CHAPTER 1

DISEASE TRANSMISSION BY CONTAMINATED WATER

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INTRODUCTION

Water is traditionally viewed as the "universal solvent" which accounts for its vital support of all living things. The property of solvency is also responsible, in the main, for the chemical quality of natural water as pertains to the dissolution of naturally occurring minerals, atmospheric gases, and organic molecules present in plant and animal residues. Natural waters are also a vehicle for suspended matter, including microbial cells.

Fresh surface waters are collectively represented by streams, rivers, lakes, ponds, and reservoirs and constitute a major source of drinking water. Unless protected, they are prone to receiving anthropogenic discharges of domestic, industrial, and agricultural wastewaters. Such adulterations alter the natural water quality, and the severity of change is dependent on the rate, extent, and composition of the waste discharges. Groundwater (subsurface water) is the most plentiful form of available freshwater. However, owing to greater inaccessibility and higher cost, groundwaters are less utilized as a water supply than surface waters.

The consequences for utilizing polluted waters as a drinking water supply are well documented historically and will be dealt with in the section "Historical waterborne disease background." Natural water should be valued both as a commodity and a habitat for aquatic life. The former consideration pertains to public health issues and the latter deals with the ecological value of natural waters.

Surface waters can be rated according to best usage with respect to drinking, bathing, shellfish rearing, fishing, and navigation purposes. A set of minimum water-quality standards defines the best usage of a water body. Waters suitable for drinking-water supplies, recreational bathing, and shellfish rearing are monitored regularly for microbiological quality. The best usage of a water body such

as a river may change along its course. Designation of a water according to best usage as a source of drinking water may imply high raw water quality but does not preclude the need for proper treatment of the water before release to consumers. Even then, faults in the water distribution system can permit access of disease-producing microbes to an otherwise-adequately treated water. Furthermore, drinking water sources and subsequent purification steps vary widely in quality among world nations. It cannot be assumed that water drawn from a faucet is totally safe to consume, especially, in lesser-developed countries and rural areas. During a visit to Canada in 1989, then-Czechoslovakian president Vaclav Havel remarked, "I was surprised to learn that I was drinking tap water. No one in Czechoslovakia would do that."[1]

Only about 2.6 percent of the global content of water constitutes fresh water (atmospheric, and both surface and subsurface water bodies). Distribution of fresh-water supplies among countries of the world is uneven and without regard to population demands. Although water is a renewable resource, loss of usable drinking-water supplies through unfavorable natural and manmade environmental changes intensifies the challenge of providing adequate and safe drinking water worldwide in the coming years. There is the anticipation of major alterations in rainfall patterns and increased frequency of catastrophic floods owing to climate change, meteoric expansion of human populations, and the likelihood of increasingly unfavorable air, soil, and water quality in populous nations such as China and India, where the focus is on competitive economic development. Compromising environmental standards, especially with respect to drinking-water quality, heightens the potential for transmission of disease-producing agents within the population. Poor sanitation is unequivocally linked to the occurrence of high rates of communicable and noncommunicable diseases worldwide.

The title of this chapter is "Disease Transmission by Contaminated Water." The classical concept of disease transmission by contaminated water is by the oral route. Other avenues of infection are possible, however. Gleeson and Gray[2] have denoted four categories of infectious behavior in humans through contact with contaminated water or lack of water:

1. *Waterborne disease*. Sickness or ailment results from ingestion of water that is harboring a pathogen.
2. *Water-washed disease*. Sickness or ailment is spread by the fecal-oral route or person-to person contact and facilitated by the lack of adequate water for personal hygiene,
3. *Water-based infection*. Sickness or ailment is caused by infection arising through ingestion of a pathogenic agent (e.g., guinea worm larvae) or invasion of the body through water contact (e.g., schistosome and other trematode larvae able to penetrate the skin of individuals in contact with water).
4. *Water-related diseases*. Sickness or ailment is facilitated by insect vectors that breed in waters (e.g., malaria mosquitoes and filariasis arthropods that carry viruses responsible for dengue ad yellow fever).

To these may be added three more:

5. *Inhalation of water aerosols contaminated by a pathogenic agent*. This could include *Legionella pneumophila*, the etiologic agent of legionellosis and Pontiac fever.
6. *Consumption of water-based foods derived from contaminated water*. Sickness might be related, for example, to ingestion of raw shellfish containing *Vibrio vulnificus* or *V. parahemolyticus*, both causative agents of diarrheal diseases.
7. *Consumption of foods that have had contact with contaminated water at some stage of production*. Sickness results from microbial contamination during production/preparation (e.g., irrigation, washing, and preservation) of food such as leafy vegetables.

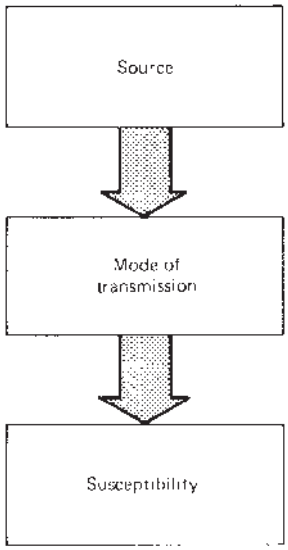
Many disease-producing viruses and bacteria have been identified in this connection, and the protozoan, *Cyclospora cayetanensis*, etiologic agent of a diarrheal disease, cyclosporiasis, with pathology resembling that of cryptosporidiosis, has been identified in imported raspberries and lettuce from South American countries.[3]

At this juncture, it is recommended that the reader consult the definition of terms in Chapter 2 in order to appreciate textural issues. Most definitions of the phrase "communicable disease" emphasize the involvement of an identifiable pathogenic agent. With any communicable disease, there is the need to transmit or communicate an infectious agent to a host by means of a vector or vehicle or person-to-person contact. Continuous propagation of the communicable disease within members of a population requires that the infectious agent be able to exit the diseased individual and find access to a healthy person. One definition of communicable disease appears in the list of definitions given in Chapter 3 and includes "toxic products" of infectious agents as an instrument of communicable diseases. Biological-based toxins alone have rarely been found to be the cause of a water-transmitted disease. However, such toxins are a potential weapon for terrorists and, notwithstanding the minimal chance of success, are an anticipated threat to water supplies.

Ingestion of chemical contaminants in water may cause acute and chronic forms of toxicity leading to the development of noncommunicable diseases in individuals. Biological agents are the cause of infectious (communicable) diseases that may or may not be contagious.

Control of Source (Agent Factors)

Certain sources of disease agents are noted in Figure 1.1. Gerstman[4] defines an agent as a biological, chemical, or physical factor whose presence or absence in varying amounts is required for the occurrence of a disease; a form of necessary factor. Gerstman identified several types of factors of varying essentiality in the propagation of a communicable disease. The agent is a necessary factor, that is, its presence in the host is required to produce a disease although its presence



Source (agent factors-physical, chemical biologic): food and infected or infested animals; poisonous plants and animals; parasites; toxic solid, liquid, and gaseous substances and natural deposits; genetic and inherited materials; ionizing and nonionizing radiations; noise.

Mode of transmission or contributing factors (environmental factors): environmental pollutants; contact; animals; personal behavior; level of hygiene, sanitation, standard of living; work, recreation, travel,home, climate.

Susceptibility (host factors): all animals or susceptibles, resulting in acute, chronic, or delayed effects, depending on portal of entry, dose, and virulence or toxicity of the agent; natural and acquired resistance of the host, and lifestyle.

Animals include humans and arthropods. *Arthropods* include insects, arachnids, crustaceans, and myriapods. *Environmental pollutants* may be transmitted by air, water, food, or contact. *Personal behavior* may involve cigarette smoking, drug use, poor nutrition, stress, lack of exercise, cultural habits, and obesity. *Physical agents* may be heat, cold, precipitation, and causes of accidents. *Biologic agents* include arthropods, helminthes, protozoa, fungi, bacteria, rickettsiae, and viruses. Chemical agents include inorganic and organic chemicals.

FIGURE 1.1 Spread of communicable and noninfectious diseases.

does not guarantee that the disease may be expressed. There is ample evidence that individuals may be carriers of a pathogenic agent (necessary factor) but not become clinically ill. The kinds of factors proposed by Gerstman are addressed elsewhere in the chapter. Elimination or control of the source and environmental exposure to disease agents or vectors is a primary step to be carried out to the extent feasible. Individuals frequently are not aware that they are being exposed to a potential source of disease, particularly when it is a minute, insidious, and cumulative substance, such as certain chemicals in the air, water, and food. An additional complication arises on the biological front when the disease agent is transmissible by more than one route. For example, many of the viral and bacterial agents of disease can be transmitted through both contaminated food and water.

In many instances, control at the source is not only possible but also practical. Measures that might be taken to reduce or eliminate the appearance of toxic substances in waters are:

1. Change the raw material or industrial process to eliminate or adequately minimize the offending substance. For example, terminate the production

of a chemical such as polychlorinated byphenyl (PCB). The U.S. Environmental Protection Agency (EPA) "zero-discharge" *goal* is a step in this direction.

2. Select the cleanest available source of drinking water, as free as possible from microbiological and toxic organic and inorganic chemicals.
3. Make available water with optimum mineral content, such as through fluoridation and water hardness control.
4. Prohibit taking of fish and shellfish from contaminated (e.g., pathogen, methylmercury, PCB) waters.
5. Regulate food production, processing, and service to ensure freedom from toxic substances and pathogens and to assure food of good nutritional content.
6. Provide decent housing in a suitable living environment.
7. Provide a safe and healthful work and recreational environment.
8. Promote recycling, reuse, and zero discharge of hazardous wastes.
9. Eliminate disease vectors (arthropods and other animals, including rodents) at the source. Practice integrated pest management.
10. Isolate infected persons and animals from others during their period of communicability and provide medical treatment to eliminate disease reservoir.
11. Educate polluters, legislators, and the public to the need for regulation and funding where indicated.
12. Adopt and enforce sound standards.
13. Support comprehensive environmental health, engineering, and sanitation planning, surveillance, and regulation programs at the state and local levels.

See also "Control of Susceptibles (Host Factors)" in this chapter.

Control of Mode of Transmission

Several types of factors may be brought into any discussion of disease expression and transmission. An environmental factor, in the context of disease transmission, would be any external physical, biological, or chemical condition, other than the agent, that contributes to the disease process.[4] As an example, several environmental factors, including high humidity, high temperature, neutral to slightly alkaline soils, presence of organic matter, variety of animal reservoirs, and infected cattle herds, appear to contribute to the high endemic rate of leptospirosis in certain tropical countries.[5] Several species of *Leptospira* are pathogenic. The causative agent of Weil's disease is the pathogenic spirochete, *Leptospira interrogans* serovar Icterohemmoragiae. The disease is one of the leading zoonoses worldwide and, while the incidence is infrequently encountered in the temperate climates (0.1 to 1 case per 100,000 individuals per year), it is more prevalent in tropical areas of high rainfall (10 to 100 cases per 100,000

individuals per year).[6] Although this chapter has the focus of water involvement within the scope of illness transmission in the environment, it is well to adopt an interconnective attitude toward the control of environmental disease transmission in general. It is necessary to continually ask the question, "Can a known pathogen or toxic substance be exposed to a susceptible population by more than one route?" Again, leptospirosis may be used to address the question. Leptospires may be found in the urine of those suffering from leptospirosis. Vehicles of transmission for this disease are urine-contaminated water, food, and direct bodily contact with contaminated materials, such as through cuts and abrasions of the skin and mucous membranes. In addition, many animals, especially rodents, are reservoirs of the leptospires. It can be appreciated, therefore, that spread of the pathogens is open to many routes of transmission.

Prevention of disease requires the continual application of control procedures such as the following 10 measures and elimination of the human element to the extent feasible:

1. Prevent the travel of disease vectors and control disease carriers.
2. Assure that all drinking water is at all times safe to drink and adequate for drinking, culinary, laundry, and bathing purposes.
3. Provide adequate spatial separation between sources of disease (and pollution) and receptors.
4. Assure that food processing, distribution, preparation, and service do not cause disease.
5. Control air, land, and water pollution, hazardous wastes, accidents, carcinogens, and toxics.
6. Prevent access to disease sources—polluted bathing waters and disease vector–infested areas.
7. Adopt and enforce environmental standards—air, water, land, noise, land use, housing.
8. Educate polluters, legislators, media, and the public to the need for regulation and funding where indicated.
9. Support comprehensive environmental health, engineering, and sanitation planning, protection, surveillance, and regulation programs at the state and local levels.
10. Adjust personal behavior to counteract cigarette smoking, poor nutrition, stress, overeating, and lack of exercise. Promote personal hygiene and handwashing to prevent person-to-person transmission of pathogenic or toxic agents.

Control of Susceptibles (Host Factors)

Host factors are personal characteristics and behaviors, genetic predispositions, and immunologic and other susceptibility-related factors that increase or decrease the likelihood of disease and may be as sufficient factors.[4] A sufficient factor is

a causal factor that, in concert with a necessary factor, is "sufficient" to ensure that a disease will develop.[4] A necessary factor is a type of causal factor that is essential to, but not solely sufficient to, ensure the expression of a disease.[4] To facilitate the understanding of these factors collectively, consider the fate of an immunocompromised person who drank contaminated tap water containing the oocysts of *Cryptosporidium hominis*, an etiologic agent of the disease, cryptosporidiosis. After about 10 days, the individual begins to express symptoms of the disease. The afflicted person would be referred to as a case. A host factor and, in this instance also a sufficient factor, is the immunocompromised state of the individual. The necessary factor was the presence of the infectious material (oocysts) in the drinking water.

Individuals most susceptible to infectious diseases, especially the illnesses responsible to opportunistic pathogens, are the very young, the elderly, those with cardiovascular and respiratory disease, the immunocompromised, those occupationally exposed to airborne and other pollutants, those who smoke heavily, the obese, and those who underexercise. There are many diseases to which all persons are considered to be generally susceptible. Among these are measles, streptococcal diseases caused by group A streptococci, the common cold, ascariasis, chickenpox, amebic dysentery, bacillary dysentery, cholera, malaria, trichinosis, and typhoid fever. There are other diseases, such as influenza, meningococcus meningitis, pneumonia, human brucellosis (undulant fever), and certain water- and foodborne illnesses, to which some people apparently have an immunity or resistance. To these should be added the noninfectious diseases such as diseases of the heart, malignant neoplasms, and cerebrovascular diseases.

In order to reduce the number of persons who may be susceptible to a disease at any one time, certain fundamental disease-prevention principles should be followed to improve the general health of the public. This may be accomplished through educational programs on personal hygiene and immunization; avoidance of smoking; maintenance of proper weight; minimal liquor consumption; and conserving or improving the general resistance of individuals to disease by a balanced diet and nutritious food, fresh air, moderate exercise, sufficient sleep, rest periods, and the avoidance of stress, fatigue, and exposure. In addition, all individuals should be educated and motivated to protect themselves to the extent feasible from biological, physical, chemical, and radiation hazards and environmental pollutants.

Immunization can be carried out by the injection of vaccines, toxoids, or other immunizing substances to prevent or lessen the severity of specific diseases. Typhoid and paratyphoid fevers, poliomyelitis, and tetanus are some of the diseases against which the armed forces are routinely immunized. Children are generally immunized against diphtheria, tetanus, pertussis (whooping cough), poliomyelitis, rubeola (measles), mumps, and rubella (German measles). Revaccination of students and others born after January 1, 1957, against measles is recommended and may be required prior to school admission. It is now possible to discontinue smallpox vaccination as a routine measure in view of the global eradication of smallpox.[7]

Typhoid bacilli may be found in the feces and urine of cases and carriers. Typhoid immunization is reported to be about 70 to 90 percent effective, depending on degree of exposure,[8,9] and then only against small infectious doses. Routine typhoid vaccination is indicated only when a person is in intimate contact with a known carrier or travels in areas where there is a recognized risk of exposure, but precautions should still be taken with water and food. Routine vaccination of sewage sanitation workers is warranted only in areas with endemic typhoid fever. There is no reason to use typhoid vaccine for persons in areas of natural disaster such as floods or for persons attending rural summer camps.[8,9] There are currently two typhoid vaccines available in the United States, an oral live-attenuated vaccine (Vivotif Berna) and an injected capsular polysaccharide vaccine (Typhim Vi). Both vaccines have been shown to protect 50 to 80 percent of recipients. Boosters are required, every five years for the oral vaccine and every two years for the injected form.[10] Before choosing to forgo typhoid vaccination, travelers should be advised that a marked increase in antibiotic resistance by *S. typhi* has been documented in recent years and that the geographic location of the more resistant strains may be related to the frequency of antibiotic use.[11]

Cholera vaccine is not available in the United States. It has not been recommended for travelers because of the brief and incomplete immunity it offers. Currently, this issue is somewhat controversial; however, it is generally agreed that effective deployment of vaccines for cholera should take place in areas or countries of high endemic level of cholera, and 50 to 70 percent of the susceptible population must be immunized. Antibiotic resistance to tetracycline has been found in some *V. cholerae* isolates. However, widespread acquisition of antibiotic resistance has not been reported as in the case of *S. typhi*. No cholera vaccination requirements exist for entry or exit of any country. Yellow fever vaccine offers protection for at least 10 years and possibly up to 35 years. A certificate of vaccination is required for entry into some countries.[10] The WHO is recommending the use of five antihelminthic agents—albendazole, mebendazole, diethylcarbamazine, ivermectin, and praziquantel—to control parasitic worm infections that affect over 25 percent of the world's population.[12]

Good housing, sanitation (water, sewerage, solid wastes, and vermin control), and personal hygiene provide long-term protection against many diseases whereas an immunization protects only against a specific disease and must be repeated to remain effective. Individual and community performance, environmental hygiene, and economic levels are also improved,[13] in addition to the quality of life. This is not to minimize the importance of immunization against the childhood diseases and epidemic control where indicated.

Typical Epidemic Control

Outbreaks of illnesses such as influenza, measles, dysentery, poliomyelitis, and other diseases can still occur. At such times, the people become apprehensive and look to the health department for guidance, assurance, and information to calm their fears.

An example of the form health department assistance can take is illustrated in the precautions released June 1, 1951, in the *Illinois Health Messenger* for the control of poliomyelitis. These recommendations predate the 1955 availability of the Salk vaccine; hence, they portray a sense of urgency. For this reason, they are instructive and are generally applicable to outbreaks of other diseases. Even though poliomyelitis is under control in the United States, experience dictates that if the vaccination program is allowed to lapse, a resurgence of the disease is apt to follow.[14]

General Precautions during Outbreaks

1. The Illinois Department of Public Health will inform physicians and the general public as to the prevalence or increase in the incidence of the disease. *Note:* Incidence and prevalence are not synonyms. Incidence refers to the number of new cases occurring in a certain population *during* a defined time period. Prevalence is the number of cases of a disease *in* a defined population at a particular point in time. The terms are illustrated later in this chapter in the section "Epidemiology and Risk."
2. *Early diagnosis* is extremely important. Common early signs of polio are headache, nausea, vomiting, muscle soreness or stiffness, stiff neck, fever, nasal voice, and difficulty in swallowing, with regurgitation of liquids through the nose. Some of these symptoms may be present in several other diseases, but in the polio season they must be regarded with suspicion.
3. All children with any of these symptoms should be isolated in bed, pending diagnosis. Early medical care is extremely important.
4. Avoid undue fatigue and exertion during the polio season.
5. Avoid unnecessary travel and visiting in areas where polio is known to be prevalent.
6. Pay special attention to the practices of good personal hygiene and sanitation:
   a. Wash hands before eating.
   b. Keep flies and other insects from food.
   c. Cover mouth and nose when sneezing or coughing.

Surgical Procedures

Nose, throat, or dental operations, unless required as an emergency, should not be done in the presence of an increased incidence of poliomyelitis in the community.

General Sanitation (Including Fly Control)

1. Although there has been no positive evidence presented for the spread of poliomyelitis by water, sewage, food, or insects, certain facts derived from research indicate that they might be involved in the spread:

a. *Water*. Drinking water supplies can become contaminated by sewage containing poliomyelitis virus. Although no outbreaks have been conclusively traced to drinking water supplies, only water from an assuredly safe source should be used to prevent any possible hazards that might exist.

b. *Sewage*. Poliomyelitis virus can be found for considerable periods of time in bowel discharges of infected persons and carriers and in sewage containing such bowel discharges. Proper collection and disposal facilities for human wastes are essential to eliminate the potential hazard of transmission through this means.

c. *Food*. The infection of experimental animals by their eating of foods deliberately contaminated with poliomyelitis virus has been demonstrated in the laboratory, but no satisfactory evidence has ever been presented to incriminate food or milk in human outbreaks. Proper handling and preparation of food and pasteurization of milk supplies should reduce the potential hazard from this source.

d. *Insects*. Of all the insects studied, only blowflies and houseflies have shown the presence of the poliomyelitis virus. This indicates that these flies might transmit poliomyelitis. It does not show how frequently this might happen; it does not exclude other means of transmission; nor does it indicate how important fly transmission might be in comparison with other means of transmission.

2. Fly eradication is an extremely important activity in maintaining proper sanitation in every community.

3. Attempts to eradicate flies by spraying effective insecticides have not shown any special effect on the incidence of polio in areas where it has been tried. Airplane spraying is not considered a practical and effective means in reducing the number of flies in a city. The best way to control flies and prevent them from spreading any disease is to eliminate fly-breeding places. Eradicate flies by:

   a. Proper spreading or spraying of manure to destroy fly-breeding places.

   b. Proper storage, collection, and disposal of garbage and other organic waste.

   c. Construction of all privies with fly- and rodent-proof pits.

   Proper sanitation should be supplemented by use of effective insecticide around garbage cans, manure piles, privies, and so on. Use effective insecticide spray around houses or porches or paint on screen to kill adult flies.

Swimming Pools

1. Unsatisfactorily constructed or operated swimming pools should be closed, whether or not there is poliomyelitis in the community.

2. On the basis of available scientific information, the State Department of Public Health has no reason to expect that closure of properly equipped

and operated swimming pools will have any effect on the occurrence of occasional cases of poliomyelitis in communities.

3. In communities where a case of poliomyelitis has been associated with the use of a swimming pool, that pool and its recirculation equipment should be drained and thoroughly cleaned. (The State Department of Public Health should be consulted about specific cleansing procedures.) After the cleaning job is accomplished, the pool is ready for reopening.
4. Excessive exertion and fatigue should be avoided in the use of the pool.
5. Swimming in creeks, ponds, and other natural waters should be prohibited if there is any possibility of contamination by sewage or too many bathers.

Summer Camps

Summer camps present a special problem. The continued operation of such camps is contingent on adequate sanitation, the extent of crowding in quarters, the prevalence of the disease in the community, and the availability of medical supervision. Full information is available from the Illinois Department of Public Health to camp operators and should be requested by the latter:

1. Children should not be admitted from areas where outbreaks of the disease are occurring.
2. Children who are direct contacts to cases of polio should not be admitted.
3. The retention of children in camps where poliomyelitis exists has not been shown to increase the risk of illness with polio. Furthermore, return of infected children to their homes may introduce the infection to that community if it is not already infected. Similarly, there will be no introduction of new contacts to the camp and supervised curtailment of activity will be carried out, a situation unduplicated in the home. This retention is predicated upon adequate medical supervision.
4. If poliomyelitis occurs in a camp, it is advisable that children and staff remain there (with the exception of the patient, who may be removed with consent of the proper health authorities). If they do remain:
   a. Provide daily medical inspection for all children for two weeks from occurrence of last case.
   b. Curtail activity on a supervised basis to prevent overexertion.
   c. Isolate all children with fever or any suspicious signs or symptoms.
   d. Do not admit new children.

Schools

1. Public and private schools should not be closed during an outbreak of poliomyelitis, nor their opening delayed except under extenuating circumstances, and then only upon recommendation of the Illinois Department of Health.

2. Children in school are restricted in activity and subject to scrutiny for any signs of illness. Such children would immediately be excluded, and parents would be urged to seek medical attention.
3. Closing of schools leads to unorganized, unrestricted, and excessive neighborhood play. Symptoms of illness under such circumstances frequently remain unobserved until greater spread of the infection has occurred.
4. If poliomyelitis occurs or is suspected in a school:
   a. Any child affected should immediately be sent home, with advice to the parents to seek medical aid, and the health authority notified.
   b. Classroom contacts should be inspected daily for any signs or symptoms of illness and excluded if these are found.

Hospitals

1. There is no reason for exclusion of poliomyelitis cases from general hospitals if isolation is exercised; rather, such admissions are necessary because of the need for adequate medical care of the patient.
2. Patients should be isolated individually or with other cases of poliomyelitis in wards.
3. Suspect cases should be segregated from known cases until diagnosis is established.
4. The importance of cases to hospitals in a community where poliomyelitis is not prevalent has not been demonstrated to affect the incidence of the disease in the hospital community.

Recreational Facilities

1. Properly operated facilities for recreation should not be closed during outbreaks of poliomyelitis.
2. Supervised play is usually more conducive to restriction of physical activities in the face of an outbreak.
3. Playground supervisors should regulate activities so that overexertion and fatigue are avoided.

