Microbiological Quality and Safety Issues in Cheesemaking

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ABSTRACT As the manufacture of cheese relies in part on the select outgrowth of microorganisms, such conditions can also allow for the multiplication of unwanted contaminants. Milk ultimately becomes contaminated with microorganisms originating from infection, the farm environment, and feedstuffs, as well as milking and processing equipment. Thus, poor sanitation, improper milk handling, and animal health issues can result in not only decreased yield and poor quality but also sporadic cases and outbreaks of dairy-related disease. The entry, establishment, and persistence of food-borne pathogens in dairy processing environments also present a considerable risk to products postprocessing. Food safety management systems coupled with regulatory policies and microbiological standards for milk and milk products currently implemented in various nations work to reduce risk while improving the quality and safety of cheese and other dairy products. With that, cheese has enjoyed an excellent food safety record with relatively few outbreaks of food-borne disease considering the amount of cheese produced and consumed worldwide. However, as cheese production and consumption continue to grow, we must remain vigilant in ensuring the continued production of safe, high-quality cheese.

The microbiological quality and safety of cheese begin with milk. With an abundance of nutrients, milk is intended to provide complete primary nutrition for young mammals. With its near neutral pH, milk also serves as an excellent growth medium for the select microorganisms utilized in the manufacture of cheese. Consequently, the same applies to contaminating microorganisms, including those associated with spoilage and defects as well as those that are pathogenic to humans. Milk, essentially sterile when secreted into the alveoli of the udder, ultimately becomes contaminated with microorganisms originating from infection, the farm environment, and feedstuffs, as well as milking and processing equipment. Generally, environmental contaminants found in soil, feces, and bedding material attach to the exterior of teats and are released into milk during collection. Some of these microorganisms may also enter the teat canal and cause infection and are thus excreted in the milk. Lastly, microorganisms adhered to the surfaces of the milking and processing equipment, present as a result of inadequate cleaning and sanitation, can also contaminate milk. Contamination is of great concern to the cheesemaker, as milk of high microbiological quality is important to achieve optimal cheese yield, quality, and safety. Many spoilage organisms are capable of producing negative changes in the organoleptic attributes of cheese through the enzymatic alteration of milk components. In addition, contaminants can access the cheese itself during manufacture and aging, resulting in additional defects and food safety concerns. This review discusses the various types of contaminating microorganisms, with a focus on those associated with quality and/or safety issues related to cheesemaking. It also covers the regulatory policies regarding milk and milk products currently implemented in various developed nations designed to increase the quality and safety of cheese and other dairy products.
When milk is hygienically collected from an uninfected herd or flock using clean milking and storage equipment, the concentration of contaminating organisms can be less than 5,000 CFU/ml (10). Populations less than 20,000 CFU/ml generally indicate good sanitary practices, whereas higher concentrations indicate deficiencies in production hygiene such that bacterial counts often correlate well with unsanitary conditions during milk collection and handling. Increases related to the outgrowth of microorganisms can occur when milk is exposed to elevated temperatures (>7°C) during storage and/or transportation. Before modern refrigeration, when milk was either not cooled at all or cooled to ambient temperature in water, the growth of gram-positive bacteria, particularly LAB, dominated (10). LAB as a group are described as gram-positive, non-motile, non-spore-forming bacteria with a strictly fermentative metabolism producing lactic acid as the major end product; they include the genera *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Lactococcus*, and *Pediococcus*, among others (11).

The outgrowth of endogenous LAB was, and still is in some areas, responsible for the production of traditional cultured dairy products such as cheese. Now that modern cheese is manufactured with select pure cultures, many wild-type LAB that do not produce significant amounts of acid during the manufacturing process are referred to as nonstarter LAB (NSLAB). NSLAB include obligately heterofermentative mesophilic lactobacilli (*Lactobacillus brevis* and *Lactobacillus fermentum*), pediococci, and enterococci, with the facultatively heterofermentative *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, and *Lactobacillus curvatus* as the most common species. In addition to their presence in raw milk, NSLAB can also be found in pasteurized milk as the result of both heat resistance and postprocessing contamination. According to Lawrence and colleagues (12), the NSLAB in Cheddar cheese vary between countries and facilities, within a single facility, and even within an individual cheese, where populations are often comprised of numerous species and strains in a dynamic state during ripening (13). Given the diverse metabolic and enzymatic activities among species, NSLAB growth can potentially impart both positive sensorial attributes and defects, including high acidity, off-flavors, and excessive gas production. Gas production by NSLAB during ripening is recognized as a cause of “slit” defects in hard and semihard cheeses such as Cheddar (12) and excessive openness in Gouda and related cheeses (9). Although gas-related defects can also result from the outgrowth of *Leuconostoc* spp. (14), they
are most often associated with heterofermentative lactobacilli. The formation of carbon dioxide is believed to result from the metabolism of carbohydrates or citrate or, most likely, from amino acids. Amino acid metabolites are also related to flavor defects described as sulfurous, phenolic, putrid, and mealy (9). The presence and growth of NSLAB in Cheddar are also associated with the formation of white spots of calcium lactate pentahydrate crystals on the cheese surface (12). The growth of NSLAB in Cheddar occurs primarily after the lactose has been metabolized by residual starter activity. Presuming that the growth substrates in cheese are limited, it may be possible to outcompete wild NSLAB through the addition of select strains to cheese milk as adjunct starter cultures in an effort to better control the ripening process (15).

The genus Enterococcus has been considered the most controversial of the LAB (16). As enterococci are normal inhabitants of the gastrointestinal tracts of both animals and humans, their presence has long been considered an indication of poor sanitation during the production and processing of dairy products. With the ability to persist in diverse environmental niches due to their high heat tolerance and ability to survive adverse environmental conditions, enterococci can also be present in pasteurized milk and cheese as postprocessing contaminants (17, 18). Some enterococci are also able to grow at 7°C with detectable proteolytic activity (19). The impact of high levels of contaminating enterococci in cheese is unclear, as the proteolytic and lipolytic activities of enterococci appear to vary by strain (4). Enterococci constitute an important part of the natural flora of many washed-rind cheeses. According to a review by Franz and colleagues (20), enterococci often contribute positively to the ripening and aroma development of many Mediterranean cheeses due to their proteolytic and esterolytic activities, as well as the production of diacetyl, and have thus been proposed for use as select starter cultures in the manufacture of various European cheeses. An additional benefit of the use of enterococci in cheese is that many strains produce bacteriocins that may help control pathogens such as Listeria monocytogenes.

In recent times, the proportions of LAB and other organisms in raw milk that are beneficial to cheese manufacture have become proportionately lower as the likely result of hygienic collection and handling practices coupled with rapid cooling and refrigerated storage (3, 19, 21). Proper cooling and storage of milk are critical in preventing the outgrowth of contaminating microorganisms, including pathogens, in milk prior to cheese manufacture if not used immediately. Thus, numerous countries have established regulations regarding the cooling and refrigerated storage of milk as shown in Table 1. Although rapid cooling and refrigerated storage hinder the outgrowth of mesophilic contaminants, including most pathogens, it favors the growth of psychrotrophic organisms, defined as those capable of growth at temperatures of <7°C regardless of optimal growth temperature. The psychrotrophic flora of raw milk is made up of predominantly gram-negative organisms (e.g., species of the genera Achromobacter, Aeromonas, Alcaligenes, Chromobacterium, Citrobacter, Enterobacter, Flavobacterium, Klebsiella, Pseudomonas, Serratia, and Yersinia), although gram-positive organisms are also encountered (e.g., Arthrobacter, Bacillus, Corynebacterium, Clostridium, Lactobacillus, Listeria, Microbacterium, Micrococcus, and Streptococcus) (19, 22). Most of these organisms in raw milk originate from soil, water, and vegetation, although fecal

<table>
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<tr>
<th>Region</th>
<th>Cooling requirements</th>
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<td>European Union (315)</td>
<td>Unless processing begins immediately after milking, or within 4 h of acceptance at the processing establishment, or the competent authority authorizes a higher temperature for technological reasons concerning the manufacture of certain dairy products, raw milk must be cooled to ≤8°C when collected daily and ≤6°C when not collected daily.</td>
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<td>United States (302)</td>
<td>Grade A raw milk for pasteurization must be cooled to ≤10°C (50°F) within 4 h of beginning milking and to ≤7°C (45°F) within 2 h of finishing and kept at ≤7°C (45°F) until it is processed. Grade B milk must be cooled to ≤10°C (50°F) within 2 h of finishing and kept at or below this temperature until it is processed. No specific federal regulation for raw milk for the production of cheese or raw milk products.</td>
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<td>New Zealand (323)</td>
<td>Unless used within 2 h from the completion of milking, raw milk used for the manufacture of raw milk products must be cooled to ≤7°C within 3 h from the completion of milking if collected daily or to ≤6°C within 2 h if collected every other day. Milk must also be no older than 48 h at the commencement of manufacture.</td>
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<td>Quebec, Canada (330)</td>
<td>Unless used within 2 h from the completion of milking, raw milk for the production of raw milk cheese aged less than 60 days must be cooled to ≤10°C (50°F) within 1 h and to ≤4°C (40°F) but &gt;0°C (32°F) within 2 h of finishing milking. The bulk milk must also be cooled to between 0 and ≤4°C within 1 h of the addition of subsequent milkings and maintained at this temperature. Milk must also be no older than 24 h at the commencement of manufacture.</td>
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material may contribute psychrotrophs to milk as well (22). When equipment is cleaned at lower temperatures and/or without sufficient concentrations of sanitizers, milk residues can form, allowing these organisms to adhere to surfaces of the milking equipment and accumulate in cracked rubber parts such as gaskets (23, 24). This, equipment may constitute a major source of contamination of milk, be it raw or pasteurized. Milk produced under sanitary conditions may contain lower proportions of psychrotrophic bacteria than milk produced under insanitary conditions (22). Some psychrotrophs, including species of the genera Arthrobacter, Microbacterium, Streptococcus, and Corynebacterium, in addition to Bacillus spores, may survive typical pasteurization time and temperature treatments (25). Although most psychrotrophs are eliminated by pasteurization, psychrotrophic bacteria often access milk from improperly cleaned and sanitized equipment. Additionally, extracellular proteases and lipases secreted by psychrotrophs in milk and milk products during refrigerated storage are heat stable and not readily inactivated by pasteurization. The presence and growth of psychrotrophs in milk and/or cheese and the production and presence of these heat-stable enzymes are responsible for numerous defects in cheese, including surface discoloration and off-odors, as well as off-flavors, including bitterness and rancidity (22).

The dominant psychrotrophic organisms encountered in raw milk are species of the genus Pseudomonas, including Pseudomonas fluorescens, P. fragi, P. putida, and, less commonly, P. aeruginosa. At low temperatures, Pseudomonas spp. have a short lag period and long stationary phase and can thus survive for long periods in milk residues (25). In addition to improper cleaning, Pseudomonas species, which are often present in water, can gain access to milk during the cleaning and/or rinsing of equipment. During refrigerated storage of milk and milk products, these motile, straight or curved gram-negative bacteria secrete heat-stable proteases and lipases. According to a review by Sorhaug and Stepaniak (25), low temperatures may actually induce the production of proteinases by Pseudomonas spp. Resulting enzymatic activity can alter cheese texture, resulting in running paste, and produce off-flavors, including bitterness and soapy rancidity (26). Proteolytic degradation of casein may also result in cheeses of higher-than-normal moisture (19), which can further exacerbate the aforementioned defects. Requiring oxygen for growth, contaminating pseudomonads often predominate on the surface of cheese, producing numerous volatile compounds (27), and are responsible for yellow to brown coloration of cheese rinds associated with bad ripening flora development as well as the water-soluble pigment pyoverdin, which fluoresces under UV light. While proper cleaning and sanitation and limiting time between milkings will help minimize contamination, milk should be processed as soon as possible to limit the growth of Pseudomonas spp. during refrigerated storage prior to cheesemaking. With water as a possible contamination source, care must also be taken to ensure that water used for brines and smear solutions is free of Pseudomonas spp. (28).

The other major group of organisms associated with water and improperly cleaned and sanitized equipment are the coliforms. Coliforms are a general group of bacteria of the family Enterobacteriaceae defined as aerobic and facultatively anaerobic, nonsporulating, gram-negative rods that ferment lactose, producing acid and gas, at 32 to 35°C in 48 h. Common genera within this group include Citrobacter, Escherichia, Enterobacter, and Klebsiella, with the last three considered fecal coliforms capable of producing acid and gas from lactose at elevated temperatures (≥44.5°C). As with the aforementioned psychrotrophs, the presence of coliforms in dairy products manufactured from pasteurized milk is typically due to postpasteurization contamination from insufficiently cleaned and sanitized equipment or from errors in the pasteurization process (17). Since some coliforms are associated with human and animal feces (e.g., Escherichia coli), these organisms often serve as indicators of fecal contamination in both raw milk and environmental testing. However, the presence of coliforms in raw milk is not always evidence of direct fecal contamination, as coliforms can rapidly build up in moist milk residues and in biofilms on milking equipment and thus contaminate milk. Coliforms are also a notable causative agent of mastitis where abnormally high levels of the contaminant can enter the milk supply. In the absence of infection, coliform concentrations exceeding 100 CFU/ml are often indicative of unhygienic production practices. Maintaining low coliform levels in cheese milk (<10 CFU/ml) is critical, as coliforms can multiply rapidly during early cheese manufacturing stages, when pH and temperature are favorable. This growth is often associated with the production of “yeasty,” “putrid,” “gassy,” and “unclean” off-flavors as well as the excessive production of carbon dioxide and hydrogen gas (29) linked to “early blowing” defects (Fig. 1). In the absence of sugar, gas formation depends on the presence of strains that can ferment citric acid, such as Enterobacter aerogenes (29). Coliforms do not grow well at low pH in higher-acid cheeses. However, it is not uncommon to find coliforms in washed-curd
cheese varieties that are lower in acid, cheeses with increasing pH during aging (e.g., surface-ripened varieties), or cheeses where the acid development was slower than normal. With residual sugar as a prime substrate, gas formation in pasta filata cheeses can lead to the presence of unwanted splits and eyes and puffy packages (28).

Although many cheeses are made from raw milk, most cheeses are made from heat-treated milk. Modern pasteurization eliminates a large proportion of spoilage bacteria and all vegetative bacteria pathogenic to humans. If produced from high-quality raw milk and subsequently handled under hygienic conditions, pasteurized milk should have a very low total bacterial count (15). Milk pasteurization requirements as described by the Codex Alimentarius Commission in the Code of Hygiene Practice for Milk and Milk Products (30) are the same as those observed in the United States and European Union (EU), where legal pasteurization requires specific time-temperature combinations, typically 72°C for 15 s or 63°C for 30 min or any other approved equivalent combination. Pasteurized milk products must also show a negative alkaline phosphatase test. Pasteurization does not completely inactivate populations of non-spore-forming thermoduric bacteria which survive 63°C for 30 min or bacterial endospores which survive heat exposures of 80°C for 10 min (31). Most, if not all, gram-negative bacteria (especially psychrotrophs) are destroyed along with many gram-positive organisms, while some thermoduric species of Enterococcus, Streptococcus (notably S. thermophilus), Microbacterium, Lactobacillus, Mycobacterium, and Corynebacterium may survive (32). Most nonsporulating thermoduric bacteria, including species of Micrococcus, Streptococcus, and Microbacterium as well as Corynebacterium, are not commonly associated with major cheese defects. Milder heat treatments such as thermization (e.g., 63°C for 10 to 15 s) are also effective in the destruction of large proportions of spoilage bacteria and are capable of reducing pathogen populations to various degrees. Milk for cheese manufacture is often thermized upon receipt at the cheese factory to eliminate spoilage bacteria, including most psychrotrophs (9), in an effort to minimize associated flavor and textural defects in cheese (10). In some countries, such as Canada, significant amounts of cheese are made from thermized milk, as this treatment results in less inactivation of enzymes and NSLAB important in cheese flavor development (10).

