anaerobic sludge blanket reactor and baffled stabilization pond	Mean 0.99 ova/L
Egypt	Trickling filter effluent	Mean 0.09 ova/L (82%)
	Trickling filter and gravel bed hydroponic	0 ova/L
India	Sedimentation	Mean 16 ova/gal (52%)
	Sedimentation	90%
	Activated sludge Plant A	Mean 0.6 ova/L (91.8%)
	Activated sludge Plant B	100%
	Activated sludge	100%
	Trickling filter plant A	92.5%
	Trickling filter plant B	100%
	Biological disc	60%
	Aerated lagoon	100%
	Oxidation ditch (pilot scale)	100%
	Stabilization pond system 1	6 ova /L (68.4%)
	Stabilization pond system 2	100%
	Stabilization pond system 3	100%
	Stabilization pond system 4	100%
Morocco	Sedimentation	0 ova/L
	High-rate algal pond	0 ova/L
	Sewage irrigation	37% (ova not detected*)
	Primary waste stabilization pond	0 ova/L
	Secondary waste stabilization pond	0 ova/L
Puerto Rico	Primary sedimentation	13.8 ova/L (66%)

* Data on the occurrence and removal of *Ascaris* sp. ova are normally presented as occurrence and removal of helminth ova. Some publications refer to *Ascaris* spp., others to *Trichuris* spp., and yet others to mixtures of both or mixtures of various intestinal helminth ova.

Table 37-3 Viability of *Trichuris* sp. ova in sewage, sludge, and water

Country	Sample Type	Percentage of Viable *Trichuris* sp. Ova
Brazil	Untreated sludge	50%
	Anaerobic treated sludge	20%
Mexico	Flocculated prim. sludge and thickened (15 days)	98%
	Flocculated prim. sludge and sedimentation	Mean 7 viable ova/g TS
	Flocculated primary sludge and sedimentation	Ova detected*
	Flocculated primary sludge and sedimentation + 15% CO at 21.3°C / 2 hr	Inactivated*
	Flocculated primary sludge and sedimentation + 15% CO at 23.8°C / 2 hr	Inactivated*
South Africa	Seawater for 2 days (laboratory scale)	Less resistant than *Ascaris*

TS = total solids.

* Data on the occurrence and removal of *Ascaris* sp. ova are normally presented as occurrence and removal of helminth ova. Some publications refer to *Ascaris* spp., others to *Trichuris* spp., and yet others to mixtures of both or mixtures of various intestinal helminth ova.

MODES OF TRANSMISSION

T. trichiura is transmitted when infective ova are ingested by a susceptible human host. Because of the requirement for external maturation of ova to infectivity in the environment, contact with recently voided feces is not a risk. In endemic areas, major routes of transmission include

- defecation by infected individuals, especially children, in backyards or compounds, which leads to the contamination of soil, fingers, hands, and cooking utensils, and other surfaces
- use of untreated human feces as fertilizer (night soil), especially for crops that receive minimal heating prior to consumption
- defecation by agricultural workers in or near to the fields in which they work (defecating in or near flowing water is often seen as being more sanitary than defecating into latrines where often no water supply is available for hand washing)
- use of untreated sewage effluent for irrigating crops and untreated and treated sludge for fertilizing crops which receive minimal heating prior to consumption
- consumption of contaminated unfiltered drinking water or communal drinking water
- redistribution of fertile ova to other uncontaminated sites associated with human activities by coprophagous animals such as pigs, dogs, cats, and chickens which feed on human feces

METHODS FOR DETECTING THE AGENT

Detection of *T. trichiura* ova, either in feces or in the environment, is by the morphological and morphometric examination of suspect organisms by bright field micros-

copy. In human beings, serology has, on occasion, been used to attempt to confirm or exclude infection.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Trichuris ova can occur commonly in the environment, densities being dependent upon the level of infection in the indigenous population. *Trichuris* ova can be found in surface water, groundwater, and seawater but not in treated drinking water. They can also be present in feces, night soil, sewage, sludge, soil, and on crops and beaches.

The life span of adult female *T. trichiura* is between 1 and 8 years. In endemic areas, where reinfection is common, susceptible hosts can excrete fertile ova for extended periods of time. In nonendemic areas, the period of communicability lasts as long as the last fertilized, egg-laying female worm(s) is resident in the intestine.

Based primarily upon cases with positive tests for eggs, the prevalence of *Trichuris* is 902 million (17 percent) worldwide. Prevalence increases rapidly with age in early childhood and attains a plateau in adulthood, remaining high and relatively constant in endemic regions. Socioeconomic and ethnic differences in behavior can influence exposure to infective *Trichuris* eggs. The average worm burden of *Trichuris* peaks in the child-age classes and shows a marked decline in adulthood. *Trichuris* worm burdens follow an aggregated or overdispersed frequency distribution with most individuals harboring few or no worms and few individuals harboring many. Significant correlations between the worm burdens of *Trichuris* before and after anthelmintic treatment can be observed from reinfection studies.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Because the embryo develops into the infective larva outside the host, *Trichuris* ova are well suited to prolonged survival in the environment. Embryo development and survival in the environment depend upon aerobic metabolism; however, ova can also survive in anaerobic conditions. Under conditions of ambient temperature (22 to 35ºC), moisture, shade from ultraviolet (UV) light, and oxygen, *Trichuris* ova take 11 to 38 days to become infective. At 15ºC, *Trichuris* ova may take 4 to 6 months to develop to infectivity. Infective *Trichuris* ova can remain viable for several months, with about 20 percent remaining viable for more than 18 months. However, exposure for short periods of time (hours) to temperatures above 37°C will kill developing embryos. The viability of *Trichuris* spp. ova exposed to a variety of environments is summarized in Table 37-3.

There is a need to address the concentrations of viable ova remaining in conventionally treated sludge samples, which can be used as fertilizer for domestic and agricultural purposes. In Europe, there is a move towards some form of heat treatment of sludge as a minimum before sludge application to agricultural land, used to grow food crops, is allowed. In the United Kingdom, currently, there is a voluntary agreement on a ban upon the use of untreated sludge on land used for growing food crops.

DOCUMENTED WATERBORNE OUTBREAKS

Although associated with foodborne outbreaks of disease, there is no documented waterborne outbreak of trichuriasis in association with treated public drinking water supplies.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Effective coagulation and filtration processes remove ova.

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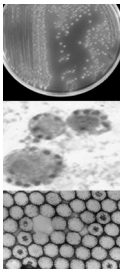


IV

Introduction to Viral Pathogenic Agents

Adenoviruses
Astroviruses
Emerging viruses
Enteroviruses and Parechoviruses
Hepatitis A virus
Hepatitis E virus
Human Caliciviruses (Noroviruses and Sapoviruses)
Reoviruses
Rotaviruses

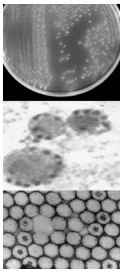
Viruses are the smallest and most basic of known life forms. Their extremely small size (18–120 nm in diameter) and resistance to chemical and environmental degradation present great challenges to the drinking water treatment industry. Viruses consist only of nucleic acid and a protein shell (or capsid) and cannot replicate independently. Their simple genetic system is a single-stranded or double-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Because of their inability to autonomously reproduce, viruses must take over a living cell and usurp cellular machinery in order to replicate. After replication, and the subsequent death of the host cell, viral particles are spread to neighboring cells, resulting in the infection of the individual.

VIRUSES IN THE ENVIRONMENT

More than 120 different enteric viruses are known to infect humans. Enteric viruses are excreted in high numbers (from 10^7 to 10^{11} per gram) in the feces of infected individuals and may directly or indirectly contaminate water intended for drinking. These viruses are commonly isolated in domestic wastewater, even after disinfection. Once in the environment, they can survive for long periods of time, up to several months under cool and moist conditions. The enteric viruses include the enteroviruses, rotaviruses, hepatitis A and E, noroviruses, adenoviruses, reoviruses, and many emerging or previously unidentified viruses. These viruses are predominantly transmitted by

the fecal–oral route, infect the gastrointestinal or respiratory tracts, and are capable of causing a wide range of illness, including diarrhea, fever, hepatitis, paralysis, meningitis, and heart disease. Despite causing a variety of illness in humans, many viral infections are remarkably asymptomatic (with no clinical symptoms). Even in asymptomatic infections, however, virus particles can be shed in the environment. Virus detection and identification in water supplies has been limited by the difficulties and expense of conventional cell culture assays. However, advances in molecular techniques including polymerase chain reaction (PCR) for DNA viruses and reverse transcription–PCR (RT-PCR) for RNA viruses have provided simpler and more rapid methods for viral monitoring of water samples.

The following chapters discuss the groups of viruses identified as sources of waterborne disease outbreaks or those that have the potential to cause outbreaks.



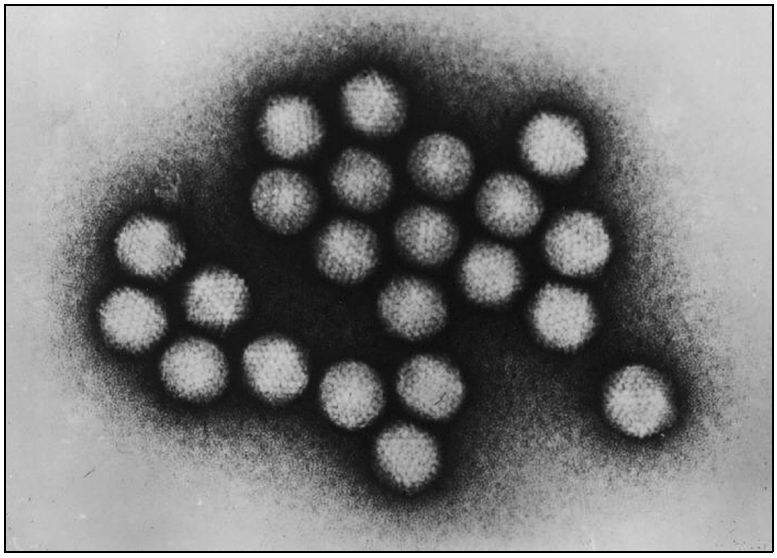
Chapter 38

Adenoviruses

Carlos Enriquez and Jeanette Thurston-Enriquez

DESCRIPTION OF THE AGENT

Adenoviruses belong to the Adenoviridae family, which includes human (49 serotypes), simian (27 serotypes), bovine (10 serotypes), equine (1 serotype), porcine (4 serotypes), ovine (1 serotype), and canine (3 serotypes) viruses. These agents are approximately 70 to 100 nm in diameter (Figure 38-1). The adenovirus genome consists of double-stranded deoxyribonucleic acid (DNA) surrounded by a nonenveloped protein capsid.



Source: G. William Gary Jr., Centers for Disease Control and Prevention.

Figure 38-1 Transmission electronmicrograph of andenovirus

DESCRIPTION OF THE DISEASE

Human adenoviruses may cause acute respiratory disease, pneumonia, epidemic conjunctivitis, and acute gastroenteritis in children. Most clinical conditions are caused by approximately 30 percent of all types of adenoviruses. Respiratory disease caused by adenovirus is observed mostly among children. It is characterized by general malaise, fever, nasal congestion, and cough. The most prevalent adenoviruses associated with respiratory infection are types 1, 2, 5, and 6. Waterborne conjunctivitis caused by adenoviruses may occur sporadically or in large groups. The most common adenoviruses causing this type of conjunctivitis are serotypes 3 and 7, but other types have been implicated as well. Swimming pool conjunctivitis is observed mostly among young adults and children during the summer. Conjunctivitis sometimes accompanies respiratory symptoms. Recovery without complications usually follows this condition. Although many adenoviruses replicate efficiently in the intestine, only the enteric adenoviruses 40 (Ead 40) and 41 (Ead 41) have been recognized as important causes of gastroenteritis in children, similar in importance to astrovirus and rotovirus. Adenoviral gastroenteritis occurs mostly in children under the age of 2, particularly during the first year of life. The incubation period is 1 to 3 days, and symptoms are watery diarrhea, sometimes with vomiting. Unlike rotaviral infections, enteric adenoviral illness does not vary by season.

RESERVOIRS FOR THE AGENT

Human adenovirus typically is not pathogenic to animals, and animal adenovirus is only pathogenic to the species of origin. However, asymptomatic infections with human adenovirus type 12 has been documented in simian species, and antibodies to bovine, simian, and canine adenoviruses have been found in humans.

MODE OF TRANSMISSION

Adenoviruses can enter a susceptible host by mouth, nose, or eye membranes. Adenoviruses may be shed for months or years, because of low levels of virus replication in tonsils, adenoids, and intestines of infected individuals. Hurst et al. (1988) suggested that enteric adenoviruses could be transmitted through recreational or drinking water. The enteric nature of the adenoviruses 40 and 41, their presence only in the gastrointestinal tract, and their extensive distribution suggest that water may play a role in the transmission of these agents.

METHODS FOR DETECTING THE AGENT

Nonenteric adenoviruses can be grown in Hep-2 cells, and both enteric and nonenteric adenoviruses can be grown in human primary liver carcinoma cells (PLC/PRF/5) with the production of cytopathic effect (CPE). Successful growth of enteric adenoviruses 40 and 41 occurs in a cell line derived from a human colon carcinoma (CaCo-2 cells). Polymerase chain reaction (PCR) and gene probe techniques have been used to detect adenoviruses from environmental water. A promising new method for the detection of infectious human adenoviruses 2 and 41 was recently reported. A reverse transcription–PCR (RT-PCR) procedure was used to detect enteric adenovirus-specific mRNA in cell culture assays. AD2 and AD41 were detected within 6 and 24 hours after inoculation of 10E+6 infectious units per cell culture assay. Levels as low as 5 infectious units were detected within 3 days postinfectivity assay. Comparison of this mRNA RT-PCR to traditional cell culture demonstrated that this method is very specific, sensitive, and rapid for the detection of infectious adenoviruses in water.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

A comparative study of CPE, immunofluorescence, and in situ DNA hybridization suggested that 80 percent of infectious adenoviruses in raw sewage may be enteric adenoviruses. The number of indigenous adenoviruses detected in primary sewage sludge was 10 times greater than the number of enteroviruses. In addition, a greater number of adenoviruses than enteroviruses have been found consistently in raw sewage around the world.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

The stability of enterovirus 70 and human adenovirus type 19 were compared under different conditions. Drying these agents at room temperature on ophthalmic instruments resulted in a 99.999 percent (5-log) reduction of enterovirus 70. In contrast, the titer of adenovirus 19 decreased less than 90 percent (1 log) after 11 days under the same conditions. Studies have shown that adenovirus type 2 survived longer than poliovirus 2, vaccinia virus, coxsackievirus B3, and herpes virus. Adenovirus type 5 survived longer than both poliovirus 1 and echovirus 7 in tap water, at either 4 or 18°C. The Ead 40 and Ead 41 survive longer than polio 1 in primary and secondary wastewater. The survival of these two agents was even higher when compared to hepatitis A virus (HAV) and polio in tap and seawater. Evidence shows higher thermal stability of the enteric adenoviruses compared with polio 1 when Ead 40 and polio 1 were held at 50, 65, and 80°C in phosphate-buffered saline. The inactivation rate of poliovirus 1 was higher at the three tested temperatures. In addition to thermal stability, Ead 40 shows some resistance at low and high pH levels. An experiment evaluated the stability of this virus at pH values of 3.5, 9.5, 10, and 10.5. No reduction of Ead 40 infectivity was observed after 45 minutes at either pH 3.5, 9.5, or 10. However, after 45 minutes at pH 10.5, Ead 40 suffered a reduction of 2.7 log. Other data have indicated that adenovirus type 5 was sensitive to high pH values, and found that the infectivity of this agent decreased steadily at pH higher than 9.0, with rapid inactivation at pH 10.