WATERBORNE DISEASES

General

Disease agents spread by water and food have in common the capability to incapacitate large groups of people and sometimes result in serious disability and death. The World Health Organization estimates that 80 percent of all diseases are attributable to inadequate water or sanitation and that 50 percent of hospital beds worldwide were occupied by people afflicted with water-related diseases.[15] During the period 1920 to 2000, there were 1,836 waterborne outbreaks representing 882,592 cases of illness in the United States.[16] The number of deaths recorded for

the period was well under 1 percent, however. Most waterborne disease fatalities occurred before 1940 and were attributable to typhoid fever.[17] The finding probably reflects the unavailability of antibiotics during the early time frame. Diseases of a waterborne nature appear when disregard of known fundamental sanitary principles occurs, hence, in most cases are preventable. As often occurs, very young, elderly, immunocompromised, and critically ill persons with some other illness succumb with the added strain of a water- or foodborne illness. These groups of disease-sensitive people are thought to make up 20 to 25 percent of the population of the United States.[18]

Water- and foodborne diseases are sometimes referred to as the intestinal or filth diseases because they are frequently transmitted by food or water contaminated with excreta. Raw drinking water and improperly protected and treated surface and groundwater supplies may be polluted by excreta or sewage, which is almost certain to contain pathogenic microorganisms with potential to cause illness in consumers. In the United States, community waterborne outbreaks during the period 1981 to 1990 predominantly associated with inadequately treated surface water and deficiencies in the distribution system whereas untreated groundwaters were the major source of waterborne diseases for persons utilizing private water sources.[19]

Survival of Pathogens

Survival periods for selected pathogens in surface and groundwater are given in Table 1.1. The survival of pathogens is quite variable and affected by the type of organism, the presence of other antagonistic organisms, the soil characteristics, temperature, moisture, nutrients, pH, and sunlight. Table 1.1 is intended only as a comparative measure of survivability among pathogens. The amount of clay and organic matter in the soil affect the movement of pathogens, but porous soils, cracks, fissures, and channels in rocks permit pollution to travel long distances.

Some organisms are more resistant than others. Soil moisture of about 10 to 20 percent of saturation appears to be best for survival of pathogens; drier conditions increase die-off.

Nutrients may increase survival of some organisms, although elevated metabolism in vegetative cells and the germination of spores may produce the opposite effect. Typically, pH is not a major factor. As would be expected, survival of some pathogenic bacteria at very low pH (e.g., pH 2.5–3) is poor in certain media.[20,21] When pH values are below the isoelectric point of both bacteria and viruses, surface charge will be positive and, although controversial, may promote aggregation and adsorption of cells to predominantly negatively charged particulate matter and produce a protective effect against the potentially harmful effects of high hydrogen ion. In addition, hydrogen ion may effect the solubilization of nutrients. Viruses appear stable over the pH range of 3 to 9. Exposure to sunlight increases the death rate. Low temperatures favor survival.[22,23] The survival of pathogens in soil, on foods, and following various wastewater unit treatment processes, as reported by various investigators, is summarized by Bryan[24] and others.[25] Most

TABLE 1.1 Survival of Certain Pathogens in Water

	Survival Time[a]	
	In Surface water	In Groundwater
Coliform bacteria	—	7–8 days[b]
Cryptosporidium spp. oocyst	18+ months at 4°C	2–6 months, moist[c]
Excherichia coli	—	10–45 days[b]
Entamoeba histolytica	1 month[d]	
Enteroviruses	63–91+ days[e]	
Giardia lamblia cyst	1–2 months, up to 4[f]	
Leptospira interrogans serovar Ichterohemorrhagiae	3–9 days[g]	
Franciscella tularensis	1–6 months[g]	
Rotaviruses and reoviruses	30 days–1+ years[e]	
Salmonella faecalis	—	15–50 days[b]
Salmonella paratyphi	—	60–70 days[b]
Salmonella typhi	1 day–2 months[g]	8–23 days[b]
Salmonella typhimuriun	—	140–275 days[b]
Shigella	1–24 months[g]	10–35 days[b]
Vibrio cholerae	5–16 days[g] 34 days at 4°C[g] 21+ days frozen[g] 21 days in seawater[d]	
Viruses (polio, hepatitis, other enteroviruses)	—	16–140 days[b]
Enteroviuses[h]	38 days in extended aeration sludges at 5°C, pH 6–8; 17 days in oxidation ditch sludges at 5°C, pH 6–8	
Hepatitis A[i]	1+ years at 4°C in mineral water, 300+ days at room temperature	
Poliovirus[i]	1+ years at 4°C in mineral water, not detected at room temperature	

[a] Approximate.

[b] Guidelines for Delineation of Wellhead Protection Areas, Office of Ground-Water Protection, U.S. Environmental Protection Agency, Washington, DC, June 22, 1987, pp. 2–18. *Source:* Matthess et al., 1985. G. Matthess, S.S.D. Foster and A.Ch. Skinner, Theoretical background, hydrogeology and practice of groundwater protection zones, IAH International Contributions to Hydrogeology 6 (1985).

[c] A. S. Benenson (Ed.), *Control of Communicable Diseases in Man*, 15th ed., American Public Health Association, Washington, DC, 1990, p. 113.

[d] B. K. Boutin, J. G. Bradshaw, and W. H. Stroup, "Heat Processing of Oysters Naturally Contaminated with *Vibrio cholerae*, Serotype 01," *J. Food Protection*, **45**, 2 (February 1982): 169–171.

[e] G. Joyce and H. H. Weiser, *J. Am. Water Works Assoc*. (April 1967): 491–501 (at 26°C and 8° C).

[f] S. D. Lin, "*Giardia lambia* and Water Supply," *J. Am Water Works Assoc*. (February 1985): 40–47.

[g] A. P. Miller, *Water and Man's Health*, U. S. Administration for International Development, Washington, DC, 1961, reprinted 1967.

[h] G. Berg et al., "Low-Temperature Stability of Viruses in Sludges," *Appl. Environ. Microbiol*., **54**, 839 (1988); *J. Water Pollut. Control Fed*. (June 1989): 1104.

[i] E. Biziagos et al., "Long-Term Survival of Hepatitis A Virus and Poliovirus Type 1 in Mineral Water," *Appl. Environ. Microbiol*., **54**, 2705 (1988); *J. Water Pollut. Control Fed*. (June 1989): 1104.

enteroviruses pass through sewage treatment plants, survive in surface waters, and may pass through water treatment plants providing conventional treatment. According to WHO, water treatment plants maintaining a free residual chlorine in the distribution system of at least 0.5 mg/l for at least 30 minutes and low turbidity [less than 1 nephelometric turbidity unit (NTU)] in the finished water can achieve satisfactory virus inactivation. Other approved disinfection treatment (e.g., ozonation) can accomplish satisfactory virus destruction.

Substance Dose to Cause Illness

The development of illness is dependent on the toxicity or virulence of a substance or pathogen, the amount of the substance or pathogen ingested (at one time or intermittently), and the resistance or susceptibility of the individual. The result may be an acute or long-term illness. Sometimes two or more substances may be involved to produce a synergistic, additive, or antagonistic effect. The microbial modes of disease transmission include ingestion of a pathogen or toxin in contaminated water or food, contact with an infected person or animal, or exposure to an aerosol containing the viable pathogen.

If the dose of a chemical substance administered to a series of animals is plotted against the effect produced, such as illness, and increased doses produce no increases in illnesses, the substance is said to cause "no effect." If increased doses cause increasing illnesses, the substance has "no threshold." If increased doses cause no apparent increases in illnesses at first but then continuing increased doses show increasing illnesses, the dose at which illnesses begin to increase is referred to as the substance "threshold." Below that dose is the "no-observed-effect" range. Variations between and within animal species must be considered.

Table 1.2 contains a list various microorganisms and the approximate infectious dose required to cause disease. Bryan[24] has summarized the work of numerous investigators giving the clinical response of adult humans to varying challenge doses of enteric pathogens. For example, a dose of 10^9 *Streptococcus faecalis* was required to cause illness in 1 to 25 percent of the volunteers, 10^8 *Clostridium perfringens* type A (heat resistant) to cause illness in 26 to 50 percent of the volunteers, and 10^9 *C. perfringens* type A (heat sensitive) to cause illness in 76 to 100 percent of the volunteers.

If one were to consume 16 ounces of water containing a pathogen having a high infectious dose value (pathogen A) and the same amount of water containing a pathogen of low infectious dose value, it might be concluded that illness would be less likely through infection with pathogen A than pathogen B. Such thinking contains several fallacies, however. Pathogen infectious dose data should be used only as a guide and must be tempered in the knowledge that many variables influence the host-parasite relationship.[26] In any specific situation, virulence of the pathogen, physiological state of the pathogen, distribution of the infective units (pathogen) in a unit volume (in this case water), susceptibility of the host (infant, young, old, healthy, sick, immunocompromised), and route of infective

TABLE 1.2 Substance Dose to Cause Illness

Microorganism	Approximate Number of Organisms (Dose) Required to Cause Disease
Campylobacter jejuni[a]	10^2 or less
Coxiella burneti[b]	10^7
Cryptosporidium[c]	10^1–10^2 oocysts
Dracunculus, Ascaris, Schistosoma	1 cyst, egg, or larva
Entamoeba histolytica[d]	10–20 cysts, one in a susceptible host
Escherichia coli[b]	10^8
Giardia lamblia[c–f]	5–10^2 cysts
Salmonella typhi[b,g]	10^5–10^6
Salmonella typhimurium[g]	10^3–10^4
Shigella[b,g]	10^1–10^2
Staphylococcus aureus[b]	10^6–10^7 viable enterotoxin-producing cells per gram of food or milliliter of milk
Vibrio cholerae[b,g]	10^6–10^9
Virus, pathogenic	1 plaque-forming unit (PFU) or more

[a]Robert V. Tauxe et al., *"Campylobacter* Isolates in the United States, 1982–1986," *MMWR CDC Surveillance Summaries* (June 1988): 9.
[b]H. L. Dupont and R. B. Hornick, "Infectious Disease from Food," in *Environmental Problems in Medicine*, W. C. McKee (Ed.), Charles C. Thomas, Springfield, IL, 1974.
[c]R. M. Clark et al., "Analysis of Inactivation of *Giardia lamblia* by Chlorine," *J. Environ. Eng*. (February 1989): 80–90.
[d]*Guidelines for Drinking Water Quality*, Vol. 2, World Health Organization, Geneva, 1984, p. 44.
[e]Up to 10 cysts from beaver to human and 1 to 10 cysts to cause human to human infection.
[f]R. C. Rendtorff, "Experimental Transmission of *Giardia lamblia," Am. J. Hyg*., **59**, 209 (1954).
[g]Eugene J. Gangarosa, "The Epidemiologic Basis of Cholera Control," *Bull. Pan Am. Health Org*., **8**, 3 (1974).

contact (ingestion, inhalation, cutaneous) influence the inception of disease. The experimental conditions pertinent to the determination of infectious dose levels is important. The nature of the host subjects (human volunteers, monkeys, mice, or other), health status of the host subjects, protocol for introducing the pathogen dose to the subjects (oral, injection, aerosolization), and frequency of exposure of the host subjects to the pathogen challenge are all important to the interpretation of infectious dose values.

The low infectious dose for pathogenic viruses and protozoa would appear to suggest that viral infections ought be readily spread through drinking water, food, shellfish, and water-contact recreational activities. Fortunately, the tremendous dilution that wastewater containing viruses usually receive on discharge to a watercourse and the treatment given drinking water greatly reduce the probability of an individual receiving an infectious dose. However, some viruses do survive and present a hazard to the exposed population. Not all viruses are pathogenic in the sense that their obligate destruction of host cells to sustain replication and release of new virus particles may not trigger clinical symptoms of disesase in the host. Nonetheless, heretofore unknown insidious relationships between

viruses and their effects on hosts are becoming better understood, resulting in recognition of the pathogenicity of viruses thought to be innocuous.

Data on infectious doses for many important environmentally transmitted diseases are lacking. Obtaining estimates of infectious doses is time consuming, animal or human subject intensive, and costly. An indication of the difficulty involved may be imagined in economics of testing for the effect of chemicals as given by Kennedy:[27] "A typical chronic toxicology test on compound X, done to meet a regulatory requirement with an adequate number of animals and an appropriate test protocol, costs $250,000 to 300,000" and requires 2 to 3 or more years to complete.

Information concerning the *acute* effect of ingestion of toxic substances is available in toxicology texts.[28]

Summary of Characteristics and Control of Water- and Foodborne Diseases

In view of the fact that water- and foodborne diseases result in discomfort, disability, or even death, a better understanding of their source, method of transmission, control, and prevention is desirable. Although not mutually inclusive throughout, several of the infections transmitted by contaminated food and water are caused by the same pathogenic agents. The primary focus of attack is the gastrointestinal tract.

Special attention should be paid the subject of gastroenteritis. It is a vaguely understood disease with a complex epidemiology, often without a known causal pathogen or chemical instigator. Three types of gastroenteritis may be distinguished by the pathological response to the presence of an infectious agent: (1) noninflammatory, (2) inflammatory, and (3) invasive (Table 1.3).[29] Yet, different forms of gastroenteritis typically display common symptoms such as watery diarrhea, vomiting, intestinal and stomach cramps, and muscular aches, all of which create a nausea in the victim. The purging of the gastrointestinal tract that takes place removes or inactivates the normal barriers to infection and changes the unshielded epithelium that alters the host defenses, causing malabsorption and nutrient loss. The severity of the symptoms somewhat characterizes the nature of its etiology as do the complications that accompany protracted illnesses.

There are acute and chronic forms of gastrointestinal diseases. The number of cases worldwide of gastrointestinal illnesses are estimated to be from 6 billion to 60 billion of which over 2 million directly result in death.[30] Acute forms of gastroenteritis outbreaks in countries of the world have a storied history, some of which are noted elsewhere in this chapter. The symptoms of gastroenteritis appear frequently among diseases associated with different source pathogens. This is borne out in Table 1.4, which contains a comprehensive grouping and summary of the characteristics and control of a number of these diseases for easy reference.

Although comprehensive, the body of information should not be considered exhaustive or terminally complete, rather the table should serve as an orientation

TABLE 1.3 Forms of Gastroenteritis, Symptoms, and Causative Agents

Gastroenteritis	Symptoms	Responsible Organisms
Noninflammatory gastroenteritis	Diarrhea and/or vomiting, no fecal leukocytes, no blood in stool, usually no fever.	Bacteria: *Staphylococcus aureus*,[a] *Bacillus cereus*,[a] *Clostridium perfringens*,[a] *Clostridium botulinum*[a] Viruses: noroviruses Protozoa: *Giardia lamblia (intestinalis), Cryptosporidium parvum* Algae: *Pfiesteria* spp[a].
Inflammatory gastroenteritis	Diarrhea and/or vomiting, fecal leukocytes present, usually severe fever, no blood in stool.	Bacteria: *Vibrio cholerae*,[b] enteropathogenic *Escherichia coli* (EPEC), enteroaggregative *E. coli* (EAggEC), *Clostridium difficile, Shigella* spp, enterotoxigenic *E. coli* (ETEC) Viruses: rotavirus, Caliciviruses[b] Protozoa: *Entamoeba dispar*
Invasive gastroenteritis	Invasion past epithelial layer of GI tract, may not have any diarrhea or vomiting, dysentery may be present (mucus containing bloody feces), fecal leukocytes present, fever: may not have any GI tract problems but instead severe systemic problems.	Bacteria. *Salmonella* spp., *Campylobacter jejuni*, enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), *Vibrio vulnificus, Yersinia* spp., *Franciscella tularensis, Bacillus anthracis, Helicobacter pylori* Viruses: unknown Protozoa: *Entamoeba histolytica*

Source: MWH, *Water Treatment: Principles and Design*, 2nd ed., John Wiley & Sons, Hoboken, NJ, 2005.

[a]These microorganisms grow on food or in the environment and produce toxins that, when ingested, cause gastroenteritis a few hours later (only *Pfiesteria* spp. is of concern to drinking water).
[b]Often cited as not causing a fever.

to a complex field requiring much further study. There are likely many bacterial toxins, bacteria, viruses, protozoa, helminths, chemicals, and other agents that are not suspected or that are not examined for or discovered by available laboratory methods. Emerging infectious diseases worldwide are becoming recognized, particularly among the viruses, and will undoubtedly expand the list.

The primary bacterial pathogens, which have been historically linked to waterborne disease, are well known. However, a less-recognized occurrence in the

TABLE 1.4 Characteristics and Control of Water- and Foodborne Diseases

	Disease	Specific Agent	Reservoir	Common Vehicle	Symptoms in Brief	Incubation Period	Prevention and Control
Bacterial Toxins	Botulism food poisoning	*Clostridium botulinum* and *C. parabotulinum* that produce toxin	Soil, dust, fruits, vegetables, foods, mud, fish, animal and human feces	Improperly processed canned and bottled foods containing the toxin, also other foods	Gastrointestinal pain, diarrhea or constipation, prostration, difficulty in swallowing, double vision, difficulty in respiration	2 hr–8 days, usually 12–36 hr	Boil home canned nonacid food 5 min; thoroughly cook meats, fish, foods held over. Do not taste suspected food. Store fish at $\leq 38^{\circ}$F.
	Staphylococcus food poisoning	Staphylococci that produce entero-toxin, *Staphylococcus aureus*. (Toxin is stable at boiling temperature.)	Skin, mucous membranes, pus, dust, air, sputum, and throat	Contaminated custard pastries, cooked or processed meats, poultry, dairy products, hollandaise sauce, salads, milk	Acute nausea, vomiting, and prostration; diarrhea, abdominal cramps. Usually explosive in nature, followed by rapid recovery of those afflicted.	1–6 hr or longer, average 2–4 hr	Refrigerate promptly prepared food in shallow containers at a temperature below 45°F. Discard leftover food. Avoid handling food. Educate foodhandlers in personal hygiene and sanitation.
	Clostridium perfringens food poisoning	*Clostridium perfringens* (*C. welchi*), a sporeformer. (Certain spores are heat resistant.)	Soil, gastrointestinal tract of man and animals, cattle, poultry, pigs, vermin, and wastes	Contaminated food, inadequately heated meats, including roasts, stews, beef, poultry, gravies, improperly held or cooled food	Sudden abdominal pain, then diarrhea and nausea Ingestion of large numbers of vegetative cells that grow in intestine and form spores. Cast off cell releases toxin causing symptoms.	8–22 hr, usually 10–12 hr	Cook foods thoroughly, cool rapidly, and refrigerate promptly foods not consumed. Cool foods in shallow containers, cut up large pieces. Reheat thoroughly to 165°F before reserving. Educate cooks.

TABLE 1.4 ***(continued)***

	Disease	Specific Agent	Reservoir	Common Vehicle	Symptoms in Brief	Incubation Period	Prevention and Control
	Bacillus cereus food poisoning—diarrheal type	*Bacillus cereus*, toxin heat labile	Spores found in wide variety of cereals, spices, vegetables, and milk	Inadequately refrigerated cooked foods and subsequently inadequately reheated	Diarrhea, cramps; vomiting sometimes	6–16 hr	Prevent food contamination. Cool food rapidly in shallow containers, reheat rapidly.
	Bacillus cereus food poisoning—vomiting type	*Bacillus cereus*, toxin heat stable	Same as diarrheal	Boiled and fried rice	Vomiting, diarrhea, nausea, sometimes	1–6 hr	Same as diarrhea. Some spores, if present in large numbers may survive Ultra high temperture (UHT) and High temperature-short time (HTST) pasteurization.
	Gastroenteritis, dermatitis, central nervous system disorders	Cyano-bacteria	Nutrient-rich surface waters, aquatic sediments, soils	Ingestion and body contact involving waters containing dense cyanobacterial cell mass	Tremors, hypersalivation, ataxia, diarrhea (ingestion); rash, eye irritation, asthma (skin contact)	7–14 days	Water treatment may involve microscreening, coagulation, filtration; distribution system should be monitored for accumulation of cell residues and flushed
Bacteria	Salmonellosis (*Salmonella* infection)	*Salmonella typhimurium, S. newport, S. enteritidis, S. montevideo*, others	Hogs, cattle, and other livestock, poultry, pets, eggs, carriers, powdered eggs, turtles, animal feed, and rodents	Contaminated sliced cooked meat, salads, uncooked meats, equipment, warmed-over foods, milk and milk products, water, eggs	Abdominal pain, diarrhea (persists several days), chills, fever, vomiting, and nausea	6–48 hr, usually 12–24 hr	Protect storage of food. Thoroughly cook food. Eliminate rodents, pets, and carriers. Similar measures as in *Straphylococcus*, Poultry, water, and meat sanitation. Do not eat raw eggs or ground beef, Refrigerate foods.

Bacteria	Typhoid fever	Typhoid bacillus, *Salmonella typhi*	Feces and urine of typhoid carrier or patient	Contaminated water, milk and milk products, shellfish, and foods; flies	General infection characterized by continued fever, usually rose spots on the trunk, diarrheal disturbances	Average 14 days, usually 7–21 days	Protect and purify water supply. Pasteurize milk and milk products. Sanitary sewage disposal. Educate food-handlers. Food, fly, shellfish control. Supervise carriers. Personal hygiene. Isolate patients.
	Paratyphoid fever	*Salmonella paratyphi* A, *S. schott mulleri* B. *S. hirschfeldii* C	Feces and urine of carrier or patient	Contaminated water, milk and milk products, shellfish, and foods; flies	General infection characterized by continued fever, diarrheal disturbances, sometimes rose spots on trunk, other symptoms	1–10 days for gastroenteritis; 1–3 weeks for enteric fever	Similar preventive and control measures as in typhoid fever and salmonellosis
	Shigellosis (Bacillary dystentery)	Genus, *Shigella*, i.e., *flexneri, sonnei, boydii, dysenteriae*	Feces of carriers and infected persons	Contaminated water or foods, milk and milk products, flies, person-to-person	Acute onset with diarrhea, fever, tenesmus, frequent stools containing blood and mucus	1–7 days, usually less than 4 days	Food, water, sewage sanitation as in typhoid. Pasteurize milk (boil for infants). Control flies; supervise carriers. Personal hygiene.
	Cholera	*Vibrio comma*	Feces, vomitus; carriers	Contaminated water, raw foods, flies, shellfish	Diarrhea, rice-water stools, vomiting, thirst, pain, coma	A few hours-5 days, usually 3 days	Similar to typhoid. Quarantine. Isolate patients. Vaccine of limited value.

(*continues*)

TABLE 1.4 ***(continued)***

	Disease	Specific Agent	Reservoir	Common Vehicle	Symptoms in Brief	Incubation Period	Prevention and Control
Bacteria	Melioidosis	*Pseudomonas pseudomallei*	Rats, guinea pigs, cats, rabbits, dogs, horses	Contact with or ingestion of contaminated excreta, soil, or water	Acute diarrhea, vomiting, high fever, delirium, mania	Less than 2 days or longer	Destroy rats. Protect food. Thoroughly cook food. Control biting insects. Personal hygiene.
	Brucellosis (Undulant fever)	*Brucella melitensis*-goat, *B. abortus*-cow, *Br. suis*-pig	Tissues, blood, milk, urine, infected animals	Raw milk from infected cows or goats; also contact with infected animals	Insidious onset, irregular fever, sweating, chills, pains in joints and muscles	5–21 days or longer	Pasteurize all milk. Eliminate infected animals. Handle infected carcasses with care.
	Streptococcal infections	*Streptococcus pyogenes*	Nose, throat, mouth secretions	Contaminated salads or milk products	Sore throat and fever, sudden in onset, vomiting	1–3 days	Pasteurize all milk. Inspect contacts. Same as staphylococcus
	Diphtheria	*Coryne-bacterium diphtheriae*	Respiratory tract, patient, carrier	Contact and milk or milk products	Acute febrile infection of tonsils, throat, and nose	2–5 days or longer	Pasteurize all milk. Disinfect utensils. Inspect contacts. Immunize.
	Tuberculosis	*Mycobacterium tuberculosis (M. tuberculosis and M. bovis)*	Respiratory tract of man, rarely cattle	Contact, also eating and drinking utensils, food, and milk	Cough, fever, fatigue, pleurisy	4–6 weeks	Pasteurize all milk, eradicate TB from cattle. Skin test. Control contacts and infected persons. Selective use of BCG.

Bacteria	Tularemia	Francisella	Rodents, rabbits, horseflies, wood ticks, dogs, foxes, hogs	Meat of infected rabbit, contaminated water, handling wild animals	Sudden onset, with pains and fever, prostration	1–10 days, average of 3	Thoroughly cook meat of wild rabbits. Purify drinking water. Use rubber gloves (care in dressing wild animals).
	Campylobacter enteritis	*Campylobacter jejuni*	Chickens, swine, dogs, cats, man, raw milk, contaminated water	Undercooked beef, chicken, also pork Raw milk, contaminated water	Watery diarrhea, abdominal pain, fever, chills, nausea, vomiting, blood in stool	1–10 days 2–5 days average	Thoroughly cook chicken and pork and properly refrigerate. Treat water. Prevent cross-contamination.
	Vibrio para-haemolyticus gastroenteritis	*Vibrio para-haemolyticus*	Marine fish, shell-fish, mud, sediment, salt water, brackish and fresh water	Raw seafoods or seafood products; inadequately cooked seafoods, and cross-contamination between raw and cooked products and sea water	Nausea, headache, chills, fever, vomiting, severe abdominal cramps, watery diarrhea, sometimes with blood	2–48 hr, usually 12–24 hr	Properly cook all seafood (shrimp 7 to 10 min). Avoid cross-contamination or contact with sea water or preparation surfaces used for uncooked foods. Refrigerate prepared seafoods promptly if not immediately served.
	Diarrhea enteropath-ogenic (Traveler's diarrhea)	Enteropath-ogenic *Escherichia coli* invasive and entero-toxigenic strains	Infected persons	Food, water, and fomites contaminated with feces, raw or under-cooked meat	Fever, mucoid, occasionally bloody diarrhea; or watery diarrhea, cramps, acidosis, dehydration	12–72 hr	See Typhoid. Scrupulous hygiene and formula sanitation in hospital nursery. Food sanitation, thorough cooking.