Heat-resistant sporulating bacteria are ubiquitous in the farm environment, accessing milk via teats contaminated by soil and bedding material. Those that originate in contaminated feeds, notably silage, are subsequently excreted in the feces and thus contaminate milk during collection. These organisms are also associated with contaminated milking machines, pipelines, gaskets and fittings, pump and receiver vessels, bulk tank surfaces, and tank outlet valves (33). Heat-resistant sporulating bacteria have even been isolated from extreme environments such as hot (80°C) alkaline solutions in reuse clean-in-place systems (34). The two most-discussed genera of spore-forming bacteria related to dairy products are Clostridium and Bacillus.

Clostridium spp. are obligate anaerobes and require the complete absence of oxygen for growth. With only certain strains capable of psychrotrophic growth, and the inability of spores to germinate in milk, clostridia are rarely implicated in the spoilage of dairy products (26). However, as reviewed by van den Berg and colleagues (9), certain species of Clostridium (Clostridium sporogenes, C. butyricum, and, most importantly, C. tyrobutyricum) are responsible for the defect known as “late blowing” in brine-salted hard and semihard cheeses such as Grana, Gouda, and Emmental (Fig. 2). These cheeses are particularly susceptible, as the relatively high initial pH and low salt, resulting from the slow diffusion after brining, allow for the growth of Clostridium. The defect, which typically appears after 1 to 4 months of aging, is characterized by the production of rancid off-flavors and excessive gas from the fermentation of lactic acid and the resulting production of butyric acid, acetic acid, carbon dioxide, and hydrogen gas. Although it is difficult to identify the exact number of spores related to gas

FIGURE 1 “Early blowing” gas defect in cheese at dehooping. doi:10.1128/microbiolspec.CM-0011-2012.f1
defects, as populations are not constant, studies have shown visual gas formation in naturally contaminated Gouda cheese after 8 weeks of aging with levels of C. tyrobutyricum of only 0.4 most probable number (MPN)/g (35). The actual number of spores necessary to cause blowing can vary according to the cheese type, shape, size, pH, ripening time, temperature, and structure. In the case of a single manufacturer, changes in pH and salt in moisture throughout the seasons or from batch to batch can also contribute to the variation in the observation as well as the extent of visible defects. Silage is considered the most important source of Clostridium spores which survive passage through the animal and are excreted in the feces where fecal contamination of the cow’s teats results in subsequent contamination of milk. Limiting the number of spores in milk is critical to prevention, and as a result, target spore levels as low as <1 spore per 10 ml of milk have been suggested for the production of high-risk cheeses such as Gouda (9, 36). In order to reduce the risk of milk contamination, levels in contaminated feeds must be kept to a minimum. The presence and concentration of clostridial spores in feeds are a function of the conditions encountered during ensiling where the viability of clostridia is a function of dry-matter content and pH. Although high-quality dry silages containing greater than 55% dry matter are inhibitory to clostridia, when the dry matter content is 50% or less, inhibition of Clostridium growth can be guaranteed only by rapid acid production and the subsequent drop in pH (37). Because bacterial spores have a higher density than the bacteria themselves, they can be removed via bactofugation or double bactofugation, a method that employs a type of centrifuge called a bactofuge, or through microfiltration of the skim milk fraction (9). Certain cheeses recognized with the appellation d’origine contrôlée, such as Emmental (Swiss), are produced from fresh raw milk using no milk treatment or additives. This in effect mandates that cheese milk come from animals that have not been fed silage.

In contrast to Clostridium, Bacillus spp. are aero-

bic, and many species are capable of psychrotrophic growth and thus more commonly found in milk and milk products. Of the Bacillus species, Bacillus cereus, B. licheniformis, B. mycoides, B. circulans, and B. coagulans are frequently isolated from pasteurized milk (1). As reviewed by Svensson and colleagues (38), spores of B. cereus frequently contaminate milk via teats contaminated by soil, feces, and bedding material and to some extent via feed and pasteurization and milking equipment. Proteolytic and lipolytic activities of B. cereus limit the shelf life of pasteurized milk and give rise to defects such as “bitty cream,” sweet curdling, and various off-flavors (39). Moreover, psychrotrophic Bacillus spp. secrete heat-resistant extracellular proteinases, lipases, and phospholipases which could also give rise to additional defects in cheese, including decreases in yield, texture and body defects, and the development of bitterness and various off-flavors, including rancidity (22, 33). Since it is unlikely that psychrotrophic sporeformers can be eliminated completely from raw milk, it is imperative to maintain healthy animals and clean equipment and to ensure rapid cooling of milk (33).

Various teat contaminants, including Pseudomonas spp., Streptococcus uberis, and Streptococcus dysgalactiae, as well as the coliforms E. coli and Klebsiella spp., can enter the teat canal and infect the interior tissues, resulting in inflammation referred to as mastitis. These organisms are therefore considered environmental mastitis pathogens. Conversely, pathogens such as Staphylococcus aureus, Streptococcus agalactiae, and Corynebacterium bovis are contagious and are transmitted from animal to animal. Although less common, and in some cases extremely rare, several human pathogens have been associated with mastitis, including coagulase-positive staphylococci (notably S. aureus), Corynebacterium diphtheriae, Streptococcus pyogenes, B. cereus, L. monocytogenes, Streptococcus equi subsp. zooepidemicus (40), Leptospira interrogans, Mycobacterium tuberculosis, Campylobacter jejuni, B. anthracis, and Brucella abortus. Since high levels of mastitis-related organisms can be transmitted to milk during infection, cases caused by human pathogens are of

FIGURE 2 “Late blowing” gas defect in aged cheese. doi:10.1128/microbiolspec.CM-0011-2012.f2
significant concern in terms of milk and cheese safety and are discussed in detail below.

In contrast to the majority of organisms discussed in this chapter, the presence of mastitis pathogens is indirectly linked to the quality of milk and cheese. During the inflammatory process, damage to epithelial cells by leukocytes, notably polymorphonuclear leukocytes, or by invasion of epithelial cells by pathogenic bacteria results in a decrease in the cells’ capacity for synthesis, resulting in decreased milk yield and decreases in lactose content as a result of osmoregulation. Inflammation also results in widened endothelial and epithelial junctions (41), ultimately resulting in a transfer of nutrients between blood and milk and vice versa (42). The influx of blood components in milk brings additional plasminogen (present in high levels in blood), which can be activated by plasminogen activators synthesized by polymorphonuclear leukocytes. Milk composition is affected by the increase in plasmin and nonplasmin proteolytic degradation of casein which can occur in the udder prior to milking when temperatures are more conducive to enzymatic activity (43). This degradation results in a decrease in intact αs- and β-caseins (43), with an increase in the γ-caseins and minor caseins (42). Such degradation can lead to reductions in casein available for curd formation, thereby reducing curd tension and curd firmness (44). Decreased curd tension increases the loss of fat, protein, and total solids in the whey at cutting and draining and thus decreases cheese yield (45, 46, 47, 48). It is generally accepted that high somatic-cell counts (SCCs) delay starter culture growth (49) and inhibit acid production (44), thus prolonging the fermentation process in cultured dairy products. Additionally, higher pH associated with high-SCC milk (50) and the reduction in available lactose may also affect the acidification profile. Elevated pH may also affect clotting enzymes whose activity is pH dependent (46), increasing clotting time and the rate of curd firming (47, 51). In experimental Cheddar cheese, total solids, fat, protein, and fat in dry matter all decrease with increasing SCC, while protein in dry matter and moisture-nonfat substance increase (52). Cheese composition, and therefore cheese quality, is greatly affected by increasing SCC in milk used for manufacture. According to a review by Coulon and colleagues (332), cheese manufactured from milk with SCC of >200,000 cells/ml displayed a decrease in firmness and elasticity and an increase in stickiness, which were correlated to an increase in cheese moisture. Textural problems may arise from the breakdown of αs1-casein, which is believed to be one of the major determinants of cheese texture (53). The secretion of macrophage-derived lipolytic enzymes during prolonged mammary gland infections can also damage the milk fat globule membrane, making it more susceptible to lipoprotein lipase activity (54) elevating the production of free fatty acids, which can result in “rancid” and “oxidized” off-flavors in cheese.

Yeasts and Molds

Although yeasts are found in freshly drawn milk, the spoilage of dairy products by yeasts and molds is usually a result of postpasteurization contamination, as both are ubiquitous airborne contaminants and are common in the dairy plant environment (28). Growth of yeasts and molds in milk gives rise to fruity and dusty odors, respectively (312). In cheese, they can metabolize lactic acid and hydrolyze protein, releasing ammonia and amino acids, resulting in an increase in pH as well as characteristic proteolysis-induced changes in texture. Although some airborne yeasts, such as Debaryomyces hansenii, Candida spp., and Kluyveromyces marxianus var. lactis, often contribute positively to the flavor of certain cheeses (26), characteristic heterofermentative metabolic spoilage by yeasts can produce ethanol and related fermented off-flavors as well as gas-related defects from the production of carbon dioxide. Yeast proteolysis can also produce odors reminiscent of rotten eggs, while lipolytic activity can lead to rancidity from the production of free fatty acids. Contamination with halotolerant yeasts, such as D. hansenii, present in brine or raw milk can result in the development of undesirable brown spots on blue cheese (55). A major factor contributing to yeast on cheese is a wet surface where yeast contamination can produce soft white spots. With brine solutions as a common contamination source, brined cheeses tend to be more susceptible to yeast contamination (28).

Molds require oxygen for growth and will therefore grow on most cheese surfaces exposed to air, including gaps in vacuum packaging and exposed surfaces in brined cheese. As with yeasts, some fungi, including Geotrichum candidum, Penicillium camemberti, and Penicillium roqueforti, are utilized intentionally in the manufacture of cheeses, including blue and surface-mold-ripened varieties. Despite their intended presence in the ripening of these cheeses, defects can still occur. For example, undersalting combined with insufficient draining, among other things, can cause excessive growth of G. candidum and hinder the implantation of P. camemberti, resulting in a defect referred to as “toad skin” in surface-mold-ripened cheeses. Similarly, undersalting can favor the implantation of Rhizomucor, producing the “cat hair” defect in this
cheese variety (56). Common contaminating molds isolated from cheese that may be undesirable include species of *Cladosporium* (especially the black mold *Cladosporium cladosporioides*), *Aspergillus*, *Fusarium*, *Mucor*, *Scopulariopsis*, *Verticillium*, and, most commonly, *Penicillium*, especially the blue mold *Penicillium commune* (28). As air contaminants, *Penicillium roqueforti* var. *roqueforti*, *P. commune*, *Penicillium palitans*, and *Penicillium solitum* are common contaminants of the semihard cheeses Norvegia and Jarlsberg (57, 58). According to Spinnler and Leclercq-Perlat (59), several species of *Penicillium* with grey, blue, or green spores are common environmental contaminants of surface-mold-ripened cheeses. Additionally, *Penicillium funiculosum* and *Penicillium bruneoviolaceum* may also produce purple and brown blots, respectively, in this cheese type. *Cladosporium herbarum*, a common contaminant of cold rooms, ceilings in ripening rooms, and air conditioning ducts, can develop dark green or black spots on white mold-ripened cheeses when the surface pH is abnormally high. Growth of contaminating *Penicillium* species, including *P. commune* and *Penicillium nalgiovense*, may also produce brown spots in blue cheese (55). Surface discoloration caused by mold growth is also a problem with smear-ripened cheeses, where, in addition to discolorations caused by penicillia, species of *Fusarium* can form slimy yellow growths on Tilsit-type cheeses, for example (60). In addition to visual defects, unwanted mold growth on cheese is often associated with musty and bitter flavors, while certain highly proteolytic molds cause sporadic spoilage and ammonia production in surface-ripened cheeses (26). One such mold, *Scopulariopsis brevicaulis*, is associated with wrapping papers stored under humid conditions and produces beige to purple blots on white mold-ripened cheese (59). In cheeses such as Gouda, surface mold growth negatively affects both the quality and appearance. To combat mold contamination and for appearance, some modern manufacturers apply plastic polymer coatings that serve as a physical barrier. These films may also be formulated to contain mold inhibitors such as sorbates and natamycin (9, 28). Air filtration, UV irradiation, and regular sanitation with fungicides can aid in the control of airborne contaminants. Proper ventilation also helps to remove moisture produced during processing and prevent condensation and subsequent mold growth on surfaces.

**Milk Quality Tests**

The most common method for enumerating bacteria present in raw milk is referred to as the standard plate count (SPC). In general, the SPC indirectly measures the number of aerobic bacteria that are capable of growth at 32°C. It is often recommended that producers aim to keep counts at <5,000 CFU/ml; counts of >10,000 CFU/ml are usually indicative of a problem. In a study by D’Amico and colleagues (61), 79.6% of raw bulk tank cheese milk samples analyzed had SPCs of <10,000 CFU/ml, while 43.2% had SPCs of <1,000 CFU/ml and 16.9% had SPCs of <100 CFU/ml. An expansion of this study in 2008 (62) that included additional artisan cheese operations revealed that 86% of raw cheese milk samples contained <10,000 CFU/ml, while 42% contained <1,000 CFU/ml.

The coliform count, as the name suggests, identifies coliform bacteria in milk and is used as an indication of the effectiveness of milking hygiene and the cleanliness of the animal’s environment. As previously stated, coliform counts should be <10 CFU/ml, although levels of <100 CFU/ml are often considered acceptable for the manufacture of cheese. Counts between 100 and 1,000 CFU/ml often indicate poor milking hygiene, while counts exceeding 1,000 indicate that bacterial multiplication is occurring somewhere along the line. In the aforementioned studies of raw milk destined for artisan cheese manufacture in Vermont, 61 to 68% of samples tested contained <10 CFU/ml, with 84 to 92% of samples containing <100 CFU/ml, as recommended by the Specialty Cheesemakers Association for raw milk for cheese (61, 62). Both studies found no significant differences in coliform levels between milking species (cow, goat, or sheep).

Other informative numbers often used to aid in diagnosing issues include the preliminary incubation (PI) count and the laboratory pasteurized count (LPC). Essentially, the PI count, which is used to identify psychrotrophic bacteria, is an SPC (incubation at 21°C) that is performed on milk that has been incubated at 21°C for 18 h. Although PI counts of <1,000 CFU/ml are recommended for keeping the quality of fluid milk, up to 50,000 CFU/ml is considered acceptable. The LPC is the SPC of milk that has been pasteurized at 62.8°C for 30 min, thereby allowing for the enumeration of thermoduric bacteria. As previously discussed, the presence of high levels of thermoduric bacteria is often associated with improperly cleaned and/or sanitized equipment. Since most coliform organisms do not survive pasteurization, milk with a high coliform count and SPC but a normal LPC would indicate poor milking hygiene. Typical LPC targets are less than 100 to 200 CFU/ml, while an LPC of <10 CFU/ml indicates excellent equipment hygiene. Testing for yeasts and molds can also be done when necessary. This test is a plate count that is conducted using media formulated to prevent the growth of bacteria. The
plates are also incubated at room temperature (\(\sim 25^\circ C\)) for 5 days to aid in isolating slow-growing fungi.

Since a single quality indicator value is not very useful diagnostically, it is best for producers to establish consistent average counts and identify sporadic “spikes.” In an effort to adjust for these anomalies when establishing average values, producers may want to consider utilizing geometric means as opposed to standard means. The geometric mean is a log transformation of data obtained over a period of time that tends to dampen the effects of outlying data. The EU employs this method to take into account seasonal variations because regulatory samples are taken multiple times over a 2- or 3-month period. Once average counts are established, significant variations can indicate issues and help elucidate the cause.

**MICROBIOLOGICAL SAFETY ISSUES RELATED TO CHEESEMAKING**

With the same routes of contamination as spoilage organisms, poor sanitation, improper milk handling, and animal health issues can result in both sporadic cases and outbreaks of dairy-related disease. Although many milk contaminants are pathogenic to humans, as shown in [Table 2](#), there are relatively few outbreaks of food-borne illness attributed to cheese considering the amount produced and consumed worldwide. While most organisms found in raw milk are successfully eliminated by pasteurization, the risk of food-borne illness applies to products manufactured from raw and heat-treated milks, especially in the case of inadequate or faulty processing, which may not adequately inactivate pathogens. The entry, establishment, and persistence of food-borne pathogens in dairy processing environments also present a risk of postprocessing contamination of dairy products.

