
5 Water Contaminants

ARE WE TO WAIT UNTIL ALL FROGS “CROAK”?*

Are we to wait until all frogs “croak”?

The earliest chorus of frogs—those high-pitched rhapsodies of spring peepers, those “jug-o-rum” calls of bullfrogs, those banjo-like bass harmonies of green frogs, those long and guttural cadences of leopard frogs, their singing—a prelude to the splendid song of birds beside an otherwise still pond on an early spring evening—heralds one of nature’s most dramatic events: metamorphosis. This metamorphosis begins with masses of eggs that soon hatch into gill-breathing, herbivorous, fishlike tadpole larvae. As they feed and grow, warmed by the spring sun, almost imperceptibly a remarkable transformation begins. Hind legs appear and gradually lengthen. Tails shorten. Larval teeth vanish, and lungs replace gills. Eyes develop lids. Forelegs emerge. In a matter of weeks, the aquatic, vegetarian tadpole (should it escape the many perils of the pond) will complete its metamorphosis into an adult, carnivorous frog.

This springtime metamorphosis is special. This anticipated event (especially for the frog) marks the end of winter, the rebirth of life, a rekindling of hope (especially for mankind). This yearly miracle of change sums up in a few months each spring what occurred over 3000 million years ago, when the frog evolved from its ancient predecessor. Today, however, something is different, strange, and wrong with this striking and miraculous event.

In the first place, where are all the frogs? Where have they gone? Why has their population decreased so dramatically in recent years? The second problem is that this natural metamorphosis process (perhaps a reenactment of some Paleozoic drama whereby, over countless generations, the first amphibian types equipped themselves for life on land) now demonstrates aberrations of the worst kind, of monstrous proportions and with dire results for frog populations in certain areas. The U.S. Environmental Protection Agency has received reports about deformed frogs in certain sections of the United States, particularly Minnesota, as well as in Canada and parts of Europe.

Most of the deformities have been in the rear legs and appear to be developmental. The question is why? Researchers have noted that neurological abnormalities have also been found. Again, the question is why?

Researchers have pointed the finger of blame at parasites, pesticides and other chemicals, ultraviolet radiation, acid rain, and metals. Something is going on. What is it? We do not know!

* Adapted from Spellman, F.R., *The Science of Water*, 3rd ed., CRC Press, Boca Raton, FL, 2015.

The next question, then, is what are we going to do about it? Are we to wait until all the frogs croak before we act—before we find the source, the cause, the polluter—before we see similar results in other species ... maybe our own?

The final question is obvious: When frogs are forced by mutation into becoming something else, is this evolution by gunpoint?

Are humans holding the gun?

WATER CONSTITUENTS

To prevent the croaking of frogs and the rest of us, it is extremely important (to all of us) to ensure that we are ingesting water and other fluids and foods that will do us no harm. With regard to our drinking water supplies and other water or fluid usage, there are a number of steps we can take to ensure our health and well-being, and many of them are discussed in this book, including pretreatment, treatment, and posttreatment technology that works (when properly applied and operated) to ensure our safety. Before we discuss reverse osmosis (RO) system applications, how the technology is used to clean up our drinking water, and its other important uses, we must have some basic appreciation for and knowledge of the constituents and contaminants contained or possibly contained in our water.

On a personal note, in the not too distant past, while teaching a college classroom full of environmental health, science, and engineering undergraduate and graduate students, I was lecturing on water quality. Specifically, I was discussing the major water constituents and contaminants that can be present in many sources of freshwater selected to be used for drinking water. In my mind, the only thing worse than presenting a monotone lecture for three hours is having to *listen* to the same. So, to capture students' attention and to ensure that something (anything) I present to them registers on their brain cells and keeps them off their iPads and awake, I have found that storytelling (with silly embellishments that they can relate to) is a great attention getter; it actually works.

In the beginning minutes of this particular lecture session, when I mentioned that there is no such thing as naturally pure water at ground level on Earth, I was instantly flagged down (as I knew I would be) by one of my super-smart (really—all my students are smart) undergrad students who was stirring up that butterfly effect of flapping wings we all feel even when flapped at a great distance from us. However, instead of flapping filamentous wings in South America, she was flapping both arms to get my total attention. This she did because I usually notice what a student who sits directly in front of me during a lecture is doing, or not doing.

So, I paused and acknowledged that the over-eager student had the floor. I asked if she had a question (don't they all)?

"No, no, not a question ... but I completely disagree [I love when this happens] with your statement that there is no such thing as perfectly clear or pure rainwater."

"Oh," I said. "Please explain what you mean."

I noticed that she had the full attention of all 78 students, all of whom were eagerly awaiting her explanation. They were hooked; it does not get any better than this.

The student stated, "Well, my family and I collect rainwater in barrels that we have placed below our house gutters and downspouts."

“Yes,” I said.

“Yes,” she replied. “You see, my family and I do not like to drink tapwater or that phony bottled water, so we collect and drink pure rainwater.”

“What makes you think rainwater is pure?” I asked.

“Well, professor, any fool [giggles and laughter from the students; a deadpan look from me], make that *anyone*, knows that water from the heavens above has not had the opportunity to be contaminated from exposure to human pollution.”

“Hmmm. I see. Well, have you ever tested your collected rainwater to determine its purity?”

“Nope, no need. When it falls from the sky, well, it just has to be clean and pure and wholesome—the purest form of water available on the Earth. The fact is that evaporation of the water due to the sun allows any contaminants to be left behind. Rainwater is much better than any of that water that is treated at the local waterworks or bottled water that is supposedly taken from some pure mountain stream.”

“I see,” I said. To myself I thought: Let her dig the hole deeper so that only I can bale her out with the facts ... and some common sense.

She continued, “We also use the rainwater for our garden vegetables so that they are not contaminated by that horrible tapwater stuff. You know, they add disinfectant to it. It just about gags a maggot to drink it, and it stinks terrible.”

I have to admit that after this particular comment I stood in front of that classroom full of bright-eyed and bushy-tailed students almost in total shock. Almost. Now I am certain that there are those out there in la-la land reading this account and wondering how I was going to counter-argue the student’s assertion that rainwater is the purest form of water available on Earth. Well, in the first place, at my advanced age and having been the recipient of countless amounts of real-world experience, both good and bad, I had learned not to argue with anyone. Why should I? Experience has taught me that calmly showing and telling can be a hundred times more effective as a training tool than any form or amount of argument. Thus, after regaining my cool, I turned to my usual friends to help me show the student and her fellow students the error of her statements. The friends? Well, for me that could only be the Rabbit and the Grasshopper, as well as, in this particular instance, the addition of Mr. Squirrel, of course.

As a learned rebuttal, I related the following to that classroom of some of America’s finest.

A RECENT RABBIT AND GRASSHOPPER CONVERSATION

Grasshopper stated to his friend Rabbit: “You know, dear friend, I am careful, down-right cautious, about the water I drink and wallow in at times, including rainwater.”

Rabbit replied, “I’ve always preferred rainwater myself because it is so pure.”

While they continued their discussion, Mr. Squirrel scampered from a copse of vine-maple below them, and Grasshopper and Rabbit watched him move off to the left. Mr. Squirrel, seemingly in a hurry, was constantly jerking his head to the right, over his shoulder. He dashed toward the marshy bank and stopped to sniff the ground. Some noise or an odor carried on the wind suddenly seemed to startle Mr. Squirrel, and he caught sight of Grasshopper and Rabbit. He scurried toward them and away from the tall marsh grass.

Mr. Squirrel said, “Hi, friends! What you up to?”

Grasshopper and Rabbit replied in unison that, “We were just discussing the purity of rainfall for drinking and just generally wallowing around in.”

Mr. Squirrel looked at his two friends in wonder while scratching at some itch near his bushy tail. “The purity of rainwater? Gee, the only concern I ever have about rainwater is will there be enough of it? Will it rain enough for me to quench my thirst? I’m not worried about purity. If it’s wet, that’s all that matters to me.”

“Well,” Rabbit stated, “I just think that the water we take from humans’ water bottles and sinks and hoses and puddles is not as good as basic rainwater. Rainwater is the purest form of water available on Earth, or so they say. Except for Grasshopper, that is. He thinks I might be mistaken about the purity of rainwater. What do you think, Mr. Squirrel?”

Looking all bright-eyed and bushy-tailed (remember this saying originated with the accepted description of a squirrel) and full of energy, vigor, and more energy, Mr. Squirrel replied: “Hey, wet is wet, and I love to drink up rainwater, any and all rainwater. Who cares about purity?”

After deliberate and well-practiced thumping of his foot, Rabbit replied to his friend, Mr. Squirrel: “Grasshopper has told me that, although rainwater is considered the purest form of water, it is often much less than pure when it reaches the surface of the Earth. As rain moves through the atmosphere, it picks up particles and impurities that are in the air. Chemicals and pollutants are among the impurities that can be picked up by the raindrops. These chemicals can drastically change the purity of water as it falls toward the ground.”

“Wow,” said Mr. Squirrel, while looking for the nearest puddle to quench his thirst.

Rabbit asked, “So, what do you think now, Mr. Squirrel?”

Pausing a moment to scratch again and having spotted a puddle a few feet away, Mr. Squirrel said, “Right on, Grasshopper!” and scampered away to the puddle.

Classroom stories like this one actually work and can deliver the intended message. For some reason, the students understand and get the message better. I have found out that one good Grasshopper, Rabbit, and Mr. Squirrel story is better than any lecture I have ever given or ever could give. There has never been a failure to communicate with my students.

Let’s fast forward to our discussion of water constituents. Natural water, whether rainwater or not, can contain a number of substances, or what we call impurities or *constituents*. When a particular constituent can affect the quality or health of the water user, it is called a *contaminant* or *pollutant*. It is these contaminants that the environmental specialist or waterworks operator tries to prevent from entering the drinking water supply.

SOLIDS

Other than gases, all contaminants of water contribute to the solids content. Natural waters carry a lot of dissolved solids and non-dissolved solids. The non-dissolved solids are nonpolar substances and relatively large particles of materials, such as *silt*, that will not dissolve. Classified by their size and state, by their chemical characteristics,

and by their size distribution, solids can be dispersed in water in both suspended and dissolved forms. With regard to size, solids in water can be classified as

- Suspended solids
- Settleable solids
- Colloidal solids
- Dissolved solids

Total solids are those solids, both suspended and dissolved, that remain behind when the water is removed by evaporation. Solids are also characterized as being *volatile* or *nonvolatile*. Colloidal solids are extremely fine suspended solids less than 1 micron in diameter that can still make water cloudy; they are so small they will not settle even if allowed to sit quietly for days or weeks.

Note: Though not technically accurate from a chemical point of view because some finely suspended material can actually pass through the filter, *suspended solids* are defined as those that can be filtered out in the suspended solids laboratory test. The material that passes through the filter is defined as *dissolved solids*.

Turbidity

One of the first characteristics people notice about water is its *clarity*. Turbidity is a condition in water caused by the presence of suspended matter, resulting in the scattering and absorption of light rays. In plain English, turbidity is a measure of the light-transmitting properties of water. Natural water that is very clear (low turbidity) allows one to see images at considerable depths. High-turbidity water appears cloudy. Even water with low turbidity, however, can still contain dissolved solids. Dissolved solids do not cause light to be scattered or absorbed; thus, the water looks clear. High turbidity causes problems in water treatment because the components that cause high turbidity can cause taste and odor problems and will reduce the effectiveness of disinfection.

