CHAPTER 2

Foodborne Infectious and Microbial Agents

LEARNING OBJECTIVES

1. Describe the Tree of Life and explain the differences between prokaryotic (procaryotic) and eukaryotic (eucaryotic) cell types.
2. Name and describe the main taxonomic categories of microorganisms and the important foodborne pathogens in each category.
3. Briefly describe how microorganisms acquire new traits, and how these traits contribute to their survival and pathogenicity.
4. Discern the differences between foodborne illnesses, diseases, infections, toxicoinfections, intoxications, and poisonings.
5. Describe the steps in the cycle of parasitism and infection, and define key terms used in the processes of the cycle.
6. Describe common signs and symptoms of diseases caused by foodborne pathogens and, in general terms, the mechanisms of pathogenicity involved.
7. Identify factors or circumstances that may increase pathogen virulence or reduce host resistance to a foodborne infection.
8. Explain the importance of serotyping and other classification schemes for the identification and control of pathogens in foods, particularly for the genera *Escherichia* and *Salmonella*.
9. For each important foodborne pathogen, identify the major food groups most often associated with the disease(s) it causes.
10. List the important bacterial pathogens that cause foodborne illnesses, and identify their common and unique reservoirs and/or sources of food contamination.
11. Name the viruses that are most frequently transmitted by foods, and explain how foods are most likely contaminated with these viruses.
12. List important protozoans that may be transmitted by foods, and identify their primary reservoirs.
13. Describe the sources of parasitic helminths in foods and their relationships to humans and animals.

14. Understand the nature of prions and the diseases they cause, and explain how they may enter the human food chain.
15. Differentiate between emerging and contemporary foodborne diseases, and give examples of how various factors contribute to the emergence of foodborne diseases.

TYPES OF INFECTIOUS AND MICROBIAL AGENTS

The oldest and most adaptable forms of life on earth are microorganisms. They occupy every part of the biosphere, the theoretical boundaries of life extending several miles above and below the earth's surface. Over the course of 3.8 billion years of evolution, microorganisms have adapted to survive under very diverse environmental conditions, and they have developed complex microbial communities and ecosystems. Furthermore, these adaptations have been essential to the evolution and survival of other organisms such as plants and animals. The relationships of microorganisms with plants and animals are frequently symbiotic. This may include mutualism, commensalism, and/or parasitism. The principal differences between these three types of symbiotic relationships are related to the differences in benefits between the organisms. With mutualism, both species (e.g., plant/animal and microorganism) benefit somehow by the relationship, whereas with commensalism, one species benefits from the relationship, while the other species neither benefits nor is harmed by it. In contrast, parasitism is where one species (e.g., microorganism) benefits and the other species (e.g., plant or animal) is somehow harmed by the relationship.

The adaptation of microorganisms to environmental conditions results in some becoming pathogenic (i.e., able to cause disease) in plants and animals. These adaptations can be measured in two general time frames: (1) millions of years, resulting in the creation of totally new species; and (2) days and years, leading to new variants in a species or strain (Groisman and Casadesus 2005). The underlying mechanisms of such microbial adaptations are in the genes, specifically from changes in the coding and/or regulatory control of DNA (or RNA in the case of certain viruses). The genetic makeup of microorganisms is variable and subject to rapid change by a number of different mechanisms. And because under ideal conditions the reproduction/multiplication time of most microorganisms is measured in minutes, genetically new populations of microorganisms can evolve in a short time, at least compared with the evolution of more complex organisms. These new variants and strains of microorganisms may be endowed with genes to survive new environments (including in foods or within human hosts), develop resistance to antibiotics, or become increasingly pathogenic. Additional information about DNA and the genes of pathogens is presented later in Chapter 7.

Despite the vast number of microbial species on earth, a relatively small number are pathogenic to animals—including humans. The pathogenic agents of greatest concern in foods are taxonomically classified as bacteria, viruses, fungi, protozoa, helminths, and prions. Except for the viruses and prions, these organisms are represented in the Bacteria and Eucaryota domains of the phylogenetic Tree of Life, depicted in Figure 2-1. Although this classification scheme is speculative, it is based on the sequencing of nucleic acids (ribosomal RNA) and regarded as more relevant in terms of phylogeny than simple phenotypic characteristics. In recent years, more than 70 phylum-level bacterial lines have been identified, but only about 7 phyla contain human pathogens (Pace 2008). The phyla with human pathogens are also represented by a large body of scien-

tific literature that includes phenotypic characteristics derived from microscopy and culturing studies. Before the advent of gene sequencing, phenotypic characteristics were the sole basis for the taxonomic classification of microorganisms, and such characteristics still remain important in the identification and understanding of microorganisms and their pathogenicity.

The taxonomic classification of microorganisms is debatable among scientists and scholars, particularly at the phyla and class levels. Nevertheless, the classification of microorganisms is important for reasons other than scientific curiosity, a fact that will become increasingly apparent in the practice of food safety. In general, an agreed-upon classification system permits the precise identification of foodborne pathogens, which in turn assists in the epidemiology of foodborne diseases and the development of prevention strategies for food safety.

Bacteria

The bacteria are single-celled microorganisms that represent a major domain on the theoretical Tree of Life. They are diverse and ubiquitous microorganisms with genetic identities and phenotypic characteristics that distinguish them from the other domains. Their cellular composition and structures are "prokaryotic" in nature. Prokaryotic cells are characterized by the absence of a

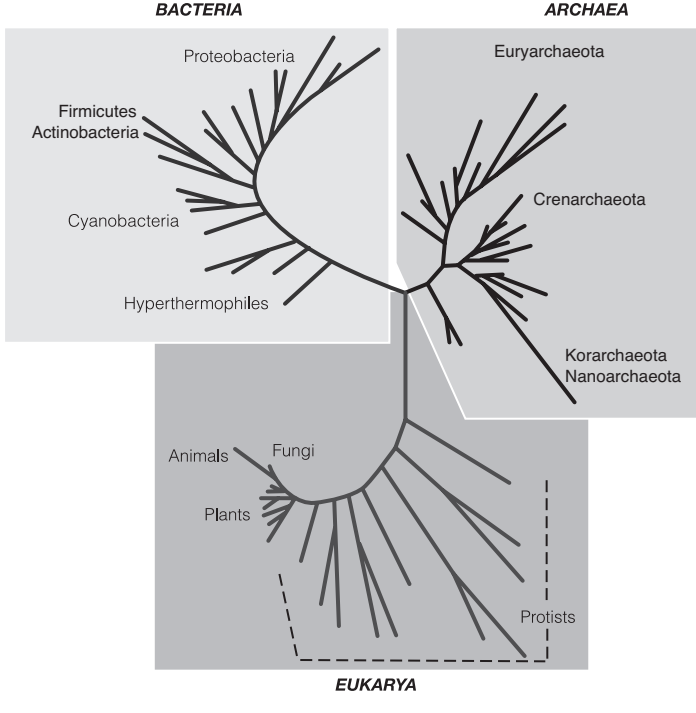

Figure 2-1 The Phylogenetic Tree of Life*

nuclear membrane and organelles, presence of a cell wall, division by binary fission, and other characteristics exhibited in Figure 2-2. In addition to a circular DNA chromosome, bacteria have circular snippets of DNA called plasmids in their cytoplasm that may also confer traits such as resistance to antibiotics, expanded metabolic functions, and greater pathogenicity. The chromosome and plasmids can be inherited by successive generations of bacteria (called vertical transfer). Furthermore, the plasmids and chromosomal DNA may be transferred between bacterial cells (called horizontal transfer) by processes known as transformation and conjugation. Transformation involves the uptake of DNA by a viable bacterial cell from one that has ruptured. Conjugative transfer of DNA occurs between bacterial cells through appendages called pili. A third means of DNA transfer between bacterial cells is called transduction and involves bacteriophages, that is, viruses that infect bacterial cells.

Bacterial cells have a variety of morphologies, or shapes. The shapes of greatest concern in food safety are the bacillus (rod), coccus (sphere), and spiral. Cell sizes of bacteria (see Figure 2-3) can range from an extremely small 0.2 μm to a relatively large 750 μm (Schulz and Jorgensen 2001). The bacterial cell sizes most encountered with food safety range close to the rod-shaped *Escherichia coli*, about 1 μm in width and 2 μm in length. The individual coccus of *Staphylococcus aureus* is about 0.6 μm in diameter, though this bacterium is usually associated with larger grape-like clusters of cocci. External cellular structures such as the flagellum are not present in all bacteria, and the biochemical composition of the cell walls and capsules can vary among different species. Researchers can use these different characteristics to identify and classify bacterial species or strains. More details on such characteristics are provided later for individual species of food

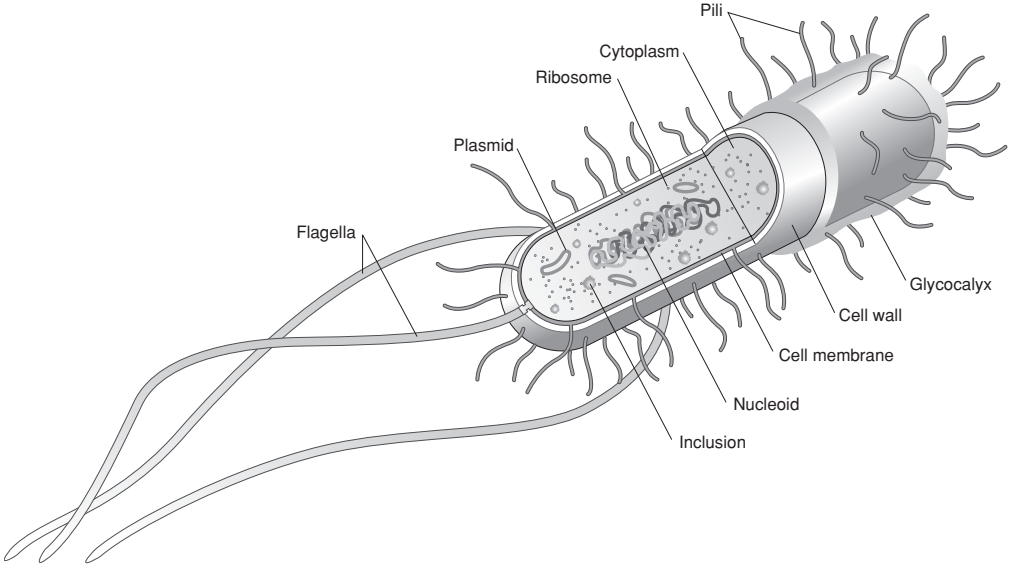

Figure 2-2 Idealized Prokaryotic Cell Structure

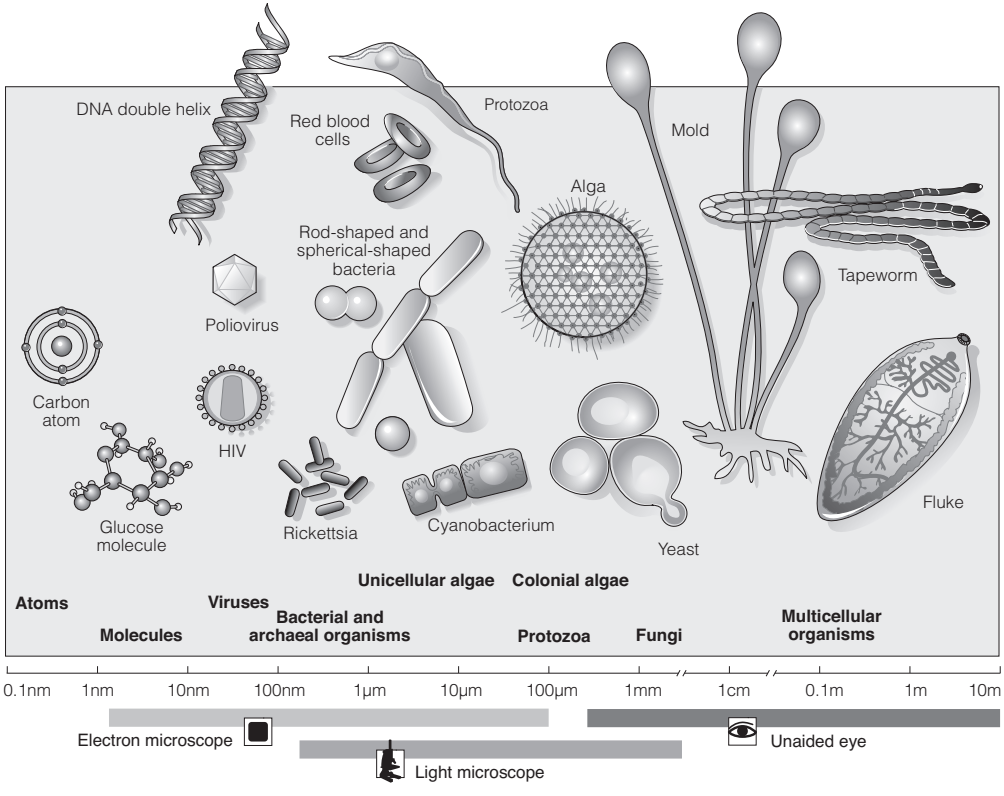

Figure 2-3 Size Comparison among Various Atoms, Molecules, and Microorganisms (not drawn to scale)

safety importance. For now, some important bacterial species and their taxonomic classifications are listed in Table 2-1.

Various species of bacteria have different requirements for growth in terms of nutrients, temperature ranges, moisture content, and oxygen availability. All of these requirements are important with regard to food safety, in particular for destroying or controlling the growth of bacteria. The bacteria that can grow under full oxygen conditions are called aerobes, whereas bacteria that can grow only under anoxic conditions are called obligate anaerobes. Species that can grow with or without the presence of oxygen are facultative. A few species such as *Campylobacter jejuni* are microaerophilic, meaning they can use oxygen only under reduced conditions (<21% oxygen). Bacteria are also categorized by their temperature growth ranges and optima: psychrophilic (~4°C optimum), mesophilic (~39°C optimum), and thermophilic (~60°C optimum). Water availability to bacteria is determined not only by the amount of H_2O, but by the osmotic effect of solutes (e.g., salts and sugars) dissolved in the water. With the exception of some species, most bacteria of food safety concern are not very tolerant of high osmotic conditions.

Table 2-1 Taxonomic Classification of Important Foodborne Bacterial Pathogens

Phylum	*Class*	*Order*	*Family*	*Genus*	*Species*
Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	*Campylobacter*	*jejuni*
	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	*Aeromonas*	*hydrophila*
		Enterobacteriales	Enterobacteriaceae	*Escherichia*	*coli*
				Salmonella	*enterica* *bangori*
				Shigella	*sonnei* *dysenteriae* *flexneri* *boydii*
				Yersinia	*enterocolitica*
		Vibrionales	Vibrionaceae	*Vibrio*	*parahaemolyticus* *vulnificus* *cholerae*
Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	*cereus*
			Listeriaceae	*Listeria*	*monocytogenes*
			Staphylococcacea	*Staphylococcus*	*aureus*
		Lactobacillales	Streptococcaceae	*Streptococcus*	*pyogenes*
	Clostridia	Clostridiales	Clostridiaceae	*Clostridium*	*botulinum* *perfringens*

Source: Compiled from Garrity GM, Lilburn TG, Cole JR, Harrison SH, Euzeby J, Tindall BJ. 2007. Taxonomic outline of the bacteria and archaea, release 7.7 March 6, 2007. Michigan State University Board of Trustees. Available from: http://www.taxonomicoutline.org/

The family Enterobacteriaceae is arguably the most important group of bacteria in food safety. This family consists of approximately 44 genera and 176 species, many of them living symbiotically within the intestines of animals as commensal microorganisms (Brenner and Farmer 2005). However, several species are enteric pathogens that are frequently responsible for foodborne disease outbreaks and are also detected in clinical samples from patients with infections. Whereas most Enterobacteriaceae can thrive within intestinal tracts of animals, some species also survive and multiply in the environment. This makes them problematic because of possible food contamination. On the other hand, nonpathogenic Enterobacteriaceae are useful sanitary indicators for food safety. A few species have evolved to live solely in the environment (including plants, soil, and water) and have less value as sanitary indicators. Additional information about the classification of Enterobacteriaceae and their use as sanitary indicators is provided in a Chapter 7.

Viruses

Viruses must have access to a host cell to replicate and propagate. In other words, they are obligate intracellular parasites. This is because viruses are essentially nucleic acids (DNA or RNA) packaged in proteins that form a capsid (Figure 2-4) and have neither the molecular machinery nor the resources necessary for metabolism and independent replication. Yet, viruses are the most prolific and abundant microorganisms on earth. Viruses accomplish this feat by infecting host cells and exploiting their metabolism and resources to replicate and disseminate to new host cells. The nucleic acid packaged in the virus directs the host cell to divert its energy and resources to serve the virus's needs. Viruses have a very specific range of hosts, and they have preferences for certain cell types within a multicellular host. Virtually every organism is a host to some viruses, but the virus must have the correct proteins for access to the host cell and the correctly encoded nucleic acids to direct the host cell's activity.

The classification of viruses is challenging because they are not "living" in the same sense as other microorganisms, and they vary greatly in structure, nucleic acid content, and hosts. Two schemes for virus classification are employed: (1) the Baltimore Classification, named after the Nobel Prize Winner David Baltimore; and (2) the International Committee on Taxonomy of Viruses (ICTV), established in the early 1990s to standardize virus taxonomy. The Baltimore Classification scheme assigns viruses to groups on the basis of nucleic acid structure and other characteristics, whereas the ICTV scheme attempts to assign viruses using taxon structures similar to cellular organisms (e.g., species, genus, family, subfamily, and order). To avoid confusion, the classification of viruses is discussed as necessary in a later section on foodborne viruses.

Protozoans

The protozoans are located under the domain of Eucarya on the phylogenetic Tree of Life. These organisms, as well as the others under the domain Eucarya, are characterized by eukaryotic cells. As illustrated in Figure 2-5, eukaryotic cell structures have many differences from prokaryotic cells, including the presence of a membrane-bound nucleus, a cytoskeleton, and other structural and chemical differences. A single kingdom does not adequately categorize all protozoans. The principal reason is the extreme diversity of these eukaryotic microorganisms, being neither

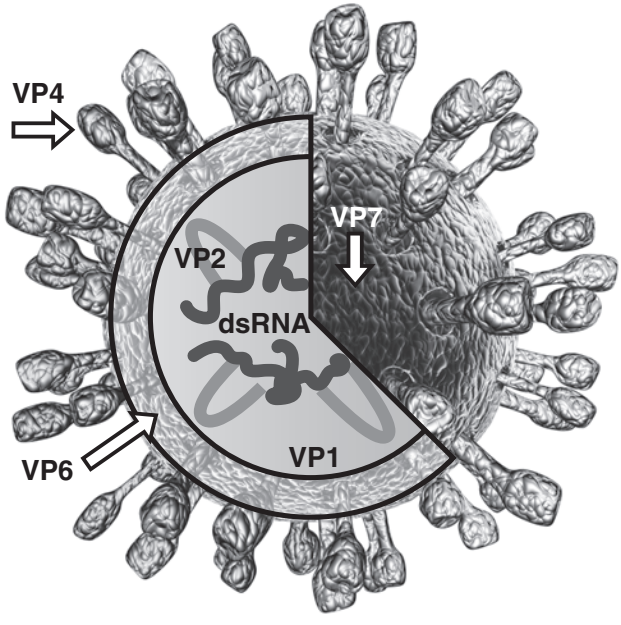

Figure 2-4 Diagram of a Rotavirus. *Source:* Photo © Psim/Dreamstime.com. *Note:* Double-stranded RNA (dsRNA) virus. Viral structural proteins are represented by VP1 through VP7.

animals (Animalia), plants (Plantae), nor fungi (Fungi). In the recent past, protozoans belonged to a single kingdom called Protista that seemed like the default category for eucaryotes. Under this conventional scheme, the protozoans were further classified using simple but useful criteria such as morphology, motility, host ranges, geographic distribution, and types of diseases caused. The advent of sophisticated molecular techniques that can reveal phylogenetic relationships has placed the classification of protozoans into a state of flux, leading to scientific debates about their interrelationships.

Regardless of the issues with protozoan taxonomy and phylogenetics, certain characteristics in terms of their pathogenesis and relevance to food safety can be generally described. Protozoans are unicellular organisms, most motile, with eukaryotic characteristics. Their morphologies differ among genera and species, and the protozoan morphology can also change during certain stages in their life cycle. One stage involves the development of cysts that can survive harsh environmental conditions. Most protozoans are free-living in the environment, while some also require multiple hosts to complete their life cycles. They reproduce by either binary fission or sexual fusion of cellular forms called gametes. Larval forms of the protozoan develop in animals called intermediate hosts, whereas the adult forms sexually reproduce in animals called definitive hosts. The active feeding stages of protozoans are usually called trophozoites. In most cases, the stage ingested from contaminated food is the cyst, and the stage that causes human disease is the trophozoite.

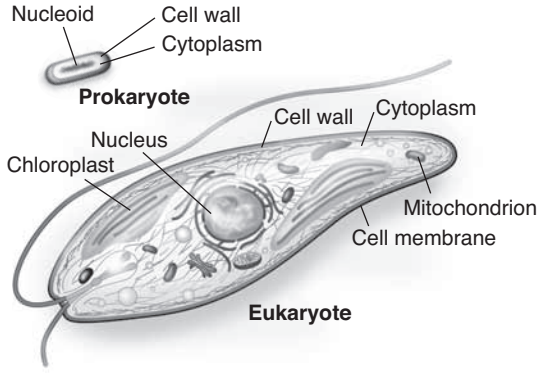

Figure 2-5 Differences Between Prokaryotic and Eukaryotic Cells

Fungi

The fungi represent a kingdom under the domain of Eucarya. Fungi reproduce through the development of either sexual or asexual spores that are easily and ubiquitously disseminated by the wind. Unlike bacterial spores, fungal sexual spores are not very resistant to heat. Fungal vegetative cells are more closely related to animals than plants and other microorganisms, and their morphology can be either unicellular or multicellular with filamentous threads called hyphae. When hyphae grow and overlap, they form visible tufts called mycelium. In their unicellular form, fungi are referred to as yeasts, while the filamentous forms are called molds when visible to the naked eye. Mushrooms are actually fungi where the mycelium differentiates into a fruiting body called a basidiocarp.

Although fungi are essential to life on earth as decomposers, only a relatively few are pathogenic to animals—including humans. These infections (called mycotic infections) and diseases (called mycoses) are rarely acquired from food, except in circumstances where the host is severely immunocompromised. Like animals, fungi are heterotrophic (i.e., must feed on preformed organic material), but unlike animals, fungi digest their food extracellularly with exoenzymes. Therein lies the primary hazard associated with fungi in foods: Many of the substances produced by fungi are inherently toxic, collectively called mycotoxins. These substances can contaminate foods. In the case of edible fungi such as mushrooms, toxic substances may be found within the basidiocarp. The toxic hazards associated with fungi in foods are discussed further in a section on mycotoxins in Chapter 3.

Helminths

The term *helminths* is used to describe a broad variety of parasites colloquially called worms. The classification of helminths varies greatly among textbooks, and some of the phyla

encompassed by the term *helminth* are phylogenetically distant from one another in the Tree of Life. Three phyla of helminths are important to food safety:

1. Phylum Plathyhelminthes, commonly known as cestodes, flatworms, or tape worms
2. Phylum Nematoda, commonly known as nematodes or roundworms
3. Phylum Annelida, including the commonly known trematodes or flukes

The helminths differ from protozoans by their size and complexity of biology. Helminths are multicellular organisms. Adults of most helminth species are macroscopic, capable of being seen without a microscope, though several helminth stages of development can be microscopic, for example, the egg and larval stages. Helminths usually have complex life cycles involving multiple intermediate and/or definitive hosts, and they have organ systems that permit functions such as sexual reproduction, sometimes as hermaphrodites. In lesser developed countries, the population is heavily burdened by these parasitic worms, whereas developed countries such as the United States have largely controlled helminth infections in human populations. However, the opportunity for reemergence of helminth infections always exists, either from lax food safety practices, international travel, and/or importation of unsafe foods.

Prions

At one time, viruses were thought to be the smallest and simplest infectious agents. This changed with the discovery of prions. Essentially, prions are rogue proteins that can be transmitted through the consumption of prion-infected animals. Once consumed, these prions corrupt other proteins by inducing protein misfolding. This results in plague formation within critical neurologic tissues and organs such as the brain. The most infamous example in recent years is bovine spongiform encephalopathy, otherwise known as BSE or "mad cow disease." Several types of prions in other animals have been identified that pose a potential threat to the food chain of humans.

FOODBORNE INFECTIONS VS. INTOXICATIONS, A.K.A. POISONING

Chapter 1 briefly introduced the difference between a foodborne "illness" and "disease." More specifically, *disease* is a condition that has been diagnosed by a clinician with a specific understanding of the etiology and biology involved. On the other hand, the term *illness* is more subjective and implies a person is "unwell" without necessarily knowing the causes or understanding the biology (Helman 1981). By extension of logic, a *foodborne illness* happens whenever a person feels sick after consuming food items, whereas a *foodborne disease* is a specific and confirmed case of disease (with a known etiology) associated with the ingestion of food.

In simplest terms, a foodborne infection is the establishment of a host–parasite relationship where the parasite entered the host by the ingestion of food. In contrast, foodborne intoxication results from an acutely toxic dose of a chemical substance in the food. The chemical substance may be a biological toxin produced by a microorganism, an inherent toxin in the tissues of a food animal or plant, or a toxicant in the environment, including food containers. The differences between foodborne infections and intoxications are discussed later in more detail.

Laypersons and the popular press sometimes refer to foodborne illnesses as "food poisoning." This colloquial term does not properly convey the etiology and prevention of a foodborne disease—or even whether it is an infection or intoxication. Yet, the term is often used and is misleading, making it difficult to educate the public about good food safety practices. For food safety professionals, the distinction between the various terms is important to understand the causes and prevention of foodborne illnesses and diseases.

Over the years, a condition called a toxicoinfection has been recognized. This term is used to describe disease from microorganisms producing toxins in the lumen of the gut after being consumed—as opposed to foodborne intoxications where the toxin is preformed in the food prior to consumption. Several bacteria are known to cause toxicoinfections and are covered in this chapter.