**Pathogens Considered Low Risk in Cheesemaking**

The historical public health concerns associated with milk and milk products have been previously described in great detail in a comprehensive review by Ryser (40). Prior to World War II, the primary milk-borne diseases in the United States included typhoid (Salmonella enterica serovar Typhi) and scarlet fever (S. pyogenes) in addition to sporadic outbreaks of diphtheria (C. diphtheriae), poliomyelitis (poliovirus), and nonpulmonary tuberculosis (Mycobacterium bovis). However, with the gradual implementation of pasteurization, coupled with the modernization of milk production practices, the threat of these diseases was essentially eliminated (40). Additionally, the development and implementation of human immunization programs have all but eliminated dairy products as a source of C. diphtheriae infections in industrialized countries. The incidence of Brucella

<table>
<thead>
<tr>
<th><strong>Organism</strong></th>
<th><strong>Disease</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Gastroenteritis, invasive infections</td>
</tr>
<tr>
<td>EHEC</td>
<td>Toxicoinfection, hemorrhagic colitis, hemolytic-uremic syndrome (HUS) in children; thrombocytopenic purpura in adults</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>Gastroenteritis, invasive infections, typhoid fever (S. Typhi)</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>Gastroenteritis, invasive infections</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td><em>Brucella spp.</em></td>
<td>Brucellosis, undulant fever</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Gastroenteritis, invasive infection, Guillain-Barré syndrome, colitis, septicemia</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Gastroenteritis, invasive infections</td>
</tr>
<tr>
<td><strong>Gram-positive bacteria</strong></td>
<td><strong>Disease</strong></td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>Gastroenteritis, invasive infection, emetic intoxication</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>Gastroenteritis, invasive infections</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
<td>Botulism</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Emetic intoxication</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>Sore throat</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>Scarlet fever/sore throat</td>
</tr>
<tr>
<td><em>Streptococcus equi subsp. zooepidemicus</em></td>
<td>Pharyngitis, n hipmetic sequelae</td>
</tr>
<tr>
<td><em>Corynebacterium diphtheriae</em></td>
<td>Diphtheria</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Listeriosis</td>
</tr>
<tr>
<td><em>Mycobacterium bovis and M. tuberculosis</em></td>
<td>Tuberculosis</td>
</tr>
<tr>
<td><strong>Rickettsiae</strong></td>
<td><strong>Q fever</strong></td>
</tr>
<tr>
<td><em>Coxiella burnetii</em></td>
<td>Q fever</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td><strong>Enteric infection</strong></td>
</tr>
<tr>
<td><em>Enterovirus, including polioviruses, rotaviruses, coxsackieviruses</em></td>
<td>Enteric infection</td>
</tr>
<tr>
<td><em>Hepatitis virus</em></td>
<td>Infectious hepatitis</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td><strong>Mycotoxins</strong></td>
</tr>
<tr>
<td><strong>Molds</strong></td>
<td>Mycotoxins</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td><strong>Amebiasis</strong></td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>Amebiasis</td>
</tr>
<tr>
<td><em>Giardia lamblia</em></td>
<td>Giardiasis</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>Toxoplasmosis</td>
</tr>
<tr>
<td><em>Cryptosporidium parvum</em></td>
<td>Invasive infection, gastroenteritis</td>
</tr>
</tbody>
</table>

*Adapted from references 26 and 312.*

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**TABLE 2** Human pathogens associated with milk and milk products

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**ASMscience.org/MicrobiolSpectrum 9**
melitensis and Brucella abortus, the causative agents of brucellosis, has also been drastically reduced in industrialized nations as a result of animal immunization programs and the routine slaughtering of infected animals. Dairy-related cases of brucellosis are further prevented through milk pasteurization and the aging of cheeses made from raw or unpasteurized milk (40). Tuberculosis screening and control programs for cattle in the United States, Canada, and Western Europe have also limited the incidence of M. bovis in cattle and thus in the raw milk supply and dairy products. Because certain wild animals are also susceptible to M. bovis infections, total eradication in dairy animals is unlikely, which may present a particular problem in the manufacture of products from unpasteurized milk. Although incidence in dairy products is rare, it is important to note that numerous early studies have detailed the survival of M. bovis in cheeses prepared from naturally contaminated milk, where M. bovis reportedly survived at least 47 days in Camembert and Muenster cheeses, 62 days in Cheddar cheese, and 232 days in Tilsit cheese (40). Similarly, early studies also indicated that S. Typhi could survive both in and on cheese. For example, S. Typhi survived for 3 months and at least 10 months in Cheddar cheese made from experimentally inoculated milk and ripened at 15 and 5°C, respectively. With the contamination of raw milk, pasteurized milk, and other dairy products linked to symptomatic or asymptomatic carriers of S. Typhi, pasteurization and improved sanitary standards have drastically reduced the number of typhoid cases and human carriers and thus the contamination of milk and cheese (40).

One interesting organism of historical concern is Coxiella burnetii, the causative agent of Q fever and the organism whose heat resistance formed the basis for modern pasteurization requirements. According to Ryser (40), concern regarding Q fever as a milk-borne illness comes from the presence of C. burnetii in milk from infected milking animals and elevated titers of antibody to the organism observed in consumers who regularly drink unpasteurized milk. Q fever is usually transmitted to humans through aerosols or contaminated environments. The role of consumption of unpasteurized milk in acquisition of Q fever is controversial, as experimental evidence to support a causal relationship is scant. Unpasteurized milk and cheese have frequently been found to harbor viable C. burnetii but have rarely been implicated in transmission (63), and many associations between the consumption of these products and infection are only epidemiological (40, 64, 65). In a comprehensive review of the literature, Cerf and Condron (66) conclude that Q fever likely results only from inhalation of C. burnetii and sometimes through arthropod bites. The authors add that serological conversion following the ingestion of contaminated milk or milk products may result from the ingestion of live or dead cells and that seroconversion may indicate infection but not necessarily clinical disease. The risk of Q fever through the ingestion of cheeses from raw or pasteurized milk could therefore be considered of minimal concern. In fact, Cerf and Condron (66) state that Q fever should not even be considered a food-borne disease.

Between 1920 and 1960, a few small sporadic outbreaks of shigellosis resulting from Shigella dysenteriae infection were documented in the United States. Outbreaks generally involved raw milk contaminated by a human carrier that was then held without refrigeration for several hours. No additional cases of milk-borne shigellosis have been reported in the United States since (40). Cheese-related outbreaks appear to be even rarer, with one notable outbreak of Shigella sonnei in 1982 linked to French cheeses resulting in at least 50 cases of illness in Scandinavia (40, 67). As with other organisms of historical concern, improvements in personal hygiene standards coupled withpasteurization and refrigerated storage minimize the risk of milk- and cheese-borne shigellosis.

Unfortunately, despite the tremendous improvements made in dairy production, pathogens continue to emerge, as do public health concerns, although many are extremely rare in cases of cheese-borne illness. From 1973 to 1992, 46 raw milk-associated food-borne disease outbreaks resulting in 1,733 illnesses were reported to the Centers for Disease Control and Prevention (CDC), with Campylobacter and Salmonella species causing a majority of cases (68). In 1987, unpasteurized milk and milk products in final package form intended for human consumption were banned from interstate commerce (333). Although these products are still permitted for intrastate sale in some states, the mean annual number of reported outbreaks attributed to milk decreased dramatically following the ban (68). Campylobacter contamination of milk typically occurs through fecal contamination of milk during collection, though subclinical mammary gland infections have been described. As a human pathogen, C. jejuni is associated with invasive infection, gastroenteritis, Guillain-Barré syndrome, colitis, and septicemia (26), which typically occurs in children. Following numerous outbreaks of campylobacteriosis associated with consumption of raw or inadequately pasteurized milk both domestically and abroad, C. jejuni emerged as an important milk-borne
pathogen. As shown in Table 3, C. jejuni is not as hardy as other milk-borne pathogens and is quite sensitive to salt, acidity, heat, and the presence of oxygen and needs a rather high water activity (a_w) for growth and survival. It is therefore not surprising that this organism shows limited survival in cheese challenge studies. Very few outbreaks of C. jejuni infection associated with cheese consumption have been reported compared to those attributed to unpasteurized milk. According to the Wisconsin Department of Health and Family Services, an outbreak in 2006 involving over 100 suspected cases of illness was attributed to consumption of contaminated raw milk cheese curds manufactured by an unlicensed cheesemaker (http://www.campylobacterblog.com/campylobacter-watch/bad-cheese-curdst-now-responsible-for-over-100-illnesses/). In 2007, 67 members of an insular religious community suffered gastrointestinal illness after eating fresh cheese made from unpasteurized milk at a local fair. The cheese was produced at an activity station at the fair by adding rennet extract to the raw milk, producing a soft cheese in 5 to 6 h which was then served at a banquet the same evening. Although all samples of leftover cheese and unpasteurized milk from the dairy tested negative for Campylobacter, an epidemiological association was made between illness and consumption of the fresh cheese (69). Queso fresco purchased in Tijuana, Mexico, was implicated in an outbreak of gastroenteritis in San Diego County, CA, in 2003. Queso fresco was again implicated in 10 cases of campylobacteriosis in Oregon in 2008 (http://www.washoe.gov/foodborneoutbreaks/Default.aspx) and in 2010 when a small child developed campylobacteriosis after eating homemade queso fresco sold door to door in Nevada. The implicated cheese was made in a home, otherwise known as “bathtub cheese,” instead of under local and federal food safety standards (http://www.washoe.co.us/health/pr/pr.html?year=2010&month=5&article=5702#a5702). Given the results of scientific challenge studies and the lack of outbreaks attributed to licensed domestic producers, properly manufactured cheese is considered a highly improbable vehicle for campylobacteriosis (40).

Previously discussed as a spoilage organism, B. cereus is also pathogenic to humans when present at extremely high levels, causing both diarrheal and emetic syndromes. The diarrheal syndrome results from a heat-labile enterotoxin that is presumably produced during growth of vegetative cells within the small intestine, whereas the emetic syndrome results from ingesting a heat-stable toxin preformed in food. B. cereus spores are unique in that they are among the few pathogens which will survive pasteurization and grow at low temperatures. In a comprehensive review, Ryser (40) explains that the presence of enterotoxin in pasteurized milk is typically associated with B. cereus populations greater than 10^7 CFU/ml. At this level, milk frequently shows obvious spoilage and thus would not be used for cheese manufacture. This, in addition to the inability to germinate in raw milk, may explain the lack of milk-borne cases of B. cereus poisoning. Outbreaks of cheese-borne B. cereus food poisoning are virtually nonexistent for a case traced to feta cheese in Canada. In fact, most dairy-associated outbreaks have been small and typically linked to contaminated nonfat dry milk used as

<table>
<thead>
<tr>
<th>Organism</th>
<th>Minimum a_w</th>
<th>Minimum–maximum temp (°C)</th>
<th>Maximum salt (%)</th>
<th>Minimum–maximum pH</th>
<th>Oxygen needs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>0.92</td>
<td>4–55</td>
<td>18</td>
<td>4.3–9.3</td>
<td>Aerobe</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>0.987</td>
<td>30–45</td>
<td>1.5</td>
<td>4.9–9.5</td>
<td>Microaerophile</td>
</tr>
<tr>
<td>Clostridium botulinum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type A, and proteolytic B and F</td>
<td>0.935</td>
<td>10–48</td>
<td>10</td>
<td>4.6–9.0</td>
<td>Anaerobe</td>
</tr>
<tr>
<td>Type E, and nonproteolytic B and F</td>
<td>0.97</td>
<td>3.3–45</td>
<td>5</td>
<td>5.0–9.0</td>
<td>Anaerobe</td>
</tr>
<tr>
<td>Pathogenic Escherichia coli</td>
<td>0.95</td>
<td>7.0–49.4</td>
<td>6.5</td>
<td>4.0–9.0</td>
<td>Facultative anaerobe</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>0.92</td>
<td>–0.4–45</td>
<td>10</td>
<td>4.4–9.4</td>
<td>Facultative anaerobe</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>0.94</td>
<td>5.2–46.2</td>
<td>8</td>
<td>3.7–9.5</td>
<td>Facultative anaerobe</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>0.96</td>
<td>6.1–47.1</td>
<td>5.2</td>
<td>4.8–9.3</td>
<td>Facultative anaerobe</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth</td>
<td>0.83</td>
<td>7–50</td>
<td>25</td>
<td>4.0–10.0</td>
<td>Facultative anaerobe</td>
</tr>
<tr>
<td>Toxin production</td>
<td>0.85</td>
<td>10–48</td>
<td>10</td>
<td>4.0–9.8</td>
<td>Facultative anaerobe</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>0.945</td>
<td>–1.3–42</td>
<td>7</td>
<td>4.2–10.0</td>
<td>Facultative anaerobe</td>
</tr>
</tbody>
</table>

*Adapted from reference 331.*
an ingredient (40). Because such high concentrations of *B. cereus* are necessary to induce illness, dairy-related outbreaks of *B. cereus* poisoning are readily prevented by minimizing contamination of raw milk at the farm level through general hygiene measures related to feeding, milking, and milk storage. In fermented dairy products, pH serves as a significant hurdle to the growth of *B. cereus*, with only minimal growth observed at pH values lower than 5; thus, the pH of properly manufactured dairy products is often low enough to inhibit spore germination and growth of vegetative cells. It is therefore believed that low levels of *B. cereus* in properly fermented dairy products are of minimal public health concern (40).

*Clostridium botulinum* is the causative agent of botulism, a rare and fatal disease that results from the ingestion of minute amounts of preformed neurotoxin produced by all strains of this organism (40). Although *C. botulinum* is rarely found in raw milk, at least 20 human outbreaks of botulism have been linked to milk or milk products, most of which have been related to cheese and cheese products (70) where in many cases the presence of *C. botulinum* in cheese resulted from cross-contamination. In the remaining cases where milk was the likely source, products were often prepared at home, packaged under anaerobic or low-oxygen conditions, and/or temperature abused (40, 70). Ripening temperatures in the range of 12 to 25°C employed for many cheese varieties are suitable for spore germination, growth, and toxin formation (70). Although cheese does not spoil as readily at increased temperatures and enjoys a longer shelf life than fluid milk, intrinsic cheese factors such as a$_w$, pH, lactate levels, and fat concentration in addition to competitive organisms all aid in controlling *C. botulinum* growth and toxin formation (70). For example, hard cheeses do not generally pose a risk due to their relatively low a$_w$ and pH, while soft and semisoft cheeses with a higher a$_w$ rely on the combined inhibitory effects of pH and temperature. Although young cheeses with a pH around 5 are not generally considered a botulism hazard, the increase in pH observed in surface-ripened cheeses may eventually support growth and toxin production (70) in the absence of oxygen. However, as is the case with the other pathogenic sporulating bacterium *B. cereus*, *C. botulinum* is not considered a major pathogen of concern in most cheeses.

Surveys conducted in numerous countries have shown that the psychrotrophic pathogen *Yersinia enterocolitica* is relatively common in raw milk, with reported prevalence rates ranging from 10 to 81.4% (67). Despite this prevalence, *Y. enterocolitica* is not generally regarded as a cause of milk-borne illness because of the low incidence of strains that are pathogenic to humans in the raw milk supply. As a postpasteurization contaminant *Y. enterocolitica* has been isolated from, and associated with outbreaks linked to, pasteurized milk (40, 67). According to Ryser (40), yersiniosis, the disease caused by infection with *Y. enterocolitica*, varies depending on the strain and amount of the organism consumed as well as the immune status of the person infected, with gastroenteritis as the most common manifestation. This illness is normally self-limiting and of short duration, with symptoms subsiding after 1 to 3 days, although generalized septicemia, the second major manifestation of yersiniosis, can occur in the severely immunocompromised with a mortality rate of more than 50% (40). With yersiniae rarely recovered from cheese, or any fermented dairy product for that matter, and the lack of reported outbreaks, it is suggested that fermented dairy products manufactured under good sanitary conditions can be considered of minimal risk. With swine as the major source of strains pathogenic for humans and the high incidence of yersiniae in dairy processing facilities, it is imperative to minimize cross contamination and the risk of postprocessing contamination (40).