Color

Water can be colored, but often the color of water can be deceiving. In fact, color is considered an aesthetic quality of water and has no direct health impact. Many of the colors associated with water are not “true” colors but the result of colloidal suspension. This *apparent color* can be attributed to dissolved tannin extracted from decaying plant material. *True color* is the result of dissolved chemicals, most often organics that cannot be seen.

DISSOLVED OXYGEN

Gases can be dissolved in water; for example, gases such as oxygen, carbon dioxide, hydrogen sulfide, and nitrogen dissolve in water. Gases dissolved in water are important. Carbon dioxide, for example, is important because of the role it plays in pH and alkalinity. Carbon dioxide is released into the water by microorganisms and

TABLE 5.1
Common Metals Found in Water

Metal	Health Hazard
Barium	Circulatory system effects and increased blood pressure
Cadmium	Concentration in the liver, kidneys, pancreas, and thyroid
Copper	Nervous system damage and kidney effects; toxic to humans
Lead	Same as copper
Mercury	Central nervous system (CNS) disorders
Nickel	CNS disorders
Selenium	CNS disorders
Silver	Gray skin
Zinc	Taste effects; not a health hazard

consumed by aquatic plants. Dissolved oxygen (DO) in water is of most importance to waterworks operators because it is an indicator of water quality. Just as solutions can become saturated with solute, this is also the case with water and oxygen. The amount of oxygen that can be dissolved at saturation depends on the temperature of the water. In the case of oxygen, however, the effect is just the opposite of other solutes. The higher the temperature, the lower the saturation level; the lower the temperature, the higher the saturation level.

METALS

Metals are constituents or impurities often carried by water. At normal levels, most metals are not harmful, but a few metals can cause taste and odor problems in drinking water. Some metals may be toxic to humans, animals, and microorganisms. Most metals enter water as part of compounds that ionize to release the metal as positive ions. Table 5.1 lists some metals commonly found in water and their potential health hazards.

ORGANIC MATTER

Organic compounds contain the element carbon and are derived from material that was once alive (i.e., plants and animals). Organic compounds include fats, dyes, soaps, rubber products, plastics, wood, fuels, cotton, proteins, and carbohydrates. Organic compounds in water are usually large, nonpolar molecules that do not dissolve well in water. They often provide large amounts of energy to animals and microorganisms.

INORGANIC MATTER

Inorganic matter or inorganic compounds are carbon free, not derived from living matter, and easily dissolved in water. Inorganic matter is of mineral origin and includes acids, bases, salts, etc. Several inorganic components are important in establishing and controlling water quality.

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TABLE 5.2
Relative Strengths of Acids in Water

Acid	Formula
Perchloric acid	HClO ₄
Sulfuric acid	H ₂ SO ₄
Hydrochloric acid	HCl
Nitric acid	HNO ₃
Phosphoric acid	H ₃ PO ₄
Nitrous acid	HNO ₂
Hydrofluoric acid	HF
Acetic acid	CH ₃ COOH
Carbonic acid	H ₂ CO ₃
Hydrocyanic acid	HCN
Boric acid	H ₃ BO ₃

Acids

An acid is a substance that produces hydrogen ions (H⁺) when dissolved in water. Hydrogen ions are hydrogen atoms that have been stripped of their electrons. A single hydrogen ion is nothing more than the nucleus of a hydrogen atom. Lemon juice, vinegar, and sour milk are acidic or contain acid. The common acids used in treating water are hydrochloric acid (HCl), sulfuric acid (H₂SO₄), nitric acid (HNO₃), and carbonic acid (H₂CO₃). Note that in each of these acids, hydrogen (H) is one of the elements. The relative strengths of acids in water, listed in descending order of strength, are classified in Table 5.2.

Bases

A base is a substance that produces hydroxide ions (OH⁻) when dissolved in water. Bitter things, such as lye or common soap, contain bases. The bases used in waterworks operations are calcium hydroxide, Ca(OH)₂; sodium hydroxide, NaOH; and potassium hydroxide, KOH. Note that the hydroxyl group (OH) is found in all bases. In addition, note that bases contain metallic substances, such as sodium (Na), calcium (Ca), magnesium (Mg), and potassium (K). These bases contain the elements that produce the alkalinity in water.

Salts

When acids and bases chemically interact, they neutralize each other. The compounds (other than water) that form from the neutralization of acids and bases are salts. Salts constitute, by far, the largest group of inorganic compounds. A common salt used in waterworks operations, copper sulfate, is utilized to kill algae in water.

pH

pH is a measure of the hydrogen ion (H⁺) concentration. Solutions range from very acidic (having a high concentration of H⁺ ions) to very basic (having a high concentration of OH⁻ ions). The pH scale ranges from 0 to 14, with 7 being the neutral

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value. The pH of water is important to the chemical reactions that take place within water, and pH values that are too high or low can inhibit the growth of microorganisms. High pH values are considered basic, and low pH values are considered acidic. Stated another way, low pH values indicate a high H^+ concentration, and high pH values indicate a low H^+ concentration. Because of this inverse logarithmic relationship, there is a tenfold difference in H^+ concentration.

Natural water varies in pH depending on its source. Pure water has a neutral pH, with an equal number of H^+ and OH^- . Adding an acid to water causes additional positive ions to be released, so the H^+ ion concentration goes up and the pH value goes down. Changing the hydrogen ion activity in solutions can shift the chemical equilibrium of water. Thus, pH adjustment is used to optimize coagulation, softening, and disinfection reactions, as well as for corrosion control. To control water coagulation and corrosion, it is necessary for the waterworks operator to test for the hydrogen ion concentration of the water to get the pH. In coagulation tests, as more alum (acid) is added, the pH value is lowered. If more lime (alkali, or base) is added, the pH value is raised. This relationship is important, and if good floc is formed the pH should then be determined and maintained at that pH value until there is a change in the new water.

ALKALINITY

Alkalinity is defined as the capacity of water to accept protons (positively charged particles); it can also be defined as a measure of the ability of the water to neutralize an acid. Stated in even simpler terms, alkalinity is a measure of the capacity of the water to absorb hydrogen ions without a significant pH change (i.e., to neutralize acids). Bicarbonates, carbonates, and hydrogen cause alkalinity compounds in a raw or treated water supply. Bicarbonates are the major components, because of the action of carbon dioxide on the basic materials of soil; borates, silicates, and phosphates may be minor components. Alkalinity of raw water may also contain salts formed from organic acids, such as humic acid. Alkalinity in water acts as a *buffer* that tends to stabilize and prevent fluctuations in pH. It is usually beneficial to have significant alkalinity in water because it would tend to prevent quick changes in pH. Quick changes in pH interfere with the effectiveness of the common water treatment processes. Low alkalinity also contributes to the corrosive tendencies of water. When alkalinity is below 80 mg/L, it is considered to be low.

HARDNESS

Hardness may be considered a physical or chemical parameter of water. It represents the total concentration of calcium and magnesium ions, reported as calcium carbonate. Hardness causes soaps and detergents to be less effective and contributes to scale formation in pipes and boilers. Hardness is not considered a health hazard; however, water that contains hardness must often be softened by lime precipitation or ion exchange. Low hardness contributes to the corrosive tendencies of water. Hardness and alkalinity often occur together because some compounds can contribute both alkalinity and hardness ions. Hardness is generally classified as shown in Table 5.3.

TABLE 5.3
Water Hardness Classification

Classification	mg/L CaCO_3
Soft	0–75
Moderately hard	75–150
Hard	150–00
Very hard	Over 300

IMPORTANT PROPERTIES OF WATER

SOLUBILITY

Compounds that can form hydrogen bonds with water tend to be far more soluble in water than compounds that cannot form hydrogen bonds.

SURFACE TENSION

Water has a high surface tension. Surface tension governs surface phenomena and is an important factor in physiology.

DENSITY

Density is mass per unit volume. Water has its maximum liquid density at 4°C. When water freezes, the resulting ice floats.

BOILING POINT

In general, the boiling point increases with molecular weight, but hydrogen bonding increases the boiling point of water above that predicted based on molecular weight alone.

HEAT CAPACITY

Heat capacity is the amount of energy required to raise the temperature of a substance 1°C. Water has a higher heat capacity than any other liquid except for ammonia. This attribute allows organisms and geographical regions to stabilize temperature.

HEAT OF VAPORIZATION

Heat of vaporization is the energy required to change a liquid to a vapor. Water has a higher heat of vaporization than any other material. This attribute affects the transfer of water molecules between surface water and the atmosphere.

LATENT HEAT OF FUSION

The heat of fusion is the energy required to change a substance from a solid (ice) to a liquid (water).

PHASE TRANSITIONS OF WATER

A phase transition is the spontaneous conversion of one phase to another that occurs at a characteristic temperature for a given pressure. For example, at 1 atm, ice is the stable phase of water below 0°C, but above this temperature the liquid is more stable. A phase diagram of water, for example, is a map of the ranges of pressure and temperature at which each phase of the water is the most stable.

WATER MICROBIOLOGY

Microorganisms are significant in water and wastewater because of their roles in disease transmission and they are the primary agents of biological treatment.

Note: To have microbiological activity, the body of water or wastewater must have the appropriate environmental conditions. The majority of wastewater treatment processes, for example, are designed to operate using an aerobic process. The conditions required for aerobic operation include (1) sufficient free, elemental oxygen; (2) sufficient organic matter (food); (3) sufficient water; (4) enough nitrogen and phosphorus (nutrients) to permit oxidation of the available carbon materials; (5) proper pH (6.5 to 9.0); and (6) lack of toxic materials.

MICROBIOLOGY: WHAT IS IT?

Biology is generally defined as the study of living organisms (i.e., the study of life). Microbiology is a branch of biology that deals with the study of microorganisms so small in size that they must be studied under a microscope. Microorganisms of interest to water and wastewater practitioners and those involved in the operation of RO systems include bacteria, protozoa, viruses, algae, and others. With regard to microbes, the primary concern is how to control microorganisms that cause waterborne diseases (waterborne pathogens) to protect the consumer (human and animal).

WATER AND WASTEWATER MICROORGANISMS

Microorganisms of interest to water and wastewater operators include bacteria, protozoa, viruses, algae, rotifers, fungi, and nematodes. These organisms are the most diverse group of living organisms on Earth and occupy important niches in the ecosystem. Their simplicity and minimal survival requirements allow them to exist in diverse situations. Because microorganisms are a major health issue, water treatment specialists are concerned about controlling the waterborne pathogens (e.g., bacteria, virus, protozoa) that cause waterborne diseases. The focus of wastewater operators, on the other hand, is on the millions of organisms that arrive at the plant with the influent. The majority of these organisms are nonpathogenic and beneficial to plant

operations. From a microbiological standpoint, the mix of microorganism species depends on the characteristics of the influent, environmental conditions, process design, and mode of plant operation. This mix may also include pathogenic organisms responsible for diseases such as typhoid, tetanus, hepatitis, dysentery, and gastroenteritis, among others.

To understand how to minimize or maximize the growth of microorganisms and control pathogens, one must study the structure and characteristics of the microorganisms. The sections that follow will look at each of the major groups of microorganisms (those important to water/wastewater operators) with regard to their size, shape, types, nutritional needs, and control.

