

Chapter 2

Selected Pathogens of Concern to Industrial Food Processors: Infectious, Toxigenic, Toxicoinfectious, Selected Emerging Pathogenic Bacteria

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Abstract This chapter, written by several contributing authors, is devoted to discussing selected microbes of contemporary importance. Microbes from three categories are described by the following: (1) infectious invasive agents like *Salmonella*, *Listeria monocytogenes*, and *Campylobacter*; (2) toxigenic pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium botulinum*; and (3) toxico-infectious agents like enterohemorrhagic *Escherichia coli* and *Clostridium perfringens*. In addition, emerging pathogens, like *Cronobacter* (*Enterobacter*) *sakazakii*, *Arcobacter* spp., and *Mycobacterium avium* subspecies *paratuberculosis* are also described.

In most cases, the discussion includes a description of the organism itself, economic impact of the organism (due to disease, loss of market share, etc.), disease syndromes/infectious process, infectious dose, reservoirs (where the organism originates in the food processing chain), foods associated with the organism, and the occurrence of the organism in food processing environments.

2.1 Introduction

This chapter will not address all the pathogenic microbes that are of concern in all foods or all food processing environments. However, selected pathogens will be described which illustrate typical organism types (i.e., infectious, toxigenic, toxico-infectious) of common concern in food manufacturing environments. A few selected emerging foodborne pathogens will also be discussed. Detailed reviews and descriptions of foodborne pathogens can be found in a number of references (Doyle, 1989; Jay et al., 2005; Doyle et al., 1997). The later part of this chapter will address selected emerging microbial pathogens of concern.

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Post-process contamination from the factory environment is a very common (and in this authors opinion – the most common) means by which commercially processed foods are contaminated (Kornacki, 2000; Allan et al., 2004; Reij and Den Aantrekker, 2004). Examples of post process contamination from the food processing environment are illustrated in Table 2.1. In this book we refer to “commercially processed” foods as those which have been modified from the raw state into a ready-to-eat format in an industrial manufacturing environment. Environmental contamination can come from ingredients used in processing, whether directly or indirectly, worker’s hands, shoes, walls, floors, and a myriad of other sources. This chapter is devoted to discussing selected microbes of contemporary importance. These microbes fit into three categories which include

- (1) infectious invasive agents like *Salmonella* (Section 2.2), *Listeria monocytogenes* (Section 2.3), *Campylobacter* (Section 2.4), and enteroinvasive *Escherichia coli*,
- (2) toxigenic pathogens like *Staphylococcus aureus* (Section 2.5), *Bacillus cereus* (Section 2.6), and *Clostridium botulinum* (Section 2.7). (The growth and production of pre-formed toxin in foods is a particular concern with enterotoxin producing strains of *Staphylococcus*, *B. cereus*, and *C. botulinum*.)
- (3) toxico-infectious agents like enterotoxigenic and enterohemorrhagic *E. coli* (Section 2.8) and *Clostridium perfringens* (Section 2.9) are described.

In addition emerging pathogens, like *Cronobacter* (*Enterobacter*) *sakazakii* (Section 2.11), *Arcobacter* spp. (Section 2.10), and *Mycobacterium avium* sub-species *paratuberculosis* (Section 2.12), are also described.

Infectious vs. toxigenic bacterial pathogens

In general, infectious pathogens may enter the body and invade or colonize host tissues. This requires some time (e.g., usually greater than 8 h for onset of illness). Toxigenic pathogens create food “poisoning” situations by producing an enterotoxin in the food. Incubation times for onset of disease from toxigenic microbes are often shorter than for invasive pathogens and can be as little as 1 h, as in the case of staphylococcal enterotoxin-induced illness. The short incubation time in comparison to the infectious pathogens results because the agent of illness, the toxin, is pre-formed in the food and ingested. Illness is not contingent upon the organism migrating to the intestinal tract implanting and growing. Selected examples of invasive and infectious foodborne pathogens and their importance in various foods follow.

2.2 *Salmonella*, an Infectious Invasive Agent

Salmonella spp. are an example of an “invasive” infectious pathogen and are second only to the thermophilic *Campylobacter* spp. (e.g., *jejuni*, *coli*) in the number of foodborne disease cases per year attributed to bacteria (Table 2.2).

Table 2.1 Examples of outbreaks attributed to environmental contamination

Product	Pathogen	Comment
Tuna salad	<i>C. jejuni</i>	Probably chicken handled in same kitchen
Ice cream	<i>S. enteritidis</i>	Pasteurized ice cream mix in tanker truck previously used for transporting raw liquid eggs
Infant formulae	<i>S. ealing</i>	Contamination from the processing environment, insulation material of the drying tower
Soft cheese	<i>S. beria</i>	Cheese ripening in buckets previously used for chicken carcasses
Cooked sliced ham	<i>S. typhimurium</i>	Cooked ham placed into containers previously used for curing
Chocolate	<i>S. Napoli</i>	Possibly contaminated water used in double-walled pipes, tanks
Chocolate	<i>S. eastbourne</i>	Contamination from the processing environment
Butter	<i>L. monocytogenes</i>	Contamination from the processing environment
Hot dogs	<i>L. monocytogenes</i>	Contamination from the processing environment
Canned salmon	<i>C. botulinum</i>	Contamination from the processing environment, cooling water
Lasagne	<i>S. aureus</i>	Growth of <i>S. aureus</i> in the processing equipment, improper cleaning
Different foods	<i>E. coli</i> O157:H7	Contaminated meat grinder and equipment at retail level
Chocolate milk	<i>Y. enterocolitica</i>	Probably during manual mixing of pasteurized milk and chocolate
Canned meat	<i>S. typhi</i>	Use of non-potable water for can cooling
Crabmeat	<i>S. aureus</i>	Contamination during manual picking of cooked meat
Canned mushrooms	<i>S. aureus</i>	Possible growth of <i>S. aureus</i> in the brine bath before canning
Flavored yogurt	<i>E. coli</i> O157:H7	Pump previously used for raw milk
Pastry	<i>S. Enteritidis</i> PT4	Equipment previously used for raw eggs or insufficiently cleaned piping and nozzles used for cream
Yeasts	<i>S. münchen</i>	Contamination from the processing environment
Pasteurized milk	<i>S. typhimurium</i>	Possibly cross-connection between raw and pasteurized milk
Pasteurized milk	<i>E. coli</i> O157:H7	Contamination from pipes and rubber seals of the bottling line
Pasteurized milk	<i>B. cereus</i>	Filling equipment
Pasteurized milk	<i>Y. enterocolitica</i>	Post-process contamination
Mexican type cheese	<i>L. monocytogenes</i>	Contamination from the processing environment

Adapted from Reij and Aantrekker (2004); and ICMSF (2002).

Table 2.2 Estimated annual foodborne disease from selected pathogens

Bacterium	Number of total illness	Total illness (%)	Hospitalized (%)	Deaths (%)	Number of deaths
<i>Campylobacter</i>	2 M	14.2	17.3	5.5	99
<i>Salmonella</i> (non-typhoidal)	1.3 M	9.7	25.6	30.6	553
<i>Listeria monocytogenes</i>	2,493	0.0	3.8	27.6	499
<i>E. coli</i> O157:H7	62,458	0.5	3.0	2.9	52
<i>Clostridium perfringens</i>	248,520	1.8	0.1	0.4	7
<i>Staphylococcus</i>	185,060	1.3	2.9	0.1	2
<i>Shigella</i>	89,648	0.6	2.0	0.8	14

Adapted from Mead et al. (1999).

M = million.

2.2.1 *Salmonella: The Organism*

Salmonella species are gram-negative, rod-shaped, usually motile, members of the taxonomic family, Enterobacteriaceae. Despite great advances in molecular genetic approaches to identification and characterization these organisms are still *serologically defined*, i.e., by their somatic (O) and (usually) flagellar (H) and sometimes capsular (Vi) antigens. Over approximately 2,400 different serotypes are known to exist. The nomenclature of this microbe has gone through a number of changes resulting in some confusion. In this author’s opinion it is easiest to refer to the serotype designation (e.g., *Salmonella* serotype Typhimurium) as opposed to other nomenclatural approaches (e.g., *Salmonella enterica* serovar Typhimurium).

2.2.2 *Cost*

Costs that are difficult to measure include pain and suffering, death, and loss of a company’s reputation. Other costs may include lost market share, lost jobs or reduced wages, lawsuits from shorted customers, the price to remanufacture products that have been destroyed, the cost to recondition contaminated product (if possible and allowed), and lawsuits from stricken individuals or class actions (see Chapter 1). The United States Department of Agriculture (USDA) estimated that 696,000–3,800,000 cases of non-typhoid, foodborne Salmonellosis occurs annually with an estimated cost of 0.9–12.2 billion dollars (Buzby and Roberts, 1996).

2.2.3 *Disease Syndromes*

Salmonella can cause a number of disease syndromes including typhoid fever from *Salmonella typhi* (rarely found in foods produced in the United States). However,

other strains of *Salmonella* cause gastroenteritis, bacteremia, and enteric or paratyphoid fever (Hanes, 2003). Onset times typically range from 18 to 36 h (IAFP, 1999). Symptoms include abdominal pain, diarrhea, occasionally with mucous or blood. Nausea and vomiting often occur but are rarely severe or protracted. A fever of 38–39°C is common, often after a chill. In many instances the disease resolves within 48 h. However, it can last with diarrhea and low-grade fever for 10–14 days. In severe cases dehydration may lead to hypotension, cramps, oliguria, and uremia. Symptoms are often more severe in infants and adults over 60 years of age. Fatalities rarely exceed 1% of the affected population and are generally limited to infants, elderly, and debilitated individuals (Hanes, 2003). Nevertheless, *Salmonella* infection accounts for more foodborne deaths (31%) in the United States than any other foodborne pathogen (Mead et al., 1999). Furthermore, multi-drug-resistant *Salmonella* DT104 has been associated with double the hospitalization rate and ten times the case fatality rate of other foodborne *Salmonella* (Hanes, 2003).

The presence of viable salmonellae in the gastrointestinal tract indicates that the organism survived a variety of non-specific host defenses including lactoperoxidase in saliva, stomach acidity, mucous secretions from intestinal goblet cells, and sloughing of luminal epithelial cells. In addition, they must survive non-specific phagocytic cells, immune responses associated with specific T and B lymphocytes, Peyer's patches, and complement inactivation (D'Aoust, 1991). Once they have survived these conditions, they attach to intestinal tissues and mesenteric lymph follicles resulting in enterocolitis. Endotoxin is produced, leukocytes move into the infected tissues, and increased mucous secretion occurs. Mucosal inflammation results from the release of prostaglandins by leukocytes which also activates adenyl cyclase in intestinal epithelial cells causing increased fluid secretion into the intestinal lumen and resulting in diarrhea. Septicemia and other chronic conditions result when host defenses fail to keep these invasive *Salmonella* in check (D'Aoust, 1991).

2.2.4 Infectious Dose

The infectious dose appears to be very low as evidenced by some foods implicated in foodborne disease with only a few cells recovered. An example of this occurred in a 1994 frozen dessert-associated outbreak wherein the level of *Salmonella* serotype Enteritidis was reported to have a most probable number (MPN) range of 4 cells per 1,000 g to 46 cells per 100 g with a median of 93 cells in 1,000 g (Vought and Tatini, 1998). The 95% confidence interval for these MPN values was from < 1 cell per 1,000 g to 2.4 cells/g. The number of *Salmonella* serotype Enteritidis cells per serving was estimated at 25 cells. In this study, the infective dose appeared to be less than 28 cells. Evidence from other studies indicates that from 1 to 10 cells may constitute an infectious dose in some circumstances (D'Aoust et al., 1985 and Kapperud et al., 1990).

2.2.5 Reservoirs

Salmonellae are widespread in the natural environment and a number of these are host specific (e.g., *Salmonella* serotype Pullorum in chickens, *Salmonella* serotype Cholera-Suis in pigs). In many countries poultry remain the dominant reservoir, although pork, beef, and mutton have served as vehicles of infection. The eggborne pandemic of *Salmonella* serotype Enteritidis phage type 4 in Europe and phage type 8 in North America illustrates the importance of poultry products as vehicles of human salmonellosis.

2.2.6 Foods Associated with Human *Salmonella* spp. Infection

Salmonella spp. have a long history of food contamination and have caused illness from ingestion of a wide variety of foods. These organisms have been a particular concern with foods of animal origin (e.g., meat, poultry, eggs, and dairy products). Dry foods and fruit and vegetableborne outbreaks have also occurred. One multi-state cantaloupeborne outbreak affected more than 25,000 across 30 states (Ries et al., 1990).

Major outbreaks have occurred with chocolate, milk powder, potato salad, egg salad, raw milk, mustard dressing, salad base, cheddar cheese, liver pate, aspic glaze, pasteurized milk, egg drink, cuttlefish, cooked eggs, cantaloupes, fruit soup, mayonnaise, paprika chips, ice cream, and alfalfa sprouts (D'Aoust, 1997). In the 1994 frozen dessert-associated outbreak mentioned above, an estimated 224,000 people were infected with *Salmonella* serotype Enteritidis. The source of the organism was traced to contaminated post-pasteurized ice cream mix which had been shipped in tank trucks previously used to transport raw eggs (Hennessy et al., 1996).