(*continues*)

TABLE 1.4 ***(continued)***

	Disease	Specific Agent	Reservoir	Common Vehicle	Symptoms in Brief	Incubation Period	Prevention and Control
Bacteria	Yersiniosis	*Yersinia entercolitica, Yersinia pseudotuberculosis*	Wild and domestic animals, birds, man, surface water	Raw milk and milk products, seafoods, raw and rare meats, infected food-handlers, contaminated water	Diarrhea, cramps, fever, headache, vomiting, skin rash, pseudo-appendicitis	3–7 days, usually 2–3 days	Sanitary disposal of human, dog, and cat feces. Safe water. Pasteurize milk. Food sanitation. Wash hands. Organism grows at 40°F. Thoroughly cook food.
	Listeriosis	*Listeria monocytogenes*	Goats, cattle, man, fowl, soil, water, sewage	Raw milk, contaminated pasteurized milk and milk products, contaminated vegetables	Fever, headache, nausea, vomiting, meningeal symptoms	Probably a few days-3 weeks	Avoid contact with infected persons and aborted animal fetuses, raw milk and meats. Listeria grows at 37° to 113°F.
	Vibrio vulnificus gastroenteritis	*Vibrio vulnificus*	Oysters, sea water, sediment, plankton	Raw or lightly cooked seafood, i.e., oysters	Fever, chills, vomiting, nausea, diarrhea	16 hr	Same as *Vibrio parahaemolyticus* gastroenterits.
Rickettsias	Q Fever	*Coxiella burneti*	Dairy cattle, sheep, goats, ticks	Slaughterhouse, dairy employees, handling infected cattle: raw cow and goat milk, dust and aerosols from urine and feces	Heavy perspiration and chills, headache, malaise	2–3 weeks, average 20 days	Pasteurize milk and dairy products. Eliminate infected animal reservoir. Clean slaughterhouse and dairies. Keep down dust from dried wastes.

Viruses	Chorio-meningitis, lymphocytic Infectious hepatitis	*choriomenin-gitis* virus (LCMV) Hepatitis A virus	House mice urine, feces, secretions Feces from infected persons	Contaminated food Water, food, milk, oyster, clams, contacts, person-to-person, fecal-oral	Fever, grippe, severe headache, stiff neck, vomiting, somnolence Fever, nausea, loss of appetite; possibly vomiting, fatigue, headache, jaundice	8–13 days 10–50 days, average 30–35 days	Eliminate or reduce mice. General cleanliness. Sanitation. Sanitary sewage disposal, food sanitation, personal hygiene. Coagulate and filter water supply, and plus 0.6 mg/l free Cl_2. Obtain shellfish from certified dealers. Steam clams 4 to 6 min. Exclude ill workers.
	Gastroenteritis, viral	Rotaviruses, Nor-virus agent, echo- and coxsackie-viruses, and others	Man, feces from infected foodhandler or sewage	Water, food including milk, possibly fecal-oral or fecal-respiratory route, ice, clams	Nausea, vomiting, diarrhea, abdominal pain, low fever	24–72 hr, 24–48 hr, 3–15 days	Same as hepatitis A.
	Amebiasis (Amebic dysentery)	*Entamoeba histolytica*	Bowel discharges of carrier, and infected person; possibly also rats	Cysts, contaminated water, foods, raw vegetables and fruits, flies, cockroaches	Insidious and undetermined onset, diarrhea or constipation, or neither; loss of appetite, abdominal discomfort; blood, mucus in stool	5 days or longer, average 2–4 weeks	Same as Shigellosis. Boil water or coagulate, set, filter through diatomite 5 gpm/ft^2, Cl_2. Usual Cl_2 and high-rate filtration not 100% effective. Slow sand filtration plus Cl_2, or conventional RSF OK. Pressure sand filtration ineffective. Also sanitation and personal hygiene.

(*continues*)

TABLE 1.4 ***(continued)***

	Disease	Specific Agent	Reservoir	Common Vehicle	Symptoms in Brief	Incubation Period	Prevention and Control
Portozoa	Giardiasis	*Giardia lamblia*	Bowel discharges of carrier and infected persons; dog, beaver	Cysts, contaminated water, food, raw fruits; also hand-to-mouth route	Prolonged diarrhea, abdominal cramps, severe weight loss, fatigue, nausea, gas; fever is unusual.	6–22 days, average 9 days	Same as amebiasis.
	Cryptos-poridiosis	*Cryptos-poridium* spp	Farm animals, man, fowl, cats, dogs, mice	Contaminated water, food, fecal-oral, person-to-person	Mild flulike symptoms, diarrhea, vomiting, nausea, stomach pain	2–21 days, average 2–10 days	Avoid untreated water, also ice, unpasteurized milk, salads in areas of poor hygiene.
	Balantidiasis	*Balantidium coli*	Swine, man, and other animals	Ingestion of cysts in infected feces	Mild diarrhea, nausea, dysentery, vomiting	Unknown, a few days	Same as cryptosporidiosis, and Shigellosis.
	Primary amebic meningoen-cephalitis (PAM)	*Naegleria fowleri*	Warm freshwater bodies and swimming pools	Nasal tissue contact with water through inhalation during swimming and diving in surface waters and swimming pools	Sudden headache, vomiting, fever, nausea, pharyngitis; late stages include confusion, lethargy, neck stiffness, coma	3–7 days	Drinking water supplies may be disinfected with chlorine or ultraviolet irradiation at proper dosage. Swimming pool water may be sand filtered and disinfected.

	Granulomatous amebic encephalitis (GME), acanthamoeba keratitis	*Acanthamoeba*	Soils, dusts, all forms of natural waters; swimming pools, spas, air conditioners	Abrasions, skin cuts, nasal passages, eyes	Eye pain, redness, blurred vision (keratitis); see symptoms for PAM (GME)	>10 days to weeks	Similar to protection against *Naegleria* spp.
Bacteria	Leptospirosis (Weil's disease)	*Leptospira* interrogans with 27 serovars	Urine and feces of rats, swine, dogs, cats, mice, foxes, sheep	Food, water, soil contaminated with excreta or urine of infected animal, contact	Fever, rigors, headaches, nausea, muscular pains, vomiting, thirst, prostration, jaundice	4–19 days, average 9 to 10 days	Destroy rats. Protect food. Avoid polluted water. Treat abrasion of hands and arms. Disinfect utensils, treat infected dogs.
	Trichinosis (Trichiniasis)	*Trichinella spiralis*	Pigs, bears, wild boars, rats, foxes, wolves	Infected pork and pork products, bear and wild boar meat	Nausea, vomiting, diarrhea, muscle pain, swelling of face and eyelids, difficulty in swallowing	2–28 days, usually 9 days	Thoroughly cook pork (150°F), pork products, bear and wild boar meat. Destroy rats. Feed hogs boiled garbage or discontinue feeding. Store meat 20 days at 5°F or 10 days at −10°F.

(*continues*)

TABLE 1.4 ***(continued)***

	Disease	Specific Agent	Reservoir	Common Vehicle	Symptoms in Brief	Incubation Period	Prevention and Control
Helminths	Schistosomiasis (Bilharziasis)[a] (blood flukes)	*Schistosoma haematobium, S. mansoni, S. japonicum, S. intercalatum*	Venous circulation of man; urine, feces, dogs, cats, pigs, cattle, horses, field mice, wild rats, water buffalo	Cercariae-infested drinking and bathing water (lakes and coastal sea waters)	Dysenteric or urinary symptoms, rigors, itching on skin, dermatitis; carrier state 1 to 2 years and up to 25 years. Swimmer's itch schistosomes do not mature in man.	4–6 weeks or longer	Avoid infested water for drinking or bathing; coagulation, sedimentation, and filtration plus Cl_2 1 mg/l; boil water; impound water 48 hr, Cl_2. Slow sand filtration plus Cl_2. 1 mg/l $CuSO_4$ to kill cercariae and 20 mg/l to kill snails. Drug treatment available.
	Ascariasis (intestinal roundworm)	*Ascaris lumbricoides*	Small intestine of man, gorilla, ape	Contaminated food, water; sewage	Worm in stool, abdominal pain, skin rash, protuberant abdomen, nausea, large appetite	About 2 months	Personal hygiene, sanitation.[b] Boil drinking water in endemic areas. Sanitary excreta disposal.
	Echinococcosis (Hydatidosis)	*Echinococcus granulosus*, dog tapeworm	Dogs, sheep, wolves, dingoes, swine, horses, monkeys	Contaminated food and drink; hand to mouth; contact with infected dogs	Cysts in tissues: liver, lung, kidney, pelvis, may give no symptoms, may cause death	Variable, months to several years	Keep dogs out of abattoir and do not feed raw meat. Mass treatment of dogs. Educate children and adults in the dangers of close association with dogs.

Helminths	Taeniasis (pork tapeworm) (beef tapeworm)	*Taenia solium* (pork tapeworm), *T. saginata* (beef tapeworm)	Man, cattle, pigs, buffalo, possibly rats, mice	Infected meats eaten raw, food contaminated with feces of man, rats, or mice	Abdominal pain, diarrhea, convulsions, insomnia, excessive appetite	8–10 weeks	Thoroughly cook meat. Control flies. Properly dispose of excreta. Foodhandler hygiene. Use only inspected meat. Store meat as for trichinosis.
	Fish Tapeworm (broad tapeworm)	*Diphyllo-bothrium latum*, other	Man, frogs, dogs, cats, bears	Infected freshwater fish eaten raw	Abdominal pain, loss of weight, weakness, anemia	3–6 weeks	Thoroughly cook fish, roe, (caviar). Proper excreta disposal.
	Dracontiasis (Guinea worm disease)	*Dracunculus medinensis*, a nematode worm	Man	Water contaminated with copepods-*Cyclops*; larvae from infected persons	Blistering of feet, legs, and burning and itching of skin; fever, nausea, vomiting, diarrhea; worms from skin	About 12 months	Use only filtered or boiled water in endemic areas for drinking, or a safe well-water supply. Treat water from unsafe source with temephos, Abate®. Health education.
	Paragonimiasis (lung flukes)	*Paragonimus ringeri, P. westermani, P. kellicotti*	Respiratory and intestinal tract of man, cats, dogs, pigs, rats, wolves	Contaminated water, freshwater crabs or crayfish	Chronic cough, clubbed fingers, dull pains, diarrhea	Variable	Boil drinking water in endemic areas. Thoroughly cook freshwater crabs and crayfish.[b]
	Clonorchiasis[a] (liver flukes)	*C. sinensis, Opisthorchis felineus*	Liver of man, cats, dogs, pigs	Contaminated freshwater fish	Chronic diarrhea, night blindness	Variable	Boil drinking water in endemic areas. Thoroughly cook fish.[b]
	Fascioliasis (sheep liver flukes)	*Fasciola hepatica*	Liver of sheep	Sheep liver eaten raw	Irregular fever, pain, diarrhea	Several months	Thoroughly cook sheep liver.[b]

(*continues*)

TABLE 1.4 ***(continued)***

	Disease	Specific Agent	Reservoir	Common Vehicle	Symptoms in Brief	Incubation Period	Prevention and Control
Helminths	Trichuriasis (whipworm)	*Trichuris trichiura*	Large intestine of man	Contaminated food, soil	No special symptoms, possibly stomach pain	Long and indefinite	Sanitation, boil water, cook food well, properly dispose feces.[b]
	Oxyuriasis (pinworm, threadworm, or enterobiasis)	*Oxyuris vermicularis*, or *Enterobius vermicularis*	Large intestine of man, particularly children	Fingers, ova-laden dust, contaminated food, water, sewage; clothing, bedding	Nasal and anal itching, diarrhea	3–6 weeks; months	Wash hands after defecation. Keep fingernails short. Sleep in cotton underwear. Sanitation.
	Fasciolopsias[a] (intestinal flukes)	*Fasciolopsis buski*	Small intestine of man, dogs, pigs	Raw freshwater plants, water, food	Stomach pain, diarrhea, greenish stools, constipation, edema	6–8 weeks	Cook or dip in boiling water roots of lotus, bamboo, water chestnut, caltrop.
	Dwarf tapeworm (rat tapeworm)	*Hymenolepis nana* (*diminuta*)	Man and rodents	Food contaminated with ova, direct contact	Diarrhea or stomach pain, irritation of intestine	1 month	Sanitary excreta disposal, personal hygiene, food sanitation, rodent control. Treat cases.
	Anisakiasis	Nematodes of Anisakides family	Marine mammals and fish: rockfish, salmon, cod, tuna	Contaminated fish eaten raw or under-cooked	Stomach pain, nausea, vomiting, confused with appendicitis	Hours	Do not eat raw fish. Cook fish to 140°F or freeze to −4°F for 60 hr to kill larvae.

Poisonous Plants and Animals	Ergotism[c]	Ergot, a parasitic fungus (*Claviceps purpurea*)	Fungus of rye and occasionally other grains	Ergot-fungus contaminated meal or bread	Gangrene involving extremities, fingers, and toes; or weakness and drowsiness, headache, giddiness, painful cramps in limbs	Gradual, after prolonged use of diseased rye in food	Do not use discolored or spoiled grain (fungus grows in the grain). Meal is grayish, possibly with violet-colored specks.
	Rhubarb poisoning	Probably oxalic acid	Rhubarb	Rhubarb leaves	Intermittent cramplike pains, vomiting, convulsions, coma	2–12 hr	Do not use rhubarb leaves for food.
	Mushroom poisoning	Phalloidine and other alkaloids: also other poisons in mushroom	Mushrooms—*Amanita phalloides* and other Amanita	Poisonous mushrooms (*Amanita phalloides, Amanita muscaria*, others)	Severe abdominal pain, intense thirst, retching, vomiting, profuse watery evacuations	6–15 hr or 15 min–6 hr with muscaria	Do not eat wild mushrooms; warn others. *Amanita* are very poisonous, both when raw or cooked.
	Favism[a]	Poison from *Vicia faba* bean, pollen	*Vicia faba* Plant and bean	The bean when eaten raw, also pollen	Acute febrile anemia with jaundice, passage of blood in urine	1–24 hr	Avoid eating bean, particularly when green, or inhalation of pollen. Toxin not destroyed by cooking.
	Fish poisoning	Poison in fish, ovaries and testes, roe (heat stable)	Fish: pike, carp, sturgeon roe in breeding season	Fish: tedrodon, meletta, clupea, pickerel eggs, mukimuki	Painful cramps, dyspnea, cold sweats, dilated pupils, difficulty in swallowing and breathing	30 min–2 hr or longer	Avoid eating roe during breading season. Heed local warnings concerning edible fish.

(*continues*)

TABLE 1.4 ***(continued)***

	Disease	Specific Agent	Reservoir	Common Vehicle	Symptoms in Brief	Incubation Period	Prevention and Control
Poisonous Plants and Animals	Ciguatera poisoning	Toxin concentrated in tropical reef fish flesh, possibly from toxic dinoflagellate; also roe	Warm-water fish, possibly barracuda, snapper, grouper, amberjack, sea bass	Warm-water fish caught near shore from Pacific and Caribbean, coral reef fish	Progressive numbness, tetanuslike spasms, heavy tongue; facial stiffness; also nausea, vomiting, diarrhea, dryness of the mouth, abdominal cramps	1–8 hr, usually 3–5 hr	Avoid warm-water fish caught near shore in Pacific and Caribbean. The toxin ciguatera is not destroyed by cooking; toxin is not poisonous to fish.
	Shellfish poisoning (Paralytic)	Neurotoxin produced by *Gonyaulax catenella* and *G. tamarensis*	Clams and mussels feeding on specific dinoflagellates	Mussels and clams, associated with so-called "red tides"	Respiratory paralysis: in milder form, trembling about lips to loss of control of the extremities and neck. Fish kills and mass deaths in seabirds.	5–30 min and longer, up to 12 hr	Obtain shellfish from certified dealers and from approved areas. Monitor plankton in coastal waters. Toxin not destroyed by routine cooking.
	Scombroid fish poisoning	Scombrotoxin (histamine-like toxin)	Scombridea family primarily tuna, bluefish, amberjack	Fish that have been held at room temperature forming toxic histamine in muscle	Headache, burning mouth, nausea, vomiting, diarrhea, tingling of fingers, fever, cramps	Several minutes to 1 hr	Gut fish immediately after catch and refrigerate at 32°F or on ice. Toxin heat stable.
	Snakeroot poisoning	Trematol in snakeroot (*Eupatorium urticaefolium*)	White snakeroot jimmy weed	Milk from cows pastured on snakeroot	Weakness or prostration, vomiting, severe constipation and pain, thirst; temperature normal	Variable, repeated with use of the milk	Prevent cows from pasturing in wooded areas where snakeroot exists.

	Potato poisoning	*Solanum tuberosum*; other *Solanum*	Sprouted green potatoes	Possibly green sprouted potatoes	Vomiting, diarrhea, headache, abdominal pains, prostration	A few hours	Do not use sprouts or peel of sprouted green potatoes.
	Water-hemlock poisoning	Cicutoxin or resin from hemlock (*Cicuta maculata*)	Water hemlock	Leaves and roots of water hemlock	Nausea, vomiting, convulsions, pain in stomach, diarrhea	1–2 hr	Do not eat roots, leaves, or flowers of water hemlock.
Chemical Poisons	Antimony poisoning	Antimony	Gray-enameled cooking utensils	Foods cooked in cheap enameled pans	Vomiting, paralysis of arms	5 min–1 hour	Avoid purchase and use of poor-quality gray-enameled, chipped enamel utensils.
	Arsenic poisoning	Arsenic	Arsenic compounds	Arsenic-contaminated food or water	Vomiting, diarrhea, painful tenesmus (a cumulative poison)	10 min and longer	Keep arsenic sprays, etc., locked; wash fruits, vegetables. Avoid substances with concentrations greater than 0.05 mg/l.
	Cadmium poisoning	Cadmium	Cadmium-plated utensils	Acid food prepared in cadmium utensils	Nausea, vomiting, cramps, diarrhea	15–30 min	Watch for cadmium-plated utensils, racks, and destroy. Inform manufacturer.
	Cyanide poisoning	Cyanide, sodium	Cyanide silver polish	Cyanide-polished silver	Dizziness, giddiness, dyspnea, palpitation, unconsciousness	Rapid	Select silver polish of known composition. Prohibit sale of poisonous polish.

(*continues*)

TABLE 1.4 ***(continued)***

	Disease	Specific Agent	Reservoir	Common Vehicle	Symptoms in Brief	Incubation Period	Prevention and Control
Chemical Poisons	Fluoride or sodium fluoride poisoning	Fluoride or sodium fluoride	Roach powder	Sodium fluoride taken for baking powder, soda, flour	Acute poisoning, vomiting, abdominal pain, convulsions; paresis of eye, face, finger muscles, and lower extremities; diarrhea	Few minutes-2 hr	Keep roach powder under lock and key; mark "Poison"; color the powder, apply with care, if use is permitted.
	Lead poisoning	Lead	Lead pipe, sprays, oxides, and utensils, lead-base paints	Lead-contaminated food or acid drinks; toys, fumes, paints, drinking water	Abdominal pain, vomiting, and diarrhea (a cumulative poison), mental retardation, birth defects, fatigue, anemia	30 min and longer	Do not use lead pipe; Pb < 0.015 mg/l. Wash fruits. Label plants. Avoid using unglazed pottery. Test imported pottery. Screen child. Remove lead paint.
	Mercury poisoning	Mercury—methyl mercury and other alkyl-mercury compounds	Contaminated silt, water, aquatic life	Mercury-contaminated food, fish	Fatigue, mouth numbness, loss of vision, poor coordination and gait, tremors of hands, blindness, paralysis	2–30 min or longer	Keep mercuric compound under lock and key. Do not consume: fish with concentrations of mercury more than 0.5 ppm, water with more than 0.002 ppm, food with more than 0.05 ppm. Eliminate discharges to the environment.

Chemical Poisons	Methyl chloride poisoning	Methyl chloride	Refrigerant, methyl chloride	Food stored in refrigerator having leaking unit	Progressive drowsiness, stupor, weakness, nausea, vomiting, pain in abdomen, convulsions	Variable	Use nontoxic refrigerant, or ice, water, brine, dry ice.
	Selenium poisoning	Selenium	Selenium-bearing vegetation	Wheat from soil containing selenium, also other plants and water	Gastrointestinal, nervous, and mental disorders; dermatitis in sunlight	Variable	Avoid semiarid selenium-bearing soil for growing of wheat, or water with more than 0.05 mg/l Se.
	Zinc poisoning	Zinc	Galvanized iron	Acid food made in galvanized iron pots and utensils	Pain in mouth, throat, and abdomen followed by diarrhea	Variable, short	Do not use galvanized utensils in preparation of foods or drink, or water with more than 5.0 mg/l zinc.
	Methemo-globinemia	Nitrate nitrogen, plus nitrite	Groundwater; shallow dug wells, also drilled wells	Drinking water from wells high in nitrates	Vomiting, diarrhea, and cyanosis in infants	2–3 days	Use water with less than 45 mg/l NO_3 for drinking water and in infant formula. Properly develop and locate wells.
	Sodium nitrite poisoning	Sodium nitrite	Impure sodium nitrate and nitrite	Sodium nitrate taken for salt, cured meats	Dizziness, weakness, stomach cramps, diarrhea, vomiting, blue skin	5–30 min	Use USP sodium nitrate in curing meat. Nitrite is poisonous, keep locked.

(*continues*)

TABLE 1.4 ***(continued)***

	Disease	Specific Agent	Reservoir	Common Vehicle	Symptoms in Brief	Incubation Period	Prevention and Control
Chemical Poisons	Copper poisoning	Copper	Copper pipes and utensils	Carbonated beverages and acid foods in prolonged contact with copper	Vomiting, weakness, diarrhea	1 hr or less	Do not prepare or store acid foods or liquids or carbonated beverages in copper containers. Cu should not exceed 0.3 mg/l. Prevent CO_2 backflow into copper lines in soft drink machines.
	Reference: Material safety data sheets-Infectious substances. 2001. Public Health Agency of Canada (available at: http://www.phac-aspc.gc.ca/msds-ftss/msds106e.html); Centers for Disease Control and Prevention. 2007. Acanthamoeba infection, Department of Health and Human Services (available at http://www.cdc.gov/NCIDOD/DPD/parasites/acanthamoeba/factsht_acanthamoeba.htm); Martinez, A. J. Free-living amebas: *Naeglaria, Acanthamoeba*, and *Balamuthia*, Medmicro chapter 81 (available at http://www.gsbs.utmb.edu/microbook/ch081.htm)						

Source: This figure represents a summary of information selected from: I. G. M. Dack, *Food Poisoning*, 251 pp., University of Chicago Press, 1956. 2. C. E. Dolman, "Bacterial Food Poisoning," 46 pp., *Canad. Pub. Health J. Assoc*., 1943. 3. V. A. Getting, "Epidemiologic Aspects of Food-Borne Disease," 75 pp., *New Eng. J. Med*., 1943. 4. F. A. Korff, "Food Establishment Sanitation in a Municipality," *Am. J. Pub. Health* **32**, 740 (1952). 5. P. Manson-Bahr, *Synopsis of Tropical Medicine*, 224 pp., Williams & Wilkins Co., Baltimore, 1943. 6. New York State Department of Health, *Health News*. 7. Miscellaneous military and civilian texts and reports. 8. R. P. Strong, *Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases*, 2 vols., Blakiston Co., Philadelphia, 1942. 9. *The Control of Communicable Diseases in Man*, American Public Health Association, Washington, D.C., (Sept. 1944, Revised May 1945, 1946, 1952, 1971, 1980, 1990.) Copyright 1946, Joseph A. Salvato, Jr., MCE.) More complete characteristics, preventive and control measures, and modes of transmission, other than food and water, have been omitted for brevity as has been the statement "epidemiological study" and "education of the public" opposite each disease under the heading "Prevention and Control." Milk and milk products are considered foods. Under "Specific Agent" and "Common Vehicle" above, only the more common agents are listed.

[a]Does not originate in the U.S.

[b]Take same precautions with drinking, culinary, and bathing water as in Schistosomiasis.

[c]Many other fungi that produce toxin are associated with food and feedstuffs. The mycotoxins cause illness in humans and animals; see text. For more information see F. L. Bryan, *Diseases Transmitted by Foods*. DHEW, PHS, Atlanta, Ga., 1971, 58 p., *Procedure for the Investigation of Foodborne Disease Outbreaks*, and *Procedures to Investigate Waterborne Illness*, International Association of Milk, Food, and Environmental Sanitarians, P.O. Box 701, Ames, Ia. 50010, 1988, and Morbidity and Mortality Weekly Report(s), U.S. DHHS, PHS, CDC, Atlanta, Ga.

present-day human population is the increasing number of infections caused by bacteria not normally considered highly virulent. These organisms, sometimes considered secondary pathogens, are opportunistic bacteria that, under certain conditions, can cause infections through contact in some way with water.[31] Certain groups of people notably, infants, elderly, immunocompromised, transplant recipients, and convalescents, are at greatest risk of susceptibility to infection by these organisms. A summary of some important opportunistic bacterial pathogens appears in Table 1.5. Several of the bacterial species listed in the table are relatively newly discovered and responsible for specific pathological problems. Two such organisms are *Helicobacter pylori* and *Legionella pneumophila*.

Gastrointestinal disturbances are so commonplace in the human experience in wealthy countries that they are essentially an accepted fact of life, hence, usually receive little medical attention and go unreported. However, in undeveloped lands, gastrointestinal diseases are a ravishing scourge that accounts for numerous deaths, especially, among children. Of an estimated 2.2 million deaths from diarrheal-type diseases, 1.8 million of these involve children under five years of age.[32] To grasp the importance of safe drinking water on reduction of child mortality in various countries of the world, examine the comparative data in Figure 1.2.[33] The occurrence of a large number of diarrheal cases indicates that there has been a breakdown in hygiene or in the sanitary control of water or food and may forewarn impending cases of salmonellosis, typhoid fever, dysentery, or other illness.