Note: In a water environment, water is not a medium for the growth of microorganisms but is instead a means of transmission (that is, it serves as a conduit; hence, the name *waterborne*) of the pathogen to the place where an individual is able to consume it and thus begin an outbreak of disease (Koren, 1991). This is contrary to the view taken by the average person. That is, when the topic of waterborne disease is brought up, we might mistakenly assume that waterborne diseases are at home in water. Nothing could be further from the truth. A water-filled ambience is not the environment in which the pathogenic organism would choose to live, if it had such a choice. The point is that microorganisms do not normally grow, reproduce, languish, and thrive in watery surroundings. Pathogenic microorganisms temporarily residing in water are simply biding their time, going with the flow, waiting for their opportunity to meet up with their unsuspecting host or hosts. To a degree, when the pathogenic microorganism finds its host or hosts, it is finally home or may have found its final resting place. The good news is that when we incorporate RO membranes in the source feedwater system that supplies our potable water systems, because the membranes are semipermeable and not porous, they are able to screen pathogenic microorganisms and particulate matter from our water supplies. This is the case whether the pathogen is right at home or not; the bottom line is we do not want them in our homes (Spellman, 1997).

KEY TERMS

Algae, simple—Plants, many microscopic, containing chlorophyll. Freshwater algae are diverse in shape, color, size, and habitat. They are the basic link in the conversion of inorganic constituents in water into organic constituents.

Algal bloom—Sudden spurts of algal growth which can affect water quality adversely and indicate potentially hazardous changes in local water chemistry.

Anaerobic—Able to live and grow in the absence of free oxygen.

Autotrophic organisms—Produce food from inorganic substances.

Bacteria—Single-celled microorganisms that possess rigid cell walls. They may be aerobic, anaerobic, or facultative. They can cause disease, but some are important in pollution control.

Biogeochemical cycle—The chemical interactions among the atmosphere, hydrosphere, and biosphere.

Coliform organisms—Microorganisms found in the intestinal tract of humans and animals. Their presence in water indicates fecal pollution and potentially adverse contamination by pathogens.

DID YOU KNOW?

Reverse osmosis membranes provide between 4- and 5-log (i.e., 99.99 to 99.999%) removal of viruses normally associated with waterborne disease (Lozier et al., 1994).

Denitrification—The anaerobic biological reduction of nitrate to nitrogen gas.

Fungi—Simple plants lacking the ability to produce energy through photosynthesis.

Heterotrophic organism—Organisms that are dependent on organic matter for foods.

Prokaryotic cell—The simple cell type, characterized by a lack of nuclear membrane and the absence of mitochondria.

Virus—The smallest form of microorganisms capable of causing disease.

MICROORGANISM CLASSIFICATION AND DIFFERENTIATION

The microorganisms we are concerned with are tiny organisms that make up a large and diverse group of free-living forms; they exist as single cells, cell bunches, or clusters. Found in abundance almost anywhere on Earth, the vast majority of microorganisms are not harmful. Many microorganisms, or microbes, occur as single cells (unicellular), others are multicellular, and still others (viruses) do not have a true cellular appearance. A single microbial cell, for the most part, exhibits the characteristic features common to other biological systems, such as metabolism, reproduction, and growth.

CLASSIFICATION

Greek scholar and philosopher Aristotle classified animals based on fly, swim, and walk/crawl/run. For centuries thereafter, scientists simply classified the forms of life visible to the naked eye as either animal or plant. We began to have trouble differentiating microorganisms, though, so this system of classification had to be revised. The Swedish naturalist Carolus Linnaeus organized much of the current knowledge about living things in 1735. The importance of organizing or classifying organisms cannot be overstated, for without a classification scheme it would be difficult to establish a criteria for identifying organisms and to arrange similar organisms into groups. Probably the most important reason for classifying organisms is to make things less confusing (Wistreich and Lechtman, 1980). Linnaeus was quite innovative in his classification of organisms. One of his innovations still with us today is the *binomial system of nomenclature*. Under the binomial system, all organisms are generally described by a two-word scientific name: *genus* and *species*. Genus and species are groups that are part of a hierarchy of groups of increasing size, based on their taxonomy:

Kingdom
Phylum
Class
Order
Family
Genus
Species

Using this system, a fruit fly might be classified as

Animalia
Arthropoda
Insecta
Diptera
Drosophilidae
Drosophila
melanogaster

This means that this organism is the species *melanogaster* in the genus *Drosophila* in the family Drosophilidae in the order Diptera in the class Insecta in the phylum Arthropoda in the kingdom Animalia.

To further illustrate how the hierarchical system is exemplified by the classification system, the standard classification of the mayfly is provided below:

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Ephemeroptera
Family	Ephemeridae
Genus	<i>Hexagenia</i>
Species	<i>limbata</i>

Utilizing this hierarchy and Linnaeus’ binomial system of nomenclature, the scientific name of any organism includes both the generic and specific names. To uniquely name a species, it is necessary to supply both the genus and the species; for our examples, those would be *Drosophila melanogaster* for the fruit fly and *Hexagenia limbata* for the mayfly. The first letter of the generic name is usually capitalized; for example, *E. coli* indicates that *coli* is the species and *Escherichia* (abbreviated to *E.*) is the genus. The largest, most inclusive category is the kingdom. The genus and species names are always in Latin, so they are usually printed in italics. Some organisms also have English common names. Microbe names of particular interest in water/wastewater treatment include

- *Escherichia coli* (a coliform bacterium)
- *Salmonella typhi* (the typhoid bacillus)
- *Giardia lamblia* (a protozoan)

TABLE 5.4
Simplified Classification of Microorganisms

Kingdom	Members	Cell Classification
Animal	Rotifers	
	Crustaceans	
	Worms and larvae	
Plant	Ferns	Eucaryotic
	Mosses	
Protist	Protozoa	Prokaryotic
	Algae	
	Fungi	
	Bacteria	
	Lower algae forms	

- *Shigella* spp.
- *Vibrio cholerae*
- *Campylobacter*
- *Leptospira* spp.
- *Entamoeba histolytica*
- *Cryptosporidia*

Note: *Escherichia coli* is commonly referred to as simply *E. coli*, and *Giardia lamblia* is usually referred to by only its genus name, *Giardia*.

Generally, we use a simplified system of microorganism classification in water science by breaking down the classification into the kingdoms of Animalia, Plantae, and Protista. As a general rule, the animal and plant kingdoms contain all of the multicell organisms, and the protist kingdom includes all single-cell organisms. Along with a microorganism classification based on the animal, plant, and protist kingdoms, microorganisms can be further classified as being *eucaryotic* or *prokaryotic* (see Table 5.4).

Note: A eucaryotic organism is characterized by a cellular organization that includes a well-defined nuclear membrane. The prokaryotes have a structural organization that sets them off from all other organisms. They are simple cells characterized by a nucleus *lacking* a limiting membrane, an endoplasmic reticulum, chloroplasts, and mitochondria. They are remarkably adaptable and exist abundantly in the soil, sea, and freshwater.

DIFFERENTIATION

Differentiation among the higher forms of life is based almost entirely on morphological (form or structure) differences; however, differentiation (even among the higher forms) is not as easily accomplished as we might expect, because normal variations among individuals of the same species occur frequently. Because of this variation, even within a species, securing accurate classifications when dealing with

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TABLE 5.5
Forms of Bacteria

Form	Technical Name		Example
	Singular	Plural	
Sphere	Coccus	Cocci	<i>Streptococcus</i>
Rod	Bacillus	Bacilli	<i>Bacillus typhosis</i>
Curved or spiral	Spirillum	Spirilla	<i>Spirillum cholera</i>

single-celled microscopic forms that present virtually no visible structural differences becomes extremely difficult. Under these circumstances, it is necessary to consider physiological, cultural, and chemical differences, as well as structure and form. Differentiation among the smaller groups of bacteria is based almost entirely on chemical differences.

BACTERIA

The simplest wholly contained life systems are bacteria or prokaryotes, which are the most diverse group of microorganisms. They are among the most common microorganisms in water. They are primitive, unicellular (single-celled) organisms possessing no well-defined nucleus and presenting a variety of shapes and nutritional needs. Bacteria contain about 85% water and 15% ash or mineral matter. The ash is largely composed of sulfur, potassium, sodium, calcium, and chlorides, with small amounts of iron, silicon, and magnesium. Bacteria reproduce by binary fission.

Note: *Binary fission* occurs when one organism splits or divides into two or more new organisms.

Bacteria, once considered the smallest living organism (although now it is known that smaller forms of matter exhibit many of the characteristics of life), range in size from 0.5 to 2 μm in diameter and about 1 to 10 μm long.

Note: A *micron* is a metric unit of measurement equal to 1/1000 of a millimeter. To visualize the size of bacteria, consider that about 1000 average bacteria lying side by side would reach across the head of a straight pin.

Bacteria are categorized into three general groups based on their physical form or shape (although almost every variation has been found; see Table 5.5). The simplest form is the sphere. Spherical-shaped bacteria are called *cocci* (meaning “berries”). They are not necessarily perfectly round but may be somewhat elongated, flattened on one side, or oval. Rod-shaped bacteria are called *bacilli*. Spiral-shaped bacteria, called *spirilla*, have one or more twists and are never straight. Such formations are usually characteristic of a particular genus or species. Within these three groups are many different arrangements. Some exist as single cells; others as pairs, as packets of four or eight, as chains, or as clumps.

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Most bacteria require organic food to survive and multiply. Plant and animal material that gets into the water provides the food source for bacteria. Bacteria convert the food to energy and use the energy to make new cells. Some bacteria can use inorganics (e.g., minerals such as iron) as an energy source and can multiply even when organics (pollution) are not available.

Bacterial Growth Factors

Several factors affect the rate at which bacteria grow, including temperature, pH, and oxygen levels. The warmer the environment, the faster the rate of growth. Generally, for each increase of 10°C, the growth rate doubles. Heat can also be used to kill bacteria. Most bacteria grow best at neutral pH. Extreme acidic or basic conditions generally inhibit growth, although some bacteria may require acidic conditions and some alkaline conditions for growth.

Bacteria are aerobic, anaerobic, or facultative. If *aerobic*, they require free oxygen in the aquatic environment. *Anaerobic* bacteria exist and multiply in environments that lack dissolved oxygen. *Facultative* bacteria (e.g., iron bacteria) can switch from aerobic to anaerobic growth or grow in an anaerobic or aerobic environment.

Under optimum conditions, bacteria grow and reproduce very rapidly. As noted previously, bacteria reproduce by *binary fission*. An important point to consider with regard to bacterial reproduction is the rate at which the process can take place. The total time required for an organism to reproduce and the offspring to reach maturity is the *generation time*. Bacteria growing under optimal conditions can double their number about every 20 to 30 minutes. Obviously, this generation time is very short compared with that of higher plants and animals. Bacteria continue to grow at this rapid rate as long as nutrients hold out—even the smallest contamination can result in a sizable growth in a very short time.

Note: Even though wastewater can contain bacteria counts in the millions per milliliter, in wastewater treatment under controlled conditions bacteria can help to destroy and identify pollutants. In such a process, bacteria stabilize organic matter (e.g., activated sludge processes) and thereby assist the treatment process in producing effluent that does not impose an excessive oxygen demand on the receiving body. Coliform bacteria can be used as an indicator of pollution by human or animal wastes.