2.2.7 Dry Foods

Dry products are not often associated with microbial contamination problems. However, salmonellae have been a particular concern with some dry foods and dry food production environments. Control of these organisms is a priority among industries that produce dried foods such as dry milk, infant formula, chocolate, dry soup mixes, and rendered animal proteins (an ingredient in animal feed and pet food). Human outbreaks of disease have been reported with dry milk, chocolate, and even paprika potato chips, dry cereal, and peanut butter (D'Aoust et al., 1975; Greenwood and Hopper, 1983; Kapperud et al., 1990; Lehmacher et al., 1995; Weissman et al., 1977; CDC, 2007, 2009). *It is important to note that simply because a microbe cannot grow in a low water activity food, it may still survive for some time.* Factors that influence microbial growth survival and death are discussed in Chapter 5.

2.2.8 Food Processing Environments

A wide variety of food factory environments may be contaminated with this microbe due to their widespread occurrence in the natural environment and likely presence in some raw ingredients which may enter factory environments. In the author's experience, birds, which are frequent carriers of this organism, may find roosts on factory roofs or roof-associated structures (e.g., air intakes for air handling units). Most food processing facilities have flat roofs and many are not adequately sloped to drains resulting in collection of standing water. Standing water on roof tops will permit the growth of salmonellae to high numbers. Entry of salmonellae in the factory environment may occur through inappropriately sealed roof top-associated penetrations. Other routes of entry are described in Chapter 4. In the author's experience, it is rare that the post-cook side of a factory is contaminated with more than one serotype of *Salmonella*. Most often the *Salmonella* serotype found on the finished side of the facility is its "signature" organism or "house-bug." It appears that each environment selects for the strain which has likely adapted to its environment. However, exceptions to this observation have been noted and the presence of multiple serotypes suggests multiple sources for the microbe. The author has observed sporadic detection of a specific serotype of *Salmonella* in some dry product processing environments for 10 or more years.

2.3 *L. monocytogenes*, an Infectious Invasive Agent

The very widespread (some say ubiquitous) distribution of this organism in the natural environment coupled with its resistance to freezing, growth in the presence of 10% salt, survival in concentrated brine solutions, and its ability to grow at 1–45°C (optimum at 35–37°C) makes control of this organism in the processing environment challenging. Eradication of this organism from ready-to-eat meat and poultry processing environments is unlikely given current technology (Tompkin et al., 1999). Hence, implementation of rigorous controls is essential to prevent processed food contamination.

2.3.1 The Organism

L. monocytogenes is a gram-positive, short (0.4–0.5×0.5–2 μm) non-spore-forming, rod-shaped, microaerobic bacterium that exhibits tumbling motility. The organism appears translucent with a "characteristic" blue-green sheen when observed under oblique lighting. However, some technicians are better than others at visualizing this phenomenon. It is typically weakly β-hemolytic on horse blood agar and exhibits a characteristic CAMP reaction on sheep blood agar when streaked perpendicularly to *S. aureus* (enhanced hemolysis) and *Rhodococcus equi* (hemolysis not enhanced). Other than *Listeria seeligeri*, the remaining *Listeria* spp.

Table 2.3 Key reactions of *Listeria*

Camp reaction (enhanced hemolysis)				
Species	<i>Staphylococcus</i> streak	<i>Rhodococcus equi</i> streak	Rhamnose	Xylose
<i>monocytogenes</i>	+	— ^a	+	—
<i>ivanovii</i>	—	+	—	+
<i>innocua</i>	—	—	Variable	—
<i>welshimeri</i>	—	—	Variable	+
<i>seeligeri</i>	+	—	—	+
<i>grayi</i> ^b	—	—	Variable	—

^aRare strains may show a region of enhanced hemolysis near both the *Staphylococcus* and the *Rhodococcus* streaks

^bThis species is mannitol positive

yield different CAMP reactions (Table 2.3). Typical *L. monocytogenes* isolates ferment rhamnose, dextrose, esculin, and maltose but not xylose and mannitol (Datta, 2003). The CAMP reaction and key biochemical reactions have been traditionally used to confirm cultures. However, numerous *Listeria* identification test kits are now available that greatly expedite this process without the use of the CAMP reaction.

2.3.2 Cost

Approximately 2,518 cases of foodborne listeriosis, including ~500 fatalities, occur annually in the United States at an estimated cost of \$2.3 billion, making listeriosis the second most costly foodborne illness after salmonellosis which some have estimated at 2.33 billion dollars (Mead et al., 1999; Buzby and Roberts, 1996). Consequently, foodborne listeriosis has been targeted by many public health programs, most notably Healthy People 2010 – a comprehensive nationwide health promotion and disease prevention program developed by the Department of Health and Human Services to reduce bacterial infections and enhance life expectancy/quality.

2.3.3 Disease Syndromes

Two types of listeriosis are recognized – (a) an invasive form that can be life-threatening in newborn infants, the elderly, and immunocompromised adults and (b) a less common self-limiting gastrointestinal illness. In the gastrointestinal form, flu-like symptoms (e.g., diarrhea, vomiting, fever) may occur 18–24 h after ingestion of the contaminated food. In contrast, invasive listeriosis has an onset time of 3 to as long as 70 days after which adults typically experience septicemia, meningitis, or endocarditis, whereas unborn fetuses develop abscesses in their liver, lungs,

and other organs that often result in spontaneous abortion and stillbirth. Surviving children may be seriously ill with meningitis and neurological impairment.

2.3.4 The Infectious Process

Once the bacterium enters the host's monocytes, macrophages, or polymorphonuclear leukocytes, it is bloodborne (septicemic) and can grow. Its intracellular presence with phagocytic cells also permits access to the brain and probably transplacental migration to the fetus in pregnant women. The pathogenesis of *L. monocytogenes* centers on its ability to survive and multiply in phagocytic host cells (FDA "Bad Bug Book" <http://vm.cfsan.fda.gov/~mow/chap6.html>).

2.3.5 Infectious Dose

The infectious dose of the organism, as with other pathogens, will be related to the virulence of the particular strain and the host's susceptibility. In fact there is an estimated 2,584-fold greater risk of Listeriosis among transplant patients than in healthy individuals under the age of 65 (ILSI, 2005).

Controversy exists about the infectious dose. It is clear that most Listeriosis results from ingestion of very high numbers of the organism with 82.9% of cases attributed to ingestion of foods with greater than 1×10^6 cfu, it remains unknown if there is a minimum level that can cause illness (FDA, USDA, 2003; <http://www.fda.gov/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/ucm183966.htm>). However, the ICMSF has reported that "Epidemiologic data indicate that foods involved in listeriosis outbreaks are those in which the organism has multiplied and in general have contained levels well in excess of 100 cfu/g and proposed that the concentration of *L. monocytogenes* in frankfurters not exceed 100 cfu/g at the time of consumption" (ICMSF Volume 7, 2002a, p. 294). Nevertheless because a single microbe has the potential to cause illness (likely in a very debilitated host). The US government has tolerance of negative in 25 g or 0.4 cfu/g.

In addition the 2003 FDA/USDA Listeria risk assessment (<http://www.fda.gov/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/ucm183966.htm>) indicated that up to 10 million-fold differences in risk between various foods. The highest risks were found for deli meats and frankfurters with very low risks found for cultured milk products, process cheese, hard cheese, ice cream, and other frozen dairy products.

A consequence of this risk assessment has been a proposed tolerance in selected ready-to-eat foods of <100 cfu/g *L. monocytogenes* (FDA, 2008a, b). Essentially these draft guidelines, if approved, will be for foods in which *L. monocytogenes* cannot grow due to low pH (≤ 4.4), low water activity (≤ 0.92), or formulated to prevent *L. monocytogenes* from growing. The documents recognize that frozen

foods will prevent *Listeria* growth and would be acceptable if they contain less than 100 cfu/g of *L. monocytogenes* if consumed in the frozen state. Hence, the proposed tolerance level may apply to ice cream that is consumed in the frozen state but may not apply to frozen items which must be thawed prior to consumption. Nevertheless non-exempt foods with less than 100 cfu/g made under insanitary conditions would still, likely, be subject to regulatory consequences.

2.3.6 Reservoirs and Implicated Foods

Listeria is widespread in the natural environment having been found in soil, water, sewage, decaying vegetation, humans, domestic animals (including pets), raw agricultural commodities, food processing environments, and the home (Ryser and Marth, 1999). The microbe has been found in a wide variety of foods including meats, poultry, dairy products, vegetables, and seafoods. In fact, invasive outbreaks of Listeriosis have been reported from consumption of a variety of products including raw milk, sour milk, cream, cottage cheese, pasteurized milk, cheese (blue-mold, hard, Brie de Meaux, Vacherin Mont d'Or, Mexican-style, Pont l'Eveque, raw milk cheese), butter, pork, pork tongue, delicatessen turkey meat, Pâte, processed meats, pork tongue in aspic (jelly), Rillettes, mousse, raw vegetables, coleslaw, shellfish, shrimp, and raw eggs (Norton and Braden, 2007).

Since 1998, over 130 recalls involving more than 80 million of pounds of ready-to-eat meat and poultry products were issued due to *Listeria* contamination with three of these recalls related to major outbreaks of listeriosis. Virtually all of these recalls have been attributed to post-processing contamination at the manufacturing facility.

2.3.7 Food Processing Environments

The extremely widespread nature of the organism in the environment, its psychrotrophic growth, resistance to other stress as compared to other non-spore-forming pathogens (e.g., freezing, salt, heat) makes it a particularly difficult microbe to control in a wide variety of food processing facilities. A review of the incidence and control of *Listeria* in food processing facilities can be found in Kornacki (2007).

2.4 *Campylobacter*, an Infectious Invasive Agent

Campylobacter species are enteric pathogens and are considered one of the leading foodborne disease agents in the United States causing an estimated 2.1–2.4 million cases of gastroenteritis annually (Altekruse et al., 1999).

2.4.1 The Organism

Campylobacter species are gram-negative, spiral-shaped rods and typically motile. The *Campylobacter* genus consists of 14 species. The most common foodborne species are *Campylobacter jejuni* and *Campylobacter coli*. Members of the genus are susceptible to environmental stresses and are considered to be relatively fragile. Because of these concerns, this organism can be difficult to isolate in the laboratory.

2.4.2 Costs

Like any foodborne disease agent, it can be difficult to measure the exact costs related to an outbreak, especially those related to pain and suffering, losses related to the reputation of the manufacturer, market share loss, but also lost jobs, and lawsuits. According to the United States Department of Agriculture, campylobacteriosis costs in the United States are estimated to be 1.2–6.6 billion dollars annually (Buzby and Roberts, 1996).

2.4.3 Disease Syndromes

The most common type of illness caused by *Campylobacter* spp. is gastroenteritis referred to as campylobacteriosis. Enteric symptoms are caused by a thermolabile toxin (CJT) (Ray, 1996). This toxin is similar to cholera toxin and the LT toxin of enterotoxigenic *E. coli* (Smith, 1995). Symptoms of campylobacteriosis include diarrhea, abdominal pain or cramps, headache, muscle pain, and fever (Jay et al., 2005). Diarrhea may also be bloody. The onset of symptoms typically occur within 2–5 days of exposure and illness usually lasts 7–10 days, with relapses occurring in 25% of cases (US FDA, 2009). Infections are usually self-limiting, and treatments typically include fluid and electrolyte replacement. Antibiotic treatments may be used in severe cases (Altekruse et al., 1999). Most common groups afflicted include children less than 5 years of age and young adults 15–29 years. The fatality rate is approximately 0.1%. Over the last several years, it has been shown that males are afflicted more often than females with campylobacteriosis (Franco and Williams, 1994; CDC, 2004).

A serious neurological complication called Guillain–Barré syndrome (GBS) can occur in a small percentage of patients following infection with some *C. jejuni* strains. GBS is an acute inflammation of peripheral nerves. GBS may cause fever, pain, and weakness and can also lead to paralysis (Keener et al., 2004). *C. jejuni* serovar O:19 and serotypes associated with Guillain–Barré syndrome are considered by the International Commission on Microbiological Specifications for Foods (ICMSF) under the category “Severe Hazard-Restricted Populations” (ICMSF, 2002a).

It is estimated that about a quarter of GBS patients experienced a recent *C. jejuni* infection in the months preceding onset of GBS symptoms. Most GBS patients recover fully; however, about 20% continue to suffer varying degrees of disability (Hughes and Cornblath, 2005), and 3–8% die (Smith, 1995).

There is an increasing rate of antibiotic-resistant *Campylobacter*. The rate is highest in the developing world. Resistance to ciprofloxacin, azithromycin, and fluoroquinolone has been noted (Altekruse et al., 1999).

2.4.4 Infectious Dose

The infectious dose of *Campylobacter* has been shown to be low. Levels at or below 500 organisms have been shown to cause illness (Keener et al., 2004; Smith, 1995). *C. jejuni* accounts for approximately 99% of cases (FDA), even though other species can cause human illness.

2.4.5 Reservoirs

The primary ecological niche for campylobacters is the intestinal tract of warm-blooded animals. Campylobacters replicate almost exclusively within these hosts (Ketley, 1997). The reservoir for infection comprises a wide variety of both wild and domestic animals (Skelly and Weinstein, 2003). *C. jejuni* is commonly found in the intestinal tract of birds, cattle, and sheep; whereas, *C. coli* is most often associated with pigs (Doyle, 1944). Domestic pets such as dogs and cats can serve as important reservoirs for campylobacter with reported isolation rates as high as 66% in healthy cats and 34% in healthy dogs (Moreno et al., 1993). Other species such as rodents, flies, and insects may also harbor campylobacters and may serve as vectors of disease for other hosts. Many avian species have been associated with high rates of colonization of *C. jejuni* including both wild and domestic birds.