Bacteria are prokaryotic, microscopic organisms, typically unicellular with morphologies described as coccoidal (ovoid), bacillary (rodlike), spiral (vibroid or helical), and filamentous. Typical eubacterial single-cell dimensions average 0.5 to 1 μm in diameter by 1 to 5 μm in length. Bacterial physiologies are more varied among the species than those of any group of microorganisms that supports the notion that plant and animal life on earth as we know it would not be possible without the bacteria. Unfortunately, the typical notoriety that bacteria in general have among the uninformed is that bacteria are "germs" and, therefore, are synonymous with disease. *Rickettsias* are obligate, intracellular parasitic bacteria not cultivatable outside host cells. Unlike viruses, they are retained by the Berkefield filter.[34] Their sizes average 0.3 to 0.7 μm by 1 to 2 μm.

Viruses are submicroscopic, genetic parasitic elements consisting of a nucleic acid (DNA or RNA) core surrounded by a protein coat, fall in the size range of 10 to 100 nm, pass through filters that retain bacteria, are visible only with the aid of an electron microscope, and can replicate only following invasion of living (host) cells. Viruses responsible for diseases transmitted by the water route are all RNA viruses, and most are geometrically icosahedral (ovoid) and small (about 30 nm) in size. Virus particles (virions) maintain infectiousness outside the host. Although all viruses require a host for sustaining replication of virions, expression of a clinical disease does not always take place. Animal enteric viruses do not appear to be readily transmissible to humans, although hepatitis A virus has been shown to pass from chimpanzees to humans. There are more than 100 types of human enteric viruses excreted in large numbers from the gastrointestinal tract.

TABLE 1.5 Opportunistic and "Modern" Bacterial Pathogens Transmitted by the Water Route

Disease and /or Conditions	Specific Agent	Reservoir	Common Vehicle	Symptoms	Incubation Period	Prevention and Control
Varied infections (urinary, eye) and abscesses (lung, brain), septicemia	*Acinetobacter* spp.: *calcoaceticus-baumannii* complex	Soil, seawater, freshwater, estuarine water, wastewater, contaminated food	Finished waters withhigh bacterial levels and low disinfectant residuals	Multifactorial according to body site affected	6-12 days	Adequate water treatment; maintain chlorine residual in distribution system
Gastrointestinal maladies; septicemia	*Aeromonas* spp.: *hydrophila, sobria, caviae*	Freshwater, marine water, estuarine water, wastewater, sludges, sediments	Finished waters with high bacterial levels	Diarrhea, vomiting	1-2 days	Adequate water treatment; maintain chlorine residual in distribution system
Gastroenteritis	*Campylobacter* spp.: *jejuni, coli, upsaliensis*	Contaminated water, wastewater, wastewater effluent	Contaminated water facilitating a zoonosis especially involving poultry consumption	Diarrhea, fever, cramps, tiredness, occasional vomiting	2-5 days	Disinfect water to effectively minimize residual *E. coli* numbers
Septicemia, pneumonia, infant meningitis, endocarditis	*Flavobacterium* spp.: *meningosep-ticum, breve, odoratum*	Soil, natural and finished waters, plumbing systems, hospital water fixtures	Water supply by ingestion or bodily contact	Unavailable	Unavailable	Tight control of finished water quality and provide well maintained distribution system

Gastritis, peptic and duodenal ulcer disease, stomach adeno-carcinoma	*Helicobacter* sp.: *pylori*	Uncertain presence in water distribution system	Possible contaminated food and water	Chronic indigestion, heartburn	5-10 days	Maintain adequate disinfectant residual in finished water
Enterocolitis, urinary and respiratory infections including hypersensitivity pneumonitis	*Klebsiella* spp.: *pneumoniae, oxytoca, rhinoscleromatis, planticola, ozaenae terrigena*	Certain industrial wastes, especially of paper-making and sugar refining; fruits and vegetables, plant matter	Surface water and unprotected ground water; water filters	Diarrhea, abdominal cramps; fever, dry cough, bloody sputum	1-3 days (chronic respiratory disease)	Maintain adequate disinfectant residual in distribution system and conduct periodic flushing to eliminate biofilms
Central nervous system, bone, soft tissue infections; endocarditis (HIV/AIDS patients highly susceptible	*Mycobacterium avium* complex (*M. avium* and *M. intracell-ulare*)	All environments (soil, clean and polluted waters), animals	Water and soil by ingestion, inhalation of aerosols	Abdominal pain, fatigue, diarrhea, anemia	Typically very long and dependent on health status	Water treatment coagulation and filtration for suspended matter removal, maintain effective disinfectant residuals

(*continues*)

TABLE 1.5 ***(continued)***

Disease and /or Conditions	Specific Agent	Reservoir	Common Vehicle	Symptoms	Incubation Period	Prevention and Control
Infant diarrhea, bacteremia, eye infections, cystic fibrosis, folli- colitis, osteo- myelitis, malig- nant external otitis	*Pseudomonas* sp.: *aeruginosa*	Surface water, groundwater, bottled water, distilled water, seawater, soils, vegetation	Water and food by ingestion, bodily contact in bathing waters and spas, hospital environments	Multifactorial; coughing, chest pain, fatigue (cystic fibrosis); pimply rash; ear ache	1-10 days (infections and rashes)	Control growth of *Pseudomonas* spp. in all phases of water treatment and in the distribution system maintaining chlorine residual of 0.5 mg/l throughout
Legionellosis, Pontiac fever	*Legionella* sp.: *pneumophila*	Surface water, cooling water towers, evaporative condensers, whirlpools, hot water tanks, fountains, water distribution systems	Inhalation of water mists emanating from cooling waters, condensers, spas	Pneumonia, anorexia, malaise, headache (legionellosis); influenza-like symptoms (Pontiac fever)	2-10 days (legionellosis); 1.5-3 days (Pontiac fever)	Clean sediments from hot water tanks and cooling towers; hold temperature range of 71–77°C in hot water tanks; maintain steady disinfectant levels in whirlpools and cooling towers

Cystitis, septicemia, central nervous system infections	*Serratia* spp.:*marcescens, odorifera, rubidaea* subgroup *liquefaciens*	Surface water, groundwater, soil, decaying vegetation, insects, decaying meat, sour milk	Hospital sump water, medical solutions, dialysis effluents	Fever, urinary discomfort; vomiting, headache	Not clearly defined; 1-7 days (dose related septicemia	Maintain adequate disinfectant residuals and reduce sediment accumulation in pipes and storage reservoirs
Skin infections, bacteremia, urinary tract infections, nosocomial infections	*Staphylococcus* spp.: *epidermis saprophyticus*	Warm-blooded animal surfaces, wastewater, stormwater	Contaminated bathing water in contact with open skin lesions, eyes, and ears	Sudden vomiting (poisoning); inflammation, boils (dermal)	1-6 hours (poisoning); 5-10 days (dermal)	Distribution system maintenance in the manner required for minimizing conditions favorable to any of the opportunistic pathogens
Gastroenteritis	*Plesiomonas* sp.: *shigelloides*	Domestic animals, fish, amphibians	Contaminated drinking and recreational water	Fever, chills, diarrhea, nausea, vomiting	1 day	Effective water disinfectant residual; proper food handling

Source: Information presented in Table 1.5 was retrieved from chapters 5, 6, 11–16, 18, 20 in ref. 30; ref. 28; R. Webber, Communicable Disease Epidemiology and *Control*, CAB International, Wallingford, UK, 1996. and Internet sources.

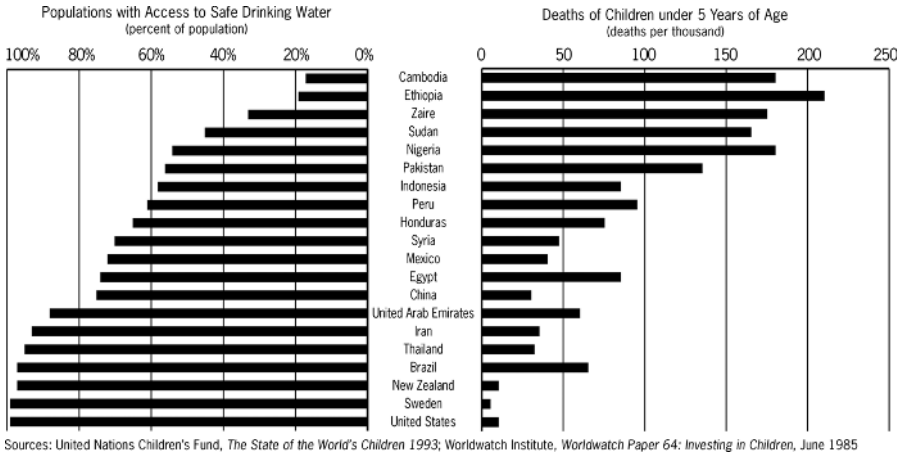


FIGURE 1.2 Relationship between availability of safe drinking water and mortality of children below age five. (*Source*: T. E. Ford and R. R. Colwell, A Global Decline in Microbiological Safety of Water: A Call for Action, American Academy of Microbiology, 1996. Washington, DC.)

The following groups of enteric viruses have been implicated or suspected to be transmitted by contaminated water: enteroviruses (including polioviruses and four subsets of enterovirus [A, B, C, D]), coxsackievirus A viruses, parechoviruses [1-3], hepatitis A (HAV) virus, hepatitis E (HEV) virus, caliciviruses (Noroviruses and Sapoviruses), rotaviruses, adenoviruses, and astroviruses.

Algae are chlorophyllous microorganisms ranging from microscopic unicellular to "seaweed"-size multicellular forms. Their oxygenic capability in performing the light reaction in photosynthesis is the major source of atmospheric oxygen. Various types of algae serve as sources of food and pharmaceutical agents. Although pathogenic algae are relatively rare, certain of the marine dinoflagellates (e.g., *Gonyaulax* spp.) are producers of saxitoxin and gonyautoxin, two of the most virulent nonprotein neurotoxins of record. *Gambierdiscus toxicus* is a tropical marine, benthic dinoflagellate, that synthesizes ciguatoxin, a polycyclic ether compound that creates imbalance in sodium concentration in the axons and nerve terminals causing influx of water and swelling. Ciguatera is a foodborne illness in humans caused by eating marine species that have accumulated cells of G. toxicus by ingestion.[35]

Protozoa are aerobic or anaerobic protists having a true nucleus (eukaryotic). They reproduce usually by fission. They are classically described as simple, unicellular microorganisms, some of which feed on particulate organic matter, including bacteria, and others that utilize soluble organic matter. Motility may be by protoplasmic streaming (amoeba), flagellation, or the synchronize thrashing of cilia. Free-living forms may utilize soluble nutrients or ingest particulate matter (e.g., bacteria). Several pathogenic forms exist such as *Giardia* sp. and *Cryptosporidium* sp. species, that are responsible for waterborne, communicable diseases. Protozoa range in size from approximately 5 to 100 μm in size. Giardia cysts are 8 to 18 μm in length and 5 to 12 μm in width and Cryptosporidium 3 to 5 μm in size.

Fungi are principally aerobic, achlorophyllous microorganisms represented by single and multicellular forms. Most notable of the multicellular fungi are the filamentous varieties known as molds. Filaments (hyphae) are typically on the order of 5 to 10 μm in diameter and many millimeters in length. Molds are important as degraders of complex animal and vegetative matter in nature but become a nuisance in food spoilage and as producers of allergens via sporulation. Many fungi cause diseases in both plants and animals. Certain of the higher fungi, notably the edible mushrooms, are important foodstuffs, as are the yeasts used in bread making and the brewing of alcoholic beverages. Some of the most valuable antibiotics used for medical therapy are synthesized by fungi.

Helminths include intestinal worms and wormlike parasites: the roundworms (nematodes), tapeworms (cestodes), and flatworms or flukes (trematodes). The eggs are about 40 μm or larger in size.

Poisonous plants contain toxic substances that may cause illness or even death when consumed by humans or other animals. Poisonous animals include fish whose flesh is poisonous when eaten in a fresh and sometimes cooked state. (Poisonous flesh is not to be confused with decomposed food.) Acute toxins,

such as paralytic shellfish neurotoxins, pose the threat of severe illness or, in rare occasions, death when consumed along with shellfish meats by humans, especially children and the immunocompromised, and by other animals. As already noted, the toxic substance (e.g., saxitoxin) present in some poisonous shellfish flesh results from the filtration of toxigenic marine dinoflagellates, Gonyaulax spp., and appears to be heat stable. Inorganic chemical elements of greatest concern as a seafood hazard appear to be cadmium, lead, and mercury. The long-term effects are nephropathy, anemia and central nervous system disorders, and retardation; the latter two effects associated with lead and mercury are especially dangerous to the human fetus and neonatal stages.[36] Organic contaminants of fish flesh of particular concern are polychlorinated biphenyls, doxins, chlorinated insecticides, and furans as pertains to their potential as carcinogens and teratogens.

Illnesses associated with the consumption of poisonous plants and animals, chemical poisons, and poisonous fungi are not strictly communicable diseases but more properly noninfectious or noncommunicable diseases.

Vehicle or Means by Which Waterborne Diseases Are Spread

The means by which waterborne disease agents are transmitted to individuals include drinking, bathing in swimming pools and recreational waters, showering (mists), natural aerosols, contaminated hand towels and wash cloths, contaminated water (fish and shellfish), produce irrigated or washed with contaminated water, contact with water containing invasive parasites, and bites of insects that spend at least a part of the life cycle in water. The lack of potable water for bathing, household cleanliness, and food preparation also contributes to poor personal hygiene and sanitation and to the spread of disease. In addition, contagious diseases of individuals, originally produced by contact with contaminated water, may then be passed to another person. The discussions that follow will cover the role of water as a source of disease-producing organisms and poisonous substances.

The reporting of waterborne illnesses has, with rare exceptions, been very incomplete. Various estimates have been made in the past, indicating that the number reported represented only 10 to 20 percent of the actual number.

Hauschild and Bryan,[37] in an attempt to establish a better basis for estimating the number of people affected, compared the number of cases initially reported with either the number of cases identified by thorough epidemiologic investigations or the number estimated. They found that for 51 outbreaks of bacterial, viral, and parasitic disease (excluding milk), the median ratio of estimated cases to cases initially reported to the local health authority, or cases known at the time an investigating team arrived on the scene, was 25 to 1. On this basis and other data, the annual food- and waterborne disease cases for 1974 to 1975 were estimated to be 1,400,000 to 3,400,000 in the United States and 150,000 to 300,000 in Canada. The annual estimate for the United States for 1967 to 1976 was 1,100,000 to 2,600,000.[37] The authors acknowledge that the method used to arrive at the estimates is open to criticism. However, it is believed that the

estimates come closer to reality than the present CDC reporting would indicate, particularly to the nonprofessional. The estimates would also serve as a truer basis for justifying regulatory and industry program expenditures for waterborne illness prevention, including research and quality control.

Historical Waterborne Disease Background

Prior to the mid-1800s, understanding the connection between routes of disease transmission and the causes of illness was greatly hampered by the ignorance of mankind concerning the existence and role of pathogenic agents. Two centuries separated the seminal discoveries of the basic biological cell, including the existence of microbial beings, and the demonstration that certain microorganisms were at the root of disease formation and decay. Prior to the formative years of the field of microbiology, civilization regarded the onset of infections as the curse of some undefined phenomenon of fouled air (miasma), and treatments of the sick were largely relegated to the practice of quarantine or administering of harsh chemical potions. Pollution of water sources was rampant. Some chose to intuitively avoid contact with such waters, not because of any knowledge of the presence of disease-producing agents, but because of the intolerable offensive odors. Indeed, such philosophy was espounded by Dr. John Sutherland, a Scottish physician, when asked in 1854 to comment on the origins of the London Asiatic cholera epidemic of 1853 to 1854: "There is no sufficient proof that water in this state [of impurity] acts specifically in generating cholera" [but] "use of water containing organic matter in a state of decomposition is one predisposing cause of cholera.[38]

Diseases such as cholera, typhoid, typhus, and dysentery were common in the United States, Europe, and other parts of the world prior to the 20th century. Three classical waterborne disease outbreaks are summarized next.

Asiatic cholera produced two epidemics in London in the years 1849 and 1853, both of which were investigated by John Snow, a physician in the twilight of his life, who came to believe that the feces of cholera patients were the source of the disease.[28] It was the Italian physician, Filippo Pacini of Florence,[39] however, who actually observed the cholera vibrio in the intestinal tissue specimens of a deceased victim with the aid of a microscope and deduced the relationship between the bacteria and the disease. Snow noted that the Broad Street well in the SoHo district of London—specifically, St. James Parish, Westminster—served an area where 616 people had died during a 15-week period, and the death rate for St. James Parish was 220 per 10,000, compared to 9 and 33 per 10,000 in adjoining subdistricts.

Snow found that a brewery on Broad Street employing 70 workmen had no deaths. The brewery had its own well, and all the workers had a daily allotment of malt liquor. It can be reasonably assumed that these workers did not drink any water. In contrast, at a factory at 38 Broad Street, where only water from the Broad Street well was available, 18 of 200 workers died (900 per 10,000). But in a nearby workhouse, which had its own water supply in addition to the city supply, there were only 5 deaths among 535 inmates.

Snow's investigation included a follow-up on each death. He spotted the location of each on a map with relation to the Broad Street well and inquired of the work and activities of each person, their habits and customs, and source of drinking water. The one common factor was consumption of water from the Broad Street well. With this information in hand, he convinced the Board of Guardians of St. James Parish to have the handle of the pump removed, and the epidemic was brought under control.

A survey was made to determine the cause and source of the epidemic. The house at 40 Broad Street nearest the well was suspected as the source; there had been four fatal cases of cholera at the house. A privy emptying into a cesspool, which served more like a tank, overflowed to a drain passing close to the well.

On further investigation, including excavations, it was found that the Broad Street well was a brick-lined dug well with a domed brick top 3 feet, 6 inches below the street. The well was 28 feet, 10 inches deep and 6 feet in diameter, and contained 7 feet, 6 inches of water. The house drain, 12 inches wide with brick sides 12 inches high and stone slab top and bottom, passed within 2 feet, 8 inches of the brick lining of the well. The drain, on a very flat grade, was 9 feet, 2 inches above the water level in the well and led to a sewer. The mortar joints of the well lining and the drain were completely disintegrated. It was found on inspection after excavation that the drain was like a "sieve and through which house drainage water must have percolated for a considerable period" into the well, as indicated by black deposits and washout of fine sand. The drain received wastewater from 40 Broad Street in addition to the overflow from a cesspool in the basement, over which there was a privy.[40]

In another study in 1854, Snow found that a low incidence (37 per 10,000 residences) of cholera fatalities occurred in one part of London supplied by the Lamberth Company with water from the River Lea, a tributary of the River Thames, with an intake more than 38 miles upstream from London. People supplied by the Southwark & Vauxhall Company received water taken from the heavily wastewater-polluted Thames River, opposite the location of Parliament, with a very high incidence of cholera and a death rate of 315 per 10,000 residences. Snow compared the income, living conditions, work, and other characteristics of the people in the two areas and found that source of water was the main variable and, hence, the cause of the illness. The study involved approximately 300,000 people and laid the basis for future epidemiologic studies.

Today, John Snow is considered the epidemiological giant of his time. However, his views on the transmission of cholera did not go unchallenged during his active investigations. William Farr, a professional epidemiologist, was lukewarm to Snow's findings of 1849 and, although he accepted that an association existed between cholera illness and the south district water supply of London, clung to the view that the cholera epidemic of 1849 was responsible to "spread by atmospheric vapours" and the consequences of the lower elevation of water pipes in the soil carrying water from the lower Thames as opposed to that of the upstream region.[41] Farr also contended that the cholera agent was heavier than water and,

therefore, would be expected to be of higher concentration in pipes of lower elevation than those of higher elevation. Interestingly, in 1866 a cholera epidemic occurred in the Whitechapel area of London that was traced to water supplied by the East London Water Company whose source was the River Lea. William Farr pronounced, "Only a very robust scientific witness would have dared to drink a glass of the waters of the [river] Lea," on which note Farr's notions that air, not water, was the cause of London's infamous cholera epidemics came to an end.[42] Snow was immersed in the study of anesthesiaology in his final days and died from complications of a stroke at the age of 45; quite possibly brought on by his self-committed experimentation with chloroform, ether, and other noxious agents in the quest for useful anesthetics. Epidemics of cholera persisted in London after Snow's death. The poor water quality of the Thames is evident from the account of a large pleasure craft that capsized on a Sunday afternoon in the mid 1800s with its passengers thrown into the river; no one drowned, but most died of cholera within a few weeks, thereafter.[43]

In still another instance, Robert Koch (1843–1910), an eminent German physician, unaware of Pacini's earlier discovery, observed the cholera bacillus under similar pathological conditions in Alexandria, Egypt, in 1883.[44] In 1884, Koch succeeded in isolating and culturing the organism from the stools of advanced cholera patients in Calcutta, India. Closer to home in 1892, Koch investigated the incidence of cholera in two adjacent cities in Germany that pumped drinking water out of the Elbe River. Hamburg pumped water from a point upstream and Altona, a suburb, took water downstream from the city sewer outfalls, but the outbreak occurred in Hamburg upstream. However, the water in Altona was filtered through a slow sand filter, whereas the water in Hamburg was not. Koch isolated *Vibrio cholerae* from the polluted Elbe River, proving the relationship between polluted water and disease. There were 8,605 deaths in Hamburg, a death rate of 1,342 per 100,000. The death rate in Altona was 234 per 100,000.

Water treatment, specifically the application of a disinfectant, notably chlorine, has practically eliminated cholera, typhoid, and dysentery in developed areas of the world. The conquest of these and other waterborne diseases parallels the development of microbiology and sanitary engineering, as well as immunization; water treatment, including chlorination, proper excreta, and wastewater disposal; and education in hygiene and public health. However, waterborne diseases still occur with viral gastroenteritis (nonspecific gastroenteritis being more common), infectious hepatitis A, giardiasis, and cryptosporidiosis. As noted elsewhere, absence of potable water and latrines is associated with high diarrheal illness and mortality rates among children under five in developing countries. The major concerns in developed countries today are the chronic and degenerative diseases, including those associated with the ingestion of trace amounts of toxic organic and inorganic chemicals, but it is also essential that the safeguards found effective in preventing waterborne diseases be maintained and strengthened to prevent their recurrence.

Waterborne Disease Outbreaks Given the vulnerability of surface waters to pollution, it may be surprising to learn that in every decade since 1920,

contaminated groundwaters in the United States have been responsible for more waterborne outbreaks than contaminated surface waters, and that during the period 1971 to 2000, waterborne outbreaks have declined in untreated ground waters, whereas disinfected groundwaters have accounted for 38 percent of the groundwater-related waterborne outbreaks during that time frame.[16] Most recently, however, a waterborne outbreak suspected to involve a *Salmonella* sp. was believed to be linked to the undisinfected, deep-well, groundwater system serving Alamosa County, Colorado, in the United States. On March 19, 2008, at least 33 confirmed cases of salmonella infections were recorded, and the Colorado Department of Health issued a "bottled water" advisory. The source of the contamination was unknown, but a cross-connection with a wastewater line or a violated storage water tank was suspected. The following day, the number of confirmed and suspected salmonella cases rose to 79. Two days later, 139 people were reported ill from salmonella infections, and the city declared a state of emergency. By Sunday, March 28, the suspected case load had reached 276, with 10 people hospitalized. Laboratory-confirmed-cases numbered 72 and a candidate pathogen, *Salmonella enterica* serotype Typhimurium, was isolated from the stools of confirmed victims.[45] The "boil order" was lifted on April 11, 2008 and Alamosa likely will be required to comply with U.S. EPA Groundwater Disinfection Rule as published in the Federal Register on November 8, 2006 concerning disinfection of groundwater public drinking water supplies. It was reported on April 20, 2008 that 411 salmonella cases, of which 112 were confirmed and 18 hospitalized, included one death not proven responsible to infection by salmonella.[46]

Waterborne outbreaks occur more frequently in noncommunity water systems than in community water systems; however, the number of cases associated with community water systems is usually larger than in noncommunity water systems. In the period 1991 to 2000, the annual average of waterborne outbreaks in noncommunity water systems was approximately eight compared to six outbreaks for community water systems. The median number of illness cases associated with the noncommunity and community outbreaks was 112 and 498, respectively.[16] Although waterborne diseases account for only a very small percentage of all human illness in the United States and other industrialized countries, this advantage can only be maintained by the continued reduction in biological and chemical pollution of our surface and groundwaters and by complete and competent treatment of drinking water. A case in point is the cryptosporidiosis outbreak that occurred in Milwaukee, Wisconsin, in 1993, resulting in an estimated 403,000 cases of watery diarrhea.[47] Although in excess of 100 deaths have been stated in various media sources, 54 deaths were *officially* reported in the 4-year post-outbreak period, of which 85 percent involved AIDS patients;[48] testimony to the ravishing effect of infectious diseases on immunocompromised individuals. The magnitude of the Milwaukee incident is such that it represented 93 percent of the total 173 waterborne disease outbreaks during the period 1991 to 2000. The total cost of the Milwaukee outbreak was estimated

to be $96.2 million (1993 U.S. dollars), with about $31.7 million in medical expenses and about $64.6 million in productivity losses.[49]

Between 1946 and 1980, a total of 672 waterborne disease outbreaks were reported, with 150,475 cases. Contaminated untreated groundwater accounted for 35.3 percent of the 672 outbreaks, inadequate or interrupted treatment for 27.2 percent, distribution or network problems for 20.8 percent, contaminated untreated surface water for 8.3 percent, and miscellaneous for 8.3 percent. Forty-four percent of the outbreaks involved noncommunity water systems and accounted for 19 percent of the cases.[50]

Weibel et al.[51] studied the incidence of waterborne disease in the United States from 1946 to 1960. They reported 22 outbreaks (10 percent) with 826 cases due to use of untreated surface waters; 95 outbreaks (42 percent) with 8,811 cases due to untreated groundwaters; 3 outbreaks (1 percent) with 189 cases due to contamination of reservoirs or cisterns; 35 outbreaks (15 percent) with 10,770 cases due to inadequate control of treatment; 38 outbreaks (17 percent) with 3,344 cases due to contamination of distribution system; 7 outbreaks (3 percent) with 1,194 cases due to contamination of collection or conduit system; and 28 outbreaks (12 percent) with 850 cases due to miscellaneous causes, representing a total of 228 outbreaks with 25,984 cases.