DOCUMENTED WATERBORNE OUTBREAKS

Documented waterborne outbreaks of conjunctivitis caused by adenovirus type 3 and type 4 have been reported. The only viral waterborne outbreak from 1991 to 1992 in the United States associated with recreational waters was caused by adenovirus type 3, which affected 595 individuals causing conjunctivitis, pharyngitis, and fever. In April 1994 a waterborne outbreak of enteric viruses, including adenovirus, occurred in a Finnish municipality, where 25 to 50% of the population (1,500 to 3,000 reported cases) was affected. The outbreak was associated with polluted river water that contaminated a groundwater well.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Chlorine, chlorine dioxide, and ozone appear to be very effective for the inactivation of human adenoviruses in water. Like adenovirus type 40 (Table 38-1), adenovirus type 3 is less resistant to chlorine inactivation compared to poliovirus. $C \times T$ values for adenovirus type 3 inactivation by chlorine have been reported to range from 0.013 to 0.084 mg/L × min (high and low pH and temperature conditions). Human adenovirus types 3 and 7 were found to be inactivated by 99 percent at chlorine levels of 0.69 mg/L and 0.89 mg/L, respectively. In experiments that combined chlorine and copper or silver ions, adenovirus type 5 was more resistant than poliovirus, but less resistant than HAV and human rotavirus. $C \times T$ values range from 0.03 to 0.40 mg/L × min for 99.99 percent ozone inactivation of adenovirus type 2 at pH 7 and 5°C. Depuration of enteric adenovirus 40 from

Table 38-1 $C \times T$ values for 99.99% inactivation of enteric adenovirus type 40 by various disinfectants in buffered demand-free water

Disinfectant	pH	Temperature (°C)	$C \times T$ Value*
Chlorine	6	5	0.22
	8	5	0.24
Chlorine dioxide	6	5	1.28
	8	5	0.67
Ozone	7	5	0.07 to 0.60
UV light	7	22–25	226†

Source: Adapted from Thurston-Enriquez et al. 2003a, b.

* mg/L × min, or UV light dose (mW·s/cm^2).
† Extrapolated value based on linear regression.

artificially contaminated mussels, in a continuous flow of ozonated marine water, took much longer than the removal of poliovirus 1. However, it was depurated faster than either HAV or human rotaviruses. Unlike these oxidizing disinfectants, human adenoviruses are very resistant to inactivation by ultraviolet (UV) light (Table 38-1). Adenovirus type 40, for example, requires UV doses of 50, 109, 167, and 226 mW·s/cm^2 for 90, 99, 99.9, and 99.99 percent inactivation, respectively. In fact, adenovirus type 40 is the most UV-light-resistant virus studied to date. Comparative studies have reported that 90 percent inactivation of adenovirus type 40, adenovirus type 2, coliphage MS-2, feline calicivirus, poliovirus type 1, and echovirus type 1 requires UV doses of 50, 43, 23, 6, 8, and 8 mW·s/cm^2, respectively.

A recent pilot-scale study showed the inability of physical treatment processes (coagulation and filtration) to remove human adenoviruses. Moreover, adenoviruses that bypassed secondary and tertiary treatment were not inactivated by posttreatment UV light disinfection. Indicator bacteria; however, were reduced to levels in compliance with water recycling criteria.

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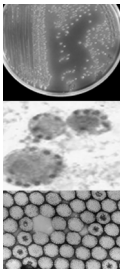


Chapter 39

Astroviruses

Kellogg J. Schwab

DESCRIPTION OF THE AGENT

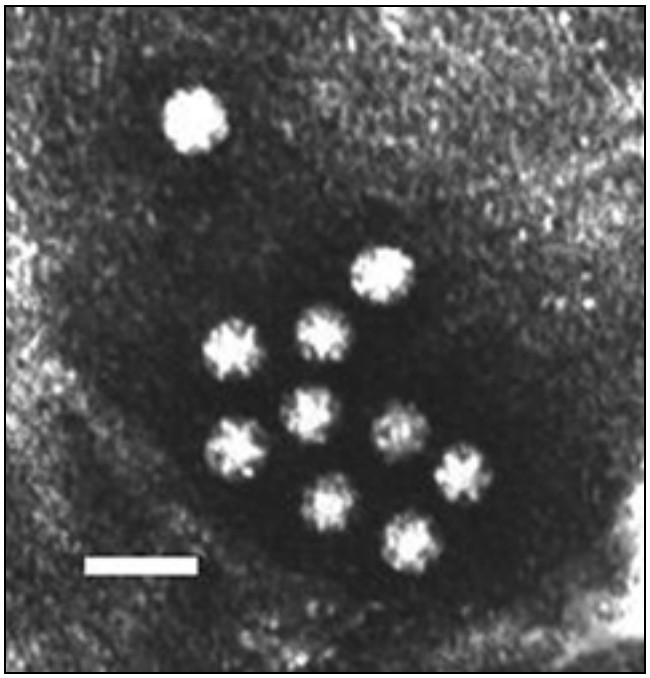
Human astroviruses, classified in genus *Mamastrovirus* (human and animal astrovirus) within the family *Astroviridae*, are small (27–43 nm in diameter), nonenveloped viruses that have a single-stranded, polyadenylated, positive-sense ribonucleic acid (RNA) genome approximately 6.8 to 7.2 kb in length (Figure 39-1). These viruses were given the name *astro* (astron, "star" in Greek) because of the characteristic five- or six-point star shape they often display when viewed by electron microscopy (EM). To date, eight human astrovirus serotypes have been described with serotype 1 the most globally prevalent, whereas the second most common serotype differs depending on the country. Serotypes 6 and 7 have rarely been detected.

DESCRIPTION OF THE DISEASE

Astroviruses have been isolated from humans as well as numerous animal species. In most species, astroviruses are found in association with gastroenteritis although extraintestinal manifestations are also observed in avian species. In humans, after a 1- to 4-day incubation period, the clinical symptoms of astrovirus infection present as self-limiting watery diarrhea that typically lasts for 2 to 3 days associated with vomiting, fever, anorexia, and abdominal pain. Protracted diarrhea and viral shedding has been observed as well as documented asymptomatic infections. While the disease is most often mild and does not result in severe dehydration, it can be more serious in immunocompromised children and adults and elderly institutionalized patients. Studies have indicated that astroviruses are common causes of diarrhea in children worldwide and that most children are infected during their first 2 years of life. Astroviruses are second only to rotaviruses as a cause of hospitalization for childhood viral gastroenteritis. Human volunteer studies have indicated that astrovirus, unlike norovirus, is of relatively low pathogenicity in adults.

RESERVOIRS FOR THE AGENT

The family *Astroviridae* includes human and animal astroviruses causing gastroenteritis in a wide variety of young mammals and birds including lambs, calves, deer, piglets, mice, kittens, and puppies. Ducklings infected with astrovirus can develop rapidly fatal hepatitis. Although the viral capsid proteins of astroviruses that infect



Source: F.P. Williams, US Environmental Protection Agency.

Figure 39-1 Negative-stain transmission electron micrograph of Astrovirus; bar equals 50 nanometers

different hosts are reportedly highly divergent, similarities among human astrovirus, feline astrovirus, and porcine astrovirus capsid sequences suggest that zoonoses (animal to human transmission) involving pigs, cats, and humans could occur. However, astrovirus infection appears to be species specific, and to date no interspecies transmission has been documented.

MODE OF TRANSMISSION

Transmission of human astrovirus infections occurs via the fecal–oral route. Contaminated food and water have been associated with outbreaks of human astrovirus-associated gastroenteritis; however, the risk attributable to food and water contamination in the transmission of human astroviruses has not been fully elucidated.

METHODS FOR DETECTING THE AGENT

Astroviruses were first observed by EM in stool specimens from infants with gastroenteritis. However, this method was relatively insensitive, and studies based on EM detection indicated that astroviruses were a rare cause of gastroenteritis. For example, hospital-based EM studies indicated that incidence rates of astrovirus infection never exceed 4 percent. The development of more advanced methods of detection such as enzyme-linked immunosorbent assays (ELISA), nucleic acid sequence-based amplification (NASBA), and reverse transcription-polymerase chain reaction (RT-PCR) have revealed that astroviruses are second only to rotavirus as the most common cause of viral gastroenteritis in children worldwide and that adults can become infected with astrovirus if exposed to large doses.

A major advance in the ability of investigators to study astroviruses came as the result of the finding that with the addition of trypsin into the assay, astroviruses could be propagated in the CaCo2 continuous line of colon carcinoma cells. Researchers have now begun to investigate how infectious astroviruses respond to environmental degradation and drinking water disinfectants using both cell culture and integrated cell culture-reverse transcription-PCR (ICC-RT-PCR) procedures.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Little information is available on the environmental occurrence of human astroviruses. However, since infected individuals may excrete large numbers of viruses in the feces, human astroviruses are present in sewage. Animal astroviruses are also present in the environment, but to date there is no indication that nonhuman astroviruses are of human health risk. A study in Finland found high concentrations of human astrovirus in raw sewage during a diarrhea epidemic in a local day-care center. In this study, the presence of astroviruses did not correlate with bacterial indicators of fecal contamination. Astroviruses have also been found in shellfish. It should be noted that since astroviruses only replicate in living host cells, the numbers of astroviruses will not increase once they are shed into the environment.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Astroviruses are nonenveloped enteric viruses that are acid stable and resistant to a range of detergents and lipid solvents. Astroviruses are heat resistant for short periods above 56°C and survive for long periods below –20°C.

A recent study investigated the persistence of human astroviruses applied onto fomites as monitored by ICC-RT-PCR. Astroviruses persisted longer than poliovirus and adenovirus but shorter that rotavirus and hepatitis A virus (HAV) on fomites, particularly at low temperature.

DOCUMENTED WATERBORNE OUTBREAKS

In addition to rotaviruses and noroviruses, astroviruses are now recognized as important etiologic agents of viral gastroenteritis in all age groups. However, astrovirus is not routinely screened for in stool or environmental samples, and data on the health impact of waterborne astrovirus are lacking. A recent study evaluated the potential impact of astrovirus in drinking water by assessing the relationship between incidence of gastroenteritis and astrovirus RNA prevalence in public drinking water systems in France. The study found 12 percent (8/68) of the analyzed water samples were positive for astrovirus and that the presence of astrovirus RNA was associated with a significant increased risk of gastroenteritis, suggesting a role for waterborne astrovirus in the endemic level of gastoenteritis in the general population. Astroviruses have also been associated with an outbreak of gastroenteritis at a wading pool in the Netherlands.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

There have been limited studies on the effectiveness of water treatment in removing or inactivating astroviruses. Human astroviruses are similar in size, morphology, and nucleic acid composition with other enteric viruses such as poliovirus, HAV, and caliciviruses and thus astroviruses most likely respond to treatment processes in a similar fashion. A recent study used ICC-RT-PCR to measure the persistence of astrovirus suspended in dechlorinated tap water and free chlorine. In dechlorinated tap water following 60 days, the reduction of astrovirus infectivity was 2 $\log_{10}$ units at 4°C and 3.2 $\log_{10}$ units at 20°C, and following 90 days there was 3.3 and 5 $\log_{10}$ units reduction

at 4°C and 20°C, respectively. Following 2-hour incubation in the presence of 0.5 or 1 mg free chlorine per liter, residual astrovirus infectivity was still found with a 2.4 and 4 $\log_{10}$ reduction in titer, respectively.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

Currently there are no national or international guidelines or monitoring recommendations for astroviruses.

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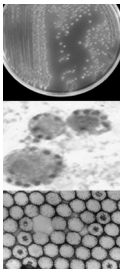


Chapter 40

Emerging Viruses

Charles P. Gerba

DESCRIPTION OF THE AGENT

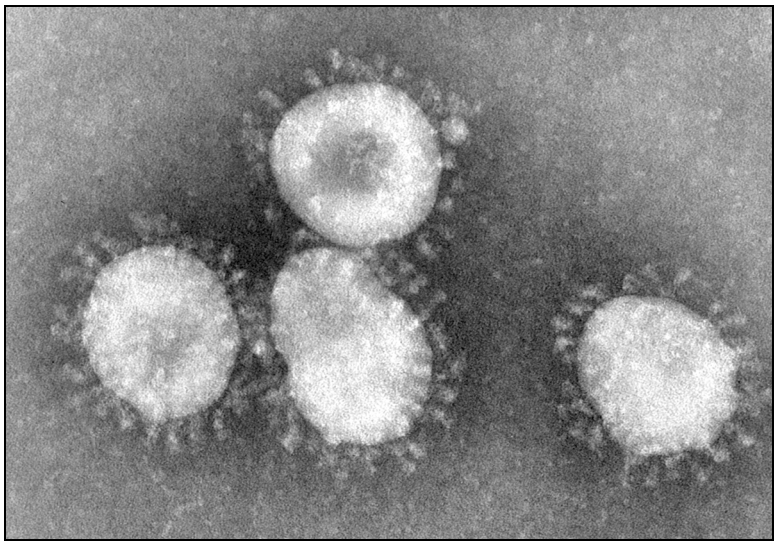
The development of molecular methods for the detection of enteric viruses has led to the continued discovery of new viruses. Several of the recently discovered enteric viruses that infect humans have been found to have unique genomes which could make them more resistant to some treatment processes than the well-studied enteric viruses. Table 40-1 contains a list of emerging viruses and their properties. While waterborne transmission has not yet been demonstrated, all are excreted in the feces of infected persons and have the potential to be transmitted by water.

Parvoviruses are single-stranded viruses, which have been associated with gastroenteritis in man; they are the smallest known human enteric viruses and have the lowest isoelectric point. They are the most resistant of the human enteric viruses to inactivation by heat. TT virus (TTV) and TTV-like mini virus (TLMV) were the first human circoviruses to be described. They both contain circular single-stranded, deoxyribonucleic acid (DNA). JC virus (JCV) is a polyoma virus etiologically associated with a fatal demyelinating disease known as progressive multifocal leukoencephalopathy (PML). Polyoma viruses are small, double-stranded DNA viruses that are very heat stable. Picobirnaviruses are small, double-stranded ribonucleic acid (RNA) viruses associated with gastroenteritis. Coronaviruses are single-stranded RNA

Table 40-1 Characteristics of emerging viruses

Virus	Type of Nucleic Acid	Size (nm)	Remarks
Parvo	ss DNA	18–25	Low isoelectric point; smallest human enteric viruses; most heat-stable enteric virus
Corona	ss RNA	100–120	Enveloped
Polyoma	ds DNA	38–43	Very heat stable
Picobirna	ds RNA	30–40	
Circo (TTV and TLMV)	Circular ss DNA	30–32	Very heat stable

ss = single stranded
ds = double stranded



Source: Fred Murphy, Centers for Disease Control and Prevention.

Figure 40-1 Coronaviruses are a group of viruses that have a halo or "corona" appearance when viewed under a microscope

viruses with a lipid membrane (enveloped) associated with respiratory illness and gastroenteritis in humans. Severe acute respiratory syndrome (SARS) is caused by a coronavirus (Figure 40-1).