Campylobacters are frequently isolated from the natural environment typically as a result of fecal contamination from colonized animal hosts. Campylobacters have been isolated from streams, rivers, lakes, sea water, and estuaries that have been tainted with fecal contamination from wild or domestic animals (Knill et al., 1982).

2.4.6 Foods Associated with Campylobacter

Campylobacter is most frequently associated with foods of animal origin. Any raw meat from an animal bred for consumption may be contaminated with campylobacters (Butzler, 2004). Raw poultry meat is usually contaminated with *Campylobacter* spp. and there is sufficient epidemiological evidence to suggest that poultry meat serves as a primary source of *Campylobacter* infection in humans (Sahin et al., 2002). The prevalence of *Campylobacter* on raw meat products from other food

animals tends to be lower than that for poultry (Murphy et al., 2006). Beef, pork, lamb, seafood, and shellfish have all been implicated in cases of campylobacteriosis (Kramer et al., 2000; Wilson and Moore, 1996). Campylobacters are not usually associated with vegetables, although they have been detected in spinach, lettuce, radish, green onions, parsley, and potatoes (Park and Sanders, 1992) and some cases of human infection from consumption of contaminated vegetables have been described (Jacobs-Reitsma, 2000). The epidemiology of human campylobacter infection is generally more often associated with numerous, sporadic small-scale infections than large-scale outbreaks. For example, in a study among university students in Georgia, 70% of campylobacter infections could be attributed to eating undercooked chicken (Tauxe, 1992). Poor kitchen hygiene may also play a role: cross-contamination events during handling of contaminated fresh chicken parts have been shown to be a risk factor for infection and illness (Luber et al., 2006). When large outbreaks occur they are most often waterborne disease outbreaks (Sacks et al., 1986; Millson et al., 1991) or are associated with consumption of raw milk or milk that has been contaminated post-pasteurization (Jones et al., 1981; Morgan et al., 1994; Riordan et al., 1993).

2.4.7 *Campylobacter and Poultry*

The strong association between campylobacter and poultry warrants further discussion of specific concerns in the poultry production and processing environments. *C. jejuni* has been isolated from the reproductive tracts of broiler breeder hens (Hiett et al., 2002) and there is some recent evidence supporting vertical transmission (Cox et al., 2002); the significance of this source for the contamination of chicken flocks is a point of conjecture (Callicott et al., 2006). *C. jejuni* is not usually isolated from the production environment during the first 2 weeks after the chicks are placed. However, by the third or fourth week of production most flocks are contaminated and the pathogen generally spreads rapidly through most members of the flock (Stern and Line, 2000). The intestinal tract of the chickens may harbor up to 10^7 cfu *C. jejuni* g⁻¹ with no apparent harm to the host (Stern et al., 1988). The stresses associated with cooping and transport of chickens from the farm to the processing facility and holding the birds prior to processing have been demonstrated to increase *Campylobacter* populations on the birds (Stern et al., 1995). During processing, the poultry carcasses may become contaminated with intestinal contents and as a result most raw poultry products are contaminated with *C. jejuni* (Stern, 1992).

To significantly reduce the load of campylobacters entering the poultry processing facility, intervention on the farm during production is required. Carryover of the same strain of *Campylobacter* from flock to subsequent flock reared in the same house is thought to be a relatively infrequent event (Shreeve et al., 2002). Potential on-farm intervention strategies employing increased biosecurity measures, litter treatment, acidified feed, probiotics, and competitive exclusion products have

met with mixed success (Wagenaar et al., 2006). Likewise vaccines have been developed for *Campylobacter* but have not proven completely efficacious. *Campylobacter* has been demonstrated to be able to form biofilms on a variety of surfaces (Joshua et al., 2006) which increase its chances of survival in the poultry production environment (Trachoo et al., 2002) and emphasizes the need for proper sanitization of water lines. Novel intervention strategies employing bacteriocins or bacteriophage may be useful in the future; however, they are not yet commercially available (Joerger, 2003; Stern et al., 2006). Effective intervention on the farm will likely require a multifaceted approach and it is unlikely that there will be any one “silver bullet” approach to eliminate *Campylobacter*.

In the poultry processing facility there are many critical control points which have been identified to reduce contamination. These include washer and product temperature controls, chemical interventions (including chlorine and trisodium phosphate among others), water replacements and counter-flow technology in the scalding and chiller, and equipment maintenance (White et al., 1997). In some European countries the concept of “scheduled processing” or keeping colonized and non-colonized flock separate during processing is seen as a promising control strategy (Wagenaar et al., 2006), but this may not be applicable to processing conditions in the United States where prevalence of *Campylobacter* in broiler flocks is very high. Freezing of contaminated poultry can also be utilized to significantly reduce *Campylobacter* populations as was recently demonstrated in Iceland (Georgsson et al., 2006); however, this is not necessarily a cost-effective strategy in the US market.

Unlike other foodborne pathogens, *Campylobacter* does not grow effectively in the environment or under normal food storage conditions (Park, 2002). There are a number of techniques to control *Campylobacter* spp. in foods including physical treatments such as heat, cold, dehydration, hydrostatic pressure, and irradiation (Alter and Scherer, 2006). Heat treatment processes designed to kill *Salmonella* and *Listeria* will eradicate *Campylobacter* spp. in similar food matrices as well (Moore and Madden, 2000). A terminal pasteurization step (e.g., irradiation or heat pasteurization) applied under controlled conditions at the processing plant may be the best means currently available for reducing campylobacteriosis (Stern and Line, 2000). *Campylobacter* are also much less tolerant to osmotic stress than other foodborne pathogens (Doyle and Roman, 1982). *Salmonella* and *Listeria* will grow in sodium chloride concentrations of 4.5 and 10%, respectively, whereas *Campylobacter* strains are not able to grow in the presence of 2% sodium chloride (Alter and Scherer, 2006). Because campylobacters are microaerobic, they are injured by oxidative stress and have an inherent sensitivity toward oxygen (Park, 2002). High hydrostatic pressure can also be used to reduce populations of campylobacters. Solomon and Hoover (2004) demonstrated that *C. jejuni* populations in inoculated milk or chicken puree could be reduced by 2–3 log units at 300–325 MPa, while treatment at 400 MPa completely inactivated the pathogen. Campylobacters are also sensitive to pH with a pH value above 9.0 or below 4.0 leading to rapid decreases in populations (Gill and Harris, 1983). Campylobacters are susceptible to most routinely used disinfectants including chlorine (Wang et al., 1983). However, the effectiveness of chlorinated water is reduced when the organisms are attached

to organic matter such as chicken carcasses (Alter and Scherer, 2006). Trachoo and Frank (2002) demonstrated that chlorine was the most effective sanitizer for inactivation of *Campylobacter* in biofilms. *Campylobacter* may not be the best choice for monitoring in processing plant environmental samples as they are typically more fragile than other foodborne pathogens or indicator organisms and should not survive a thorough cleaning and sanitization regimen appropriate for removal of more fastidious microorganisms.

2.5 *Staphylococcus*, a Toxigenic Pathogen

S. aureus is a common bacterial pathogen causing staphylococcal food poisoning (SFP) – a leading cause of foodborne intoxication worldwide – and accounts for an estimated 14% of all foodborne illnesses in the United States. SFP is not attributed to ingestion of live bacterial cells but rather acquired from ingesting one or more heat-stable pre-formed staphylococcal enterotoxins (SEs) in foods contaminated with enterotoxin producing strains of staphylococci, principally, *S. aureus*. This type of food poisoning is classified as an intoxication since it does not require growth of the bacterium in the host. Indeed, numerous outbreaks have been caused by foods in which the organism has been killed but the heat-stable toxin remained. SEs are unique because they are not destroyed by heating including canning.

2.5.1 *The Organism*

Staphylococci belong to the family Micrococcaceae. They are gram-positive spherical bacteria about 1 μm in diameter that appear as grape-like clusters under the microscope. The grape-like configuration of staphylococci helps to distinguish staphylococci from streptococci that usually form chains because they divide in one plane only. Staphylococci are catalase-positive, oxidase-negative, facultative anaerobes that grow by aerobic respiration or fermentatively with the principal end product being lactic acid. The catalase test is important in distinguishing streptococci (catalase negative) from staphylococci, which are strong catalase producers.

In 1884, Rosenbach described the two pigmented colony types of staphylococci and proposed the appropriate nomenclature: *S. aureus* (yellow) and *Staphylococcus albus* (white). The latter species was named *Staphylococcus epidermidis* in 1908. Thirty-one *Staphylococcus* spp. are currently recognized of which 15 are human pathogens. Among these, *S. aureus* and *S. epidermidis* are the most significant in their interactions with humans.

S. aureus forms a fairly large yellow colony on rich media. It is often β -hemolytic on blood agar, producing a strong zone of lysis. *S. aureus* grows in a temperature range from 15 to 45°C and at NaCl concentrations as high as 16%. It does not survive legal milk pasteurization. Nearly all strains of *S. aureus* produce the enzyme coagulase (an enzyme closely associated with toxin

producing strains). However, some coagulase negative staphylococcal strains have been isolated that also produce SET. *S. aureus* should always be considered as a potential pathogen with multi-antibiotic-resistant strains of this organism also a leading cause of post-operative infections in hospitals. Unlike *S. aureus*, *S. epidermidis* produces relatively small white colony and is non-hemolytic with nearly all strains lacking the enzyme coagulase. *S. epidermidis* is generally regarded as being non-pathogenic; however, a few strains have been linked to infections in hospital environments. Multiple antibiotic resistance characterized by resistance to methicillin is increasingly common in both *S. aureus* and *S. epidermidis*. Methicillin-resistant *S. aureus* (MRSA) causes outbreaks in hospitals and is frequently endemic in hospital environments.

2.5.2 *Staphylococcal Enterotoxin*

The current classification of staphylococcal enterotoxins (SEs) is based on antigenicity with each enterotoxin designated by letter in order of discovery. Twelve SEs have been thus far identified and include SE A, B, C₁, C₂, C₃, D, E, F, G, H, I, and J. Initially, the designation “SEF” was used to refer to an exotoxin commonly produced by isolates of *S. aureus* associated with toxin shock syndrome (TSS). However, this toxin was later designated TSS toxin 1 when it was confirmed that SEF was not emetic. The relative incidence of SEs produced by isolates of *S. aureus* varies. In general, SEA is most common having been implicated in more than 80% of all outbreaks of staphylococcal food poisoning. SED is the second most common and has been most frequently associated with egg and fish products. Few outbreaks have been traced to SEE (Jablonski and Bohach, 2001).

Production of SEs is favored at optimal growth temperature, a_w , and pH with *S. aureus* producing less or no SE under suboptimal growth conditions. SEs can be produced at 10–46.6°C with 40–45°C being best. The minimum a_w for SE production is 0.90 whereas *S. aureus* can grow at a_w values as low as 0.84. SE production is favored at pH 5.2–9.0 (optimum 6.5–7.5), whereas the range for the growth is from 4.3 to 9.4.

The SEs are quite heat resistant with SEB retaining biological activity after 16 h of heating at 60°C/pH 7.3. Furthermore, no change in serological reactivity was found in SEC after 30 min of heating at 60°C. Heating of SEA at 80°C for 3 min or at 100°C for 1 min led to a loss in serological reactivity. Retorted canned mushrooms imported from China in 1989 were shown to be contaminated with heat-stable staphylococcal enterotoxin (SET); (http://findarticles.com/p/articles/mi_m1370/is_n7_v23/ai_8017475; <http://www.cdc.gov/mmwr/preview/mmwrhtml/00001410.htm>, Brunner and Wong, 1992). SET has been reported to have a $D_{250^\circ\text{F}}$ value of about 20 min (David et al., 1996). SEs are also resistant to gamma irradiation with about 33% of SEA remaining active in a meat slurry after exposure to 8 kGy. All SEs except SEB are resistant to pepsin at pH 2.0 and therefore survive passage through the stomach.

2.5.3 Cost

S. aureus in food causes an estimated 1,513,000 cases of illnesses and 1,210 deaths annually in the United States. Costs associated with *S. aureus* infections and intoxications are estimated at \$6.8 billion from all sources of which \$1.2 billion in expenses is attributed to foodborne sources.

2.5.4 Disease Syndromes

In humans, *S. aureus* causes various suppurative (pus-forming) infections including superficial skin lesions, boils, styes, and furunculosis as well as more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis. *S. aureus* is a major cause of hospital-acquired post-operative infections and infections associated with indwelling catheters and other medical devices. *S. aureus* causes food poisoning by releasing enterotoxins into the food and toxic shock syndrome by release of superantigens into the bloodstream.

The onset of symptoms in staphylococcal food poisoning is usually rapid, typically within 1–6 h, and is influenced by individual susceptibility to the toxin, amount of contaminated food consumed, amount of toxin in the food ingested, and general health status. The most common symptoms are nausea, vomiting, abdominal cramping, diarrhea, sweating, headache, prostration, and sometimes a fall in body temperature. Some individuals may not exhibit all of these symptoms. In more severe cases, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur. Most individuals recover on their own within 2 days, but symptoms may persist for 3 or more days in severe cases. The usual treatment for healthy persons consists of bed resting and fluid replacement to counteract accompanying dehydration. Non-hospital-acquired infections can usually be treated with penicillinase-resistant β -lactams. However, hospital-acquired infections are most often caused by antibiotic-resistant strains and can only be treated with vancomycin.