Weibel et al.[51] reported the greatest number of outbreaks and cases in communities of 10,000 population or less. Wolman and Gorman stated that the greatest number of waterborne diseases occurred among population groups of 1,000 and under and among groups from 1,000 to 5,000—that is, predominantly in the rural communities.[52] Between 1971 and 1978, 58 percent of the outbreaks occurred at small, noncommunity water systems. The need for emphasis on water supply control and sewage treatment at small existing and new communities, as well as at institutions, resorts, and rural places, is apparent and was again confirmed in the 1970 PHS study,[53] a 1978 summary,[54] and others.[50] From 1971 to 1982, a total of 399 waterborne outbreaks with 86,050 cases of illness were reported to the U.S. Public Health Service. Forty percent of the outbreaks occurred at community water systems, 48 percent at noncommunity systems, and 12 percent at individual systems. Thirty-one percent involved groundwater systems serving motels, hotels, camps, parks, resorts, restaurants, country clubs, schools, day care centers, churches, factories, offices, and stores. Thirty-one percent of the total waterborne outbreaks were caused by use of contaminated untreated groundwater (wells and springs); 20 percent by inadequate or interrupted disinfection of groundwater (wells and springs); 16 percent by distribution system deficiencies (cross-connection, storage facilities, and contamination of mains and through household plumbing); 14 percent by inadequate or interrupted disinfection of surface water; 8 percent by use of contaminated untreated surface water; 4 percent by inadequate filtration, pretreatment, or chemical feed; and 7 percent by miscellaneous deficiencies.[55] In another analysis of 484 waterborne outbreaks with 110,359 cases between 1971 and 1985, the agent was bacterial in 59, parasitic in 90, viral in 40, chemical in 51, and acute gastrointestinal in 244. Community systems, noncommunity systems, and individual systems experienced 209, 217,

and 58 outbreaks, respectively. Untreated groundwater and treatment deficiencies were the major causes.[56]

Drinking water contaminated with sewage is the principal cause of waterborne diseases. The diseases that usually come to mind in this connection are bacterial and viral gastroenteritis, giardiasis, hepatitis A, shigellosis, and typhoid and paratyphoid fevers. However, nearly one-half of outbreaks involving drinking water in the United States between the years 1971 to 2002 were described as gastroenteritis of unknown origin.[18] Protozoa, bacteria, and viruses were the causative agents in 19, 14, and 8 percent of outbreaks, respectively, and chemicals were responsible for 12 percent percent. A breakdown of the various diseases of drinking water for eight decades in the United States can be found in Table 1.6.[16]

Modern day globalization presents a concern for the monitoring and control of infectious diseases. Human transport and interaction on an international scale along with transport of animals and food items enhances the threat of disease transmission. The United States must be vigilant in recognizing the risk for its citizens in contracting infectious diseases or becoming carriers as a result of travel to countries having lower standards of environmental health. [57]

Because of the supervision given public water supplies and control over a lessening number of typhoid carriers, the incidence of typhoid fever has been reduced to a low residual level. Occasional outbreaks, due mostly to carriers, remind us that the disease is still a potential threat. During the period 1967–1972, *Salmonella typhi* was isolated from 3661 individuals in the United States and, coincidentally, the number of travel-associated cases of typhoid fever rose yearly by 270%; a phenomenon believed connected in some way to Mexico.[57] Although the incidence of typhoid fever cases has decreased from approximately 1.9 per million to 1.3 per million travelers to Mexico between 1985 and 1994, of all states reporting cases of typhoid fever to the Typhoid Fever Surveillance System for the period between 1985 and 1994, California and Texas ranked one and two, respectively, with California accounting for 44% of the 2443 cases recorded.[58] United States residents with Hispanic names were found to be at higher risk of contracting typhoid fever than were others in the population.[57] In effect, globalization is likely to influence the level of endemic infectious diseases in the United States and, as noted by Mermin et al[58], will be interconnected to the incidence of infectious diseases in other countries of the world, thus underscoring the importance of achieving high standards of environmental hygiene worldwide.

The outbreaks reported below are also instructive. In 1940 some 35,000 cases of gastroenteritis and 6 cases of typhoid fever resulted when about 5 million gallons of untreated, grossly polluted Genesee River water were accidentally pumped into the Rochester, New York, public water supply distribution system. A valved cross-connection between the public water supply and the polluted Genesee River firefighting supply had been unintentionally opened. In order to maintain the proper high pressure in the fire supply, the fire pumps were placed in operation and hence river water entered the potable public water supply system. The check valve was also inoperative.

TABLE 1.6 Causes of Drinking Water Outbreaks in the United States, 1920-2000. Calderon, and M. F. Craun. 2006

Organism	Survival Time[a]	
	In Surface Water	In Groundwater
Coliform bacteria	—	7–8 days[b]
Cryptosporidium spp. oocyst	18 + months at 4°C	2–6 months, moist[c]
Escherichia coli	—	10–45 days[b]
Entamoeba histolytica	1 month[d]	
Enteroviruses	63–91 + days[e]	
Giardia lamblia cyst	1–2 months, up to 4[f]	
Leptospira interrogans	3–9 days[g]	
Pasteurella tularensis	1–6 months[g]	
Rotaviruses and reoviruses	30 days–1 + years[e]	
Salmonella faecalis	—	15–50 days[b]
Salmonella paratyphi	—	60–70 days[b]
Salmonella typhi	1 day–2 months[g]	8–23 days[b]
Salmonella typhimurium	—	140–275 days[b]
Shigella	1–24 months[g]	10–35 days[b]
Vibrio cholerae	5–16 days[g] 34 days at 4°C[g] 21 + days frozen[g] 21 days in seawater[d]	
Viruses (polio, hepatitis, entero)	—	16–140 days[b]
Enteroviruses[h]	38 days in extended aeration sludges at 5°C, pH 6–8; 17 days in oxidation ditch sludges at 5°C, pH 6–8	
Hepatitis *A*[i]	1 + years at 4°C in mineral water, 300 + days at room temperature	
Poliovirus[i]	1 + years at 4°C in mineral water, not detected at room temperature	

[a] Approximate. See also refs. [27–30].

[b] *Guidelines for Delineation of Wellhead Protection Areas*, Office of Ground-Water Protection, U.S. Environmental Protection Agency, Washington, DC, June 22, 1987, pp. 2–18. *Source:* Matthess et al., 1985.

[c] A. S. Benenson (Ed.), *Control of Communicable Diseases in Man*, 15th ed., American Public Health Association, Washington, DC, 1990, p. 113.

[d] B. K. Boutin, J. G. Bradshaw, and W. H. Stroup, "Heat Processing of Oysters Naturally Contaminated with *Vibrio cholerae*, Serotype 01," *J. Food Protection*, **45**(2), 169–171 (February 1982).

[e] G. Joyce and H. H. Weiser, *J. Am. Water Works Assoc*., April 1967, pp. 491–501 (at 26°C and 8°C).

[f] S. D. Lin, "*Giardia lamblia* and Water Supply," *J. Am Water Works Assoc*., February 1985, pp. 40–47.

[g] A. P. Miller, *Water and Man's Health*, U. S. Administration for International Development, Washington, DC, 1961, reprinted 1967.

[h] G. Berg et al., "Low-Temperature Stability of Viruses in Sludges," *Appl. Environ. Microbiol*., **54**, 839 (1988); *J. Water Pollut. Control Fed*., June 1989, p. 1104.

[i] E. Biziagos et al., "Long-Term Survival of Hepatitis A Virus and Poliovirus Type 1 in Mineral Water," *Appl. Environ. Microbiol*., **54**, 2705 (1988); *J. Water Pollut. Control Fed*., June 1989, p. 1104.

At Manteno State Hospital in Illinois, 453 cases of typhoid fever were reported, resulting in 60 deaths in 1939.[59] It was demonstrated by dye and salt tests that sewage from the leaking vitrified clay tile hospital sewer line passing within a few feet of the drilled well-water supply seeped into the well. The hospital water supply consisted of four wells drilled in creviced limestone. The state sanitary engineer had previously called the hospital administrator's attention to the dangerously close location of the well to the sewer and made several very strong recommendations over a period of eight years, but his warning went unheeded until after the outbreak. Indictment was brought against three officials, but only the director of the Department of Public Welfare was brought to trial. Although the county court found the director guilty of omission of duty, the Illinois Supreme Court later reversed the decision.

An explosive epidemic of infectious hepatitis in Delhi, India, started during the first week of December 1955 and lasted about six weeks. About 29,300 cases of jaundice had developed in a total population of 1,700,000 people. (The authorities estimated the total number of infections at 1,000,000.) No undue incidence of typhoid or dysentery occurred. Water was treated in a conventional rapid sand filtration plant; however, raw water may have contained as much as 50 percent sewage. Inadequate chlorination (combined chlorine), apathetic operation control, and poor administration apparently contributed to the cause of the outbreak, although the treated water was reported to be well clarified and bacteriologically satisfactory.[60]

Waterborne salmonellosis in the United States is usually confined to small water systems and private wells.[61] However, an outbreak of gastroenteritis in Riverside, California, in 1965 affected an estimated 18,000 persons in a population of 130,000. Epidemiologic investigation showed that all cases harbored *Salmonella typhimurium*, serological type B and phage type II, which was isolated from the deep-well groundwater supply. There was no evidence of coliform bacteria in the distribution system, although 5 of 75 water samples were found positive for *S. typhimurium*, type B, phage II. The cause was not found in spite of an extensive investigation.[62]

Of potential for causing protozoal infections in humans are the species *Entamoeba histolytica, Giardia lamblia, Cryptosporidium parvum* and *C. hominis, Cyclospora cayetanensis, Enterocytozoon bieneusi, Isopora belli* and *I. hominis*, and *Balantidium coli*.[63] *E. histolytica, G. lamblia, C. parvum* and *C. hominis*, and *C. cayetanensis* have all been implicated in diseases of the water route. The remaining organisms stated above are intestinal parasites so there is potential for their transmission by contaminated water. Nonetheless, present-day concerns center on three genera, namely *Giardia, Cryptosporidium*, and *Cyclospora*. Also of interest are the free-living amoebae, *Naegleria* spp., especially, *N. fowleri*, the etiologic agent of an explosive disease of the central nervous system termed primary *amebic meningoencephalitis (PAM)* and *Acanthamoeba* spp., which are also free-living amoebae and causative agents of *granulomatous amebic encephalitis (GAE)* and *acanthamoeba keratitis* (see Table 1.4).

In 1974 to 1975, a waterborne outbreak of giardiasis occurred in Rome, New York.[64] About 5,357 persons out of a population of 46,000 were affected. The source of water was an upland surface supply receiving only chlorine–ammonia treatment, which confirmed the inadequacy of such treatment to inactivate the Giardia cyst. The coliform history was generally satisfactory. Other early giardiasis outbreaks in the United States occurred in Grand County (1973, 1974, 1976)[65] and near Estes Park (1976)[65], Colorado; Camas, Washington (1976) [66,67]; Portland, Oregon(presumptive, 1954-55)[68]; Unita Mountains, Utah(1974)[69]; Berlin, New Hampshire (1976)[70]; and in areas of California and Pennsylvania.[71] Between 1969 and 1976 a total of 18 outbreaks with 6,198 cases were reported. An additional 5 outbreaks with approximately 1,000 cases were reported in 1977. There were 42 outbreaks reported with 19,728 cases between 1965 and 1980.[72] A total of more than 90 outbreaks occurred through 1984. Acceptable turbidity and coliform tests are important for routine water quality control, but they do not ensure the absence of *Giardia* or enteric viruses; complete water treatment is necessary.

The reporting of outbreaks of waterborne giardiasis has become more common in the United States, Canada, and other countries of the world. The source of the *G. lamblia* cyst is humans, and possibly the beaver, muskrat, and other wild and domestic animals, probably infected from our waste. The *Giardia* stool positive rate may range from 1 to 30 percent, depending on age and the indigenous level of personal hygiene and sanitation, with the higher rate in day care centers and institutions.[73] Infected individuals may shed 10^6 cysts per gram of stool for many years. The cyst is resistant to normal chlorination, similar to the cyst of *E. histolytica*. Conventional rapid sand filtration of surface water—including coagulation, flocculation, and sedimentation, slow sand filtration, and diatomaceous earth filtration followed by disinfection—is considered effective in removing the *Giardia* cyst.[74] Prolonged protected sedimentation and a filter press using special cellulose sheets (reverse osmosis) to remove 1-μm-size particles is also reported to be effective.[75] Pressure sand filtration is not reliable and should not be used, as the cyst penetrates the filter. Experimental results show that 2.5 mg/l (free) chlorine for 10 minutes killed all cysts at pH 6 at a water temperature of 60°F (15°C), but 60 minutes was required at pH 7 and 8, and 1.5 mg/l at 77°F (25°C) in 10 minutes at pH 6, 7, and 8; at 42°F (5°C), 2 mg/l killed or inactivated all cysts in 10 minutes at pH 7 and in 30 minutes at pH 8. [76] A total chlorine residual of 6.2 mg/l after 30 minutes at pH 7.9 and 37°F (3°C) also inactivated *G. lamblia*. A temperature of 131°F (55°C) will destroy the cyst, but boiling is advised.

Cryptosporidium parvum (Type 1) and *C. hominis*, are both infectious apicoplexan protozoan parasites of humans. The first human cases of the disease were reported in 1976. [77] Infection occurs by the ingestion of oocysts that have been excreted in the feces and the disease, cryptosporidiosis, is usually spread by the fecal-oral route, but has also been implicated as the cause of food- and waterborne illness.[78] The incubation period is in the range of 2 to 14 days.[78] It is still often overlooked or not identified, contributing to the problem of underreporting of the disease. However, new molecular and clinical diagnostic tests are in use. The organism is found in the fecal discharges of humans and many wild and

domestic animals, including cattle, deer, muskrats, raccoons, foxes, squirrels, turkeys, pigs, goats, lambs, cats, and dogs and zoonotic transmission to humans has been documented. The oocyst, 3 to 6 μm in diameter, survives 18 months or longer at 39°F (4°C), however, inactivation can be exacted at 45°C (20 minutes), 64.2°C (5 minutes), 72.4°C (1 minute), and −20°C (3 days).

Conventional rapid sand filtration, including coagulation, should remove 90 to 100 percent of the *Cryptosporidium*. The oocysts may be inactivated in the presence of a free chlorine residual of 2 mg/l (two days) at 20°C; 2 mg/l (one day) at 30°C, and 10 mg/l (less than six hours) at either temperature under chlorine-demand free conditions.[79] Circumstances contributing to the resistance of oocysts to chlorine in real-world conditions include presence of chlorine-consuming organic matter, protection of oocysts by clumping, and protection of oocysts by adsorption to particulate matter. Other chemicals, such as hydrogen peroxide (6 to 7.5 percent) and ammonia (5 percent), can be effective. Ultraviolet irradiation presents the interesting effect of being able to curtail infective capability in oocysts irradiated at low dosage (99 percent at 1 mWs/cm^2 at 20°C), however, prevention of excystation required 230 mWs/cm^2 at 20°C.[80] Cyclosporiasis is a diarrheal disease with symptoms closely resembling cryptosporidiosis, including watery diarrhea without blood, which may last for an extended period of up to 40 days. Other symptoms are anorexia, nausea, vomiting, pronounced flatulence, stomach cramps, and abdominal bloating. The incubation period is similar to that of cryptosporidiosis. The causative agent is *Cyclospora cayetanensis*—an intestinal parasite with many of the characteristics of *Cryptosporidium* spp. and viewed as an emerging, opportunistic waterborne pathogen.

In this vein, increased numbers of immunocompromised people in the population since the AIDS epidemic appears to be a root to the upwelling of disease incidence by organisms such as *Cyclospora* sp. and the collection of intracellular parasites making up the Microsporididea.[81] The oocysts of *C. cayetanensis* are larger (8–10 μm in diameter and approximately the size of *Giardia* spp. cysts) than those of *Cryptosporidium* spp. However, this feature has not deterred much past misdiagnosis of diseases caused by the misinterpretation of *Cyclospora* sp. for *Cryptosporidium* spp. One important difference between the cycle of cryptosporidiosis and cyclosporiasis is that the latter is not transmitted person to person, owing to the need for oocysts of *Cyclospora* sp. to spend an extended amount of time outside the human host in order to sporulate; a condition essential for the oocysts to become infectious upon transfer to another human. Detection of *Cyclospora* sp. oocysts, which autofluoresce a bright blue by epifluorescence microscopy, involves laboratory techniques similar to those described for *Cryptosporidium* spp.[82] Inactivation of the oocysts of *Cyclospora* sp. is difficult. Organisms die quickly at −70°C; at −20° and −15°C, survival is one day and two days, respectively.

Information on the effect of chemical disinfectants on the oocysts of *Cyclospora* sp. is little known. On the one hand, there is the general belief that oxidants such as chlorine are ineffective, at least at the concentrations employed

in water and wastewater treatment. On the other hand, disinfection combined with secondary wastewater treatment may be sufficient to remove *Cyclospora* sp.[83] At present, there is the tendency to infer that inactivation steps effective for containment of *Cryptosporidium* spp. ought to prevail with *Cyclospora* sp. Incidence of cyclosporiasis in the United States up to the present is rare, and, when suspected, is often without the presence of the tell-tale oocysts.

Legionnaires' disease is caused by *Legionella pneumophila*. Another form is Pontiac fever, which typically has a shorter incubation period and results in mild, influenzalike symptoms. The organism has been readily isolated from surface waters and adjacent soils. Other sources are cooling towers and evaporative condensers, hospital hot-water systems, whirlpools, showerheads, domestic hot-water tanks, hot- and cold-water distribution systems, humidifiers, and open water-storage tanks. The organism is primarily spread by aerosols and, to a much lesser extent, water ingestion. It is a major problem in hospitals. Person-to-person spread has not been documented.[84] A water temperature of 68° to 114°F (20° to 45°C) or 104° to 122°F (40°to 50°C)[85] appears to be most favorable for organism survival. The critical temperature is believed to be 97°F (36°C). The organism has been found in hot-water tanks maintained at 86° to 129°F (30° to 54°C) but not at 160° to 172°F (71° to 77°C).[86] The FDA recommends a minimum temperature of 166°F (75°C).

Suggested *Legionella* control measures include 1 to 2 ppm free residual chlorine at water outlets, including daily testing; maintenance of continuous chlorination and hot water temperature; annual cleaning and disinfection of the cold-water system.[87,88] Consensus data suggests that 140°F (60°C) is the minimal temperature for thermal disinfection of hot water plumbing systems and that this temperature should be used in flushing outlets, faucets, and shower heads for a period in excess of 30 minutes and maintained to prevent reestablishment of L. *pneumophila*.[89] It should be noted that scalding is a potential hazard at the recommended thermal inactivation temperature. It has been suggested that 4 to 6 mg/l residual chlorine, maintained in the facility for 6 hours, is sufficient for disinfection, however, this level of disinfectant is difficult to maintain in hot water and may cause problems with patients having transplant surgery.[90] In view of the different findings, laboratory monitoring of the water in the distribution system for *L. pneumophila* is also suggested.

Control and Prevention of Waterborne Diseases

Many health departments, particularly on a local level, are placing greater emphasis on water quality and food protection at food-processing establishments, catering places, schools, restaurants, institutions, and the home and on the training of food management and staff personnel. An educated and observant public, a systematic inspection program with established management responsibility, coupled with a selective water- and food-quality laboratory surveillance system and program evaluation, can help greatly in making health department food protection programs more effective. It is necessary to remain continually alert because

waterborne diseases have not been eliminated and other diseases, previously considered not typically transmissible or thought to be transmissible by the water route, are being discovered.

In the general sense, Lashley[91] outlines preventive measures to be taken to control waterborne disease, including the safeguard of drinking water, recreational water, and more stringent actions for the protection of immunocompromised persons. Immunocompromised individuals should not rely on tap waters without additional home treatment such as boiling for one minute or treatment with certain filters. The CDC AIDS Hotline (1-800-342-2437) is available for additional information on this subject. Immunocompromised persons should be especially careful about exposure to fecal matter, young animals—which are more apt to be carriers of infectious disease organisms that are especially difficult (e.g., *Cryptosporidium* and *Cyclospora* agents)—and travel to countries with low-grade sanitation.

Prevention of Waterborne Diseases

A primary requisite for the prevention of waterborne disease at the community level is the ready availability of an adequate supply of water that is of satisfactory sanitary quality for meeting microbiological, chemical, physical, and radiological standards. The prevailing scheme in the water treatment industry for the establishment of a reliable water purification system is the multiple-barrier concept.[92] The multiple-barrier plan for the treatment of water is, in effect, a fail-safe program for ensuring the safety of the consumer of finished water, should a step in the overall process fail. The barriers thus proposed are (1) source water protection, (2) water treatment plant processes, (3) disinfection practices, (4) distribution systems, (5) security, and (6) education. Protection of source water deals with the selection and developing of the raw water supply and safeguarding the watershed from infiltration of pollution. Water treatment plant processes entails the appropriate and proper unit operations and the necessary measures to maintain plant functions. Disinfection practices assume the maintenance of an adequate disinfectant residual throughout the distribution system for destruction of pathogenic agents arising from the untreated source water and faults within the distribution system. The distribution system includes inspection and remediation of piping and inline storage facilities. Security involves the physical watch on the treatment system against the possibility of unlawful entry, with the intent to disrupt or compromise treatment operations and goal of producing quality water. Education embraces the training of water treatment personnel and informing public officials and the public at large of any emergency measures required, owing to interruptions in operations that may affect water quality and quantity. Publicly owned water companies are preferred because they usually provide water of satisfactory quality and quantity and are under competent supervision. It is important that the finished water be convenient, attractive, and palatable to inspire public confidence in the product and dissuade alternate choices of expensive bottled waters or the selection of some other source water, such as a nearby well or spring of

doubtful quality. Although excellent water service, especially in municipalities, is generally available in the United States and in many developed areas of the world, consumers and public officials must not have tended to become complacent. Many of the older water treatment facilities have distribution systems in dire need of replacement. The American Society of Civil Engineers in 2001 acknowledged the need for replacement of aged facilities in 54,000 water treatment plants in the United States at a cost of $11 billion, not including the additional cost to meet new drinking water standards.[93] Compounding the problem is the shrinking availability of revenues within the tax structure of communities such that, in some instances, funds may have to be diverted from maintenance, operation, and upgrading of the water supply system in order to cover other expenses. It is also sometimes forgotten that in developing areas of the world, a convenient, safe, and adequate water supply, in addition to affording protection against waterborne diseases, makes possible good personal hygiene, including hand washing, sanitation, household cleanliness, and clean food preparation. In addition, it obviates the need to wade in schistosome-snail-infested streams to undertake the laborious and time-consuming task of transporting water (see the section "Schistosomiasis," later in this chapter). An interesting sidelight is the controversy that emerged over the construction of the Aswan High Dam in the early 1960s. A large impoundment was formed on the Nile River to serve both as a water supply and flood control. It had been argued that the dam lowered the downstream level of the Nile River and, combined with large-scale irrigation, brought increased incidence of schistosomiasis. This may not be the case. With the improved level of sanitation, clean water, and medical facilities, schistosomiasis has actually been reduced from over 40 percent in predam years to 10.7 percent in 1991.[94]

Adequate drinking water statutes and regulations and surveillance of public water supply systems are necessary for their regulatory control. This is usually a state responsibility, which may be shared with local health or environmental regulatory agencies. The EPA recommendations for a minimum state program include [95]:

1. A drinking-water statute should define the scope of state authority and responsibility with specific statutory regulations and compliance requirements. Regulations should be adopted for drinking-water quality standards; water-supply facility design and construction criteria; submission, review, and approval of preliminary engineering studies and detailed plans and specifications; approval of a water-supply source and treatment requirements; establishment of a well construction and pump installation code; operator certification; provision for state laboratory services; and cross-connection and plumbing control regulations.
2. The surveillance of public water-supply systems should involve water quality sampling—bacteriological, chemical, and radiological, also turbidity and residual chlorine; supervision of operation, maintenance, and use of approved state, utility, and private laboratory services; cross-connection control; and bottled and bulk water safety.

3. Surveillance and disease prevention are recommended with periodic, onsite fact finding as part of a comprehensive sanitary survey of each public water-supply system, from the source to the consumer's tap, made by a qualified person to evaluate the ability of the water supply system to *continuously* produce an adequate supply of water of satisfactory sanitary quality. The qualified person may be a professionally trained public health, sanitary, or environmental engineer, or a sanitarian, to make sanitary surveys of the less complex water systems such as well-water supplies. The EPA suggests that the sanitary survey, as a minimum, cover quality and quantity of the source; protection of the source (including the watershed and wellhead drainage area); adequacy of the treatment facilities; adequacy of operation and operator certification; distribution storage; distribution system pressure; chlorine residual in the distribution system; water quality control tests and records; cross-connection control; and plans to supply water in an emergency. The WHO has similar suggestions.[96]

Details concerning water supply quality and quantity, source protection, design, and treatment are given in Chapters 1 and 2 of the water and wastewater volume *of Environmental Engineering*, *Sixth* Edition (Wiley, 2009).