Destruction and Removal of Bacteria

In water and wastewater treatment, the destruction of bacteria is usually referred to as *disinfection*. Disinfection does not mean that all microbial forms are killed. That would be *sterilization*. Instead, disinfection reduces the number of disease-causing organisms to an acceptable number. Growing bacteria are generally easy to control by disinfection; however, some bacteria form survival structures known as *spores*, which are much more difficult to destroy. It is these survival structures that necessitate the use of RO membranes (and other membrane technologies) to remove them. RO systems are highly effective in removing bacterium such as *Campylobacter*, *Salmonella*, *Shigella*, and *E. coli*.

Note: Inhibiting the growth of microorganisms is termed *antiseptis*, whereas destroying them is called *disinfection*.

Waterborne Bacteria

All surface waters contain bacteria, and these waterborne bacteria are responsible for infectious epidemic diseases. Bacterial numbers increase significantly during storm events when streams are high. Heavy rainstorms increase stream contamination by washing material from the ground surface into the stream. After the initial washing occurs, few impurities are left to be washed into the stream, which may then carry relatively “clean” water. A river of good quality shows its highest bacterial numbers during rainy periods; however, a much-polluted stream may show the highest numbers during low flows because of the constant influx of pollutants. Water and wastewater operators are primarily concerned with bacterial pathogens responsible for disease. These pathogens enter potential drinking water supplies through fecal contamination and are ingested by humans if the water is not properly treated and disinfected.

Note: Regulations require that owners of all public water supplies collect water samples and deliver them to a certified laboratory for bacteriological examination at least monthly. The number of samples required is usually in accordance with federal standards, which generally require that one sample per month be collected for each 1000 persons served by the waterworks.

PROTOZOA

Reverse osmosis systems have a very high effectiveness in removing protozoa. Protozoans (or “first animals”) are a large group of eucaryotic organisms of more than 50,000 known species belonging to the kingdom Protista. They have adapted a form of cell that serves as the entire body; in fact, protozoans are one-celled, animal-like organisms with complex cellular structures. In the microbial world, protozoans are giants, many times larger than bacteria. They range in size from 4 to 500 μm . The largest ones can almost be seen by the naked eye. They can exist as solitary or independent organisms, such as the stalked ciliates (e.g., *Vorticella* sp.) (Figure 5.1), or they can colonize (e.g., the sedentary *Carchesium* sp.). Protozoa get their name because they employ the same type of feeding strategy as animals; that is, they are *heterotrophic*, meaning that they obtain cellular energy from organic substances such as proteins. Most are harmless, but some are parasitic. Some forms have two life stages: *active trophozoites* (capable of feeding) and *dormant cysts*.

The major groups of protozoans are based on their method of locomotion (motility). The Mastigophora are motile by means of one or more *flagella*, the whip-like projection that propels the free-swimming organisms (*Giardia lamblia* is a flagellated protozoan). The Ciliophora move by means of shortened modified flagella called *cilia*, which are short hair-like structures that beat rapidly and propel them through the water. The Sarcodina rely on *amoeboid movement*, which is a streaming or gliding action; the shape of amoebae changes as they stretch and then contract to move from place to place. The Sporozoa, in contrast, are nonmotile; they are simply swept along, riding the current of the water.

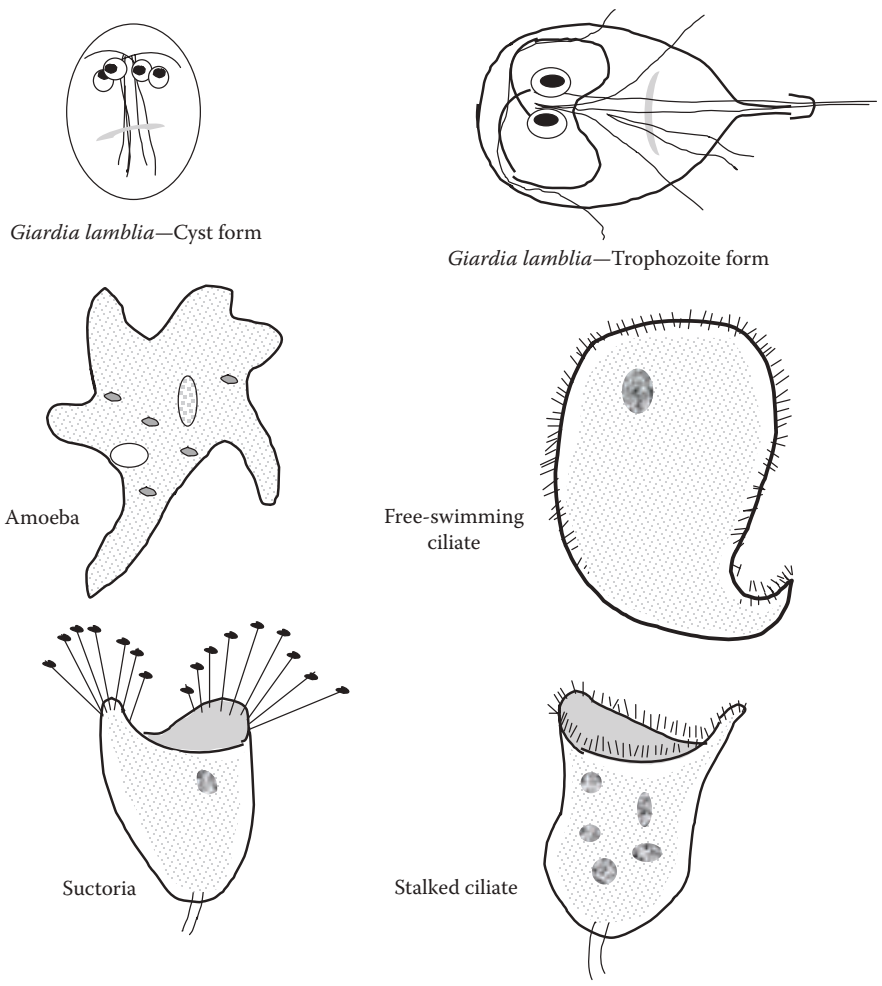


FIGURE 5.1 Protozoa.

Protozoa consume organics to survive; their favorite food is bacteria. Protozoa are mostly aerobic or facultative with regard to their oxygen requirements. Toxic materials, pH, and temperature affect protozoan rates of growth in the same way as they affect bacteria.

Most protozoan life cycles alternate between an active growth phase (*trophozoites*) and a resting stage (*cysts*). Cysts are extremely resistant structures that protect the organism from destruction when it encounters harsh environmental conditions—including chlorination.

Note: Protozoans that are not completely resistant to chlorination require higher disinfectant concentrations and longer contact time for disinfection than normally used in water treatment.

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DID YOU KNOW?

Nonpathogenic intestinal protozoa do not harm the body, even in people with weak immune systems.

The protozoans and associated waterborne diseases of most concern to waterworks operators include

- *Entamoeba histolytica* (amoebic dysentery)
- *Giardia lamblia* (giardiasis)
- *Cryptosporidium* (cryptosporidiosis)

In wastewater treatment, protozoa are a critical part of the purification process and can be used to indicate the condition of treatment processes. Protozoa normally associated with wastewater include amoebae, flagellates, free-swimming ciliates, and stalked ciliates.

Amoebae are associated with poor wastewater treatment of a young biosolids mass (see Figure 5.1). They move through wastewater by a streaming or gliding motion. Moving the liquids stored within the cell wall effects this movement. They are normally associated with an effluent high in biochemical oxygen demand (BOD) and suspended solids.

Flagellates (flagellated protozoa) have a single, long, hair-like or whip-like projection (flagellum) that is used to propel the free-swimming organisms through wastewater and to attract food (see Figure 5.1). Flagellated protozoans are normally associated with poor treatment and a young biosolids. When the predominant organism is flagellated protozoa, the plant effluent will contain high levels of BOD and suspended solids.

The *free-swimming ciliated protozoan* uses its tiny, hair-like projections (cilia) to move itself through the wastewater and to attract food (see Figure 5.1). This type of protozoan is normally associated with a moderate biosolids age and effluent quality. When the free-swimming ciliated protozoan is the predominant organisms, the plant effluent will normally be turbid and contain a high amount of suspended solids.

The *stalked ciliated protozoan* attaches itself to the wastewater solids and uses its cilia to attract food (see Figure 5.1). The stalked ciliated protozoan is normally associated with a plant effluent that is very clear and contains low levels of both BOD and suspended solids.

DID YOU KNOW?

Giardia lamblia is the most important cause of waterborne disease outbreaks in the United States.

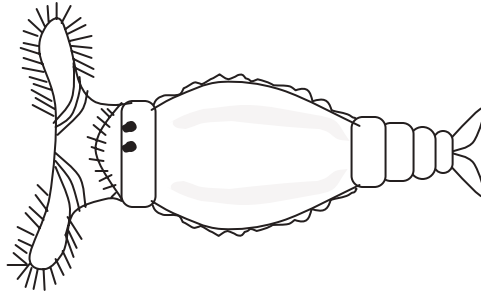


FIGURE 5.2 *Philodina*, a common rotifer.

Rotifers make up a well-defined group of the smallest, simplest multicellular microorganisms and are found in nearly all aquatic habitats (see Figure 5.2). Rotifers are a higher life form associated with cleaner waters. Normally found in well-operated wastewater treatment plants, they can be used to indicate the performance of certain types of treatment processes.

PATHOGENIC PROTOZOA

As mentioned, certain types of protozoans can cause disease. Of particular interest to the drinking water practitioner are *Entamoeba histolytica* (amoebic dysentery and amoebic hepatitis), *Giardia lamblia* (giardiasis), *Cryptosporidium* (cryptosporidiosis), and the emerging *Cyclospora* (cyclosporiasis). Sewage contamination transports eggs, cysts, and oocysts of parasitic protozoa and helminths (tapeworms, hookworms, etc.) into raw water supplies, leaving water treatment (in particular, filtration) and disinfection as the means by which to diminish the danger of contaminated water for the consumer.

Entamoeba histolytica is an amoeboid protozoan that lives in anaerobic environments. Like other pathogenic protozoa, *Entamoeba* is capable of forming cysts that can remain dormant for extended periods of time in the water. These cysts spread to new hosts when we ingest contaminated water.

To prevent the occurrence of *Entamoeba histolytica*, *Giardia*, and *Cryptosporidium* spp. in surface water supplies and to address increasing problems with waterborne diseases, the U.S. Environmental Protection Agency (USEPA) implemented the Surface Water Treatment Rule (SWTR) in 1989. The rule requires both filtration and disinfection of all surface water supplies as a means of primarily controlling *Giardia* and enteric viruses. Since implementation of the SWTR, the USEPA has also recognized that *Cryptosporidium* species are agents of waterborne disease. In its 1996 series of surface water regulations, the USEPA included *Cryptosporidium*.

To test the need for and the effectiveness of the USEPA's SWTR, LeChevallier et al. (1991) conducted a study on the occurrence and distribution of *Giardia* and *Cryptosporidium* organisms in raw water supplies to 66 surface water filter plants. These plants were located in 14 states and one Canadian province. A combined immunofluorescence test indicated that cysts and oocysts were widely dispersed

DID YOU KNOW?