Basic Pathogenesis of Foodborne Infections

To understand foodborne infections, it is necessary to understand basic infectious disease transmission and pathogenesis (i.e., origin and development of disease). First, in the strictest biological sense, an infection is a symbiotic relationship between a parasite and host. When the nature of this relationship causes harmful changes in the host, an infectious disease develops. Thus, the term *infection* describes the establishment of a host–parasite relationship, whereas *disease* is when the parasite causes clinical manifestations in the host. In such cases, a parasite is also called a *pathogen*.

Quite often, bacteria are not referred to as parasites because they are rarely obligate parasites, that is, they do not always require a host to survive and reproduce. In fact—depending on the species—a great number of pathogenic bacteria can also survive and multiply outside of a host under favorable environmental conditions for various periods of time. These survival characteristics allow some pathogens to be foodborne rather than being transmitted solely through person-to-person contact. Health professionals typically use the term *parasite* for protozoans and helminths because these organisms are often obligate parasites (though some are free-living or remain viable in foods), and they possess a complex unicellular or multicellular biology similar to animals.

As illustrated in Figure 2-6, the cycle of parasitism and infection can be described in three general steps:

1. Access to the host
2. Establishment and pathogenesis (leading possibly to a carrier state or disease pathology)
3. Egress and further transmission of pathogens

Access to the Host

Before a pathogen can gain access to a host, it must be transmitted from a reservoir or other source. A reservoir is part of the pathogen's normal habitat, where it can reproduce and sustain species survival while awaiting an opportunity for transmission to a host. Several modes of transmission exist: person-to-person contact, consumption of contaminated food and water, handling of fomites (inanimate objects), vectors (other organisms), and through the air from aerosols or droplets. These modes of transmission are not necessarily mutually exclusive for most pathogens,

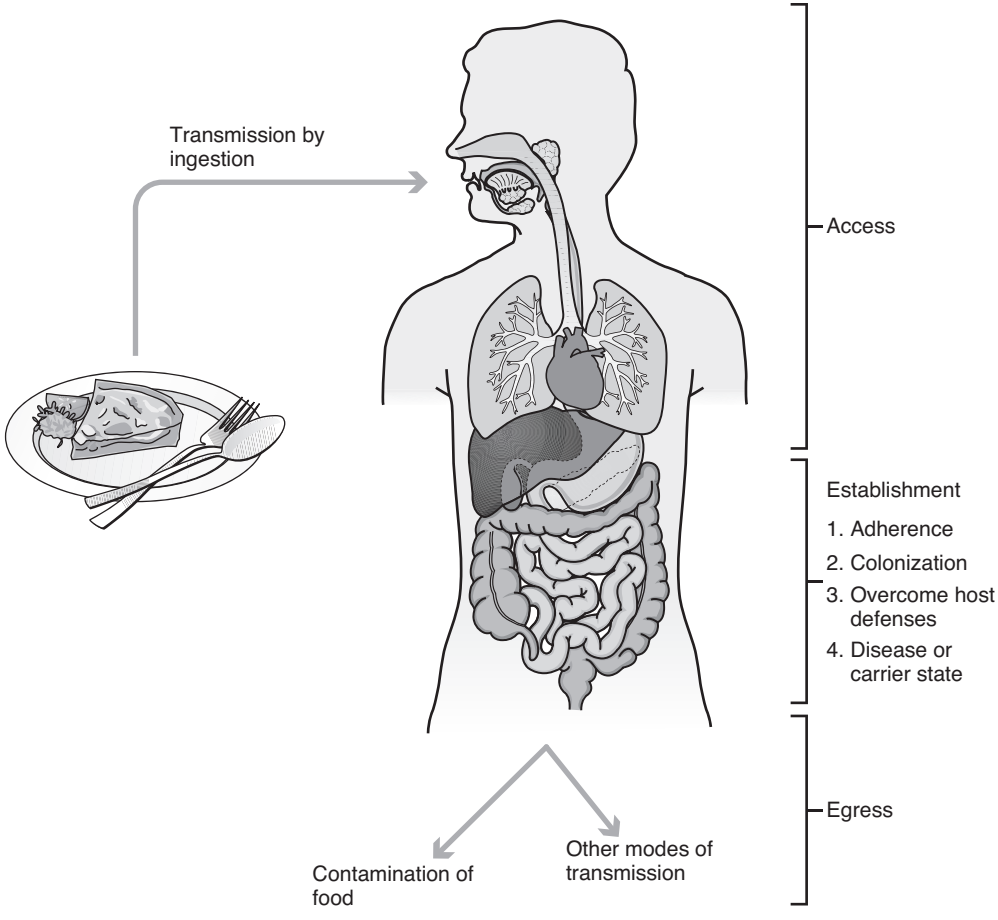

Figure 2-6 Cycle of Parasitism and Infection for Foodborne Disease

including the foodborne pathogens, and may involve a chain of transmission. For example, sputum or sneezing from an infected food handler can contaminate servings of food to further spread infection. Similarly, a rodent (vector) can transmit a pathogen by contaminating foodstuffs with feces and urine. And inanimate objects such as contaminated utensils and unclean dishes can facilitate communicable disease transmission among a population. Likewise, with certain infections, an initial case of foodborne infection can also spread through a population by person-to-person transmission.

Pathogen access to a host can occur through several transmission routes or portals of entry: inhalation, oral, ocular, through the skin (e.g., cuts, needles, bites), and sexual contact. Obviously, for food safety, the oral or ingestion route is the utmost concern. The majority of food-

borne pathogens need to access the gastrointestinal tract to cause disease, but a few only need to access the mucous membranes, for example, the agent of strep throat (*S. pyogenes*). To gain access to the gastrointestinal tract, a pathogen must survive passage through the extremely acidic environment of the stomach, which has a normal pH range of 1 to 5, and the presence of digestive enzymes such as pepsin. Several factors can help a pathogen while passing through the harsh environment of the stomach. One factor is the protective effects of food. Pathogens suspended in lipophilic vehicles are protected to various degrees from the killing effectiveness of an acid barrier (Todd et al. 2008b). Foods such as ice cream and fat-laden desserts provide good vehicles for pathogen protection. Several species of pathogens also have an acid tolerance response (ATR) for short periods of time; this is an adaptive response involving the production of specialized proteins that prevent cellular damage from environmental stress (O'Driscoll, Gahan, Hill, 1996). Such short-term adaptive responses are driven by molecular signals that turn on or off in response to environmental conditions. As discussed earlier, these responses have evolved in microorganisms as a result of selection pressures that favored strains with such molecular mechanisms.

Establishment and Pathogenesis

Establishment of an enteric pathogen requires its adhesion and colonization to the single layer of cells in the intestine called the mucosal epithelium, depicted in Figure 2-7. Several pathogen and host factors collectively determine whether adhesion and colonization will be successful. Among the host factors are barriers such as secreted mucins and other compounds that form a mucous layer and also provide an antimicrobial effect (Boirivant and Strober 2007). Other host factors include innate immune responses involving cytokines (signaling molecules), lymphocytes that can pass into the intestinal lumen, and a network of lymphatic vessels and nodes in the lamina propria, the mucosal layer of various cells under the epithelium (Magalhaes, Tattoli, Girardin 2007). Unlike adaptive immunity that requires prior exposure to the pathogen, the responses by innate immunity provide a relatively rapid and broad barrier for the epithelium.

Before an enteric pathogen can successfully adhere to and colonize the intestine, it must possess specialized biomolecular "traits." Adherence to the mucosal epithelium involves the binding of specialized proteins (and other molecules) on the pathogen called adhesins with receptors that span the cell membranes of the epithelium. In bacteria, most adhesins are associated with hairlike appendages protruding from the cell wall called fimbriae; these structures are sometimes referred to as pili when describing the intercellular transfer of nucleic acids (Pizarro-Cerda and Cossart 2006). The adhesins are typically associated with the apical end of the fimbriae, and the protein structure of adhesins can be different for certain species or strains of bacteria. This is important because protein structure determines the specificity and strength of binding to a receptor on the epithelial cell membrane.

An important barrier to pathogen adhesion and colonization is an extremely large population of commensal microorganisms (approximately 10^{14} bacteria) that reside in the gut, collectively called the microbiota. These microorganisms colonize both the lumen and the mucosal epithelium. They confer benefits to the host in terms of development and digestion, but more important, they protect the intestine from pathogens by a phenomenon called microbial interference or colonization resistance (Stecher and Hardt 2008). In essence, this phenomenon results from the

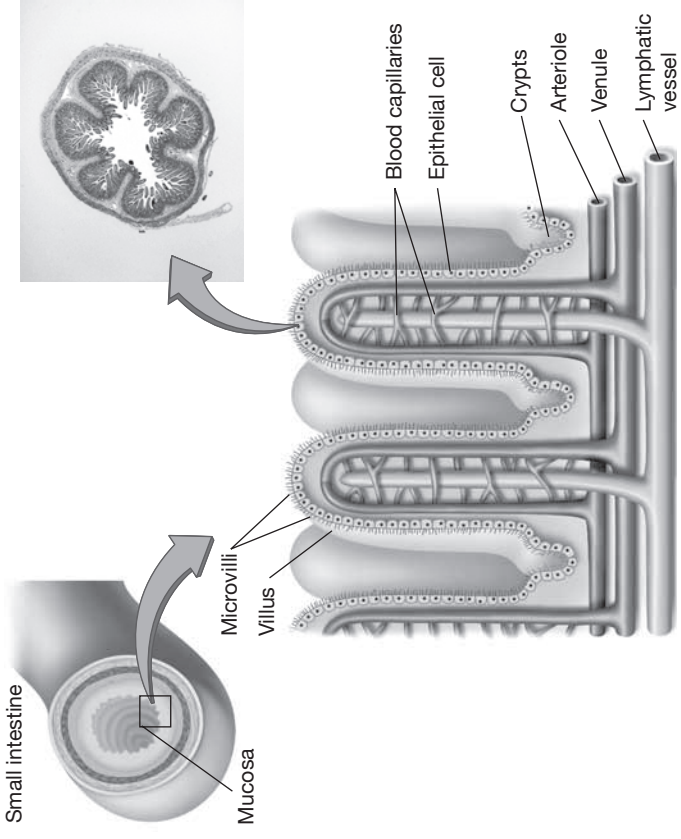

Figure 2-7 Anatomy of the Intestine. *Source:* Photo courtesy of Douglas Burrin/USDA ARS

microbiota outcompeting enteric pathogens for space and nutrients within the microbial ecology of the intestine. At the biomolecular level, a complex set of mechanisms is involved among the pathogens, microbiota, and host. Some of these biomolecular mechanisms have been described in great detail, while others are not fully understood (Magalhaes et al. 2007).

Various species of the gut microbiota also possess adhesins for colonizing the mucosal epithelium, thus creating competition for pathogens to access the epithelium. Pathogens outcompete the microbiota in a number of ways. One way is for the pathogen's adhesins to possess a unique protein structure that preferentially binds with different receptors on the surface of the epithelium (Viswanathan, Hodges, Hecht 2009). Evidence also exists that gut inflammation can disrupt colonization resistance of the microbiota; pathogens can circumvent multiple biomolecular processes involved with colonization resistance as well (Stecher and Hardt 2008). Finally, it is well established that reduction of commensal bacteria in the gut by preexisting disease or certain exposures (e.g., radiation and antibiotic therapy) can lead to an increased risk of enteric infections and to more severe cases of infection.

Colonization by the pathogens is necessary to increase their numbers. Several species of bacterial pathogens can develop an attachment matrix called a biofilm. Actually, this matrix is not a uniform film but rather a heterogeneous mixture of bacterial cells and organic and inorganic components (Donlan 2001). Through an intercellular communication process called quorum sensing, certain pathogens can induce a colony of bacteria to produce substances for biofilm development. Biofilms offer several distinct advantages for a pathogen: (1) They provide resistance to the host immune system; (2) they provide protection against antimicrobial agents; (3) they provide an environment to exchange DNA among the pathogens; and (4) they provide a protective matrix for detachments of pathogens to colonize other parts of the body (Donlan and Costerton 2002). The importance of biofilms is discussed again later with regard to sanitation and environmental surfaces.

Pathogens must overcome the immune defenses of the host before, during, and after colonization. This is accomplished by any of several tactics involving evasion, subversion, and/or exploitation of the host's innate and acquired immunity (Finlay and Falkow 1997). One tactic is the development of biofilms discussed previously. Most tactics involve biomolecular interactions that circumvent the host immune system. For example, pathogens can disguise themselves by attaching proteins on their surfaces that mimic host proteins, causing the host's antigen-recognition mechanisms to be fooled. Other mechanisms involve secretion of compounds that enzymatically degrade or destroy the host's immune components and interfere with the molecular signaling processes. The most common pathogen tactic is antigenic variation, often by a new strain of pathogen. By changing the antigens on their surfaces (Figure 2-8), pathogens can escape recognition by the host's immune system, or at least make the recognition process less efficient.

After successful colonization by a pathogen (i.e., infection), the host can suffer disease or become an asymptomatic carrier. Incubation periods can vary before any signs and symptoms appear (Figure 2-9). The most common affliction is gastroenteritis, a general term used to describe irritation or inflammation of the gastrointestinal tract. The signs and symptoms of gastroenteritis caused by different foodborne pathogens tend to overlap one another. According to the list in Table 2-2, the predominant sign is diarrhea.

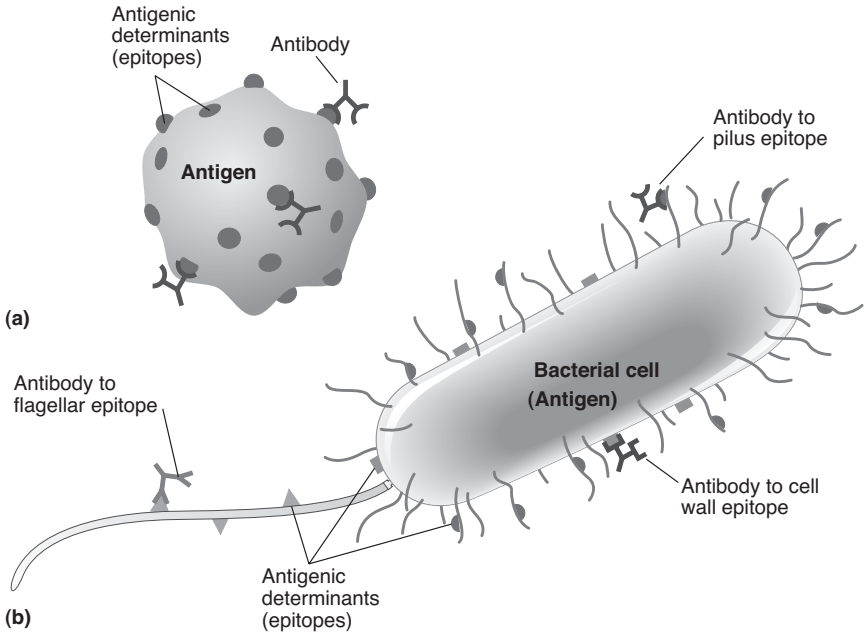

Figure 2-8 Antigens and Antigenic Determinants (Epitopes)

The internal lining of the intestine has exquisitely evolved to maximize its surface area and to facilitate nutrient absorption, while also maintaining an electrolyte balance between the lumen contents and surrounding tissues. These important functions are primarily dependent on the mucosal epithelium. When an imbalance occurs between the absorption and secretion of ions

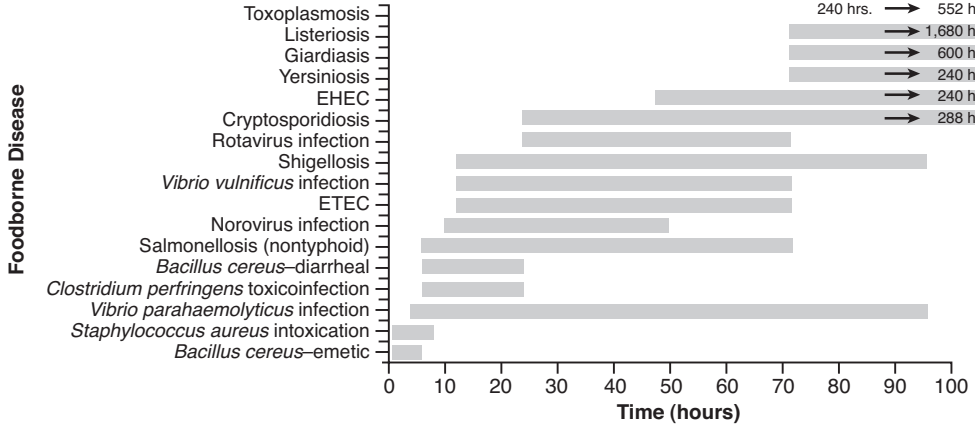

Figure 2-9 Incubation Ranges for Selected Foodborne Diseases. *Source:* Data from Heymann, 2008

Table 2-2 Signs and Symptoms of Gastroenteritis from Foodborne Infections

Disease	*Nausea or Vomiting*	*Diarrhea (Type)*	*Cramps or Pain*	*Fever*	*Other Signs, Symptoms*
Campylobacteriosis	P	P-bloody[c] (inflammatory)	P	P	—
EHEC[a] infection	P	P-severe-bloody[c] (inflammatory)	P	P/A	—
ETEC[b] infection	P/A	P-watery (noninflammatory)	P	A	—
Salmonellosis (nontyphoid)	P	P (inflammatory)	P	P	—
Shigellosis	A	P-mucus[c]-blood[c] (inflammatory)	P	P	—
Cholera	P	P-profuse watery (noninflammatory)	A	A	Severe dehydration
Yersiniosis	P	P (inflammatory)	P	P	Appendicitis-like
Listeriosis	P/A	P/A	A	P	Muscle aches Flu-like Bacteremia
Cryptosporidiosis	A	P-watery (noninflammatory)	P	P-slight	May be relapsing
Giardiasis	A	P (noninflammatory)	P	A	Gas
Norovirus infection	P	P (noninflammatory)	P	P	Myalgia Headache
Rotavirus infection	P	P-watery (noninflammatory)	P	P-low grade	Lactose intolerance

Note: P = usually present; A = usually absent; P/A = may be present or absent.
[a] Enterohemorrhagic *E. coli*.
[b] Enterotoxigenic *E. coli*.
[c] May or may not be this type of diarrhea.
Source: Compiled from AMA et al. 2004 and Navaneethan and Giannella 2008.

and solutes by the epithelial cells, excessive water moves into the lumen, resulting in diarrhea (Viswanathan et al. 2009). Although several conditions can lead to diarrhea, the most common preventable causes are enteric pathogens—including foodborne pathogens. Two major syndromes of diarrhea are recognized: (1) noninflammatory diarrhea and (2) inflammatory diarrhea (Navaneethan and Giannella 2008).

Pathogens that alter the permeability of the mucosal epithelium to ions and solutes without significant inflammation or mucosal damage cause noninflammatory diarrhea. This typically involves pathogen adherence to and colonization of the small intestine. With this type of diarrhea,

the mucosal epithelium is rarely invaded by the pathogen, except for viruses, which use a method called endocytosis. The primary mechanism for noninflammatory diarrhea is the production of target-specific exotoxins by the pathogen. Exotoxins are proteins released by pathogens that modify or destroy host cellular structures such as membranes, extracellular matrices, or other intracellular targets. Toxin structures may consist of two or more subunits and/or involve multiple molecular interactions. By altering transporter proteins and ion channels in the mucosal epithelium, some exotoxins cause diarrhea without inflammation (Navaneethan and Giannella 2008). The toxin's structure, mechanism of action, and pathogenesis are often important to classifying and identifying a strain of pathogen. Toxins that actively affect the gastrointestinal tract are collectively called enterotoxins.

As the term implies, inflammatory diarrhea is caused by molecular products from an acute inflammatory reaction of the intestinal mucosa. One cause of inflammation is the invasion of the intestinal mucosa by the pathogens. A few pathogens may actually pass through the mucosa into the bloodstream and affect other organs (e.g., *Salmonella* spp. and *Listeria monocytogenes*). The invasiveness of pathogens is enhanced by compounds appropriately called invasins. Another cause of inflammation is the production of exceptionally noxious toxins by the pathogens called cytotoxins, which severely damage host cells and induce inflammatory diarrhea (Navaneethan and Giannella 2008). Some particularly virulent pathogens cause inflammatory diarrhea by both invasiveness and the production of toxins.

Over the past few decades, research and follow-up on acute foodborne infections have revealed latent or chronic aftereffects called sequelae among a small percentage of victims (Rees et al. 2004). Among these conditions are neurologic disorders; inflammatory diseases of the bowel and joints, such as colitis and arthritis; kidney and vascular damage; and other gastrointestinal disorders (Lindsay 1997). These chronic conditions can appear weeks or years after the acute infection and may last for many years after onset, possibly leading to premature death. The particular species and strains of pathogens, along with host predisposition, can play a role in the risk of sequelae.

An important concept associated with foodborne infection (and infectious diseases in general) is the nature of pathogen virulence and host resistance. Simply stated, virulence is the capacity of a given pathogen to produce disease. Conversely, resistance is the capacity of a given host to resist disease. Virulence and resistance can be viewed abstractly as a battle between pathogens and hosts with an array of different weapons available to each one. For the pathogen, the arsenal of weapons includes adhesins, invasins, toxins, antigenic factors, dissemination to other tissues, antibiotic resistance, and molecular signals for immune system circumvention. These virulent properties or capabilities of the pathogen are referred to as virulence factors, and they are the products of specific sets of genes within the pathogen's DNA.

For the host, the defensive weapons can be classified as either nonspecific host resistance or specific host resistance. The most important defense within the realm of nonspecific host resistance is the health and nutritional status of the host. A healthy and well-nourished host is better equipped to defend against and eliminate a pathogen. On the other hand, preexisting disease and/or malnourishment can negatively affect everything from immune system function to the microbiota of the intestine. Certain drugs and medications can also have the same effect. The specific host resistance includes the synthesis of immunoglobulins and/or the development of cellular immunity. Much of the specific host resistance is determined by the host's genes. Low host

resistance is equivalent to host susceptibility to infection. Both nonspecific and specific host resistance can be affected by age, with the very young and very old being most susceptible to infections because of either undeveloped (younger) or diminished (older) host resistance.

A related and important concept to food safety is the infectious dose or infective dose of a pathogen. In theory, this is the minimum number of pathogens (of a specific strain and species) necessary to cause an infectious disease in a host. However, a review of literature on infective dose does not reveal a formal definition in any medical texts (Johnson 2003). The difficulties with trying to determine an infective dose for a pathogen are numerous. Foremost, the variability associated with pathogen virulence and host resistance is huge—especially when different strains of pathogens and the heterogeneous makeup of human hosts are taken into account. Experiments to determine infective dose using similar strains of pathogens and inbred animals still yielded great variability, and the results could not be extrapolated to humans (Johnson 2003). It has been hypothesized that much of this variability can be explained by the biomolecular mechanisms of pathogenesis, but research has not adequately tested this hypothesis (Schmid-Hempel and Frank 2007).

Although extreme variability hinders establishment of an infective dose for pathogens, epidemiologic studies of foodborne illness outbreaks have provided estimates for several pathogens. Despite the shortcomings of determining infective doses, these estimates do convey the relative virulence of foodborne pathogens in healthy individuals. Furthermore, the concept of infective dose is helpful in risk assessments and determining acceptable levels of pathogens in foods (Teunis, Nagelkerke, Haas 1999). It is important to remember that infective doses can be much lower for very young or senior people, and many medical conditions or concurrent exposures, some discussed earlier, can lower host resistance and hence the infective dose.

Egress and Transmission

The final step of the parasitism and infection cycle is egress and transmission: leaving the primary host to infect other hosts. The principal means of egress for foodborne pathogens is through defecation, usually diarrhea, and sometimes vomiting. Other body fluids may also be contaminated with the more invasive pathogens. To a great degree, inducing diarrhea is a survival mechanism for enteric pathogens because it provides the pathogens with an opportunity to leave the host in greater numbers, increasing the chances of transmission and access to other hosts. The number of pathogens expelled commonly ranges from 10^5 to 10^9 infectious cells or viral particles per milliliter (mL) or gram (g) of feces, sometimes as high as 10^{11} cells or particles per mL or g (Todd et al. 2008b).

During the incubation and convalescent phases of a disease, when a host may not exhibit disease symptoms, large numbers of pathogens can still be shed in the feces. These hosts are incubatory and convalescent carriers of disease-causing microorganisms, capable of contaminating foodstuffs and transmitting the disease to others. In situations where individuals have a great resistance to a pathogen, they can become subclinically or asymptomatically infected for indefinite periods of time. These individuals apparently do not suffer from disease but remain disease carriers capable of shedding the pathogen and transmitting it to others. They are referred to as asymptomatic carriers. The first confirmed case of an asymptomatic carrier was the villanized Mary Mallon, a.k.a. Typhoid Mary, discussed in Chapter 1. Since then, it is an established fact that a great

number of asymptomatic disease carriers exist, both in human and animal populations. Disease carriers are not easily identified but are extremely important considerations in food safety.

FOODBORNE BACTERIAL AGENTS

Pathogenic *Escherichia coli*

News reports about outbreaks caused by *Escherichia coli* have caused grave concern among consumers and public health officials over the past few decades. Despite the notoriety that has become associated with *E. coli*, this species of bacterium usually resides peacefully within our intestines as part of the microbiota. The strain or serotype of *E. coli* determines its virulence and pathogenicity. Simply stating that *E. coli* is a hazard without stipulating a strain or serotype is a failure to understand the complexity of this bacterium's interaction with a host. The various schema used to classify *E. coli* must be introduced to better understand this phenomenon. Before this can be done, however, some definitions about the differences within a bacterial species must be provided.