Schistosomiasis

Schistosomiasis is a largely endemic disease in parts of Africa but also occurs in areas of Asia and South America. If known preventive precautions are not taken, the global prevalence of schistosomiasis, spread by freshwater snails and estimated at 300 million or more cases, is expected to increase as new impoundments and irrigation canal systems are built. Cooperation in the planning through the construction phases in endemic areas, or potentially endemic areas, between the health and water resources agencies can help reverse this trend. Water contact through swimming, wading, laundering, bathing, and collecting infested water and poor sanitation and hygiene are the major causes for the persistence and spread of schistosomiasis. Individuals who have or had schistosomiasis (bilharziasis) are more likely to have a urinary infection. Long-term schistosomiasis control would involve an appropriate combination of chemotherapy; mollusciciding; basic sanitation, including biological intervention and the supply of potable water at the village level; and socioeconomic development.[97] Molluscicciding is impractical where the water is used as a direct source of drinking water or where the water body and its tributaries are inaccessible or beyond control. In such cases, chemotherapy is considered the most cost-effective control *when* coupled with safe drinking water and sanitation facilities to minimize indiscriminate urination and defecation. In any case, education to prevent reinfection is necessary.[98,99] Heating water to 122°F (50°C) for 5 minutes or treating with chlorine or iodine as in drinking water and filtration through tightly woven cloth or paper (coffee) filter will remove the cercaria. Settling water for 3 days is also effective, as cercaria survives only 48 hours, but reinfestation must be prevented.

BIOTERRORISM

Bioterrorism is a disruptive and health-threating event directed at an individual, group of individuals, a community, or at-large population within a nation and is facilitated by the intentional release of a highly virulent biological agent. In this context, the term *biological agent* includes a microorganism or a biologically synthesized toxin that causes disease in man, plants, or animals or causes deteoriation of materials.[100] The use of pathogenic elements to subvert and disrupt the normal life style of innocent people has a long history.[101] As far back as the fourth century, Scythian warriors coated the tips of their arrows with human feces as a means of infecting their enemies. This is testimony to the very early suspicions about the noxious properties of excreta. In 1346, the Mongols used catapults to hurl the corpses of their dead soldiers, riddled with plague, over the walls in Kaffa, currently Theodosia. The practice of spreading infectious disease by exposure to the dead continued in the siege of the Bohemian castle at Karlstein in 1422 and the attack of the Swedes by Russians in 1710, whereupon corpses were catapulted over the city walls of Reval (Tallinn).

The selection of an agent to be used in an act of terrorism should satisfy the following properties: (1) be readily available, (2) be easy to produce on large scale, (3) be highly virulent for lethal or incapacitation purposes, (4) be of appropriate size for distribution by aerosolization and uptake by victims (penetrate defense mechanisms of the upper respiratory tract), (5) be easy to disseminate by available means, (6) be environmentally stable, and (7) be dispersible in a way that targeted individuals, but not the terrorists, suffer intended effects.[102] A list of candidate biological agents and biologically produced toxins for application in bioterroristic attacks is given in Table 1.7. The categories mainly reflect high level of priority for prepardness (category A), need for improved awareness, surveillance measures, and laboratory diagnosis (category B), and need for continued review of potential threat to the public (category C). Many of the typical vehicles and vectors of infectious disease transmission may be deployed in acts of terrorism. Several of the prominent bacterial agents high on the list of potential bioweapons are the cause of zoonotic infections.

An interesting approach has been made to quantitatively evaluate the usefulness of a biological agent as a weapon of bioterror by calculation of the agent's weapon potential (WP):

$$WP = [V_{BW} SC/T] \times XD$$

where: V_{BW} = virulence of a bioweapons derived from F_{SI}/I where F_{SI} is the fraction of symptomatic infections for a given inoculum, I.
S = stability of biological agent when released
C = communicability by host to host transfer
T = time
X = terror modifier based on judgment that the agent could cause panic and social disruption

TABLE 1.7 Biological Agents Categorized According to Level of Concern as Threats to Human Welfare.

Biologic Agent	Disease(s)
Category A Agents	
Variola virus	Smallpox
Bacillus anthracis	Anthrax
Yersinia pestis	Plague
Clostridium botulinum toxin	Botulism
Francisella tularensis	Tularemia
Ebola virus	Ebola hemorrhagic fever
Marburg virus	Marburg hemorrhagic fever
Lassa virus	Lassa fever
Junin virus	Argentine hemorrhagic fever
Other arenaviruses	
Category B Agents	
Coxiella burnetti	Q fever
Brucella species	Brucellosis
Burkholderia mallei	Glanders
Venezuelan equine encephalitis virus	Venezuelan encephalomyelitis
Eastern equine encephalitis virus	Eastern equine encephalomyelitis
Western equine encephalitis virus	Western equine encephalomyelitis
Others include:	
Ricin toxin from *Ricinus communis*	*Salmonella* species
Epsilon toxin of *Clostridium perfringens*	*Shigella dysenteriae*
	Escherichia coli O157:H7
Staphylococcus enterotoxin B	*Vibrio cholerae*
	Cryptosporidium parvum (now *hominis*)
Category C Agents	
• Nipah virus	
• Hantaviruses	
• Tickborne hemorrhagic fever viruses	
• Tickborne encephalitis viruses	
• Yellow fever	
• Multidrug-resistant tuberculosis	

Source: Centers for Disease Control and Prevention, 2000a, pp. 5–6.
M. Cohen, "Bioterrorism in the Context of Infectious Diseases," in F. R. Lashley and J. D. Durham (Eds.), *Emerging Infectious Diseases—Trends and Issues*, Springer Publishing Company, New York, 2007, pp. 415–442

D = deliverability of the agent that is a function of technical capabilities of the user and biological characteristics of the agent

Currently, availability of essential data and the necessity to make assumptions for terms in the equation limit the applicability of the equation for its intended purpose.[103]

Natural pathogens and even normally nonpathogenic agents, earmarked as potential terror weapons, may be genetically altered to improve virulence, nullify protection of the individuals that may have been immunized against terror agent, resist chemotherapy (antibiotic or antiviral treatments) applied to attack victims, and, possibly, alter the bodily regulatory functions of victims.[103]

Following the attacks in New York and Washington on September 11, 2001, letters containing *Bacillus anthracis* (anthrax) spores were mailed to various locations in the United States. This led to 11 inhalation and 7 cutaneous cases of anthrax, resulting in the death of 5 individuals due to inhalation anthrax. DNA sequencing of the anthrax DNA has led to the conclusion that the origin of the infectious material contained in the letters was a U.S. military laboratory.As such, the possibility existed that an employee of the laboratory was involved and that the laboratory harboring anthrax was in violation of the Biologic and Toxin Weapons Convention.[104] It remains to be determined whether these terrorist attacks were related and to identify the perpetrators. As of early 2008, 9,100 persons were interviewed and the Department of Justice had not named any suspects.[105] More recently, four suspects were placed under watch by the FBI, and the source of the anthrax used in the letters of 2001 was narrowed to the U.S. Army's biological weapons research facility at Ft. Detrik, Maryland. On August 6, 2008, it was concluded by the Justice Department, based on documents provided by federal investigators, that a mentally disturbed microbiologist employed at the U.S. Army biological weapons laboratory and who committed suicide one week earlier, acted alone in the 2001 anthrax letter attacks.

Critical microbiological agents in the United States are endemic but of low incidence in disease manifestation, and each new case reported should serve as an alert for investigation, especially in areas were the disease is nonendemic.[106] Several of the major agents will be briefly discussed next. Due to the significant pathogenicity of each of these agents, individuals seeking to employ their use, especially in large amounts, would require substantial knowledge, expertise, and laboratory equipment as well as protection against accidental exposure (e.g., vaccination or antibiotics).

Smallpox

Smallpox, a disease that has killed approximately 300 million people worldwide in the twentieth century alone, and is now globally nonexistent, may have been one of the first microbial agents to be used as a weapon. During the 1800s, North American Indians were deliberately given blankets contaminated with the virus[107] by European settlers. Smallpox virus comprises two strains: variola major, a highly virulent form that produces a high mortality among cases of the disease

and variola minor, which causes a milder form of the disease resulting in under 1 percent fatalities among cases. The only remaining stocks of the variola virus are currently being held in secure locations in the United States and Russia. The WHO voted to postpone a decision on the remaining variola stocks until 2002, raising the possibility of their misappropriation and use as weapons.[108] The scientific community has requested that the available virus stocks be maintained and no further action on the part of WHO has been taken. Some have questioned the grounds for maintaining smallpox stocks. The likelihood of a rebirth of a vaccination program is minimal leading to the conviction that the only purpose the stocks could serve is for bioweapons research. This raises the question of accidental release, improper disposal of hazardous materials, and laboratory mishandling.[104] Variola virus satisfies a number of the prerequisites for an ideal bioterror agent. Since immunization against smallpox was halted in 1976, following a successful worldwide eradication program that saw the last known case of smallpox in 1977, a significant number of the U.S. population would be at risk from a bioterrorism attack. Although individuals vaccinated prior to 1976 may retain immunity to smallpox, the level of protection is currently unknown. Smallpox is generally fatal in about 30 percent of infections of unvaccinated individuals.[109]

Given these uncertainties and the significant health risk of smallpox, the United States and other countries are currently increasing the production of smallpox vaccine. In the wake of concerns for the deployment of variola virus in a bioterror attack, The Advisory Committee on Immunization Practices formulated an interim smallpox release plan, guidelines and a revision of vaccine recommendations in 2001 and reiterated recommendations in 2003.[102] However, approximately 1 in 1 million people exhibit serious and potentially fatal complications following vaccination. Thus, if the entire U.S. population were to be vaccinated, we might expect 100 to 300 deaths from the vaccine. To avoid this situation, one strategy that is being considered for a bioterrorism attack is to limit vaccination to individuals who have come in contact with the initial (index case) infected individual. Vaccination and training of primary health care workers and physicians who are most likely to see the first cases in an attack will also be an important aspect for countering the use of viruses and bacteria as weapons.

Anthrax

Anthrax is a zoonotic disease caused by *Bacillus anthracis*, the facultatively anaerobic, gram positive, nonmotile, endospore-forming bacillus isolated by Robert Koch in 1877 and used by Koch to demonstrate for the first time the relationship between an infectious agent and the etiology of disease. Many domestic and wild animal species have been demonstrated to harbor the anthrax bacillus. Three forms of the disease may be expressed and each is related to the points of entry of the bacterial spores into the body: cutaneous, gastrointestinal, and pulmonary.[102] Cutaneous anthrax in humans occurs through handling of

infected animal meat or hides. Anthrax spores gain entry through skin abrasions or cuts. In fact, the term *anthrax* derives from the Greek word for "coal" and reflects the blackened nature of advanced skin lesions produced by infected individuals. It is far less fatal (under 1 percent) than the gastrointestinal and pulmonary form, which may exceed 50 percent. Gastrointestinal anthrax results from the ingestion of spores and if the disease reaches the septicemia stage, fatality rates are as high as 90 percent. Pulmonary anthrax, while normally rare, poses the greatest risk to humans that have inhaled the spores. Initiating the disease requires a high infectious dose, however, the incubation period is short (on the order of two days) followed by rapidly progressing symptoms culminating in cardiovascular arrest. Fortunately, *B. anthracis* responds readily to antibiotic therapy, most notably, penicillin. Antibiotics such as amoxicillin, ciprofloxacin, and doxycycline are effective against the inhalation form of anthrax; however, they must be administered prior to spore germination, which can occur within 48 to 72 hours following exposure and must be continued for several months.

The level of naturally occurring, human anthrax in the United States is nearly nil, having fallen steadily from about 130 cases in 1920. The last reported incidence of naturally occurring anthrax was a cutaneous case in 1989; however, in 2006, a pulmonary case developed in New York City.

As already noted, anthrax poses a major concern for use in bioterrorism. The endospore stage of the organism confers longevity for the organisms in the environment and represents an advantage to its use as a bioweapon. In fact, it is believed that during World War I, Germany intentionally infected sheep to be shipped to Russia for the purpose of infecting the Russian military. Gastrointestinal anthrax has been reported in the former Soviet Union, but never in the United States. Inhalation of anthrax spores, resulting in the full-blown pulmonary disease, is highly fatal when untreated—and sometimes even with treatment. Of the 18 cases of pulmonary anthrax recorded in the United States for the entire twentieth century, greater than 75 percent of them were fatal. The anthrax bacillus synthesizes four major virulence factors: a antiphagocytic polysaccharide capsule and three separate proteins (exotoxins) that act to induce an endema in the infected localities of the body and cause macrophages to elicit tumor necrosis factor and interleukin 1, which promotes sudden death in the pulmonary disease. An anthrax vaccine is available and is generally effective, although it is currently in limited supply (and mostly dedicated to military rather than civilian use). It has also been observed to cause side effects. Animal vaccines are available, also, however, disease incidence in herds has been so meager that farmers are reluctant to have their animals vaccinated.

It will be important to be able to rapidly monitor and analyze the genetic properties of different anthrax strains and to develop new antibiotics. Another promising avenue stems from the recent identification of the receptor for anthrax lethal factor toxin[110] as well as high-resolution structural determination of lethal factor[111] and edema factor.[112] These molecules represent potential targets for rational drug design of new antibacterial compounds to combat this disease.

Plague

The etiologic agent of plague is the gram negative, facultatively anaerobic, nonmotile, coccobacillus, *Yersinia pestis*. Plague is a vectorborne disease that manifests itself in three clinical forms; bubonic, septicemic, and pneumonic. Bubonic plague has the greatest notoriety, having been the cause of great historic pandemics, such as the sixth-century pandemic that killed 100 million people and the fourteenth-century "Black Death" pandemic that claimed 40 million people.[113,102] The bubonic form of plague has a 75 percent fatality rate. No bacterial disease in history has been more devastating. *Y. pestis* is a zoonotic pathogen, and the reservoirs of *Y. pestis* are various rodents. Infected rodents transmit the pathogen to other animals, most notably domestic rats, through the bite of fleas. Domestic rats are susceptible to the plague and will die. In areas of poor sanitation and living conditions, as characterized much of Europe and Asia in the Dark and Middle Ages, domestic rat populations abounded among human squalor. As domestic rat populations dwindled, owing to loss of members to the plague, fleas carrying *Y. pestis* infected humans. The flea carries a high density of *Y. pestis* following a blood meal on an infected rat and can deposit the bacteria at the site of a human bite, both by regurgitation and fecal deposition.

The term *bubonic* comes from the word "bubo," which refers to the enlarged nodule that forms as a result of *Y. pestis* growth in lymph nodes. The human (host) defense system, through the action of polymorphonuclear leucocytes and macrophages, attack the infectious bacteria. Bacteria phagocytized by macrophages produce toxins that spare them from enzymatic destruction. Other bacteria (e.g., *Legionella pneumophila*) have similar defense strategies. The bacteria contained in the macrophages survive and grow and are delivered to lymph nodes and various organs of the body by the macrophages in the bloodstream. The hemorrhaging (gangrene) that occurs beneath the skin over various parts of the body appears dark—hence the term *Black Death* (recall a similar visible effect to the lesions developed in anthrax infections). More fatal than the bubonic form of plague is pneumonic plague; a manifestation of the disease caused by the migration of the infectious bacteria to the lungs. Untreated pneumonic plague is 100 percent fatal. Septicemic plague, which results either upon inoculation of the bacteria directly into the blood stream or as secondary complications from bubonic or pneumonic forms, progresses from the multiplication of the infectious bacteria in the bloodstream and is essentially always fatal.

As a bioweapon, it is likely that an attack would involve dissemination of the infectious bacteria in aerosol form. The respiratory consequences of inhalation would be expressed as pneumonic plague, which is the most contagious form of plague. *Assuming* the availability of swift medical attention and effective hospital care, the fatality rate from such an attack might be held to 25 percent of the infected portion of the population. First indications of an attack would be a burst in incidence of the disease, especially in places free of animal reservoirs such as a metropolitan area. The incubation period of the disease would be short, likely in the range of two to four days. Despite the high fatality rate and contagious

nature of *Y. pestis*, the organism has a relatively short-lived existence in the free state, disfavoring its use as a terror agent for causing widespread panic.

Tularemia

Like *Y. pestis*, the etiologic agent of tularemia, *Franciscella tularensis*, is a gram negative, nonendospore-forming, coccobacillus. It is a strict aerobe and nonmotile, having many natural arthropod and animal reservoirs and not limited to a particular group of related species. Transmission of the infectious bacterium may occur by several routes:

- Insect bite
- Contaminated aerosols
- Contact with infected animal carcasses, hides, or fluids
- Contaminated water, food, or soil

It is not contagious; person-to-person transmission has not been demonstrated. Virulence of the organism varies among the subspecies, and type A, the North American variety, is the most virulent. There are six clinical manifestations of the disease, of which three are described here: ulceroglandular, pneumonic, and typhoidal. Ulceroglandular infection results from the bite of an insect, often a tick, or a scratch from an animal. The infectious bacteria initiate ulcer formation at the point of entry to the body and in various organs accessed through travel in the bloodstream. The pneumonic form of the disease results from inhalation of the infectious bacteria during handling of infected animals. Advanced symptoms include fatigue, malaise, atypical pneumonia signs, and, possibly respiratory failure. Pneumonic tularemia can develop in any of the other forms of tularemia. Typhoidal tularemia results from ingestion of the infectious bacteria and the symptoms resemble gastroenteritis-type diseases (i.e., vomiting, diarrhea, and abdominal pain). Typhoidal tularemia usually follows in pneumonic cases and is the most fatal form of the disease, with fatality rates as high as 35 percent in untreated cases.

The attractiveness of *F. tularensis* as a bioterror agent is its high rate of infectivity, high virulence, low infectious dose (25 to 50 percent rate of infection in exposed individuals when 10 organisms are presented by the respiratory route), and ease of dissemination by aerosolization. Incubation periods vary from 3 to 15 days, however, clinical symptoms typically appear in 3 to 5 days. There is ample evidence of the interest in *F. tularensis* as a bioweapon, having been studied by both the Japanese and United States during World War II and the Soviet Union into the 1990s.[29]

Glanders

Glanders is a disease occurring mostly in horses and rarely encountered in the United States. The disease in humans is very rare; however, one case was reported

in the United States in 2000. The etioloigic agent of the disease is *Burkholderia mallei*, a gram negative, strictly aerobic, nonmotile bacillus, previously assigned to the Genus *Pseudomonas*. Several *Burkholderia* species are responsible for respiratory-type diseases including melioidosis (see Table 1.4). Glanders infection can be by the cutaneous (skin lesion), inhalation (upper respiratory and pulmonary), or bloodstream (septicemic) routes. Cutaneous infection produces swelling and sores at the site of inoculation within 1 to 5 days. Upper pulmonary invasion induces such symptoms as development of mucus and discharges from the nose and eyes. Pulmonary infection affects the lungs and the symptoms are edema, abscesses, and pneumonia. The incubation period is 10 to 14 days. Septicemia results in fevers, chills, sweating, chest pain, diarrhea, and fatigue, culminating in death within 7 to 10 days. Fatality rates as high as 95 percent occur in untreated events. Therapeutic measures are not well developed, owing to inexperience with the disease, but some recommendations on antibiotic therapy have been made. Several antibiotics are effective against the organism in vitro. Transmission by person to person is rare; however, there are documented cases of sexual transmission. Susceptible animals contract the disease through contaminated water.

Aerosolization of the bacterium is the anticipated form of bioweaponry. The glanders organism was deployed successfully by the Germans in World War I to infect enemy horses and mules. The Japanese intentionally infected both horses and humans in China during World War II.[114,115]

Botulism

The disease derives mainly from ingestion of foods containing an extremely potent neurotoxin produced by the strictly anaerobic, gram positive, endospore-forming, bacillus *Clostridium botulinum*. Spores of *C. botulinum* may gain entry to the body through wounds, ingestion, and inhalation. In these cases, neurotoxin formation would occur *in vivo* during and following spore germination. Intestinal botulism occurs in infants and adults. Inhalation is the mode of infection by intentionally dispersed, aerosolized spores, and by the snorting of spore-containing cocaine. Several forms of the toxin exist, assigned class A status by the CDC. The toxin consists of light (some number of peptides) and heavy (large quantity of proteins) chains. The mode of action of the botulinum toxin begins with the attachment of the heavy toxin chain to axon terminals. Briefly, toxin gains access to the neuron and the light chain penetrates synaptic cells. Through proteolytic action on a protein required for release of acetylcholine, muscle contraction is inhibited. Clinical manifestations of botulism may initially involve interruption in bowel functions, blurred vision, and dry mouth proceeding in advanced stages to paralysis of voluntary muscles, including those controlling the diaphragm. Respiratory arrest follows.

The lethal dose of the toxin to a 150-pound adult human being is approximately 0.15 μg, which explains its appeal as a bioweapon. It is deliverable in particulate form. Botulinum toxin is very unstable, however. In fact, several

bacterial toxins are labile and would be short-lived upon release to the natural environment. Hence, if selected to inflict intentional harm to humans, the preferred delivery vehicle would be food rather than water. Although use of the toxin intentionally on mass scale is rare, such attempts by the cult Aum Shinrikyo took place in Tokyo, Japan, and at U.S. military sites in 1990 and 1995. Fortunately, the group lacked microbiological and technological expertise to deliver the bioweapon successfully.[116]

Tetanus or Lockjaw

This disease develops upon contamination of a wound or burn with soil, street dust, or animal excreta containing endospores of the bacterium, *Clostridium tetani*. Morphological characteristics of the organism are essentially similar to those of *C. botulinum*. The bacillus lives in the intestines of domestic animals. Gardens that are fertilized with manure, barnyards, farm equipment, and pastures are particular sources of danger owing to presence of endospores. The tetanus toxins are tetanolysin and tetanospasmin; the latter a neurotoxin and the known active participant in the pathology of the disease. The toxin is slightly less potent than botulinum toxin, requiring about 0.175 μg to be fatal to a 150-pound adult, but is still a powerful inhibitor of the nervous system. Fatality rates in the United States range from 18 to 25 percent; however, in lands where treatment is less effective, fatality can be 50 percent. There is a tetanus antitoxin that can be used after infection, however, preventative vaccination is much more effective. Older adults (over 50) especially should be revaccinated against tetanus.

Tetanospamin is taken up at the nerve axon, as in the case of botulinum toxin, but is delivered across the synapses to points directly on the central nervous system, as opposed to peripheral regions in the case of botulinum toxin. The effect of the toxin is to interfere with the release of neurotransmitters resulting in muscle contractions and spasms. The incubation period is 1 to 3 weeks.

In summary, use of pathogens as weapons is no longer theoretical. Strategies to counteract their use and defend against their presence are currently in place or under discussion. Research involving the synthesis of a reporter protein for use in a toxin detection system is underway at the Lawrence Livermore National Laboratories in California. Continued efforts in this arena will likely stimulate the development of improved treatments for many known and little understood infectious diseases that will likely plague mankind for the foreseeable future.

NONINFECTIOUS AND NONCOMMUNICABLE DISEASES AND CONDITIONS ASSOCIATED WITH THE WATER ENVIRONMENT

Background

The terms *noncommunicable* and *noninfectious* are used interchangeably. The noncommunicable diseases are the major causes of death in developed areas of the world, whereas the communicable diseases are the major causes of death in

the developing areas of the world. The major noncommunicable disease deaths in the United States in 1988 were due to diseases of the heart, malignant neoplasms, cerebrovascular diseases, accidents, atheriosclerosis, diabetes mellitus, and chronic liver disease and cirrhosis (accounting for 73 percent of all deaths). An analysis of mortality due to noncommunicable diseases in five subregions of the Americas in 1980 showed 75 percent of the total mortality attributed to noncommunicable diseases in North America (United States and Canada); 60 percent in Temperate South American countries (Argentina, Chile, and Uruguay); 57 percent in the Caribbean area (including Cuba, the Dominican Republic, and Haiti); 45 percent in Tropical South America (including the Andean countries, Brazil, French Guiana, Guyana, Paraguay, and Suriname); and 28 percent in Continental Middle America (Central America, Mexico, and Panama).[117] The mortality can be expected to shift more to noncommunicable causes in the developing countries as social and economic conditions improve and communicable diseases are brought under control. Major diseases of developing countries are gastrointestinal, schistosomiasis, malaria, trachoma, and malnutrition.

Treatment of the environment supplements treatment of the individual but requires more effort and knowledge. The total environment is *the most important determinant of health*. A review of more than 10 years of research conducted in Buffalo, New York, showed that the overall death rate for people living in heavily polluted areas was twice as high, and the death rates for tuberculosis and stomach cancer three times as high, as the rates in less polluted areas.[118] Rene Dubos points out that "many of man's medical problems have their origin in the biological and mental adaptive responses that allowed him earlier in life to cope with environmental threats. All too often, the wisdom of the body is a shortsighted wisdom."[119]

Whereas microbiological causes of most communicable diseases are known and are under control or being brought under control in many parts of the world (with some possible exceptions such as malaria and schistosomiasis), the physiologic and toxicologic effects on human health of the presence or absence of certain chemicals in air, water, and food in trace amounts have not yet been clearly demonstrated. The cumulative body burden of all deleterious substances, especially organic and inorganic chemicals, gaining access to the body must be examined both individually and in combination. The synergistic, additive, and neutralizing effects must be learned in order that the most effective preventive measures may be applied. As noted earlier, chemicals contributed to 12 percent of drinking water outbreaks during the period 1971 to 2002, which is greater than the fraction attributed to viruses.[18] Some elements, such as fluorine for the control of tooth decay, iodine to control goiter, and iron to control iron deficiency anemia, have been recognized as being beneficial in proper amounts. But the action of trace amounts ingested individually and in combination of the pollutants shown in Figure 1.3 and other inorganic and organic chemicals is often insidious. Their probable carcinogenic, mutagenic, and teratogenic effects are extended in time, perhaps for 10, 20, or 30 years, to the point where direct causal relationships with

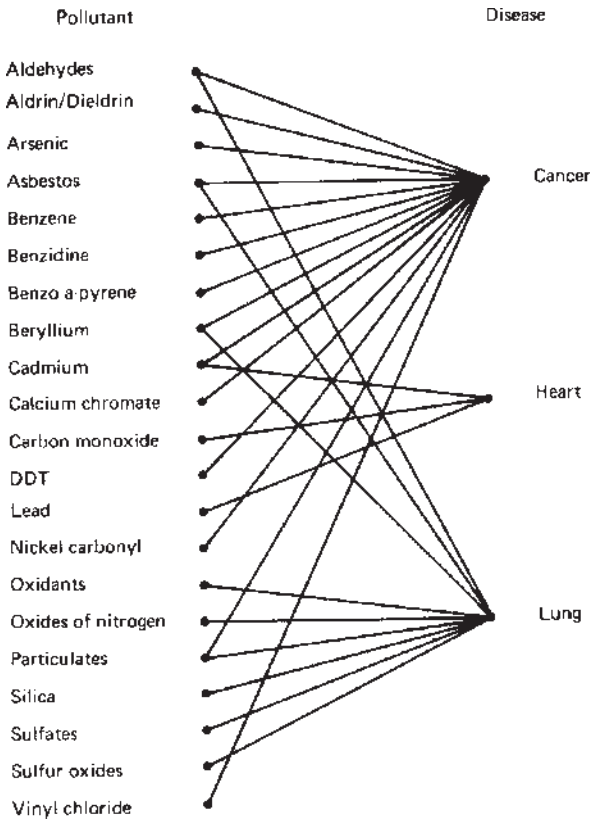


FIGURE 1.3 Known or suspected links between selected pollutants and disease. (*Source*: First Annual Report by the Task Force on Environmental Cancer and Heart and Lung Disease, Printing Management Office, U.S. Environmental Protection Agency, Washington, DC, August 7, 1978.)

morbidity and mortality are difficult, if not impossible, to conclusively prove in view of the many possible intervening and confusing factors.