2.5.5 Toxic Dose

Many factors contribute to the development of SFP and the degree of severity including susceptibility of the individual to SE, the total amount of food/toxin ingested, the type of toxin, and the overall health of the infected person with children and elderly individuals being more susceptible. SEB causes more severe symptoms than SEA as is now considered a potential bioterrorism threat if inhaled. Despite these differences, a basal level of approximately 1 ng of SE per gram of contaminated food is sufficient to induce symptoms of SFP. This toxin level is reached when *S. aureus* populations exceed 10^5 cfu/g in contaminated food.

2.5.6 Reservoirs

Staphylococci are commonly found in air, dust, sewage, water, milk, and food as well as on food contact surfaces and equipment. Humans and animals are the primary reservoirs for staphylococci with these organisms isolated from the nasal passage, throat, hair, and skin of more than 50% of healthy individuals. Higher isolation rates are common among those who associate with or who come in contact with sick individuals and hospital environments. Equipment and environmental surfaces can also serve as sources of *S. aureus*, although food handlers are the primary source of contamination in outbreaks of SFP. SFP is caused by ingesting enterotoxins produced in food by certain strains of *S. aureus*, usually because the food has not been kept sufficiently hot ($\geq 60^{\circ}\text{C}$, 140°F) or cold ($\leq 7.2^{\circ}\text{C}$, 45°F).

Most SFP outbreaks are caused by food contamination during processing, preparation, and packaging. *S. aureus* is commonly found in the nose, mouth, and throat of humans and transmission to foods may occur via purulent discharges such as from an infected finger or even from normal skin since 30–50% of all healthy individuals harbor the organism and 15–35% are persistent carriers. This is one reason why individuals with open sores or infected cuts should not handle food.

S. aureus competes poorly with the native microflora in most raw foods; however, products containing higher levels of salt provide *S. aureus* with a competitive edge. Consequently, products most frequently incriminated in SFP outbreaks are cooked or otherwise processed high-protein foods that have come in contact with worker's hands and then were either served after being temperature abused or served after improper heating/refrigerated storage. Foods most frequently implicated in outbreaks include meat and meat products (particularly ham due to the high salt content); poultry and egg products; salads such as egg, tuna, chicken, potato, and macaroni; bakery goods such as cream-filled pastries, cream pies, and chocolate eclairs; sandwich fillings; and milk and dairy products. Foods that require considerable handling during preparation and that are kept at slightly elevated temperatures after preparation are most frequently involved in staphylococcal food poisoning. Hence, adherence to stringent hand washing and sanitation practices in food preparation areas along with proper storage temperatures is critical in minimizing contamination along with subsequent growth of *S. aureus* to potentially toxic levels.

2.6 *B. cereus*, a Toxigenic Pathogen

2.6.1 The Organism

B. cereus is a gram-positive, spore-forming rod-shaped microorganism. It is closely related to *Bacillus anthracis*, a serious human and animal pathogen and to *Bacillus thuringiensis*, an insect pathogen (Helgason et al., 2000; Erickson and Kornacki, 2003). Together with *B. cereus* variety *mycoides*, these four form the *B. cereus* group

of organisms (Bennett and Belay, 2001). The spores of *B. cereus* can survive most cooking processes. Mainly prolific as an aerobic vegetative cell, survival in anaerobic conditions is also possible. Some psychrotrophic strains are known (Bennett and Belay, 2001). Germination of spores to high populations of viable cells may produce a toxin in foods or in human intestines, causing gastrointestinal illness. *B. cereus* enterotoxin has also been associated with endophthalmitis (eye infection) (Wong, 2001).

2.6.2 Factors Related to Cost

The CDC has estimated that 27,360 cases of *B. cereus*-induced foodborne illness occur each year in the United States (Mead et al., 1999). However, the number of reported incidents of *B. cereus* foodborne illness ranges from 6 to 50 incidents per year and most of these data are from cases associated with outbreaks. Active surveillance for *B. cereus*-induced illness is not done in the United States. The illness is self-limiting and usually not severe. Furthermore, not all state public health laboratories routinely test for *B. cereus*. People may thus become ill and not consult a doctor due to the relatively mild symptoms with the result being under reporting of the actual number of illnesses (MMWR, 1994). Thus, many sporadic cases go undetected. Consequently, the financial costs are difficult to estimate.

2.6.3 Symptoms

B. cereus strains can produce two types of foodborne illness: diarrheal and emetic. The diarrheal illness is often associated with meat products, soups, potatoes and other starchy vegetables, puddings, and sauces. Onset times may occur 8–16 h after ingestion of food containing the microorganisms and/or toxin. Abdominal pain, diarrhea, and possibly nausea and vomiting may ensue. Illness usually lasts for 12–14 h and complications are rare. Tripartite hemolysin BL has been identified as a diarrheal toxin while cereulide is known as an emetic toxin. These two are the only specifically identified *B. cereus* toxins to date (Schoeni and Wong, 2005). The emetic illness may result in diarrhea and abdominal cramps but is most often characterized by nausea and vomiting. The onset of symptoms may occur only 1–5 h after ingestion and symptoms may continue for 6–24 h. Rice dishes and pasta products held at improper temperatures and allowed to cool slowly are often associated with this type of illness.

Cereulide is known to survive autoclave treatments (Lui, 2001) which are similar to some retort processes. In fact, a document entitled, *B. Cereus* at <http://www.nzfsa.govt.nz/science/data-sheets/bacillus-cereus.pdf> cites a report of Jenson and Moir (1997) who indicate that the emetic toxin of *B. cereus* can survive 90 min at 126°C.

2.6.4 Infectious Dose

B. cereus can produce both heat-stable and heat-labile toxins. However, a non-biological assay does not yet exist for the emetic toxin nor has the FDA recommended use of the commercially available kits for detection of the diarrheal toxin until further validations are done (Rhodehammel and Harmon, 2001). The number of viable cells generated by the species is the best way to estimate their toxicity. *B. cereus* intoxication usually requires high cell numbers ($>10^5$ cfu/g) in healthy adults. Methods for recovery of the microbes can be found in the FDA Bacteriological Manual and the Compendium of Methods for the Microbiological Examination of Foods, among others.

2.6.5 Reservoirs

B. cereus is widely distributed in soil, vegetation, and a variety of foods. It is present in the intestinal flora of about 10% of healthy adults and can be found in dairy products, meats, spices, dried products, and cereals, particularly rice (Hammack et al., 1990).

2.6.6 Foods Associated with *B. cereus*

Fried rice is a common source of illness caused by *B. cereus*. It is frequently present in uncooked rice, their heat-resistant spores can survive and germinate after cooking and a heat-stable enterotoxin may be produced that can survive further heating such as stir frying. *B. cereus* food poisoning associated with fried rice was the cause of two outbreaks at child day care centers in Virginia in 1993 (MMWR, 1994). Toxin production is enhanced by the presence of protein such as eggs or meat. Foods with high fat content may also have a protective effect. Therefore, the enterotoxin may be present in the food or it may be produced after ingestion within the small intestine.

2.6.7 Food Processing Environments

B. cereus is ubiquitous in soil and raw vegetables and therefore should be expected to be present in the environments of many food production facilities. The production of stress-resistant spores may make this microorganism difficult to control in a factory environment. Kornacki isolated the organism from a food service refrigerator where it was the apparent source of a foodborne illness and from a salad production environment during the course of a food factory risk assessment (Kornacki, 2009, Personal Communication). The first recorded outbreak of foodborne disease from the consumption of raw, sprouted seeds was in 1973 and this was from soy, mustard, and cress grown in home-sprouting packs which were contaminated with

B. cereus (Food Safety Australia, November, 2000). As stated earlier, most human gastrointestinal disease attributed to *B. cereus* intoxication has been traced to improper preparation and storage of foods. Delay in the cooling process due to voluminous containers is often a factor. It is advisable to hasten the cooling process by distribution to smaller containers and immediate refrigeration. For further information a recent review article can be referenced (Schoeni and Wong, 2005).

2.7 *C. botulinum*, a Toxigenic Pathogen

2.7.1 *The Organism*

C. botulinum, a gram-positive, anaerobic, rod-shaped bacterium, consists of four physiological diverse groups (groups I–IV) that share the common feature of producing the extremely potent botulinum neurotoxins. These neurotoxins are in turn differentiated on the basis of their serological reaction and are classified as types A–G. Foodborne botulism is an intoxication involving the consumption of food containing botulinal toxin produced during the growth of these organisms in food. Groups responsible for foodborne botulism in humans include group I (all type A strains and proteolytic strains of types B and F) and group II (all type E strains and nonproteolytic strains of types B and F) whereas groups III (strains C and D) and IV (strain G) affect primarily non-human animal hosts.

2.7.2 *Disease Incidence and Syndrome*

Foodborne botulism is a severe but rare disease. In the United States, there were 444 foodborne botulism outbreaks reported from 1950 to 1996 (CDC, 1998). Higher incidences have been reported in countries (Poland and Russia) where economic conditions have contributed to an increased reliance on home bottling/canning of foods. In the United States, it has been estimated that the cost per case of botulism is approximately \$30 million compared to *L. monocytogenes* or *Salmonella* with an average cost per case of \$10,000–12,000 (Setlow and Johnson, 1997).

Botulinal intoxication can range from a mild illness, that may be disregarded or misdiagnosed, to a serious disease that can be fatal within 24 h. Rapidity of onset and severity of disease depend on the rate and amount of toxin absorbed with roughly half the annual cases of foodborne botulism being attributed to type A strains and types B and E responsible for the remaining cases. Lethal doses of type A botulinal neurotoxin for humans (1 µg/kg) have been estimated from primate data; however, type A is considered more lethal than types B and E (Shapiro et al., 1998). Relative ease of passage through the intestinal wall affects toxicities. Following absorption from the gastrointestinal tract, the water-soluble neurotoxic proteins (zinc-containing endopeptidases) are carried by the bloodstream and irreversibly bound to peripheral nerve endings where the release of acetylcholine is

blocked. Signs and symptoms of botulism develop 12–72 h after consumption of the toxin-containing food and include nausea, vomiting, fatigue, dizziness, headache, dryness of skin, mouth and throat, constipation, paralysis of muscles, double vision, and difficulty breathing. Paralysis of the respiratory muscles can result in death if not treated, although the mortality rate is less than 10%. Treatment includes administration of equine antitoxin and supportive care with up to 95% of patients requiring hospitalization and 62% of patients needing mechanical ventilation. Recovery may take weeks to months (Shapiro et al., 1998).

2.7.3 Reservoirs and Prevalence in Foods

C. botulinum is widely dispersed in the environment (soils, sediments, and the gastrointestinal tracts of animals) by virtue of their ability to produce resistant endospores; however, the spore load as well as the predominant type varies with the geographic region. In the United States, the spores of type A are found most commonly west of the Rocky Mountains and the neurotoxin of this type accounts for 85% of foodborne outbreaks west of the Mississippi. In the Eastern United States, type B spores are most prevalent and consequently there has been a 60% incidence in type B outbreaks east of the Mississippi River. Implicated foods in the United States are vegetables, particularly “low-acid” vegetables such as beans, peppers, carrots, and corn; however, where once outbreaks were most commonly associated with home-preserved foods, non-preserved foods and public eating places have become more often involved. In Canada and Alaska, most foodborne outbreaks have resulted from type E toxin and have been associated with native and Eskimo foods. Similarly in Russia and Japan, neurotoxin type E contaminated pickled and home-preserved fish have been the leading vehicles because the principal habitat of type E spores is freshwater and brackish marine habitats. In European countries such as Poland, France, Germany, Hungary, Portugal, the former Czechoslovakia, and Belgium, the foods most often implicated have been home-preserved meats such as ham, fermented sausages, and canned products, and the predominant type has been B. Whereas European type B strains are predominantly nonproteolytic, American type B strains have been predominantly proteolytic.

2.7.4 Physiological Characteristics of *C. botulinum*

Due to the presence of exogenous proteases, protein breakdown products, in addition to sugars, may be used for growth by proteolytic *C. botulinum*. Under these conditions, off-odors are produced concurrently with protein breakdown that fortunately serve to alert the consumer that the product is spoiled. In contrast, spoilage odors may not be present in foods where growth of non-proteolytic strains of *C. botulinum* has occurred (Lynt et al., 1975).

In addition to growth substrates, proteolytic and nonproteolytic strains of *C. botulinum* also differ in their minimum and optimal growth temperatures. In the case of proteolytic strains, the optimal temperature for growth is 37°C, with growth occurring between 10 and 48°C. Nonproteolytic strains, however, have a lower optimal growth temperature (30°C) but more importantly may grow at temperatures as low as 3°C (Graham et al., 1997). Consequently, there has been considerable concern raised that nonproteolytic organisms may grow and produce toxin in refrigerated foods that receive minimal processing and have extended shelf lives.

Tolerance to pH, salt, and water activity by the vegetative cells also differ between the proteolytic and nonproteolytic strains. Conditions that favor growth of proteolytic strains include low acid (pH above 4.6), low salt (below 10%), and relatively high moisture (a_w above 0.94). Growth of nonproteolytic strains, on the other hand, requires higher pH (above 5.0) and moisture (a_w above 0.97), but lower salt (above 5%) contents (Kim and Foegeding, 1993).

In addition to the differences in tolerance of vegetative cells to environmental conditions, differences in spore resistance exist between the *C. botulinum* groups. $D_{100^\circ\text{C}}$ values for spores from proteolytic and nonproteolytic strains are approximately 25 min and less than 0.1 min, respectively. Consequently, the high heat resistance by spores of proteolytic strains represents a major concern in the processing of low-acid canned foods.

Botulinum toxin synthesis and activation is a complex process that is highly regulated by nutritional and environmental conditions. In general, toxin production onsets during late log and early stationary phases; however, toxin production varies among strains (Bradshaw et al., 2004).