DESCRIPTION OF THE DISEASE

Parvoviruses, coronaviruses, and picobirnaviruses have all been associated with gastroenteritis. Picobirnaviruses are largely associated with gastroenteritis in acquired immunodeficiency syndrome (AIDS) patients and a cause of traveler's diarrhea. Coronaviruses (including SARS) have also been associated with respiratory illness. JC virus has been associated with PML in AIDS patients and colon cancer. TT virus was originally isolated from patients with hepatitis of unknown etiology; however, the role of TTV and TLMV in human disease is still uncertain.

RESERVOIRS FOR THE AGENT

No animal reservoirs of the emerging enteric viruses are currently known, although viruses in the same genus or family have been detected in animals for all of the emerging viruses.

MODE OF TRANSMISSION

All of the emerging viruses have been detected in feces or sewage suggesting fecal–oral transmission is possible, although respiratory route of infection is also possible. JC virus infects the kidneys and is excreted in the urine.

METHODS FOR DETECTING THE AGENTS

The polymerase chain reaction (PCR) or the reverse transcription-PCR (RT-PCR) used for DNA or RNA virus detection, respectively, has been used to detect JC virus, TT virus, and SARS in sewage. The SARS virus detected in sewage by RT-PCR was not

found to be infectious in cell culture. Methods are available to detect all of the emerging viruses by PCR or RT-PCR. SARS and two other human coronaviruses, as well as JC virus, can be grown in cell culture.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

JC virus, TTV, and coronavirus infections are common in the human population. Prevalence of TT virus has been found to range from 21 to 74 percent, indicating that it is a common infection. In a study in Spain, it was found that 41 percent of children tested were excreting JC virus.

No animal reservoirs of the emerging enteric viruses are currently known, although viruses in the same genus or family have been detected in animals for all of the emerging viruses.

JC virus has been detected in sewage throughout the world. TT virus has been detected in sewage in India. SARS virus was detected in sewage from a hospital in which infected patients were treated.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Because of the lack of cell culture assays to assess infectivity, no information exists on the survival of parvoviruses, picobirnaviruses, and TT virus in the environment. The animal parvoviruses, polyomaviruses, and circoviruses are known to be very heat stable. JC virus survives for many days in sewage. Parvoviruses are the most heat stable enteric viruses known. Human coronavirus 229E does not survive as long as poliovirus type I in water and sewage. It can survive as long as 14 days at 5°C. SARS appears capable of survival in the environment for 3 to 4 days.

DOCUMENTED WATERBORNE OUTBREAKS

No waterborne disease outbreaks have been documented for any of the emerging enteric viruses. Parvoviruses have been associated with two foodborne outbreaks.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

No information on the effectiveness of water treatment processes on any of the human emerging viruses is available. The simian polyoma virus SV40 was found to be more sensitive to chlorine inactivation than the enteroviruses. The small, double-stranded RNA of picobirnaviruses may make them more resistant to ultraviolet (UV) light disinfection than single-strand enteric RNA viruses.

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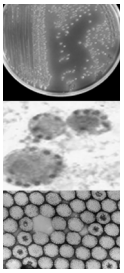

Chapter 41

Enteroviruses and Parechoviruses

Charles P. Gerba

DESCRIPTION OF THE AGENT

Enteroviruses and parechoviruses are in the genus of the Picornaviridae family, which are among the smallest ribonucleic acid (RNA) viruses. "Pico" means small RNA virus. Enteroviruses are divided into the following five groups: poliovirus (3 types), enterovirus A (12 types), enterovirus B (37 types), enterovirus C (11 types), and enterovirus D (2 types). There are currently three known types of parechovirus. Parechovirus has recently been recognized as a new genus in the family Parechovirus based on distinctive molecular and biological properties. Echovirus types 22 and 23 have been placed in this group and renamed parechoviruses 1 and 2. These viruses have been associated with gastroenteritis, mycocarditis, and encephalitis. All enteroviruses and parechoviruses are nonenveloped, icosahedral in shape, and 25 to 30 nm in diameter. They contain a genome of single-stranded RNA of the positive sense, which acts like messenger RNA (mRNA). A characteristic of enteroviruses is their resistance to high and low pH. They are stable at pH 3 to 5 for 1 to 3 hours and can tolerate pH 10 to 11 for minutes. They are resistant to all known antibiotics and chemotherapeutic agents. Alcohol (70 percent), Lysol (5 percent), 1 percent quaternary ammonium compounds (Roccal), or similar laboratory disinfectants are not effective. They are also insensitive to ether and detergents that destroy lipid-containing viruses.

DESCRIPTION OF THE DISEASE

Enteroviruses cause a wide variety of illnesses, ranging from paralytic poliomyelitis (polio) to the common cold (Table 41-1). While most infections result in asymptomatic or mild illness, these viruses can cause more serious illness, including myocarditis, diabetes, and acute febrile illness among young children and infants. They are the most common cause of aseptic meningitis in developed countries. Fatalities in more severe illnesses (meningitis, myocarditis, and paralytic disease) can range from 0.01 to 0.9 percent. Poliovirus is the most studied of the enteroviruses. Poliomyelitis has been known since ancient times, but became a major concern in the earlier part of the

Table 41-1 Human enteroviruses and parechoviruses and clinical illness

Virus		Clinical Illness
Poliovirus	3 types	Paralysis Aseptic meningitis Febrile illness
Enterovirus A	12 types	Paralysis Aseptic meningitis
Coxsackievirus	A 2–7 A 8–16	Hand, foot, and mouth disease Encephalitis
Enterovirus	71 76	Hepangina Exanthema Diarrhea
Enterovirus B	37 types	Aseptic meningitis Paralysis
Coxsackievirus	B1–B6, A9	Exanthems Respiratory diseases
Echovirus	1–9, 11–21, 24–33	Diarrhea
Enterovirus	69, 73–78	Pericarditis Myocarditis Febrile illness
Enterovirus C	11 types	Paralysis Aseptic meningitis
Coxsackievirus A	1, 11, 13, 15, 17–22, 24	Myocarditis Encephalitis
Enterovirus D		Pneumonia Acute hemorrhagic conjunctivitis
Enterovirus	68, 70	
Parechoviruses	1–3	Pericarditis Herpangina Respiratory disease

20th century when epidemics primarily affecting young children became common during the summer and fall. The virus can cause permanent nerve damage that paralyzes arms, legs, or breathing. Most symptomatic infections only cause influenza-like or respiratory illness. Symptoms include aseptic meningitis, fever, rash, and severe headache, followed by stiffness. Only a small percentage of cases (0.1 to 2 percent) develop into paralytic disease. Poliovirus vaccine and large-scale vaccination programs have eradicated paralytic poliomyelitis from the Western Hemisphere. The World Health Organization (WHO) is hoping to eliminate the disease from the human population in the next few years.

The incubation period for enteroviruses varies greatly and may range from 1 to 35 days. Shorter incubation periods (2 to 3 days) are typical for respiratory-tract infections. Laboratory diagnosis has usually been by isolation of the virus from stool, rectal swabs, throat swabs, or spinal fluid following inoculation into primate cell cultures. Serological typing with antisera pools or genome sequencing is used to identify the specific enterovirus. Persons with enterovirus infections usually excrete the virus several days before clinical symptoms, during the acute illness, and often for several

weeks afterwards. The period of communicability is potentially long. Enteroviruses are also excreted by persons who have asymptomatic infections.

In temperate climates, enterovirus infections peak in the late summer and fall. Enterovirus infections are greater in lower socioeconomic groups and are very common in young children. The incidence of children excreting enteroviruses has been observed to range from 2 percent in late fall to 40 percent in the summer.

RESERVOIRS FOR THE AGENT

Humans are the only known natural host for enteroviruses. Although serologically distinct enteroviruses have been found in many animals, humans do not usually have recognizable infections with "animal" enteroviruses. Some laboratory animals, however, are susceptible to infection with human enteroviruses, including primates and neonatal mice for the coxsackieviruses.

MODE OF TRANSMISSION

The fecal–oral route or the respiratory route may transmit enteroviruses. One route may be more predominant than another, depending on the serotype. All enteroviruses are believed to be capable of transmission by the fecal–oral route, with the possible exception of enterovirus type 70. This type, which causes acute hemorrhagic conjunctivitis, may be transmitted only by direct contact with contaminated hands and fomites. Most transmission is believed to be person-to-person. Outbreaks of coxsackie- and echoviruses from water and food have been documented.

METHODS FOR DETECTING THE AGENT

Enteroviruses can be grown in primate or human animal cell cultures with the production of cytopathogenic effects (CPE), although they may not all grow in the same cell line. Enteroviruses in water or other environmental samples are usually isolated by the production of CPE in the buffalo green monkey kidney (BGMK) cell line. Many of the enteroviruses also can be isolated by the plaque-forming unit (PFU) method. The use of the reverse transcription polymerase chain reaction (RT-PCR) to detect the viral genome in cell culture of non-cytopathogenic enteroviruses has led to increasing reports of enteroviruses in drinking water in developing countries.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Because enteroviruses grow easily in cell culture, they have been the human viruses most commonly isolated from the environment. The concentration of enteroviruses in raw sewage has been reported to vary from a few hundred per liter to more than 100,000/L in some parts of the world. The concentration of enteroviruses in sewage is greater during the late summer and fall, and depends on the incidence rates in the community and the sanitation condition within the population. The average concentration of enteroviruses in raw sewage in the United States is estimated to be approximately 7,000/L.

Coxsackieviruses are the enteric viruses most often isolated from water, including treated drinking water. Enteroviruses are not completely removed by domestic sewage treatment, including standard disinfection, and can usually be isolated in sewage effluent. Enteroviruses have been isolated in almost any source exposed to human fecal contamination, including groundwater, marine waters, and sediments, shellfish, crabs, crops irrigated with sewage, domestic solid waste, soil, aerosols, and where spray irrigation of sewage is practiced.

The use of integrated cell culture RT-PCR to detect the presence of noncytopathogenic enteroviruses in cell culture has led to increasing reports of enteroviruses in drinking water in developing countries. Several recent studies in the United States have reported the detection of enteroviruses in groundwater ranging from 8 to 32 percent of the samples tested.

The incidence of the virus in human fecal material depends on the same factors that influence its occurrence in sewage. The incidence in young children, which is higher than adults, may range from 10 to 40 percent during the summer months in some age groups.

Although animals are not the normal hosts of enteroviruses, they have been isolated from the stools of dogs, cats, and other domestic animals. They are not known to cause disease in these animals. Their occurrence is thought to be related to the often-close association with these animals in developing world households.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

The survival of enteroviruses in the environment has been shown to depend on many factors (e.g., temperature, sewage pollution, bacteria, and adsorption to solids [clays and sediments]). Typically, the lower the temperature, the longer enteroviruses will survive. Enteroviruses may survive for years below 5°C in the environment.

DOCUMENTED WATERBORNE OUTBREAKS

Before the advent of the poliovirus vaccine, several published reports attributed outbreaks in part to contaminated drinking water. The studies, however, were not adequate to be conclusive. Coxsackieviruses have been associated with two meningitis recreational outbreaks, and an epidemiological study of bathers in surface waters found a significant increased risk of enteroviral infection in children. Echovirus 30 was associated with an outbreak of gastroenteritis, and echovirus 9 with meningitis, both related to swimming pools. There have recently been outbreaks of echovirus 30 and coxsackievirus B4 meningitis reported from Europe associated with drinking water, but the epidemiological evidence remains scanty.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Enteroviruses have been the most studied of all enteric viruses for their ability to be removed by water treatment processes. Enteroviruses appear to be capable of being removed easily by conventional water treatment involving disinfection (99.99 percent or greater). Coxsackieviruses appear to be more resistant to ultraviolet (UV) light disinfection than the other enteroviruses.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

The European Economic Community (EEC) has had an enterovirus standard for bathing waters of no plaque forming units per 10 L since at least 1985. The EEC has also recommended that enteroviruses be absent in 10-L volumes of drinking water. The state of Arizona, under its wastewater reuse standards, previously had an allowable limit of one enteric virus per 40 L of full-body contact water. There are no national guidelines.

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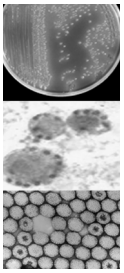


Chapter 42

Hepatitis A Virus

Mark D. Sobsey

DESCRIPTION OF THE AGENT

Hepatitis A virus (HAV) is a major cause of infectious hepatitis and the major cause of enterically transmitted infectious hepatitis in the United States. HAV is a member of the Picornaviridae family and the only member of the hepatovirus genus. There is only one serotype of HAV, but like other viruses with a single-stranded ribonucleic acid (RNA) genome, there is considerable genetic variability in the form of four genogroups of humans and three genogroups of nonhuman primates and further genetic differences within these genogroups.

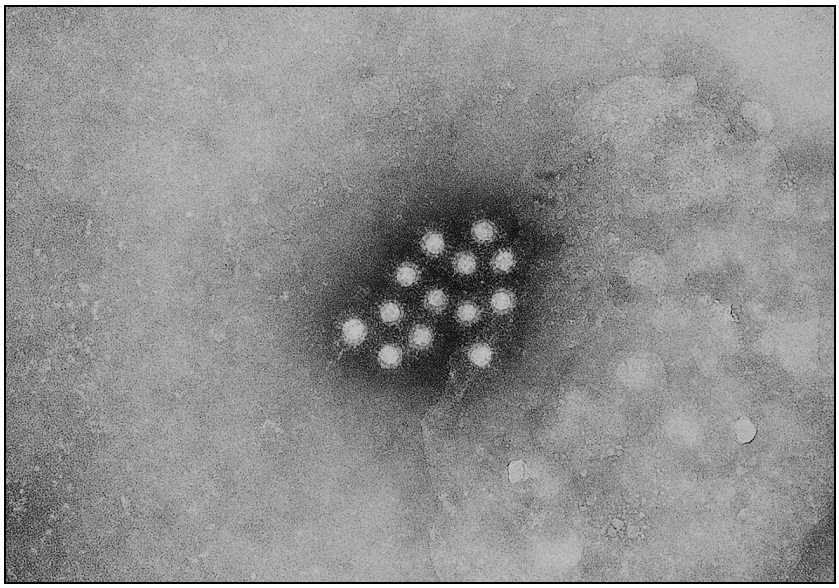
HAV is a nonenveloped or "naked," icosahedral virion of about 27 nm in diameter (Figure 42-1). Virions consist of single-stranded, plus-sense RNA about 7,500 nucleotides long, with a poly(A) tract on the 3' end, within a capsid (protein coat) containing four structural proteins, designated VP1, VP2, and VP3, and the putative VP4.

HAV is one of the most resistant viruses to a variety of physical, chemical, and biological agents. Compared to most other viruses, it is quite stable in water up to a temperature of 60°C, or even 80°C if stabilized with high concentrations of divalent cations. It is very stable to pH extremes, tolerating pH levels as low as 1 and as high as 10 for hours. It can be inactivated by strong oxidizing agents, such as free chlorine and ozone, and by powerful alkylating agents, such a gluteraldehyde. Compared to other enteric viruses, HAV is quite resistant to a variety of proteolytic enzymes, including those produced by naturally occurring microbes in water and soil.

DESCRIPTION OF THE DISEASE

HAV causes the disease known as "infectious hepatitis," which is an acute inflammation of the liver. Hepatitis E virus (HEV), a currently unclassified virus described in chapter 43, also causes infectious hepatitis like that of HAV and can also be waterborne. HEV is rare in the human population of the United States although widespread in many other regions of the developing world. However, HEV strains genetically similar to those in humans have been found in swine, rats, and several other animals in the United States and other parts of the world.