There are an estimated 2 million recognized chemical compounds and more than 60,000 chemical substances in past or present commercial uses. Approximately 600 to 700 new chemicals are introduced each year, but only about 15,000 have been animal tested with published reports. Limited trained personnel and laboratory facilities for carcinogenesis testing in the United States by government and industry will permit testing of no more than 500 chemicals per year. Each animal experiment requires 3 to 6 years and a cost of more than $300,000.[120] Another estimate is $500,000 just to establish the carcinogenicity of one compound with the National Cancer Institute test protocol, requiring at least two species of rodents and 3 years' time.[121] A full toxicologic test, including those for carcinogenicity, can take five years and cost in excess of $1.25 million for

each compound. The chemicals are viewed by Harmison[122] as falling into four groups: (1) halogenated hydrocarbons and other organics, (2) heavy metals, (3) nonmetallic inorganics, and (4) biological contaminants, animal and human drugs, and food additives.

In group 1 may be polychlorinated biphenyls (PCBs); chlorinated organic pesticides such as DDT, Kepone, Mirex, and endrin; polybrominated biphenyls (PBBs); fluorocarbons; chloroform; and vinyl chloride. These chemicals are persistent, often bioaccumulate in food organisms, and may in small quantities cause cancer, nervous disorders, kidney and brain damage, and toxic reactions. A recently recognized undesirable role for pharmaceuticals, herbicides, and pesticides in natural waterways is as endocrine disruptors.[123] The extraordinary production and use of these compounds, coupled with their persistence through wastewater treatment processes, has resulted in long residence times of such materials in the environment. Aquatic life have been impacted through the ability of endocrine disruptor-active compounds to mimic hormonal control of reproductive systems, organ development, and sensory functions. There is concern that contaminants falling into the category of endocrine disruptors may exist in finished drinking waters. The route by which herbicides and pesticides may gain entry to natural waters is through agricultural runoff. PCBs are no longer manufactured, but their residues are still present in aquatic sediments and the tissues of aquatic vertebrates and invertebrates. Other chlorinated compounds may appear in soils and waters from leaking storage drums, uncontained industrial lagoons, and accidental landfill leachates.

Another group of nine chlorinated compounds that may appear in drinking water as a consequence of the use of chlorine as a post water treatment disinfectant is the haloacetic acids or disinfection byproducts (DBP). Trihalomethanes are a subset of the haloacetic acids that are regarded as the major carcinogens among DBP in relation to colon and rectal cancers[124] and reproductive disorders including spontaneous abortions, fetal deaths, miscarriages, and birth defects.[119] Precursors to the formation of DBP are naturally occurring organic molecules present in raw water supplies. Unlike the plethora of organic substances referred to in the AP report, DBP are regulated in the drinking water standards. However, only five of the nine DPB compounds are monitored.

Group 2 includes heavy metals such as lead, mercury, cadmium, barium, nickel, vanadium, selenium, beryllium. These metals do not degrade; they are very toxic and may build up in exposed vegetation, animals, fish, and shellfish. Some of them (e.g., lead, mercury, cadmium, and beryllium) have no role in human metabolism and are inhibitors of enzymes at very low concentrations. As poisons, they can affect the functions of various organs (e.g., kidney, liver, brain) and damage the central nervous system, cardiovascular system, and gastrointestinal tract. Children and pregnant women are especially vulnerable. The levels of heavy metals in drinking water are highly regulated. Heavy metals variably appear in many manufactured products, including metal goods and electronic devices, as well as naturally occurring minerals and coal deposits. Hence, there is ample opportunity for contamination of natural waters through runoff from insecure

toxic waste containment sites, improper disposal and storage, and anthropogenic discharges such as power plant emissions.

Group 3 represents nonmetallic inorganics such as arsenic (metalloid) and asbestos, which are carcinogens.

Group 4 includes biological contaminants such as aflatoxins and pathogenic microorganisms; animal and human drugs such as diethylstilbestrol (DES) and other synthetic hormones; and food additives such as red dye No. 2. An Associated Press report released March 9, 2008 (available at http://www.metrowestdailynews.com/homepage/x1574803402), outlined the appearance of antibiotics, hormonal preparations, personal care chemicals, antidepressants, cholesterol control and cardiovascular medications, and pain relievers in ultra-small concentrations (ppb and ppt) in drinking-water samples from 24 of 28 metropolitan areas of the United States. All of these chemical substances are undetectable by the human senses.

Evaluation of the toxicity of existing and new chemicals on workers, users, and the environment and their release for use represent a monumental task, as already noted. Monitoring the total effect of a chemical pollutant on humans requires environmental monitoring and medical surveillance to determine exposure and the amount absorbed by the body. The sophisticated analytical equipment available can detect chemical contaminants in the parts-per-billion or parts-per-trillion range. Mere detection does not mean that the chemical substance is automatically toxic or hazardous. But detection does alert the observer to trends and the possible need for preventive measures. Short-term testing of chemicals, such as the microbial Ames test, is valuable to screen inexpensively for carcinogens and mutagens. The Ames test determines the mutagenic potential of a chemical based on the mutation rate of bacteria that are exposed to the chemical. However, positive results suggest the need for further testing, and negative results do not establish the safety of the agent. Other tests use mammalian cell cultures and cell transformation to determine mutagenicity.

Prevention and Control

Prevention of the major causes of death, such as diseases of the heart, malignant neoplasms, cerebrovascular disease, accidents, and other noninfectious chronic and degenerative diseases, should now receive high priority. Prevention calls for control of the source, mode of transmission, and/or susceptibles as appropriate and as noted in Figure 1.1.

The prevention and control of environmental pollutants generally involves the following three procedures:

1. *Eliminate or control of the pollutant at the source*. Minimize or prevent production and sale; substitute nontoxic or less toxic chemical; materials and process control and changes; recover and reuse; waste treatment, separation, concentration, incineration, detoxification, and neutralization.
2. *Intercept the travel or transmission of the pollutant*. Control air and water pollution and prevent leachate travel.

3. *Protect humans by eliminating or minimizing the effects of the pollutant*. This affects water treatment, air conditioning, land-use planning, and occupational protection.

At the same time, the air, sources of drinking water, food, aquatic plants, fish and other wildlife, surface runoff, leachates, precipitation, surface waters, and humans should be monitored. This should be done for potentially toxic and deleterious chemicals, as indicated by specific situations. Table 1.4 also lists characteristics of noninfectious diseases due to the ingestion of poisonous plants and animals and chemical poisons in contaminated water or food.

INVESTIGATION OF A WATER DISEASE OUTBREAK

General

The successful outcome in the investigation of any disease outbreak, no matter the source, depends on expedient execution of a preplanned process. Extensive investigations are economically burdensome to all parties involved, and the target of the study (e.g., a municipal water supply) in the end is faced with a public-relations problem in winning back the confidence of the community concerning the safety of the drinking water.

Hunter[125] delineated a nine-step "cradle to grave" program for the conduct of a waterborne outbreak study (Figure 1.4).

Each of the steps in the chronology of an investigation is elaborated on in the following sections. Although investigation of a waterborne incident is described here, the steps put forth would be applicable to a foodborne outbreak, also. Details on foodborne outbreaks are presented in Chapter 3.

Preparation Requisite to the investigation of an elevated incidence of disease, there must be in place a team of individuals having the collective expertise to handle all phases of the study. Ideally, this would include an epidemiologist, field engineer, preferably trained in matters of public health, and assistants. Each of the individuals must have an assigned role to play in the team effort to characterize an outbreak and provide suggestions to solve the problem. Responsible leadership, typically under direction of an epidemiologist, must be established in order to monitor and coordinate team activities and seek approval of the plan from pertinent pubic officials.

Detection The first stage of a potential outbreak event is the unusual level of sick individuals in the population requiring medical attention within a short time frame. Similarity in patient symptoms and results of laboratory examinations of specimens may provide preliminary evidence of the possibility of an outbreak. However, it is imperative that prompt reporting of laboratory data to public health authorities take place in order that there be an evaluation and dispensing of information to appropriate individuals to confirm the existence of an outbreak.

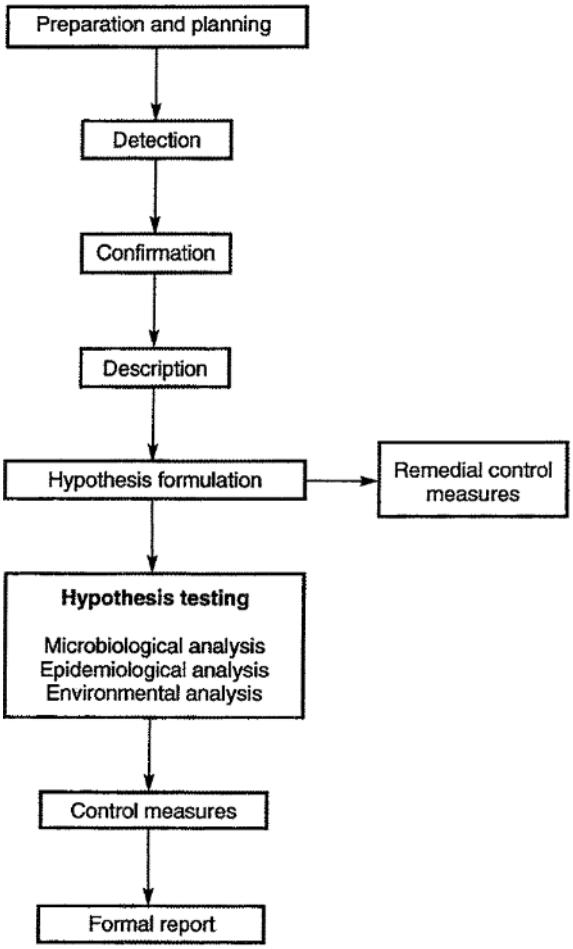


FIGURE 1.4 Flow diagram depicting the incremental steps in the investigation of a waterborne outbreak. *Source: P. R. Hunter, Waterborne Disease-Epidemiology and Ecology, John Wiley & Sons, New York, 1997*.

Hunter[125] cautions that many variables contribute to the inefficiency of identifying the existence of a waterborne disease, including difficulty in assembling patient data, proper diagnosis and laboratory testing for etiologic agents of prospective diseases, and underestimates of the number of afflicted people. For these and other reasons, much time and effort can be lost between the onset of illness in the population and the resolution of an outbreak.

Confirmation A redoubling of the effort on the part of authorities to substantiate from all information received that, indeed, an outbreak has occurred. This will involve a review of physician and laboratory records and ensuring that proper reporting of data to public health bureaus has taken place.

Description Upon confirmation that an outbreak has occurred, the investigating team should be activated and initial steps undertaken. It is not a simple matter to quickly determine the cause of illness due to water, food, or other vehicle, but a preliminary study of the symptoms, incubation periods, food and water consumed, housing, bathing area, and sanitary conditions may provide early clues and form a basis for formulating a quick response control action.

What is to be considered an outbreak case? The answer will require a preliminary set of parameters with which to define the case (e.g., limits of time regarding onset of the illness, symptoms of the illness, geographical boundaries of the affected area, and microbiological description of the disease etiology). The more rigid the definitions of parameters, the more likely it is that fewer cases will qualify for inclusion in the outbreak. However, parameter definitions should be flexible in relation to the availability of new information over time.

Following agreement on definition of a case, quantitative accounting of the number of cases involved is in order. Reliability of physician diagnoses and the collection of completed questionnaires of the type presented in Figure 1.5 are important. The information gathered from questionnaires contributes to the medical survey. If it appears that the number of completed questionnaires is insufficient, similar kinds of information can be collected and tabulated in the field when assistance is available. The tabulation horizontal headings would include the following seven categories:

1. Names of persons served food and/or water;
2. Age(s);
3. Ill—yes or no;
4. Day and time ill;
5. Incubation period in hours (time between consumption of ingestibles and first signs of illness);
6. Foods and water served at suspected meals—previous 12 to 72 hours (foods eaten are checked)
7. Symptoms—nausea, vomiting, diarrhea, blood in stool, fever, thirst, constipation, stomach ache, sweating, sore throat, headache, dizziness, cough, chills, pain in chest, weakness, cramps, other

Other analyses may include a summary of persons showing a particular symptom such as vomiting, diarrhea, and nausea, as shown in Figure 1.5, or those using a specific facility for calculation of incidence rates. For complete investigation details, consult references as appropriate.[126–129]

A common method of determining the probable offending water is a tabulation as shown in Figure 1.6, which is made from the illness questionnaire provided in Figure 1.5 or similar version. Comparison of the attack rates for each water will usually implicate or absolve a particular water. The water implicated is that showing the highest percentage difference between those who ate the specified water and became ill and those who did not eat the specified water and

Please answer the questions below to the best of your ability. This information is desired by the health department to determine the cause of the recent sickness and to prevent its recurrence. Leave this sheet, after you have completed it, at the desk on your way out. (If mailed, enclose self-addressed and stamped envelope and request return of completed questionnarie as soon as possible.)

1. Check any of the following conditions that you have had:

Nausea	Fever	Sore throat	Cough	Chills
Vomiting	Constipation	Headache	Pain in chest	Weakness
Diarrhea	Stomach ache	Dizziness	Laryngitis	Cramps
Thirst	Sweating	Paralysis	Bloody stool	Other

2. Were you ill? ..... Yes .....No.
3. If ill, first became sick on: Date.....Hour..... A.M. / P.M.
4. How long did the sickness last ? .....
5. Check below (✓) the food eaten at each meal and (×) the food not eaten. Answer even though you may not have been ill.

Meal	Tuesday	Wednesday	Thursday
Breakfast	Apple juice, Corn flakes, oatmeal, fried eggs, bread, coffee, milk, water	Orange, pancakes, wheaties, syrup, coffee, milk, water	Grapefruit, Wheatina, shredded wheat, boiled egg, coffee, milk, water
Lunch	Baked salmon, creamed potatoes, corn, apple pie, lemonade, water	Roast pork, baked potatoes, peas, rice pudding, milk, water, chef salad	Swiss steak, home fried potatoes, turnips, spinach, chocolate pudding, orange drink, milk, water
Dinner	Gravy, hamburger steak, mashed potatoes, salmon salad, cookies, pears, cocoa, water	Roast veal rice, beets, peas, jello, coffee, water	Fruit cup, meatballs, spaghetti, string beans, pickled beets, sliced pineapple, tea, coffee, milk

6. Did you eat food or drink water outside? ..... If so, where and when?
.....
7. Name..... Tel..... Age ..... Sex.....
8. Remarks (Physician's name, hospital).....
..... Investigator .....

FIGURE 1.5 Questionnaire for illness from food, milk, or water.

became ill (Figure 1.6). The sanitary survey is important to the interpretation of an environmental sample and determining a sound course of action and should include a study of all environmental factors that might be the cause or may be contributing to the cause of the disease outbreak. These should include water supply, food, housing, sewage disposal, bathing, insects, rodents, pesticide use, food handlers and other workers, practices, procedures, and any other relevant factors. Each should be considered responsible for the illness until definitely ruled

DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
CENTERS FOR DISEASE CONTROL
CENTER FOR INFECTIOUS DISEASES
ATLANTA, GEORGIA 30333

INVESTIGATION OF A WATERBORNE OUTBREAK

Form Approved
OMB No. 920-0004

1. Where did the outbreak occur?
_____(1-2) City or Town _____ Country _____

2. Date of outbreak : (Date of onset of 1st case)
_____ (3-8)

3. Indicate actual (a) or estimated (a) numbers :
Persons exposed _____ (9-11)
Persons ill _____ (12-14)
Hospitalized _____ (15-16)
Fatal cases _____ (17)

4. History of exposed persons :
No., histories obtained _____ (18-20)
No. persons with symptoms _____ (21-23)
Nauses _____ (24-26) Diarrhea_____ (33-35)
Vomiting _____ (27-29) Fever _____ (36-38)
Cramps _____ (30-32)
Other, specify (39) _____

5. Incubation period (hours):
Shortest ____ (40-42) Longest ____ (43-45)
Median _____ (46-48)

Shortest ____ (49-51) Longest ____ (52-54)
Median _____ (55-57)

7. Epidemiologic date (e.g., attack rates (number ill / number exposed) for persons who did not eat or drink specific food items or water, attack rate by quantity of water consumed, anacdotal information) * (58)

ITEMS SERVED	NUMBER OF PERSONS WHO ATE OR DRANK SPECIFIED FOOD OR WATER				NUMBER WHO DID NOT EAT OR DRINK SPECIFIED FOOD OR WATER			
	ILL	NOT ILL	TOTAL	PERCENT ILL	ILL	NOT ILL	TOTAL	PERCENT ILL

8. Vehicle responsible (item incriminated by epidemiologic evidence) : (59-60) _____

9. Water supply characteristics

(A) Type of water supply** (61)
- ☐ Municipal or community supply (Name _____)
- ☐ Individual household supply
- ☐ Semi-public water supply
  - ☐ Institution, school, church
  - ☐ Camp, recreational area
  - ☐ Other, _____
- ☐ Botteled water

(B) Water source (*check all applicable*):

☐ Well	a	b	c	d
☐ Spring	a	b	c	d
☐ Lake, pond	a	b	c	d
☐ River, stream	a	b	c	d

(C) Treatment provided (*circle treatment of each source checked in B*):
- a. no treatment
- b. disinfection only
- c. purification plant - coagulation, settling, filtration, disinfection (*circle those applicable*)
- d. other _____

10. Point where contamination occurred: (66)
☐ Raw water source ☐ Treatment plant ☐ Distribution system

*See CDC 52.13 (Formerly 4.245) Investigation of a Foodborne Outbreak, Item 7.
**Municipal or community water supplies are public or investor owned utilites, Individual water supplies are wells or springs used by single residences. Semipublic water systems are individual-type water supplies serving a group of residences or locations where the general public is likely to have access to drinking water. These locations include schools, camps, parks, resorts, hotels, industries, subdivisions, trailer parks, etc., that do not obtain water from a municipal water system but have developed and maintain their own water supply.

CDC 52.12 (f. 4.461)
Rev. 7-81

This report is authorized by law (Public Health Service Act, 42 USC 241)
While your response is voluntary, your cooperation is necessary for the understanding and control of the disease.

FIGURE 1.6 Investigation of waterborne outbreak.

11. Water specimens examined: (67)
(*Specify by "X" wheather water examined was original (drunk at time of outbreak) or check-up (collected before or after outbreak occurred*)

ITEM	ORIGINAL	CHECK UP	DATE	FINDINGS		BACTERIOLOGIC TECHNIQUE (e.g., fermentation tube, membrane filter)
				Quantitative	Quantitative	
Examples: Tap water	X		6/12/74	10 focal coliforms per 100ml.		
Raw water		X	6/2/74	23 total coliforms per 100 ml.		

12. Treatment records: (*Indicate method used to determine chlorine residual*):
Example: Chlorine residual – One sample from treatment plant effluent on 6/11/74 – trace of free chlorine
Three samples from distribution system on 6/12/74 - no residual found

13. Specimens from patients examined (stool, vomitus, etc.) (68)

SPECIMEN	NO. PERSONS	FINDINGS
Example: Stool	11	8 *Salmonella typhi*
		3 negative

14. Unusual occurrence of events :
Example: Repair of water main 6/11/74; pit contaminated with sewage, no main disinfection. Turbid water reported by consumers 6/12/74.

15. Factors contributing to outbreak (*check all applicable*):
☐ Overflow of sewage ☐ Interruption of disinfection ☐ Improper construction, location of well/spring
☐ Seepage of sewage ☐ Inadequate disinfection ☐ Use of water not intended for drinking
☐ Flooding, heavy rains ☐ Deficiencies in other treatment processes ☐ Contamination of storage facility
☐ Use of untreated water ☐ Cross-connection ☐ Contamination through creviced limestone of fissured rock
☐ Use of supplementary source ☐ Back-siphonage ☐ Other (specify) _____
☐ Water inadequately treated ☐ Contamination of mains during construction or repair _____

16. Etiology: (69-70) (71)
Pathogen _____ Suspected 1
Chemical _____ Confirmed . . . . . 2 (*Circle one*)
Other _____ Unknown 3

17. Remarks: *Briefly describe aspects of the investigation not covered above, such as unusual age or sex distribution; unusual circumstances leading to contamination of water; epidemic curve; control measures implemented; etc. (Attack additional page if necassary)*

Name of reporting agency: (72)

Investigating Official: | Date of investigation:

Note: Epidemic and Laboratory assistance for the investigation of a waterborne outbreak is available upon request by the State Health Department to the Centers for Disease Control, Atlanta, Georgia 30333.

To improve national surveillance, please send a copy of this report to: Centers for Disease Control
Attn: Enteric Disease Branch, Bacterial Diseases Division
Center for Infectious Diseases
Atlanta, Georgia 30333

Submitted copies should include as much information as possible, but the completion of every item is not required.

CDC 52.12 (f. 4.461) (BACK)
7-81

FIGURE 1.6 (*continued*)

out. Other chapters in this book dealing with water and wastewater treatment, residential housing, food protection, recreational areas, and so on may be useful. Table 1.4 should be referred to for guidance and possible specific contributing causes to an outbreak and their correction.

A form for use in an environmental field investigation is presented in Figure 1.7. Water system, food service, housing, and swimming-pool sanitary survey report forms are usually available from the state or local health

Date ..... Investigator .....
Name of place ..... Owner .....
Population ..... Manager .....
Onsets—day and hours ..... Incubation period .....
Number afflicted ..... Number hospitalized ..... Number deaths .....
Outbreak: explosive ..... gradual ..... undetermined .....
Samples collected .....

Underline symptoms most commonly reported:

Diarrhea, constipation, abdominal pains, stomach cramps, muscular cramps, prostration, high temperature, painful straining at stool or in urination, sore throat, chills, thirst, sweating, vomiting, nausea, swelling of face and eyelids, laryngitis, cough, pain in chest, enlarged tonsils or adenoids, pains in joints, eye movement difficult, swallowing difficult, headache, dizziness, other .....

Water

1. Water sources and treatment .....
2. Method of serving water .....
3. Interconnections: toilet ..... washbasin ..... bath tubs ..... tubs ..... other .....
4. Recent repairs .....
5. Cross-connections, with other supplies .....
6. Changes in water taste ..... color ..... odor .....

Milk and food

7. Source of milk (pasteurized) .....
8. Method of handling milk .....
9. Use of leftover foods .....
10. Source of fowl, meats, ice cream, shellfish, pastries .....
11. Food refrigeration and storage .....
12. Food handling and preparation .....
13. Ice source and handling .....
14. Thawing foods protected .....
15. Dressings, sauces, etc. .....

Food handlers

16. Recent illness in food handlers .....
17. Hand-washing facilities .....
18. No. pyogenic skin infections .....
19. Personal hygiene .....

Kitchen and dining hall

20. Storage and use of insecticides ..... rat poison ..... roach powder ..... water paint ..... silver polish .....
21. Garbage storage and disposal .....
22. Prevalence of rodents and insects .....
23. Fly breeding controlled .....
24. Dish cleansing and disinfection .....
25. Premises and equipment clean .....
26. Food service well organized .....

Other

27. Housing overcrowding .....
28. Bathing beach or swimming pool operation, water source .....
29. Medical and nursing care .....
30. Other .....

Remarks (Comment on unsatisfactory items and probable cause, general impressions, etc.):

FIGURE 1.7 Outbreak investigation field summary.

department to assist in making a complete epidemiologic investigation. A WHO publication also has a water system reporting form[130,131] and the EPA has an evaluation manual.[132]

Laboratory results are the key to confirming the cause of disease cases. It may be necessary to ask physicians to obtain specimens from patients considered to be presumptive cases where such sampling had not been done. Also, a reexamination

of physician records may be warranted against the possibility that certain patients were overlooked.

Once individuals are identified as cases, personal history of each of the cases must be obtained. In addition to the usual descriptors (e.g., name, age, sex, etc.), personal information relevant to the case definition is needed. Accessory data may be collected on cases (e.g., information about whereabouts and activities leading up to the occurrence of disease symptoms). Such information is useful to establish the incubation period for the disease and to compare the evaluation with published incubation periods for suspected etiologic agents. The medical survey should assist in developing a clinical picture to enable identification of the disease and its causative agent. Typical symptoms, date of onset of the first case, date of onset of last case, range of incubation periods, number of cases, number hospitalized, number of deaths, and number exposed are usually determined by the epidemiologist. To assemble this information and analyze it carefully, a questionnaire should be completed, by trained personnel if possible, for each person available or on a sufficient number of people to give reliable information (see Figure 1.5).