Although parasites and viruses can cause potentially serious health problems, they are each responsible for less than 1% of all dairy-related illnesses (40). Often transmitted via the fecal-oral route, viruses are obligate intracellular parasites, dependent on a living host in order to replicate, and are therefore only transmitted by, and not replicated in, food and water (71). Tick-borne encephalitis virus infects dairy animals and is subsequently shed in their milk (71), with raw milk of various species implicated in outbreaks of tick-borne encephalitis virus infection (72). As reviewed by Jooste and Anelich (73), investigations utilizing a variety of viruses have demonstrated their survival in raw, boiled, and pasteurized milks as well as cottage and Cheddar cheeses. However, according to O’Mahoney et al. (74), viruses that may be present in milk do not generally pose a public health hazard.

According to Ryser (40), cryptosporidiosis as a milk-borne disease is growing outside the United States, with outbreaks linked to consumption of raw milk and improperly pasteurized milk. However, aside from *Cryptosporidium parvum*, protozoa, including *Cyclospora* and *Giardia*, have not been linked to documented outbreaks of milk- or cheese-borne illness. Despite the fact that *S. zooepidemicus* is a causative agent of subacute and chronic mastitis in milking animals, human infections caused by this organism are generally uncommon and are generally acquired through the consumption of raw milk. One outbreak was, however, linked to fresh queso blanco which was illegally prepared from raw milk and consumed without aging (40).
Mycotoxins can be present in natural cheese as a result of their presence in cheese milk or from mold growth on finished cheese. Raw milk from all major milking species may contain mycotoxins, particularly aflatoxins. According to a comprehensive review by Galvano and colleagues (75), aflatoxin B1 from feed is metabolically converted to aflatoxin M1 (AFM1) and subsequently excreted in milk. Thus, the most important preventative measure is the exclusion of aflatoxin-contaminated feeds, and many countries have established limits for aflatoxins in animal feed. Galvano et al. (73) concluded that although AFM1 is fairly stable during the ripening and storage of numerous cheeses, the levels of AFM1 in milk and milk products do not seem to pose a serious health hazard. As previously reviewed by others (67, 76), some ripening molds have also been shown to produce mycotoxins, including isolates of *P. camemberti* and *P. roqueforti*. It was reported that in most cases either the toxin is produced at very low levels in cheese, if at all, or the toxic compounds produced are of very low toxicity. In addition to limiting mold contamination of feed, growth of toxigenic fungi on cheese can be controlled by careful cleaning and disinfection and the use of filtered and/or treated air.

One often overlooked health concern related to cheese consumption is that of biologically active amines formed by microorganisms through the decarboxylation of amino acids. The most toxic of the biogenic amines in cheese are histamine, tyramine, and β-phenylethylamine (77). Various amounts of histamine have been detected in a wide range of cheeses, and histamine poisoning has been associated with Gouda, Swiss, Cheddar, Gruyère, and Cheshire cheeses (77, 78). However, the true incidence of cheese-borne illness attributed to biogenic amines is unknown because the disease is mild, easily misdiagnosed, and therefore likely underreported. Numerous histamine-producing strains have been identified, including, but not limited to, *Streptococcus faecium*, *Streptococcus mitis*, *Streptococcus lactis*, *L. casei*, *Lactobacillus acidophilus*, *L. fermentum*, *Lactobacillus helveticus*, *Lactobacillus bulgaricus*, *L. plantarum*, *Enterococcus faecalis*, *Bacillus macerans*, and propionibacteria (77, 78). Studies on a histamine-producing strain of *Lactobacillus buchneri* in Gouda reveal that increases in ripening temperature, pH, and salt concentration all appear to increase histamine production. Conversely, the availability of substrate may limit the accumulation of biogenic amines in cheese (78). Although most cheeses do not usually contain biogenic amines, they can accumulate to elevated levels during extended aging (77). The use of high-quality milk is essential in the prevention of such accumulation, especially in the case of cheeses manufactured from raw or unpasteurized milk.

### Pathogens of Concern in Cheesemaking

Beginning in the 1970s, changes in agricultural practices and food processing operations and the globalization of the food supply have resulted in the emergence of newly recognized food-borne pathogens in the United States (79), including *L. monocytogenes*, multidrug-resistant *Salmonella* species, and Shiga toxin-producing strains of *E. coli* (STEC).

#### Staphylococcus aureus

Following the marked decrease in incidence of milk-borne typhoid and scarlet fever, staphylococcal poisoning emerged as the major milk-borne illness from the late 1930s through the 1960s (40). Staphylococcal food poisoning occurs not as the result of the ingestion of the organism itself but through ingestion of preformed heat-stable enterotoxins produced by some strains of *S. aureus* (80). Although two additional coagulase-positive species (*Staphylococcus hominis* and *Staphylococcus intermedius*) and 10 coagulase-negative species contain toxigenic strains, the overwhelming majority of reported cases of staphylococcal food poisoning have been linked to *S. aureus* (40). Once ingested, staphylococcal enterotoxins (SEs) act on emetic receptors in the intestinal wall, producing nausea, vomiting, retching, diarrhea, and abdominal cramps within 1 to 6 h following ingestion of the contaminated food. Recovery generally takes 1 to 2 days, with infection rarely resulting in complications or hospitalization (40). In addition to the classical types (SEA, SEB, SEC, SED, and SEE), extensive sequence data have led to the discovery of novel SEs and staphylococcal enterotoxin-like superantigens whose potential roles in staphylococcal food poisoning in many cases have yet to be confirmed (81).

A major causative agent of mastitis, *S. aureus* is one of the most common contagious pathogens infecting dairy cows, and it is commonly isolated from the raw milk of common milking species. Average incidence rates for raw cow milk are in the range of 20 to 30%, while the incidence for goat and sheep milk is typically somewhere between 30 and 40% (61, 82, 83, 84). Some surveys, however, report prevalence rates as high as 75 and 96.2% for cow milk and goat milk, respectively (85, 86). With 30 to 50% of the population asymptptomatically carrying *S. aureus* in their nostrils and on skin and hair, milk and milk products can also become contaminated before or after heat treatment during processing and handling (80). Results of a study by Tondo and colleagues (87) suggest that personnel may not play a major role in the contamination of dairy products with *S. aureus*, especially compared to raw milk. Equipment
and machinery are not identified as potential sources of contamination. Most outbreaks linked to the use of raw milk have been traced to mastitis, whereas contamination of processed products occurs postpasteurization through improper handling and human transmission (40). The proportion of dairy-related illnesses from staphylococcal poisoning in the United States has decreased substantially in the past 40 years as a result of increased monitoring of mastitis in dairy cattle coupled with improved sanitation and the implementation of pasteurization (40). Despite similar improvements, *S. aureus* was reported as the leading cause of food-borne disease related to milk and milk products in France (88), possibly resulting from the use of raw milk.

As shown in Table 3, *S. aureus* has been shown to grow in foods over a wide range of *a*<sub>w</sub> levels. While growth limits vary in foods, *S. aureus* has been shown to grow in laboratory media at *a*<sub>w</sub> levels as low as 0.86 (89). Enterotoxin production under various *a*<sub>w</sub> levels differs by toxin type, with SEA (90) and SED (91) produced over the range of *a*<sub>w</sub> levels suitable for *S. aureus* growth, while SEB and SEC are more sensitive to changes in *a*<sub>w</sub> (90, 91). *S. aureus* is also quite acid tolerant, with some strains capable of growth in acidic environments as low as pH 4.0 (92). Acid tolerance varies depending on the particular acid in use: acetic and propionic acids, which have higher *p*K<sub>a</sub> values, are more inhibitory than lactic acid (93). Some strains are more sensitive to acidification when the salt concentration is high, resulting in lower *a*<sub>w</sub> (93). While SE production has been observed at pH values as low as pH 4.8 in buffered laboratory media, most strains produce SEs around pH 5.1 and higher (94). Once formed, SEs are resistant to the heat treatment and low pH conditions that destroy the bacteria that produce them (80).

Despite the presence of *S. aureus* in market cheeses of all varieties (see, for example, reference 95), notable outbreaks related to cheese are relatively rare, as *S. aureus* is generally considered a poor competitor in the presence of active starter culture. The inhibitory effect of LAB is likely linked to nutritional factors, including the production of inhibitory metabolites, rather than just simple nutritional competition (93). LAB antagonism not only may hinder growth and survival but also may interfere with the modulation of the expression of virulence factors, thus limiting its pathogenicity (93). Although the organism is readily inactivated by standard heat treatments, outbreaks related to cheese manufactured from pasteurized milk occur as a result of SE production prior to heat treatment or as a result of postpasteurization contamination. One such U.S. outbreak occurred in 1981, with 16 cases of staphylococcal food poisoning linked to the consumption of a pasteurized milk cheese (96). In this case, milk was contaminated postpasteurization prior to the addition of starter culture, allowing for *S. aureus* growth and resulting in SE production (80). An outbreak of staphylococcal poisoning sickening 42 persons was linked to Cheddar, Monterey, and kuminost cheeses manufactured from pasteurized milk contaminated postpasteurization. Delayed starter activity and abnormal acid development likely contributed to pathogen growth and subsequent toxin production (40, 97, 98). Contaminated raw milk has also been implicated in cheese-borne infections, including 28 cases of illness traced to raw milk cheese in Scotland, where SEA was present in the incriminated cheese in the absence of culturable organisms. Contamination was associated with postinfection carriage and clinical illness in sheep on the farm. Follow-up investigations revealed that *S. aureus* isolates capable of producing SEA were sporadically present in the bulk tank milk for nearly 2 years in the absence of clinical illness (99). Although the details are unclear, Minas-type cheese heavily contaminated (7 to 8 log CFU/g) with *S. aureus* strains capable of producing enterotoxins A, B, D, and E (100) was implicated in outbreaks in Brazil in 1987 (100) and 1993 (101). Additional outbreaks have been linked to the consumption of raw milk Vacherin Mont-d’Or in Switzerland and Stilton in England (86).

The results of numerous investigations identify SEC as a common SE elaborated in milk and by strains isolated from milk (102, 103, 104, 105). Similarly, several investigators identified SEC and SED, either alone or in combination, as the most common toxin types elaborated (106, 107, 108). This is important to note, as strains producing SEA and SED were implicated in the majority of staphylococcal food poisoning outbreaks and cases in the United Kingdom (109, 110) and France (111), including those associated with the consumption of raw milk cheese.

Overall, initial milk population levels appear to have the strongest influence on levels attained in cheese (112), while subsequent growth is influenced by starter activity and acidification. Thus, the ratio of LAB to *S. aureus* determines the efficiency of inhibition (93). Therefore, the best protection from enterotoxin production in cheese of all varieties begins with ensuring that populations of *S. aureus* in milk, both raw and pasteurized, are kept low (under 100 CFU/ml [112]). Average *S. aureus* contamination levels in milk used for raw
Milk cheesemaking reported in the literature range from 20 to 250 CFU/ml (61, 62, 85), while the Specialty Cheesemakers Association suggests a limit of 500 CFU/ml. Use of active starter cultures and rapid acidification inhibits the growth and thus helps control enterotoxin production thereafter. In general, raw milk contaminated with low levels of S. aureus does not necessarily present a food safety risk, as virulence lies in the production of heat-stable enterotoxins, which generally occurs when populations exceed 5 log CFU/ml. The behavior and enterotoxin production of S. aureus in cheese vary depending on the interaction of numerous factors, including cheese type, activity, amount and type of starter culture utilized, competition for nutrients, and decreasing pH as well as the production of H2O2, undissociated weak acids, and antistaphylococcal substances (89, 93). Without the addition of starter bacteria, S. aureus naturally occurring in raw milk (at low initial levels) may exhibit growth during manufacture of rennet-coagulated fresh soft cheese (e.g., Tenerife), carrying over into young cheese. S. aureus levels typically decline shortly thereafter in the presence of dense populations of other raw milk organisms (113). Growth appears to be limited in fresh soft cheeses prepared by lactic coagulation from the activity of starter culture (114). Vernozy-Rozand and colleagues (115) observed the marked decline in S. aureus counts during the draining, salting, and ripening of goat’s milk lactic cheese. Negligible levels of SEA and a decline in S. aureus population levels were detected from initial milk populations of 5 to 6 log CFU/ml, with population declines attributed to low pH and the presence of salt. S. aureus has been shown to survive better during the manufacture and ripening of Camembert-type cheese, in which it is likely that the increase in pH provides a favorable environment for the organism. While SEA could be detected in cheeses when counts exceeded 6 log CFU/g of cheese, SEA was undetectable in most cheeses when initial counts in milk were ≤3 log CFU/ml (116). S. aureus has also been shown to survive during the manufacture and ripening of hard cheeses such as Manchego (117), resulting in SEA and SED production in cheeses made from milk with initial counts of >4 log CFU/ml. This observation was attributed to S. aureus multiplication prior to pH reaching inhibitory levels as the result of active starter culture. Thus, ensuring low initial levels of S. aureus in milk (≤3 log CFU/ml) may also aid in preventing the formation of toxin (SEC [114]).

Between 1 January 2004 and 31 December 2006, the U.S. Food and Drug Administration (FDA) tested a total of 17,324 cheese samples as part of the Domestic and Imported Cheese and Cheese Products Compliance Program. S. aureus was the most commonly detected pathogen of concern, being present in 6.9% of the 3,449 cheese samples tested. Overall, contamination rates were similar for domestic and imported cheeses, ranging from 5.5 to 9.8% throughout the sampling period. A substantial proportion of cheeses testing positive were Mexican-style soft cheeses (38.1%), with the vast majority coming from Mexico or Central America (86.8%). Despite the presence of elevated levels of S. aureus in some cheese samples, including two samples of goat cheese with levels of 18,000,000 MPN/g, the detection of enterotoxin was not reported. The lack of detectable enterotoxin in the cheeses tested as part of the FDA compliance program could be explained by any one of the aforementioned factors. It is also possible that the strains detected in these cheeses were not toxigenic or produced toxins not detected by the methods employed (118). Although the toxigenicity is not reported in their review, Koutsa and colleagues report S. aureus prevalence rates ranging from 0 to 25% in various cheese types in Italy, Egypt, Brazil, and Germany (86).

Salmonella

Salmonellae are facultatively anaerobic, straight, usually motile (by peritrichous flagella) gram-negative rods belonging to the family Enterobacteriaceae (119). According to the latest nomenclature guidelines published by the World Health Organization Collaborating Centre for Reference and Research on Salmonella, which reflect recent advances in taxonomy (120), the genus Salmonella consists of only two major species: Salmonella enterica and S. bongori. Recent unpublished data suggest that S. subterranea does not actually belong in the genus Salmonella (120). Salmonella enterica is further subdivided into six subspecies with numerous synonyms: Salmonella enterica subsp. arizonae, Salmonella enterica subsp. diarizonae, Salmonella enterica subsp. indica, and Salmonella enterica subsp. salamae (121). Salmonellae are further subdivided in the Kauffmann-White’s scheme into over 2,500 serotypes based on antigenic characterization (119). The greatest number of serotypes belongs to S. enterica subsp. enterica, often named for the geographic location of an outbreak (e.g., Montevideo, referred to as S. Montevideo), or by their antigenic formula. Despite the vast number of serotypes, all salmonellae are considered pathogenic, producing infections from mild gastroenteritis to typhoid fever. In some cases, septicemia can occur as a complication of gastroenteritis, which can be fatal in immunocompromised patients.
hosts. Prolonged septicemic infections can result in localized tissue and organ infections, especially in those previously damaged or diseased (40). The severity and duration of symptoms depend upon the type and amount of organisms consumed, as well as host susceptibility, with illness typically lasting between 2 and 6 days (122). One biochemically distinct serovar, S. Typhi, is responsible for bacteremia-related enteric fever referred to as typhoid fever.