Within any designation of a species, a certain amount of biological variation is expected. A strain of bacterium is a group that has distinct physiologic and/or biochemical characteristics in common but different from other groups within a species. Such groupings have traditionally been phenotypic (observed properties) based on biochemical testing, but recent advances in DNA sequencing (i.e., genomics) may eventually redefine the concept of strains and species (Konstantinidis, Ramette, Tiedje 2006). A serotype or serovar is "a group of intimately related microorganisms distinguished by a common set of antigens" (Medline Plus, Merriam-Webster Medical Dictionary). An antigen is any chemical substance, either alone or attached to another molecule, capable of evoking an immune response. The result of this immune response is the production of specialized proteins called antibodies that will bind to specific sites on the antigenic molecules called determinants or epitopes (see Figure 2-8). In the case of bacteria such as *E. coli*, components of the cell wall, flagellum, and capsule contain molecules capable of evoking an immune response. The antigenic molecules of the cell wall are lipopolysaccharides, while the flagellar antigens are proteins; capsular antigens are most often polysaccharides. The structural configuration and chemical composition of these antigenic molecules may vary greatly, and each variation can elicit production of highly specific antibodies. The antigens are classified by location using the letters O, H, and K for the cell wall, flagellum, and capsule, respectively (Figure 2-10). More than 200 O-type antigens and about 30 H-type antigens have been recognized for the bacterium *E. coli* (Kaper, Nataro, Mobley 2004). Serotype groupings often encompass strains of bacteria and vice versa, that is, common antigens may overlap with several observed physiologic properties. For example, the notorious serotype *E. coli* O157:H7 also has several different strains.

The pathogenic *E. coli* strains are further classified by properties such as their virulence factors, disease syndromes and pathology they cause, and by different effects they have on cell cultures. Table 2-3 summarizes the classification of pathogenic *E. coli* using this scheme. The table does not include the pathogenic *E. coli* responsible for extraintestinal diseases. Of the six groups, the Enterohemorrhagic *E. coli* (EHEC) strains are most important in North America, the United Kingdom, and Japan (Kaper et al. 2004). The EHEC strains most responsible for outbreaks

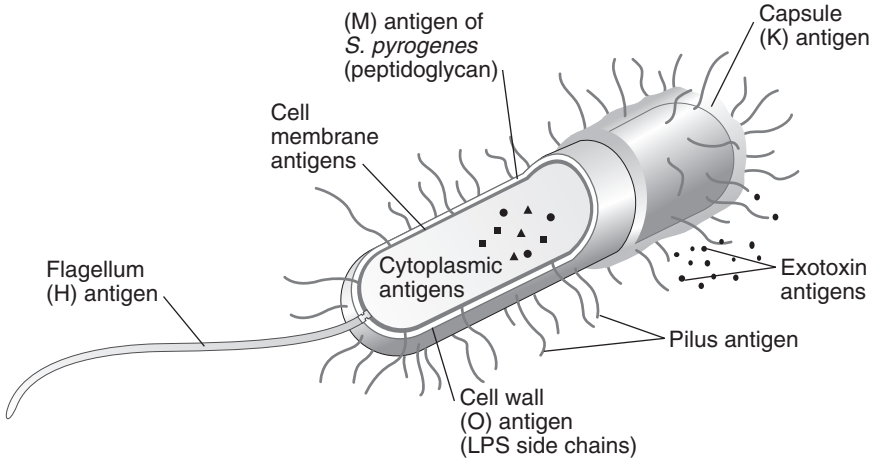

Figure 2-10 The Various Antigens Possible on a Bacterial Cell

belong to serotype *E. coli* O157:H7 (Figure 2-11), which has been identified in many serious foodborne disease outbreaks and food recalls in the United States. Only 20% of *E. coli* O157:H7 cases are assumed to be associated with outbreaks; the majority of cases (80%) are sporadic, that is, not associated with outbreaks (Mead et al. 1997).

Foodborne disease outbreaks of *E. coli* O157:H7 have been linked to beef more than any other single food (Figure 2-12). Among beef products, ground meat represents the greatest risk of disease. The disease risk from hamburger was modeled and quantitatively related to multiple factors, but the following factors were predicted to have the greatest influence on risk: (1) concentration of *E. coli* O157:H7 in cattle feces; (2) host susceptibility, greatest with children and others with weakened resistance; and (3) the degree of beef carcass contamination with *E. coli* O157:H7 (Cassin et al. 1998). Risk mitigation strategies were also modeled, and the preventive measures with the greatest influence on reducing disease risk were the following: (1) temperature storage control to limit microbial growth (80% risk reduction), (2) preslaughter screening of cattle (46% risk reduction), and (3) consumer cooking practices (16% risk reduction). Other meats have also been linked to *E. coli* O157:H7 outbreaks, and in recent years, an increasing number of outbreaks have been associated with vegetables and fruits. The contamination of produce most likely results from poor manure waste management, contaminated water runoff, and/or livestock access to fields and harvest sites. Farm workers and equipment could also be a source of contamination.

A 1993 outbreak was a landmark event for EHEC infections, bringing attention to the problems of contaminated meat—and the seriousness and sometimes fatal consequences of infection. Among the 501 confirmed *E. coli* O157:H7 cases, 151 people (31%) were hospitalized, and 45 (9%) suffered from hemolytic uremic syndrome (HUS); 3 children also died (Bell et al. 1994). All EHEC strains produce toxins consisting of A and B subunits that are similar to those of *Shigella* species, the causative agents of bacillary dysentery. Consequently, the EHEC toxins are called

Table 2-3 Classification of Pathogenic *E. coli* Strains

Pathogenic Strains	*Clinical Features*	*Virulence Factors and Pathology*	*Reservoirs and Transmission*
Enterohemorrhagic *E. coli* (EHEC) (includes *E. coli* O157:H7)	Bloody diarrhea. May have sequelae such as hemolytic uremic syndrome (HUS).	Affects the large intestine; produces large quantities of Shiga-like toxins. All toxins consist of A and B subunits. Effacement of microvilli occurs after attachment.	Cattle are the primary reservoir, but other animals and humans may be sources. Most often transmitted by contaminated foods. Low infective dose: $10–10^2$ colony forming units (CFUs).
Enteroinvasive *E. coli* (EIEC)	Acute dysenteric-type diarrhea, bloody or nonbloody.	Strains generally do not produce enterotoxins, but they invade and multiply in the colonic epithelium.	Humans are the established reservoir. Limited evidence suggests foodborne transmission.
Enteropathogenic *E. coli* (EPEC)	Acute, watery diarrhea with mucus. May be persistent and severe among infants, whom are predominantly affected.	Strains generally do not produce enterotoxins. After localized colonization of the intestinal mucosa, they cause attachment-effacement lesions.	Humans are the established reservoir. Transmission occurs mostly from infant formula and foods, fomites, and hands.
Enterotoxigenic *E. coli* (ETEC)	Profuse, watery, and noninflammatory diarrhea. Often self-limiting in adults but can be persistent and severe in children.	Colonizes the small intestine but does not invade the intestinal cells. Produces one or two enterotoxins, which are categorized into two groups: heat-labile toxins (LT) and heat-stabile toxins (ST).	Strains are very species-specific for hosts. Humans are the reservoir for human ETEC disease. Major cause of diarrhea in lesser developed countries, including traveler's diarrhea. Contaminated food and water are primary modes of transmission. Infective dose is $10^8–10^{10}$ CFUs in adults.
Enteroaggregative *E. coli* (EAEC)	Watery diarrhea with mucus, seen mostly in infants and children. Infections may be asymptomatic.	Adheres to intestinal epithelium with a characteristic biofilm incorporating aggregations of bacteria and mucus. One or two enterotoxins may be produced.	Humans are probably the reservoir. Recognized as an important cause of infant diarrhea among lesser developed countries. Food and water are probably the main modes of transmission.
Diffuse-Adhering *E. coli* (DAEC)	Watery diarrhea with mucus in toddlers and preschool children.	Little is known about the virulence and pathology, except for the characteristic pattern of adherence to HEp-2 cells in culture.	Little is currently known about the reservoirs and mode of transmission. Occurs mostly in lesser developed countries.

Source: Compiled from Kaper et al. 2004, Heymann 2008, Donnenberg and Nataro 2000.

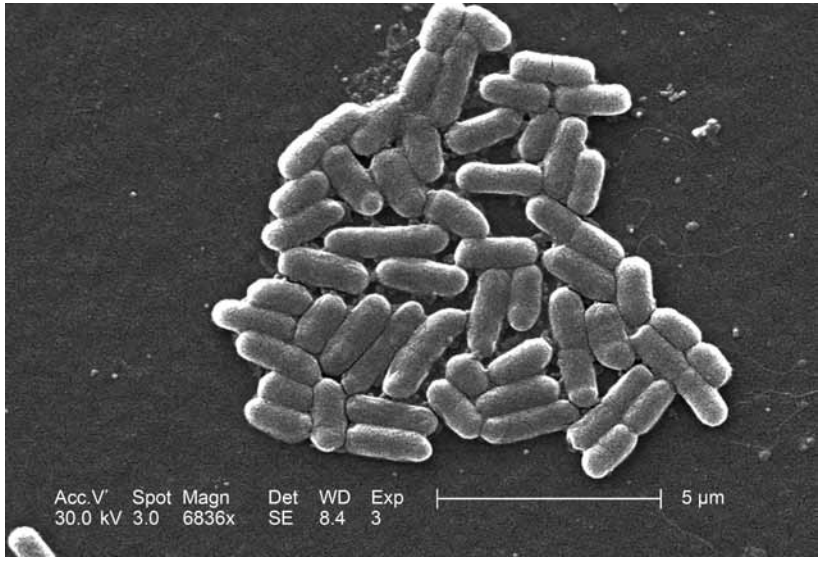

Figure 2-11 Scanning Electron Micrograph of *E. coli* O157:H7 Bacteria. *Source:* Courtesy of Janice Haney Carr/CDC

Shiga-like toxins. After intimate attachment of EHEC to the epithelial cells, the toxin's B subunit binds to the host cell membrane, and the entire toxin is internalized by endocytosis. Once inside the host cell cytoplasm, the A subunit of the toxin interferes with protein synthesis by binding to certain ribosomes. For some victims, the shiga-toxins become attached to receptors on white blood cells and travel to other organs, including the kidneys, brain, and pancreas. Once transported to distant organs, the toxins bind more strongly to the organ's cells and become transported into the cytoplasm, where they interfere with protein synthesis and cause cellular injury and death. In the case of HUS, the formation of blood clots and the destruction of red blood cells in the small vessels of the kidney lead to renal injury or failure. Children are particularly susceptible to HUS.

The Enteroinvasive *E. coli* (EIEC) strains generally do not produce enterotoxins and are distinct from other pathogenic *E. coli* because they enter and multiply in colonic epithelial cells and further invade adjacent epithelial cells in a manner similar to another invasive bacterium called *Shigella*. The result is cell and tissue damage leading to voluminous diarrhea, either bloody or nonbloody. The Enteropathogenic *E. coli* (EPEC) also generally do not produce enterotoxins, but they cause diarrhea by producing lesions on the intestinal mucosa. Enterotoxigenic *E. coli* (ETEC) adhere to and colonize the small intestine by fimbrial colonization factors. Once attached, the ETEC produce one or two enterotoxins that cause the cellular damage and diarrhea. The last two categories of pathogenic *E. coli* (EAEC and DAEC) are not well understood, but they seem more important in lesser developed countries and/or with infant diarrheal diseases.

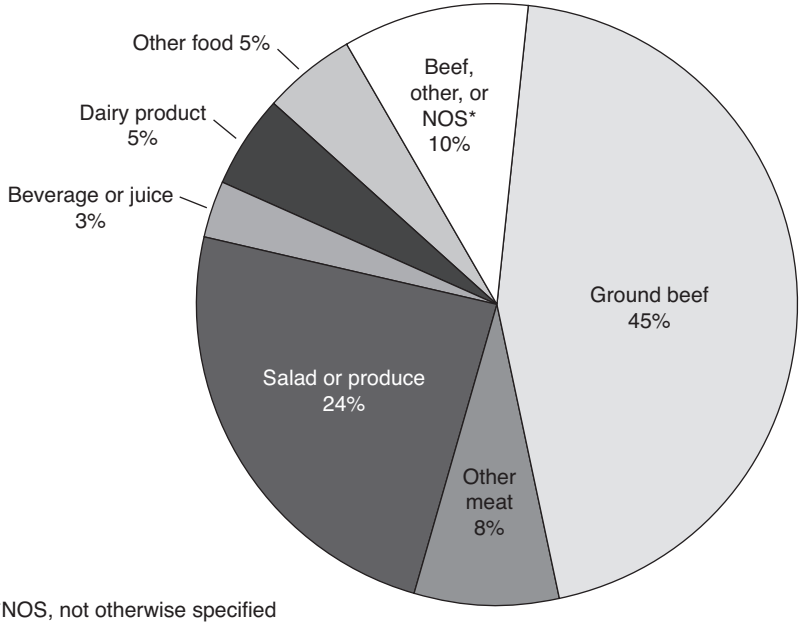

Figure 2-12 Food Categories Implicated in 267 *E. coli* O157:H7 Outbreaks—U.S. from 1990–2006. *Source:* Data from: Center for Science in the Public Interest

Human carriers are the primary reservoirs of non-EHEC infections and probably play a significant role in food and water contamination. Most non-EHEC infections are presumed to be endemic in the lesser developed countries, but the investigation and publication of outbreaks in these countries are lacking to make any scientific conclusions (Todd et al. 2008b). Compared with EHEC infections, the infective doses of non-EHEC infections are considerably higher, around 10^6 to 10^8 colony-forming units (CFUs). This suggests that foods must either become heavily contaminated or be kept at unsafe temperatures to allow pathogen growth and multiplication. The pathogens EIEC and ETEC are often associated with enteric infections known as Traveler's Diarrhea, a syndrome attributed to those who visit foreign countries and return home ill. Foodborne outbreaks of non-EHEC have been documented in the United States, but many of them involved imported foods (Naimi et al. 2003).

Figure 2-13 illustrates the foodborne transmission of pathogenic *E. coli*.

Salmonella Species and Serotypes

The name *Salmonella* has become commonplace in the vocabularies of many Americans because of unfortunate and highly publicized outbreaks of salmonellosis. In fact, the number of reported salmonellosis cases has been increasing worldwide, ironically more among the developed

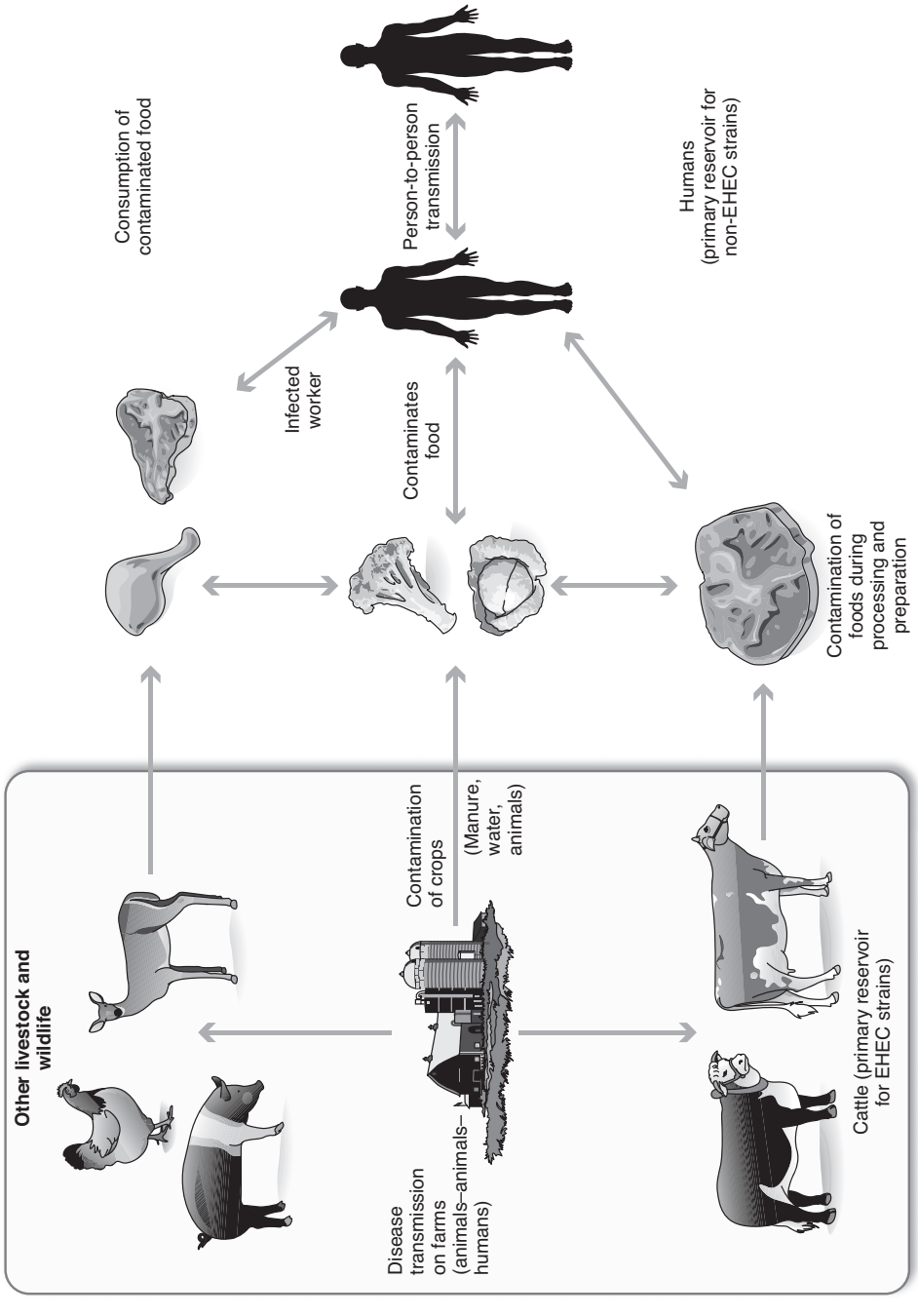

Figure 2-13 Foodborne Transmission of Pathogenic *E. coli* Strains

or industrialized countries; it was one of the first diseases classified as an emerging infection. The name *Salmonella* originated with a veterinarian, Dr. Daniel Elmer Salmon, whose research assistant discovered the bacterium in 1885. Since that time, more than 2,500 serotypes (or serovars) of *Salmonella* (Figure 2-14) have been recognized (Centers for Disease Control and Prevention [CDC] 2008). Despite this great number of serotypes, the genus consists of only two species: *S. enterica* and *S. bangori*. The species *S. enterica* consists of six subspecies (see Table 2-4) and includes most serotypes involving animal and human infections. For the unenlightened, the naming convention for *Salmonellae* can be confusing. Nevertheless, taxonomic classification and serotyping are very important to salmonellosis control, so the topic needs to be introduced.

The naming of *Salmonella* serotypes is done by international agreement but is still evolving (Brenner et al. 2000). *Salmonellae* are first grouped by O antigen similarities and then further classified based on their H antigens. Additional classification can be pursued using antimicrobial resistance, phage typing, plasmid profiling, and other methods. In the early years of *Salmonella* research, a species or serotype was named after the disease it caused or for the animal from which it was isolated. In later years, a serotype was named after the place from where it was first isolated, a dubious honor if associated with a salmonellosis outbreak. Naming convention is such that the genus, species, and subspecies names are italicized, and the serotype names are not italicized but capitalized, that is, *S. enterica* ssp. *enterica* ser. Typhimurium. For convenience, it is acceptable to use only the genus and serotype; for example, *Salmonella* Typhimurium is an acceptable alternative.

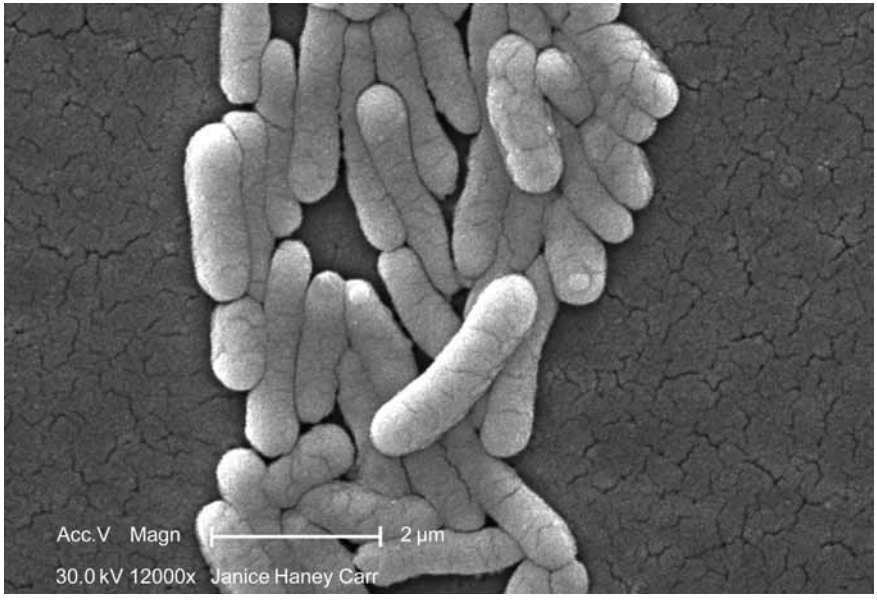

Figure 2-14 Scanning Electron Micrograph of *Salmonella Typhimurium. Source:* Courtesy of Janice Haney Carr/CDC

Table 2-4 Subspecies of *S. enterica* and Serotyping Designations

Serotyping Designation	Salmonella enterica *Subspecies*
I	*Salmonella enterica* ssp. *enterica*
II	*Salmonella enterica* ssp. *salamae*
IIIa	*Salmonella enterica* ssp. *arizonae*
IIIb	*Salmonella enterica* ssp. *diarizonae*
IV	*Salmonella enterica* ssp. *houtenae*
VI	*Salmonella enterica* ssp. *indica*

Source: CDC, 2003.

For the purposes of consistency and international health, the Centers for Disease Control and Prevention (CDC) has adopted the Kaufman-White scheme for serotype designation. In this scheme, serotypes are designated using a standardized formula, albeit in many cases the serotype names are still used (CDC 2003). The formula designation begins by specifying the subspecies using the Roman numeral shown in Table 2-4 (for serotyping purposes, the species *S. bangori* is designated by Roman numeral V). Next, the O and H antigens are listed using the following formula and format:

Subspecies O antigens: Phase 1 H antigen: Phase 2 H antigen

Unlike the *E. coli* serotyping, the O antigens are designated as numbers (some may still use letters), and the H antigens can be designated as numbers, letters, or an alphanumeric combination. The O and H antigens may have several factors overlapping with a particular serotype; in these cases, the factors are separated by commas, and if an H phase is absent, it is designated by a hyphen (-). Following are a couple of examples using the formula designation (see CDC 2008 for additional information):

Serotype Name Convention	Serotype Formula Designation
S. enterica spp. *enterica* ser. Typhimurium (or *Salmonella* Typhimurium)	I 4,5,12:i:1,2
S. enterica spp. *enterica* ser. Enteritidis (or *Salmonella* Enteritidis)	I 9,12:g,m:-

Based on pathogenicity, an important historical and clinical distinction is made between two designated classes of salmonellosis: Typhi and non-Typhi salmonellosis. The serotypes *S. enterica* ssp. *enterica* ser. Typhi (or *S.* Typhi) and *S. enterica* ssp. *enterica* ser. Paratyphi (or *S.* Paratyphi) are responsible for the serious diseases of typhoid fever and paratyphoid fever, respectively. The historical significance of typhoid fever was introduced in Chapter 1. This disease is systemic and characterized by fever, headache, and a number of other signs and symptoms with varying degrees

of severity, depending on the virulence of a particular strain and the patient's resistance. Unlike most other enteric diseases, typhoid fever causes constipation more often than diarrhea. Before antibiotics were widely available, the case-fatality rate of typhoid fever was 10% to 20%, but with proper antibiotic therapy, the case-fatality rate drops below 1% (Heymann 2008).

In the twenty-first century, typhoid fever occurs mostly in lesser developed countries, and it is considered endemic in regions of Asia, Africa, and Latin America. In endemic regions, cases of typhoid fever can often be mild or asymptomatic and without systemic involvement. This makes the control of disease transmission very difficult because of unwary human carriers. Human typhoid fever is associated exclusively with human carriers and usually with contaminated food or water, particularly in areas with poor sanitation, raw milk, and untreated water. Paratyphoid fever is generally milder compared with typhoid fever. Several special serotype designations have been established (A, B, and C) for *S.* Paratyphi, but all serotypes of paratyphoid fever have a similar clinical presentation. The geographical occurrence and transmission of paratyphoid fevers are also similar to typhoid fever.

Outbreaks or cases of *Salmonella* infections not caused by *S.* Typhi or *S.* Paratyphi are generally referred to as "non-Typhi" salmonellosis (henceforth referred to simply as salmonellosis). The infective dose of non-Typhi *Salmonella* varies greatly with the serotype and host resistance, with a range as wide as 10^1 to 10^9 CFUs (Todd 2008b). The infection process for *Salmonella* serotypes occurs relatively rapidly. After ingestion, the bacilli colonize sites on the epithelium of the intestine, and within a manner of minutes, they invade cells of the intestinal mucosa, where they rapidly multiply. The preferential sites of involvement include the ileum, appendix, and right colon (Lamps 2003). In approximately 2% of cases, the bacteria invade the bloodstream (septicemia) and other organ systems. Among the signs and symptoms of enteric disease are diarrhea, fever, vomiting, and abdominal cramps. Toxins have been implicated in the disease process, but they have been difficult to isolate and study. In most cases, treatment involves supportive care and possibly rehydration and electrolyte replacement. Although the disease is usually self-limiting, severe cases can mimic ulcerative colitis or Crohn disease, and extraintestinal cases may require specific antibiotic therapy. However, the use of antibiotics in mild cases is believed to contribute to antibiotic-resistant strains. The convalescent carrier times can be quite prolonged, lasting months and possibly a year in some cases. This underscores the importance of medical clearance and good personal hygiene among food workers who have recently recovered from salmonellosis.