Although most people who contract giardiasis recover naturally within a week or two, the illness sometimes lingers for up to a year.

in the aquatic environment. *Giardia* species were detected in more than 80% of the samples. *Cryptosporidium* species were found in 85% of the sample locations. Taking into account several variables, *Giardia* or *Cryptosporidium* species were detected in 97% of the raw water samples. After evaluating their data, the researchers concluded that the SWTR might have to be upgraded (subsequently, it has been) to require additional treatment.

Giardia

Giardia lamblia (also known as the hiker's/traveler's scourge or disease) is a microscopic parasite that can infect warm-blooded animals and humans. Although *Giardia* was discovered in the 19th century, not until 1981 did the World Health Organization (WHO) classify *Giardia* as a pathogen. An outer shell called a *cyst* allows *Giardia* to survive outside the body for long periods of time. If viable cysts are ingested, *Giardia* can cause the illness known as *giardiasis*, an intestinal illness that can cause nausea, anorexia, fever, and severe diarrhea. The symptoms last only for several days, and the body can naturally rid itself of the parasite in 1 to 2 months; however, for individuals with weakened immune systems, the body often cannot rid itself of the parasite without medical treatment.

In the United States, *Giardia* is the most commonly identified pathogen in waterborne disease outbreaks. Contamination of a water supply by *Giardia* can occur in two ways: (1) by the activity of animals in the watershed area of the water supply, or (2) by the introduction of sewage into the water supply. Wild and domestic animals are major contributors to the contamination of water supplies. Studies have also shown that, unlike many other pathogens, *Giardia* is not host specific. In short, *Giardia* cysts excreted by animals can infect and cause illness in humans. Additionally, in several major outbreaks of waterborne diseases, the *Giardia* cyst source was sewage-contaminated water supplies.

Treating the water supply, however, can effectively control waterborne *Giardia*. Chlorine and ozone are examples of two disinfectants known to effectively kill *Giardia* cysts. Filtration of the water can also effectively trap and remove the parasite from the water supply. The combination of disinfection and filtration is the most effective water treatment process available today for prevention of *Giardia* contamination.

In drinking water, *Giardia* is regulated under the SWTR. Although the SWTR does not establish a maximum contaminant level (MCL) for *Giardia*, it does specify treatment requirements to achieve at least 99.9% (3-log) removal or inactivation of *Giardia*. This regulation requires that all drinking water systems using surface water or groundwater under the influence of surface water must disinfect and filter the water. The Enhanced Surface Water Treatment Rule (ESWTR), which includes *Cryptosporidium* and further regulates *Giardia*, was established in December 1996.

Giardiasis

Giardiasis is recognized as one of the most frequently occurring waterborne diseases in the United States. *Giardia lamblia* cysts have been discovered in places as far apart as Estes Park, Colorado (near the Continental Divide); Missoula, Montana; Wilkes-Barre, Scranton, and Hazleton, Pennsylvania; and Pittsfield and Lawrence, Massachusetts, just to name a few (CDC, 1995).

Giardiasis is characterized by intestinal symptoms that can last a week or more and may be accompanied by one or more of the following: diarrhea, abdominal cramps, bloating, flatulence, fatigue, and weight loss. Although vomiting and fever are often listed as relatively frequent symptoms, people involved in waterborne outbreaks in the United States have not commonly reported them. Although most *Giardia* infections persist for only 1 or 2 months, some people experience a more chronic phase that can follow the acute phase or may become manifest without an antecedent acute illness. Loose stools and increased abdominal gassiness with cramping, flatulence, and burping characterize the chronic phase. Fever is not common, but malaise, fatigue, and depression may ensue; for a small number of people, the persistence of infection is associated with the development of marked malabsorption and weight loss (Weller, 1985). Similarly, lactose (milk) intolerance can be a problem for some people. This can develop coincidentally with the infection or be aggravated by it, causing an increase in intestinal symptoms after ingestion of milk products.

Some people may have several of these symptoms without evidence of diarrhea or have only sporadic episodes of diarrhea every three or four days. Still others may have no symptoms at all. The problem, then, may not be one of determining whether or not someone is infected with the parasite but how harmoniously the host and the parasite can live together. When such harmony does not exist or is lost, it then becomes a problem of how to get rid of the parasite, either spontaneously or by treatment.

Note: Three prescription drugs are available in the United States to treat giardiasis: quinacrine, metronidazole, and furazolidone. In a recent review of drug trials in which the efficacies of these drugs were compared, quinacrine produced a cure in 93% of patients, metronidazole cured 92%, and furazolidone cured about 84% of patients.

Giardiasis occurs worldwide. In the United States, *Giardia* is the parasite most commonly identified in stool specimens submitted to state laboratories for parasitologic examination. During a 3-year period, approximately 4% of 1 million stool specimens submitted to state laboratories tested positive for *Giardia* (CDC, 1979). Other surveys have demonstrated *Giardia* prevalence rates ranging from 1 to 20%, depending on the location and ages of persons studied. Giardiasis ranks among the top 20 infectious diseases causing the greatest morbidity in Africa, Asia, and Latin America; it has been estimated that about 2 million infections occur per year in these regions (Walsh, 1981). People who are at highest risk for acquiring *Giardia* infection in the United States may be placed into five major categories:

1. People in cities whose drinking water originates from streams or rivers and whose water treatment process does not include filtration, or where filtration is ineffective because of malfunctioning equipment
2. Hikers, campers, and those who enjoy the outdoors
3. International travelers
4. Children who attend daycare centers, daycare center staff, and parents and siblings of children infected in daycare centers
5. Homosexual men

People in categories 1, 2, and 3 have in common the same general source of infection; that is, they acquire *Giardia* from fecally contaminated drinking water. The city resident usually becomes infected because the municipal water treatment process does not include the filter necessary to physically remove the parasite from the water. The number of people in the United States at risk (i.e., the number who receive municipal drinking water from unfiltered surface water) is estimated to be 20 million. International travelers may also acquire the parasite from improperly treated municipal waters in cities or villages in other parts of the world, particularly in developing countries. In Eurasia, only travelers to Leningrad appear to be at increased risk. In prospective studies, 88% of U.S. and 35% of Finnish travelers to Leningrad who had negative stool tests for *Giardia* on departure to the Soviet Union developed symptoms of giardiasis and had positive test for *Giardia* after they returned home (Brodsky et al., 1974). With the exception of visitors to Leningrad, however, *Giardia* has not been implicated as a major cause of traveler's diarrhea, as it has been detected in fewer than 2% of travelers developing diarrhea. Hikers and campers, however, risk infection every time they drink untreated raw water from a stream or river. Persons in categories 4 and 5 become exposed through more direct contact with feces or an infected person by exposure to the soiled diapers of an infected child in cases associated with daycare centers or through direct or indirect anal–oral sexual practices in the case of homosexual men.

Although community waterborne outbreaks of giardiasis have received the greatest publicity in the United States, about half of the *Giardia* cases discussed with the staff of the Centers for Disease Control and Prevention over a 3-year period had a daycare exposure as the most likely source of infection. Numerous outbreaks of *Giardia* in daycare centers have been reported. Infection rates for children in daycare center outbreaks range from 21 to 44% in the United States and from 8 to 27% in Canada (Black et al., 1981). The highest infection rates are usually observed in children who wear diapers (1 to 3 years of age). In a study of 18 randomly selected daycare centers in Atlanta, 10% of diapered children were found to be infected. Transmission from this age group to older children, daycare staff, and household contacts is also common. About 20% of parents caring for an infected child become infected.

Local health officials and managers of water utility companies need to realize that sources of *Giardia* infection other than municipal drinking water exist. Armed with this knowledge, they are less likely to make a quick (and sometimes wrong) assumption that a cluster of recently diagnosed cases in a city is related to municipal

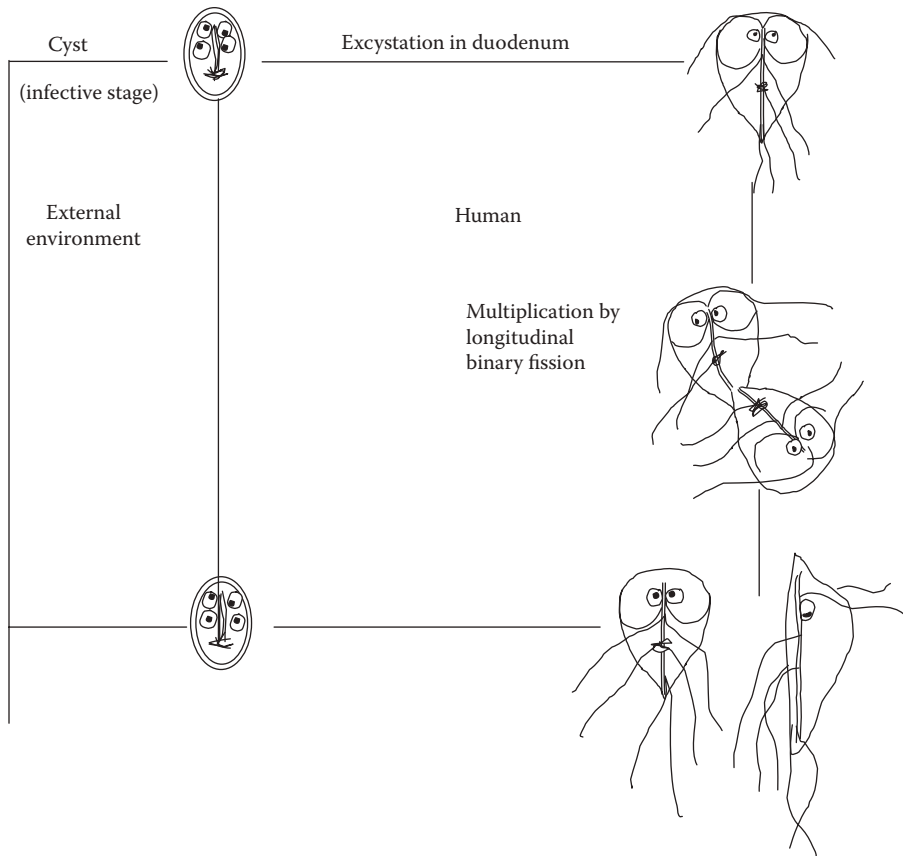


FIGURE 5.3 Life cycle of *Giardia lamblia*.

drinking water. Of course, drinking water must not be ruled out as a source of infection when a larger than expected number of cases is recognized in a community, but the possibility that the cases are associated with a daycare center outbreak, drinking untreated stream water, or international travel should also be entertained.

To understand the finer aspects of *Giardia* transmission and strategies for control, the drinking water practitioner must become familiar with several aspects of the biology of the parasite. Two forms of the parasite exist: a *trophozoite* and a *cyst*, both of which are much larger than bacteria (see Figure 5.3). Trophozoites live in the upper small intestine, where they attach to the intestinal wall by means of a disc-shaped suction pad on their ventral surface. Trophozoites actively feed and reproduce at this location. At some time during the trophozoite's life, it releases its hold on the bowel wall and floats in the fecal stream through the intestine. As it makes this journey, it undergoes a morphologic transformation into the egg-like cyst. The cyst, about 6 to 9 μm in diameter and 8 to 12 μm in length, has a thick exterior wall that protects the parasite against the harsh elements that it will encounter outside the body. This cyst form of parasite is infectious to other people or animals. Most people

become infected either directly (by hand-to-mouth transfer of cysts from the feces of an infected individual) or indirectly (by drinking feces-contaminated water). Less common modes of transmission included ingestion of fecally contaminated food and hand-to-mouth transfer of cysts after touching a fecally contaminated surface. After the cyst is swallowed, the trophozoite is liberated through the action of stomach acid and digestive enzymes and becomes established in the small intestine.