Salmonella spp. can also infect dairy cattle and other ruminant animals, resulting in symptomatic or asymptomatic fecal shedding (40). Therefore, the intestinal tracts of dairy cows serve as a major reservoir for human food-borne salmonellosis via fecal contamination (122). Although uncommon, some strains have been associated with mastitis and can therefore be shed in milk (123). Salmonella incidence rates for raw milk in Europe range from 0 to 2.9% (21, 82, 124). While low incidence has been reported in Canada (0.17% [125]), higher isolation rates, from 1.5 to 8.9% of cow milk samples, have been reported in the United States (126, 127, 128, 129, 130, 131), varying with geographic location. No Salmonella was detected in 234 samples of raw milk intended for the production of raw milk cheese collected over two manufacturing seasons (61, 62). Incidence data for small-ruminant milk are limited, but the detection of Salmonella spp. has been reported for goat milk (132). Data on incidence of Salmonella spp. in cheese are also limited. A total of 3,520 cheese samples were analyzed by the FDA for the presence of Salmonella spp. between 1 January 2004 and 31 December 2006 as part of the Domestic and Imported Cheese and Cheese Products Compliance Program, with 1.3% testing positive. In general, the majority of samples came from soft or soft-ripened cheeses produced in Mexico or Central America (118).

Numerous documented outbreaks of nontyphoidal salmonellosis have been linked to the consumption of both raw and pasteurized milk and milk products (40, 133). According to Headrick and colleagues (68), salmonellae were responsible for more than one-quarter (331 cases) of the 46 raw fluid milk-associated disease outbreaks reported in the United States from 1973 to 1992, second only to Campylobacter species. Both pasteurized milk (134) and inadequately pasteurized milk (135) have also been linked to outbreaks of salmonellosis. Aside from milk, numerous outbreaks of salmonellosis involving cheese have been reported since 1976 (40). Overall, outbreaks of cheese-borne salmonellosis are generally the result of a few common practices which represent focal points in the control of salmonellae in both raw and pasteurized milk cheese. Notable examples include the use of raw (122, 136, 137, 138, 139, 140, 141) or inadequately pasteurized (142) milk coupled with noncompliance with good manufacturing practices and inadequate control programs (123). Raw milk can be contaminated by an infected herd (122, 143), while pasteurized milk and cheese can be cross contaminated by equipment (144) as well as by other farm animals, such as chickens (145). Numerous outbreaks have also resulted from contamination by ill employees (143, 146). In 1998, a major Canadian outbreak of Salmonella enterica serovar Enteritidis phage type 8 which sickened nearly 700 people, most of whom were children, was associated with the consumption of contaminated pasteurized Cheddar cheese contained in prepackaged lunch products (147). Additional reported outbreaks in other countries of origin are outlined in a review by Kousta and others (86).

Salmonellae can also grow quite readily in acidic environments and at moderately low temperatures. In general, foods held below 5°C do not support the growth of salmonellae, whereas the minimal pH in which growth is observed varies depending on acid type, temperature, available oxygen, growth medium, level of inoculum, and serotype (122). Early challenge studies demonstrated survival of salmonellae in Cheddar cheese for 2 to 9 months dependent upon pH as well as the type and amount of starter culture utilized. Cheeses with abnormally high pH values as a result of starter culture failure displayed little inhibitory activity, whereas cheeses with pH values between 5.2 and 5.3 caused inactivation (148). According to reports, White and Custer (149) found that salmonellae (S. enterica serovars Newport, Newbrunswick, and Infantis), when initially present in large numbers in cheese milk (~5 log CFU/ml), can survive as long as 9 months in Cheddar cheese (40, 122). Similar results were obtained by Park and colleagues (150), who found that salmonellae survived up to 7 and 10 months at 13°C and 7°C, respectively. It is likely that pathogen growth and survival were related to the relatively high cheese pH values (~5.75 and 5.9). Moreover, the cheese in the latter study was of high moisture (~43%). Goepfert and others (151) examined the fate of S. Typhimurium during the manufacture and aging of stirred-curd Cheddar cheese from cheese milk inoculated with 1 to 3 log CFU/ml. Following an initial increase in cell numbers during manufacture, the number of salmonellae decreased by a factor of 10,000 after 10 to 12 weeks at 13°C and 14 to 16 weeks at 7.5°C. It is important to note that the mean pH of the cheese after overnight pressing in this study was 5.1, far lower than
in the aforementioned studies. Moisture and salt contents, however, were not provided. An important factor to consider when reviewing challenge studies is that most have employed nonstressed cultures which do not account for the adaptive responses to acidity and osmolarity that may promote growth and survival in environments hostile to nonadapted cells (152). Acid-adapted cells of S. Typhimurium display increased resistance to inactivation by organic acids commonly present in cheese, including lactic, propionic, and acetic acids. Acid-adapted cells have also shown enhanced survival during milk fermentation by LAB compared to that of nonadapted cells. Similarly, acid adaptation promotes persistence of S. Typhimurium as a surface contaminant in Cheddar, Swiss, and mozzarella cheeses when held at 5°C (152). Cold-adapted S. Enteritidis has also been shown to survive and grow during the storage of both low- and full-fat cream cheese (153).

**Escherichia coli**

*E. coli* organisms are facultatively anaerobic, gram-negative rods of the family *Enterobacteriaceae* and are commonly found in soil and water. *E. coli* organisms also constitute part of the normal intestinal flora of humans and other warm-blooded animals (119). While many strains are innocuous inhabitants, some are capable of causing disease in humans. Strains of *E. coli* are serotyped on the basis of three major surface antigens: O (somatic), H (flagellar), and K (capsular). Specific serotypes and serogroups are often associated with certain clinical syndromes and therefore serve as identifiable chromosomal markers that correlate with specific virulence factors (154).

Currently there are at least six classes of *E. coli* known to cause food-borne gastrointestinal disease in humans (155) categorized by their virulence properties, pathogenic mechanisms, clinical syndromes, and O:H serogroups (154). Enteropathogenic *E. coli* is typically associated with infant diarrhea in developing countries, characterized by attaching and effacing lesions resulting from the intimate bacterial adherence to epithelial cells and degeneration and effacement of intestinal cell microvilli (156). Enterotoxigenic *E. coli* (ETEC) is also associated with infant diarrhea in developing countries as well as traveler’s diarrhea in individuals traveling to developing countries. In addition to a variety of intestinal colonization factors, ETEC strains produce heat-stable and/or heat-labile (similar to the cholera toxin) enterotoxins that stimulate the lining of the intestines, resulting in excessive fluid secretion, thus producing diarrhea (155). Although outbreaks of illness attributable to ETEC are extremely rare, imported surface-mold-ripened semisoft cheese was implicated in a multistate outbreak of ETEC illness (157). Enteroinvasive *E. coli* is so named for its ability to invade, proliferate in, and subsequently spread between the epithelial cells of the colon, damaging the epithelial lining. This generally results in watery diarrhea often containing blood, mucus, and polymorphonuclear leukocytes typical of *Shigella* infections (158). Enteraggregative *E. coli* utilizes fimbriae to bind to the cells of the small intestine in small clumps, hence its pathotype name. Subsequent production of a heat-stable enterotoxin and a cytotoxin are believed to cause diarrhea in young children (158). Enteraggregative *E. coli* is increasingly recognized as a cause of persistent diarrhea in adults in both developing and industrialized nations (155). Similarly, diffusely adherent *E. coli*, initially defined based upon the diffuse pattern of adherence on cultured epithelial cells, has been implicated as a cause of human diarrhea (155).

STEC is so named for the presence of a specific virulence marker responsible for the production of verotoxins (toxic to Vero cells in tissue culture) similar to the toxin produced by *S. dysenteriae* and thus often referred to as Shiga-like toxins Stx1 and Stx2. The genes encoding these toxins are carried on bacteriophages and could conceivably be acquired by *E. coli* of any serotype. Recent research has demonstrated that exposure to certain antibiotics not only results in increased toxin production but also leads to mobilization of phage (159). Although more than 200 serotypes of *E. coli* have been shown to produce Shiga-like toxin, the majority of these are thought not to be pathogenic in the absence of other virulence factors. Therefore, the ability to produce toxin alone may be insufficient to cause disease (156). The virulence of STEC is multifactorial, beginning with colonization of the bowel via the action of one or more adhesins, followed by the release of toxins and other proteins which may assist with bacterial survival and multiplication. The production and release of toxins into the gut lumen cause intestinal damage followed by systemic sequelae (156). This variability in virulence between STEC strains has been observed among isolates obtained from unpasteurized dairy products (160).

Enterohemorrhagic *E. coli* (EHEC) is a subset of the broader STEC category displaying increased virulence with potentially high mortality (156). Following an incubation period ranging from 3 to 9 days, EHEC infections manifest as three principal illnesses: hemorrhagic colitis, HUS, and thrombotic thrombocytopenic purpura (161). Hemorrhagic colitis is characterized by the sudden onset of extremely painful abdominal
cramps, followed by watery diarrhea which later becomes grossly bloody (158). HUS is a disease triad of acquired hemolytic anemia, thrombocytopenia, and renal failure (158) resulting from toxin-mediated damage to the kidneys. Endothelial damage triggers the clotting mechanism that may block capillaries of the kidneys and other organs, leading to the buildup of waste products in blood (158), often requiring dialysis and blood transfusions. HUS is thought to complicate approximately 10% of EHEC infections, with a mortality rate between 2 and 10% (156), often resulting from the development of cardiovascular and central nervous system diseases or conditions such as heart failure, coma, seizures, and hypertensive encephalitis (158). While serotype O157:H7 is the most common and recognized serotype, nonmotile variants (O157:NM) lacking a flagellar antigen, as well as serotypes O26:H11, O103:H2, O2:H5, and O111, are occasionally isolated from patients with hemorrhagic colitis and HUS (162).

There are four principal routes of EHEC infection: direct contact with infected animals, person-to-person transmission, transmission through the environment, and food-borne transmission. First recognized as a food-borne pathogen in 1982 following two outbreaks of hemorrhagic colitis in Oregon and Michigan attributed to the consumption of undercooked beef patties (163), EHEC has emerged as a major pathogen of concern across almost all major food categories. In 1996, *E. coli* O157:H7 was added to the CDC’s Foodborne Diseases Active Surveillance Network (FoodNet). Based on surveillance data, an estimated 62,000 food-borne cases occur annually in the United States, resulting in approximately 1,800 hospitalizations and 52 deaths (164). Food-borne outbreaks of *E. coli* O157:H7 have involved a wide range of food products. Those of bovine origin (165), including raw and pasteurized milk and milk products (166, 167) and ground beef (including that from cow and cattle [168]), are most commonly implicated. From 1982 to 2002, a total of 183 food-borne *E. coli* O157 outbreaks were reported in the United States. While ground beef serves as the vehicle of infection most often identified, seven (4%) outbreaks were attributed to dairy products, four of which were linked to the consumption of unpasteurized fluid milk (334). Similarly, Wachsmuth and colleagues (169) reported that raw milk was responsible for 5% of O157:H7 outbreaks in the United States from 1982 to 1995. The first domestic outbreak linked to the consumption of cheese occurred in 1998 in Wisconsin, where vats used to make raw milk Cheddar cheese were inadvertently used to make fresh cheese curds. The fresh curds, incorrectly labeled “pasteurized,” were distributed and sold in six Wisconsin counties, sickening 55 people (170). Around this time, cheese made with unpasteurized milk was the source of 6 cases of gastroenteritis in England (171), including one case of HUS in a 12-year-old boy (172). Then in early 1999, three people from northeast England fell ill with *E. coli* O157 infections after consuming Cotherstone cheese made from unpasteurized cow milk (173), although samples obtained from the dairy herd, slurry, and cheese production environment were all negative for *E. coli* O157 (174). That same year, 60 cases had been confirmed in an outbreak of *E. coli* O157 infection in North Cumbria associated with the consumption of milk from a local farm resulting in 27 hospitalizations, including three cases of HUS (175, 176). In 2003, 13 cases of *E. coli* O157:H7 infection were linked to the consumption of Gouda cheese in Alberta, Canada, and resulted in 2 cases of HUS. The source of contamination at the farm was never confirmed, as samples of raw milk, well water, cow feces, and environmental swabs collected at the cheese plant were all negative for *E. coli* O157:H7 at the time the investigation was conducted (177). In addition to cow milk, raw goat milk was recently implicated in an outbreak of *E. coli* O157:H7 affecting five people in British Columbia, Canada (178). A raw goat cheese was also linked to O157 illnesses in France (86). Although raw milk and milk products have been implicated in outbreaks, it appears as though contamination of milk may be uncommon (179). In late 2010, 2 multi-state outbreaks of *E. coli* O157:H7 were linked to consumption of raw milk cheeses produced in the United States. Consumption of Dutch-style Gouda cheese manufactured from raw milk was linked to 38 illnesses across 5 states, including 15 reported hospitalizations and 1 case of HUS (http://www.cdc.gov/ecoli/2010/cheeseO157/index.html). The second outbreak, which consisted of 8 cases of illness in 4 states, was linked to soft cheeses made from raw cow, goat, and sheep milk produced on a small rural farm in Washington State. FDA follow-up investigations revealed problems related to the sanitation of the facility, its employees, equipment, and utensils as well as problems with facility construction and maintenance (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm237381.htm).

Although *E. coli* O157:H7 is not considered a causative agent of intramammary infection, EHEC is a commensal inhabitant of the bovine gastrointestinal tract. Dairy cattle are thus a major reservoir of STEC, including serotype O157:H7 (180). Studies have demonstrated the ability of *E. coli* O157:H7 to survive on
pastures in soil and manure for extended periods (181, 182, 183). Contaminated animal drinking water and birds may also serve as common vehicles for infection and a potential intervention site for on-farm control of dissemination (184, 185).

The presence of E. coli O157:H7 on a dairy farm does not necessarily correlate to pathogen presence in raw milk. Wells and colleagues (180) isolated O157:H7 from only 1 of 23 (4.3%) raw milk samples from a case farm in Wisconsin. Incidence also appears to be fairly low (0.75%) in the bulk tank milk of cull cows (186). Higher isolation rates of 3.8% in South Dakota and western Minnesota (126) and 2.4% in Pennsylvania (127) have been reported for STEC. Although potentially pathogenic, none of the isolates in any of the aforementioned studies were of serotype O157:H7. Similarly, no O157: H7 were found in surveys conducted in southeast Scotland (179), Italy (187), and The Netherlands (188) involving 500, 100, and 1,011 raw milk samples, respectively. Early investigations revealed that E. coli O157:H7 can readily contaminate raw milk on the farm, with contamination rates of 4.2 to 10% reported in the United States and 2% in Canada (158, 189). Surveys examining the prevalence of E. coli O157:H7 in small-ruminant milk are scant. Foschino and others (132) reported a 1.7% incidence rate for E. coli O157:H7 in raw goat milk in Italy, which, when present, was observed at low levels (1.5 CFU/ml). Non-O157 STEC has also been detected in both goat and sheep milk, at incidence rates of 16.3 and 12.7%, respectively (83). An incidence of 0.75% was reported for raw bulk tank cheese milk in 2006 in Vermont (61), where a single isolate was obtained from a sample of goat milk. Detection following enrichment suggested a contamination level of <1 CFU/ml. Two years later, an expansion of this study failed to isolate E. coli O157:H7 from 101 samples of raw milk used in the production of artisan cheese (62). Although rare, milk and milk products can become contaminated as a result of postprocessing contamination (167, 188, 190). However, it has been suggested that this serotype may be unable to survive in dairy processing environments and may be readily eliminated by sanitation practices and is therefore not widely disseminated (191).