The importance of animal reservoirs of infection should not be overlooked where small-scale water systems are involved. Table 1.4 contains in condensed form symptoms and incubation periods of many diseases that, when compared to a typical clinical picture, may suggest the causative organism and the disease. A high attack rate, 60 to 80 percent, for example, would suggest a virus (Norovirus) as the cause of a foodborne outbreak.[133]

Finally, all data collected in the description phase of the investigation are analyzed and charted in various ways to obtain a picture of the outbreak. Visual aids will be areal maps, graphs displaying the chronology of case densities over time with subplots according to age, sex, ethnicity, and so on. A simple bar graph, with hours and days (possibly weeks) as the horizontal axis and number who are ill each hour or other suitable interval plotted on the vertical axis, can be made from the data. The time between exposure to or ingestion of water and illness or first symptoms or between peaks represents the incubation period. The average incubation period is the sum of the incubation periods of those ill (time elapsing between the initial exposure and the clinical onset of a disease), divided by the number of ill persons studied. The median, or middle, time may be preferable when incubation periods vary widely. The shape of the curve is useful in revealing the period of primary infection as may be due to point source infection vs. person-to-person contact. Extended case-time plots may be biomodal, indicating a point-source outbreak and a secondary person-to-person outbreak. Good data presentation adds to the strength of the investigation and the location of "hot spots" that may reveal points of interest in the drinking-water distribution system subject to possible contamination.

Hyothesis Formulation The data collected and analyzed in connection with the "Description" are used to formulate hypotheses concerning the events responsible for the outbreak and make preliminary recommendations for remedial control measures. More than one hypothesis is possible. The outbreak may be

responsible to a point-source or person-to-person contact. Furthermore, if it is envisioned that a point-source is possible, it will be necessary to determine the point of access by disease-producing agents to the finished water. For example, an infectious agent believed responsible for a waterborne outbreak may be associated with a cross-connection somewhere in the distribution system or regrowth in an activated carbon filter at the treatment plant compounded by ineffective disinfectant residual in the finished water. Knowledge of past outbreaks and epidemiology of the suspected infectious agent, combined with the total of current data logs and analyses of the outbreak in question will serve to identify the hypothesis with greatest likelihood explaining the outbreak. Publications summarizing disease outbreak investigation procedures are very helpful.[134–137]

Remedial Control Measures During hypothesis formulation, implications as to the cause of the outbreak may emerge, justifying a simultaneous review of options for remedial control measures. Since the hypothesis advanced has not been proven at this point, any remedial actions called for must be directed at immediate protection of the public. Where a danger in the drinking water supply is envisioned, decisions are limited to disconnecting the purveyor from the users, issuing a boil order, or supplying an auxiliary source of safe drinking water. In the example of the Alamosa, Colorado, outbreak, residents were advised not to use tap water for potable uses on the day bacterial contamination was discovered and to bring large containers to obtain safe water from distribution centers located within the community. Bottled water was supplied mostly to schools. Main flushing following superchlorination took place in stages, beginning six days from the time the outbreak was announced and residents were asked to refrain from using tap water for drinking and cooking at that time. Water authorities should not be required to undertake expensive repair and retrofitting of the treatment system before it is definitely ascertained that there is a physical problem in need of attention. The mere enactment of precautionary measures will prescribe a liability, both in terms of monetary cost and public relations.

Hypothesis Testing This is the important "proof" step in the investigatory program. All parties affected and the rest of the community will anxiously await the final word on the cause of the outbreak. All evidence obtained during the investigation is evaluated in an acceptable plan for testing a particular hypothesis. The evidence presented is the sum total of microbiological, epidemiological, and environmental findings collected during the course of the investigation. The most definitive microbiological evidence is the unequivocal identification of the waterborne disease agent in case specimens and samples taken at the source of the outbreak, however, the latter may be difficult to accomplish. New methodologies are available to aid in rapid detection of suspected pathogenic agents in environmental samples including water. A brief description of the procedures is given in the following section. Epidemiological evidence arises from the results of retrospective studies conducted on known cases and randomly selected control subjects within the affected community. Environmental evidence pertains to

results of a sanitary survey. The sanitary survey should cover all factors that may potentially impact on operational and quality control issues associated with the treatment and distribution of the community water supply. It is very helpful to have personnel knowledgeable about the water field involved in the environmental investigation. Upon obtaining positive identification of the etiologic agent of a communicable disease, the number of confirmed cases should be made known to the state health department and to the national Centers for Disease Control.

Control Measures These are the repairs and installation of facilities and equipment necessary to safeguard the water supply from repeated microbial violations of the system. Successful establishment of the cause and source of the waterborne outbreak pays dividends, not only in returning the community to normal use of its water supply but also easing the tensions of individuals upon which the onus for correcting defects and bearing the financial burden is leveled.

Formal Report The published written report should chronicle the essentials of the waterborne outbreak. The report should be fully detailed and include the cause, laboratory findings, transmission, incidence, case by dates of onset, average incubation period and range, typical symptoms, length of illness, age and sex distribution, deaths, secondary attack rate, and recommendations for the prevention and control of the disease, so as to be of use to various professional, political, and technical members in the community workforce. Copies of the report should be sent to the state health department and the Public Health Service. The press should be carefully briefed to avoid misinterpretation and dissemination of misinformation to the community. Effort should be made to use the report as an instructional tool for the education of students in the community and geographically dispersed parties through scientific reporting.

Samples and Specimens

The prompt collection of samples and specimens for laboratory examinations is a necessary part of the investigation of any disease outbreaks. Although not often done, isolating the incriminating organism from the persons made ill and the alleged outbreak source, producing the characteristic symptoms in laboratory animals or human volunteers, and then isolating the same organisms from human volunteers or laboratory animals will confirm the field diagnosis and implicate the responsible vehicle. In the early stages of the field investigation, it is very difficult to determine just what samples to collect. It is customary, therefore, to routinely collect samples of water from representative places and available samples of all leftover milk, drinks, and food that had been consumed and place them under seal and refrigeration. Sterile spatulas or spoons boiled for 5 minutes can be used to collect samples. Sterile wide-mouth water bottles and petri dishes make suitable containers. In all cases, aseptic technique must be used. Since examination of all the food may be unnecessary, it is advisable, after studying the questionnaires and

accumulated data, to select the suspicious foods for laboratory examination and set aside the remaining food in protected sterile containers under refrigeration at a temperature of less than 40°F (4°C) for possible future use. Laboratory procedures should be followed for collection, preservation, and shipment of all specimens and samples.

Samples of water should be collected directly from the source, storage tanks, high and low points of the distribution system at times of high and low pressure, kitchens, and taps near drinking fountains for chemical and bacterial examinations. It should be remembered that the time elapsing before symptoms appear is variable and depends on the causative agent and size of dose, the resistance of individuals, and the amount and kind of food or drink consumed. For example, an explosive outbreak with a very short incubation period of a few minutes to less than an hour would suggest a chemical poisoning. Antimony, arsenic, cadmium, cyanide, mercury, sodium fluoride, sodium nitrate, or perhaps shellfish poisoning, favism, fish poisoning, and zinc poisoning are possibilities. An explosive outbreak with an incubation period of several hours would suggest botulism or fish, mushroom, potato, rhubarb-leaf, shellfish, chemical, or staphylococcus food poisoning. An incubation period of 6 to 24 hours would suggest botulism, mushroom poisoning, rhubarb poisoning, salmonella infection, or streptococcus food poisoning. An incubation period of one to five days would suggest ascariasis, botulism, diphtheria, amebic dysentery, bacillary dysentery, leptospirosis, paratyphoid fever, salmonella infection, scarlet fever, streptococcal sore throat, or trichinosis. For other diseases with more extended incubation periods, refer to Table 1.4. The laboratory examinations might be biologic, toxicologic, microscopic, or chemical, depending on the symptoms and incubation period.

The CDC[138] classifies outbreaks of unknown etiology into four subgroups by incubation period of the illnesses: less than 1 hour (probable chemical poisoning), 1 to 7 hours (probable *Staphylococcus* food poisoning), 8 to 14 hours (probable *C. perfringens* food poisoning), and more than 14 hours (other infectious or toxic agents).

The sanitary and medical surveys may involve the swimming pool or bathing beach. In that case, samples should be collected at the peak and toward the end of the bathing period for examinations.

Laboratory analyses for water samples should include the standard plate count (heterotrophic plate count), in addition to the test for coliform bacteria, since large bacterial populations may suppress the growth of coliform organisms. Where large volumes of water are needed, use 2- to 5-gallon sterile containers and store at 41°F (5°C). Sampling for recovery of viruses and *Giardia or Entamoeba* cysts may require special on-site filters and equipment.[139]

It is customary to notify the laboratory in advance that an outbreak has occurred and that samples and specimens will be delivered as soon as possible. All should be carefully identified, dated, sealed, and refrigerated. A preliminary report with the samples and specimens, including the probable cause, number

ill, age spread, symptoms, incubation period, and so on, will greatly assist the laboratory in its work.

Epidemiology and Risk

In the foregoing discussion, a scheme for dealing with the orderly investigation of a waterborne disease outbreak was presented. Central to the conduct of the investigation is the team of workers appropriately trained to perform specific roles. One such team member, if available, and a likely leader of the group, is the epidemiologist. *Epidemiology* literally translated is "study of epidemics." In the broader sense, it is the science (with considerable art) of defining the causes of disease distribution within a population and the causal factors that made the disease possible. A causal factor is an event, condition, or characteristic that increases the likelihood of a disease.[4]

Environmental epidemiology is the study of environmental factors that influence the distribution and determinants of disease in human populations.[26] In the context of a waterborne outbreak, the epidemiologist is interested in learning the susceptibility of the population under the sphere of influence of a water transmitted disease, what regions or groups of people in the population are at the greatest risk, how the disease will manifest itself temporally and spatially in the population, commonalities, and differences among the individuals listed as having been symptomatically affected and not affected, and something of the risk to the population under the conditions of exposure to water.

During the course of the investigation of a waterborne outbreak, a descriptive epidemiologic study will be undertaken with the collection of data sets obtained from laboratory, hospital and physician, environmental, and residential records and field surveys. The emphasis will be put on establishing the veracity of the outbreak, containing the spread of the disease through emergency measures, and characterizing the event in support of formulating a hypothesis on the cause of the outbreak. A follow-up to the descriptive epidemiologic study would be an analytical epidemiologic exercise involving a case-control study to identify causal factors to the outbreak. A case-control study is an observational study in which a group of persons with a disease (cases) and a group of persons without the disease (controls) are identified without knowledge of prior exposure history and are compared with respect to exposure history.[140]

If the selection of control participants is truly random, some of the subjects selected to be controls may also have expressed the illness. Selection of individuals making up the control group is not a simple process and, as with the convening of any sample of people intended to be representative of a particular population, bias is inevitable. Bias impacts the strength of the study results. The object of the exercise is to analyze the behaviors of both groups prior to the outbreak so that a determination can be made about the importance of the water as a condition to developing the disease. For this, a simple approximation of the essentiality of the water to the infectious outcome is obtained by computing an odds ratio. A 2×2 square is constructed by pairing the number of people that

consumed and did not consume water against the number of those people who became ill and did not become ill.

The following is a hypothetical example involving collected data on the population associated with the waterborne outbreak:

52 people drank contaminated water and became clinically ill. (a)

32 people drank contaminated water and did not become ill. (b)

21 people did not drink contaminated water and became ill. (c)

64 people did not drink contaminated water and did not become ill. (d)

The 2×2 table is constructed to display the data as given.

	Drank water	Did not drink water
Became ill	52	21
Did not become ill	32	64

$$\text{Calculation of the odds ratio (OR): } \frac{a/c}{b/d} = \frac{52/21}{32/64} = 4.95 = 5$$

The OR clearly establishes a strong connection between exposure (water) and the prevalence of disease.

In an actual study, there may be a number of possible sources for the disease agent including food, insects, and personal associations, to name a few. With the category of food, many subsets are possible, including salads, meats, breads, juices, milk, and so on. Each of the sources deserves consideration as a vehicle or vector, depending on the nature of the suspected disease agent. Case-control studies can be constructed to test any and all of the potential sources of the disease agent. The odds ratios can then be statistically analyzed to narrow the field of suspected sources. Usually, the statistical evaluation is performed at the 95 percent confidence level ($p < 0.05$).

In the previous example of a case-control study in connection with a waterborne outbreak, cases of the disease had been established. Now consider a situation where the town health officer released advance information to a population of people that a wastewater cross-connection was found to have leaked at some point in the distribution system. These conditions may provide the opportunity for a cohort study, which is an observational study in which two or more groups of persons who are free of disease and differ by extent of exposure to a potential cause of a disease are compared over time with respect to the incidence of the disease.[140] In our example, this would be a prospective investigation of a group (cohort) of healthy people known to have been exposed to contaminated water. The object of the study would be to follow the course of events to evaluate the appearance of illness in the exposed population and determine if consuming the contaminated drinking water posed a risk for illness. In the cohort study, it

is of interest to determine the incidence of disease in the exposed group vs. the unexposed group. To do this, a 2 × 2 table is constructed as previously illustrated and a relative risk (RR) is determined. Relative risk cannot be established for a case-control study because members of the case-control population are not random samples of the *entire* community population.

To illustrate the calculation of RR, a hypothetical situation is presented below. The same data as for the case-control study was used for comparative purposes:

52 people drank contaminated water and became clinically ill. (a)

32 people drank contaminated water and did not become ill. (b)

21 people did not drink contaminated water and became ill. (c)

64 people did not drink contaminated water and did not become ill. (d)

The 2 × 2 table is constructed to display the data.

	Drank water	Did not drink water
Became ill	52	21
Did not become ill	32	64

Calculation of the RR value involves the ratio of the exposed group as a proportion of the population examined to the unexposed group as a proportion of the population examined:

$$\text{RR} = \frac{a/(a+b)}{c/(c+d)} = \frac{52/(52+32)}{21/(21+64)} = \frac{0.62}{0.24} = 2.6$$

The RR establishes that the relative risk of becoming ill for the group of people exposed to contaminated water as opposed to the group of people not exposed to contaminated water is 2.6.

Two types of information regarding disease in a population that can be helpful to an epidemiological study are incidence rate and prevalence rate. Incidence rate is defined as the number of new cases per unit of person-time at risk. For example, suppose the waterborne outbreak used in the previous examples occurred in a stable community of 10,000 people. Following the outbreak, the number of new cases occurring over a five-year period was 30 per 10,000 people. These new cases might have nothing to do with consuming water, but the waterborne incident might have established some carriers of the disease within the population that could contribute to the infection of others. In this example, the incidence rate of the disease in the community would be 6 cases per 10,000 people-years; the expression *people-years* arriving from the normalization of the 30 disease cases over a five-year period.

Prevalence rate is something different from incidence rate because prevalence rate concerns the actual number of disease cases in a community. In the case

of the waterborne outbreak, there were 73 cases of the disease. Supposing that secondary infections occurred among the population to add another 43 cases of the disease bringing the total to 116 cases of the disease for the year. In the community of 10,000 people, the prevalence rate of the disease for the year of the outbreak would be 1 percent.

The incidence rate can be determined for both the exposed and unexposed individuals identified with the waterborne outbreak above. Looking at the data, we find that 52 people became sick out of 84 people that drank water and 21 people became sick out of 85 people that did not drink water. The incidence rate for the two subgroups of individuals is 62 percent and 25 percent, respectively. From these data, an attributable risk can be determined by subtracting the incidence rate of nondrinkers from drinkers of the water, which would be 37 percent.

Incidence measures reflect the level of infectivity of the causative agent of the disease. They do not establish the virulence of the causative agent because virulence relates to the damage produced as a result of the infection. Damage resulting from infection of an individual can range from a few mild symptoms to life-threatening symptoms, depending on many contributing factors (e.g., health and nutrition status, age, infectious dose of the pathogen received, how the pathogen was received, genetic disposition and others). In the study of an outbreak, a case is defined not by the severity of the infection but by the fact that an infection occurred.

The subject of risk assessment has advanced considerably in the last 20 years. Mathematical models have been constructed to estimate the probability of infection using databases of human exposure. Before models could be formulated it was necessary to ascertain the variables of the infection process. In the case of microbial risk assessment, such variables might include etiologic disease agent identification, human health effects manifested through infection, dose-response data relating dose received and probability of infection/disease in the target population, physiology of host-parasite relations, and epidemiological data.[26]

Molecular Detection of Waterborne Pathogens

Water, especially drinking water, when under suspicion of the transmission of pathogens, requires laboratory examination for proof of contamination. Cultural methods may prove inadequate for the isolation of pathogens, may produce uncertain results, or may be too time-consuming to support ongoing epidemiological investigations. During the past three decades, environmental laboratories have exploited molecular-based protocols to gain insight into the presence of sundry infectious bacteria, viruses, and protozoa in aquatic environments and water supplies. These techniques can be useful to investigations of disease outbreak, especially, where no cultural evidence can be obtained to show the existence of an infectious agent. In fact, a fundamental challenge in proving the hypothesis that a disease outbreak has occurred is to establish conclusively that the suspected agent of disease existed at the suspected source of the disease. A broad range of sophisticated laboratory techniques, such as fluorescent antibody,

enzyme-linked immunosorbent assay (ELISA), fluorescent *in situ* probe (FISH), flow cytometry, and the polymerase chain reaction (PCR), are available to provide answers not possible by classical measures. From these has emerged a branch of epidemiology called molecular epidemiology. Routine use of molecular tools is nonexistent in many health laboratories, however, owing to the requirement for relatively expensive equipment, need to employ technicians knowledgeable about molecular techniques, and the technical issues surrounding detection of specific genomes present in very low levels in water. Despite these apparent limitations to adopting molecular techniques for routine surveillance of pathogens in water-quality-control laboratories, molecular protocols have been used to detect a wide range of pathogenic agents in waters.

A brief introduction to molecular methods for microbiological investigation in the water environment is given based on descriptions by Rochelle and Schwab.[141]

Sample Collection Proper procedures for obtaining water samples are independent of the intended use of water. However, taking advantage of the sensitivity of molecular detection implies that the target organism is probably in very low in concentration, else it might be prudent to employ a cultural technique (assuming the target microorganism or virus is in a viable/recoverable state). Therefore, sample volumes earmarked for molecular applications are usually large and will require concentration of contents.

Sample Concentration Large water samples are processed by filtration procedures applicable to bacteria, protozoa, or viruses.

Nucleic Acid Extraction The material of interest to be assayed by molecular techniques is deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Extraction of nucleic acids from filtered/centrifuged biomass containing the target organism of interest may take place directly or following repeated elution and centrifugation steps (principally required for virus recovery). Ideally, the extraction step will be minimally time consuming, produce a high yield of intact nucleic acid, and preclude carryover of inhibitory substances inimical to the polymerase chain reaction (PCR) analysis. Special procedures can be introduced prior to nucleic acid extraction for removal of inhibitors. Published protocols and commercial kits may be used for postextraction purification of nucleic acids to eliminate inhibitors.

Methods of Detection The basic approach to assaying purified target nucleic acid is the application of PCR. The purpose of PCR is to amplify the nucleic acid of the target organism so that workable quantities of product become available for subsequent sequence analysis. It is important that the PCR procedure be sensitive and specific. PCR assays are typically operated in three cycles of temperature to accommodate three steps:

1. Denaturation of the double-stranded, target DNA (92°–94°C)
2. Annealing of specific primers to the single-strand form (denatured) of the target DNA at some prescribed or trial-and-error temperature (45°–55°C)

3. Complementary strand synthesis by primer extension of each of the single strands produced by step 1 at a temperature of 75°–80°C

The three-step procedure is repeated usually 30 to 40 times in order to obtain exponential copies of PCR product. The two important variables in successful use of PCR as a tool are primer synthesis or selection and PCR operating conditions. These two factors dictate the level of specificity and sensitivity that can be obtained by PCR and are instrumental in facilitating the detection of target nucleic acids at refined taxonomic levels.

Evaluation of PCR Products The purpose of amplifying target nucleic acids present in the environmental sample is to be able to subject a sufficient quantity of the representative material (PCR product) to a laboratory procedure for the determination of the microbial agent that it represents. Classic procedures for this purpose involve application of a series of concentrations of the PCR products to an agarose gel electrophoresis slab along with a molecular marker. Various amplified gene fragments migrate through the gels in proportion to their molecular weights. The separated gene fragments can then be confronted with an oligonucleotide probe specific for the organism of interest in relation to its possible presence in the original water sample. Oligonucleotide probes are conjugated with a reporter molecule (typically a fluorogenic compound) that under appropriate conditions (fluorescent lighting) signals hybridization with a complementary (target) nucleic acid fragment.

Two areas of interest in connection with molecular detection of specific microbial agents in environmental samples are robustness of the detection effort and the level or density of the target microbe in the representative environmental sample. In the former, since molecular detection is a gene-based exercise, it stands to reason that the more types of gene fragments that are available as probes, the more information that can be learned about the genome of the target organism. The technique that makes use of the multiple probe approach is the microarray. The microarray is a glass microscope slide that serves as a solid support for the spotting of literally thousands of genes or gene fragments—in this example, oligonucleotide probes—that serve to test hybridization potential with amplified gene fragments (PCR products) of unknown identity. The nucleotide sequence of the probe is known and representative of specific microbes. The location of each of the probes on the glass slide is carefully recorded, so when hybridization with unknown PCR products (amplicons) is indicated by reporter signals, the strain, species, and genus identity of the unknown amplicon can be learned.

Quantification of the target microbe in the environment with the aid of a PCR instrument must involve procedural modifications and special equipment in order to measure the level of production of PCR products. Fluorogenic probes and a fluorescence detection device are used to track the formation of PCR product formation. Quantitative PCR (qPCR) is still relatively new, and advances are being made to increase its utility. The following brief description is based on methodology described by Grove.[142] In the qPCR process, two fluorogenic probes anneal

to the template nucleic acid between the primers. As the nucleic acid polymerase extends the primer, the probe is displaced, and the polymerase cleaves the fluorogenic dye. Released dye is freed from the quencher and a fluorescent signal is produced. The detection device consists of a multiwell thermal cycler connected to a laser and a charge-coupled optics system. A fiber optic inserted through a lens is positioned over each of the wells, and a laser beam is directed through the fiber to excite fluorochrome in the PCR fluid present in wells. Fluorescence emissions are sent through the fiber to the CCD camera, mathematically analyzed by the system software, and the data are computerized.

Obtaining quantitative data on the original sample requires construction of a calibration curve. This is done by preparing dilutions of a known quantity of nucleic acid and performing PCR. Emissions data are obtained for each dilution of the nucleic acid and plotted against thermal cycle numbers. A series of curves result, and a line is drawn through the curves parallel to the thermal cycle numbers (x axis) at a height just above the background fluorescence (Figure 1.8). Another line is drawn perpendicular to the thermal cycles (x axis) at the intersection of the parallel line and each of the curves representing the nucleic acid dilutions. The thermal cycle number corresponding to each curve is the threshold cycle (Ct). The calibration curve is a plot of each Ct value against the corresponding nucleic acid concentration in the dilution series. The Ct is inversely proportional to the copy number (concentration) of nucleic acids in the dilution series, so a straight line should result. The actual concentration of nucleic acid in the unknown sample is determined by obtaining a Ct value under identical conditions of PCR operation

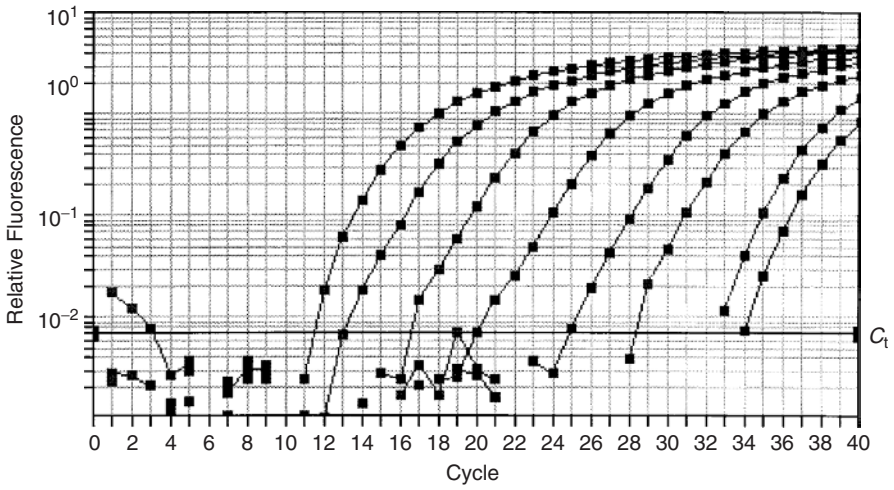


FIGURE 1.8 Family of fluorescence emission curves prepared from dilutions of nucleic acid for the determination of threshold cycle values. (*Source*: *D. S. Grove, "Quantitative Real-Time Polymerasae Chain Reaction for the Core Facility Using TaqMan and the Perkin-Elmer/Applied Biosystems Division 7700 Sequence Detector," J. Biomol. Tech, 10 (1999): 11–16.*)

as took place for the known dilution series and the nucleic acid concentration represented by the Ct value is read from the calibration curve.

Quality control and assurance is uppermost in all phases of PCR methodology. Prospective analysts should be aware of the U.S. Environmental Protection Agency publication "Quality assurance/quality control guidance for laboratories performing PCR analyses on environmental samples," available at http://www.epa.gov/nerlc/cwww/qa_qc_pcr10_04.pdf.

Advances in molecular methods of detecting and quantifying microorganisms should be powerful assets to modern environmental epidemiology. The potential exists for analyzing samples for the presence of suspect pathogens in water supplies with far greater certainty than can occur by conventional methods.

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