Although infection after ingestion of only one *Giardia* cyst is theoretically possible, the minimum number of cysts shown to infect a human under experimental conditions is 10 (Rendtorff, 1954). Trophozoites divide by binary fission about every 12 hours. What this means in practical terms is that if a person swallowed only a single cyst then reproduction at this rate would result in more than 1 million parasites 10 days later—1 billion parasites by day 15.

The exact mechanism by which *Giardia* causes illness is not yet well understood, but it apparently is not necessarily related to the number of organisms present. Nearly all of the symptoms, however, are related to dysfunction of the gastrointestinal tract. The parasite rarely invades other parts of the body, such as the gall bladder or pancreatic ducts. Intestinal infection does not result in permanent damage.

Note: *Giardia* has an incubation period of 1 to 8 weeks.

Data reported by the CDC indicate that *Giardia* is the most frequently identified cause of diarrheal outbreaks associated with drinking water in the United States. The remainder of this section is devoted specifically to waterborne transmissions of *Giardia*. *Giardia* cysts have been detected in 16% of potable water supplies (lakes, reservoirs, rivers, springs, groundwater) in the United States at an average concentration of 3 cysts per 100 L (Rose et al., 1983). Waterborne epidemics of giardiasis are a relatively frequent occurrence. In 1983, for example, *Giardia* was identified as the cause of diarrhea in 68% of waterborne outbreaks in which the causal agent was identified. From 1965 to 1982, more than 50 waterborne outbreaks were reported (CDC, 1984). In 1984, about 250,000 people in Pennsylvania were advised to boil drinking water for 6 months because of *Giardia*-contaminated water.

Many of the municipal waterborne outbreaks of *Giardia* have been subjected to intense study to determine their cause. Several general conclusions can be made from data obtained in those studies. Waterborne transmission of *Giardia* in the United States usually occurs in mountainous regions where community drinking water obtained from clear running streams is chlorinated but not filtered before distribution. Although mountain streams appear to be clean, fecal contamination upstream by human residents or visitors, as well as by *Giardia*-infected animals such as beavers, has been well documented. Water obtained from deep wells is an unlikely source of *Giardia* because of the natural filtration of water as it percolates through the soil to reach underground cisterns. Wells that pose the greatest risk of fecal contamination are poorly constructed or improperly located ones. A few outbreaks have occurred in towns that included filtration in the water treatment process but the filtration was not effective in removing *Giardia* cysts because of defects in filter construction, poor maintenance of the filter media, or inadequate pretreatment of the water before filtration. Occasional outbreaks have also occurred because of accidental cross-connections between water and sewage systems.

Note: From these data, we can conclude that two major ingredients are necessary for a waterborne outbreak: *Giardia* cysts must be present in untreated source water, and the water purification process must fail to either kill or remove *Giardia* cysts from the water.

Although beavers are often blamed for contaminating water with *Giardia* cysts, the suggestion that they are responsible for introducing the parasite into new areas seems unlikely. Far more likely is that they are also victims: *Giardia* cysts may be carried in untreated human sewage discharged into the water by small-town sewage disposal plants or originate from cabin toilets that drain directly into streams and rivers. Backpackers, campers, and sports enthusiasts may also deposit *Giardia*-contaminated feces in the environment which are subsequently washed into streams by rain. In support of this concept is a growing amount of data indicating a higher *Giardia* infection rate in beavers living downstream from U.S. national forest campgrounds when compared with beavers living in more remote areas that have a near zero rate of infection.

Although beavers may be unwitting victims of the *Giardia* story, they still play an important part in the contamination scheme because they can (and probably do) serve as amplifying hosts. An *amplifying host* is one that is easy to infect, serves as a good habitat for reproduction of the parasite, and, in the case of *Giardia*, returns millions of cysts to the water for every one ingested. Beavers are especially important in this regard, because they tend to defecate in or very near the water, which ensures that most of the *Giardia* cysts excreted are returned to the water.

The microbial quality of water resources and the management of the microbially laden wastes generated by the burgeoning animal agriculture industry are critical local, regional, and national problems. Animal wastes from cattle, hogs, sheep, horses, poultry, and other livestock and commercial animals can contain high concentrations of microorganisms, such as *Giardia*, that are pathogenic to humans.

The contribution of other animals to waterborne outbreaks of *Giardia* is less clear. Muskrats (another semiaquatic animal) have been found in several parts of the United States to have high infection rates (30 to 40%) (Frost et al., 1984). Recent studies have shown that muskrats can be infected with *Giardia* cysts from humans and beavers. Occasional *Giardia* infections have been reported in coyotes, deer, elk, cattle, dogs, and cats (but not in horses and sheep) encountered in mountainous regions of the United States. Naturally occurring *Giardia* infections have not been found in most other wild animals (bear, nutria, rabbit, squirrel, badger, marmot, skunk, ferret, porcupine, mink, raccoon, river otter, bobcat, lynx, moose, and big-horn sheep) (Frost et al., 1984).

Scientific knowledge about what is required to kill or remove *Giardia* cysts from a contaminated water supply has increased considerably. We know, for example, that cysts can survive in cold water (4°C) for at least 2 months and that they are killed instantaneously by boiling water (100°C) (Frost et al., 1984). We do not know how long the cysts will remain viable at other water temperatures (e.g., 0°C or in a canteen at 15 to 20°C), nor do we know how long the parasite will survive on various environmental surfaces, such as under a pine tree, in the sun, on a diaper-changing table, or in carpets in a daycare center.

The effect of chemical disinfection (chlorination, for example) on the viability of *Giardia* cysts is an even more complex issue. The number of waterborne outbreaks of *Giardia* that have occurred in communities where chlorination was employed as a disinfectant process demonstrates that the amount of chlorine used routinely for municipal water treatment is not effective against *Giardia* cysts. These observations have been confirmed in the laboratory under experimental conditions (Jarroll et al., 1979). This does not mean that chlorine does not work at all. It does work under certain favorable conditions. Without getting too technical, gaining some appreciation of the problem can be achieved by understanding a few of the variables that influence the efficacy of chlorine as a disinfectant:

- *Water pH*—At pH values above 7.5, the disinfectant capability of chlorine is greatly reduced.
- *Water temperature*—The warmer the water, the higher the efficacy. Chlorine does not work in ice-cold water from mountain streams.
- *Organic content of the water*—Mud, decayed vegetation, or other suspended organic debris in water chemically combines with chlorine, making it unavailable as a disinfectant.
- *Chlorine contact time*—The longer that *Giardia* cysts are exposed to chlorine, the more likely it is that the chemical will kill them.
- *Chlorine concentration*—The higher the chlorine concentration, the more likely it is that chlorine will kill *Giardia* cysts. Most water treatment facilities try to add enough chlorine to give a free (unbound) chlorine residual at the customer tap of 0.5 mg per liter of water.

These five variables are so closely interrelated that improving one can often compensate for another; for example, if chlorine efficacy is expected to be low because water is obtained from an icy stream, the chlorine contact time or chlorine concentration, or both, could be increased. In the case of *Giardia*-contaminated water, producing safe drinking water with a chlorine concentration of 1 mg per liter and contact time as short as 10 minutes might be possible if all the other variables are optimal—a pH of 7.0, water temperature of 25°C, and total organic content of the water close to zero. On the other hand, if all of these variables are unfavorable—pH of 7.9, water temperature of 5°C, and high organic content—chlorine concentrations in excess of 8 mg per liter with several hours of contact time may not be consistently effective. Because water conditions and water treatment plant operations (especially those related to water retention time and, therefore, to chlorine contact time) vary considerably in different parts of the United States, neither the USEPA nor the CDC has been able to identify a chlorine concentration that would be safe yet effective against *Giardia* cysts under all water conditions. For this reason, the use of chlorine as a preventive measure against waterborne giardiasis generally has been utilized under outbreak conditions when the amount of chlorine and contact time have been tailored to fit specific water conditions and the existing operational design of the water utility.

The bottom line is that filtration, reverse osmosis, and boiling are more effective at killing *Giardia*. In an outbreak, for example, the local health department and water utility may issue an advisory to boil water, and they may increase the

DID YOU KNOW?

Scientists estimate that between 1% and 5% of Americas are infected with *Cryptosporidium* at any one time.

chlorine residual at the consumer's tap from 0.5 mg/L to 1 or 2 mg/L. Also, if the physical layout and operation of the water treatment facility permit, they may increase the chlorine contact time, as well. These are emergency procedures intended to reduce the risk of transmission until a filtration device can be installed or repaired or until an alternative source of safe water (a well, for example) can be made operational.

The long-term solution to the problem of municipal waterborne outbreaks of giardiasis involves improvements in and more widespread use of filters in the municipal water treatment process. The sand filters most commonly used in municipal water treatment today cost millions of dollars to install, which makes them unattractive for many small communities. The pore sizes in these filters are not sufficiently small to remove *Giardia* (6 to 9 μm by 8 to 12 μm). For the sand filter to remove *Giardia* cysts from the water effectively, the water must receive some additional treatment before it reaches the filter. The flow of water through the filter bed must also be carefully regulated.

An ideal prefilter treatment for muddy water would include sedimentation (a holding pond where large suspended particles are allowed to settle out by the action of gravity) followed by flocculation or coagulation (the addition of chemicals such as alum or ammonium to cause microscopic particles to clump together). The sand filter easily removes the large particles resulting from the flocculation–coagulation process, including *Giardia* cysts bound to other microparticulates. Chlorine is then added to kill the bacteria and viruses that may escape the filtration process. If the water comes from a relatively clear source, chlorine may be added to the water before it reaches the filter.

The successful operation of a complete waterworks operation is a complex process that requires considerable training. Troubleshooting breakdowns or recognizing the potential problems in the system before they occur often requires the skills of an engineer. Unfortunately, most small water utilities with water treatment facilities that include filtration cannot afford the services of a full-time engineer. Filter operation or maintenance problems in such systems may not be detected until a *Giardia* outbreak is recognized in the community. The bottom line is that, although filtration is the best protection against waterborne giardiasis that water treatment technology has to offer for municipal water systems, it is not infallible. For municipal water filtration facilities to work properly, they must be properly constructed, operated, and maintained.

Whenever possible, persons outdoors should carry drinking water of known purity with them. When this is not practical and when water from streams, lakes, ponds, or other outdoor sources must be used, time should be taken to properly disinfect the water before drinking it.

Cryptosporidium

Ernest E. Tyzzer first described the protozoan parasite *Cryptosporidium* in 1907. Tyzzer frequently found a parasite in the gastric glands of laboratory mice. Tyzzer identified the parasite as a sporozoan but of uncertain taxonomic status, and he named it *Cryptosporidium muris*. Later, in 1910, after more detailed study, he proposed *Cryptosporidium* as a new genus and *muris* as the type of species. Amazingly, except for developmental stages, Tyzzer's original description of the life cycle (see Figure 5.4) was later confirmed by electron microscopy. In 1912, Tyzzer described another new species, *Cryptosporidium parvum* (Tyzzer, 1912).