Clinicians now recognize several possible sequelae associated with salmonellosis that appear after acute infection has been resolved. One prominent aftereffect is postinfectious irritable bowel syndrome (PI-IBS), which is prevalent an average 15% among those who have recovered from an intestinal infection (Smith and Bayles 2007). The signs and symptoms of PI-IBS can be intermittent and include abdominal pain, diarrhea, fever, and other conditions, sometimes resulting in abdominal abscesses. The other bacteria also often associated with foodborne disease outbreaks and PI-IBS are species of *Campylobacter* and *Shigella*. Another possible sequela associated with postsalmonellosis is an autoimmune disease known as Reiter's syndrome (Lindsay 1997). This condition results when the body's immune system attacks its own tissues. The symptoms often include arthritis, eye irritation, urinary tract problems, and other symptoms. These symptoms usually occur within 3 weeks following the infection.

Compared with other pathogens in the family Enterobacteriaceae, the reservoirs of *Salmonella* encompass a greater variety of warm- and cold-blooded animals. Isolates of *Salmonella* have been cultured from the intestinal tracts of domestic and wild birds, hoofed animals, cats, dogs, rodents, reptiles, and even insects. Animal-to-human contact frequently causes transmission of salmonellosis. For foodborne transmission of salmonellosis, animals used for meat are the most important, though any animal (e.g., pests) that contaminates food-contact surfaces may be a source of foodborne transmission. Most outbreaks of salmonellosis have been traced to red meats, poultry, eggs, and unpasteurized milk products. Beef has been implicated most often in individual outbreaks, but chickens and eggs combined represent the most frequent transmission vehicles of salmonellosis, and they are major contributors to the salmonellosis pandemic (Molbak, Olsen, Wegener 2005). International trade has transported infected breeding fowl and eggs to other geographic areas, and the scope of contamination problems increased with transformation of the poultry industry into one of higher production and volume. Another contributing factor is the contamination of chicken feed with *Salmonella*. The result is inoculation of uninfected birds and the spread of infection among the flocks. Eggs were implicated in 80% of the reported outbreaks in the United States by *Salmonella* Enteritidis from 1985 through 1999 (Patrick et al. 2004). Risk modeling of eggborne *S.* Enteritidis infections (including sporadic and unreported cases) for the year 2000 was estimated to be between 81,535 and 276,500 cases (Schroeder et al. 2005).

Although most cases of salmonellosis are linked to meat and poultry products, the two largest outbreaks of salmonellosis in the United States were caused by contaminated dairy products. In 1985, pasteurized milk from a dairy in Illinois was somehow cross-contaminated with raw milk. This resulted in more than 16,000 culture-confirmed salmonellosis cases by an antibiotic-resistant strain of *S.* Typhimurium (Ryan et al. 1987). The total number of cases was estimated to be nearly 200,000 people. At that time, it was the largest reported outbreak of salmonellosis, as indicated by the spike in Figure 2-15. The exact source of *S.* Typhimurium was never definitively determined, but evaluations of the dairy plant operations revealed several opportunities for contamination of the pasteurized milk with raw milk. The largest reported outbreak of salmonellosis to date occurred in 1994 from contaminated ice cream. In this outbreak, an estimated 224,000 persons were infected with *S.* Enteritidis (Hennessy et al. 1996). Investigators of the outbreak concluded that the ice cream was contaminated with *S.* Enteritidis from pasteurized ice cream premix. The premix was transported in tank trailers previously used to carry nonpasteurized liquid eggs, the most likely source of *S.* Enteritidis. This incident highlighted the importance of cross-contamination in salmonellosis and the need for safety management of the entire food supply chain.

Whereas the majority of salmonellosis cases are associated with foods of animal origin, an increasing number of outbreaks have been linked to fruits, vegetables, and processed products. Outbreaks have been associated with produce such as alfalfa sprouts, tomatoes, peppers, cantaloupes, and lettuce (Beuchat 2006). Furthermore, *Salmonella* contamination has been detected in a great variety of fresh produce items, including cabbage, cauliflower, cilantro, parsley, green onions, strawberries, and many others (Buck, Walcott, Beuchat 2003). In February 2009, an outbreak of *Salmonella* Saintpaul involving 228 cases from 13 states was traced to contaminated alfalfa sprouts (CDC 2009d). Previous outbreaks since 1995 have also been linked to

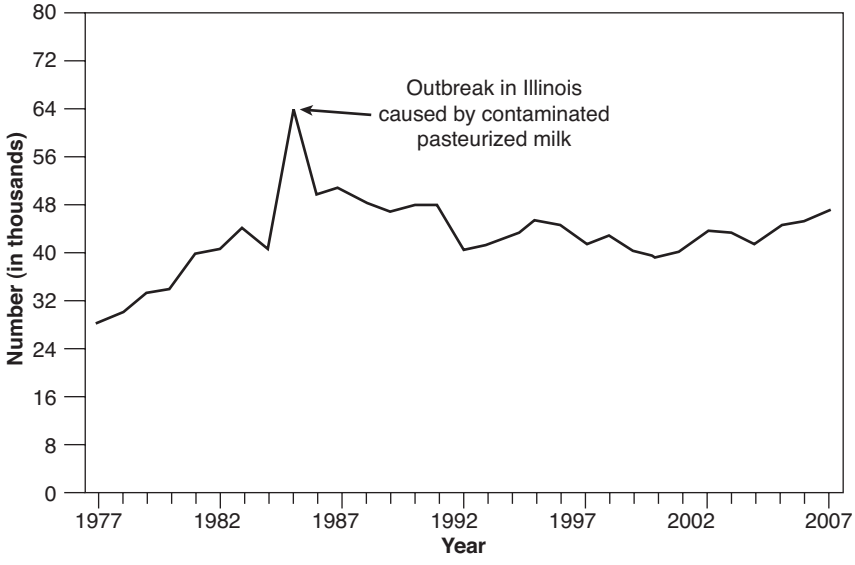

Figure 2-15 Salmonellosis. Number of Reported Cases, by Year—United States, 1977–2007.
Source: Courtesy of CDC, 2009.

lightly cooked or raw alfalfa sprouts. Products like alfalfa seeds can be contaminated from poorly managed manure, contaminated runoff or irrigation water, fecal matter from uncontrolled domestic or wild animals, and improperly cleaned and maintained farm equipment. Sometimes plants can internalize pathogens like *Salmonella* within their seeds or other edible parts. This suggests that simply washing a vegetable or fruit may not be adequate for prevention of the disease.

Salmonella contamination has also been found in various processed foods. Products such as orange juice, snack foods, and peanut butter are just a few examples. Often a single ingredient can be contaminated with *Salmonella* and subsequently contaminate multiple products. An outbreak in 2009 of *S.* Typhimurium in peanut butter and other peanut-containing products is an example of "ingredient-driven" outbreaks (CDC 2009c). As of April 20, 2009, a total of 714 cases of salmonellosis from 46 states were reported to the CDC in this outbreak. Along with bulk containers of peanut butter, other products associated with this outbreak included peanut-containing items like snack crackers, cookies, cakes, pies, brownies, cereal, ice cream, and even pet foods. Other products such as breads may also be contaminated by ingredients (e.g., eggs) and cause disease if cooking temperatures are not sufficient to kill the *Salmonella* (Lu et al. 2004).

Figure 2-16 illustrates how contamination of foods with *Salmonella* is possible almost anywhere in the food supply chain. This is a topic of discussion in Chapter 4 on prevention principles and in Chapter 8 on safety management of the food supply.

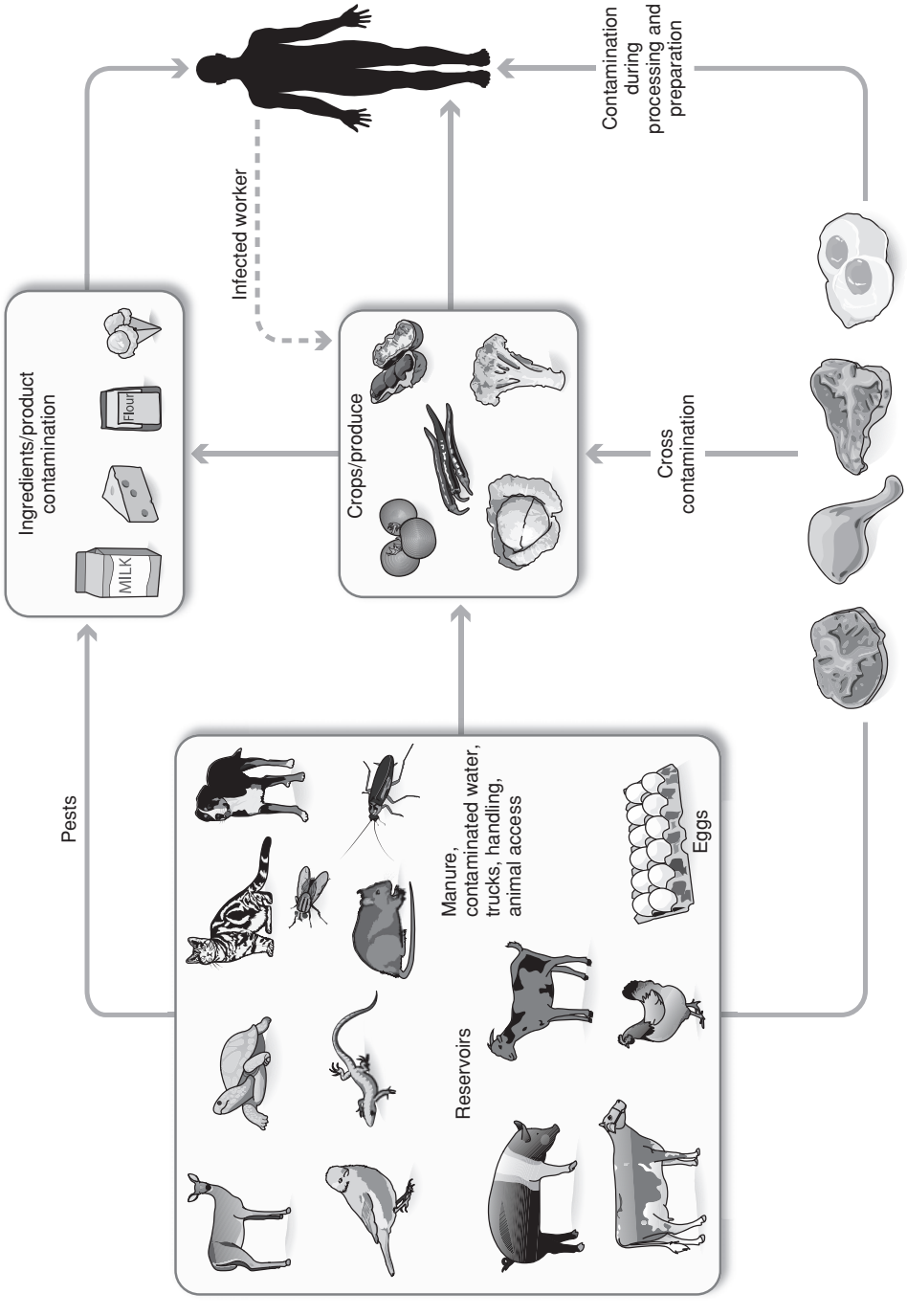

Figure 2-16 Transmission of Foodborne Salmonellosis

Shigella Species

Dysentery is a disease characterized by frequent, watery diarrhea, often with mucus and/or blood; abdominal cramps; and possibly including fever, nausea, vomiting, and ulceration of the intestinal mucosa. It has been the scourge of humanity since ancient times and traveled with armies around the world, often causing more morbidity and mortality than inflicted by the enemy. One form of the disease is caused by an amoeba (amoebic dysentery) and another is caused by a bacillus (bacillary dysentery). The causative organism of classic bacillary dysentery is *Shigella dysenteriae*. This organism is most often—but not exclusively—encountered in lesser developed countries and is responsible for devastating outbreaks and epidemics. Major complications such as HUS and intestinal perforations are possible, and the fatality rate is as high as 20% among hospitalized cases (Heymann 2008).

Shigellosis refers to all enteric infections caused by *S. dysenteriae* and the other species *S. flexneri*, *S. boydii*, and *S. sonnei*. Approximately 44 serotypes have been established among these four species of *Shigella*. The pathogenicity and geographic distribution of the species and serotypes are quite different. Figure 2-17 shows the most common *Shigella* isolate reported by public health laboratories in the United States is *S. sonnei*, followed by *S. flexneri* (CDC 2006). For a significant number of *Shigella* isolates, the species was not identified. The infective dose for shigellosis is very low compared with other foodborne bacterial pathogens, with infective doses among the species generally ranging from <10 to 10^3 CFUs (Todd et al. 2008b). The pathogen attaches to the intestinal epithelium and invades the cells, spreading to other adjacent cells of the epithelium. A potent enterotoxin called shiga-toxin is produced by the more virulent strains. Mild cases are very common with some strains, in which case recovery typically happens without medical intervention. Convalescent carriers may continue shedding the pathogen in their feces from days to months, transmitting the pathogen to others via person-to-person contact and vehicles such as food and water. Crowded and unsanitary conditions, such as refugee camps and overloaded day care centers, are breeding sites for the pathogens. Depending on the pathogen strain and host susceptibility, sequelae such as PI-IBS and aseptic or reactive arthritis can occur following recovery from infection (Lindsay 1997; Smith and Bayles 2007).

Humans (and other nonhuman primates) are the primary reservoirs for *Shigella* species. The bacteria do not survive long in the environment, unless they are within a moist and protective medium, such as certain foods. Transmission by person-to-person contact and fomites are important modes for the propagation and continuation of shigellosis epidemics within a community. Drinking water contaminated by feces is probably the most common mode of transmission in lesser developed countries, whereas food is the likely primary mode of transmission in developed countries, thanks in great part to safer water supplies and sewage treatment. Because humans are reservoirs of *Shigella* species, the infected food worker is the likely source of contamination for foodborne outbreaks (Todd et al. 2008b). But food can also become contaminated with *Shigella* species by animals, insects, and water that have come in contact with the feces of infected persons. Foods of greatest concern are those handled and eaten raw, or handled after cooking by an infected preparer. Figure 2-18 illustrates these pathways of contamination and transmission.

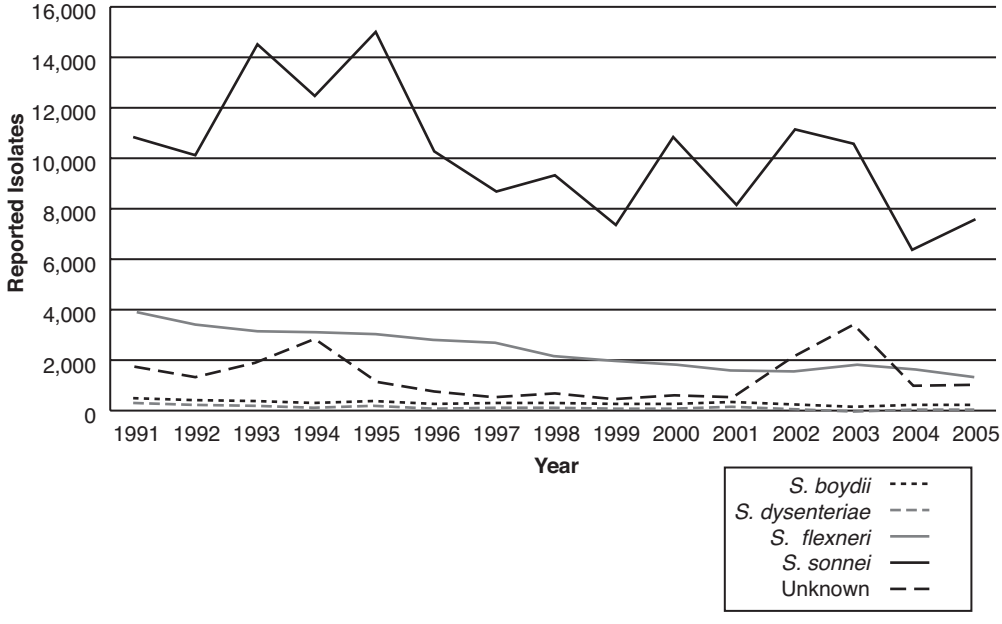

Figure 2-17 Laboratory-Confirmed Shigella Isolates Reported to CDC by Species and Year for 1991–2005. *Source:* Data from CDC, 2008

Two particularly noteworthy outbreaks demonstrate the importance of food worker hygiene and sanitary conditions in the prevention of foodborne shigellosis. In the summer of 1987, a festival and gathering occurred in western North Carolina. An outbreak of diarrheal disease caused by an antibiotic-resistant strain of *S. sonnei* infected more than half of the estimated 12,700 participants (Wharton et al. 1990). Investigation by public health officials determined multiple modes of transmission were responsible for this massive outbreak: food, water, and person-to-person transmission. The gathering had 47 communal kitchens with questionable or no handwashing facilities, shallow trench latrines nearby, no refrigeration, and probably infected food handlers. The water supply was also likely contaminated. After disbanding of the gathering, the attendees dispersed and transmitted the infection nationwide, causing at least three outbreaks in other states. Another noteworthy outbreak occurred in 2000 from a commercially produced dip (Kimura et al. 2004). A total of 406 cases of infection by *S. sonnei* were detected nationwide and linked to the contaminated dip. Investigators traced the source of contamination to a sick worker who used his bare hands to help mix the cheese ingredient for the dip. Other settings where outbreaks of shigellosis have occurred include day care centers, airline flights, and cruise ships (CDC 2006; Todd et al. 2007b).

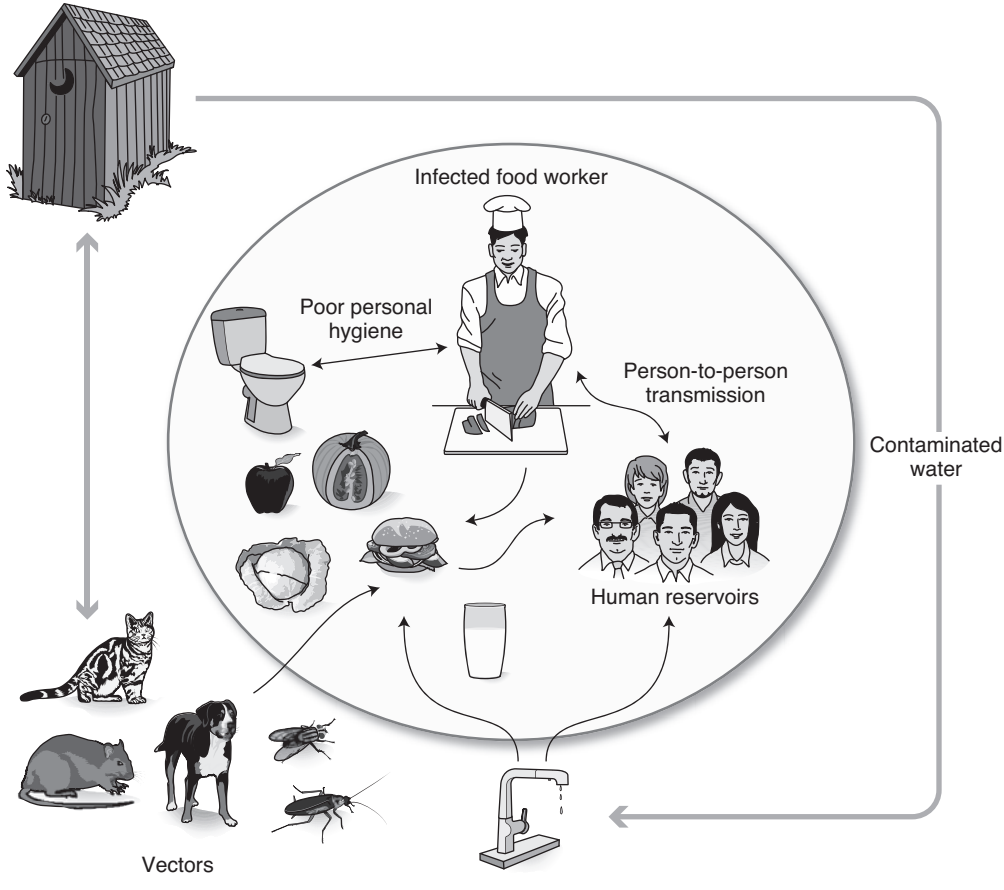

Figure 2-18 Transmission of Shigellosis

Yersinia Species

Yersiniosis is an enigmatic disease compared with other diseases caused by members of the family Enterobacteraciae because several disease states of yersiniosis are possible. After colonization in the small intestine, the *Yersinia* bacteria invade the intestinal mucosa and cause tissue destruction. The most common signs and symptoms are diarrhea, low-grade fever, and abdominal cramps. Vomiting and bloody diarrhea may also be present. Many infected individuals are asymptomatic, while others can develop serious or life-threatening states of disease. In these more serious states, the bacteria invade and colonize the lymphoid tissue and spread to the lymph nodes and beyond, possibly causing septicemia and affecting other organs. Cases of septicemia can have high case-fatality rates (Bottone 1997). The disease may display a variety of manifestations, such

as pseudo appendicitis. Reactive arthritis and other sequelae are also risks within a year following acute yersiniosis (Ternhag et al. 2008).

The most deadly species of *Yersinia* is the etiologic agent of plague (*Y. pestis*)—normally transmitted by infected fleas—not food. Another species, *Y. pseudotuberculosis*, is believed to cause epizootic diseases among wild and domestic animals and incidentally infects humans, but knowledge about the transmission of *Y. pseudotuberculosis* is limited. The most important species with regard to foodborne transmission is *Y. enterocolitica*. This bacterium is unique among the Enterobacteriaceae because of its psychrophilic nature, that is, it is capable of reproducing at refrigeration temperatures. Approximately 60 serotypes of *Y. enterocolitica* have been established, though most of them are nonpathogenic (Bottone 1999). The infection is more common in cooler climates such as northern Europe and Japan. In the United States, reported outbreaks and cases are infrequent compared with EHEC and *Salmonella* infections. Prior to 1983, only three well-documented outbreaks of yersiniosis were reported in the United States, and they were linked to contaminated and/or unpasteurized milk, bean sprouts soaked in contaminated water, and tofu (Bottone 1997). More recently, cases of foodborne yersiniosis have been linked to pork products, principally chitterlings, a dish made from the large intestines of pigs (Jones 2003).

Swine are the principal reservoir of *Y. enterocolitica*. Other potential reservoirs include a variety of mammals and birds. However, the majority of pathogenic strains are associated with the pig, and the occurrence of yersiniosis among humans is more prevalent in areas where the pig is a main source of meat (Bottone 1997). Within the United States, the percentage of pig farms with at least one positive test for *Y. enterocolitica* varied by state from 33% to 57% (Wesley, Bhaduri, Bush 2008). Higher rates of positive tests may be found among slaughtered pig carcasses because contamination from the intestines occurs during evisceration procedures. Despite these seemingly high rates of positive tests, the reported human cases of yersiniosis in the United States are comparatively low. Since *Y. enterocolitica* can grow at refrigeration temperatures, the control of foodborne yersiniosis is greatly dependent on good sanitary practices and thorough heating (i.e., milk pasteurization) and/or cooking of foods, particularly pork products.

Campylobacter Species

As one author describes it, *Campylobacter* has gone from complete "obscurity to celebrity" status in 30 years (Butzler 2004). In 1906, British veterinary surgeons isolated these spiral-shaped microorganisms from pregnant sheep, and because of their spiral morphologies (Figure 2-19), the bacteria were considered a member of the genus *Vibrio* until 1963 (Zilbauer et al. 2008). Difficulties in culturing *Campylobacter* over the years delayed its confirmation as a cause of human diarrhea until the 1970s. Since then, *Campylobacter* has been identified as a common cause of diarrheal disease around the world (Petri et al. 2008), and global estimates suggest it may be the leading bacterial cause of outpatient visits for diarrhea in children younger than 5 years (Lanata, Mendoza, Black 2002). In the United States, surveillance programs estimated a decline in the incidence of campylobacteriosis by 32% from 1996 to 2008 (CDC 2009e). Factors attributed to this decline include programs aimed at reducing the rates of infection among chicken flocks. Even with the decline, campylobacteriosis remains a prominent foodborne infection in the United States.

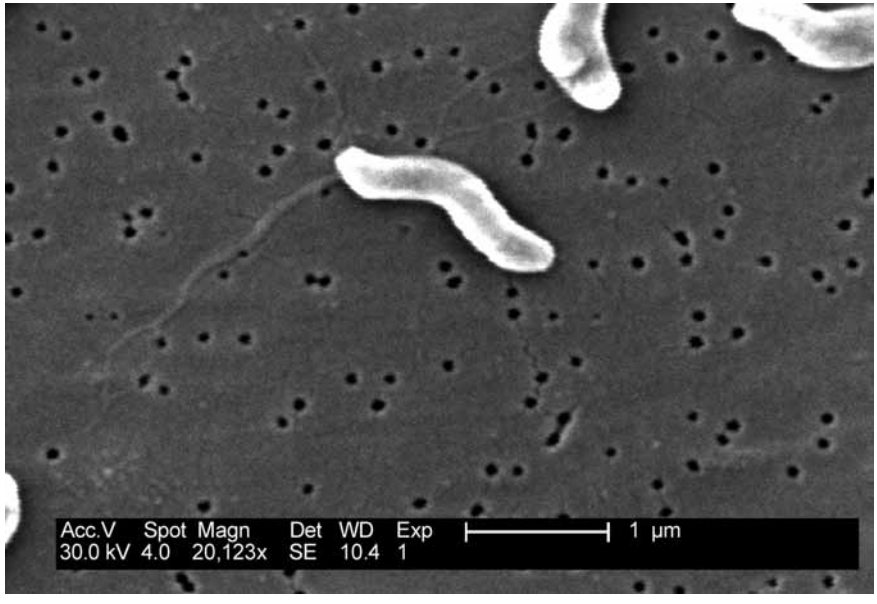

Figure 2-19 Scanning Electron Micrograph of *Campylobacter jejuni. Source:* Courtesy of Dr. Patricia Fields, Dr. Collette Fitzgerald, and Janice Carr/CDC

Among the several species of *Campylobacter* that can cause human disease, *C. jejuni* and *C. coli* are the most important worldwide. About 90% of human campylobacteriosis cases are caused by *C. jejuni*, making it most important in terms of control (Janssen et al. 2008). Serotyping methods for *C. jejuni* are expensive, can be technically difficult to perform, and may yield strains that are difficult to categorize or type. Hence, DNA-based methods and subtyping schemes are often preferable (Altekruse et al. 1999). The infective dose for *Campylobacter* species can be as low as 500 to 800 CFUs (Young, Davis, Dirita 2007). After passage through the stomach, which can greatly reduce the number of viable organisms, the *Campylobacter* colonize the intestine, where they cause diarrhea by either releasing a toxin (secretory or noninflammatory diarrhea) or by invading and damaging the epithelial tissue, resulting in inflammatory diarrhea (Janssen et al. 2008). In most cases, the disease is self-limiting, and the majority of people recover without medical intervention or antibiotic therapy. Some individuals infected with *Campylobacter* become asymptomatic carriers. A small percentage of cases may require antibiotic therapy, and a few cases may have complications arising from septicemia and colonization at extraintestinal sites (Janssen et al. 2008; Zilbauer et al. 2008). Sequelae such as reactive arthritis, PI-IBS, and a neurologic condition known as Guillain-Barré syndrome (GBS) are special concerns with campylobacteriosis. GBS is an autoimmune condition that causes weakness, paralysis, and respiratory insufficiency, possibly requiring artificial ventilation. Among the multiple causes of GBS, *C. jejuni* is the most frequently identified pathogen known to induce it (Zilbauer et al. 2008).