Infectious hepatitis is caused by ingestion of fecal matter containing the virus, including fecally contaminated food and water. Infection is thought to begin in epithelial cells of the intestinal tract, although the replication site has not been identified. The virus apparently spreads in the bloodstream to the liver where it destroys parenchymal



Source: Betty Partin, Centers for Disease Control and Prevention.

Figure 42-1 Electron micrograph of the hepatitis A virus

cells, is excreted in bile, and shed in feces. The incubation period averages about four weeks, ranging from 2 to 6 weeks, and symptoms typically last 4 weeks with a range of 2 weeks up to 6 months.

The mortality rate of hepatitis A (HA) is low, less than 0.3 percent in the United States, but can be higher in more susceptible members of the population and in other countries where additional factors contribute to poor health. The ratio of symptomatic to asymptomatic (subclinical or inapparent) infection varies with age. More than 90 percent of infections in infants and young children are asymptomatic. Symptomatic infection increases with age, reaching 70 percent by adulthood. The severity of illness also increases with age, with most adults experiencing jaundice.

HA has an abrupt onset with clinical symptoms of malaise, loss of appetite, dark urine, nausea, and vomiting. Scleral icterus (yellowing of the whites of the eyes) often occurs, as does jaundice and a tender liver. Shedding of the virus in feces, which can reach a billion virions per gram, begins during the incubation period and as early as 2 weeks before the onset of symptoms. Fecal shedding of viruses declines during the acute phase of illness, but can continue for weeks. Fecal material from HAV cases is infectious for weeks and highest in infection risk before clinical illness occurs. Virus is present in blood, serum, and saliva, but at much lower levels than in feces. Liver damage results not only in jaundice, but also increased levels of certain serum enzymes indicative of liver damage, primarily alanine and aspartate aminotransferase (ALT and AST). Liver damage is reversible, and the virus disappears from the body and recovery is complete.

Serum antibodies indicative of an immune response begin to appear about 4 weeks after infection. Initially, the levels of immunoglobulin M (IgM) are higher than the levels of immunoglobulin G (IgG), and this is diagnostic for current or recent infection. Over several months, levels of IgM wane and levels of IgG rise, which indicates past or historical infection. Immunity is considered to be lifelong. In the United States, as in and other developed countries, HA infection is declining, with an estimated 61,000 new cases in 2003 and 33 percent of Americans ever infected (antibody

positive). Seroprevalence is less than 10 percent in young children and rises with age to about 50 percent in young adults. In developing countries with poor sanitation and hygiene, HAV infection is acquired early in life and seroprevalence approaches 100 percent in young adults.

RESERVOIRS FOR THE AGENT

Humans are the main reservoir for HAV. Nonhuman primates can be experimentally infected with human isolates of HAV, and they harbor their own unique genotypes or strains of HAV. Natural infection of humans with nonhuman primate strains of HAV may be possible. The primary endemic source of HA in the United States is inapparent or asymptomatic infection among children. Such unrecognized infection facilitates transmission to their adult contacts within and between households, in childcare centers, and in other settings. Decreasing the burden of HA in the United States is now based on widespread vaccination of children at least 2 years old. Dramatic decreases of HA have occurred in the United States, especially in the age groups and regions for which routine childhood vaccination is recommended.

MODE OF TRANSMISSION

Hepatitis A is transmitted primarily by ingesting feces containing HAV. In addition to direct and indirect personal contact, the virus is transmitted in fecally contaminated water, foods, fomites, and other objects. Less commonly, HAV is parenterally transmitted by infectious blood, serum, and therapeutic factors from blood, such as clotting factors. HAV transmission by exposure to respiratory exudates or urine is not likely. Water is an important vehicle of HAV transmission, but the other routes, especially food, account for the majority of reported cases of illness from outbreaks.

METHODS FOR DETECTING THE AGENT

Detecting HAV in water and other environmental samples is difficult and requires special techniques. In feces and other clinical samples containing high virus concentrations, HAV antigens can be detected by immunoassays, but laboratory diagnosis of suspected cases of HAV is primarily by detection of IgM antibodies in serum. HAV can be recovered and concentrated from water and other environmental samples by methods developed and used for other enteric viruses. HAV from clinical and environmental samples grows poorly and without cytopathogenic effects (CPE) in known cell cultures, so cell culture isolation is unreliable and often difficult, even for samples known to be contaminated. The best cell cultures for HAV isolation are primary monkey kidney and FRhK4 (fetal rhesus monkey kidney derived) cells, but typically, several weeks or even months are required to achieve and confirm virus isolation. Confirmation of HAV isolation requires additional analytical methods, such as immunofluorescence assay, nucleic acid hybridization, or nucleic acid amplification by reverse transcription-polymerase chain reaction (RT-PCR). HAV in water can be detected by RT-PCR amplification, followed by nucleic acid hybridization and/or nucleotide sequencing after special processing of sample concentrates. This is now the method of choice.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Persons actively infected or ill with HAV are major reservoirs and shed the virus in feces. Infected nonhuman primates also may be sources of HAV exposure. Once a person recovers from HAV infection or illness, virus shedding ceases. There is no evidence

of chronic carriage, but long-term virus shedding occurs for some immunocompromised persons and some infants. Endemic transmission of HAV in the United States is often by children with asymptomatic infection. Epidemic transmission in the United States is often by fecally contaminated food. Other important settings for HAV transmission are overcrowded communities with poor sanitation and hygiene, such as those found in developing countries and underserved areas of the United States (e.g., Native American reservations in the Southwest); male homosexual and intravenous drug-use communities; institutions such as mental-health facilities, prisons, and jails; and facilities for nonhuman primates, such as zoos and animal research facilities. There is some evidence for increased risk of occupational exposure to HAV among sewer workers, but recent epidemiological studies in the United States suggest no increased risk of HAV infection in this country.

Besides direct and indirect person-to-person contact, major sources of HAV exposure are fecally contaminated water and food, especially in areas highly endemic for HAV. Water and fresh produce contaminated with sewage or other sources of human fecal waste, and foods, such as salads and sandwiches, prepared by infected persons are significant sources of HAV exposure. Of particular risk are bivalve molluscan shellfish (e.g., oysters, clams, and mussels) harvested from fecally contaminated waters and eaten raw or only partially cooked. Bathing in fecally contaminated waters also is a risk.

HAV infection and illness in the United States has been declining, especially since the introduction of a vaccine in 1995. In 2003 there were 7,653 reported cases of HAV and an estimated 61,000 total new cases in the United States, which are new lows for annual rates. HAV vaccine is not universally required. It is an expensive, killed-virus vaccine. Vaccination is recommended for children over 2 years of age in communities with high HA rates and persons in high-risk groups, including, international travelers to hyperendemic regions, men who have sex with men, illicit drug users, persons with chronic liver disease, persons receiving clotting factor concentrates, and lab researchers who work with HAV.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

HAV is one of the more persistent enteric viruses in the environment. Inactivation rates in feces, sewage, water, soil, and sediments are so slow that infectious viruses may persist for months to years, especially at lower temperatures. Fresh, frozen, and partially cooked shellfish have been vehicles of HAV because the viruses are highly protected and persistent in shellfish tissues and are not readily degraded or eliminated by live shellfish. HAV is relatively resistant to high temperatures and survives some pasteurization conditions. It is resistant to drying and desiccation and survives on fomites and other surfaces. It is also resistant to proteolytic enzymes and persists in microbially active water, soil, and sediments. HAV adsorption to soils is between the most highly and poorly adsorbed enteric viruses, but it survives longer than many other enteric viruses in soils and thus can contaminate groundwater sources of drinking water. Like other enteric viruses, HAV is not completely removed by sewage treatment processes, including disinfection. It is relatively resistant to combined chlorine, with $C \times T$-99.99 (disinfectant concentration by contact time for 99.99 percent inactivation) values in the hundreds to thousands of milligrams per minute per liter.

DOCUMENTED WATERBORNE OUTBREAKS

Waterborne outbreaks of HA from either drinking water or recreational water are now rare in the United States. This may be due to declining infections in the population as well as improved management practices for drinking water supplies. The last reported outbreaks from drinking water occurred in 1992. Historically, most drinking

water outbreaks of HA are due to untreated or inadequately treated groundwater that becomes contaminated from deficient on-site sewage treatment and disposal systems, usually septic tank–soil absorption systems.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Removal of HAV by conventional physicochemical water treatment processes of coagulation-flocculation and filtration is similar to that of other enteric viruses, with reductions up to 99 percent. Disinfection of water with free chlorine, chlorine dioxide, ozone, and ultraviolet (UV) light radiation can achieve up to 99.99 percent inactivation of HAV under optimum conditions, $C \times T$-99.99 values are <20 mg-min/L for free chlorine, <1 mg-min/L for ozone, and ≤40 mg-min/L for chlorine dioxide. If HAV is protected within organic matter or other particles, however, rates of inactivation can be dramatically reduced and $C \times T$ values can be more than tenfold higher than for dispersed virions. UV radiation inactivates HAV better than some other enteric viruses, with 99.99 percent reduction at a dose of less than 20 mW-sec/cm^2. Like other enteric viruses, HAV is relatively resistant to combined chlorine. For monochloramine, HAV $C \times T$-99.99 values are in the thousands of milligrams per minute per liter. A small fraction of HA virions are highly resistant to monochloramine, probably because they are aggregated. Overall, complete conventional treatment of surface water should reduce HAV by 99.99 percent, as required by the Surface Water Treatment Rule (SWTR). Under optimum conditions, substantial (>99.99 percent) inactivation of HAV is achievable in groundwater by optimized disinfection processes.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

Direct monitoring of HAV and other enteric viruses is not recommended for routine water quality management. The World Health Organization (WHO) *Guidelines for Drinking-water Quality* (GDWQ) recognize the importance of HAV as a waterborne viral pathogen, but it is not singled out for special treatment. Instead, it is expected that HAV, other enteric viruses, and other pathogens can be controlled in drinking water to an acceptable level of risk by an integrated approach to water supply management within a risk assessment framework. Recommended management to achieve acceptably low pathogen risk is based on water safety plans that address source water protection, effective treatment options, maintaining distribution system integrity, and the ability to rapidly respond to changing conditions for different sources of ground- and surface water.

Currently, national guidelines or standards for drinking water quality in the United States do not specify requirements to monitor or treat for HAV. The Information Collection Rule (lCR) in support of the Enhanced Surface Water Treatment Rule (ESWTR) required monitoring for enteric viruses in drinking water supplies from surface sources serving 100,000 people or more. However, the methods used in the ICR did not reliably detect HAV. The current SWTR based many of the $C \times T$ requirements for enteric virus disinfection on HAV inactivation, although inactivation of *Giardia* cysts and *Cryptosporidium* oocycts is usually the more stringent and therefore controlling basis for the $C \times T$ requirements of this rule.

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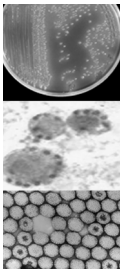

Chapter 43

Hepatitis E Virus

Charles P. Gerba

DESCRIPTION OF THE AGENT

Hepatitis E virus (HEV) is one of the most common causes of hepatitis is the world. HEV has been responsible for massive waterborne outbreaks in the devolving world, but none have ever been documented in developed countries. HEV is an icosahedral, nonenveloped, and positive-sense single-stranded, ribonucleic acid (RNA) virus, 27 to 34 nm in diameter. The virus is in the Picornaviridae family, and was once classified with the caliciviruses, but has been removed from that genus and is currently unclassified. Recent phylogenetic analysis indicates four principal genotypes exist.

HEV is believed to be more liable than hepatitis A virus (HAV). HEV does not survive exposure to high concentrations of salt, including cesium chloride, or freeze-thawing. The viral particles tend to lose their integrity after routine laboratory procedures, whereas in the environment they are more stable and survive exposure to extreme conditions. Although there have been reports of HEV growth in cell culture, these reports have not been reproduced. The lack of a reliable cell culture for growth and assay of the virus has limited our knowledge on the resistance of the virus to water treatment.

DESCRIPTION OF THE DISEASE

The acute clinical features are indistinguishable from hepatitis A. Anorexia, abdominal pain, and jaundice are the main features but are usually mild and illness is self-limiting. There appears to be no chronic or carrier state. However, mortality rates are much greater (2 to 3 percent) compared to HAV. The illness is particularly severe during the third trimester of pregnancy with case fatality rates ranging from 10 to 24 percent. Symptomatic disease occurs most commonly in young adults.

RESERVOIRS FOR THE AGENT

Various species of nonhuman primates are known to be susceptible to HEV, at least under experimental conditions. Antibodies to HEV have been detected in pigs, chickens, and rats. A virus closely related to HEV has been isolated from swine in the United States. The common prevalence of HEV antibodies in persons who raise swine and the recent documentation of HEV from consumption of undercooked pork suggest that HEV is a zoonotic (animal-to-human transmission) virus.

MODE OF TRANSMISSION

Fecal–oral transmission of HEV is well documented. Water appears to be a major route of transmission for HEV, although foodborne (raw shellfish, deer meat) transmission has been documented. Person-to-person transmission is less than 2 percent and does not appear to be a significant mode of transmission. The low titer of infectious virions shed in the feces during infection may account for the decreased spread of HEV among humans.

METHODS FOR DETECTING THE AGENT

Currently HEV cannot be routinely cultured in cell culture using human or animal cell lines. Detection is by direct observation of virions by transmission electron microscopy or reverse transcription-polymerase chain reaction (RT-PCR). The virus can be concentrated from water by adsorption-elution from charcoal or microporous filters and detected by RT-PCR.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

HEV has been detected in sewage in both developed and developing countries including Spain, France, Sweden, Greece, the United States, and India. In sewage in India it was detected in 10 percent of the samples compared to 24 percent for HAV. In Barcelona, Spain, 43 percent of the urban sewage samples tested positive for HEV over an 8-year period. Antibodies have been detected in rats, chicken, and swine, indicating that the virus is zoonotic.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

No information exists on the survival of HEV in the environment.

DOCUMENTED WATERBORNE OUTBREAKS

Numerous large waterborne outbreaks in the Indian subcontinent and Africa have been documented. Waterborne outbreaks involving 10,000 to 80,000 persons have been reported from the consumption of contaminated drinking water. Waterborne outbreaks have been documented in most of Asia, the Middle East, Africa, and Mexico.