2.7.5 Detection of *C. botulinum* Neurotoxins

To date, the mouse bioassay remains the only validated method for food analysis of botulinal neurotoxins. In this assay, the toxin (minimum detection limit, 0.03 ng) is detected by injection of a food extract into mice, which are then observed for characteristic symptoms of botulism and ultimately death over a 48-h period. In vitro assays, such as the standard enzyme-linked immunosorbent assay, often lack specificity and exhibit poor sensitivity compared to the mouse bioassay (Sharma and Whiting, 2005).

2.7.6 Control Treatments

Control of *C. botulinum* in foods may be exerted at three levels: inactivation of *C. botulinum* spores or inhibition of germination; inhibition of *C. botulinum* growth and toxin formation; and inactivation of *C. botulinum* neurotoxin. Thermal processing is the most common method used to inactivate spores of *C. botulinum* with the most heat-resistant spores (group I) having $D_{121^\circ\text{C}}$ values of 0.1–0.2 min and

therefore serving as the target organism. It is important to be aware that thermal destruction of *C. botulinum* spores does not follow first-order kinetics, indicating that some spores are more heat resistant than others (Peleg and Cole, 2000). Moreover, heat resistance of spores is greater at higher pH values (Mafart et al., 2001) and fat contents (Molin and Snygg, 1967) and lower sodium chloride concentrations (Juneja and Eblen, 1995) and water activities (Murrell and Scott, 1966). Spores are of particular concern in the commercial stabilization of canned low-acid foods, hence the canning industry has adopted a 12-D process as the minimum thermoprocess (Hauschild, 1989). For the less heat-resistant spores of group II strains, moderate temperatures (40–50°C) may be combined with high pressures of up to 827 mPa (Reddy et al., 1999). Lower heat treatments (pasteurization) in combination with other control measures, such as refrigeration, are used for perishable vacuum-packaged foods. Avoidance of conditions or compositions that increase spore germination may also be considered in control of *C. botulinum* in foods. For example, germination is similar in aerobic and anaerobic conditions and will occur from 1 to 40°C but not at 50°C (Plowman and Peck, 2002). Furthermore, formulating products with ingredients that lack L-alanine and L-lactate (essential germinants) or sodium bicarbonate and sodium thioglycollate (accelerants of germination) is desirable (Plowman and Peck, 2002). Such a case was demonstrated in potato and broccoli purees whose lag times for growth of *C. botulinum* were much longer than seen with mushroom purees (Braconnier et al., 2003).

Targeting the primary factors that control growth of *C. botulinum*, temperature, pH, water activity, redox potential, and oxygen level have been used for control of this pathogen in foods. The critical level of oxygen that will permit growth of proteolytic *C. botulinum* is 1–2% but this level depends on other conditions such as a_w , pH, or redox potential (Kim and Foegeding, 1993; Johnson, 1999). When control by these parameters is inadequate, inhibitory substances (nitrites, sorbates, parabens, nisin, phenolic antioxidants, polyphosphates, ascorbates, EDTA, metabisulfite, *n*-monoalkyl maleates and fumarates, and lactate salts) may be added to the food system (Kim and Foegeding, 1993). Efficacy of these antimicrobials added to or found naturally in foods, however, may be reduced by fat (Glass and Johnson, 2004). Growth of competitive and growth-promoting microorganisms in foods, on the other hand, has a very significant effect on the fate of *C. botulinum*. While acid-tolerant molds can provide an environment that enhances the growth of *C. botulinum*, lactic acid bacteria can inhibit growth of *C. botulinum* largely not only by reducing the pH but also by the production of bacteriocins (Rodgers et al., 2003). Ideally, an appropriate degree of protection against growth and toxin production by *C. botulinum* would employ multiple barriers. Irregardless, challenging foods with spores of *C. botulinum* to determine whether toxin is produced in optimal conditions or during temperature abuse is desirable to evaluate the safety of that food.

Although only minute amounts of botulinal neurotoxin need be present to observe a toxic response, protective measures for inactivation of the agents could ensure that they do not reach those toxic doses. In addition to high sensitivity to temperatures above 55°C, botulinal neurotoxins are sensitive to protease activity and oxidizing agents (i.e., chlorine) (Siegel, 1993).

2.8 Enterohemorrhagic *E. coli*, a Toxicoinfectious Pathogen

E. coli O157:H7 and other enterohemorrhagic *E. coli* produce a toxin(s) after it implants in the colon and colonizes it resulting in illness. Pre-formed toxins have not been shown to occur in foods or cause human disease. Hence this organism is considered to be “toxicoinfectious” agent in this chapter, as opposed to an invasive pathogen (such as *Salmonella* spp.). However, some evidence for an invasive mechanism has been reported (Doyle et al., 1997). It is a difficult organism to manage from a public health standpoint, because of its low infectious dose which may be, in part, related to its substantial acid tolerance and ability to survive low pHs sometimes found in the stomach.

The CDC estimates that *E. coli* O157:H7 sickens over 73,000 individuals per year. Despite the relatively low number of illnesses compared to other foodborne microbes, there are an estimated 2,100 hospitalizations and 61 deaths (CDC), putting it fourth in the number of annual foodborne illness related deaths below non-typhoidal *Salmonella* (553 deaths), *L. monocytogenes* (499 deaths), and *Campylobacter* (99 deaths) (CDC; Mead et al, 1999).

2.8.1 The Organism

E. coli is a gram-negative, non-spore-forming short rod-shaped bacterium capable of growth and gas production at 45.5°C (except when testing water, shellfish, and shellfish harvest water, which use 44.5°C) in lactose-containing medium which also exhibit the characteristic biotype 1 (+,+,−,−) or biotype II (−,+,−,−) standing for positive “+” or negative “−” reactions on Indole, Methyl Red, Voges-Proskauer, and Citrate (IMViC) reactions, respectively (Kornacki and Johnson, 2001). Most *E. coli* strains are harmless inhabitants of the gastrointestinal tract of man and animals. However, several foodborne pathogenic strains of *E. coli* are known to exist (Kornacki and Marth, 1982, Doyle et al., 1997b). In 1982 a particularly severe strain, with the “157” O-antigen and the “7” H-antigen, was isolated from clinical samples of individuals with gastrointestinal illness associated with the consumption of undercooked hamburgers from two fast food restaurants in Oregon and Michigan in which over 700 persons in four states were infected including 51 cases of HUS and four deaths (Besser et al., 1999; Feng, 1995). There was also an outbreak associated with consumption of raw milk that year (Mortimore and Wallace, 1998). This organism is distinguished from other *E. coli* strains by their inability to ferment sorbitol and their lack of production of β -glucuronidase (Besser et al., 1999). Early evidence with one outbreak-associated strain suggested that they may not be able to grow at 45.5°C (Doyle and Schoeni, 1994). Palumbo et al. (1995) reported that 18 of 23 strains were capable of growth at 45°C in EC broth in a circulating water bath. It is not clear whether those strains of *E. coli* O157:H7 that are capable of growth at 45°C would be capable of growth in typical coliform waterbath held at 45.5 $\pm$ 0.2°C, however. Nevertheless, another strain assayed with a temperature gradient

incubator was not recoverable at such temperatures and the authors concluded that “The temperature range for growth of *E. coli* O157:H7 is inconsistent with that of other fecal coliforms, suggesting that this pathogen is excluded with standard enumeration procedures used for foods and water.” (Raghubeer and Matches, 1990). These data indicate that the 45.5°C incubation temperature used in the identification of generic *E. coli* cannot be expected to be a reliable means to isolate *E. coli* O157:H7. Furthermore Tuttle et al. (1999) found that the MPN of *E. coli* (performed at 45.5°C) did not show *any* correlation with the presence of *E. coli* O157:H7. The authors speculated that this may be a result of the heterogeneity of distribution of *E. coli* O157:H7 in ground beef.

2.8.2 Cost

Buzby and Roberts (1996) estimated that illness due to *E. coli* O157:H7 costs \$300–700 million annually in the United States. However, the non-calculable cost associated with human suffering can be staggering given the seriousness of some forms of the disease its potential sequelae (e.g., hemolytic uremic syndrome – HUS).

2.8.3 Disease Syndromes

Some individuals can be infected but remain asymptomatic (Doyle et al., 1997). However, human illness from *E. coli* O157:H7 can result in nonbloody diarrhea and hemorrhagic colitis. In 3–5% of cases hemolytic uremic syndrome (HUS) may result.

2.8.3.1 Onset Time

Onset times of 3–4 days have been reported (Besser et al., 1999). The ICMSF indicated a range of 3–9 days (ICMSF, 2002b). However, the CDC (2004) in their document entitled, “Diagnosis and management of foodborne illnesses: A primer for physicians. Second Revision” has indicated that a 1- to 8-day incubation period is possible. This document was produced collaboratively by the CDC and the American Medical Association, the American Nurses Association, the FDA’s Center for Food Safety and Applied Nutrition, and the US Department of Agriculture and also appears in another publication authored by all the above (http://www.amaassn.org/ama1/pub/upload/mm/36/2004_food_introclin.pdf). Elsewhere CDC (2000) stated a 1- to 10-day incubation period may occur. The average onset time is 4 days (ICMSF, 2002b). This time frame appears to be consistent with gastrointestinal transport times of solid food and data from animal studies. It is difficult to imagine a shorter onset time than one entire 24 h day with a contaminated *solid* food because some hours, perhaps 10–12 h in some instances, can be expected

for the microbe to traverse the relatively harsh environments of the stomach (perhaps 4–6 h for large particles), and small intestine (perhaps another 6 h), where growth would not be expected to occur, before it reaches the colon where attachment, proliferation, colonization, toxin production, and effacement of colonic microvilli can begin.

2.8.3.2 Attachment and Colonization

The ability of the organisms to adhere to intestinal epithelial cells and colonize the gut is “undoubtedly one of the key determinants of virulence” (Patton and Patton, 1998). Ritchie et al. (2003) showed that infant rabbits were useful models for investigation of the intestinal stage of enterohemorrhagic *E. coli* pathogenesis. Data from this model indicated that diarrhea and *inflammation in the colon were dependent on colonization* (Ritchie et al., 2003). Interestingly they found that *colonization without persistent diarrhea resulted when a Stx₂ non-producing isogenic mutant strain was used*. Infant rabbits developed severe diarrhea 2–3 days post-intragastric inoculation with a high inoculum (5×10^8 cfu) of this organism (per 90 g body weight). They also developed diarrhea when given 100 µg of Stx₂/kg on days zero and one, but not in controls given heat-inactivated Stx₂. These data suggest that attachment, colonization, and toxin production are required for disease symptoms. *E. coli* O157:H7 levels associated with intestinal colonization in animal models appear to be very high in photomicrographs where colonization of the entire crypt length of the cecum and colon of gnotobiotic pigs was noted at the fourth day (Francis et al., 1986). Ritchie et al. (2003) recovered 1×10^8 /g *E. coli* O157:H7/g of colonized intestine.

It should be noted that growth of *E. coli* O157:H7 from about 10 cells/ml (1–10 cells/g) is considered to be a high level in ground beef product (ICMSF, 2002b) to the late log/early stationary phase occurring in 17.5 h under ideal aerated conditions in nutritious medium (Brain–Heart Infusion) at 37°C (Palumbo et al., 1995). This is also consistent with what one would expect for the time to produce one visible colony from a single cell on nutritious solid media under optimum laboratory conditions. Furthermore, McIngvale et al. (2002) found that the highest amount of toxin mRNA was detected from late log-phase and early stationary-phase cells corresponding to 10^8 – 10^9 cfu/ml. Thus it seems logical that colonization of the human colon should therefore take longer than 17 h once the microbe has implanted on colonic epithelial cells after passage from mouth to the colon, given a contamination level of 10 cells or less per gram of product. If the results from the infant rabbit model can be extrapolated to humans then inflammation would not be expected to occur until after 17.5 h in humans after the organism embedded in large particles of a solid food has traversed the mouth, stomach, and small intestine to the colon; a process that may take an additional 10–12 h thus resulting in hypothetical minimal onset time of 27.5–29.5 h in this example.

Furthermore genes involved in the attaching effacing lesion were shown to be regulated by a quorum (cell population)-based sensing mechanism (Sperandio et al., 1996). Data derived from a study of *E. coli* O157:H7 growth in BHI by Palumbo

et al. (1995) indicated that toxin production did not occur at a detectable level until about the mid-log phase of growth (about 8 h) when incubated at 37°C under ideal aerated conditions in the laboratory. The amount of toxin produced correlated to the population of *E. coli* O157:H7, with the maximum amount of toxin being detected when the population reached the stationary growth phase (about 17.5 h in the example given). *E. coli* O157:H7 growth (and therefore colonization) in the suboptimal (e.g., anaerobic, variable nutrition, presence of competitors) environment of the human colon is apt to be much slower than under optimal controlled conditions in a laboratory. Not surprisingly, Tamplin (2002) demonstrated that the rate of inoculated *E. coli* O157:H7 growth (in beef) decreased as the ratio of background flora to *E. coli* increased. It seems possible, though unlikely, that shorter onset times shorter than 24 h may occur with liquid foods or small particles that could be expected to pass through the gastrointestinal tract more quickly.

However, this author has not seen examples of shorter onset times in the literature. Onset times shorter than 24 h should be rare indeed.

The reported onset time between the onset of HUS or TTP-like illness is 4–15 days (Griffin et al., 1988). HUS and TTP-like illness (the role of *E. coli* in TTP has been questioned in recent times-editor) resulting from *E. coli* O157:H7 need not be preceded by diarrhea, although that would be more common (Griffin et al., 1988).