Figure 2-20 illustrates the transmission cycle of foodborne campylobacteriosis.

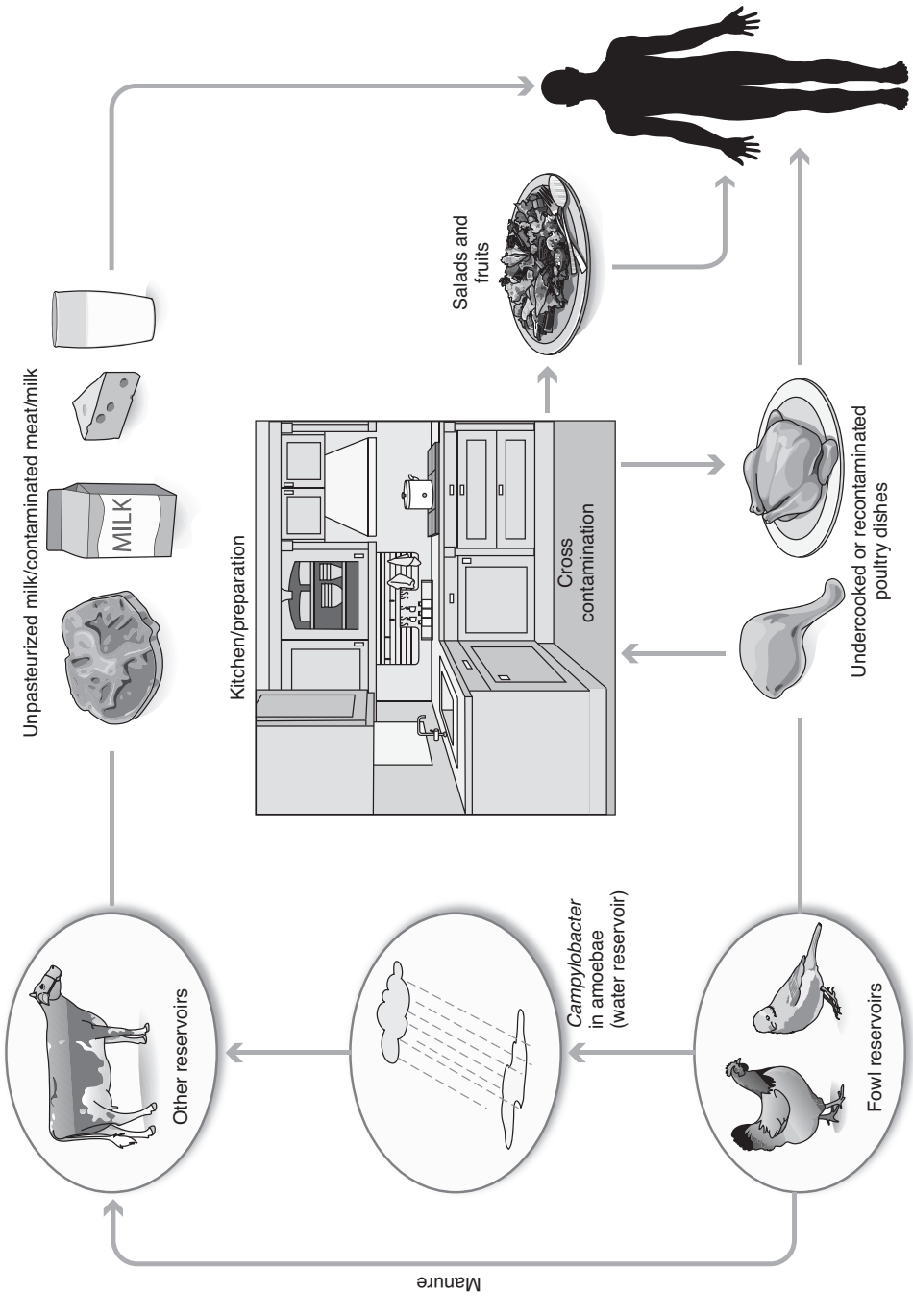

Figure 2-20 Transmission of Foodborne Campylobacteriosis

Reservoirs of *Campylobacter* include a variety of birds and mammals. Chickens are particularly important reservoirs, not only as a source of foodborne infection but also as a source of infection to other farm animals. Normally found in the intestinal tracts, *Campylobacter* are very sensitive to oxygen (microaerophilic) and do not survive long in unfavorable environments. Recent evidence suggests, however, that *Campylobacter* can survive in protozoans such as amoebae living in water and, hence, the waterborne amoebae can be a reservoir of infection for other animals (Young et al. 2007); this same mechanism of survival has been found with other foodborne pathogens (Bleasdale et al. 2009). The formation of biofilms and a state of dormancy known as viable but nonculturable (VBNC) may also contribute to *Campylobacter* survival in the environment, but the relative importance of these factors remains controversial (Murphy, Carroll, Jordan 2006). Whatever the relative importance of the aforementioned survival mechanisms, epidemiologic studies of campylobacteriosis outbreaks have confirmed the prime importance of contaminated, undercooked chicken and the cross-contamination of other foods during preparation and handling (CDC 1998).

A variety of foods has been implicated in epidemiologic studies of campylobacteriosis. The most important categories are poultry, red meats, and raw milk (Altekruse et al. 1999). Figure 2-21 illustrates the types of foods implicated in 145 confirmed and suspected campylobacteriosis outbreaks in the United States from 1990 through 2006. An important observation of these data is that dairy products represent 36% of the total food types, with unpasteurized dairy products—predominantly raw milk—representing the single largest category. The likely *Campylobacter* contamination source for raw milk is infected cows, or the contamination of dairy equipment, and/or the mixing of contaminated milk with uncontaminated milk (CDC 2009a). Of the meats, chicken is implicated most often (17%). This corresponds with surveys indicating retail chicken meats have had historically high rates (up to 98%) of contamination with *C. jejuni* (Altekruse et al. 1999). More recently, efforts by the U.S. poultry industry to reduce chicken infections and carcass contamination have dramatically reduced *Campylobacter* loading on chicken carcasses (Stern and Pretanik 2006). One particularly interesting and important observation in Figure 2-21 is the large percentage of salads, fruits, and mixed-ingredient dishes implicated as vehicles for *Campylobacter* transmission. Because *Campylobacter* species are readily destroyed at cooking temperatures, a likely explanation for this observation is the cross-contamination of cooked food and produce items (most salads and fruits are eaten uncooked) by raw meats and/or contaminated surfaces in the food preparation area.

Listeria monocytogenes

The bacterium *Listeria monocytogenes* (Figure 2-22) is the etiology of listeriosis—a disease that rightfully frightens pregnant women and immunocompromised individuals. In pregnant women, the disease can result in serious outcomes such as abortion, stillbirth, premature birth, and neonatal infections, along with other serious or life-threatening conditions to newborns. Older adults and others who are immunocompromised are at increased risk of complications such as meningitis and septicemia. Among healthy and intermediate-age individuals, the disease is usually limited to gastroenteritis, diarrhea, vomiting, fatigue, and headache, though some relatively young adults may also experience meningitis and septicemia (Drevets and Bronze 2008).

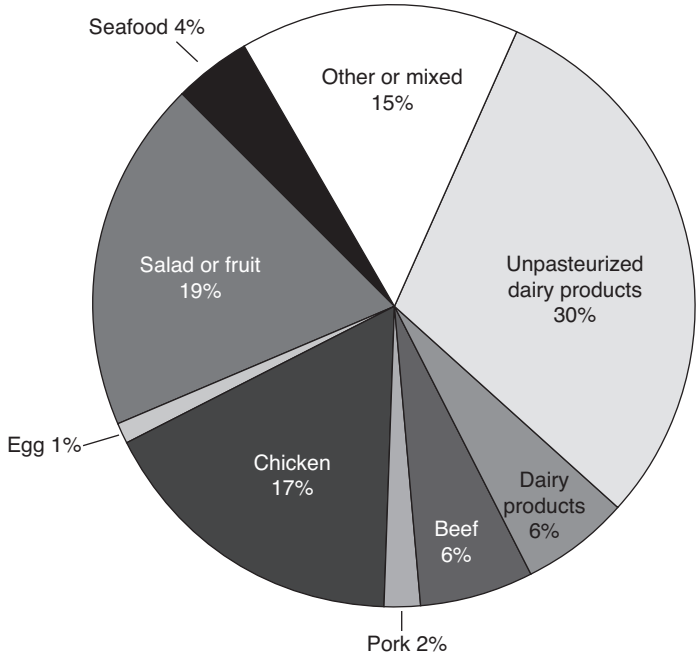

Figure 2-21 Food Categories Implicated in 145 Campylobacteriosis Outbreaks—U.S. from 1990–2006. *Source:* Data from Center for Science in the Public Interest

Fortunately—compared with other foodborne bacterial infections—the incidence of listeriosis is low in the general population. But certain subpopulations (e.g., those discussed previously) are at increased risk of infection and severe consequences; this risk merits the placement of listeriosis on a priority list of public health problems.

The infective dose of *L. monocytogenes* is relatively high compared with other infectious agents. Outbreaks have been associated with levels approximating 10^4 to 10^6 CFUs/g of *L. monocytogenes* (Food and Drug Administration [FDA] et al. 2003). Of course, individuals with impaired immune systems are at increased risk of infection and, hence, have lower infective doses. The subject of infective doses for *L. monocytogenes* has been extensively debated, and new risk assessment approaches advocate using dose-response curves, where the statistical probability of becoming infected increases with the dose of bacteria. (This subject is discussed later under risk assessment.) After access to the body through the consumption of contaminated food, the *L. monocytogenes* bacteria invade the mucosal epithelium and lymphatics of the intestine. The invasive bacteria spread cell to cell in the host using the host cells' membranes as a protective vacuole. Infected lymph nodes disseminate bacteria via blood to other organs. The bacteria are uniquely capable of crossing key protective barriers in the body, for example, the maternofetal barrier and

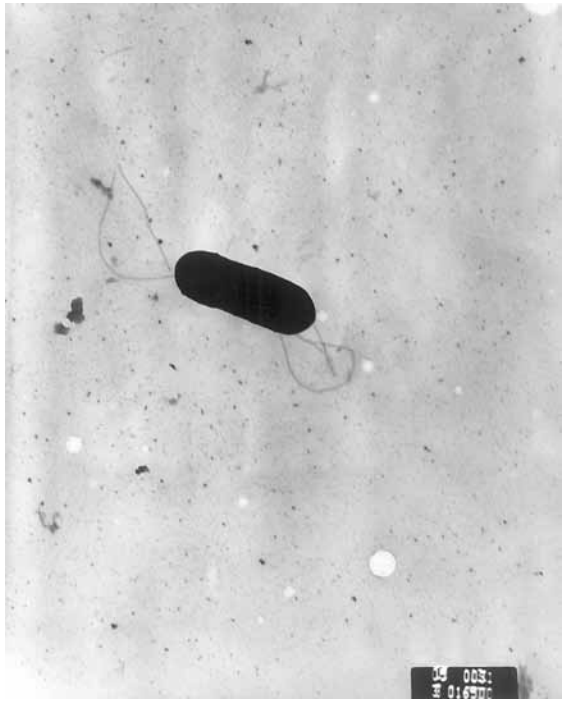

Figure 2-22 Electron Micrograph of *Listeria monocytogenes. Source:* Courtesy of Elizabeth White, Dr. Balasubr Swaminathan, Peggy Hayes/CDC

blood–brain barrier (Drevets and Bronze 2008). This leads to the complications discussed earlier associated with pregnancy and the nervous system.

More than a dozen serotypes of *L. monocytogenes* have been established; nearly all (95%) of human infections are caused by four serotypes, designated as 1/2a, 1/2b, 1/2c, and 4b (Heymann 2008). Because listeriosis is primarily a zoonotic disease, the reservoirs include many species of animals. Yet, the *Listeria* bacteria are found throughout nature in soil, decaying vegetation, water, and other materials and surfaces that come in contact with animals and their feces. This includes food preparation surfaces and processing equipment. The ability of *L. monocytogenes* to produce biofilms contributes to its tenacity and persistence in natural and human environments. Other important survival factors include its ability to grow at a relatively wide range of pH and temperatures—even at refrigeration temperatures—and in relatively high concentrations of salt (Gandhi and Chikindas 2007). Figure 2-23 illustrates the various ways that *L. monocytogenes* contaminates the outdoor/indoor environment and enters the food handling chain. Despite the fact that heat processing (e.g., cooking and pasteurization) of foods usually kills *L. monocytogenes*, it is frequently detected in a wide variety of finished food products, suggesting that postprocessing contamination and cross-contamination are important contributors to foodborne transmission.

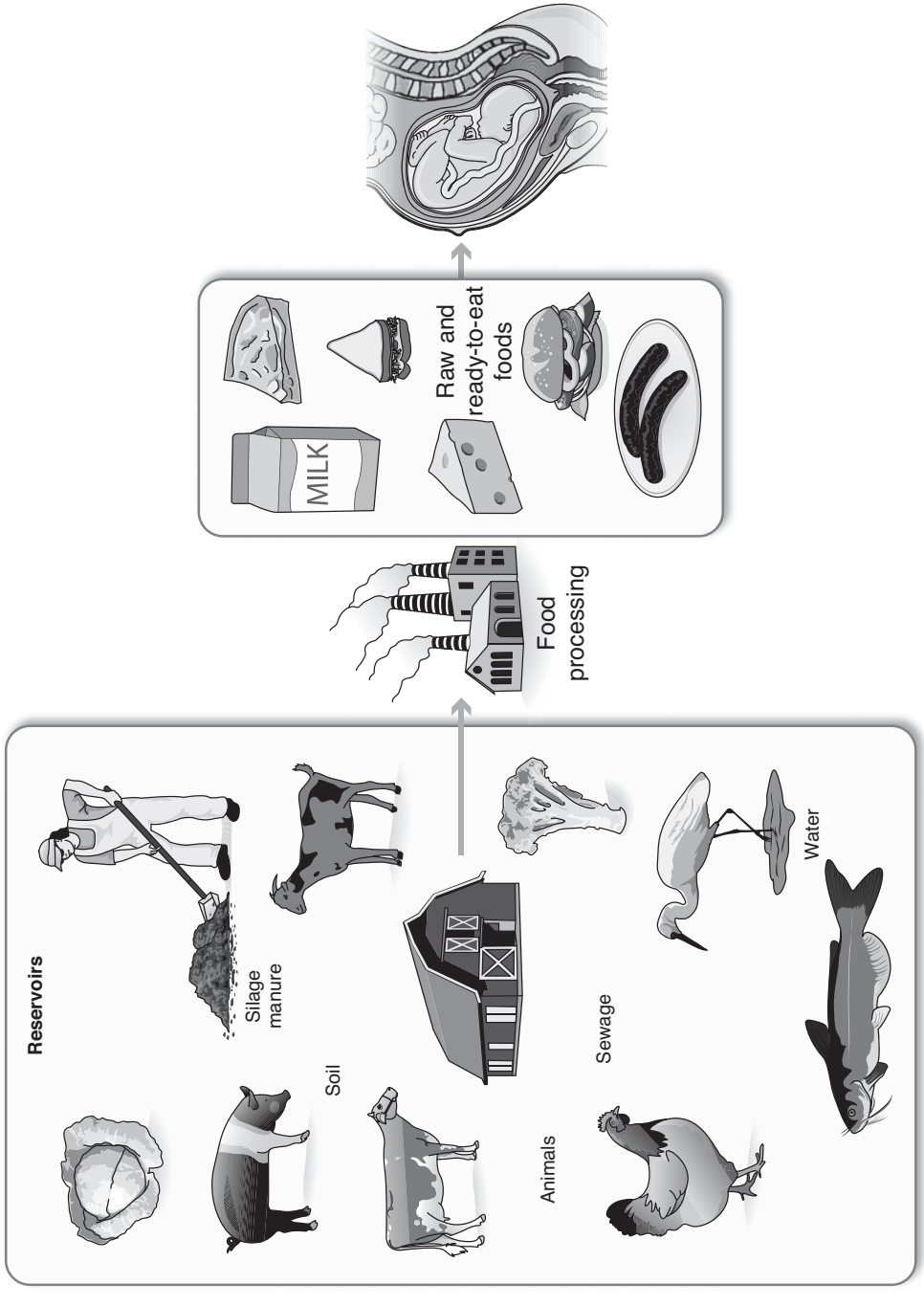

Figure 2-23 Transmission of Foodborne Listeriosis

Indeed, the establishment of *L. monocytogenes* in the food-processing environment has been clearly established as related to food contamination during production, even over prolonged periods of time (Tompkin 2002).

Outbreaks of listeriosis have been most commonly linked to a variety of meat and dairy products, seafood, and vegetables (Farber and Peterkin 1991; Ramaswamy et al. 2007). In recent years, ready-to-eat (RTE) foods such as deli products have been linked to listeriosis outbreaks. Table 2-5

Table 2-5 Food Categories Used in the *Listeria monocytogenes* Risk Assessment

SEAFOOD
Smoked Seafood (finfish and mollusks)
Raw Seafood (finfish, mollusks, and crustaceans)
Preserved Fish (dried, pickled, and marinated finfish)
Cooked Ready-to-Eat Crustaceans (shrimp and crab)
PRODUCE
Vegetables (raw)
Fruits (raw, dried)
DAIRY
Fresh Soft Cheese (queso fresco, queso de Crema, Queso de Puna)
Soft Unripened Cheese, >50% moisture (cottage cheese, cream cheese, ricotta)
Soft Ripened Cheese, >50% moisture (brie, camembert, feta, mozzarella)
Semi-soft Cheese, 39–50% moisture (blue, brick, Monterey, Muenster)
Hard Cheese, <39% moisture (cheddar, Colby, parmesan)
Processed Cheese (cheese foods, spreads, slices)
Pasteurized Fluid Milk
Unpasteurized Fluid Milk
Ice Cream and Other Frozen Dairy Products
Cultured Milk Products (yogurt, sour cream, buttermilk)
High Fat and Other Dairy Products (butter, cream, other miscellaneous milk products)
MEAT
Frankfurters (reheated)
Frankfurters (not reheated)
Dry/Semi-Dry Fermented Sausages
Deli Meats (cooked, ready-to-eat)
Pâté and Meat Spreads
COMBINATION FOODS
Deli-type Salads (fruit, vegetable, meat, pasta, egg, or seafood salads)

Source: FDA, 2003.

lists RTE food categories used in assessing the relative risks to the public health from foodborne *L. monocytogenes*. All these foods can be considered risky for susceptible individuals, but the deli meats represent the highest risk on a per annum and per serving basis in the United States (FDA et al. 2003). If control measures involving the processing and retail handling of deli products are not strictly followed, the risks of listeriosis increase greatly (Lianou and Sofos 2007). However, the risks of listeriosis are even greater if deli products are not properly stored (refrigerated at proper temperatures) and handled in the home (Yang et al. 2006).

Vibrio Species

Multiple species of bacteria belonging to the genus *Vibrio* have been implicated and confirmed as an etiology of human infections, but the three most important from a food safety perspective are *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* (Figure 2-24). The dreaded disease cholera is caused by different strains of *V. cholerae*, which is categorized by serotypes (predominantly type O1 and O139). Further categorization is done by biotypes on the basis of biochemical testing and the production of cholera toxin (toxigenic), a protein that causes voluminous diarrhea and dehydration. Without oral rehydration solution (ORS) therapy, the mortality from cholera would be greater than the currently reported rate of 0–9% (Heymann 2008). From the nineteenth century to the current date, seven pandemics of cholera have swept across the globe causing great morbidity, particularly in the lesser developed countries. With international laws and mandatory reporting of cholera, the world community has attempted to stem the tide of this communicable disease. But the enforcement of international laws is difficult because environmental conditions within sovereign countries are largely responsible for the persistence of cholera (Jones 1999).

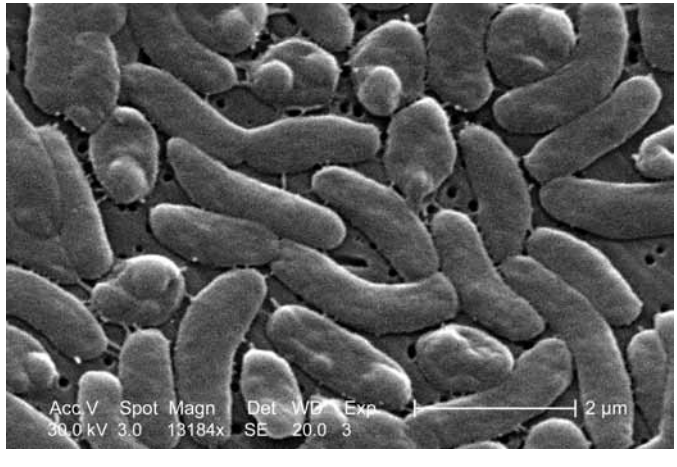

Figure 2-24 Scanning Electron Micrograph of *Vibrio vulnificus. Source:* Courtesy of Janice Carr/CDC

Unlike the other *Vibrio* pathogens, the principal reservoir for toxigenic *V. cholerae* is humans. Therefore, eliminating fecal-oral routes of transmission in humans is critical to reducing the incidence of cholera in a community. This involves the assurance of safe drinking water and proper sewage disposal, and excluding the contamination of foods, which frequently occurs from using contaminated water to wash or prepare raw or cooked foods. Beverages and ice prepared from contaminated water are especially risky vehicles of transmission. Another important source of cholera transmission is the consumption of raw seafood (or cross-contamination of cooked seafood) harvested from waters contaminated by inadequately treated sewage or runoff. Although the global leading cause of cholera epidemics is water contamination, food transmission accounted for 32% and 71% of outbreaks in South America and East Asia, respectively (Griffith, Kelly-Hope, Miller 2006). In developed and industrialized countries, where water and wastewater treatment are more common, the consumption of raw seafood and unwashed vegetables is the riskiest transmission vehicle for cholera. Still, increasing international travel and frequent importation of foods make cholera a concern for all countries.

The *Vibrio* species are marine organisms. Being halophilic (salt loving), the habitat of species like *V. parahaemolyticus* and *V. vulnificus* is primarily brackish estuarine waters; they cannot survive in the deep ocean because of the extreme pressures. In terms of ecology, these organisms live in the water column during warmer months and survive winters in the sediments. During the warmer months, *Vibrio* species attach themselves to the surfaces of zooplankton and shellfish, and they become part of the microbiota within shellfish digestive tracts. Their habitat and association with shellfish explain why *Vibrio* infections are seasonal and almost always associated with seafood and, to a lesser degree, contact with seawater.

In January 2007, all cases of *Vibrio* illnesses became nationally notifiable to the CDC, regardless of the species involved. The reports from these cases are compiled, maintained, and periodically analyzed by the CDC. In 2007, a total of 549 *Vibrio* illnesses (excluding toxigenic *V. cholerae* infections) was reported to the CDC. The breakdown of these cases by *Vibrio* species and complications are shown in Table 2-6. A total of 160 *Vibrio* specimens from patients in Table 2-6 were obtained from wound sites. Nearly all *Vibrio* species are capable of causing wound infections, usually following exposure to seawater and/or the handling of seafood; several species also cause septicemia. Among those nonwound infections, 89% reported eating seafood within 7 days prior to illness. The most frequently reported single seafood item eaten was oysters (58%), followed by finfish (13%), and clams (11%); the oysters and clams were eaten raw by 97% and 83%, respectively, of the cases. Other types of shellfish implicated in past outbreaks include mussels, crabs, lobsters, and shrimp. Among the finfish, specialty dishes such as sushi are particularly risky because the fish is eaten raw.

As with many gastrointestinal infections, the symptoms of vibriosis include diarrhea, cramps, nausea, and weakness; some may experience chills, vomiting, and headaches. In the case of *V. vulnificus* infection, the severity of disease is much greater, as indicated by the complication rates in Table 2-6. Acquired primarily by eating seafood, the majority of *V. vulnificus* infection cases develop complications and require hospitalization. More than 50% of septicemic cases may be fatal, and 90% of the hypotensive cases may die (Heymann 2008). Hence, prompt public health action is necessary to minimize the threat of seafood contaminated with *V. vulnificus*.

Table 2-6 Number of *Vibrio* Illnesses (excluding toxigenic *V. cholerae*) by Species and Complications from the United States, 2007

	Complications					
	Patients		*Hospitalized*		*Deaths*	
Vibrio *Species*	*N*	%	*n/N*	%	*n/N*	%
V. alginolyticus	100	18	15/90	17	0/88	0
V. cholerae (nontoxigenic)	49	9	21/43	49	3/37	8
V. damsela	2	<1	0/2	0	0/2	0
V. fluvialis	19	3	3/17	18	1/17	6
V. hollisae	6	1	4/6	67	0/6	0
V. mimicus	10	2	4/9	44	0/9	0
V. parahaemolyticus	232	42	52/221	24	0/218	0
V. vulnificus	95	17	87/94	93	30/83	36
Species not identified	23	4	7/18	39	1/16	6
Other	4	1	2/3	67	0/3	0
Multiple species	9	2	4/9	44	1/9	11
Total	549	100	199/512	39	36/488	7

Source: Courtesy of CDC, 2008b.