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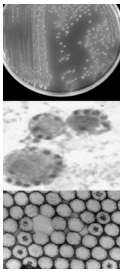


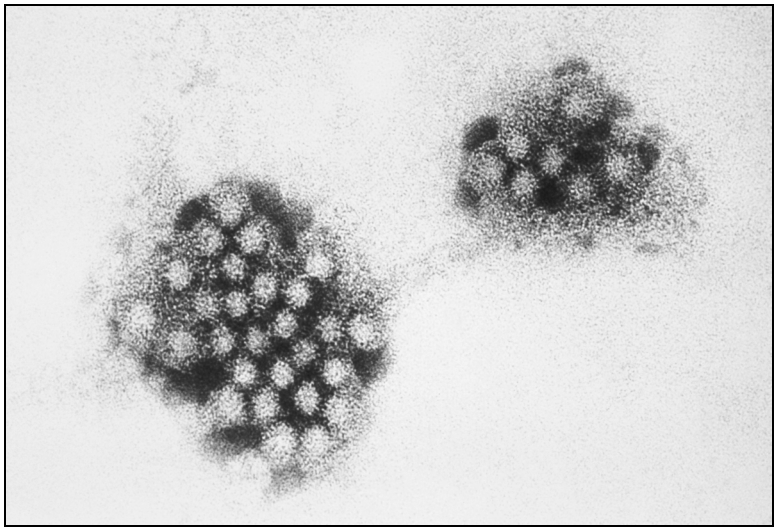
Chapter 44

Human Caliciviruses (Noroviruses and Sapoviruses)

Kellogg J. Schwab and Christon Hurst

DESCRIPTION OF THE AGENT

Caliciviruses are small (27–30 nm in diameter), nonenveloped, icosahedral viruses containing a single-stranded, positive-sense ribonucleic acid (RNA) genome, approximately 7,000–8,000 nucleotides in length (Figure 44-1). Viruses in the *Caliciviridae* family are divided into four genera: norovirus (NoV), sapovirus (SoV), lagovirus, and vesivirus. In this classification, which is based on genome organization and sequence comparisons, all human caliciviruses are grouped within the first two genera and most animal caliciviruses are grouped within the other two genera. Most virions of sapoviruses have typical calicivirus morphology with a "Star of David" appearance, while the majority of norovirus virions have an irregular surface appearance and thus were referred to as small, round-structured viruses (SRSVs). As a further complication in calicivirus taxonomy, noroviruses and sapoviruses were given the temporary names "Norwalk-like viruses" and "Sapporo-like viruses" by the International Committee on Taxonomy of Viruses in 1998 until the names norovirus and sapovirus were selected in 2002. For norovirus investigations, this has resulted in dozens of manuscripts using different terminology (including but not limited to winter vomiting disease, SRSV, Norwalk virus, "Norwalk-like viruses" and norovirus) to describe what are now officially known as noroviruses. It is hoped that the viral nomenclature has finally been resolved. SoVs predominantly cause gastroenteritis in young children and have been associated with sporadic outbreaks in adults. NoVs are the most common cause of acute nonbacterial gastroenteritis worldwide, infecting all age groups. The vast majority of the water-related human calicivirus scientific literature has focused on noroviruses, and thus this genus will be the focus for the remainder of this chapter. Noroviruses can be classified into at least five genetic groups. Two of these groups, denoted genogroups I and II (GI and GII), contain the majority of the human norovirus strains while genogroups III and V contain animal noroviruses and genogroup IV contains a few additional human strains. There are more than 100 strains of circulating human NoVs. Human NoVs



Source: Centers for Disease Control and Prevention.

Figure 44-1 Electron micrograph of Norovirus with 27–32 nm-sized particles

have been difficult to characterize because researchers cannot grow them in cell culture and they lack a suitable animal infectivity model. NoVs that infect cattle, pigs, and mice have been described in the literature and although genetically similar to human NoVs, to date none of these animal NoVs have shown zoonotic (animal to human) transmission. A mouse NoV has recently been propagated in cell culture, opening a new avenue for infectivity and disinfection research on NoVs.

DESCRIPTION OF THE DISEASE

Illnesses due to NoVs are characterized by median incubation periods of 24 to 48 hours and a median duration of 12 to 48 hours. A high percentage of patients suffer the rapid onset of explosive diarrhea, vomiting, or both. Symptoms are typically self-limiting and not life threatening with adequate fluid replacement to prevent dehydration, which is the only treatment needed. Strains of NoVs can be highly infectious and spread rapidly. Volunteer studies have revealed that ingestion of as few as 10 virus particles is capable of inducing an infection. These studies also documented attack rates greater than 50 percent and sometimes as high as 90 percent. Asymptomatic infection also occurs and can result in continued virus transmission. Reports in the literature have identified that NoVs attach to potential host cells in the gut only if the individual expresses specific, genetically determined carbohydrates. These reports have contributed to a breakthrough in understanding NoV host-susceptibility factors.

RESERVOIRS FOR THE AGENT

For NoVs associated with human disease, the only reservoir appears to be humans. Recent reports have indicated that asymptomatic individuals can excrete large amounts of virus particles, making infection control even more problematic.

MODE OF TRANSMISSION

NoVs are predominantly transmitted via the fecal–oral route. Viruses have been transmitted through contamination of private wells, small water systems, community

water systems, recreational waters, and ice cubes. Cold food items (deli meat, pasta salads, cake frosting) contaminated by infected food handlers and consumption of contaminated raw and cooked shellfish (predominantly bivalve mollusks) have been documented as vehicles for the transmission of these viruses.

METHODS FOR DETECTING THE AGENT

Norwalk virus (NV), the reference strain for GI noroviruses, was first isolated from a 1968 outbreak of epidemic gastroenteritis in an elementary school in Norwalk, Ohio. The virus was observed using immunoelectron microscopy (IEM) in specimens from volunteers that were fed stool filtrates from the school outbreak. IEM uses sera from convalescent patients as a source of specific antibodies to agglutinate the virions and make them more easily recognizable. A more recent advance using solid-phase immunoelectron microscopy, fixes the antibodies onto the electron microscopy grid providing a platform to capture virus particles for subsequent IEM detection. These methods have serious limitations because specific hyperimmune animal sera are not available for many of the viruses, and human sera contain a wide spectrum of antibodies. Detection of viruses by IEM requires approximately 10^5 virus particles/mL; the concentration of NoVs present in environmental samples often are below this detection level, limiting the diagnostic value of IEM.

Immunologic assays, such as the radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), have been developed using human volunteer reagents to detect both antibody and virus. All these solid-phase assays are based on the differential binding of virus antigen (present in stool) to wells in micro titer plates coated with convalescent-phase or preinfection serum.

The cloning and subsequent sequencing of NV nucleic acid genomic material unequivocally classified NV as a human calicivirus in the family *Caliciviridae*. Following the successful expression and self-assembly of the Norwalk virus capsid protein into stable virus-like particles (VLPs), sensitive and specific ELISAs using hyperimmune antisera were developed. ELISAs using these new reagents have significantly improved the sensitivity of detecting these viruses over older methods using human reagents. However, the ELISAs using hyperimmune animal serum are relatively type-specific, detecting only those strains most closely related to the strain used to produce the serum although cross-reactive antibodies are being developed.

Reverse transcription-polymerase chain reaction (RT-PCR) assays are the most sensitive diagnostic methods currently available for the detection of NoVs. Primer pairs to amplify a number of different regions in the viral genome have been used diagnostically. Unfortunately, the genetic diversity of NoVs has prevented the development of a universal primer pair that can amplify all such viruses. Techniques to overcome the diversity of NoVs resulting in improved RT-PCR amplification continue to be developed.

Because nonspecific amplicons that are the same approximate size as virus-specific amplicons may be generated from environmental samples, a confirmatory test should be performed following the RT-PCR reaction. Hybridization assays (dot/slot or Southern blot hybridization) are the most common confirmatory assays used. They also increase the sensitivity of virus detection by 10- to 100-fold over that obtained with agarose gel electrophoresis alone. However, the genetic diversity of NoVs limits the usefulness of hybridization assays. The use of as many as ten different oligoprobes was not sufficient to confirm all PCR results obtained using a panel of different NoV strains. The development of NoV reverse line blot hybridization has alleviated many of these problems. Direct sequencing of PCR products is an alternate, although more labor intensive, approach to confirm PCR results.

Stool and environmental samples contain a variety of substances that may inhibit reverse transcription or PCRs. When working with stool and environmental

samples, the use of appropriate controls is essential to detect any inhibition. The addition of low concentrations of an internal standard RNA control allows the detection of inhibitory substances without compromising the sensitivity of the RT-PCR assay. False-positive results due to carryover contamination may occur due to the sensitivity of RT-PCR assays. Physical separation of pre- and post-PCR working areas, dedicated reagents and equipment for pre-PCR sample processing, and appropriate controls for each PCR reaction to prevent such occurrences should be followed.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

NoVs that infect humans appear to have humans as their only reservoir. Many newly discovered animal NoVs are being identified, but to date, no animal NoV has been linked to an illness in humans. NoVs can only replicate within host cells and thus do not replicate in the environment.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Because human NoVs cannot be grown in cell culture, nothing is known about survival of intact and potentially infectious human NoVs in the environment. Although NoVs are unique in that their capsid is comprised of only one major protein, it is thought that NoV resistance to environmental degradation might be similar to other nonenveloped, single-stranded RNA viruses such as poliovirus and hepatitis A virus. Human volunteer studies have shown that NV retained infectivity for volunteers following exposure to pH 2.7 for 3 hours at room temperature, treatment with 20 percent ether at 4°C for 18 hours, or incubation at 60°C for 30 minutes. The development of a mouse NoV cell culture assay has potential to enhance our understanding regarding the persistence of NoVs in the environment.

DOCUMENTED WATERBORNE OUTBREAKS

The development of newer assays (ELISAs and RT-PCR) has increased the accuracy of disease surveillance for NoVs to the point that the majority of gastroenteritis cases which otherwise might have been considered of "unknown etiology" are now attributable to NoVs with an estimated 23 million cases per year in the United States. Almost all outbreaks of nonbacterial gastroenteritis are a result of NoV infection. Outbreaks of waterborne viral gastroenteritis have been associated with private wells, small water systems, community water systems, groundwater contamination, and even ice. Several outbreaks have been associated with recreational waters, and outbreaks have also occurred when food has been washed with contaminated water. One such outbreak involved over 1,500 cadets and staff at the U.S. Air Force Academy. In that instance, celery had been soaked for 1 hour in water from a hose used earlier in the day to clear clogged drains in the kitchen after sewage had backed up.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Very little information has been reported on the survival characteristics of NoVs during standard water treatment processes. In one study, human volunteers were given NV treated with chlorine. A dose of 3.75 mg/L, resulting in no free residual Cl, failed to inactivate Norwalk virus while a dose of 10 mg/L, with a free residual Cl level of 5–6 mg/L, apparently did inactivate the virus. The study concluded that NV was very resistant to Cl as compared to other indicator viruses. Because an infectivity model has not been developed for human NoVs, the effectiveness of water treatment procedures in eliminating or inactivating these important human pathogens is difficult to determine.

Researchers are attempting to identify specific human intestinal cells that will support the growth of human NoVs to establish such an infectivity model. In addition, continued studies using NV VLPs in filtration experiments show great promise for the use of VLPs as model systems for enteric virus transport through treatment systems.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

There are no guidelines for monitoring human caliciviruses in water at this time.

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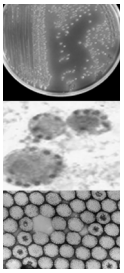


Chapter 45

Reoviruses

Syed A. Sattar and V. Susan Springthorpe

INTRODUCTION

The name "reo" derives from the fact that these viruses are found in the respiratory and Enteric tracts of humans and are orphans due to a lack of association with a specific human disease. They belong to the genus *Orthoreovirus* in the family Reoviridae. They were first isolated from the respiratory and gastrointestinal tracts of humans in 1951 and are commonly found in sewage and fecally-polluted waters; they are perhaps among the viruses most commonly isolated from water.

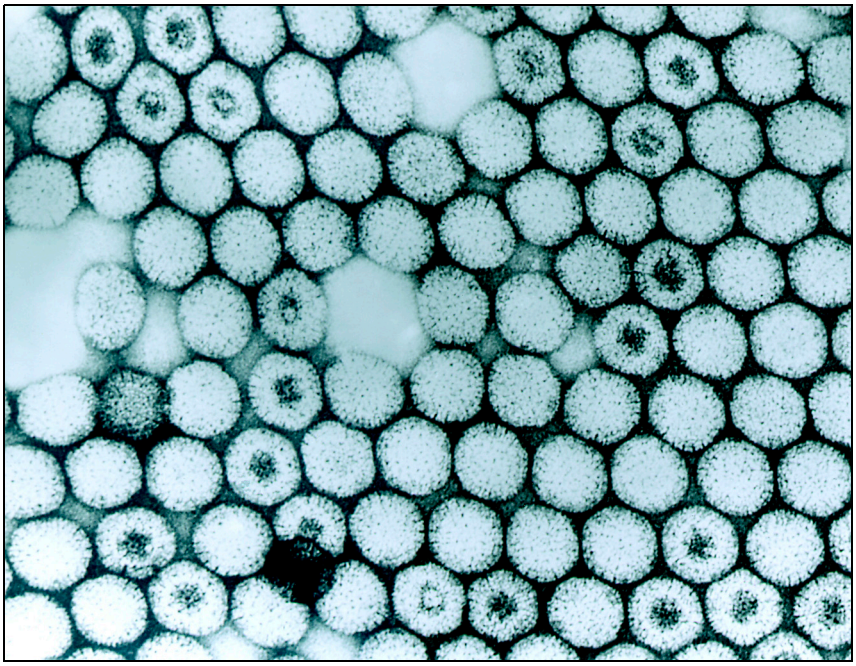
ASSOCIATION OF REOVIRUSES WITH DISEASE IN HUMANS AND ANIMALS

Reovirus infections in humans occur early in life, and most cases are subclinical or very mild. Reports of the association of reoviruses with biliary atresia, juvenile onset diabetes, fever, exanthema (rash), respiratory disease, diarrhea, hepatitis, pneumonia, eye infections, encephalitis, meningitis, myocarditis, etc., remain unconfirmed and uncommon. In some cases reovirus is isolated in conjunction with other infectious agents (e.g., Giordino et al. 2005; Hermann et al. 2004) where it is unclear whether its presence is coincidental, causal, or secondary to and facilitated by other infections. Thus, it is not surprising that there are no reports of waterborne outbreaks of reovirus disease. Reoviruses are very widely distributed in nature and seem to be uniquely successful viruses. Apart from humans, reoviruses have been found in many other mammals, birds, reptiles, fish, invertebrates, fungi, and plants.

The three serotypes of reoviruses of humans and other mammals are indistinguishable, whereas avian reoviruses are antigenically distinct from mammalian types. In poultry, reoviruses can cause arthritis and other diseases (Nibert et al. 1996). Avian reoviruses can also induce immunosuppression in chickens by destroying B cells (Sharma et al. 1994). Human reoviruses are pathogenic in newborn mice, and some plant reoviruses can infect certain insects, but it is not yet known if reoviruses of other animals can cross species barriers to infect humans.

BASIC CHARACTERISTICS AND PROPAGATION

Virions are icosahedral in shape with a double protein shell (Figure 45-1). They are 75–82 nm across and lack an envelope. The genome is double-stranded ribonucleic



Source: Erskine Palmer, Centers for Disease Control and Prevention.

Figure 45-1 Transmission electron micrograph of reovirus type 3

acid (RNA) with 10 segments. Reoviruses can grow in experimental animals or cell cultures, but not in embryonated eggs. Replication occurs exclusively in the cytoplasm, and complete particles can be assembled as early as 6 to 8 hours after infection.

Mammalian reoviruses can be grown in kidney cells from a variety of animals. L cells, derived from the mouse, are often used for this purpose. For virus isolation from human clinical specimens, bovine kidney cells (MDBK) are regarded as most sensitive. Monkey kidney cells (LLC-MK_2, MA-104, Vero, or BGM) are usually used for reovirus isolation from environmental samples. Cell lines that are not normally permissive for replication of particular reoviruses sometimes can be made so by the addition of protease enzymes (Golden et al. 2002).

Reoviruses produce a highly distinctive and characteristic cytopathology. The large cytoplasmic inclusions initially appear as dense cytoplasmic granules and develop into large, irregular shaped masses in the perinuclear region. They are readily recognized before cell destruction occurs and are diagnostic of reoviruses even if the numbers are too low to be readily confirmed with electron microscopy. As the cytopathology progresses, the cells tend to curl up without becoming refractile. Part of the affected cell remains attached to the surface of the culture vessel, and the rest shows "flagging." To the uninitiated, advanced reovirus cytopathology may appear as nonspecific degeneration of cells in culture.