Although the exact infectious dose for E. coli O157:H7 is unknown, it is thought to be as low as <100 organisms (171). Challenge studies have shown that E. coli O157:H7 can grow at temperatures as low as 7°C in milk (188) and likely survives during refrigerated storage in highly acidic environments such as those found in yogurt (192, 193), sour cream, buttermilk (194), apple cider (195), and cheese, among others. It is believed that the ability of E. coli O157:H7 to induce an adaptive tolerance response when exposed to mild acid conditions may confer a higher resistance to stronger acid conditions upon subsequent exposure (196). Therefore, induction of an adaptive tolerance response following exposure to the mild acid conditions found in cheese may promote greater resistance to acid during passage through the stomach, thus reducing the infectious dose (197). An alternate sigma factor, encoded by rpoS, regulates genes required for acid tolerance, allowing E. coli O157:H7 to survive at pH values as low as 2.5 for more than 2 h (156). The rpoS-regulated proteins also cross protect E. coli O157:H7 against subsequent heat and salt challenges.

Numerous challenge studies have been conducted to examine the behavior of E. coli O157:H7 in cheeses. E. coli O157:H7 has been shown to survive the processing conditions used in the manufacture of feta (192, 198), Colby, Romano (192), Camembert (198), smear rind (197), and Cheddar (199, 200, 201) cheeses. Subsequent reductions in populations observed during the aging (<30 days) of Colby and Romano were attributed to prolonged exposure to pH, presence of starter culture, temperature, and salt concentration (192). Similar reductions from high initial milk inocula to undetectable levels in cheese within 60 days of storage were observed in brined cheeses such as feta and Teleme (202). However, studies on Cheddar (199, 200, 201) indicate that when present, even at low levels, in raw milk, this pathogen can survive the cheese manufacturing process and remain viable in finished cheese during the aging period well beyond 60 days. Although levels fell below the detection limit in cheese with an initial milk inoculum of 1 CFU/ml, Reitsma and Henning (200) detected viable E. coli O157:H7 in Cheddar for up to 130 days through enrichment and up to 158 days in cheese manufactured from milk inoculated at 1,000 CFU/ml. With an initial raw milk inoculation rate of ~33 CFU/ml, Schlesser and colleagues (201) reported enumerable levels of E. coli O157:H7 after 210 days of aging, with the detection of viable cells out to 240 days. Similarly, in another study (199) with an initial raw milk inoculation rate of ~20 CFU/ml, levels of E. coli O157:H7 remained above the detection limit (≥5 CFU/ml) for up to 180 days, with the detection of viable cells after more than 1 year of aging. A similar observation was reported by Schlesser et al. (201) using higher inoculation levels (~1,000 to ~100,000 CFU/ml). D’Amico and colleagues (199) examined the behavior of E. coli O157:H7 during the manufacture and aging of a Gouda-type cheese, reporting that pathogen levels dropped below
the cultural detection limit through direct plating (≥ 5 CFU/ml) after 120 days and remained detectable by enrichment and agar plating for an average of 300 days. The authors noted that moisture-nonfat substance, an indicator of the potential enzymatic and microbial activity during cheese ripening, was positively correlated with pathogen levels and duration of survival in both cheese types.

Population inactivation during aging of smear-rind cheese, whose pH increases following surface growth, is thought to result from the activity of antimicrobial substances produced by the surface flora (197). While *E. coli* O157:H7 may survive the initial steps in manufacture, exposure to elevated temperatures (80°C) during the stretching of mozzarella cheese (203) and the cooking of cottage cheese (204) results in complete pathogen inactivation. Studies have also shown that heating raw milk to 65°C for 17.6 s is sufficient for the destruction of *E. coli* O157:H7 (205). In addition to these findings, Bowen and Henning (206) failed to recover *E. coli* O157:H7 in 50 retail samples of natural cheese. Moreover, no *E. coli* O157 was detected in 153 soft and semisoft cheeses made with raw cow, ewe, and goat milk in a survey conducted in Belgium (207). Between 1 January 2004 and 31 December 2006, the FDA examined 3,360 cheese samples for the presence of EHEC, of which only 3 (0.09%) tested positive, including samples of imported Mexican-style soft cheese and imported soft-ripened cheeses (118).

**Listeria monocytogenes**

Listeriae are short, gram-positive, non-spore-forming, facultatively anaerobic rod-shaped bacteria with rounded ends, appearing almost coccoid at times (208). Among the currently recognized species of listeriae are *Listeria welshimeri*, *L. murrayi*, *L. grayi*, *L. innocua*, *L. ivanovii*, and *L. monocytogenes*; recent additions to the list include *L. rocourtiae* (209), *L. marthii* (210), *L. fleischmannii* (336), and *L. weihenstephanensis* (337). Although there have been isolated cases of *L. ivanovii* causing disease in humans (211, 212), *L. ivanovii* is typically pathogenic only to nonhuman mammals. *L. monocytogenes* is pathogenic to both humans and animals (208) and is therefore of concern with regard to human food-borne illness and thus the focus of this section. The likelihood of any one food contaminated with low numbers of *L. monocytogenes* (<100 CFU/g) resulting in illness is considered to be remote, and foods containing such levels pose little risk (213). In fact, it is likely that healthy people consume foods contaminated with *L. monocytogenes* on a regular basis with no ill effects, which is realized in the relatively low incidence of reported illnesses compared to its incidence in at-risk foods. While listeriosis is rare, accounting for an estimated 0.02% of food-borne illnesses per year, it is responsible for 27.6% of resulting deaths (164). The high mortality of this pathogen can be attributed to its target population, as listeriosis typically affects hosts with suppressed immune function, including the elderly, fetuses, cancer patients, human immunodeficiency virus patients, organ transplant recipients, and individuals receiving corticosteroid therapy. More recently, the growing number of people with predisposing factors has increased the size of the at-risk population (214). In fact, the first documented food-borne outbreak of listeriosis is believed to have occurred in Germany between 1945 and 1952 (215), reflected in upwards of 100 stillbirths documented at a single clinic (216). Investigation showed that the isolates obtained from the milk of a cow with atypical mastitis and stillborn infants of a mother who had consumed the raw milk of that cow were of the same serotype (216).

Although mastitis resulting from *L. monocytogenes* infection is quite uncommon, the organism’s presence in raw milk is not. Combined data from numerous surveys conducted worldwide suggest that approximately 2.2 to 3.8% of raw cow milk is likely to contain *L. monocytogenes* (215, 217). While some surveys have failed to detect any *L. monocytogenes* in bulk tank milk (218), the incidence rate in cow milk reported in surveys conducted throughout the United States ranges from 2.8 to 7% (126, 127, 130, 131, 218, 219). Outside the United States, reported prevalence ranges from 1 to 5.3% throughout Europe (124, 220, 221, 222, 223, 224, 335) and Ontario, Canada (125). Higher incidence rates, up to 33.3% (222), have been reported for dairy silo milk due to the longer storage time and the commingling of milk from numerous producers (224). In a study of farmstead raw milk cheese producers, *L. monocytogenes* was isolated from 3 of 133 raw milk samples. The three isolates were obtained from two cow farms, with two of the three isolates coming from the same farm 10 weeks apart. Contamination levels were <1 CFU/ml (225). Two years later, no *L. monocytogenes* was detected among 101 raw milk samples collected over the summer artisan cheese production season (62). While both goats (226) and sheep (227) are known carriers of *L. monocytogenes*, contamination of small-ruminant milk appears to be less common, with average incidences of 1.6 and 2.4% reported for sheep and goat milk, respectively (215, 220, 227).

Raw milk is most likely contaminated via various farm sources during milking. *L. monocytogenes* occurs
widely in dairy farm environments (228, 229), although prevalence on sheep and goat farms may be lower than on bovine farms (29). Contamination of the farm environment with L. monocytogenes likely begins with animals initially exposed via contaminated feedstuffs (229), which are likely contaminated through soil (229). Feedstuffs such as stock straw (230) and improperly fermented (pH > 5) silage (hay, grass, or corn), which can harbor a diverse group of strains (228, 231, 232), has long been considered an important source of L. monocytogenes on the farm and in cases of listeriosis in ruminants (215, 232, 233). Once ingested, L. monocytogenes can be shed in the feces of healthy dairy goats, sheep (229), and cows for months to several years (218), dispersing a diverse population of subtypes, including bovine clinical isolates (228), into the soil and farm environment (229). Fecal shedding can also contaminate water tanks, milk socks, and silage through the spreading of manure as fertilizer (234), further contributing to a contamination cycle of fecal spread and oral infection (228). Interestingly, L. monocytogenes became a major food-borne concern beginning in 1981 following 41 cases of listeriosis reported in Canada linked to coleslaw produced from cabbage harvested from fields fertilized with untreated sheep manure obtained from a farm with a history of ovine listeriosis (215, 235).

In 1985, the first major documented outbreak of listeriosis was attributed to the consumption of cheese and resulted in 142 cases of illness and 88 deaths. Cases were linked to consumption of a specific brand of soft, unripened Mexican-style cheese sold as queso fresco (215, 216). Although the implicated cheese was reportedly manufactured from pasteurized milk, the presence of high levels of the enzyme alkaline phosphatase in cheese samples produced over 6 months indicated the presence of raw milk used in cheesemaking. Plant records indicated that the quantity of raw milk coming into the plant exceeded the capacity of the pasteurizer, suggesting that the milk used for cheesemaking either was improperly pasteurized or had raw milk added directly to it (216, 236). Contaminated raw milk was also implicated in 33 cases of listeriosis resulting in 11 deaths associated with the consumption of Brie de Meaux, a French surface-ripened soft cheese (215, 237). Two years later, the French National Research Center identified 14 cases of listeriosis over a 4-month period traced to Pont-l’Évêque, a soft, washed-rind cheese produced from raw milk in Normandy (215). Mexican-style cheese was once again implicated in an outbreak of listeriosis in North Carolina in 2000. Case control studies implicated homemade raw milk Mexican-style soft cheese purchased in unlabeled packages from small Hispanic markets or street vendors or through door-to-door sales. The outbreak strain was traced back to the raw milk from the bulk tank of a manufacturing-grade dairy that admitted to illegally selling the raw milk (238). Mexican-style cheese produced in Mexico and illegally distributed in Texas resulted in yet another outbreak of listeriosis in 2003. Five of the six women affected reported consuming queso fresco which was made from raw milk in Mexico and sold at flea markets and by unlicensed street vendors in the United States (216).

Although listeriae are readily inactivated by typical pasteurization parameters, pasteurized milk and milk products have been implicated in outbreaks and sporadic cases of listeriosis as the result of postprocessing contamination. For example, 49 cases of listeriosis, with a case fatality rate of 29%, were attributed to the consumption of pasteurized milk in Massachusetts in 1983 (239). Pasteurized milk was again implicated in an outbreak of listeriosis in July of 1994. Postpasteurization contamination of chocolate milk due to poor sanitation practices at the dairy followed by extensive temperature abuse promoted excessive pathogen outgrowth in unopened containers (240). Raw milk is also not the only source of L. monocytogenes in cheese and cheese-related outbreaks. For example, 122 cases of listeriosis, including 33 deaths, were attributed to the consumption of contaminated Vacherin Mont-d’Or between 1983 and 1987 (237). Although this cheese is traditionally produced from raw milk, the 40 producers manufacturing this cheese in Switzerland converted to the use of pasteurized milk in 1983, prior to the outbreak, suggesting environmental contamination (216). In support of this theory, it was reported that cheeses were commonly transferred between aging caves and that wooden hoops were shared and returned to different producers without prior disinfection. Follow-up investigations revealed that half of the cellars were contaminated with one or both of the outbreak phage types (216). Consumption of goat milk anari-type soft cheese produced from raw milk in England resulted in a case of meningitis in a previously healthy 40-year-old woman in London in 1988. Based on the inability to isolate listeriae from raw milk and the high cook temperature (85°C) used in its manufacture, environmental recontamination was believed to have occurred (241). Most recently, a commercial acid curd cheese manufactured from pasteurized milk caused a large outbreak of listeriosis in Germany from October 2006 through February 2007, with a reported fatality rate of 14%. Unopened samples of the implicated cheese contained

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From late 2009 to early 2010, a multinational outbreak of listeriosis was linked to consumption of Quargel, a sour milk curd cheese. The outbreak, which involved 2 separate \textit{L. monocytogenes} clones, resulted in 34 cases of invasive listeriosis, with 8 deaths \cite{243}. According to published reports, the producer claimed that failure in warning and control systems had been the cause of the contamination of the 8 brands of its Quargel cheese. Apparently beetles had climbed through an open window and contaminated machines used to make the cheese (http://austrianindependent.com/news/Business/2010-03-01/1273/Shelby_an_admits_firm). More recently, a vat gasket was identified as a possible source of contamination of pasteurized milk used in the production of a Mexican-style cheese that was implicated in a multistate outbreak of listeriosis among pregnant Hispanic women \cite{244}.

A quantitative risk assessment conducted in the United States identified ready-to-eat (RTE) foods contaminated postprocessing as the cause of most cases of food-borne listeriosis \cite{245}, as listeriae, including \textit{L. monocytogenes}, are common contaminants of food processing facilities. Some strains of \textit{L. monocytogenes} have shown long-term persistence in processing environments \cite{225, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255}, possibly as a result of physical adaptation to the range of environmental conditions encountered (low temperature, wide pH range, fluctuating moisture, etc. \cite{255}). Persistent strains have also been shown to be significantly more adept in forming biofilms than sporadically isolated strains \cite{246}. These biofilms, typically formed in joints, valves, and gaskets, as well as in the pits and cracks of corroded surfaces, are more resistant to adverse environmental conditions \cite{257}. Persistent strains may therefore constitute part of the resident microflora not eliminated by cleaning and sanitation of these potential niches \cite{255}, which may indicate that prerequisite programs such as sanitation standard operating procedures and good manufacturing practices are inadequate \cite{255}. Once strains of \textit{L. monocytogenes} have become established, the environment can serve as a source of food product contamination \cite{250, 252, 254, 257, 258, 259} and possibly human listeriosis \cite{215, 222, 228, 236, 241}.

As with other food processing plants, \textit{L. monocytogenes} is regularly isolated from dairy processing and cheesemaking environments \cite{225, 247, 253, 258, 260, 261, 262, 263, 264, 265, 266}. Contamination rates reported for \textit{Listeria} spp. and \textit{L. monocytogenes} in various types of dairy processing plants range from 7.7 to 76.2\% and 7.7 to 30.2\%, respectively \cite{264, 267, 268, 269}. However, rates as high as 100\% for \textit{Listeria} spp. have been reported for dairy processing plants with adjacent farms \cite{225, 264}. In fact, dairy processing facilities with a contiguous farm may be at higher risk of contamination than those facilities without an on-site dairy farm \cite{264}: farm sources of \textit{Listeria} spp. such as dairy cattle, raw milk, and silage can subsequently contaminate dairy processing environments \cite{270}. High site isolation rates (57.9\%) within plants have been reported for on-farm dairy processing facilities \cite{264}. Although D’Amico and Donnelly \cite{225} reportedly detected listeriae in each farmstead cheese processing plant at least once in their study, the overall incidences of \textit{L. monocytogenes} and \textit{Listeria} spp. in environmental samples \((n = 705)\) were only 2.1 and 6.7\%, respectively. Similarly, the mean frequencies of \textit{L. monocytogenes} and \textit{Listeria} sp. isolation were quite low, at 1.9 and 7.3\%, respectively. The overall prevalence of \textit{Listeria} spp. in farmstead dairy processing plants manufacturing cultured dairy products, including cheese, may be comparatively low \cite{248}, as substantially higher site isolation rates, as high as 64.5\%, have been reported for other dairy processing facilities \cite{267, 269, 271}. \textit{L. innocua} is often the most prevalent species isolated \cite{238, 263, 264, 268}, followed by \textit{L. monocytogenes}. The results of a study by D’Amico and Donnelly \cite{225} where \textit{L. monocytogenes} was isolated from less than one-half of plants positive for other species question whether monitoring for \textit{Listeria} spp. is a good predictor of \textit{L. monocytogenes} contamination as previously suggested \cite{260}. The authors noted, however, that all processing environments from which \textit{L. monocytogenes} was isolated were also positive for other \textit{Listeria} species.