For almost 50 years, Tyzzer's discovery of the genus *Cryptosporidium* remained (like himself) relatively obscure because it appeared to be of no medical or economic importance. Slight rumblings of the importance of the genus began to be felt in the medical community when Slavin (1955) wrote about a new species, *Cryptosporidium melagridis*, associated with illness and death in turkeys. Interest remained slight even when *Cryptosporidium* was found to be associated with bovine diarrhea (Panciera et al., 1971). Not until 1982 did worldwide interest focus on the study of organisms in the genus *Cryptosporidium*. At that time, the medical community and other interested parties were beginning a full-scale, frantic effort to find out as much as possible about acquired immune deficiency syndrome (AIDS), and the CDC reported that 21 AIDS-infected males from six large cities in the United States had severe protracted diarrhea caused by *Cryptosporidium*. It was in 1993, though, that *Cryptosporidium*—"the pernicious parasite"—made itself and Milwaukee famous (Mayo Foundation, 1996).

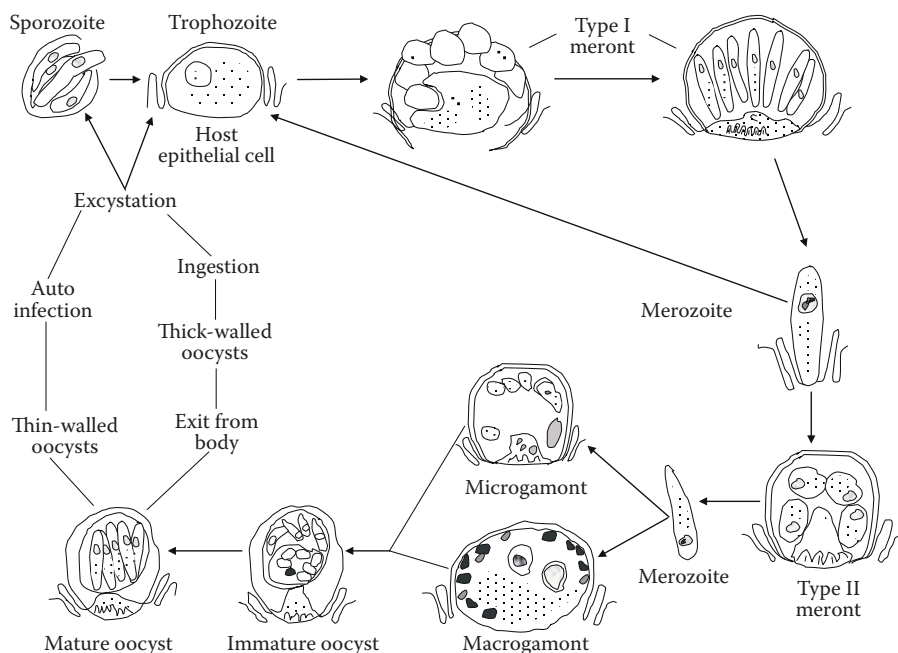


FIGURE 5.4 Life cycle of *Cryptosporidium parvum*.

Note: The *Cryptosporidium* outbreak in Milwaukee caused the deaths of 100 people—the largest episode of waterborne disease in the United States in the 70 years since health officials began tracking such outbreaks.

The massive waterborne outbreak in Milwaukee (more than 400,000 persons developed acute and often prolonged diarrhea or other gastrointestinal symptoms) increased interest in *Cryptosporidium* at an exponential level. The Milwaukee incident spurred both public interest and the interest of public health agencies, agricultural agencies and groups, environmental agencies and groups, and suppliers of drinking water. This increase in interest level and concern spurred new studies of *Cryptosporidium*, with an emphasis on developing methods for recovery, detection, prevention, and treatment (Fayer et al., 1997).

The USEPA is particularly interested in this pathogen. In its reexamination of regulations on water treatment and disinfection, the USEPA issued a maximum contaminant level goal (MCLG) and contaminant candidate list (CCL) for *Cryptosporidium*. Its similarity to *Giardia lamblia* and the need for an efficient conventional water treatment capable of eliminating viruses forced the USEPA to regulate surface water supplies in particular. The proposed Enhanced Surface Water Treatment Rule (ESWTR) included regulations from watershed protection to specialized operation of treatment plants (certification of operators and state overview) and effective chlorination. Protection against *Cryptosporidium* included control of waterborne pathogens such as *Giardia* and viruses (De Zuane, 1997).

Cryptosporidium Basics

Cryptosporidium is one of several single-celled protozoan genera in the phylum Apicomplexa (all referred to as coccidian). *Cryptosporidium* along with other genera in the phylum Apicomplexa develop in the gastrointestinal tract of vertebrates through all of their life cycle; in short, they live in the intestines of animals and people. This microscopic pathogen causes a disease called *cryptosporidiosis*. The dormant (inactive) form of *Cryptosporidium* is called an *oocyst* and is excreted in the feces (stool) of infected humans and animals. The tough-walled oocysts survive under a wide range of environmental conditions.

Several species of *Cryptosporidium* were incorrectly named after the host in which they were found, and subsequent studies have invalidated many species. Now, eight valid species of *Cryptosporidium* (see Table 5.6) have been named. Upton (1997) reported that *C. muris* infects the gastric glands of laboratory rodents and several other mammalian species but (even though several texts state otherwise) is not known to infect humans. *C. parvum*, however, infects the small intestine of an unusually wide range of mammals, including humans, and is the zoonotic species responsible for human cryptosporidiosis. In most mammals *C. parvum* is predominantly a parasite of neonate (newborn) animals. Upton pointed out that, even though exceptions occur, older animals generally develop poor infections, even when unexposed previously to the parasite. Humans are the one host that can be seriously infected at any time in their lives, and only previous exposure to the parasite results in either full or partial immunity to challenge infections.

TABLE 5.6
Valid Named Species of *Cryptosporidium*

Species	Host
<i>Cryptosporidium baileyi</i>	Chicken
<i>Cryptosporidium felis</i>	Domestic cat
<i>Cryptosporidium meleagridis</i>	Turkey
<i>Cryptosporidium murishouse</i>	House mouse
<i>Cryptosporidium nasorium</i>	Fish
<i>Cryptosporidium parvum</i>	House mouse
<i>Cryptosporidium. serpentis</i>	Corn snake
<i>Cryptosporidium wrairi</i>	Guinea pig

Source: Fayer,R.,Ed.,*Cryptosporidium and Cryptosporidiosis*,
CRC Press, Boca Raton, FL, 1997. With permission.

Oocysts are present in most surface bodies of water across the United States, many of which supply public drinking water. Oocysts are more prevalent in surface waters when heavy rains increase runoff of wild and domestic animal wastes from the land or when sewage treatment plants are overloaded or break down. Only laboratories with specialized capabilities can detect the presence of *Cryptosporidium* oocysts in water. Unfortunately, current sampling and detection methods are unreliable. Recovering oocysts trapped on the material used to filter water samples is difficult. When a sample has been obtained, however, determining whether the oocyst is alive and if it is *C. parvum* and thus can infect humans is easily accomplished by looking at the sample under a microscope.

The number of oocysts detected in raw (untreated) water varies with location, sampling time, and laboratory methods. Water treatment plants remove most, but not always all, oocysts. Low numbers of oocysts are sufficient to cause cryptosporidiosis, but the low numbers of oocysts sometimes present in drinking water are not considered cause for alarm in the public.

Protecting water supplies from *Cryptosporidium* demands multiple barriers. Why? Because *Cryptosporidium* oocysts have tough walls that can withstand many environmental stresses and are resistant to chemical disinfectants such as chlorine that are traditionally used in municipal drinking water systems.

Physical removal of particles, including oocysts, from water by filtration is an important step in the water treatment process. Typically, water pumped from rivers or lakes into a treatment plant is mixed with coagulants, which help settle out particles suspended in the water. If sand filtration is used, even more particles are removed. Finally, the clarified water is disinfected and piped to customers. Filtration is the only conventional method now in use in the United States for controlling *Cryptosporidium*.

Ozone is a strong disinfectant that kills protozoa if sufficient doses and contact times are used, but ozone leaves no residual for killing microorganisms in the distribution system, as does chlorine. The high costs of new filtration or ozone

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treatment plants must be weighed against the benefits of additional treatment. Even well-operated water treatment plants cannot ensure that drinking water will be completely free of *Cryptosporidium* oocysts. Water treatment methods alone cannot solve the problem; watershed protection and monitoring of water quality are critical. As mentioned earlier, watershed protection is another barrier to *Cryptosporidium* in drinking water. Land use controls such as septic system regulations and best management practices to control runoff can help keep human and animal wastes out of water.

Under the Surface Water Treatment Rule of 1989, public water systems must filter surface water sources unless water quality and disinfection requirements are met and a watershed control program is maintained. This rule, however, did not address *Cryptosporidium*. The USEPA has now set standards for turbidity (cloudiness) and coliform bacteria (which indicate that pathogens are probably present) in drinking water. Frequent monitoring must occur to provide officials with early warning of potential problems to enable them to take steps to protect public health. Unfortunately, no water quality indicators can reliably predict the occurrence of cryptosporidiosis. More accurate and rapid assays of oocysts will make it possible to notify residents promptly if their water supply is contaminated with *Cryptosporidium* and thus avert outbreaks.

The bottom line is that the collaborative efforts of water utilities, government agencies, healthcare providers, and individuals are needed to prevent outbreaks of cryptosporidiosis.

Cryptosporidiosis

Cryptosporidium parvum is an important emerging pathogen in the U.S. and a cause of severe, life-threatening disease in patients with AIDS. No safe and effective form of specific treatment for cryptosporidiosis has been identified to date. The parasite is transmitted by ingestion of oocysts excreted in the feces of infected humans or animals. The infection can therefore be transmitted from person-to-person, through ingestion of contaminated water (drinking water and water used for recreational purposes) or food, from animal to person, or by contact with fecally contaminated environmental surfaces. Outbreaks associated with all of these modes of transmission have been documented. Patients with human immunodeficiency virus infection should be made more aware of the many ways that *Cryptosporidium* species are transmitted, and they should be given guidance on how to reduce their risk of exposure (Juranek, 1995).

Since the Milwaukee outbreak, concern about the safety of drinking water in the United States has increased, and new attention has been focused on determining and reducing the risk of acquiring cryptosporidiosis from community and municipal water supplies. Cryptosporidiosis is spread by putting something in the mouth that has been contaminated with the stool of an infected person or animal. In this way, people swallow the *Cryptosporidium* parasite. As mentioned earlier, a person can become infected by drinking contaminated water or eating raw or undercooked food contaminated with *Cryptosporidium* oocysts, by direct contact with the droppings of infected animals or stools of infected humans, or by hand-to-mouth transfer of oocysts from surfaces that may have become contaminated with microscopic amounts of stool from an infected person or animal.

The symptoms may appear 2 to 10 days after infection by the parasite. Although some persons may not have symptoms, others have watery diarrhea, headache, abdominal cramps, nausea, vomiting, and low-grade fever. These symptoms may lead to weight loss and dehydration. In otherwise healthy persons, these symptoms usually last 1 to 2 weeks, at which time the immune system is able to defeat the infection. In persons with suppressed immune systems, such as persons who have AIDS or who recently have had an organ or bone marrow transplant, the infection may continue and become life threatening.