2.8.3.3 Hemorrhagic Colitis

Symptoms of hemorrhagic colitis are characterized by a prodromal phase which includes crampy abdominal pain, followed 1–2 days later by nonbloody diarrhea which progresses within 1–2 days to bloody diarrhea lasting 4–9 days (Doyle et al., 1997; Griffin et al., 1988).

2.8.3.4 HUS

The prodrome, described briefly above, is bloody diarrhea. This can then be followed by acute nephropathy, seizures, coma, and death. The disease is characterized by hemolytic anemia, thrombocytopenia, and renal failure. Among those who have been colonized with *E. coli* O157:H7 approximately 3–5% develop HUS (Tuttle et al., 1999; ICMSF, 2002b). A mortality rate of 4% can still be expected in HUS patients, even in the presence of meticulous care (Besser et al., 1999).

2.8.3.5 TTP

Thrombotic Thrombocytopenic Purpura-like illness is similar to HUS but includes fever and central nervous system disorder (ICMSF, 2002b). TTP tends to be diagnosed in adults and some feel that post-diarrheal TTP is likely to be the same disorder as HUS (Besser et al., 1999).

2.8.4 Pathogenic Mechanisms

The pathogenic mechanisms of *E. coli* O157:H7 are not fully elucidated. The virulence mechanism associated with hemorrhagic colitis is a combination of attaching and effacing adherence to the colon. These strains produce one or both of two toxins (Stx₁ and Stx₂) related to the toxins produced by *Shigella dysenteriae*, known as Shiga toxins which act by inhibiting protein synthesis. Stx₂-producing *E. coli* O157:H7 are more common and they are often associated with severe cases of bloody diarrhea than those which produce Stx₁ or a combination of Stx₁ and Stx₂ (Ritchie et al., 2003). It has been proposed that intestinal fluid secretion and therefore diarrhea results from the selective killing of the villus tips colonic epithelial cells by Stx (Eslava et al., 2003). Damage to the underlying tissue and vasculature, perhaps by exotoxin- and endotoxin-related mechanisms, results in bloody diarrhea (Doyle et al., 1997). The Stx's then enter into the blood stream where they may damage kidney glomeruli (a characteristic of HUS) and cause other problems (Doyle et al., 1997).

A set of genes called the locus of enterocyte effacement (LEE) are considered a key pathogenicity "island" for *E. coli* O157:H7. These genes include, among others, the *eae* gene which encodes for intimin and a gene called *tir* (stands for translocated intimin receptor) which plays a critical role in the attaching/effacing lesions (Ritchie et al., 2003). The Tir protein is translocated into the enterocyte which provides a receptor for intimin which induces the production of actin filaments in the enterocyte that produce a cup-like attachment structure on the enterocyte for *E. coli* O157:H7 cells. The active portions of the Stx toxins enter the cell, are transported through the endoplasmic reticulum, and inhibit the function of the 28S rRNA thus inhibiting protein synthesis (Paton and Paton, 1998).

2.8.5 Infectious Dose

Ground beef patties with less than 700 organisms per uncooked patty have been associated with illness (CDC, 2001; Tuttle et al., 1999). In one study levels of 0.3–15 cells/g of ground beef were found in positive lots implicated in an outbreak (Doyle et al., 1997). Mead and Griffin (1998) reported doses as low as 50 cells can be infectious and in one reference the FDA has indicated that as few as 10 cells may be adequate to cause illness (FDA, 2001; <http://www.cfsan.fda.gov/~mow/chap15.html>). Consequently the meat industry has taken strenuous actions to control this in the beef supply and in the processing environment.

2.8.6 Reservoirs

The main reservoir for this organism appears to be the gastrointestinal tract of cattle. Approximately 1% of healthy cattle have the organisms. However, it has been found

in other ruminants and in dogs, horses, and birds as well (Besser et al., 1999; Meng et al., 2001).

2.8.7 Foods Associated with *E. coli* O157:H7

More disease outbreaks have been caused in the United States by undercooked ground beef than by any other food vector. However, ground beef is not the only source. Between 1982 and 1994 outbreaks were reported from consumption of contaminated ground beef (32.4%), vegetables and salad bars (5.9%), roast beef (2.9%), raw milk (2.9%), and apple cider (2.9%) (Doyle et al., 1997). In addition FDA reports that outbreaks have also occurred from alfalfa sprouts, unpasteurized fruit juices, lettuce, game meat, and cheese curds (FDA, 2001; <http://www.cfsan.fda.gov/~mow/chap15.html>). Raw milk was the vehicle in a school outbreak in Canada. Salami, sandwiches, ranch dressing have also been implicated (Besser et al., 1999). Furthermore radish sprouts were implicated in several Japanese outbreaks including one in Sakakai City where 6,000 school children were affected (Besser et al., 1999) and raw spinach has also been implicated in the United States (Grant et al., 2008).

2.8.8 Unique Acid Tolerance in Foods

E. coli O157:H7 survived with only a 2 log₁₀ reduction in 2 months in fermented sausage (pH 4.5) held at 4°C (Doyle et al., 1997). It also survived in mayonnaise (pH 3.6–3.9) for 5–7 weeks at 5°C and 1–3 weeks at 20°C when inoculated at high levels (Doyle et al., 1997). The organism was also shown to survive in apple cider (pH 3.6–4.0) for 10–31 days at 8°C and for 2–3 days at 25°C (Doyle et al., 1997).

2.8.9 Other Sources of Infection

Contaminated food and water remain the most common source of transmission of *E. coli* O157:H7. Unchlorinated water and swimming water have both been shown to be sources of outbreaks (Besser et al., 1999). However, between 1982 and 1994 person-to-person outbreaks accounted for 13.2% of outbreaks (Doyle et al., 1997).

2.8.10 Food Processing Environments

Despite the collection of hundreds of environmental swabs, there has been great difficulty finding growth niches for this organism in the meat industry (Pruett, 2004, Personal Communication; Freier, 2004, Personal Communication). This may be a consequence of the lack of psychrotrophic (e.g., capable of growth at 7°C) adapted

strains in the refrigerated environments of meat processing factories which strive to maintain temperatures of 40°F (4.4°C) in many areas or perhaps competition with other strains of *E. coli* in these environments. Many strains were shown to be capable of growth at 10°C and a few were found able to grow at 8°C (Palumbo et al., 1995) which suggests the importance of refrigeration of the product and processing environments.

Contamination of beef surfaces may occur during slaughter and processing and the organism may be transferred from the surface to the interior of the product during grinding (Tuttle et al., 1999). Some actions taken by the industry to reduce the prevalence of *E. coli* O157:H7 on carcasses include refrigeration, cleaning and sanitization in the factory, organic acid carcass rinses, carcass steam chamber treatments, and specialized automated steam trimmers which promote destruction of microbes on cut surfaces, among others.

2.9 *C. perfringens*, a Toxico-Infectious Agent

C. perfringens, as well as *C. botulinum* and *B. cereus*, are gram-positive and anaerobic *spore-forming* bacteria known to cause food poisoning. According to the Center for Disease Control and Prevention (CDC), it ranks as the third most foodborne bacterial common disease in the United States. Sometimes it is called the “food service germ” because foods served and left for long periods at room temperature have been associated with this illness. Strains of this bacterium produce a protein toxin, named *C. perfringens* enterotoxin (CPE), which is considered as the virulence factor of this food poisoning organism in the context of food poisoning. Almost all *C. perfringens*-mediated foodborne illness in the United States and other developed countries involves the “Type A” toxin. *C. perfringens* is widely distributed in the environment and frequently occurs in the intestines of humans and many domestic animals. Its spores are able to survive normal cooking and pasteurization temperatures, after which they can then germinate and multiply during slow cooling, or storage at room temperatures and/or during inadequate re-warming (Jay, 2000).

2.9.1 The Organism

C. perfringens is a gram-positive, anaerobic, spore-forming rod-shaped bacterium. It is considered an anaerobic, because it does not grow on agar plates continuously exposed to air. However, unlike most other anaerobes, such as *C. botulinum*, this bacterium tolerates moderate exposure to air.

C. perfringens is a mesophilic bacterium. The lowest temperature for growth is around 20°C and the highest is around 50°C. The optimum growth temperatures are between 37 and 45°C. The organism’s generation time at 45°C under optimal conditions can be as rapid as 7 min allowing *C. perfringens* to quickly multiply in foods where it may form discrete microscopic colonies of high population. Hence

quantitative results from sampling of such foods may differ widely from sample to sample. Many strains grow over the pH range of 5.5–8.0, but not below 5.0 or above 8.5. The required water activity (a_w) for growth and germination of spores lies between 0.97 and 0.95 with sucrose or NaCl, but can be as low as 0.93 with glycerol. Sporulation requires higher a_w than for growth. Growth is inhibited by about 5% NaCl (McClane, 2001).

The organism's ability to form heat-resistant spores also contributes to its ability to cause food poisoning. Spores of some *C. perfringens* strains can survive boiling under some conditions. However, heat resistance differs among *C. perfringens* strains. For example, a $D_{100^\circ\text{C}}$ value of 17.6 min for strain NCTC 8238 and 0.31 min for strain ATCC 362 have been reported. It appears that other stress factors, such as high pH, may cross-induce heat resistance of some *C. perfringens*. In addition, the literature reports some rare strains of *C. perfringens* isolated from canned food which are also more heat resistant than *C. botulinum* (Bradshaw et al., 1977; Adams, 1973) a fact that may have some implications for canning of foods that would permit the growth of these organisms.

2.9.2 *C. perfringens* Enterotoxin

The virulence factor of *C. perfringens* food poisoning is an enterotoxin that induces fluid secretion and electrolyte losses from the GI tract of human and animals. It is a sporulation-specific protein toxin. The CPE is synthesized during the late stage of sporulation. The toxin production peak occurs just before lysis of cell's sporangium, and the CPE is released along with spores (McClane, 2000).

C. perfringens is classified as five types (A through E) based on four toxins (alpha, beta, epsilon, and iota) produced. Almost all *C. perfringens* foodborne illness in the United States and other western countries are attributed to type A food poisoning. Necrotic enteritis (known as Darmbrand or Pig-Bel), caused by type C poisoning, is rare in industrial countries. The overwhelming association between type A isolates of *C. perfringens* and type A food poisoning may be due to its wide distribution in the environment.

Unlike the organism, the enterotoxin CPE is not heat stable. Its biological activity can be destroyed by heating for 5 min at 60°C. The toxin is also very sensitive to pH extremes.

2.9.3 Cost

CDC has indicated that *C. perfringens* type A food poisoning ranks as the third most commonly reported bacterial cause of foodborne disease in the United States. Outbreaks of type A intoxication that occurred between 1992 and 1997 involved 248,520 cases, as estimated by USDA. The annual costs of illness from *C. perfringens* infection were estimated at \$100 million.

2.9.4 Disease Syndromes

Symptoms of *C. perfringens* type A food poisoning appear between 6 and 24 h (usually 8–12 h) after eating contaminated food and then resolve spontaneously within the next 12–24 h. The symptoms consist of the sudden onset of acute abdominal pain followed by diarrhea. Nausea is common, but fever and vomiting are usually absent. Death rates from *C. perfringens* type A food poisoning are low, however, fatalities do occur in elderly or in debilitated persons. The illness occurs when people swallow these bacteria or their spores which then multiply and produce toxin in the small intestine (hence its classification as a toxico-infection in this chapter). The diagnosis is confirmed by a laboratory test on a fecal specimen, with an outbreak being confirmed by tests on suspect foods.

2.9.5 Infectious Dose

C. perfringens type A food poisoning starts when bacteria are ingested with contaminated food. Most of the bacteria (vegetative cells) are killed by gastric acid in the stomach. However, if the food has sufficiently high numbers ($>10^6$ – 10^7 cfu vegetative cells/g food), some of these bacteria may pass through into small intestine. Once present in small intestine, vegetative cells will multiply and later undergo sporulation during which CPE responsible for *C. perfringens* type A food poisoning is produced.

2.9.6 Reservoirs

C. perfringens is ubiquitous and found in soil (at levels of 10^3 – 10^4 cfu/g), decaying vegetation, dust, foods ($>50\%$ of raw or frozen meat contains some *C. perfringens*), the intestinal tract of human and other vertebrates (e.g., human feces usually contain 10^4 – 10^6 cfu/g). They are also commonly recovered from infected sites but usually as a component of a polymicrobial flora, which makes their role in pathogenesis difficult to establish.

Meats, meat products, and gravy are the most frequently implicated with food poisoning caused by *C. perfringens*. The heating procedures of such foods may be inadequate to destroy the heat-resistant endospores, and when the foods are cooled and rewarmed, the spores germinate and grow. Consequently, the USDA has published guidelines with the goal of ensuring that cooling of such products not permit more than 1 log₁₀ cfu growth of *C. perfringens* populations (USDA, 1999).

2.10 *Arcobacter*, an Emerging Pathogen

2.10.1 *Characteristics of the Organism*

Arcobacter is a member of the Epsilonbacteria group which also includes *Campylobacter* and *Helicobacter* spp. The genus *Arcobacter* contains four different species. *Arcobacter nitrofigilis* has been isolated from plant roots, and *Arcobacter cryaerophilus*, *Arcobacter skirrowii*, and *Arcobacter butzleri* have been isolated from animals. *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* have been reported to cause human and animal illnesses, whereas *A. butzleri* has been isolated most frequently from cases of human enteritis (Kiehlbauch et al., 1991; Lerner et al., 1994).