Spore Formers and Toxicoinfections

An important survival mechanism with certain bacteria is the formation of endospores—highly differentiated cellular structures formed from within the bacterial cells (Figure 2-25). These structures are often simply called spores. They contribute to bacterial survival by switching metabolism into a dormant state and forming barriers that can withstand harsh environmental conditions such as heating, drying, and some chemicals. The formation of spores is called sporulation and occurs when certain key nutrients become absent in the bacteria's environment. The dormant state of spores may last for many years, and when environmental conditions return to a favorable state, germination and outgrowth of the vegetative cells occurs, allowing resumption of multiplication. The genera of spore formers most important to food safety are *Clostridium* and *Bacillus*. These bacteria and/or their spores are very common in soil, dust, plants, and the intestinal tracts of nearly every animal species. Furthermore, the spores can become airborne and dispersed anywhere outside and within building structures. Hence, the complete elimination of spores is difficult to achieve.

Clostridium perfringens: *Toxicoinfection*

Clostridium perfringens (Figure 2-26) is responsible for the most commonly encountered foodborne toxicoinfection. Unlike foodborne infections where attachment and colonization of the

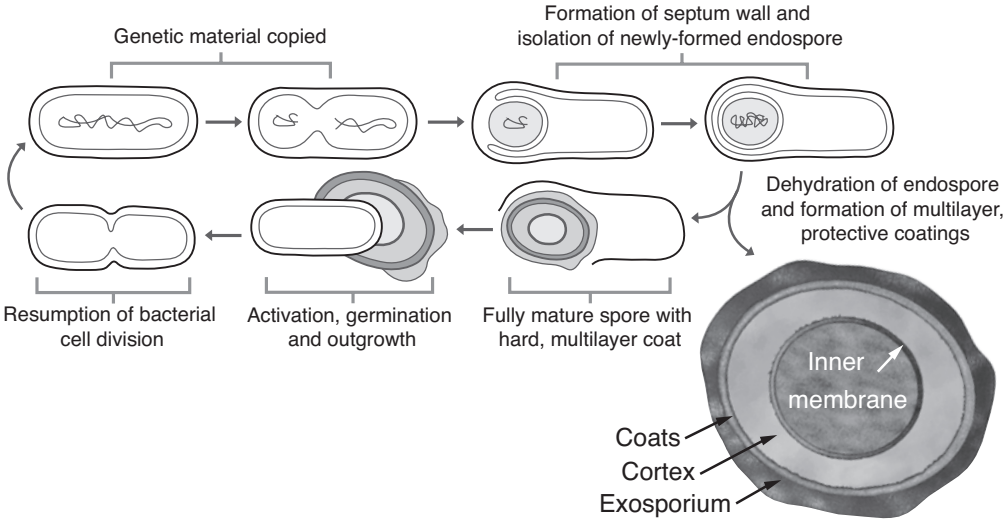

Figure 2-25 Diagram of a Bacterial Endospore. *Source:* Cross-section of bacterial spore, Courtesy of Lawrence Livermore National Laboratory

intestinal mucosa are necessary, a toxicoinfection occurs when the viable bacteria are present in large numbers within the lumen of the intestinal tract. In the case of *C. perfringens*, the disease results from a toxin released during the sporulation of vegetative cells in the gut lumen. A minimum of 10^5 CFUs/g or more of *C. perfringens* in food is believed necessary to cause illness (CDC 2009b). The host response to the toxin is typically diarrhea and abdominal cramps. The incubation period ranges from 8 to 24 hours, and symptoms usually subside 1 to 2 days later. In most cases, the disease does not progress to more serious states. However, a severe form of disease called enteritidis necroticans or "pig-bel" can occur with certain populations, usually among refugees or malnourished individuals who lack sufficient proteases to destroy the toxin. Some evidence exists that preformed toxins and/or sporulating bacterial cells in foods may also contribute to illness and to the shortening of incubation periods for some cases (Roach and Sienko 1992).

For a number of reasons, the actual incidence of *C. perfringens* foodborne disease is difficult to determine. One probable reason is that mild cases of the disease and sporadic cases are never reported. Nonetheless, from 1998 to 2002, *C. perfringens* was the third leading bacterial etiology of foodborne disease outbreaks reported in the United States (Lynch et al. 2006). In 2006, it was the second leading bacterial etiology of reported foodborne disease outbreaks (CDC 2009g). Meats and dishes with gravy, casseroles, or stews are frequently associated with *C. perfringens* disease outbreaks (CDC 2009b). The settings for a majority of these outbreaks involve large quantities of food prepared in advance at institutions (e.g., schools and prisons), gatherings (e.g., banquets, catered events), cafeterias, and restaurants. Because *C. perfringens* spores can survive

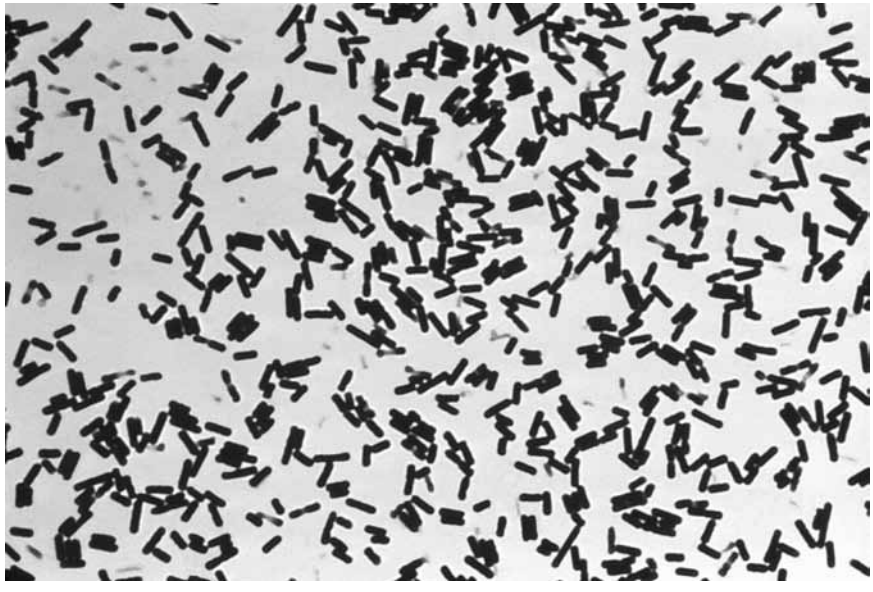

Figure 2-26 Photomicrograph of Clostridium perfringens. *Source:* Courtesy of CDC/Don Stalons

many cooking processes, the bacteria can germinate and multiply to hazardous levels from inadequate holding temperatures prior to consumption. Thus, attention to time and temperature safety guidelines are critically important to the prevention of this disease.

Bacillus cereus: *Toxicoinfection and Intoxication*

Two distinct syndromes are attributed to *B. cereus*: a diarrheal syndrome, characterized by abdominal cramps and diarrhea; and an emetic syndrome, characterized by nausea and vomiting. Each syndrome is caused by different types of toxins, though outbreaks with overlapping syndromes have occurred. Table 2-7 highlights key differences in incubation periods and disease characteristics between the two syndromes, along with those from *C. perfringens* toxicoinfection and *C. botulinum* toxicoinfection/intoxication. Note for *B. cereus* that the diarrheal syndrome is believed to be a toxicoinfection, whereas the emetic syndrome is believed to be an intoxication by preformed toxins in the foods prior to consumption. The topic of foodborne intoxication by *B. cereus* is revisited in the section on microbial toxins in Chapter 3. Unlike the toxicoinfection by *C. perfringens*, the toxicoinfection by *B. cereus* does not require sporulation to release toxins.

Like *C. perfringens*, the bacteria and spores of *B. cereus* can be found in soil, dust, and plants, but the bacteria are found less often in the intestinal tracts of animals. The spores of *B. cereus* can also survive most cooking processes and germinate later when conditions become more suitable.

Table 2-7 Comparison of Spore-Forming Bacteria and Foodborne Diseases

Disease or Syndrome	*Incubation Period*	*Signs/Symptoms & Recovery Period*	*Key Differences*
C. perfringens Toxicoinfection	8–16 hours	Watery diarrhea with abdominal cramps and nausea lasting 24–48 hours.	Sporulation in the small intestine is necessary to produce and release toxins.
B. cereus Diarrheal Syndrome (Toxicoinfection)	10–16 hours	Abdominal cramps with watery diarrhea and nausea lasting 24–48 hours.	Sporulation is not necessary; lysis of vegetative cells releases toxins.
B. cereus Emetic Syndrome (Intoxication)	1–6 hours	Sudden and severe nausea and vomiting, possibly diarrhea, typically over in 24 hours.	Toxin is preformed in foods prior to consumption.
C. botulinum Infant Botulism or Adult Intestinal Toxemia Botulism	3–30 days	Lethargy, weakness, poor head control, and other neurological signs in infants. Duration is variable.	The victim or host usually does not possess a fully developed microbiota in the gut. Spores may be ingested from non-food sources.
C. botulinum Intoxication (Foodborne Botulism)	12–72 hours	Vomiting, diarrhea, blurred vision, muscle weakness and other neurological signs, possible respiratory failure and death. Recoveries may take from days to months.	The heat labile toxin is preformed under anaerobic conditions in low-acid foods prior to consumption.

Source: Compiled from Fenicia and Anniballi 2009; Heymann 2008; Stenfors Arnensen et al. 2008.

Therefore, the times and temperatures of cooked foods are critical to inducing the germination and growth of *B. cereus* spores to hazardous levels, usually about 10^5 to 10^8 CFUs/g of food (Stenfors Arensen, Fagerlund, Granum 2008). In the United States, the food most often associated with outbreaks from *B. cereus* is fried rice. Other foods include those of plant origin, usually cooked foods and meat dishes with a variety of ingredients.

Clostridium botulinum: *Infant Botulism and Adult Intestinal Toxemia Botulism*

Although typically not described in the medical literature as a toxicoinfection, the ingestion of *C. botulinum* (and a few other *Clostridia*) spores by susceptible individuals can lead to colonization and release of neurotoxins in the large intestine by these bacteria. The potent neurotoxins are then absorbed by the intestine and transported to neuronal synapses, where the neurotoxic action takes effect. The greatest risk factor for this condition is the absence of a fully developed microbiota; apparently, the microbiota inhibits the colonization of *C. botulinum* in the gut. The most susceptible individuals are infants younger than 1 year who have not yet developed their microbiota (Fenicia and Anniballi 2009). In extremely rare cases, adults with an altered gastrointestinal

tract may also develop this type of botulism. The sources of *C. botulinum* spores include household dusts and soil or foods in contact with the soil, such as honey. Classic foodborne botulism is actually an intoxication from preformed neurotoxins and is discussed separately in the section on microbial toxins in Chapter 3.

FOODBORNE VIRUSES

In terms of food safety, several important points should be remembered about viruses. First, the estimated portion of foodborne illness outbreaks in the United States attributed to one virus alone (*Norovirus*) ranges from 28% to 67% (Mead et al. 1999; Turcios et al. 2006). This is difficult to prove, however, because most foodborne illness outbreaks have an unknown etiology, and diagnostic tests for norovirus infections are lacking. Nevertheless, on the basis of clinical and epidemiologic profiles, norovirus is believed to be the leading cause of foodborne illness (Turcios et al. 2006). Second, viruses do not replicate in food or water. A suitable and living host is necessary for viral replication. Third, infected persons shed large numbers of viruses in their stools, as many as 10^{11} particles per gram of stool, and only a few virus particles are necessary to cause infection (Koopmans and Duizer 2002). In the overwhelming majority of cases (with some exceptions), the sources of viruses that cause foodborne illness come from infected people, usually food handlers or workers, or they can come from food contacting water contaminated by feces (Todd et al. 2007a). Finally, viruses transmitted via food are not easily degraded in the environment and may remain infectious for considerable periods of time.

Enteric viruses typically cause gastroenteritis, but some viruses use the enteric route of transmission to access other organs such as the liver (e.g., hepatitis) and the nervous system (e.g., polio). Of the possible foodborne viruses, those of greatest concern in developed countries are noroviruses (formerly Norwalk-like viruses) and hepatitis A virus (Koopmans and Duizer 2002), shown in Figures 2-27 and 2-28, respectively. Other viruses are important to a lesser extent. The difficulty of ranking risks from foodborne viruses is that these viruses are also transmitted by other modes and vehicles (e.g., waterborne, person-to-person), and the proportion of viral infections directly attributable to foodborne transmission cannot be easily ascertained.

Noroviruses

Noroviruses (Figure 2-27) are considered the most common foodborne viruses. According to the ICTV, the genus *Norovirus* consists of viruses belonging to the family Caliciviridae, which are characterized by single-stranded RNA enclosed in an icosahedral-shaped capsid, without an envelope, and whose natural hosts are vertebrates. Among the different *Norovirus* species, strains, and serotypes is the well-known *Norwalk virus*, named after a 1968 outbreak at an elementary school in Norwalk, Ohio. Since that time, similar viruses were identified in numerous outbreaks, and the genus *Norovirus* was subsequently established (ICTVdB Management 2006). Among noroviruses responsible for epidemics and foodborne outbreaks, humans are the only established reservoirs.

Norovirus infections are relatively mild and self-limiting, except maybe in the very young and old, or those who are immunocompromised. Sometimes referred to as the "stomach flu," typical symptoms include nausea, vomiting, and diarrhea, and possibly abdominal cramps, muscle aches,

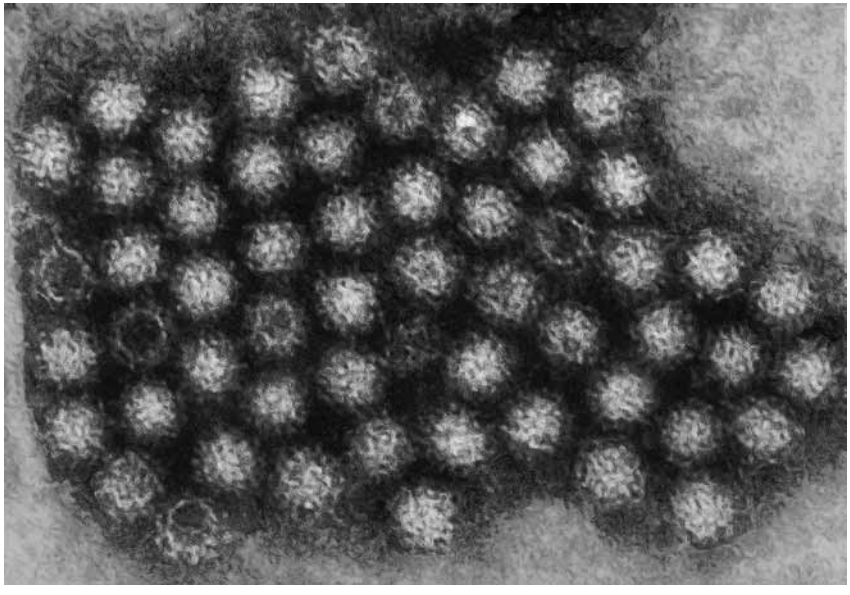

Figure 2-27 Transmission Electron Micrograph of Norovirus Particles. *Source:* Courtesy of CDC/ Charles D. Humphrey

low-grade fever, and headache. Outbreaks from noroviruses can affect large numbers of people and result in significant suffering and financial expense. Cruise ships were common settings for norovirus infection outbreaks, earning the virus the title of "Cruise Ship Virus" (CDC 2002). Several modes of transmission are involved with norovirus infections, such as person-to-person and contaminated water, but food has a prominent role in outbreaks. Three broad categories of foodborne outbreaks have been recognized (Food and Agriculture Organization [FAO]/World Health Organization [WHO] 2008):

1. Consumption of food contaminated by infected food workers
2. Consumption of contaminated molluscan shellfish
3. Consumption of contaminated produce

Infected food workers are primary sources of norovirus outbreaks. In a review of foodborne illness outbreaks where pathogen contamination was determined to be caused by food workers, norovirus was the etiologic agent most frequently reported (Todd et al. 2007b). In 47.4% of these outbreaks, the food workers had symptoms of infection while at work. Studies have also determined that individuals shed noroviruses in their stools for hours before and many days after becoming symptomatic (Todd et al. 2008a). In addition, the vomit of infected individuals can contain as many norovirus particles as loose stools (10^5–10^{11} particles/g), and noroviruses can remain viable on soiled clothing and other surfaces for many hours (Todd et al. 2008a). Because

the infective dose of norovirus is estimated between only 10 and 100 particles, secondary and tertiary cross-contamination of foods by objects and surfaces can easily lead to infections (Todd et al. 2009).

Given the great amount of virus shedding by infected food workers, and the transmissibility of norovirus, it is not surprising that foods associated with norovirus outbreaks include salads, sandwiches, and other foods that are handled (Parashar et al. 2001). Contamination of foods with noroviruses can also occur during preharvest, postharvest, and shipping. Molluscan shellfish, particularly oysters, are often associated with norovirus infections. Shellfish harvested from sewage-polluted waters are frequently contaminated with enteric viruses—including noroviruses (FAO/WHO 2008). Other foods that have been implicated in the transmission of noroviruses are produce such as raspberries and green onions. Data and knowledge on the specific sources of produce contamination are incomplete, but pre- and postharvest contact with contaminated water and/or infected workers is considered the most likely sources (FAO/WHO 2008).

Hepatitis A and E Viruses

Hepatitis is an inflammation of the liver that may have several etiologies. Viruses are common transmissible etiologies of hepatitis among people. Several forms of viral hepatitis have been identified and extensively studied (Figure 2-28). Some are transmitted by percutaneous routes (e.g., needles) or through mucosal contact with infected fluids, while others are transmitted by the fecal-oral route. Those of food safety concern include the latter route. Hepatitis A virus (HAV) is considered more serious in the developed countries because most people in lesser developed countries are infected during childhood and develop immunity by adulthood. Over several decades, HAV has been responsible for large outbreaks frequently traced back to contaminated water and food (Fiore 2004). Humans and other primates are the only reservoirs of HAV, and infected food workers represent the most common sources of food contamination with HAV (CDC 1990). Shellfish harvested from polluted waters are also important sources of HAV infection but considered less common. Yet, one of the largest recorded outbreaks of HAV involved more than 292,000 cases from people consuming clams in China (Halliday et al. 1991). Outbreaks with HAV from produce contaminated during growing, harvesting, and distribution have also occurred, but determining the sources of contamination in these circumstances is difficult at best (Fiore 2004).

Investigating outbreaks from foodborne HAV is difficult for several reasons. Foremost is the long incubation period with HAV infection that averages 28 to 30 days. Most people have difficulty recalling specific meals and food items consumed several weeks prior to the onset of disease. Even if a specific location or meal is implicated, the contaminated food items are probably no longer available for laboratory analysis, and laboratory methods for HAV detection in foods are rife with complications. Another difficulty is the confounding problem arising from sporadic cases in the community at large and other modes of transmission before and after the suspected outbreak. In 2007, only 6.5% of reported HAV cases were suspected to be from food- or waterborne outbreaks, but in 67.7% of cases, the risk factors were unknown (Daniels et al. 2009). People may also have mild cases of HAV infection and fail to seek medical attention, resulting in underreporting of cases. The geographic distribution of cases and extensive travel histories also make it difficult to pinpoint a particular source. All of the aforementioned difficulties suggest

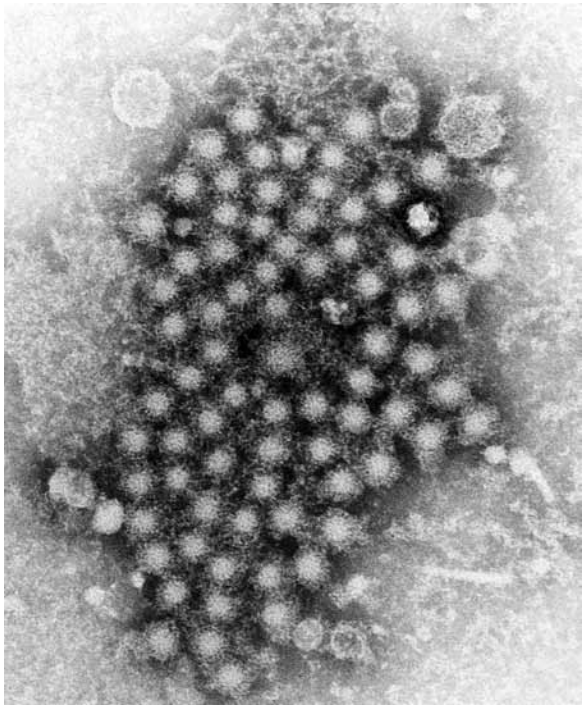

Figure 2-28 Transmission Electron Micrograph of Hepatitis Virus Particles. *Source:* Courtesy of CDC/ E.H. Cook, Jr.

that the best strategy to prevent foodborne HAV infections is consistent food handler hygiene (at work and home) and safe food practices.

The incidence of HAV infections in the United States has decreased dramatically since it last peaked in 1995 (Daniels et al. 2009). This decrease was attributed primarily to HAV immunizations administered over the following decade. In 1996, the CDC's Advisory Committee on Immunization Practices recommended HAV immunizations for adults at increased risk as a result of lifestyle and to children living in communities with high HAV infection rates. This recommendation was expanded in 1999 to include routine vaccination of children in multiple states with incident rates above certain thresholds. In 2006, the recommendation was expanded further to all children in all 50 states. As a result, the HAV incidence in 2007 was the lowest ever recorded (1.0 case per 100,000 population, see Figure 2-29).

Hepatitis E virus (HEV) infections are endemic in lesser developed countries, where poor sanitation and inadequate water and wastewater treatment are common. The disease is self-limiting but may cause complications during pregnancy, with case-fatality rates reaching 20% among infected women in their third trimester of pregnancy (Heymann 2008). The HEV is distinctly different from other viruses that cause hepatitis and has been taxonomically assigned as the only

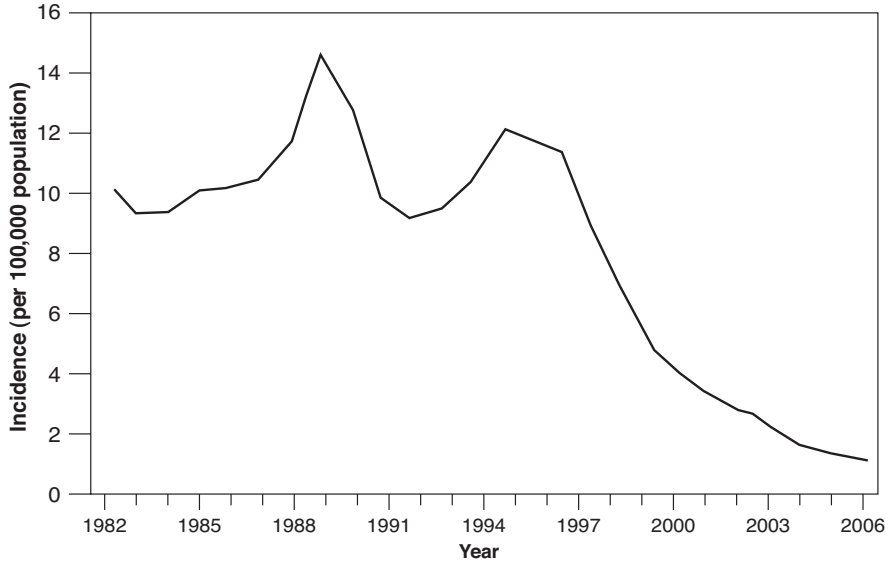

Figure 2-29 Incidence of Hepatitis A in the United States by Year. *Source:* From Daniels et al. (CDC) 2009

"species" under the genus *Hepevirus*, family Hepeviridae. Only one serotype has been identified, but sequencing of its RNA has identified four major genotypes (Mushahwar 2008). And humans may not be the only reservoirs. Research has identified HEV in domestic swine and several wild animals, suggesting that HEV infections may be zoonotic and potentially foodborne from non-human sources (Aggarwal and Naik 2009). Waterborne outbreaks of HEV infection represent the most documented mode of transmission. Despite the reported low incidence of HEV infection in the United States, the recent detection of HEV in animals used as food merits additional research and enhanced surveillance (FAO/WHO 2008).

Human Rotavirus

Diarrheal disease is a leading cause of death in the world among children. The human rotavirus (HRV) is a major contributor to this burden by causing severe dehydration. The spread of HRV in lesser developed countries is predominantly person-to-person, with waterborne and foodborne modes contributing to some degree. Within the United States, approximately 80% of infants became infected with HRV by age 5 years, but higher standards of medical care prevented most deaths. A major setting for HRV transmission and outbreaks in the United States has been day care centers. This is changing with the introduction of a HRV vaccination program in 2006 (CDC 2009f). Still, multiple and emerging strains of HRV exist, and the vaccines do not ensure protection against all of them.

Other Potential Foodborne Viruses

Several other viruses are suspected of being foodborne. Compared with the viruses discussed previously, the incidence of infection is less (or unknown), and supporting evidence for foodborne transmission is usually incomplete or undocumented. Table 2-8 lists the most common and several other possible foodborne viruses, along with the diseases they cause and the likelihood of food/water transmission. This list is not intended to be all inclusive but rather to convey the unexplored and evolving nature of many viruses (Wilhelmi, Roman, Sanchez-Fauquier 2003). Surveillance and research are necessary to identify new strains or emerging foodborne viruses in the near and distant future.

PROTOZOANS

According to a report by the International Life Sciences Institute (ILSI), the protozoans of greatest concern to food production worldwide belong to the genera *Cryptosporidium*, *Giardia*, *Cyclospora*, and *Toxoplasma* (Dawson 2003). Other pathogenic protozoans are transmitted by food and water, but they are not considered to be as important in terms of food processing and distribution.