Currently, no safe and effective cure for cryptosporidiosis exists. People with normal immune systems improve without taking antibiotic or antiparasitic medications. The treatment recommended for this diarrheal illness is to drink plenty of fluids and to get extra rest. Physicians may prescribe medication to slow the diarrhea during recovery.

The best way to prevent cryptosporidiosis is to

- Avoid water or food that may be contaminated.
- Wash hands after using the toilet and before handling food.
- Be sure, if you work in a daycare center, to wash your hands thoroughly with plenty of soap and warm water after every diaper change, even if you wear gloves when changing diapers.

During community-wide outbreaks caused by contaminated drinking water, drinking water practitioners should inform the public to boil drinking water for 1 minute to kill the *Cryptosporidium* parasite.

VIRUSES

Viruses are very different from the other microorganisms. Consider their size relationship, for example. Relative to size, if protozoans are the Goliaths of microorganisms, then viruses are the Davids. Stated more specifically and accurately, viruses are intercellular parasitic particles that are the smallest living infectious materials known—the midgets of the microbial world. Viruses are very simple life forms consisting of a central molecule of genetic material surrounded by a protein shell called a *capsid* and sometimes by a second layer called an *envelope*. Viruses occur in many shapes, including long slender rods, elaborate irregular shapes, and geometric polyhedrals (see Figure 5.5).

Viruses contain no mechanisms by which to obtain energy or reproduce on their own, thus viruses must have a host to survive. After they invade the cells of their specific host (animal, plant, insect, fish, or even bacteria), they take over the cellular machinery of the host and force it to make more viruses. In the process, the host cell is destroyed and hundreds of new viruses are released into the environment. The viruses of most concern to the waterworks operator are the pathogens that cause hepatitis, viral gastroenteritis, and poliomyelitis.

Smaller and different from bacteria, viruses are prevalent in water contaminated with sewage. Detecting viruses in water supplies is a major problem because of the complexity of the procedures involved, although experience has shown that the

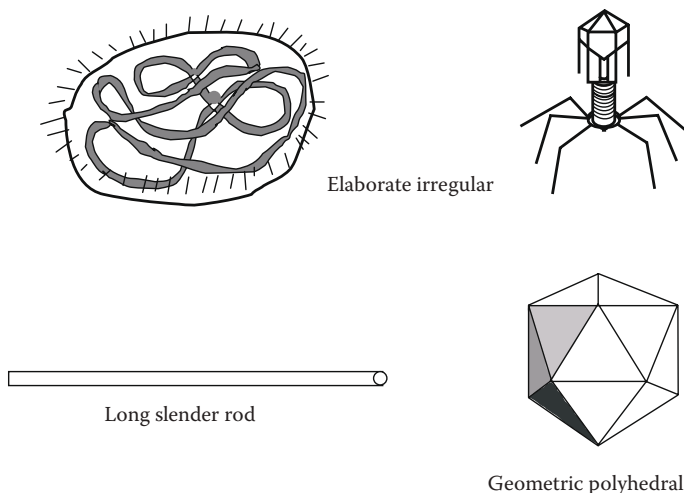


FIGURE 5.5 Virus shapes.

normal coliform index can be used as a rough guide for viruses as well as for bacteria. More attention must be paid to viruses, however, when surface water supplies have been used for sewage disposal. Viruses are difficult to destroy by normal disinfection practices, as they require increased disinfectant concentration and contact time for effective destruction. Reverse osmosis systems have a very high effectiveness in removing viruses (e.g., enteric viruses, hepatitis A, norovirus, rotavirus).

Note: Viruses that infect bacterial cells cannot infect and replicate within cells of other organisms. It is possible to utilize this specificity to identify bacteria, a procedure called *phage typing*.

FUNGI

Fungi are of relatively minor importance in water/wastewater operations (except for biosolids composting, where they are critical). Fungi, like bacteria, are extremely diverse. They are multicellular, autotrophic, photosynthetic protists. They grow as filamentous, mold-like forms or as yeast-like (single-celled) organisms. They feed on organic material.

Note: Aquatic fungi grow as *parasites* on living plants or animals and as *saprophytes* on those that are dead.

Note: De Zuane (1997) reported that pathogenic parasites are not easily removed or eliminated by conventional treatment and disinfection unit processes. This is particularly true for *Giardia lamblia*, *Cryptosporidium*, and *Cyclospora*. Filtration facilities can be adjusted with regard to depth, prechlorination, filtration rate, and backwashing to become more effective in the removal of cysts. The pretreatment of protected watershed raw water is a major factor in the elimination of pathogenic protozoa.

REFERENCES AND RECOMMENDED READING

- Abrahamson, D.E., Ed. (1988). *The Challenge of Global Warming*. Washington, DC: Island Press.
- Asimov, I. (1989). *How Did We Find Out about Photosynthesis?* New York: Walker & Company.
- Bingham, A.K., Jarroll, E.L., Meyer, E.A., and Radulescu, S. (1979). Introduction to *Giardia* excystation and the effect of temperature on cyst viability compared by exoin exclusion and *in vitro* excystation. In: *Waterborne Transmission of Giardiasis*, EPA-600/9-79-001 (Jakubowski, J. and Hoff H.C., Eds.), pp. 217–229. Washington, DC: U.S. Environmental Protection Agency.
- Black, R.E., Dykes, A.C., Anderson, K.E., Wells, J.G., Sinclair, S.P., Gary, G.W., Hatch, M.H., and Ginagaros, E.J. (1981). Handwashing to prevent diarrhea in day-care centers. *Am. J. Epidemiol.*, 113: 445–451.
- Brodsky, R.E., Spencer, H.C., and Schultz, M.G. (1974). Giardiasis in American travelers to the Soviet Union. *J. Infect. Dis.*, 130: 319–323.
- CDC. (1979). *Intestinal Parasite Surveillance, Annual Summary 1978*. Atlanta, GA: Centers for Disease Control and Prevention.
- CDC. (1983). *Water-Related Disease Outbreaks Surveillance, Annual Summary 1983*. Atlanta, GA: Centers for Disease Control and Prevention.
- CDC. (1995). *Giardiasis*. Atlanta, GA: Centers for Disease Control and Prevention.
- De Zuane, J. (1997). *Handbook of Drinking Water Quality*. New York: John Wiley & Sons.
- Fayer, R., Speer, C.A., and Dudley, J.P. (1997). The general biology of *Cryptosporidium*. In: *Cryptosporidium and Cryptosporidiosis* (Fayer, R., Ed.), pp. 1–42. Boca Raton, FL: CRC Press.
- Frost, F., Plan, B., and Liechty, B. (1984). *Giardia* prevalence in commercially trapped mammals. *J. Environ. Health*, 42: 245–249.
- Herwaldt, F.L. et al. (1997). An outbreak in 1996 of cyclosporiasis associated with imported raspberries. *N. Engl. J. Med.*, 336: 1548–1556.
- Huang, P., Weber, J.T., Sosin, D.M. et al. (1995). *Cyclospora*. *Ann. Intern. Med.*, 123: 401–414.
- Jarroll, Jr., E.L., Gingham, A.K., and Meyer, E.A. (1979). *Giardia* cyst destruction: effectiveness of six small-quantity water disinfection methods. *Am. J. Trop. Med. Hyg.*, 29: 8–11.
- Juranek, D.D. (1995). Cryptosporidiosis: sources of infection and guidelines for prevention. *Clin. Infect. Dis.*, 21: S37–S61.
- Kemmer, F.N. (1979). *Water: The Universal Solvent*, 2nd ed. Oak Brook, IL: Nalco Chemical Co.
- Kordon, C. (1992). *The Language of the Cell*. New York: McGraw-Hill.
- Koren, H. (1991). *Handbook of Environmental Health and Safety: Principles and Practices*. Chelsea, MI: Lewis Publishers.
- LeChevallier, M.W., Norton, W.D., and Less, R.G. (1991). Occurrences of *Giardia* and *Cryptosporidium* spp. in surface water supplies. *Appl. Environ. Microbiol.*, 57: 2610–2616.
- Lozier, J.C., McKim T., and Rose, J. (1994). Meeting Stringent Surface Water Discharge Requirements for Reclaimed Water with Membrane Process, paper presented at ASCE/NCEE Conf., Boulder, CO, July 11–13.
- Mayo Foundation. (1996). *The “Bug” That Made Milwaukee Famous*. Rochester, MN: Mayo Foundation.
- Metcalf & Eddy. (2003). *Wastewater Engineering: Treatment, Disposal, and Reuse*. New York: McGraw-Hill.
- Miller, G.T. (1988). *Environmental Science: An Introduction*. Belmont, CA: Wadsworth.
- Odum, E.P. (1975). *Ecology: The Link Between the Natural and the Social Sciences*. New York: Holt, Rinehart and Winston.

- Odum, E.P. (1983). *Basic Ecology*. Philadelphia, PA: Saunders.
- Panciera, R.J., Thomassen, R.W., and Garner, R.M. (1971). Cryptosporidial infection in a calf. *Vet. Pathol.*, 8: 479.
- Pennak, R.W. (1989). *Fresh-Water Invertebrates of the United States*, 3rd ed. New York: John Wiley & Sons.
- Price, P.W. (1984). *Insect Ecology*. New York: John Wiley & Sons.
- Rendtorff, R.C. (1954). The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. *Am. J. Hyg.*, 59: 209–220.
- Rose, J.B., Gerb, C.P., and Jakubowski, W. (1983). Survey of potable water supplies for *Cryptosporidium* and *Giardia*. *Environ. Sci. Technol.*, 25: 1393–1399.
- Singleton, P. and Sainsbury, D. (1994). *Dictionary of Microbiology and Molecular Biology*, 2nd ed. New York: John Wiley & Sons.
- Slavin, D. (1955). *Cryptosporidium melagridis*. *J. Comp. Pathol.*, 65: 262.
- Smith, R.L. (1974). *Ecology and Field Biology*. New York: Harper & Row.
- Spellman, F.R. (1996). *Stream Ecology and Self-Purification: An Introduction for Wastewater and Water Specialists*. Boca Raton, FL: CRC Press.
- Spellman, F.R. (1997). *Microbiology for Water/Wastewater Operators*. Boca Raton, FL: CRC Press.
- Tchobanoglous, G. and Schroeder, E.D. (1985). *Water Quality*. Reading, MA: Addison-Wesley.
- Tyzzer, E.E. (1912). *Cryptosporidium parvum* sp.: a *Coccidium* found in the small intestine of the common mouse. *Arch. Protistenkd.*, 26: 394.
- Upton, S.J. (1997). *Basic Biology of Cryptosporidium*. Manhattan: Kansas State University.
- Walsh, J.D. and Warren, K.S. (1979). Selective primary health care: an interim strategy for disease control in developing countries, *N. Engl. J. Med.*, 301: 974–976.
- Weller, P.F. (1985). Intestinal protozoa: giardiasis. *Sci. Am. Med.*, 12(4): 554–558.
- WHO. (1990). *Guidelines for Drinking Water Quality*, Vol. 2, 2nd ed. Geneva: World Health Organization.
- Wistrich, G.A. and Lechtman, M.D. (1980). *Microbiology*, 3rd ed. New York: Macmillan.