Mice are the experimental animals of choice, and upon infection they show diarrhea (with high fat content in the stools), pneumonia, myocarditis, runting, oily hair, jaundice, and involvement of the central nervous system. Infection of the bile duct in mice can lead to chronic biliary atresia and jaundice. Injury to the pancreas, pituitary gland, and thyroids also occurs in mice. Intracerebral injection of reovirus type 3 into newborn mice leads to fatal encephalitis; intramuscular injection by the same virus can cause myositis (Grandien et al. 1995). Reoviruses have been used as models for

investigating virus replication, and due to their low pathogenicity and oncolytic potential are now being investigated as potential antineoplastic agents (Russell 2002; Norman et al. 2002; Norman and Lee 2000).

SURVIVAL AND RESISTANCE TO PHYSICAL AND CHEMICAL AGENTS

Reoviruses are relatively long-lived in water. In surface waters, with an input dose of 10^4 infective units, it took more than 200 days for infectious reovirus to become undetectable (Mahnel et al. 1977). In situ survival studies in cellophane tubes indicated survival in excess of 6 months (Matsuura et al. 1988). When samples of river water were filtered or centrifuged to remove bacteria, experimentally contaminated with reovirus, and then held at low temperature (~5°C), the viruses retained their infectivity for longer than 3 years (Matsuura et al. 1988). All of these systems are to some extent artificial and may overestimate actual survival in the water column. On the other hand, viruses in natural systems can be protected by adsorption to particulate matter. The work of Lipson and Stotsky (1984) suggests that reovirus survival may vary with the type of organic and mineral material with which the virus comes into contact and that there is heterogeneity within the reovirus population.

Reoviruses are stable over a wide range of pH and can retain their infectivity even at pH 3.5. This may assist their recovery from environmental samples using common methods for virus isolation which involve exposure to a wide range of pH values. Aerosol stability of reoviruses appears favored by relative humidities above 75 percent or below 40 percent (Spendlove and Fannin 1982). This implies that recreational water users could be exposed without water ingestion, and that reovirus could also survive aerosolization during spray irrigation of crops. They are known to be relatively resistant to the levels of disinfectants normally used for water and wastewater disinfection. They are also resistant to 1 percent phenol and 1 percent hydrogen peroxide but are inactivated by 70 percent ethanol and 800 mg/L sodium hypochlorite (Drulak et al. 1984). An investigation of mutants resistant to ethanol showed that point mutations on a particular gene segment were responsible (Wessner and Fields 1993).

REOVIRUS OCCURRENCE IN WATER AND WASTEWATER

Many studies have reported the occurrence of reoviruses in natural waters. Usually such occurrence is reported against a spectrum of other virus isolations and they are rarely the specific focus of surveys. Frequently, however, they are the most numerous viruses isolated (Payment et al. 1984; Tani et al. 1995; Lee and Jeong 2004). Where few or no reoviruses are reported, one may suspect (a) failure to look for them, (b) use of a cell line that is not highly sensitive to reoviruses, or (c) use of concentration/isolation method(s) that inactivate or fail to recover small numbers of reoviruses. In addition to their isolation from river water, reoviruses have also been found in piped spring water or potable groundwater supplies (Schwartzbrod et al. 1985; Fout et al. 2003).

Reoviruses also occur in relatively high numbers in sewage, comprising 26 to 57 percent of isolates, and in slaughterhouse wastes (Palfi 1971). In Ottawa (Canada), they accounted for 57, 43, and 59 percent of virus isolates identified from samples of raw sewage, primary effluent before, and after chlorination, respectively (Sattar and Westwood 1978). At a smaller plant (Sattar et al. 1978–1979), the number of samples positive for reovirus dropped from 43.5 percent in the raw sewage to 21.7 percent in the chlorinated primary effluent. Although the total virus numbers dropped by approximately 60 percent, mixed infections made it difficult to account for the exact reductions in reovirus. Some studies (Sattar et al. 1978–1979; Sattar 1978, 1979; England 1972) did not detect seasonal patterns of reovirus recovery. However, others have suggested that reovirus isolations are seasonal, occurring with the greatest

frequency in late summer, fall, and early winter (Gilbert et al. 1976; Palfi 1971; Tani et al. 1995). Sample size, and reduced water flow at this time of year, should also be considered when evaluating possible seasonal variations in reovirus recovery.

ISOLATION OF REOVIRUSES FROM WATER AND WASTEWATER

Isolation of reoviruses from surface water will almost certainly require sample concentration. Sample volumes of 20–100 L are often used. Reoviruses can be concentrated by adsorption-elution on a variety of electronegative or electropositive matrices, by precipitation with flocculating agents, or by tangential ultrafiltration. The best methods for concentration of reoviruses from natural waters are not known because they are not usually the primary targets of virus isolations. Indeed, a recent study (Lee and Jeong 2004) comparing total culturable virus isolation with multiplex integrated cell-culture polymerase chain reaction (PCR) found them only after using an additional reovirus primer. Most isolation methods, including a tentative standard method for virus isolation, were developed primarily for enteroviruses and or adenoviruses rather than reoviruses. Any method which uses pH values above 10 for elution from the adsorption matrix may not be suitable for optimal recovery of reoviruses (Goyal and Gerba 1982). Sobsey and Glass (1984) examined the effects of water quality on enteric virus adsorption by electronegative and electropositive microporous filters and found that reoviruses adsorbed very efficiently (99 percent) to the electronegative filters but the virus was poorly (~2 percent) recovered from them by the methods used. The electropositive filters adsorbed reovirus less well (~74 percent) but more reovirus was recovered (15–27 percent). Patti et al. (1996) compared recovery of entero- and reoviruses from water samples concentrated by adsorption-elution on positively charged 1MDS filters and tangential filtration and found that both methods were essentially equivalent for reoviruses. To what extent the methods used have played a role in reovirus levels detected in surface waters is unknown, but clearly more work is needed to optimize the method for reovirus recovery.

Isolation of reoviruses from sewage and effluent requires concentration but probably of a smaller sample (1–10 L). Adsorption-elution or filtration may be used, but precipitation has also been successfully employed with these smaller volumes (England 1972; Adams et al. 1982).

For direct inoculation of primary water concentrates into cell cultures, a secondary concentration step is usually employed. It was found that hydroextraction with polyethylene glycol is suitable for isolation of reoviruses (Payment et al. 1984). Gibbs and Cliver (1965) examined three different concentration methods for detecting small numbers of reovirus.

Greater attention has been paid to the cell lines used for recovering infectious reovirus from sample concentrates. MA-104 cells appear to be more sensitive than BGM cells (Patti et al. 1996) or BSC-1 cells. Our experience suggests that even low numbers of reoviruses may be detected from natural water samples within 8 to 12 days after a first passage in cell culture using MA-104 or BSC-1 cells, or when no cytopathology is visible, in 1 to 2 additional days after blind passage. Smith and Gerba (1982) list several cell lines for reovirus isolation; they indicate the superiority of primary rhesus monkey kidney (RhMK) cells. We also found that primary HEK cells were very sensitive for reovirus isolation (Sattar 1978). However, the difficulties and costs of working with and obtaining primary cells will almost certainly be outweighed by the relative ease and consistency obtained from an established cell line. CaCo-2 cells have been proposed (Pinto et al. 1995) as a universal cell line for virus isolation because of their sensitivity to fastidious viruses. However, more work is needed to fully validate such a suggestion. Ultimately, cell line selection will be governed by both the desired target(s) for isolation

as well as individual experience, because it is well recognized that the same cell line can behave quite differently between laboratories. Longevity of cells in culture is clearly desirable for visualizing reovirus inclusions when reovirus concentrations are low, but blind passage can be utilized for cells that are subject to early demise in culture. It is also important to ensure that sera used for cell culture isolation of reovirus are free from reovirus antibodies.

It has sometimes been suggested (Muscillo et al. 2001) that the presence of other enteric virus can interfere with reovirus detection in cell cultures, but this has been refuted by the same authors. Our experience as well as that of Lee and Jeong (2004) suggests that when assessing environmental samples, dual or even triple infections with combinations of entero-, adeno-, and reoviruses in cell culture are quite common.

PCR integrated with cell culture (Spinner and Di Giovanni 2001; Lee and Jeong 2004) has been used for the detection of reoviruses. The indisputable sensitivity of PCR in conjunction with cell culture can be a useful tool to permit earlier detection of low numbers of infectious reoviruses than when cell culture is used alone. Evaluation of the primers has been performed with American Type Culture Collection (ATCC) reovirus strains as well as other human enteric viruses (Spinner and Di Giovanni 2001). Although it is assumed that only mammalian viruses will infect mammalian cell cultures, in view of the widespread nature of reoviruses in fish, invertebrates, fungi, and aquatic mammals, a much wider evaluation of the primers used may be desirable to ensure that only mammalian reoviruses are recognized from water samples.

SOURCES OF REOVIRUSES IN WATER

The source of reoviruses in surface waters is mainly fecal material discharged directly or through incomplete treatment of wastes. Studies in Japan (Matsuura et al. 1993) on reoviruses in water indicate their main source to be human excreta. Milde et al. (1995) have also conducted similar studies in Germany. While such studies are important in understanding the main sources of reoviral pollution of surface waters, any reoviruses recovered from water cannot be assumed necessarily to be of human origin. Therefore, risk assessment for contact with these viruses is difficult until more is known about their ability to cross species boundaries.

REOVIRUS ELIMINATION DURING WATER AND WASTEWATER TREATMENT

Studies which have specifically examined the degree of reovirus removal during conventional water treatment using spiking of a simulated system are lacking. However, in our own studies, we detected no reoviruses in 500–1,000-L samples of treated drinking water when reoviruses were detected in 26 or 66 percent of the raw water samples (100 L) studied. Similarly, Lee and Jeong (2004) found no reovirus in treated water where it was present in the source water. McConnell et al. (1984) examined reovirus removal by slow sand filtration and demonstrated that where such treatment is used it can remove up to 4 $\log_{10}$ units depending on water quality, flow rate, and bed construction; adsorbed reovirus was detected throughout the bed, but concentrations decreased with increasing bed depth. Some work (Sproul 1973) on inactivation of viruses by 0.5 mg/L chlorine suggests that a 4 $\log_{10}$ reduction in reovirus titer can be achieved after two to four minutes. It should be noted here that the degree of reovirus clumping may have an impact on disinfection rates.

Most studies of virus removal from wastewater during treatment do not specifically examine reductions for reoviruses. However, a treatment plant handling domestic sewage removed reoviruses less efficiently than enteroviruses (Aulicino et al. 1996). Our studies isolated reoviruses from chlorinated primary effluent at ~50 percent of the frequency that they were isolated from raw sewage. Dewatering of sludge led to only a

small drop in reovirus recovery until sludge solids were >90 percent (Ward 1983). Other factors affecting reovirus decay in sludge are discussed by Ward (1980, 1983).

CONCLUDING REMARKS

Information on reoviruses relevant to drinking water is relatively limited because they are not regarded as major human pathogens and are not known to have caused any outbreaks of waterborne disease. Since they are ubiquitous and easily recognized, they could play a role as indicators of other pathogenic viruses or for tracing viral pollution. However more work would be needed to (a) optimize methods for their recovery from natural waters and wastewaters, (b) determine whether they are seasonal or year-round contaminants, and (c) to determine if reoviruses can cross species boundaries.

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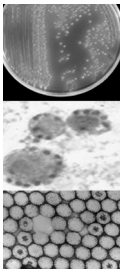


Chapter 46

Rotaviruses

Morteza Abbaszadegan

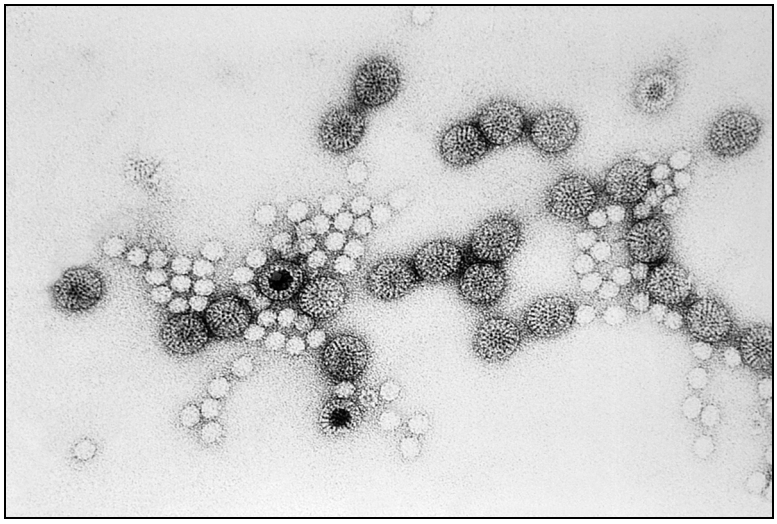
DESCRIPTION OF THE AGENT

The rotaviruses belong to a family of viruses known as Reoviridae. They are nonenveloped, icosahedral viruses that are 65 to 75 nm in diameter (Figure 46-1). The rotavirus genome is comprised of 11 segments of double-stranded ribonucleic acid (RNA) and is surrounded by a distinctive double-layer protein capsid. Rotaviruses are capable of genetic reassortment and replicate in the cytoplasm of host cells. There are six known rotavirus groups (A–F). Groups A, B, and C rotaviruses are found in humans and animals, and groups D, E, and F rotaviruses have been reported only in animals to date. Group A, which contains 9 serotypes, is the most studied and has been documented as a cause of waterborne outbreaks in humans. Group C has been found in sporadic cases and outbreaks of diarrhea in piglets and children.

DESCRIPTION OF THE DISEASE

Rotaviral infections are the cause of many serious diarrheal illnesses, mostly in infants and children under age 2. Rotavirus infections are responsible for an estimated 3.5 million cases of diarrhea and 125 deaths in infants and young children in the United States and >500,000 deaths worldwide per year. In the US, approximately 500,000 physician visits and 50,000 hospitalizations yearly are related to rotaviruses. In addition, rotaviruses account for 30–50 percent of U.S. hospitalizations for diarrhea in children younger than 5 years. Rotaviruses also infect adults and are a potential cause of traveler's diarrhea. The incubation period of rotavirus infection is less than 48 hours, and duration of illness is typically 5 to 8 days. Common clinical symptoms include vomiting, abdominal distress, diarrhea, and dehydration. Fever and respiratory involvement are often associated with the illness in children; however, adults usually have more asymptomatic infections. The virus is excreted in large numbers in the feces of infected individuals with concentrations as high as 10^{10} rotaviruses per gram of fecal material.

Rotaviruses are the leading cause of acute infantile gastroenteritis and diarrhea-related infantile deaths. The virus has also been associated with diarrhea outbreaks among the elderly and among immunocompromised patients.



Source: Centers for Disease Control and Prevention.

Figure 46-1 Electron micrograph of rotavirus

RESERVOIRS FOR THE AGENT

Humans and animals are the primary reservoirs. The virus has been isolated from humans, monkeys, cattle, sheep, mice, cats, dogs, other mammals, chickens, and turkeys. Although the various strains of rotavirus are usually associated with a specific species, reports have documented infections through interspecies transmission, including a human infection by a bovine strain of rotavirus. Additionally, recent work has shown that an isolated rotavirus nonstructural protein, alone, can produce diarrhea in mice.