Floor drains appear to be the most frequently contaminated site identified in environmental surveys \cite{225, 247, 248, 268, 269, 272, 273} and appear to be useful in assessing the presence or absence of \textit{Listeria} spp. in a general location of a plant \cite{267}. Other commonly contaminated sites include floors, coolers, and areas of pooled water \cite{225, 247, 248, 269, 273, 274}. Recovery of \textit{Listeria} spp. from surfaces in direct contact with water suggests that reducing the amount of pooled water aids in controlling pathogens in the dairy plant \cite{263}, as \textit{L. monocytogenes} can survive in aerosols created by the use of high-pressure water hoses during cleaning, thus transferring cells from reservoirs such as drains and floors throughout the environment \cite{275}. Although exact contamination patterns are difficult to determine, molecular subtyping data suggest that the contamination of food contact surfaces with \textit{Listeria} spp., including \textit{L. monocytogenes}, is typically preceded by contamination of non-food contact surfaces, which
reinforces the role of environmental control in the prevention of food product contamination. Employees can also serve as a source of Listeria contamination and dissemination, emphasizing the risks associated with rotating employees through multiple assigned duties and departments (276).

The risk of product contamination in cheese plants is thought to be greater due to increased exposure to the environment during manufacture, hooping, cutting, and packaging compared to fluid milk production. Small-scale production, by local producers, involving a large amount of hand contact could also contribute to a higher probability of contamination (277). The size and age of a cheese plant may also impact the incidence of listeriae. In a study of Listeria contamination within and between three Latin-style fresh cheese processing plants, the largest, most recently constructed plant showed the lowest frequency of contamination, while the smallest establishment showed the highest frequency, attributed to poor facility design and layout (260). Conversely, in another study, a significantly higher recovery rate was reported for larger dairy plants than for small and medium-size plants, attributable to heavier traffic and to longer operating schedules allowing for extended periods of wet conditions (269).

The ability of L. monocytogenes to survive in a cheese is determined by parameters that act as hurdles to microbial growth, including intrinsic parameters such as aw, pH, acidity, and salt content. L. monocytogenes is unusually tolerant to these environmental stresses. In addition to conditions shown in Table 3, strains have been shown to grow at temperatures between 0.4 and 45°C and can survive and grow in foods with aw and pH values between 0.90 and 0.97 and 4.3 and 10, respectively (40, 217, 278). L. monocytogenes is also capable of growing in salt concentrations up to 10% and has been shown to survive for 4 months in salt concentrations of up to 26% under refrigeration (40). Thus, it is not surprising to find L. monocytogenes in cheese. According to Brennan and colleagues (279), a series of investigations have shown incidence rates of 0, 4.2, and 4.4% for Italian, German, and other European hard cheeses, respectively. This review further demonstrates that the incidence of L. monocytogenes in various European red-smear cheeses reported by numerous investigators over a 14-year period ranged from 1.1 to 22%. According to a review by Ryser (215), FDA records revealed that the presence of L. monocytogenes was confirmed in 12 of 658 (1.82%) domestic cheese samples analyzed in 1986, whereas 108 of 2,425 (4.5%) French cheeses surveyed yielded L. monocytogenes.

Similar results were reported for Germany (4.4%), Italy (3%), and Switzerland (4.9%), with contamination most commonly observed in soft cheeses, followed by semi-soft and hard varieties. The following year in a sampling of domestic aged natural cheese manufactured from raw milk, only 1 of 181 samples tested positive. Between 1 January 2004 and 31 December 2006, the FDA examined 2,181 cheese samples for the presence of Listeria monocytogenes, of which 2.4% were positive. Approximately half of the positive samples (52%) were traced to imported Mexican-style soft cheese or soft-ripened cheeses primarily from France and Italy (118). The prevalence of L. monocytogenes in different types of cheese from various countries of origin is further detailed in a review by Kousta and colleagues (86). These reports support the general consensus that L. monocytogenes is of particular concern in higher-moisture cheeses.

As previously discussed, numerous outbreaks and sporadic cases of listeriosis have been linked to the consumption of soft cheeses (86, 88, 215, 217, 236, 237, 280, 281). The survival of L. monocytogenes in soft cheese has been demonstrated in the laboratory by numerous investigators. The lack of a curd cooking step employed in the manufacture of soft cheese, coupled with relatively high moisture, allows for the survival and growth of L. monocytogenes during manufacture. L. monocytogenes has been shown to survive longer than 90 days, with only slight reductions in population levels in cheese with considerably low pH and high salt concentrations, such as feta (pH 4.6 to 5; salt in moisture, 6.27 [56, 282]).

When L. monocytogenes was inoculated in cheese milk used for manufacture of Camembert-type cheese, counts in wedge and interior samples decreased 10- to 1,000-fold during the first 17 days of ripening, an observation attributed to the low pH (<5.5) and storage temperature (15 to 16°C) (283). Similarly, Morgan and colleagues (284) noted a decrease in, but not complete elimination of, L. monocytogenes populations during the manufacture and ripening of raw goat milk soft-ripened cheese. From a milk inoculation level of 100 CFU/ml, the number of L. monocytogenes organisms decreased until day 14 on the surface and day 28 in the interior, where it remained close to 1.5 log CFU per gram. This observation was attributed to a combined inhibitory effect of low pH and the activity of lactic starters. However, in surface-ripened cheese, growth typically initiates at pH values between 5 and 6 (198, 283, 285, 286) and parallels the increasing pH during ripening (283, 285). With an optimal pH for growth that is neutral or slightly
alkaline, an increase in pH during ripening creates a favorable environment (in terms of pH) for pathogen growth (283). If present, unlike other pathogens, L. monocytogenes can survive and continue to grow during refrigerated storage due to its psychrotrophic nature. Ryser and Marth (283) observed 2 to 3 log cycles of growth in three strains of L. monocytogenes surface inoculated onto 10-day-old Camembert-type cheese at 6°C, reaching maximum population levels of approximately 3 to 5 log CFU/g, with a final pathogen level consistent with those found in contaminated soft-ripened cheeses at retail (221). In a study by D’Amico and colleagues (285), populations of L. monocytogenes inoculated onto the surface of raw or pasteurized milk bloomy-rind cheeses at 0.2 CFU/cm² increased to 2.96 and 2.33 log CFU/g, respectively, after 60 days of holding. Similar growth was observed in cheese inoculated at 2 CFU/cm², where populations reached 4.55 and 5.29 log CFU/g for raw and pasteurized milk cheeses, respectively. No significant differences were observed in pH development, growth rate, or population levels between cheeses made from the different milk types.

Growth of L. monocytogenes during the manufacture of Colby (287) and Cheddar, including reduced-fat and stirred-curd varieties (288, 289), has been demonstrated. Although pathogen levels typically decline during aging of these cheeses, L. monocytogenes has been shown to survive well beyond the mandatory 60-day holding period. Duration of survival, however, depends on initial inoculation levels as well as moisture and salt contents. The manufacturing process of Swiss cheese, which includes the cooking of curds at 50 to 53°C, results in pathogen inactivation (290). Further reductions occur during brining and the early stages of aging, with complete inactivation following 60 to 80 days of ripening at 24°C with a milk inoculation level of ~10⁴ to 10⁵ CFU/ml (20). A similar study on hard Swiss cheese demonstrated complete inactivation of L. monocytogenes within 24 h of manufacture when manufactured from raw milk inoculated at 10⁴ to 10⁶ CFU/ml. In contrast, populations of L. monocytogenes remained detectable for >90 days in Swiss semihard cheese (291). Hard grating cheeses such as Parmesan undergo similar cooking temperatures (~52°C) for even longer durations, up to an hour, which in combination with small curd size results in the expulsion of whey, producing a dense and low-moisture cheese. These conditions, in addition to brining and aging, result in the rapid decrease in L. monocytogenes populations. L. monocytogenes remained detectable for only a few days after manufacture in cheese made with milk inoculated with 10² to 10³ CFU/ml. With higher inoculation levels, ~10⁴ to 10⁵ CFU/ml, complete inactivation occurred between 60 and 120 days depending on the strain (292). Additionally, large inocula of L. monocytogenes (4 to 5 log CFU per gram) introduced as postprocessing contaminants were unable to survive on the surface and interior of freshly manufactured hard Italian-type cheeses (215).

**REGULATORY POLICY REGARDING MILK AND MILK PRODUCTS**

Countries often differ in their food inspection and certification systems as a result of differences in the prevalence of certain food safety hazards, differing views on food safety risk management, and differences in the historical development of food control systems (293). With the globalization of the food supply, it is paramount that countries work together to provide an appropriate level of sanitary protection (ALOP) in order to facilitate fair trade while protecting public health (293). The concept that the same level of food safety can be achieved through various hazard control measures and inspection and certification systems is referred to as the principle of equivalence (294). Equivalence is determined when different safety measures applied to a specified food achieve the same level of food safety as that achieved by standard or traditional methods (295). Equivalence can also be applied to specific requirements with regard to premises and equipment, processes such as hazard analysis and critical control point (HACCP) programs, and end product microbiological limits (295).

This principle of equivalence is currently recognized in the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (296) and the Agreement on Technical Barriers to Trade (297), which encourage member countries to employ safety measures that conform to international standards unless they are deemed insufficient to achieve a country’s ALOP. Similarly, member countries are encouraged to accept as equivalent the measures and regulations of other members, provided they are satisfied that these alternative measures and regulations meet their ALOP (295). The Codex Alimentarius (food code) was created to protect consumer health and to ensure and facilitate international trade. The standards within it serve as a global reference point for food standards and codes of good practice related to hygienic processing and have become the benchmarks against which national food measures and regulations are evaluated within the legal parameters of the World Trade Organization agreements. The Codex Alimentarius Commission has
developed the *Recommended International Code of Practice General Principles of Food Hygiene* (298) as well as specific guidance on achieving the general hygiene requirements for milk products (30). These codes are intended to be used in conjunction with the *Codex Guidelines for the Establishment and Application of Microbiological Criteria for Foods* (299) and the *Codex Principles and Guidelines for the Conduct of Microbiological Risk Assessment* (300). The *Codex of Hygienic Practice for Milk and Milk Products* (30) uses a food safety objective approach, outlining hygiene principles and guidelines for the application of the principles. These guidelines do not mandate or specify the use of any one set of controls but leave it up to those responsible for ensuring the safety of the finished product to choose the most appropriate set of control measures for the particular situation. Codex Alimentarius Commission provisions for the production of milk destined for the production of raw milk products are contained within Annex I of the *Codex of Hygienic Practice for Milk and Milk Products* (30). In addition, flexibility in the application of certain requirements of the primary production of milk can be exercised on “small holder dairy farms” (where the number of animals per farmer or per herd usually does not exceed 10, milking machines are not generally used, milk is not chilled at the producer’s level, and/or the milk is transported in cans) provided that the milk is received by dairy plants and will be subjected to a combination of microbiological control measures sufficient to obtain a safe and suitable milk product. Flexibility may also apply to farms with larger numbers of animals but having similar economic constraints or limited water and/or power supplies, preventing investment in technological facilities and infrastructure. Depending on the hazard analysis performed by the manufacturer and the combination of microbiological control measures applied during and after processing of milk products, specific microbiological criteria regarding pathogens may need to be established, though specific microbiological criteria are not provided. The Codex Alimentarius Commission has also issued *Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria monocytogenes in Ready-to-Eat Foods* (301). Microbiological criteria for foods in which *L. monocytogenes* growth will not occur under the conditions of storage and use have been established as ≤100 CFU/g at the end of manufacture or port of entry (for imported products) to the point of sale. This criterion is based on the product being produced under application of the provisions of the general principles of food hygiene to the control of *L. monocytogenes* in RTE foods, with appropriate evaluation of the production environment and process control and validation that the product meets the requirements of a food in which growth of *L. monocytogenes* will not occur. The criteria for foods in which *L. monocytogenes* growth can occur under the conditions of storage and use has been established as absence in 25 g (<0.04 CFU/g) at the end of manufacture or port of entry (for imported products) to the point of sale. However, competent authorities may also choose to establish and implement other validated limits for *L. monocytogenes* levels at the point of consumption or at other points that provide an acceptable level of consumer protection.

**United States**

In 1924, the U.S. Public Health Service developed the Standard Milk Ordinance as a model regulation for voluntary adoption by state and local milk control agencies to control milk-borne disease. Adoption is voluntary, as the U.S. Public Health Service has direct legal jurisdiction only in the enforcement of milk hygiene standards for milk in interstate commerce. The goals of such a model regulation are to promote the establishment of effective and uniform milk hygiene programs in each state and locality and to encourage the application of uniform enforcement procedures. Known today as the *Grade “A” Pasteurized Milk Ordinance* (PMO), this document governs the processing, packaging, and sale of grade A milk and milk products (302). Although the FDA does not consider cheese to be a grade A product, many states regulate milk production for use in cheesemaking under the PMO. Under federal regulation, cheeses, aside from those labeled as cottage, cream, and mozzarella, can be made from grade A or grade B milk. One major difference between the grades is that the bacterial count of grade B milk is three times that of grade A. Since processors often pay premiums for low-bacterial-count milk, there is an incentive for farmers to produce milk of high quality (28). The PMO specifically describes methods recommended for proper production, transportation, processing, handling, sampling, examination, and labeling of dairy products, including guidelines for the inspection of dairy farms and processing plants (302). Regulations regarding the methods of pasteurization are very specific with regard to temperature applications and equivalent processing standards ensuring the safety of dairy products. While standard pasteurization parameters were most recently revised in the 1950s to inactivate the highly heat-stable pathogenic bacterium *Coxiella burnetii*, they remain...
effective against all recognized human pathogens. While the grade A PMO provides guidance to ensure that fluid milk is produced safely, milk intended for cheesemaking is subject to different regulations determined by individual states and not necessarily governed by the PMO (303). In fact, many states have specific regulations in place that govern the quality and/or safety of milk utilized in cheese production.

According to a review by Johnson and others (142), heat treatment of milk destined for cheesemaking was primarily focused on improving the quality and uniformity of cheese prior to the 1950s, with researchers in the United States and abroad examining the effects of various heat treatments on the overall taste and quality of cheese. In addition to improved quality, it was noted that heat treatment gave cheesemakers improved control over the cheesemaking process and was shown to destroy pathogenic bacteria to various degrees (142). During World War II, when millions of pounds of Cheddar cheese were supplied to the Allies and U.S. Armed Forces, the U.S. Department of Agriculture encouraged producers to use pasteurized milk as a means of controlling quality (142). According to a review by Fabian (304), there were 59 recorded epidemics, with 2,904 cases and 117 deaths, linked to cheese from 1883 to 1946 in North America. Interestingly, no outbreaks were reported during the 21-year period from 1893 to 1914. Thereafter, sporadic outbreaks were reported until 1932, when a major outbreak of typhoid fever was linked to the consumption of cheese produced in Quebec, resulting in 627 cases of illness and 57 deaths. In the 10-year period from 1935 to 1945, 40 epidemics were reported involving 1,741 cases and 47 deaths, which was more than twice the number reported in the previous 42 years. Although there were no outbreaks of typhoid fever in the United States linked to cheese prior to 1914, from 1914 to 1943 there were 4 epidemics, 123 cases, and 11 deaths (305). Following two major U.S. outbreaks of typhoid fever in 1944, possibly acquired from the consumption of cheese, the Surgeon General suggested that cheese be made from pasteurized milk or adequately cured to reduce the risk of illness (142, 305). Soon several states enacted legislation to help ensure the safety of cheese, requiring that milk for cheese be pasteurized or that the cheese be cured from 60 to 120 days before sale (304). New York was the first to specify that cheese, in this case Cheddar, be allowed to ripen at a temperature not less than 35°C. Shortly thereafter, in 1945, Canadian regulation specified that Cheddar be aged for a duration of 90 days at no less than 58°F for the first 10 days of said maturation and no less than 45°F for the remainder. New York City was the first city to pass sanitary regulation requiring pasteurization or aging of cheese, more specifically Cheddar and Cheddar-type cheeses. This regulation also specified that all soft cheeses were required to be made of pasteurized milk and milk products (304). At the time it was believed that the best way to obtain uniform sanitary regulations for the 48 states would be by federal regulations and the establishment of federal standards for interstate shipment of cheese to serve as a model for state regulations (304). In 1947, the FDA held a hearing to discuss newly proposed standards for several cheese varieties (142). As a result of these hearings, it was established that pathogenic microorganisms that may be found in raw milk, shed directly from infected animals or resulting from contamination during handling, are inactivated by pasteurization but not necessarily by temperatures encountered during cheesemaking (142).