Table 2-8 Known Foodborne Viruses

Virus	*Nature of Disease*	*Food/Water Transmission?*
Noroviruses	Gastroenteritis	Frequently both
Hepatitis A Virus	Hepatitis	Frequently both
Hepatitis E Virus	Hepatitis	Water, possibly food
Human Rotavirus	Gastroenteritis	Sometimes both water and food, usually person-to-person contact and fomites
Enteroviruses	Other secondary infections, e.g., neurological; poliomyelitis is the most well known	Sometimes water or food, primarily person-to-person contact
Enteric Adenoviruses	Gastroenteritis	Occasional, but fecal-oral route is involved
Astroviruses	Gastroenteritis	Occasional, but has been implicated in foodborne transmission
Sapovirus	Gastroenteritis	Occasional, possibly foodborne
Coronavirus	Gastroenteritis	Uncommon, possibly foodborne
Nipah Virus (bat virus)	Neurological	Foodborne; zoonotic disease from pigs

Source: Compiled from FAO/WHO 2008; Heymann 2008; Koopsman and Duzier 2002; and Wilhelmi et al. 2003.

Cryptosporidium Species

The species belonging to the genus *Cryptosporidium* have a wide range of hosts, infecting great numbers of mammals and some birds and reptiles. This parasite (Figure 2-30) belongs to a subclass of protozoans called Coccidia that infect hosts as sporozoites and, following sexual fusion of gametes, produce hardy oocysts. The oocysts are excreted in the feces of infected humans and animals, contaminating the water and food consumed by other hosts and restarting the cycle (Figure 2-31). These cysts are highly resistant to chlorine at levels typically found in treated water supplies. For many years, the majority of human diseases were believed to be caused by a single species, *C. parvum*, but recent molecular techniques have revealed several different genotypes and species, each often associated with specific animal hosts (Yoder, Beach, CDC 2007a). Among the reservoirs of greatest concern are humans, cattle, and other ruminants (hoofed, cud-chewing animals with multiple stomachs); reservoirs of secondary concern include dogs, cats, birds, rodents, and pigs.

The clinical manifestations of cryptosporidiosis caused by the different species are very similar. The infective dose is very low (10–30 oocysts), and infected humans can shed 10^8–10^9 oocysts in a single bowel movement (Chappell et al. 1996; Dupont et al. 1995). In healthy individuals, the disease is relatively mild with signs and symptoms that include watery diarrhea, stomach cramps, nausea and/or vomiting, and low-grade fever. The disease can have serious consequences among those who are immunocompromised, such as HIV-infected persons. The greatest risk factors for cryp-

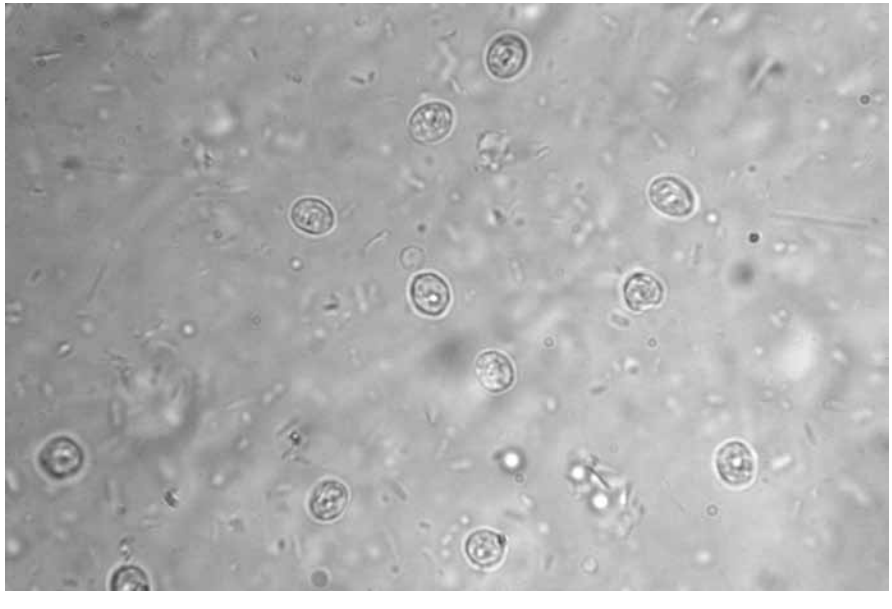

Figure 2-30 Stool Smear Micrograph of *Cryptosporidium parvum. Source:* Courtesy of Dr. Peter Drotman/CDC

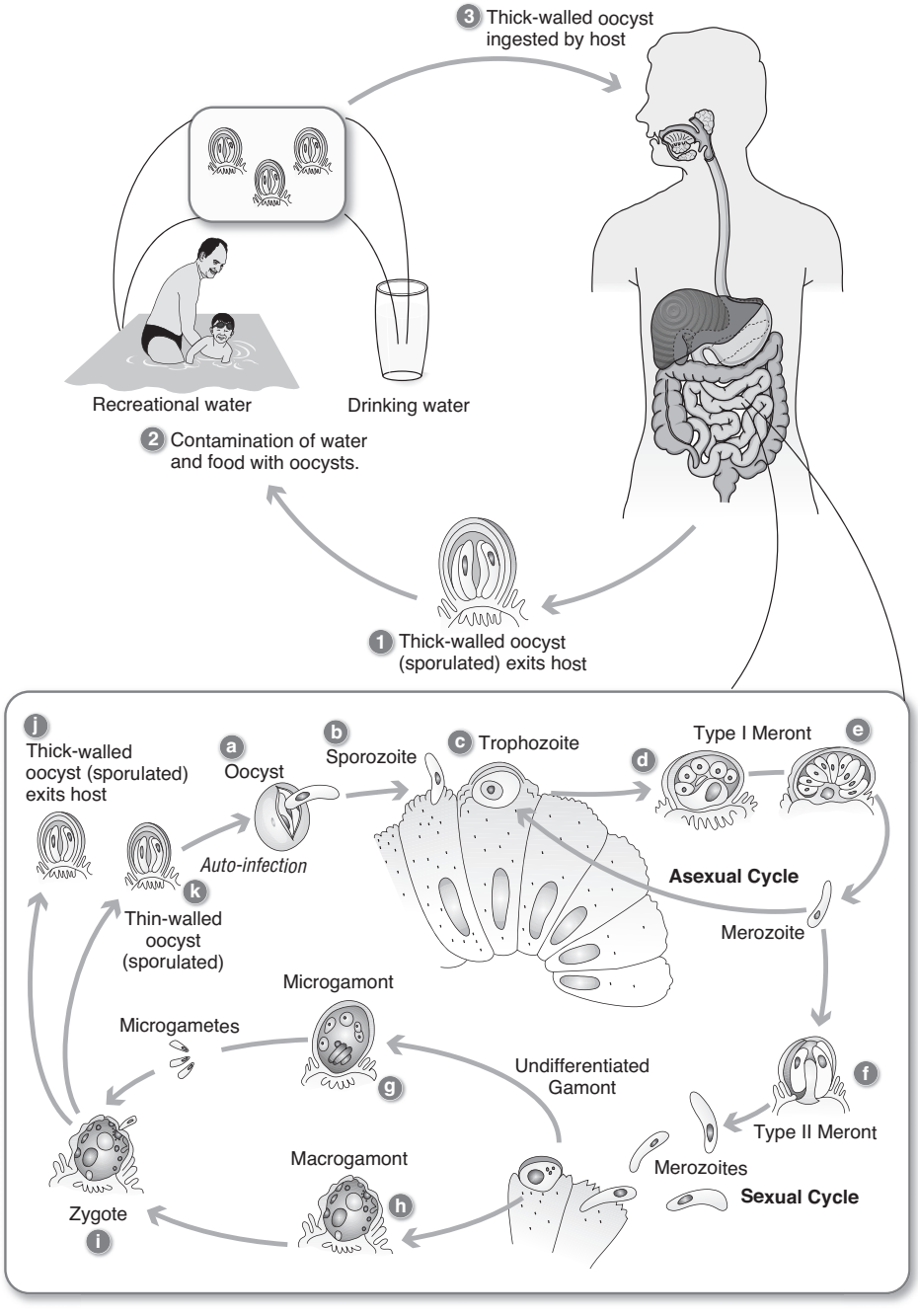

Figure 2-31 Disease Cycle of Cryptosporidiosis. *Source:* Courtesy of Alexander J. da Silva, PhD/Melanie Moser/CDC

tosporidiosis are contact with infected animals, ingestion of contaminated water, household contact with infected individuals, and international travel (Yoder et al. 2007a). Despite the large numbers of infected farm animals, foodborne cryptosporidiosis is not well documented, partly because methods to detect the oocysts in foods are not well developed (Laberge, Griffiths, Griffiths 1996).

Giardia Species

Several species of *Giardia* are capable of causing disease in humans: *G. lamblia*, *G. intestinalis*, and *G. duodenalis* (Heymann 2008). Like other protozoans, the infective stage is the trophozoite (Figure 2-32), which rapidly reproduces asexually to colonize the small intestine. They also form cysts that are excreted through defecation into the environment, permitting *Giardia* to survive for long periods of time under damp and cool conditions. The fecal-oral route of transmission is responsible for outbreaks of giardiasis through person-to-person contact, consumption of contaminated water and food, and to a lesser extent animal-to-person contact. Reservoirs of *Giardia* include many domestic and wild mammals. Historically, giardiasis was frequently associated with campers who drank contaminated water, dubbing the disease "Beaver Fever." Although many outbreaks of giardiasis have been documented over the years, including foodborne giardiasis, the relative contribution of different transmission modes to sporadic cases is unknown. To better understand its epidemiology and estimate the burden of disease, giardiasis became a nationally notifiable disease to the CDC in 2002 (Yoder et al. 2007b).

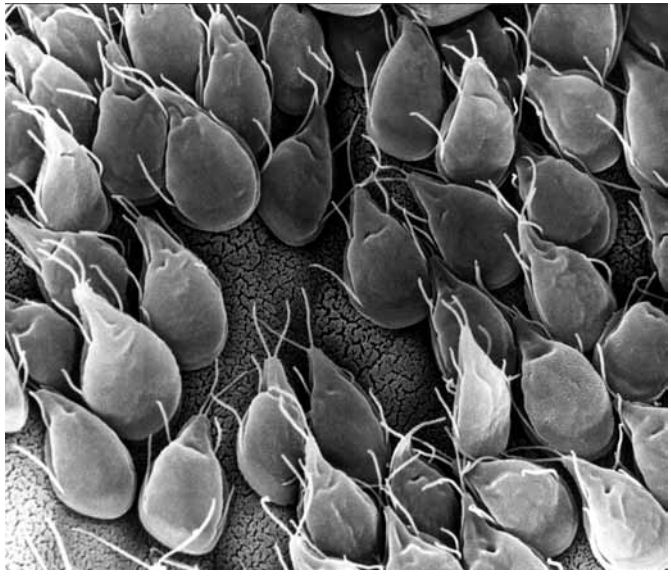

Figure 2-32 Scanning Electron Micrograph of *Giardia* trophozoites on Intestinal Epithelium of a Gerbil. *Source:* Courtesy of Dr. Stan Erlandsen/CDC

The clinical course of giardiasis is usually self-limiting, and the clinical manifestations are similar to other gastrointestinal infections, namely, diarrhea and abdominal cramps, along with bloating, malabsorption, and weight loss. Although the egg-shaped cysts and pear-shaped trophozoites of *Giardia* are easily discerned in stool specimens, many clinicians do not routinely order laboratory tests for patients with nonbloody diarrhea (Yoder et al. 2007b). Therefore, the true incidence of giardiasis in the United States is unknown.

Cyclospora cayetanensis

Among the many species of *Cyclospora* that infect animals, only *C. cayetanensis* has been associated with humans as its definitive host. The complete life cycle and biology of *C. cayetanensis* are not well understood, but its development outside of the host makes *C. cayetanensis* different from *Cryptosporidium* and *Giardia*. After the oocysts of *C. cayetanensis* are excreted in the feces of infected hosts, they must sporulate in the environment before becoming infectious to another host. This can take days to weeks (Heymann 2008). Consequently, with a longer time needed outside of the host, the role of person-to-person transmission is less compared with vehicles such as contaminated food and water. The diarrhea and other manifestations of disease can be persistent, often lasting for weeks without treatment, eventually resolving by themselves.

Cyclosporiasis is believed to be endemic in lesser developed countries, where transmission most likely occurs from the consumption of contaminated water and food. In North America, large foodborne outbreaks of cyclosporiasis have been linked to various types of imported produce (snow peas, raspberries, basil, and lettuce) (CDC 2004c). The exact sources of produce contamination were not definitively determined. Without additional research, the sources of produce contamination are speculative but probably involve contact with sewage-contaminated water.

Toxoplasma gondii

The definitive hosts of *Toxoplasma gondii* are felines, including domestic, feral, and wild cats. Humans and other animals serve as secondary and/or intermediate hosts for the parasite. Approximately 23% of adolescents and adults in the United States may have been infected with *T. gondii* (CDC 2000). For those with normal immune competence, the disease toxoplasmosis is relatively mild and self-limiting, with signs and symptoms similar to the flu, that is, fever, muscle aches, malaise. For immunocompromised individuals, the disease can be serious and even deadly. Three main routes of infection are important:

1. The ingestion of undercooked or raw meats where *T. gondii* has become encysted in the food animal's flesh
2. The accidental ingestion of *T. gondii* oocysts in cat feces (e.g., litterbox changing, gardening in contaminated soil), or by ingesting fruits and vegetables contaminated by cat feces
3. Transplacental infection to a fetus from a mother with toxoplasmosis

Infection of the fetus with *T. gondii* is potentially the most serious form of disease. Mental retardation, epilepsy and blindness are possible outcomes, and 400–4,000 cases of congenital tox-

oplasmosis are estimated to occur each year in the United States (CDC 2000). Animals used for food, whether wild or domestic, can become infected from ingesting oocysts in the soil. After excystation and further development, the *T. gondii* tachyzoites can invade other tissues in the animal and form tissue cysts that contain bradyzoites. Consumption of these tissue cysts in the meat by humans can cause toxoplasmosis. The most common meat products infected with *T. gondii* are pork.

Other Protozoans

Other protozoan diseases of foodborne importance include amebiasis and sarcocystosis. Known also as amoebic dysentery, amebiasis is cause by *Entamoeba histolytica* and characterized by the dysentery-type of diarrhea, though many cases may be comparatively mild. *E. histolytica* forms infective cysts that are passed through stools and may remain viable for weeks or months in moist environments. With humans as the only reservoirs of *E. histolytica*, food and water contaminated by human feces are the most common vehicles of transmission, followed by close personal contact in households or institutions.

Many species of the genus *Sarcocystis* that infect and parasitize animal hosts have been identified, but only two species (*S. homonis* and *S. suihominis*) are known to infect humans as their definitive host. Other species of *Sarcocystis* may infect humans accidently, but humans become a "dead-end" host for them, that is, the parasite cannot reproduce in the new host species. The life cycle of *Sarcocystis* species is complex in terms of developmental stages and intermediate and definitive hosts (Fayer 2004). Basically, humans consume undercooked meat, usually beef or pork, containing sarcocysts in the tissue. These sarcocysts release bradyzoites, and they invade the human intestinal epithelium and undergo differentiation into several forms. Ultimately, gametes fuse to form oocysts that sporulate and are shed in the feces. Ingestion of these sporocysts by livestock begins a series of stages that eventually leads to formation of sarcocysts in the muscle tissue, and the cycle repeats itself.

If humans acquire sarcocystosis from ingesting beef with *S. hominis* sarcocysts, the disease is limited to the intestinal tract, and the infections are typically asymptomatic and resolve spontaneously. Ingestion of *S. suihominis* sarcocysts in pork can result in more pronounced illness, such as bloating, diarrhea, nausea, and vomiting. Infections with nonhuman sarcocysts or sporocysts can result in muscular sarcocystosis, a relatively rare occurrence, at least in the developed countries.

FOODBORNE HELMINTHS

Approximately 300 species of helminths currently infect people around the globe (Cox 2002). Some of them have lineages linked to humans' primate evolution. Other zoonotic helminths have adapted to humans as hosts during the domestication of animals for food or companionship (Sianto et al. 2009). Humans can also become secondary or dead-end hosts for a great number of zoonotic helminths. Most of the 300 or so helminths that infect humans are rare and found only in the tropics. Their life cycles vary in complexity and may involve multiple intermediate and definitive hosts. As part of their life cycles, a few helminths access human hosts by contaminating foods with their eggs or larvae. Many helminths access their hosts primarily through other routes

but could incidentally contaminate foods to infect a human host. Because entire textbooks and journals are devoted to helminthology, the following discussion is limited to those helminths considered historically important to the North American food supply.

Trichinella Species

Trichinellosis is a nematode infection caused primarily by *Trichinella spiralis*, though several other *Trichinella* species have been identified in various hosts and different regions of the world. This parasitic roundworm has a grisly life cycle involving larvae encysting themselves in muscles and other tissues (Figure 2-33). Most often associated with the consumption of undercooked or raw pork, trichinellosis was a major concern in the United States during the mid-twentieth century. In 1943, after examining the diaphragm muscles taken from necropsies of human bodies across the country, a report published by the federal government estimated that 16% or more Americans had trichinellosis infections (Wright, Kerr, Jacobs 1943). Since then, federal regulations were developed to ensure safe pork products, dramatically reducing the number of reported trichinellosis cases (Figure 2-34). Over the decades, a link was observed between feeding hogs uncooked garbage and *Trichinella* infections. Consequently, eliminating uncooked or raw garbage from a hog's diet is an important measure in the *Trichinella* control strategy. At the consumer's level, thorough cooking of meats such as pork remains an effective control measure.

The life cycle of *Trichinella* species does not involve a free-living stage outside of the host. The adults live and mate in the mucosa of the duodenum and jejunum of the host. The resulting eggs

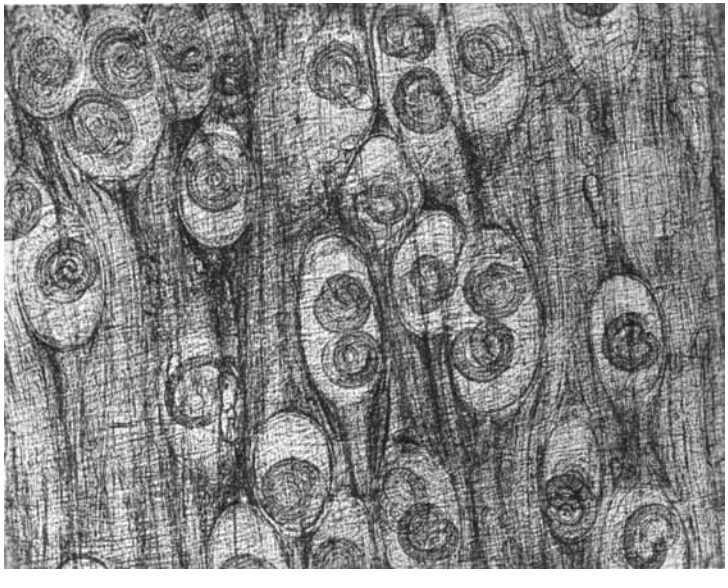

Figure 2-33 Photomicrograph of *Trichinella spirilis* Larvae Encysted in Muscle. *Source:* Courtesy of CDC

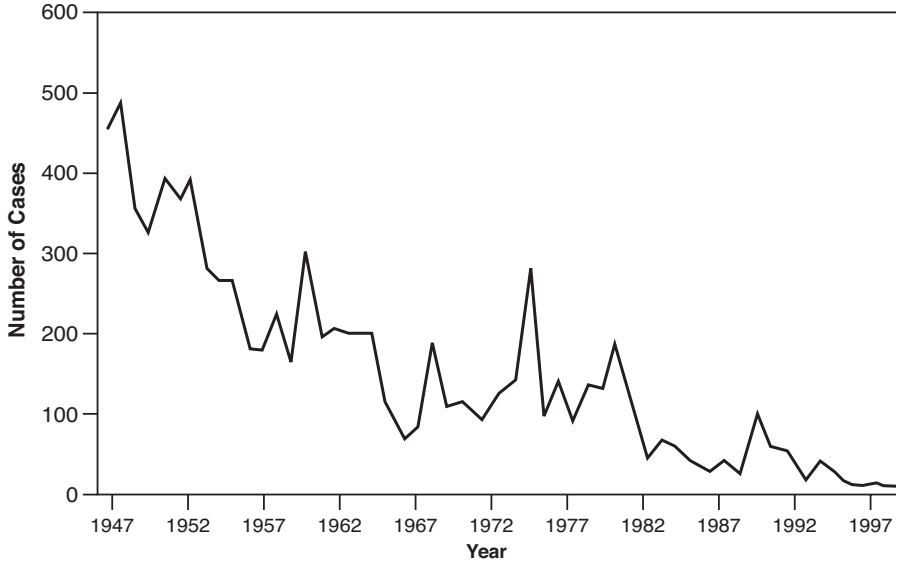

Figure 2-34 Number of Reported Trichinellosis Cases, by Year—United States, 1947–2001. *Source:* From Roy et al. (CDC) 2003

hatch in the host, and the larvae migrate through the gut wall into other tissues. Several organs, including the heart and eyes, may be affected, but only the larvae that reach the skeletal muscles of the host will ultimately survive, possibly as long as several years. They accomplish this by curling up in the muscle tissue to form a cyst (Figure 2-33). When the host animal's muscle tissue is consumed by another animal or humans, the larvae are liberated by digestive enzymes and mature in the intestines of the new host, starting the cycle over.

Domestic animals that may be hosts to *Trichinella* species include pigs, dogs, cats, rats, and occasionally horses fed items containing animal products. A great variety of wild animals such as bears, foxes, wolves, wild boars, and practically any omnivorous or predatory animal can be a potential host. Although pork was identified as the primary source of human *Trichinella* infections in the past, undercooked wild game has now become the principal source of human cases in the United States (CDC 2004d). This is partly because of the increasing popularity of bear hunting and the consumption of wild game. Furthermore, bears are frequently infected with the *T. nativa*, a species of *Trichinella* that is highly resistant to freezing and may remain viable while frozen for months or years (CDC 2004d). From 1997 to 2001, bear meat was responsible for 40% of reported cases of trichinellosis, whereas pork was responsible for 31% of reported cases; approximately 28% of reported cases were from unknown sources (Roy, Lopez, Schantz 2003).

The signs and symptoms of trichinellosis can vary by the species of *Trichinella*, but generally they coincide with the parasite's development and journey through the host. Depending on the number of larvae ingested, the incubation period can be highly variable. The first indication of

infection may be gastroenteritis (e.g., diarrhea, nausea, abdominal pain, and possible vomiting) approximately 2 days after ingestion of the contaminated meat. Invasion of the surrounding tissue by the larvae usually occurs 1 or 2 weeks following initial symptoms. Over the course of larvae migration, several clinical manifestations are possible, such as muscle soreness, fever, ocular signs, and other problems (Heymann 2008). Neurologic and cardiac complications can occur weeks later, and death from myocardial failure is possible. The disease is often misdiagnosed as illnesses such as the flu.

Taenia Species

A great variety of cestodes (tapeworms) infect animals and occasionally humans, but two species of tapeworms parasitize humans as their only definitive hosts: *Taenia saginata* and *Taenia solium*. The intermediate hosts of *T. saginata* and *T. solium* are cattle and swine, respectively. Except for differences in their anatomy and specific intermediate hosts, the life cycles of these *Taenia* species are very similar (Figure 2-35). The cycles begin by humans consuming raw or undercooked meat that contain encysted larval forms called cysticerci in the muscle flesh. The larvae develop into adult tapeworms within the human intestine, where they may live for decades, obtaining all their nourishment from the human host. The adult tapeworm infection is called taeniasis. This form of disease is often mild and variable, consisting possibly of nervousness, weight loss, and abdominal pain. Each segment of the tapeworm, called a proglottid, has reproductive organs that produce tens of thousands of eggs. The gravid proglottids and eggs are shed through human feces each day in great numbers, extensively contaminating the surrounding environment. In the soil, the eggs can survive for months with fully developed embryos. Cattle or swine consume the eggs during grazing or rooting in the soil, and the embryos hatch in the animals' intestine. From there, the embryos migrate to striated muscles and transform into cysterci, starting the cycle over again. The larval form of this infection is called cysticercosis.

The occurrence of cysticercosis is usually limited to the intermediate animal hosts. In the case of *T. solium* infections, however, it is possible for humans to acquire this very serious and sometimes fatal disease. This happens by accidental ingestion of the *T. solium* eggs, either by autoinfection (from one's self) or from contaminated soil. When humans ingest *T. solium* eggs, the cysticerci may become embedded in the muscles, ocular regions, and central nervous system—including the brain. Signs and symptoms of cysticercosis depend on the locations and numbers of the cysticerci in the tissues. With neurocysticercosis, signs and symptoms may range from headaches to seizures and stroke. Cysticercosis is a debilitating disease that merits prevention by strict adherence to sanitation and food safety practices.

From the descriptions provided previously on the life cycle of *Taenia* tapeworms, it makes sense that the disease is more common in regions where sanitation is inadequate and livestock are allowed access to human feces or human feces–contaminated soil. The occurrence of human *Taenia* infections is high in such places as Latin America, perhaps as much as 10–25% incidence in some villages, while the prevalence of cysticercosis in swine may reach 37% in some parts of eastern Tanzania, Africa (Heymann 2008). Infections by *Taenia* have become rare in North America and Europe. However, infections from imported meat are possible in these regions, and the possibility still exists for local transmission of taeniasis or cysticercosis (CDC 1992).