A. butzleri is a gram-negative, curved (vibrio-like), non-spore-forming rod. It is approximately 0.2–0.9 μm wide and 1–3 μm long. It is motile due to a single polar unsheathed flagellum. It is capable of growth at a range of temperatures from 15 to 35°C, with its optimum range from 25 to 30°C. *A. butzleri* does not grow at temperatures higher than 35°C, unlike *C. jejuni* subspecies *jejuni*, which requires a higher temperature ranging from 37 to 42°C. *Arcobacter* and *Campylobacter* species have been found at similar locations on broiler carcasses and show a close genetic relationship (Vandamme et al., 1991). Additionally, *Arcobacter* has an ability to grow under aerobic conditions in which *Campylobacter* is inhibited from growth, but capable of survival. *A. butzleri* is microaerophilic upon initial isolation, but can be grown aerobically after sub-culturing. The organism does not ferment sugars, but instead uses pyruvate as an energy source. It has few biochemical properties that may aid in its identification; however, a multiplex PCR has proven useful for the detection of all *Arcobacter* species (Wesley and Baetz, 1999).

2.10.2 *Nature of the Disease*

Marinescu et al. (1996) indicated that the biotypes and serotypes of *A. butzleri* isolates from poultry products were similar with those isolated from humans with diarrheal illness. This was the first report that implied a link between human illness and contaminated food products. In humans, *A. butzleri* and *A. cryaerophilus* have been isolated from stool samples of patients with acute diarrhea. However, the significance of *Arcobacter* spp. as a cause for human diarrhea is still unknown. This is probably due to the fact that clinical samples are not routinely tested for *Arcobacter* spp. as is commonly done for *Campylobacter* spp. or *Salmonella* spp. (Lehner et al., 2005). Patients infected with *A. butzleri* can be asymptomatic, but the most common symptom is acute watery diarrhea lasting for 3–15 days, often accompanied by abdominal pain and nausea. Limited information regarding the pathogenicity and epidemiology of *A. butzleri* is available.

2.10.3 Food and Environmental Sources

Previous studies indicate that *Arcobacter* spp. are present on many retail poultry carcasses and other meat products (Atabay and Corry, 1997; Collins et al., 1996; Festy et al., 1993; Lammerding, 1996; Marinescu et al., 1996; Schoeder-Tucker et al., 1996; Wesley, 1996). *Arcobacter* spp. have been isolated more frequently from poultry than from red meat (Wesley, 1996; Corry and Atabay, 2001) suggesting that poultry may be a significant reservoir. Most reports of *Arcobacter* in poultry meat have identified *A. butzleri*, but *A. cryaerophilus* and *A. skirrowii* have also been reported (Atabay et al., 1998). Lehner et al. (2005) summarized recent studies on the prevalence of *Arcobacter* isolated from retail raw meat products.

Currently, there are no standard isolation protocols or methods for *Arcobacter* spp., therefore the true occurrence of this pathogen in food is largely unknown. Water may play an important role in the transmission of these organisms and drinking water has been cited as a major risk factor in acquiring diarrheal illness associated with *Arcobacter* spp. (Lehner et al., 2005). *A. butzleri* is sensitive to chlorine, indicating that disinfection practices normally used in drinking water treatment would be adequate for the control of arcobacters.

While control of *Arcobacter* on the farm may reduce contamination at the processing and retail levels, the habitat of *Arcobacter* species in living birds is still unknown (Houf et al., 2002). Gude et al. (2005) reported on a study of sources of *Arcobacter* spp. in chicken rearing and processing. They concluded that *Arcobacter* species were not present in samples examined from live birds or their immediate environment, but *A. butzleri* was widely distributed throughout the abattoir environment and on poultry carcasses, usually in low numbers. In another study, with chickens, Eifert et al. (2003) reported that *A. butzleri* was capable of surviving in litter with or without birds and could therefore be problematic in operations where “built-up” litter is used. If *A. butzleri* is an environmental pathogen, then the implementation of on-farm Best Management Practices might play a substantial role in reducing its prevalence in commercial poultry.

The role of *Arcobacter* in human disease is unclear. Human exposure to this potential pathogen can occur from several sources in addition to raw meat products. Efforts to reduce or eliminate *Arcobacter* from the human food chain should be encouraged. Further studies on the ecology and epidemiology of *Arcobacter* spp. are necessary (Lehner et al., 2005).

2.11 *Cronobacter* (*Enterobacter sakazakii*), an Emerging Pathogen

2.11.1 Introduction, Background, and Bacterial Characteristics

E. sakazakii is a gram-negative, non-sporulating, rod-shaped, opportunistic bacterium that has been historically distinguished from *E. cloacae* based on its

ability to produce yellow pigment, and, as of 1980, comprised a distinct species within the genus *Enterobacter* (in the Family Enterobacteriaceae) family Enterobacteriaceae based on DNA–DNA hybridization and phenotypic characteristics (Farmer et al., 1980). The pathogen has been reported as growing between the temperatures of 4 and 47°C (Nazarowec-White and Farber, 1997b; Farmer et al., 1980) and has been implicated in sporadic cases of neonatal sepsis and meningitis, associated with necrotizing enterocolitis in infants and, in rare instances, has been implicated in infections in immunocompromised adults (Gurtler et al., 2005). Over 80 cases of *E. sakazakii*-related illness have been reported (Iversen and Forsythe, 2003; FAO/WHO, 2006). Non-pigmented isolates of *E. sakazakii* have been reported, and pigmented isolates have been known to lose pigment production following multiple transfers (Farmer et al., 1980). *E. sakazakii* produces the enzyme α -glucosidase, which has been utilized in several differential media for the presumptive detection of the pathogen (Iversen et al., 2004b; Oh and Kang, 2004; Leuschner et al., 2004; Restaino et al., 2006), although false-positives have been documented. The US Food and Drug Administration method for isolating and enumerating *E. sakazakii* from dehydrated powdered infant formula involves rehydration with water and pre-enrichment at 36°C overnight, enrichment in Enterobacteriaceae enrichment broth and incubating at 36°C overnight, streaking onto Violet Red Bile Glucose agar and incubating at 36°C overnight, picking five presumptive positive colonies that are streaked onto Tryptic Soy agar and incubating at 36°C overnight, and finally yellow pigmented colonies are confirmed by means of the API 20E biochemical identification kit (USFDA, 2002a, 2002b).

2.11.2 E. sakazakii Reservoirs and Presence in Food and the Environment

E. sakazakii is not known to have a primary reservoir and appears to be extremely widespread in nature. The pathogen has been isolated from a stirring spoon and a dish brush used to prepare infant formula (Muytjens et al., 1983), tires of a forklift in an infant formula production factory, a leaky water pipe, sugars, and gums (Olson, 2006), water (Cruz et al., 2004; Mosso et al., 1994), dust (Cruz et al., 2004), an unopened non-fat dried milk (Farmer et al., 1980), dried infant foods, milk powders, cheese products, herbs, and spices (Iversen et al., 2004a), infant weaning foods (Jung and Park, 2006), vacuum cleaner bags in homes, factories that process powdered milk, chocolate, cereal, potato flour, and pasta (Kandhai et al., 2004a), fermented bread (Gassem, 1999), a fermented beverage (Gassem, 2002), lettuce (Soriano et al., 2001); mung bean sprouts (Robertson et al., 2002), alfalfa sprouts (Cruz et al., 2004), rice starch, rice flour, and eggs (Kornacki, 1998), rice (Cottyn et al., 2001), beer mugs (Schindler and Metz, 1990), sour tea (Tamura et al., 1995), cheese, minced beef, sausage meat, and vegetables (Leclercq et al., 2002), ground meat (Nazarowec-White and Farber, 1997a), the Mexican fruit fly *Anastrepha ludens* (Kuzina et al., 2001), the stable fly larvae *Stomoxys calcitrans* (Hamilton et al., 2003), a physician's stethoscope and an uninoculated bottle of

bacterial culture medium (Farmer et al., 1980), grass silage (Van Os et al., 1996), hospital air (Masaki et al., 2001), clinical materials (Janicka et al., 1999; Tuncer and Ozsan, 1988), rats (Gakuya et al., 2001), soil (Neelam et al., 1987), rhizosphere (Emilani et al., 2001), sediment and wetlands (Espeland and Wetzel, 2001), crude oil (Assadi and Mathur, 1991), and cutting fluids (Sulliman et al., 1988). *E. sakazakii* has also demonstrated the ability to attach to bottles and enteral feeding tubes, which are used to feed infants in neonatal intensive care wards (Zogaj et al., 2003).

Reports have detailed the unusually high desiccation resistance of *E. sakazakii* (Breeuwer et al., 2003; 2004; Edelson-Mammel et al., 2005; Caubilla-Barron and Forsythe, 2006; Gurtler and Beuchat, 2007) which may contribute, in part, to its reported presence in powdered infant formulas (Biering et al., 1989; Block et al., 2002; Clark et al., 1990; Himelright et al., 2002; Iversen et al., 2004a; Muytjens et al., 1983; Muytjens et al., 1988; Simmons et al., 1989; Smeets et al., 1998; Van Acker et al., 2001) and powdered infant formula manufacturing environs (Olson, 2006).

2.11.3 Pathogenicity and Infectious Dose

The FAO/WHO has categorized *E. sakazakii* and *Salmonella* as the only two “category-A” pathogens in powdered infant formula based on their contamination risk and pathogenicity (2006). Historically, neonates and young infants have shown a greater propensity for contracting sporadic *E. sakazakii*-associated illnesses, most likely due to their immunocompromised state (Urmenyi and Franklin, 1961; Jöker et al., 1965; Monroe and Tift, 1979; Kleiman et al., 1981; Muytjens et al., 1983; Muytjens, 1985; Muytjens and Kollee, 1990; Arseni et al., 1985; Biering et al., 1989; Clark et al., 1990; Willis and Robinson, 1988; Simmons et al., 1989; Noriega et al., 1990; Gallagher and Ball, 1991; Van Acker et al., 2001; Burdette and Santos, 2000; Bar-Oz et al., 2001; Block et al., 2002; Himelright et al., 2002; Weir, 2002; Ministry of Health, New Zealand, 2005; Coignard and Valliant, 2004; Coignard et al., 2006). Infants less than 60 days old appear to be at the greatest risk of infection (FAO/WHO, 2004). Although the unusually high nutritional needs of premature neonates require supplementing their diets (traditionally accomplished with powdered infant formulas), studies are currently underway to develop and market sterile liquid infant formulas that would meet the nutritional needs of this group of infants (Olson, 2006).

The true incidence of *E. sakazakii*-associated illnesses is not known, although infections have been estimated at 1.2 cases per 100,000 infants per year, and 8.7–9.4 cases per 100,000 low and very low birth weight infants per year (Braden, 2006; Stoll et al., 2004). Mortality rates for *E. sakazakii*-associated neonatal meningitis have been estimated to be between 40 and 80% (Lai, 2001; FAO/WHO, 2006), although 94% of survivors have been reported to experience long-term neurological impairment (Drudy et al., 2006). Powdered infant formulas have been shown to contain heat-stable endotoxins, which, when consumed, may increase the chances of intestinal *E. sakazakii* invasion (Townsend et al., 2006). In an international case

study of 46 infants with invasive *E. sakazakii*-associated infections, 92% of patients, for whom feeding information was available, had consumed reconstituted powdered infant formulas (Bowen and Braden, 2006). An infectious dose for *E. sakazakii* has not yet been established and estimates range from 1 up to 1,000 cfu (Havelaar and Zwietering, 2004; Iversen and Forsythe, 2003). Non-primates are currently being examined as potential models for human pathogenicity (Lenati et al., 2006; FAO/WHO, 2006).

2.11.4 Regulation

Previous USFDA microbiological guidelines for powdered infant formula were $\leq 10,000$ cfu/g for aerobic plate count, ≤ 3.05 MPN cfu/g for coliforms (including the “so-called” fecal coliforms) and *S. aureus*, and ≤ 100 cfu/g for *B. cereus*, along with “negative” in 60×25 g samples for *Salmonella*, and negative for *L. monocytogenes* in their proposed guidelines of 1996 (USFDA, 1996). However, the United States FDA (2006) has “tentatively” dropped consideration of a requirement of testing for microbes other than *Salmonella* (“negative” in $n = 60 \times 25$ g) and *E. sakazakii* ($n = 30 \times 25$ g samples) in their 2006 proposed guidelines. The Codex Alimentarius Commission (1979) established the microbiological criteria for powdered infant formula for mesophilic aerobic bacteria ($n = 5$, $c = 2$, $m = 1,000$, and $M = 10,000$), coliforms ($n = 5$, $c = 1$, $m < 3$ MPN/g, and $M = 20$ cfu/g), and *Salmonella* negative in 60×25 g samples. (In these sampling schemes “ n ” is the number of samples taken and tested per lot, “ c ” is the number of samples allowed to be greater than “ m ” but less than “ M ,” where “ M ” represents a number associated with automatic lot rejection, see Chapter 8). Powdered infant formula performance standards (in cfu/g) for Canada have been set for the aerobic plate count ($n = 5$, $c = 2$, $m = 1,000$ /g, and $M = 10,000$ /g), *E. coli* ($n = 5$, $c = 1$, $m < 1.8$ /g and $M = 10$ /g), *Salmonella* (negative in 20 samples per lot), *S. aureus* ($n = 10$, $c = 1$, $m = 10$ cfu/g, and $M = 100$ cfu/g), *B. cereus* ($n = 10$, $c = 1$, $m = 100$ cfu/g, and $M = 10,000$ /g), and *C. perfringens* ($n = 10$, $c = 1$, $m = 100$ cfu/g, and $M = 1,000$ cfu/g) (Health Canada, 2006). The European Food Safety Authority (2004) has recommended a performance objective of the absence of *E. sakazakii* or *Salmonella* in 1, 10, or 100 kg of powdered infant formula and follow-up formulas.