MODE OF TRANSMISSION

Rotaviruses are transmitted via the fecal–oral route. They may also be transmitted via the respiratory route, but that is not yet proven. However, in a significant number of cases, the virus was isolated from the nasopharyngeal area. Most rotavirus infections and illnesses occur in the winter in temperate climates. This seasonal pattern is apparent worldwide except in the tropics where infections occur year-round.

METHODS FOR DETECTING THE AGENT

Rotavirus detection in water samples can be accomplished by cell culture assays, reverse transcription polymerase chain reaction (RT-PCR), or serological tests such as immunofluorescence, enzyme-linked immunosorbent assay (ELISA), and immunoelectromicroscopy. Group A rotaviruses can be detected using ELISA, latex agglutination, and counter-immunoelectric-osmophoresis. Non-group A rotaviruses are difficult to grow in cell culture, and only one has been successfully cultivated. Both group A and non-group A rotaviruses can be detected using an electron microscope.

OCCURRENCE OF THE AGENT IN PEOPLE, ANIMALS, AND THE ENVIRONMENT

Rotaviruses have been isolated from humans, monkeys, cattle, sheep, mice, cats, dogs, other mammals, chickens, turkeys, and the environment. The organism has been detected in fresh water and sewage. The virus is stable in feces at room temperature, with infectivity persisting for a minimum of a week. Rotavirus has been detected on contaminated surfaces in day-care centers and in wastewater at a concentration as high as 90,700 virus particles per liter. Based on a limited number of studies on the occurrence of rotavirus in raw sewage in the United States, the average concentration was reported to range from 10 to 218 virus particles per liter. The virus also has been detected in surface, ground, and recreational waters in the United States and in marine waters, including both bathing and shellfish harvesting waters.

SURVIVAL OF THE AGENT IN THE ENVIRONMENT

Rotaviruses, like most enteric viruses, are resistant to inactivation at both low (3.5) and high (10.0) pH. Rotaviruses can survive secondary sewage treatment, including disinfection processes. They also can survive in water for days to weeks depending on water quality and temperature. Although it has been difficult in some cases to prove that rotaviral outbreaks are waterborne, rotavirus group A has been documented as a cause of waterborne outbreaks in humans.

DOCUMENTED WATERBORNE OUTBREAKS

Rotaviruses have been studied in conjunction with other enteric viruses as possible sources of waterborne illness and are thought to be the cause of many gastrointestinal waterborne illnesses.

The organism has been detected in fresh water and sewage. At least nine documented waterborne outbreaks have occurred in the United States and were associated with direct fecal contamination of a water supply or improper water disinfection treatment. Rotaviruses are known to have high attack rates in adults. In an outbreak of rotaviral gastroenteritis in a community water system in Vail, Colo., the attack rate among adults was 43.8 percent.

EFFECTIVENESS OF WATER TREATMENT PROCESSES

Currently, the capability for removal of rotaviruses by conventional water treatment processes is similar to those for other enteric viruses. They are effectively removed during the processes, especially through disinfection. Rotaviruses are susceptible to disinfection in water with free chlorine, ozone, and ultraviolet (UV) radiation. They are more resistant to inactivation by UV light than some other enteric viruses, probably because they contain double-stranded RNA.

Generally, viruses are known to be substantially inactivated by free chlorine. However, they are more resistant to free chlorine than most enteric bacteria. Viruses in feces, wastewater, and tap water and viruses associated with solids are more resistant to inactivation.

NATIONAL OR INTERNATIONAL GUIDELINES AND MONITORING RECOMMENDATIONS

In the United States, rotavirus infections are monitored by the National Respiratory and Enteric Virus Surveillance System (NREVSS). National guidelines for drinking water quality do not specify requirements to monitor or treat for rotavirus.

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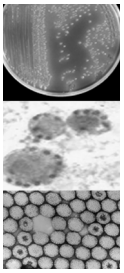

Appendix A

Additional Resources/Links

AWWA Web site: www.awwa.org

CDC waterborne disease statistics: www.cdc.gov/mmwr/PDF/SS/SS5308.pdf *or* www.cdc.gov/mmwr/preview/mmwrhtml/SS5308a4.htm.

Drugs for parasitic infections: www.medletter.com.

Giardiasis Surveillance—US, 1998–2002: www.cdc.gov/mmwr/PDF/SS/SS5401.pdf.

Recognizing Waterborne Disease and the Health Effects of Water Pollution. American College of Preventive Medicine: www.waterhealthconnection. org/index.asp.

Water Information Sharing Analysis Center (WaterISAC): www.waterisac.org

Security Resources:

American National Red Cross: www.redcross.org/news/terrorism

American Water Works Association (AWWA): www.awwa.org

Association of Metropolitan Sewer Agencies (AMSA): www.amsa-cleanwater.org

Association of Metropolitan Water Agencies (AMWA): www.amwa.net

Centers for Disease Control and Prevention (CDC): www.cdc.gov

Federal Emergency Management Agency (FEMA): www.fema.gov

National Infrastructure Protection Center (NIPC): www.nipc.gov

National League of Cities, www.nlc.org/nlc

NSF International: www.nsf.org

US Environmental Protection Agency (USEPA): www.epa.gov/

USEPA Counterterrorism: www.epa.gov/ebtpages/ecounterterrorism.html

Water Environment Federation (WEF): www.wef.org

Water Infrastructure Security Enhancements Guidance Documents, American Society of Civil Engineers: www.asce.org/static/1/wise.cfm

Water Information Sharing Analysis Center (WaterISAC): www.waterisac.org

USEPA Resources:

CCL2 Contaminant Candidate List 2 (CCL2): www.epa.gov/safewater/ccl/index.html.

USEPA's microbiology Web site: www.epa.gov.nerlcwww/online.htm *or* www.epa.gov/nerlcwww/index.html.

USEPA Long Term 2 Enhanced SWTR: www.epa.gov/safewater/lt2/pwsguide.html

USEPA's *Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance*, EPA 815-R-05-004: www.epa.gov/safewater/labcert/pdfs/manual_labcert_2005.pdf.

USEPA Microbiology Microbe Reference: www.epa.gov/microbes/

Glossary

aerobic Living or occurring only in the presence of oxygen.

aerosolization To be in water vapor.

agar A gelatinous material prepared from certain marine algae and used as a solidification agent for bacterial culture media.

agglutination Adhesion of distinct parts.

aggregation To clump or grow together.

alveolar A small cavity or pit, such as a honeycomb cell.

ambient temperature 22 to 35°C.

amebic Caused by an amoeba, as in amoebic dysentery.

ameboflagellate Amoeba that has a flagellate stage in addition to the trophozoite and cyst stages.

ameboid Shapeless or shape-changing.

amino acid An organic compound containing both an amino group (NH_2) and a carboxylic acid group (COOH).

amphotericin B Antibiotic.

anaerobic Living or occurring without the presence of oxygen.

anoxic Absence of oxygen.

antigen A substance that when introduced into the body stimulates the production of an antibody.

antihelminthic Acting to expel or destroy intestinal worms.

antimicrobial Capable of destroying or suppressing the growth of microorganisms.

AOC Assimilable organic carbon.

artifacts Unknown material, other than that being tested for.

ascariasis Infestation with nematode worms of the species *Ascaris lumbricoides*.

asymptomatic Neither causing nor exhibiting symptoms.

autochthonous Native to a particular place; indigenous.

axillae The armpit or an analogous part.

axoneme Basal portions of flagella within cytoplasm.

backsiphon Water flow in a backward direction caused by the siphon effect.

bacteria Any of numerous unicellular microorganisms of the class schizomycetes, occurring in a wide variety of forms, existing either as free-living organisms or as parasites, and having a wide range of biochemical, often pathogenic, properties.

bacteriophage A virus that infects bacteria.

bacteriostatic agent Inhibits growth or multiplication of bacteria.

Baird-Parker agar Growth medium that permits the detection, enumeration, and isolation of coagulase-positive staphylococci after 24 hours of incubation.

barrier A method or obstacle preventing passage.

biliary atresia Closure of bile ducts.

biofilm A buildup of agents.

birefringence The resolution or splitting of a light wave into two waves with mutually perpendicular vibration directions by an optically anisotropic medium, such as calcite or quartz.

BOD Biochemical oxygen demand.

boil Painful, localized, pus-filled swelling of the skin and subcutaneous tissue caused by bacterial infection.

bradyzoite Slowly multiplying forms of parasites.

CaCo-2 cell Cell line derived from colon carcinoma cells.

capsule A mucopolysaccharide layer enveloping certain bacteria.

carbol fuchsin technique, modified Used for examination of fecal specimens.

carbuncle A painful, localized, pus-producing infection of the skin and subcutaneous tissue.

casein A white, tasteless, odorless milk and cheese protein used as an ingredient in bacteriological media.

catalase An enzyme in the blood and tissues that catalyzes the decomposition of hydrogen peroxide into water and oxygen.

cecum A cavity with only one opening; the large blind pouch forming the beginning of the large intestine.

cellulitis Inflammation of subcutaneous tissue.

cercariae Free-swimming larvae of schistosomes.

cestode A flatworm of the class Cestoda, which includes the tapeworms.

cfu Colony-forming unit.

Charcot-Leyden crystal Minute crystals occurring in the sputum of patients with asthma and bronchitis.

chemorganotrophic Chemical affinity of substances for certain organs or tissues of the body.

chitinized spore coat A hard, white, horny polysaccharide that is the principal constituent of the spore coating.

chloramine Any of several compounds containing nitrogen and chlorine.

chorioretinitis Inflammation of the choroid and retina.

chromogenic colony Colony producing a pigment or coloring matter.

citrate A salt or ester of citric acid.

clearwell A reservoir of treated water.

coagulation The transformation of a liquid or solid into a soft, semisolid, or solid mass.

coccal bodies Spherical in shape.

coccobacilli Bacilli that are short and oval in shape.

coliform Of, pertaining to, or resembling the colon bacillus.

colony A group of the same kind of animals, plants, or one-celled organisms living or growing together; visible growths of microorganisms in a nutrient medium.

commensal organism Close association of two organisms in which one or both may derive some benefit but neither harms or is parasitic on the other.

community water system Water systems serving at least 25 residents year-round.

conjugation A process of sexual reproduction in which ciliate protozoans of the same species temporarily couple and exchange genetic material.

conjunctivitis Inflammation of the conjunctiva (the mucous membrane that lines the inner surface of the eyelid and the exposed surface of the eyeball).

connective tissue Tissue such as tendons and muscles.

contact time (CT) The period of disinfection in water treatment.

cribiform plate A plate that is perforated like a sieve.

cross-connection Potable and nonpotable water supplies that become connected.

cutaneous Of, pertaining to, or affecting the skin.

cyst A resting stage formed by some bacteria and protozoa in which the whole cell is surrounded by a protective layer.

cysticerci Bladderworms.

cytopathic effect The effect of pathological/morphological change.

cytoplasm The protoplasm outside a cell nucleus.

cytostome The mouth aperture (opening) of certain protozoa.

depuration Cleansing or purifying, or becoming cleansed or purified.

desiccation The process of drying out completely.

diatomaceous earth (DE) A white or cream-colored siliceous earth composed of the shells of diatoms.

diplopia A disorder of vision that causes objects to appear double.

direct plating Taking a sample aliquot from specimen and applying directly to a culture plate.

disinfection Destruction of harmful microorganisms.

disinfection by-products Compounds created by the reaction of a disinfectant with organic compounds in water.

DNA Deoxyribonucleic acid; a polymeric chromosomal constituent of living cell nuclei, consisting of two long chains of alternating phosphate and deoxyribose units twisted into a double helix and joined by hydrogen bonds between the complementary bases adenine and thymine or cytosine and guanine, each of which projects toward the axis of the helix from one of the strands where it is bonded in a sequence that determines individual hereditary characteristics.

DNA–DNA homology group Genomic species.

duodenojejunal aspirate Aspirate from the area containing duodenum and jejunum (the first portion of the small intestine).

dyspepsia Functional disturbance of the intestine, which can result in malnutrition.

ectocyst Outside the cyst.

elevated eosinophilia High counts of eosinophils.

eluted One material extracted from another, usually by means of a solvent.

embryonation To develop into the embryo stage.

empyema Pus in a body cavity, such as the pleural cavity or gallbladder.

encapsulated Enclosed by a protective coating or membrane, as in certain bacteria.

endemic Prevalent in or peculiar to a particular locality or people.

endocarditis Inflammation of the endocardium (the thin, endothelial, serous membrane that lines the interior of the heart).

endocyst Within the cyst.

endotoxin A toxin produced within a microorganism and released on destruction of the cell in which it is produced.

enteric Of or within the intestine.

enterotoxin A toxin produced by bacteria that is specific for intestinal cells and causes the symptoms of food poisoning.

enzyme-linked immunosorbent assay (ELISA) The basic system consists of antibodies bonded to enzymes that remain able to catalyze a reaction yielding a visually discernible end product while attached to the antibody. The antibody-binding sites remain free to react with their specific antigen.

eosinophilia An increase in the number of eosinophils (any microorganism, cell, or histological element easily stained by eosin dye) in the blood.

epidemiology The study of widespread, infectious diseases.

epifluorescent microscopy A microscope designed to observe organisms that have been treated with fluorescent stains or dyes.

epithelial Membranous tissue, usually in a single layer, composed of closely arranged cells separated by very little intercellular substance and forming the covering of most internal surfaces, organs, and the outer surface of an animal body.

erythema edema A redness of the skin and swelling as caused by chemical poisoning or sunburn.

erythermatous nodule A nodule that has redness associated with it.

estuary The part of the wide lower course of a river where its current is met by the tides.

etiological agent Something that causes a disease.

eugonic media Media that encourages luxuriant growth.

eukaryotic protozoa Organism with a nucleus.

eutrophic Designating a body of water in which the increase of mineral and organic nutrients has reduced the dissolved oxygen, producing an environment that favors plant over animal life.

excystation When a cyst ruptures or opens.

extraintestinal Outside the intestine.

family A taxonomic category ranking below an order and above a genus.

febrile Of, pertaining to, or characterized by fever; feverish.

fermentative Causing chemical reactions induced by living or nonliving ferments that split complex organic compounds into relatively simple substances.

filtration The process of passing a liquid or a gas through a porous substance to remove constituents such as suspended matter.

fimbriae A fringe-like part or structure.

flagellate Having a flagellum or flagella.

flagellum A long filamentous process; whiplike extensions of certain cells or unicellular organisms, usually functioning locomotion.

flocculation To form lumpy or fluffy masses.

flora Microbes collectively; the microbes of a particular environment, animal, or organ.

fluorescent antibody (FA) test A technique to identify an organism using a fluorescently labeled antibody specific to the organism.

folliculitis Inflammation of a follicle (an approximately spherical group of cells containing a cavity).

fomite An inanimate object or substance that serves to transfer infectious organisms from one individual to another.

formalin A 37 percent by weight aqueous solution of formaldehyde with some methanol.

GAC Granulated activated carbon.

GAE Granulomatous amoebic encephalitis.

gas chromatography Chromatography in which the substance to be analyzed is vaporized and diffused along with a carrier gas through a liquid or solid adsorbent for differential adsorption.

gastroenteritis Inflammation of the mucous membrane of the stomach and intestine.

genera (pl.), genus (sing.) Taxonomic categories ranking below a family and above a species.

genome The entire genetic content of an organism.