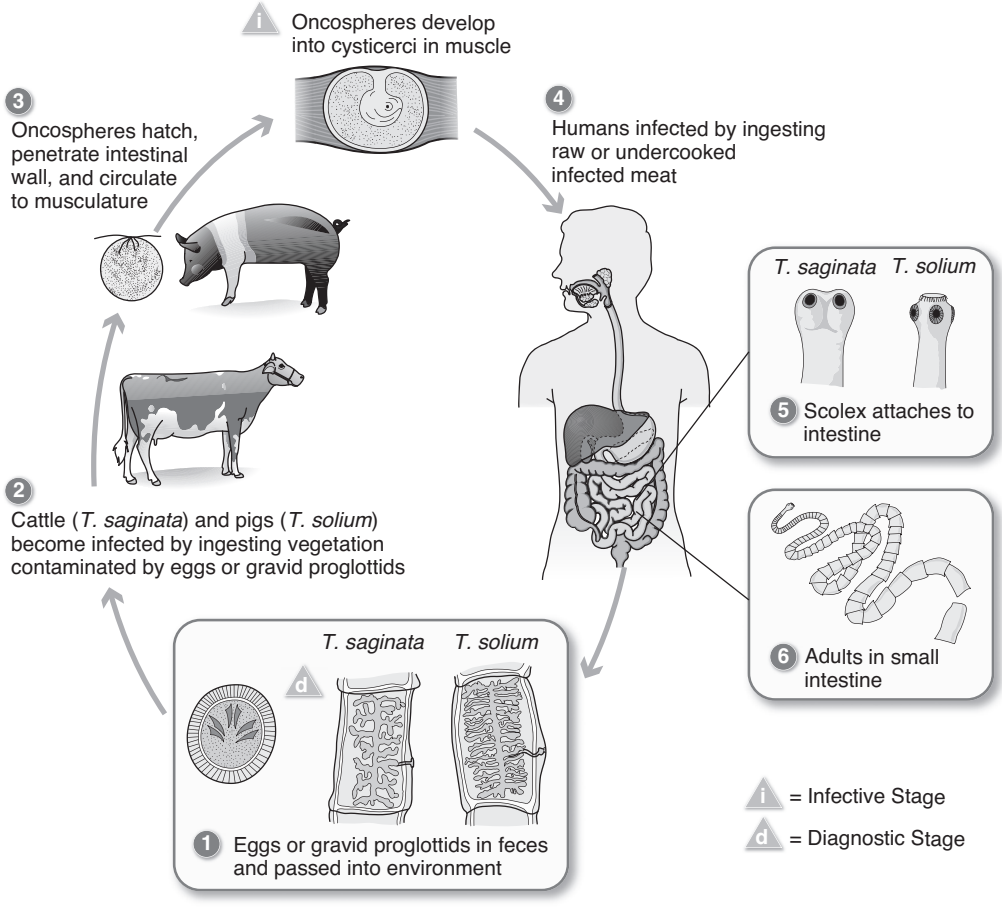

Figure 2-35 Disease Cycle of Taeniasis. *Source:* Courtesy of Alexander J. da Silva, PhD/Melanie Moser/CDC

Diphyllobothrium Species

Many different species of fish tapeworms belonging to the genus *Diphyllobothrium* have been identified to infect humans and animals (Skeríková et al. 2009). Of the several species known to infect humans, the tapeworm *Diphyllobothrium latum* is considered the most important. Figure 2-36 illustrates the life cycle of *Diphyllobothrium* species and shows at least three intermediate hosts are involved: crustaceans such copepods, freshwater fish, and/or salmon. Humans and other fish-eating mammals and birds are the definitive hosts. These hosts are also responsible for continuation of the *Diphyllobothrium* species life cycle by passing tapeworm eggs (as many as 10^6 eggs/worm/day) in feces to a body of water. In humans, the disease is called diphyllobothriasis and occurs from eating raw or undercooked fish. Cases of diphyllobothriasis by *D. latum* are usually associated with fish

caught in subarctic or temperate regions of the Northern Hemisphere, that is, northern latitudes of North America, Europe, and Asia. For many years, *D. latum* was presumed responsible for diphyllobothriasis from the consumption of raw or undercooked salmon, but in recent years, the species *D. nihonkaiense* has been increasingly identified as the etiologic agent (Arizono et al. 2009). Cases of diphyllobothriasis by *D. pacificum* have been reported in South America and Japan after consuming marine (not freshwater) fish (Scholz et al. 2009; Skeríková et al. 2009).

Other Helminths of Food Safety Concern

Several other helminths that may be transmitted by foods are included in Table 2-9. The majority of these helminths are usually encountered outside of North America. Nonetheless, with

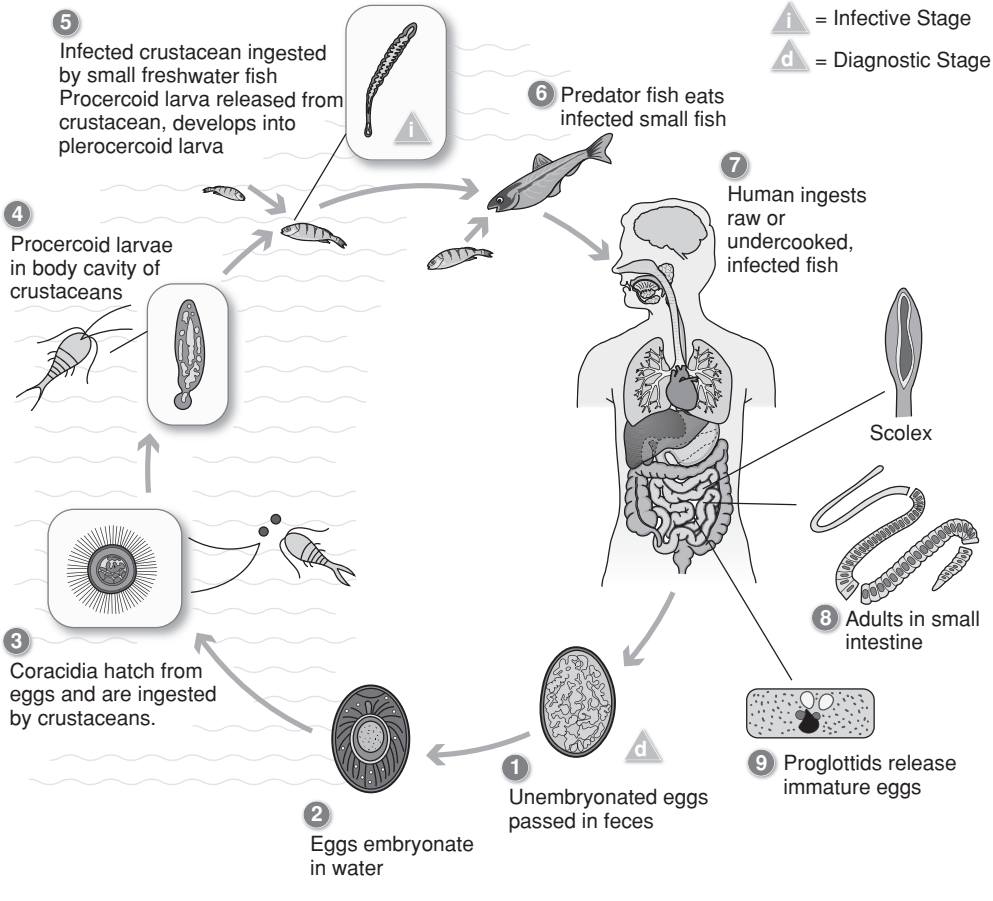

Figure 2-36 Disease Cycle of Diphyllobothriasis. *Source:* Courtesy of Alexander J. da Silva, PhD/Melanie Moser/CDC

Table 2-9 Selected Helminths of Food Safety Importance

Helminth	*Occurrence*	*Foodborne Transmission*
Trichinella spp.	Worldwide, with incidence dependent on local cooking and eating habits	Primarily from raw or undercooked pork, and from other meats, frequently from wild game
Taenia spp. (beef and pork tapeworms)	Worldwide, particularly in regions with poor sanitary conditions and with livestock having access to human feces	Consumption of cystercerci in raw or undercooked beef and pork. Cystircercosis from unintentional consumption of *T. solium* eggs
Diphyllobothrium spp. (broad or fish tapeworm)	Mostly northern hemisphere and some in the tropics	Consumption of raw or undercooked freshwater fish and salmon. To a lesser degree, marine fish consumed raw
Echinococcus granulosus	All continents except Antarctica	Contamination of food or water after contact with infected dogs
Fasciola hepatica and *F. gigantica* (agent of liver rot)	Reported in 61 countries, mostly where sheep and cattle are raised	Consumption of raw aquatic plants (watercress) containing metacercariae
Fasciolopsis buski	Southeast Asia, particularly in areas with pigs	Consumption of raw aquatic plants (water caltrop nuts, water chestnuts, water bamboo) containing metacercariae
Clonorchis sinensis (Chinese liver fluke)	Throughout China, occasionally Japan, Korea, and Southeast Asia. Human and multiple animal definitive hosts	Consumption of raw or undercooked freshwater fish containing encysted larvae
Anisakis simplex (herringworm or whaleworm) and *Pseudoterranova decipiens* (codworm or sealworm)	Throughout the world where individuals eat inadequately cooked fish, squid, and octopus	Consumption of raw or undercooked fish after larvae have migrated from fish's mesentery to muscle flesh, usually after fish die or are killed
Capillaria philipiensis	Endemic in Philippines and Thailand, but also reported in other countries	Consumption of raw, whole freshwater fish, and autoinfection
Gnathostoma spp. (agent of a visceral larva migrans)	Anyplace where dogs, cats, and large carnivores may act as definitive hosts	Consumption of undercooked fish, poultry, frogs, or snakes. Common among ethnic dishes such as sashimi, somfak, or ceviche
Ascaris lumbricoides (agent of intestinal roundworm infection)	Worldwide and common, with incidence exceeding 50% in most tropical countries	Consumption of uncooked produce from soil contaminated with human feces (and infective helminth eggs)
Trichuris trichiura (human whipworm)	Worldwide but most prominent in warm, moist regions	Consumption of human-feces-contaminated soil or vegetables

Source: Compiled from Heymann 2008; Cox 2002; Scholz et al. 2009; and CDC 1992.

an increasingly global food market and international travel, the possibility of acquiring one of these helminth infections exists, albeit the risk depends on multiple factors and circumstances. Specific measures for controlling foodborne helminths are covered in later chapters.

PRIONS

For at least 200 years, a neurodegenerative disease known as scrapie has afflicted sheep. In the 1980s, the etiologic agent was tentatively identified as a prion protein (PrP). Several variants of the transmissible PrP have been studied since then, and a collection of diseases called transmissible spongiform encephalopathies (TSE) or prion diseases was identified as afflicting several species—including humans (Chesebro 2003). As a result of additional research, the number of recognized prion diseases continues to grow (Watts, Balachandran, Westaway 2006). The animals identified to be afflicted by TSE include sheep, cattle, mink, elk, deer, and other animals experimentally inoculated.

The public became aware of prion diseases from a highly publicized epidemic of bovine spongiform encephalopathy (BSE), known as "mad cow disease." By 1992, more than 30,000 cattle per year were being identified with the disease in the United Kingdom (Brown et al. 2006). Despite intensive efforts that eventually controlled the epidemic, the disease spread across international borders, and by 2006 it was reported in 24 other countries. The first confirmed case in the United States was a dairy cow from Washington State, reported in December 2003 (CDC 2004a). It was ultimately determined that the BSE epidemic was intensified by the rendering of livestock carcasses to make feed for cattle. The prions apparently survived the ineffective rendering process and were transmitted to healthy cattle through the contaminated feed. The exportation of U.K. feed products to other countries apparently exported the disease as well.

The transmission of TSEs occurs from either consuming materials contaminated with prions or by being inoculated by prion-contaminated materials, for example, by transplanted tissues or contaminated surgical instruments (Chesebro 2003). They do not appear to be transmitted by person-to-person, animal-to-animal, or animal-to-human contact. Prions exert their detrimental effects by causing certain other proteins to misfold into a wrong shape. An accumulation of misfolded proteins in the brain leads to the formation of plaques or spongy voids (spongiform). The associated symptoms become more progressive and may take months or years before becoming clinically recognizable. In cattle with BSE, the symptoms typically include behavioral changes such as nervousness or aggression, unusual posture and incoordination, inability to rise (downer), and unexplained weight loss. Among wild animals such as deer and elk in North America, the TSE is called chronic wasting disease (CWD), a reference to the animals' emaciated appearance. In humans, the primary symptoms are dementia and ataxia. The neurologic pathology ultimately leads to an early death in all species.

Human forms of TSE are called Creutzfeldt-Jakob disease (CJD) or Gerstmann-Straussler-Scheinker syndrome, kuru, and fatal familial insomnia. Several types of CJD are known, and each has been labeled with an adjective that describes the context of its occurrence. Classic CJD (cCJD) includes familial (inherited), iatrogenic, and sporadic forms designated as fCJD, iCJD, sCJD, respectively. As their names suggest, the cCJD forms are either inherited, transmitted by tissue transplantation or surgical instruments (iatrogenic), or occur very infrequently (1 in 10^6

people) with no identifiable risk factors (sporadic). In 1994, during the BSE epidemic, the United Kingdom identified a new variant of CJD, designated as vCJD. This revelation prompted alarm among public health officials worldwide because epidemiologic and laboratory evidence suggested vCJD was transmitted from BSE-contaminated cattle products (CDC 2004b). The incidence of vCJD dropped off over a period of several years following its initial discovery. Speculation is that a tougher control on beef producers was responsible by limiting BSE in the human food chain.

Even within a species, different strains of prions have been identified, meaning that they differ by their conformational structure and behavior within the animal. Different strains or variants of prions tend to accumulate in different regions of the nervous system, or other tissues may also be involved. Because BSE can have a very long incubation period, BSE-infected cattle could be slaughtered for food before behavioral signs become obvious. Outside of the host, prions are very resistant to degradation from high temperatures, acidity, ultraviolet light, ionizing radiation, common disinfectants, and proteolytic enzymes. In other words, prions are very persistent in the environment. Unfortunately, highly sensitive and accurate methods for detecting prion infectivity in the environment have not been developed (Saunders, Bartelt-Hunt, Bartz 2008). The current food safety strategy focuses on eliminating transmission among livestock by restricting certain rendered feeds, banning certain bovine tissues from entering the human food chain, observational surveillance of cattle for infection, and occasional testing of carcasses for BSE. The risk of prions in the human food chain is difficult to assess but is generally considered to be extremely low. The concern is that complacency may lead to another epidemic similar to the past one in the United Kingdom.

EMERGING VERSUS CONTEMPORARY FOODBORNE INFECTIONS

According to the Institute of Medicine (IOM), an emerging infectious disease is one where the incidence has increased within the past two decades or poses a threat to increase in the near future (Lederberg, Shope, Oaks 1992). This includes both newly identified diseases and those previously controlled in the past (i.e., reemergence). The majority of foodborne infections discussed in this chapter fit the definition of an emerging infectious disease. But the incidence of foodborne diseases is not static and changes over time. As some diseases become controlled, others may emerge and increase in incidence. The foodborne diseases listed in Table 2-10 were once high-priority concerns in the United States because of their associated morbidity and/or mortality, but their incidences decreased greatly in the latter half of the twentieth century. Several specific control measures are credited for the reduction of these foodborne diseases (Beatty et al. 2003; Tauxe 2002). Although these foodborne diseases have been largely controlled in developed countries, their reservoirs still exist, and under the right conditions, they could reemerge. In fact, most of these diseases still occur in the United States but at very low incidence levels.

A great number of changing conditions and trends contribute to emerging foodborne diseases. Several of these trends were briefly discussed in Chapter 1. In a landmark report, the IOM identified six factors or categories that facilitate the emergence of microbial threats or diseases (Lederberg et al. 1992). Several authors have specifically analyzed the emergence of foodborne diseases in the context of these factors (Altekruse, Cohen, Swerdlow 1997; Buchanan 1997; Skovgaard 2007; Tauxe 2002). Some of their insights are shared in the following subsections on the six factors identified by the IOM.

Table 2-10 Foodborne Diseases of Concern in Early Twentieth Century United States

Disease	*Pathogenic Agent*	*Key Control Measures*
Typhoid fever	*Salmonella* Typhi	Pasteurization of milk, shellfish sanitation
Brucellosis	*Brucella abortus*	Pasteurization of milk
Q fever	*Coxiella burnetii*	Pasteurization of milk
Bovine tuberculosis	*Mycobacterium bovis*	Pasteurization of milk
Scarlet fever	*Streptococcus* spp.	Pasteurization of milk
Septic sore throat	*Streptococcus* spp.	Pasteurization of milk
Trichinellosis	*Trichinella spirilis*	Ban feeding uncooked garbage to pigs, adequate cooking of pork
Tapeworms	*Taenia* spp.	Sanitation, meat inspections
Gastrointestinal anthrax	*Bacillus anthracis*	Animal vaccinations and inspections
Botulism	*Clostridium botulinum*	Retort food canning

Source: Compiled from Beatty et al. 2003; Tauxe 2002; and Tauxe and Esteban 2007.

1. Human Demographics and Behavior

The population in the United States and other developed countries is changing demographically. A greater proportion of the population has a heightened susceptibility to foodborne infections. Individuals such as older adults, people infected with HIV, transplant recipients, and cancer/chemotherapy patients generally have less resistance to foodborne infections. Behavioral and cultural changes are reflected in the eating preferences and habits of people. More health-conscious people are eating raw fruits and vegetables, increasing the demand for fresh produce that may also become contaminated with pathogens during growing, harvesting, processing, and/or distribution. A greater percentage of people are also eating outside of the home. For many reasons related to food safety, the risks of outbreaks are greater (or more easily recognized) at sites where large quantities of food are handled, prepared, and consumed. In addition, with the population growth of developed countries attributed more and more to immigration, ethnic practices by immigrants may put them at increased risk of foodborne diseases. Examples include outbreaks from the consumption of rare pork, resulting in trichinellosis, or cheeses made from raw milk, resulting in listeriosis or brucellosis.

2. Technology and Industry

A century ago, the food production industry was not on the same scale as it is today. Technology and industry practices have changed agriculture from a widely dispersed set of company- and family-owned farms to corporate agricultural production, processing, and distribution centers. Although this change has increased food production and helps feed the world, it has also introduced and transmitted zoonotic pathogens across animal stocks and distributed them to human

consumers. The emergence of *E. coli* O157:H7 and *Salmonella* species in human populations, sometimes as massive and geographically distributed outbreaks, is reflected in this change. Food processing plants are highly productive and automated, and if adequate food safety practices are not followed, these plants can harbor foodborne pathogens and contaminate multiple lots of foods. Many food products distributed across the country are now made from ingredients provided by multiple companies. If any of these ingredients is contaminated and harbors pathogens, the pathogens are distributed along with the food product. More about this topic is discussed later in the topic of safety management of the food supply.

3. International Travel and Commerce

The world has become a smaller place in terms of international travel and commerce. Tourists and businesspeople can eat lunch on the other side of the world and return home before the next day. They can also bring back unwanted souvenirs, such as diarrheal disease and parasites, and unknowingly serve as incubatory carriers of a disease or parasite. But this phenomenon is not limited to international travelers. The exportation and importation of foods around the world are now commonplace. Foodborne diseases considered as emerging or exotic sometimes appear in developed countries from imported foods. This may be the result of poor food safety practices in the country of origin and/or contamination during shipping. International trade has also spread infected breeding stocks of animals across borders. The pandemic of salmonellosis is believed to be the result of chickens being sold for breeding purposes, particularly egg-laying hens.

4. Microbial Adaptation and Change

As discussed earlier in this chapter, microorganisms are capable of rapid evolutionary changes that result in adaptation to different environmental conditions. Some of these changes involve acquiring additional pathogenic factors, which in turn increases the virulence of foodborne pathogens. Other changes involve increased pathogen tolerances to temperature extremes, acidic conditions, desiccation, and to some disinfectants. For example, several foodborne pathogens are capable of entering a VBNC state, allowing them to survive unfavorable environmental conditions. Changes can also be very specific, such as the acid tolerance response (ATR) discussed earlier. The greatest change of concern is antibiotic resistance. Multiple strains of foodborne pathogens have been identified as resistant to various antibiotics. The principal selection pressure for antibiotic resistance among foodborne pathogens is the liberal use of antibiotics in farm animals. Patients prescribed antibiotics for foodborne diseases may also contribute to antibiotic resistance, but this selection pressure is more influential in secondary human cases and person-to-person transmission scenarios.

5. Economic Development and Land Use

Animals raised for food generate approximately 2 billion tons of manure each year in the United States. Disposal of this manure creates numerous environmental problems, but it also acts as a reservoir of zoonotic pathogens such as *E. coli* O157:H7, *Salmonella* species, *Campylobacter* species,

and others. Improper waste management of manure contributes to the spread of zoonotic pathogens among herds of farm animals. Land use and waste management are also influential in the increasing trend of foodborne infections from fresh produce. Poor waste management of manure, and animals in close proximity to field crops, can result in pre- or postharvest contamination of produce with pathogens. A similar problem is the proper treatment and disposal of human wastes. The capacity of sewage treatment plants does not always keep pace with growing human populations, especially in lesser developed countries. Even in developed countries, excessive rains (e.g., hurricanes) or equipment breakdowns can spill raw sewage onto the land and into waterways. Human pathogens in sewage can become foodborne under the right set of circumstances. Shellfish beds in local waters are particularly efficient at concentrating pathogens from raw sewage and farm runoff. Seafood harvested from these contaminated waters can be a source of several pathogens.

6. Breakdown of Public Health Measures

Believing that the war on infectious diseases was won in developed countries, the public health priority and research emphasis on infectious diseases began to wane during the mid-twentieth century. Attention and resources were directed from infectious disease control programs to higher priority chronic disease control and other programs. An institutional complacency ensued with infectious diseases, and a false sense of security developed with the public, believing that modern medicine could cure nearly any infection. The recognition of new infectious diseases and old ones reemerging came about in the latter part of the twentieth century, when several epidemics caught the public's attention, most notably the AIDS epidemic. But many public health agencies continued to operate with severely restricted budgets, especially at the state and local levels of government. During the 1990s, several states did not have personnel dedicated to a foodborne disease surveillance program. Federal and state agencies also had limited staffing to effectively conduct food safety inspections and enforcement at commercial food processing plants and restaurants. Events such as natural disasters also lead to the breakdown of public health measures, resulting in inadequate food protection and increased potential for disease transmission.

New and infrequently reported foodborne pathogens have been identified in recent years, albeit knowledge about them is limited for a number of reasons. For example, the bacteria *Aeromonas hydrophila* and *Plesiomonas shigelloides* have been associated with foodborne illnesses from the consumption of fish and shellfish, as well as other modes of transmission. Additional research is needed to characterize their pathogenicity in humans and their significance for foodborne transmission. Other emerging infections of the gastrointestinal tract continue to be identified, but new diagnostic tests and additional epidemiologic studies are needed before specific control measures can be proposed (Schlenker and Surawicz 2009). In the near and far future, humans will likely encounter new foodborne pathogens that will challenge existing food safety programs. Given the scope and magnitude of potential problems, a proactive approach is preferable to a reactive one.

Intentional Contamination of Foods

Under the auspices of the IOM, a workshop was held to address foodborne threats to health (Institute of Medicine [IOM] 2006). The ease and vulnerability of foods to deliberate adultera-

tion (i.e., terrorism) are frightening to many and a great concern to public officials. Whatever the motivation, the deliberate introduction of a pathogen or toxicant into the food supply could yield catastrophic results, both in terms of morbidity and economic losses. In fact, the IOM now includes a new category, Intent to Harm, for the emergence of infectious diseases (Smolinski, Hamburg, Lederberg 2003). This opens the possibility to a wider array of new or rarely encountered pathogens as agents of foodborne diseases. Even though a determined effort to inflict harm is difficult to thwart, food safety practices and management of the food supply must include reducing vulnerability to the deliberate introduction of harmful agents. This subject is addressed in more depth under safety management of the food supply.

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USEFUL RESOURCES

Bad Bug Book: Introduction Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. A concise and handy reference to foodborne pathogens and toxins. http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllness FoodbornePathogensNaturalToxins/BadBugBook/default.htm

Center for Science in the Public Interest, Outbreak Alert! Database. An interesting database of foodborne illness outbreaks compiled from multiple sources dating back to 1990. http://www.cspinet.org/foodsafety/outbreak/pathogen.php

Centers for Disease Control and Prevention sites:

- OutbreakNet Team. Links to Foodborne Illness A–Z, National Outbreak Reporting System (NORS), and Outbreak Surveillance Data, including by year and etiology. http://www.cdc.gov/outbreaknet/
- FoodNet Reports. Annual summaries of nine pathogens that are followed through an active surveillance system (FoodNet) involving participating states. http://www.cdc.gov/foodnet/reports.htm
- Enteric Diseases Epidemiology Branch homepage. http://www.cdc.gov/enterics/index.html
- National Surveillance Team—Enteric Diseases Epidemiology Branch. Links to National Antimicrobial Resistance Monitoring System for selected foodborne pathogens, and National Case Surveillance files for notifiable (and some other) foodborne pathogens. http://www.cdc.gov/nationalsurveillance/index.html
- Public Health Laboratory Information System, Division of Foodborne, Waterborne, and Environmental Diseases. Links to *Salmonella* and *Shigella* annual summaries. http://www.cdc.gov/ncidod/dbmd/phlisdata/d*efault.htm*
- Parasites A–Z Index, food-related parasites. http://www.cdc.gov/ncidod/dpd/food.htm
- Food Safety Office. Links to specific diseases/pathogens, foods and high-risk groups, and other resources. http://www.cdc.gov/foodsafety/
- *Morbidity and Mortality Weekly Report (MMWR)*. Important publications on current (and past) disease outbreaks and trends, including foodborne diseases. http://www.cdc.gov/mmwr/
- OutbreakNet: Foodborne Outbreak Online Database. Search OutbreakNet by year, state, location, and etiology. http://wwwn.cdc.gov/foodborneoutbreaks/

Food Safety Network, Canada. Links to food risks and other information. http://www.foodsafetynetwork.ca/aspx/public/default.aspx

Food Safety Research and Response Network. Links to foodborne pathogen research projects and publications. http://www.fsrrn.net/

FoodSafety.org. Gateway to federal food safety information. http://www.foodsafety.gov/

Michigan Department of Agriculture, FPAdvisor (Food Pathogen Advisor). A searchable database used for training exercises that allow the user to enter disease symptoms, incubation periods, foods, and other information to derive a list suspected foodborne pathogens. http://www.mda.state.mi.us/FPAdvisor/FpadvisorHelp.htm

U.S. Department of Agriculture, National Agricultural Library, Food Safety Research Information Office, Pathogens and Contaminants. Pathogen and contaminant fact sheets and links to pathogen biology. http://fsrio.nal.usda.gov/path_contam.php

World Health Organization, Food Safety. Links to microbial risks and foodborne disease. http://www.who.int/foodsafety/en/