2.11.5 Food Industry Concerns

Numerous studies have confirmed the presence of *E. sakazakii* in commercially produced powdered infant formula. Contamination incidence in international surveys have ranged from 2.4 to 14% (Muytjens et al., 1988; Iversen and Forsythe, 2004) while FDA field surveys have reported a 6.6% incidence (Zink, 2003). Contamination levels in formulas that test positive for the pathogen, however, are usually < 1.0 cfu/100 g of powdered infant formula as determined by the MPN

method. Between the years of 2002 and 2005, at least seven voluntary recalls of powdered infant formula were issued due to possible contamination with *E. sakazakii* (IBFAN, 2005). Contamination of powdered infant formula with *E. sakazakii* that occurs in manufacturing plants is expected to be a post-processing phenomenon such as might occur in the dry-mixing of finished product or during filling and packaging. Numerous ingredients added to powdered infant formulas may serve as potential sources for introducing pathogens into a processing environment, including lactose, whey protein concentrate, vegetable oil, vitamin and mineral pre-mixes, soy protein isolate, sucrose, corn syrup solids, and corn maltodextrin. Contamination appears to take place after the bactericidal heat treatment step of the hydrated dry powder base, which is a process that involves a sufficiently high temperature to destroy the pathogen. The presence of *E. sakazakii* in the processing environment, in processing equipment that may come into direct contact with the product, and in ingredients that may be mixed into the dry base powder are the three major factors outlined by the FAO/WHO as contributing to recontamination of powdered infant formulas with the pathogen (2006). Integrated Enterobacteriaceae and *E. sakazakii* testing programs of environmental samples, product contact surfaces, and finished products, detailed by the ICMSF (2002a), have been recommended by the FAO/WHO (2006) for infant formula manufacturers. The FDA is currently proposing the development of analytical techniques and a standard reporting tool to guide *E. sakazakii* investigations along with an accompanying questionnaire (Guzewich, 2006). One report from a powdered infant formula processing representative stated that proactive measures are being taken to enhance the bacteriological safety of powdered infant formulas including modifications to HACCP plans, training and awareness programs for employees, environmental monitoring and air sampling programs, plant sanitation, GMP/procedural analysis, and state-of-the-art *E. sakazakii* testing (Olson, 2006). Nevertheless the widespread nature of the organism, its ability to adapt to dry processing environments, and the statistical improbability of finding it in bulk dry ingredients (see Chapter 8) make control of *Cronobacter* spp. very difficult in the processing environment.

2.12 *M. avium* subspecies *paratuberculosis*, an Emerging Pathogen

2.12.1 The Organism

Mycobacteria are gram-positive, rod-shaped bacteria. Their unique cell wall, comprised of complex lipids, causes them to stain “acid fast.” This cell wall composition results in cell clumping and also inhibits their ability to absorb nutrients, which could account for their slow growth compared to other human pathogens (Sung and Collins, 1998). Pathogenic members of the genus *Mycobacteria* include *Mycobacterium tuberculosis*, *Mycobacterium bovis*, and *M. leprae*. Several members are opportunistic pathogens for humans and include *Mycobacterium*

kansasii, *Mycobacterium scrofulaceum*, *Mycobacterium avium-intracellulare*, *Mycobacterium fortuitum*, *Mycobacterium marinum*, *Mycobacterium ulcerans*, and *Mycobacterium smegmatis*. *Bacillus Calmette-Guerin* is an attenuated form of *M. bovis* and is used in some countries as a vaccine against *M. tuberculosis*. *Mycobacteria* do not produce typical exotoxins or endotoxins. Disease processes generally result in delayed-type hypersensitivity reactions in response to Mycobacterial proteins.

2.12.2 Disease

Mycobacterium paratuberculosis (MAP) is the etiologic agent of Johne's disease in cattle and other ruminants. Johne's disease is a chronic, progressive, and severe gastrointestinal illness (Harris and Barletta, 2001). It is characterized by chronic or intermittent diarrhea, emaciation, and death (Stabel, 1998) and has been of worldwide significance for many decades (Doyle, 1956; Harris and Barletta, 2001). The disease affects at least 10% of cows in 22% of US herds (Wells et al., 1999). However, some estimates have ranged as high as 21–54% of herds in the United States and Canada (Hermon-Taylor, 2001).

This organism causes chronic intestinal inflammation in both large and small ruminants, monogastrics (e.g., dog and pigs) and at least four types of non-human primates (Hermon-Taylor, 2001). There is evidence that MAP is associated with Crohn's disease (CD) in humans. Specifically, MAP has been cultured from intestinal tissues, breast milk, and the blood of CD patients (Chiodini et al., 1984; 1986; McFadden et al., 1987; Mishina et al., 1996; Naser et al., 2000; Hermon-Taylor, 2001; Bull et al., 2003; Naser et al., 2004). However, its role in Crohn's disease in humans is controversial (Stabel, 1998). Despite the aforementioned studies supporting an association of MAP and CD, other investigations have been unable to show any association or substantiate evidence from these previous studies (Ellingson et al., 2003; Baksh et al., 2004; Freeman and Noble, 2005; Lozano-Leon et al., 2006). The disease syndrome produced by MAP in cattle has a similar pathology as Crohn's disease in humans. In addition, isolates of MAP that have been recovered from human Crohn's lesions have induced Johne's disease in infant goats (van Kruiningen et al., 1986) and young chickens (van Kruiningen et al., 1991). Due to the conflicting scientific reports, the association of MAP with Crohn's disease is a highly debated topic.

2.12.3 Costs

The economic impact of Johne's disease to the US cattle industry has been estimated at 1.5 billion dollars per year (Jones, 1989). In one study 3% of 350 beef cattle surveyed at three slaughter facilities showed evidence of MAP infection (by fecal

culture or ileocecal lymph nodes, Rossiter and Henning, 2001). The prevalence of MAP in some herds has been estimated as high as 34% (Collins et al., 1994).

2.12.4 Reservoirs

MAP multiplies mainly in the lymphatic system and intestinal tract of infected species (Kennedy et al., 2001). “Vast numbers” of the organism were reported in feces (Doyle, 1956). In fact, fecal contamination appears to be the principal means by which this organism is transmitted through the environment (Collins, 2001; Boor, 2001). Chiodini and Hermon-Taylor (1993) indicated that millions of cells may be shed in feces of clinically or sub-clinically infected cattle. Chiodini (1989) reported that up to 10^8 cells of MAP per gram could be shed during the clinical phase of infection. This is an important factor to consider regarding potential fecal–oral contamination. Some believe that this organism may be transferred to the human population from infected animals (e.g., through raw or inadequately pasteurized milk from infected dairy cattle). The potential for other routes of human exposure and potential infection (e.g., meats, water, and the environment) has largely been ignored. Hermon-Taylor (2001) has stated that “raw and processed meats are also at risk,” despite attention largely focused on the role that milk contamination may play in MAP exposure in humans and the lack of published studies on MAP survival in meats (Collins, 2001).

2.12.5 Food Processing Issues

2.12.5.1 Heat Resistance

Milkborne transmission of tuberculosis by *Mycobacterium bovis* was common before pasteurization of fluid dairy products, widespread refrigeration and other quality enhancements became commonplace after World War II (Bryan, 1983). Early milk pasteurization parameters were based on destruction of this organism until it was realized that the rickettsial pathogen, *Coxiella burnetii* was more heat resistant. The heat resistance of MAP is considered high for a non-spore-forming organism (Stabel et al., 2001) and Sung and Collins (1998) showed that its heat resistance in milk was greater than that of *Salmonella*, *L. monocytogenes*, and *C. burnetii*. Furthermore, MAP has been isolated from pasteurized milk in the UK (Simmons, 2001). More recently Ellingson et al. (2005) showed that 2.8% of 702 pints of previously unopened pasteurized retail whole milk collected from California, Minnesota, and Wisconsin over a 12-month period contained viable MAP. MAP is considered to be an obligate intracellular pathogen; however, special laboratory medias can be used to recover the microbe when they are supplemented with Mycobactin J. Consequently, the likelihood that the microbe forms microbial growth niches in factory environments seems small, though uninvestigated. Hence the possibility of post-pasteurization contamination may be reduced compared to other organisms that

are known to form growth niches on the processing plant environment, lending some credence to those who think the microbe may survive pasteurization. Nevertheless, controversy, exists regarding whether or not the organism survives milk pasteurization despite a number of studies dealing with this issue (see Stabel, 1998 for a discussion of some of these studies). This may be due to many factors including differences in methodologies used to heat-treat milk, those used to recover the organism, the ability of the microbe to form clumps, and the low numbers (e.g., 5–8 cfu/ml) of MAP shed in raw milk of infected (clinically or asymptomatic) cows (Stable et al., 2001). Hence the risk of infection from milk may be low due to low numbers likely to be present in raw and hence pasteurized milk.

Clark et al. (2005) tested 101 cheese samples in a limited study taken over a 6-month period from Northern and Southern Wisconsin and Minnesota and found no evidence of viable MAP. They found no evidence of viable MAP in 0.17 g per sample (1 ml of a 1/6 dilution of 5 g product). However, 9.2% of the samples (6 of 65) contained *hspX* and *IS900* genetic elements consistent with the presence of MAP DNA. The presence of MAP in cooked or smoked meat products has not been investigated.

Fecal contamination of meats from the hide and intestinal tract of cattle during slaughter is a well-known phenomenon. Furthermore, *MAP* has been shown to survive for up to 152–146 days in naturally contaminated feces under different conditions (Lovell et al., 1942). Collins et al. (2001) stated that "... as with any organism found in feces, post mortem contamination of the carcass and products made from the carcass, in particular ground beef, is possible." Hence, occasional contamination of carcasses from MAP in feces will occur. Furthermore, infected animals (beef or cows) may become septicemic (Collins, 2001). Animal tissues from slaughtered cattle with Johne's disease could, therefore, have very high numbers of MAP per gram of meat tissue. It is presently unknown whether or not MAP survives meat cooking or smoke house treatments. Chapter 5 deals with the factors that influence microbial heat resistance, among other things.

2.12.5.2 Reason for Lack of Information About MAP

Until relatively recently, the scientific community has tended to ignore MAP due to the extended time and effort needed for culturing (e.g., 2 months or more to recover visible colonies) by traditional approaches. This fact resulted in reliance upon harsh sample decontamination regimes used with traditional methods to eliminate many other organisms that grow faster. These decontamination regimes also have negative effects on MAP recovery (Johansen et al., 2006) and may cause cells to become viable but non-culturable. This is inferred from the fact that the environment of granulomas, in which *M. tuberculosis* cells can exist in a dormant state in recovering tuberculosis patients, is believed to be very harsh. The environment within granulomas is characterized by low oxygen, high CO₂, acidic pH, and the presence of aliphatic organic acids. It was originally thought that *M. tuberculosis* would die quickly in the harsh environment within a granuloma, but it was later found that it can survive many years (Cunningham and Spreddbury, 1998 quoting Wayne and

Salkin, 1956). Yanmin et al. (2000) postulated that “persisting *M. tuberculosis* may exist in some physiological form in which limited metabolism, presumably with little or no cell turnover, accounting for tolerance to conventional antibiotic treatment.” Current assays were borrowed from clinical and veterinary microbiology assays developed to detect high numbers of cells in infected tissue or fecal samples. Hence, there is a need to adapt methods more suitable to recover low numbers of stressed or injured *MAP* likely to be found in food, feed, or some environmental samples. Modern molecular techniques such as PCR show some promise but results from PCR methodology, which may take but a few hours or days, have been criticized because of uncertainty whether or not positive sample assays detected living cells or merely DNA from dead cells (Stabel, 1998). The issue becomes a bit more confused when one considers that *MAP* was also detected in some milk samples by cultural methods, but not by PCR (Millar et al., 1996). These findings leave open at least three questions: (1) Were failures to recover live cells a consequence of PCR detection of dead cells? (2) Did failures to recover live cells from PCR-positive samples occur because living cells were in a viable but non-culturable state? (3) Were failures to recover live cells from PCR-positive samples and vice versa an artifact of sampling when low numbers of cells are present? A diagnostic test for *MAP* that can be used on food will most likely have to be rapid to allow testing within each product’s shelf life, differentiate live organisms from those inactivated during processing and extremely sensitive so that it can detect *MAP* with no enrichment. For these reasons, immunomagnetic capture coupled with a very sensitive PCR-based assay is currently attracting a lot of research interest.

2.12.6 Some Research Needs

Suggested research needs related to the role of *MAP* in foods include the following:

1. A better understanding of the role of *MAP* in Crohn’s disease.
2. More sensitive and rapid techniques for recovery of viable *MAP* in foods, feeds, and the environment.
3. Better understanding of the microbial ecology of the microbe both in the food manufacturing environment, the natural environment, and other foods including meats and poultry.
4. Development of a suitable surrogate microorganism capable of more rapid growth and/or detection. Use of a light emitting strain of *MAP* has also been proposed for such work (Boor, 2001).
5. Development of a suitable thermal surrogate organism, which could be used to inexpensively validate thermal processes with techniques available to a typical commercial laboratory.
6. Means and development of processing systems related to keeping this organism out of the pasteurized milk supply.

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