GN A broth used as a selective medium for cultivation of gram-negative bacteria.

gram-negative Loses the stain or becomes decolorized by alcohol in Gram's method of staining. This is a primary identifying characteristic of certain microorganisms.

granulomatous amoebic encephalitis (GAE) A meningoencephalitis caused by freshwater amoebas; may occur as a subacute or chronic disease with focal granulomatous lesions in the brain; the skin or lungs are primary sites of invasion; causative organism is an *Acanthamoeba* spp.

Guillain-Barré syndrome A syndrome described in encephalitis of virus origin consisting of absence of fever, pain or tenderness in the muscles, motor weakness, abolition of tendon reflexes, great increase in the protein in the cerebrospinal fluid without corresponding increase in cells.

HAV Hepatitis A virus.

helminth ova Eggs of the helminth (intestinal parasite).

hematogenous Containing red blood cells.

hemiparesis Muscular weakness affecting one side.

hemodialysis Dialysis of the blood.

hemolysin An agent or substance that initiates lysis of red blood cells, thereby liberating hemoglobin (a constituent of blood).

hemolytic reaction The lysis of red blood cells releasing hemoglobin. Usually a reaction due to parasitism or immunopathogenic mechanisms.

Hep-2 cells Cell line that can be used to grow certain organisms.

hepatotoxin A substance causing hepatotoxicity (the quality or condition of being toxic to the liver).

HEV Hepatitis E virus.

homologous antisera Serum containing an antibody or antibodies that is derived from a consistent source.

homology The quality or condition of corresponding in structure and evolutionary origin; having the same linear sequence of genes as another chromosome.

HPC Heterotrophic plate count.

hypochlorite A salt or ester of hypochlorous acid.

icosahedral A polyhedron having 20 faces.

IEM Immunoelectron microscopy.

IFA Immunofluorescent antibody.

immunosuppressed A reduced immune response of an organism to pathogens, also immunocompromised.

impetigo A contagious skin disease characterized by superficial pustules that burst and form characteristic thick yellow crusts.

indole A white crystalline compound, C_8H_7N, obtained from coal tar and used in diagnostic tests for certain bacteria.

infantile methemoglobinemia The presence of methemoglobin (a modified form of oxyhemoglobin) in the blood. Methemoglobin contains ferric iron with normal hemoglobin (an oxidized heme). Oxygen is held firmly so it cannot be pumped off; therefore, it does not function in respiration, resulting in anemia.

infectious Capable of causing invasion by pathogenic microorganisms of a bodily part in which conditions are favorable for growth, production of toxins, and subsequent injury to tissue.

inositol Any of nine isomeric alcohols, $C_6H_6(OH)_6$; especially one of these substances found in plant and animal tissue and classified as a member of the vitamin B complex.

isolate Something isolated from another substance.

jejunum The section of the small intestine that lies between the duodenum and the ileum.

labile Likely to undergo chemical change; unstable.

lactose A white crystalline disaccharide, $C_{12}H_{22}O_{11}$, made from whey and used as a carbon source in many microbiological growth media.

latent Present or potential but not evident or active.

leukocyte Any of the white or colorless nucleated cells occurring in blood.

LIA Lysine iron agar.

lipid Fatty or waxy material.

lipolytic Has the power to produce lipolysis, which is the decomposition or splitting up of fat.

lysed blood Blood that has gone through the lysis process. Lysis is the dissolution; a loosing, setting free, releasing of. Usually the destruction/decomposition of the blood cell.

M-Endo Liquid growth medium used for detection of coliform bacteria.

MacConkey agar Selective growth medium used for growth of many enteric bacteria.

macroinvertebrate Visible invertebrate organism.

maximum contaminant level (MCL) USEPA-established standard.

megacolon Abnormally large colon; usually due to dilatation and hypertrophy. Usually seen in children.

meningitis Inflammation of any or all of the meninges of the brain and the spinal cord, usually caused by bacterial or viral infection.

mesenteric lymph glands Lymph glands that are found in and around the mesentery. Thus, a membrane that connects portions of the intestines to the abdominal wall.

mesenteric veins Veins found in the peritoneal folds that connect the intestines to the dorsal abdominal wall.

mesophilic An organism whose optimal growth temperature is between 30 and 45°C.

metabolite Any of various organic compounds produced by metabolism.

methyl-red test A qualitative test for acid production. It is based on the use of a pH indicator, methyl red, to determine the hydrogen ion concentration present when an organism ferments glucose or other fermentable sugar.

MF Membrane filtration, a technique used to determine the number of bacteria in raw and treated water.

microbiology The science that deals with microorganisms (protists).

microvilli A microscopic projection from the surface of a cell.

MIO Motility indole ornithine.

mitochondria-bearing Organisms that carry mitochondria within the cytoplasm of their cells.

mitosis The sequential differentiation and segregation of replicated chromosomes in a cell nucleus that precedes complete cell division.

monoclonal antibodies Produced by cells that are cloned from a single hybrid cell and produce only one molecular species of antibody.

monotrichous Having one flagellum at only one pole or end, as certain bacteria.

morbidity Of, relating to, or caused by disease.

morphology The biological study of the form and structure of living organisms; the structure and form of an organism, excluding its functions.

mortality Frequency of number of deaths in proportion to a population.

motility Moving or having the power to move spontaneously.

MTF Multiple tube fermentation, a technique that requires water samples to be placed in a set of fermentation tubes that contain lactose or lauryl tryptose broth. Tubes are incubated at 35°C for 24 to 48 hours.

mucoid Any of a group of organic compounds similar to the mucins or mucoproteins, descriptive of a bacterial colony that is very sticky or stringy.

mucosal Mucous membrane.

myositis Inflammation of a voluntary muscle; seen in trichinosis or Sarcocystis infections.

necrotizing fasciitis Inflammation and necrosis of the fascia.

nematode A worm of the phylum Nematoda, having unsegmented, thread-like bodies, many of which, as the hookworm, are parasitic.

neonate A newborn.

neurocysticercosis The brain is infested with cysticerci (a form of larval tapeworm resembling Cysticercus), but having the cyst small and almost devoid of fluid.

neurotoxin A substance that can damage nerve cells.

noncommunity water system Water systems providing service to primarily transient populations, such as camps, hotels, and industries.

nonsporulating Does not produce spores.

nosocomial Disease acquired in a hospital.

obligate parasite Must have a host to complete its life cycle.

oncogenic Tending to cause the formation of tumors.

ONPG O-nitrophenyl-B-D-galactopyranoside; tests for B-D-galactosidase produced by some coliforms.

oocyst The encysted form of some sporozoan zygotes.

operculum A lid or flap covering an aperture, such as the gill cover in some fishes or the horny shell cover in snails or other mollusks.

ornithine decarboxylase Biochemical test used in differentiating coliform types.

osteomyelitis Inflammation of the bone marrow.

otitis Inflammation of the ear.

ova The female reproductive cells of animals (eggs); ovum (egg) is singular.

ovoid Lemon-shaped.

oxidase Any of various plant or animal enzymes that act as oxidants.

pandemic Widespread; general; epidemic over an especially wide geographic area.

parasite An organism that grows, feeds, and is sheltered on or in a different organism while contributing nothing to the survival of its host.

paratenic host Host of third-stage larvae that infect humans.

parenchyma The tissue characteristic of an organ, as distinguished from connective tissue.

parenchymal cell Found in the liver.

PAS stain Periodic acid-Schiff, a staining procedure used to distinguish microsporidia spores.

passeriform birds Perching birds and songbirds, such as jays, blackbirds, finches, warblers, and sparrows.

pathogen An agent that causes disease, especially a microorganism.

pathogenesis The development of a diseased or morbid condition.

pathogenicity Capable of causing disease.

PCR Polymerase chain reaction.

peptide Any of various natural or synthetic compounds containing two or more amino acids linked by the carboxyl group of one amino acid and the amino group of another.

perineum The portion of the body in the pelvis occupied by urogenital passages and the rectum, bounded in front by the pubic arch, in the back by the coccyx, and laterally by part of the hipbone.

peritoneal-dialysis effluent Fluid from the flushing of the peritoneal cavity.

peritonitis Inflammation of the membrane lining the walls of the abdominal cavity and enclosing the viscera.

PFU Plaque-forming unit.

phenospecies Species that look alike.

phototaxis The movement of an organism in response to a source of light.

pica Abnormal craving for clay, paint, chalk, or other substances not fit for food.

pico- A combining form meaning one trillionth; 10^{-12}.

plasmid A genetic element occurring outside of the nucleus that is found in the cytoplasm of some bacterial cells.

polymerase Any of various enzymes that aid in the linkage of nucleotides in the formation of DNA or RNA, with an existing strand of DNA or RNA acting as a template.

polymyxin B Antibiotic; cyclic polypeptide; causes direct membrane damage by a detergent-like action.

pour plate A bacterial culture method in which an aliquot of sample is placed in a petri dish and melted; tempered agar growth medium poured in, mixed, and allowed to solidify; and the plate then incubated at an appropriate temperature.

prepatent period The time from infection to when parasites are seen in diagnosis.

prodromal Showing the symptom of the onset of a disease.

proteolytic Able to break down proteins into simpler, soluble substances, as in digestion.

protozoa Any of the single-celled, usually microscopic organisms of the phylum or subkingdom Protozoa, which includes the most primitive forms of animal life.

pseudopod Projections of cell membranes that move a cell.

psychrophilic Thriving at relatively low temperatures, usually at or below 15°C.

pustule A slight, inflamed elevation of the skin filled with pus.

PVA Polyvinyl alcohol.

quality control A system for ensuring the maintenance of proper standards in scientific processes, especially by periodic inspection of the product and incorporation of appropriate controls.

R2A An agar; a low-nutrient medium used in heterotrophic plate counts.

reactive arthritis Inflammation of the joints; a result of injury, toxin exposure, etc.

rectal prolapse Protrusion of the rectal mucous membrane through the anus.

refractive hyaline cell wall A cell wall that has semitransparent properties and refracts light.

reverse osmosis A water filtration process using pressure to force water through a membrane.

RFLP Restriction fragment length polymorphism analysis.

ribosome A spherical cytoplasmic RNA-containing particle active in the synthesis of protein.

rifampicin An antibiotic that has antibacterial action.

rostellum Crown.

saccharolytic Has the capability of chemically splitting up sugar.

salt flotation A method by which one can concentrate parasites either through sedimentation or by flotation. Concentration is specifically designed to allow recovery of protozoan cysts, coccidian oocysts, microsporidian spores, and helminth eggs and larvae.

sanitary survey An investigation of the sanitary conditions of an area.

saprophytic A plant that derives its nourishment from dead or decaying organic matter.

schizogonic stage Asexual multiplication (merogony); multiple intracellular nuclear division precedes cytoplasmic division; reproductive stage that results in many merozoites.

schmutzdecke The carpet-like layer of bacteria, algae, and other microorganisms and inorganic and organic matter that forms on the surface of a slow sand filter and that aids in purifying the water.

scolex Head.

sedimentation The act or process of depositing sediment (material that settles to the bottom of a liquid).

selenite broth A selective enrichment medium used for the growth–isolation of *Salmonella*. Very inhibitory for most Enterobacteriaceae.

self-limiting Limiting itself, as in a disease that clears without treatment.

sepsis The presence of pathogenic organisms or their toxins in the blood or tissues.

septicemia A systemic disease caused by pathogenic organisms or their toxins in the bloodstream.

seroepidemiology Study of disease outbreak–spread using serological responses.

serological group Related organisms distinguished by their antigenic properties.

serology The medical science that deals with serums (clear yellowish fluids obtained by separating whole blood into its solid and liquid components; the fluid from the tissues of immunized animals, used especially as an antitoxin; watery fluid from animal tissue, such as that found in edema).

serotyping A test to determine a group of related microorganisms distinguished by its antigenic composition.

sp. and spp. Abbreviations for species (singular and plural, respectively).

speciation The evolutionary process by which new species are formed. Also, the classification of organisms into species using appropriate laboratory methods.

species A fundamental category of taxonomic classification, ranking after genus and consisting of organisms capable of interbreeding.

spicule A small needlelike structure or part, such as one of the silicate or calcium carbonate processes supporting the soft tissue of certain invertebrates.

spore An asexual, usually single-celled reproductive organ; a microorganism, as a bacterium, in a dormant or resting state.

sporocyst A resting cell that produces asexual plant spores; a protective case containing spores of certain protozoans; a sac-like larval stage in many trematode worms.

sporogonic state, sporogony The production of spores resulting from sexual fusion of gametes prior to multiple fission, characteristic of certain protozoans.

sporont Parasitic cells with thickened membranes seen prior to becoming sporoblasts.

sporozoite A sporozoan that has been released from a spore and is ready to penetrate a new host cell.

sporulation Producing or releasing spores.

spp. (See sp.)

stylet A slender, pointed instrument; a surgical probe.

subacute Somewhat acute (reaching a crisis rapidly).

subcutaneous Located or found just beneath the skin.

substrate The material or substance on which an enzyme acts; a surface on which a plant or animal grows or is attached.

suppurative infection An infection associated with the formation or discharge of pus.

symptomatology The medical science of disease symptoms.

tachyzoite Rapidly multiplying forms of parasites.

taxonomy The science, laws, or principles of classification; the theory, principles, and process of classifying organisms in established categories.

tegument Outer covering of an organism.

tenesmus A painfully urgent but ineffectual attempt to urinate or defecate.

tetrathionate broth A peptone-based selective broth; used for the culture of *Salmonella* and *Shigella*.

thermophilic Pertaining to organisms whose optimal temperature for growth is above 50°C.

thiosulfate-citrate-bile salts-sucrose agar Has a color indicator—bromthymol blue; a selective medium used for culture of vibrios.

titer The concentration of a substance in solution or the strength of such a substance determined by titration (the process or method of determining the concentration of a substance in solution by adding to it a standard reagent of known concentration in carefully measured amounts until a reaction of definite and known proportion is completed); the minimum volume needed to cause a particular result in titration.

TOC Total organic carbon.

toxic Of or pertaining to a toxin (a poisonous substance having a protein structure secreted by certain organisms and capable of causing toxicosis when introduced into the body tissues, but also capable of inducing a counteragent or an antitoxin); harmful, destructive, or deadly.

transient Passing through from one place to another.

trimethoprim An antibiotic.

trophozoite A vegetative stage of amoeba.

trypsin One of the proteolytic enzymes of the pancreatic juice, important in the digestive processes.

tryptophane An amino acid, $C_{11}H_{12}N_2O_2$, produced in the digestive process and essential in human nutrition.

TSI Triple sugar iron.

urea broth Hydrolysis of urea by urease releases ammonia. This causes a change in alkalinity and the color indicator (phenol red) will change from yellow to red. Urease-positive organisms produce a pink color; whereas urease-negative organisms cause no change in the broth color.

vacuole A small cavity in the protoplasm of a cell.

vasculature The arrangement of blood vessels in the body or in an organ or part of the body.

vibrio Any of various S-shaped or comma-shaped microorganisms of the genus *Vibrio*, especially *V. cholerae*, which causes cholera.

virion Infective form of a virus.

virulent Extremely poisonous or pathogenic.

virus Any of various submicroscopic pathogens consisting essentially of a core of a single nucleic acid surrounded by a protein coat, having the ability to replicate only inside a living cell.

Voges-Proskauer test Used to assist in differentiating between different Enterobacteriociae.

waterborne Found in water or transmitted through water supplies.

zoonosis, zoonotic A disease such as rabies or malaria that can be transmitted from animals to humans.

zygote The cell formed by the union of two gametes.

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