According to Boor (306), the elimination of pathogens in cheese through curing is believed to have been derived from the conclusions of a study detailing the survival of Brucella abortus in Cheddar cheese published by Gilman and colleagues in 1946. In their conclusion the authors state that “Cheddar cheese has not been proved to be a carrier for undulant fever and that reported typhoid fever epidemics have not been attributed to cheese cured for more than 63 days” (307). However, in this study, B. abortus survived up to 6 months of aging, varying with initial inoculation level. Naturally contaminated milk yielded viable organisms after 3 months or longer. Despite the obtained results, it was recommended that 60 days of aging would provide reasonable protection from Brucella abortus in Cheddar (306, 307). According to Johnson and colleagues (142), it was deemed unreasonable at this time to require the holding of cheese for a period long enough to ensure the death of all pathogens, as the exact duration of curing necessary to ensure safe cheese was unknown. It was, however, deemed reasonable to expect that cheese held for at least 60 days at temperatures no less than 35°C would be safe (142).

The final regulations governing the use of raw milk for cheesemaking, promulgated in 1949, require cheesemakers to either pasteurize milk for cheesemaking (destruction of alkaline phosphatase; commonly 62.8°C for at least 30 min or 71.1°C for 15 s) or cure the cheese for a specified amount of time as defined by its specific Standard of Identity (21 CFR 133 [308]), typically no less than 60 days at a temperature greater than 35°F (1.67°C). Currently, more than 30 natural cheese varieties can be legally made from raw milk in the United States provided that they are sufficiently aged (308).
For decades investigators have examined the survival of pathogenic microorganisms in cheese during aging. Most of the early research was focused on Cheddar cheese, likely due to its production volume at that time. According to a review by Marth (309), the survival of pathogenic bacteria, including Salmonella typhosa and S. pyogenes, in Cheddar cheese beyond 60 days at 42°F was documented in the early 1940s, prior to the institution of the specific curing requirements for cheese. The same organisms survived less than 11 weeks in Cheddar at 62°F and only 28 to 51 days in low-moisture Limburger (305). Van Slyke and Price (305) also reported on the extended survival of Mycobacterium tuberculosis (>100 days), Salmonella typhoid (3 to 10 months), and hemolytic streptococci (>160 days) in Cheddar cheese in 1949. Similarly, challenge studies conducted in the 1960s also detailed the survival of pathogens such as Salmonella typhimurium beyond the 60-day curing period (151), while Salmonella typhi inoculated into cheese milk was shown to survive in stirred-curd granular Cheddar cheese for 150 to 180 days when held at refrigeration temperatures (0°C and 4°C [148]). Despite evidence of the inadequacy of the 60-day aging rule, it remained unchallenged by regulatory bodies. However, in the 1990s, research published in the scientific literature detailed the survival of the emerging pathogen E. coli O157:H7 well beyond the mandatory 60-day holding period in Cheddar (200). In response to the detection of pathogenic microorganisms in cheese and cheese products as well as food-borne outbreaks and illnesses caused by L. monocytogenes, Salmonella, E. coli, and S. aureus, the FDA Center for Food Safety and Applied Nutrition (CFSAN) issued the Food Compliance Program for Domestic and Imported Cheese and Cheese Products in November of 1998 (310). The objectives of this program were for the FDA to conduct inspections of domestic cheese firms; to examine samples of imported and domestic cheese for microbiological contamination, presence of phosphatase, and filth; and to take appropriate regulatory action when violations were encountered. Dairy products may be considered adulterated within the meaning of section 402(a)(1) of the Federal Food, Drug, and Cosmetic Act (FD&C) [21 U.S.C. 342 (a)(1)] in that they bear or contain a poisonous or deleterious substance which may render them injurious to health based on the presence of L. monocytogenes, Salmonella species, or staphylococcal enterotoxins. The presence of L. monocytogenes, Salmonella species, or staphylococcal enterotoxin also represents a criterion for direct reference seizure to the Division of Compliance Management and Operations and for direct citation by district offices (311). Similarly, suggested limits on the number of E. coli O157:H7, S. aureus, and E. coli organisms and the presence of E. coli toxins are provided. Samples are analyzed for ETEC only when the number is ≥10⁷ CFU/g. A recommendation of legal action to CFSAN/Office of Compliance, Division of Enforcement, is warranted if E. coli O157:H7 is present; or enteropathogenic E. coli levels are greater than ≥10³ CFU/g or analysis of the cheese or cheese product demonstrates that one or more units have E. coli levels greater than ≥10⁴ CFU/g of product and a recent inspection demonstrates the existence of significant insanitary conditions (311). Inspectors are also informed to notify CFSAN when heat-stable or heat-labile toxins of E. coli are detected (310). In regard to compliance and governmental oversight, the aforementioned pathogens should be considered organisms of concern to the cheesemaker.

The FDA/CFSAN issued guidance for FDA staff regarding the administration’s current thinking on its enforcement policies for pathogens and indicators of inadequate pasteurization or postpasteurization contamination of dairy products in December 2010. The FDA reviews the available evidence on a case-by-case basis to determine whether a dairy product is adulterated and, in doing so, is guided but not bound by the guide. Under this guide the presence of Salmonella species, C. jejuni, Y. enterocolitica, Staphylococcus enterotoxin, or B. cereus enterotoxin in one or more subamples represents a criterion for direct reference seizure submission to the Division of Compliance Management and Operations, for direct reference import detention by the district, and for direct reference submission of detention without physical examination (DWPE) to the Office of Regulatory Affairs, Office of Regional Operations, Division of Import Operations and Policy. Similarly, the following represents criteria for recommending seizure, import detention, or DWPE to CFSAN/Office of Compliance/Division of Enforcement: the presence of EHEC, vegetative cells of C. botulinum, or C. botulinum toxin in one or more subsamples; the presence of E. coli at levels greater than 10 MPN per gram in two or more subsamples or greater than 100 MPN per gram in one or more subsamples; the presence of S. aureus at levels greater than or equal to 10⁴ CFU/g in one or more subsamples; or the presence of B. cereus at levels greater than or equal to 10⁴ CFU/g in one or more subsamples. This guidance document does not establish acceptable levels of pathogens, nontoxigenic E. coli, or alkaline phosphatase in dairy products, and the FDA may choose to take action regarding adulterated food that does not
meet these criteria (302). Although not legally enforceable responsibilities, the FDA/CFSAN recently issued guidance for FDA staff regarding the administration’s current thinking on its enforcement policies for *L. monocytogenes* in food, including RTE products such as cheese (313). According to the policy guide, the detection of *L. monocytogenes* in one or more subsamples of an RTE food that supports the growth of *L. monocytogenes* represents a criterion for recommending legal action to CFSAN Office of Compliance, Division of Enforcement. The FDA may regard an RTE food that supports growth of *L. monocytogenes* to be adulterated within the meaning of section 402(a)(1) of the FD&C [21 U.S.C. 342(a)(1)] when *L. monocytogenes* is present. If the pathogen is detected in RTE foods that do not support the growth of *L. monocytogenes*, staff may consult with CFSAN before recommending legal action. The FDA may regard an RTE food that does not support the growth of *L. monocytogenes* to be adulterated within the meaning of section 402(a)(1) of the FD&C [21 U.S.C. 342(a)(1)] when *L. monocytogenes* is present at or above 100 CFU/g of food. The criteria in this guidance do not establish an acceptable level of *L. monocytogenes* in food. The FDA may choose to take legal action regarding adulterated food that does not meet the criteria for recommending legal action to CFSAN. Further, the criteria in this guidance do not excuse violations of the requirement in section 402(a)(4) of the Act [21 U.S.C. 342(a)(4)] that food may not be prepared, packed, or held under insanitary conditions or of the requirements in the FDA’s good manufacturing practices regulation (21 CFR part 110). As set out in 21 CFR 110.80, food manufacturers must take “[a]ll reasonable precautions ... to ensure that production procedures do not contribute contamination from any source” (313). The FDA Food Safety Modernization Act (FSMA), the most sweeping reform of U.S. food safety laws in more than 70 years, was signed into law on 4 January 2011. The FSMA aims to ensure the safety of the U.S. food supply by shifting the focus from responding to contamination to preventing it. Although the details of several proposed rules are still being finalized as of this writing, the FSMA grants the FDA new enforcement authority to achieve higher rates of compliance with prevention and risk-based food safety standards in order to better respond to problems. In addition, the FDA has new tools to hold imported foods to the same standards as domestic foods, and the act directs the FDA to build an integrated national food safety system in partnership with state and local authorities. Under the FSMA, food producers that come under the jurisdiction of the FDA will be required to have a written hazard analysis and risk-based preventive control plan or food safety plan that includes evaluating the hazards that could affect food safety, specifying the preventive controls that will prevent or minimize the hazards, specifying how controls will be monitored to ensure that they are working, maintaining records of the monitoring, and specifying actions which will be taken to correct problems. In addition to the familiar critical control points of HACCP programs, preventive measures will include controls that were historically known as prerequisite programs such as supplier management, allergen control, good manufacturing practices, product traceability and recall, food defense, employee training, and sanitation standard operating procedures.

The FSMA also provides the FDA with important new tools for inspection and compliance, including mandated inspection frequency based on risk, access to records including food safety plans, and other required records. The FSMA also provides the FDA the tools to respond effectively when problems emerge despite preventive controls, including the authority to issue a mandatory recall and expanded administrative detention of products that are potentially in violation of the law. The FDA can also suspend registration of a facility if it determines that the food poses a reasonable probability of serious adverse health consequences or death. The FSMA also directs the FDA to establish enhanced product-tracing abilities and record-keeping requirements for facilities that manufacture, process, pack, or hold high-risk foods. More thorough and up-to-date information can be found at the FDA’s FSMA website (http://www.fda.gov/Food/GuidanceRegulation/FSMA/default.htm).

**European Union**

A preventative approach is utilized in the EU, where under the EU requirements there are specific provisions for raw milk production in relation to animal health requirements; hygiene of milking, storing, and collection operations; health and hygiene of personnel; labeling; and manufacturing as well as microbiological criteria. The general hygiene requirements for all food business operators in the EU are laid down in regulation no. 852/2004 (314) and more specifically for foods of animal origin in regulation no. 853/2004 (315), in which Annex III, Section XI, contains specific requirements for raw milk and dairy products. According to Hickey (316), the EU hygiene and food safety regulations are also implemented in England by The Food Hygiene (England) Regulations 2006, while Scotland, Wales, and
### TABLE 4 Microbiological criteria for cheese in the EU as outlined in commission regulation 1441/2007 (317)

<table>
<thead>
<tr>
<th>Microorganism or toxin</th>
<th>Food</th>
<th>Sampling plan</th>
<th>Limits</th>
<th>Stage where the criterion applies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salmonella</strong></td>
<td>Cheeses made from raw milk or milk that has undergone a lower heat treatment than pasteurization</td>
<td>5 0</td>
<td>Absence in 25 g Absence in 25 g</td>
<td>Products placed on the market during their shelf life</td>
</tr>
<tr>
<td><strong>Listeria monocytogenes</strong></td>
<td>Cheeses that are able to support the growth of <em>L. monocytogenes</em></td>
<td>5 0</td>
<td>100 CFU/g 100 CFU/g</td>
<td>Products placed on the market during their shelf life</td>
</tr>
<tr>
<td></td>
<td>Cheeses that are unable to support the growth of <em>L. monocytogenes</em></td>
<td>5 0</td>
<td>Absence in 25 g Absence in 25 g</td>
<td>Before the cheese has left the immediate control of the producer</td>
</tr>
<tr>
<td></td>
<td>Cheeses made from raw milk</td>
<td>5 2</td>
<td>10⁵ CFU/g 10⁶ CFU/g</td>
<td>Products placed on the market during their shelf life</td>
</tr>
<tr>
<td><strong>Coagulase-positive staphylococci</strong></td>
<td>Cheeses made from milk that has undergone a lower heat treatment than pasteurization and ripened cheeses made from milk or whey that has undergone pasteurization or a stronger heat treatment</td>
<td>5 2</td>
<td>100 CFU/g 1,000 CFU/g</td>
<td>Products placed on the market during their shelf life</td>
</tr>
<tr>
<td></td>
<td>Unripened soft cheeses (fresh cheeses) made from milk or whey that has undergone pasteurization or a stronger heat treatment</td>
<td>5 2</td>
<td>10 CFU/g 100 CFU/g</td>
<td>Products placed on the market during their shelf life</td>
</tr>
<tr>
<td><strong>Staphylococcal enterotoxins</strong></td>
<td>Cheeses with &gt;10⁵ CFU/g of coagulase-positive staphylococci</td>
<td>5 0</td>
<td>Not detected in 25 g Not detected in 25 g</td>
<td>Products placed on the market during their shelf life</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>Cheeses made from milk or whey that has undergone heat treatment</td>
<td>5 2</td>
<td>100 CFU/g 1,000 CFU/g</td>
<td>Products placed on the market during their shelf life</td>
</tr>
<tr>
<td></td>
<td>Products placed on the market during their shelf life</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: CFU/g = colony-forming units per gram.*
Northern Ireland have similar but separate regulations. This “farm to fork” or “stable to table” approach to food safety includes primary production (farmers) in food hygiene legislation. One key component is the requirement for food business operators (except farmers) to implement and maintain permanent procedures, based on HACCP principles in addition to good hygiene practices. Directive no. 853/2004 outlines health requirements for raw milk production and hygiene on milk production holdings not much unlike those detailed in the PMO. The directive also includes microbiological criteria for raw milk used to prepare dairy products measured as a rolling geometric average over a 2-month period, with at least two samples per month to account for seasonal variation. Somatic cells are similarly measured over a 3-month period, with at least one sample per month, unless the competent authority specifies another methodology to take account of seasonal variations in production levels. Although the limits are similar to those outlined in the PMO for prepasteurized milk, a distinction is made between raw milk for heat treatment and that for the production of raw milk products. In general, as with the PMO, no standards are provided for the presence of pathogenic bacteria, with the exception of Brucella and Mycobacterium. The PMO (302) requires prepasteurized milk to be from herds “officially free” from tuberculosis and brucellosis as certified or determined by the U.S. Department of Agriculture, whereas this requirement only applies to the sale of raw milk direct to consumers in the EU.

Additional microbiological criteria for foodstuffs are outlined in commission regulation 1441/2007 (317), as shown in Table 4. Food business operators must ensure that cheeses made from raw or heat-treated milk on the market do not contain Salmonella spp. in any of five 25-g samples. Testing for coagulase-positive staphylococci varies depending on the extent of heat treatment applied to the milk used for manufacture. This acceptable limit is similar to that of the compliance guidelines enforced in the United States, with one major exception. In the case of raw and heat-treated milk, samples must be taken at the time during the manufacturing process when the number of staphylococci is expected to be the highest, as opposed to when the cheese is already on the market. Moreover, stricter limits are placed on cheeses manufactured from heat-treated and pasteurized milks.

Allowable limits of L. monocytogenes in cheese under EU regulation, in accordance with Codex Alimentarius Commission regulations, differ between cheeses that can and cannot support the growth of this pathogen (other than those intended for infants and for special medical purposes). For RTE foods that support Listeria growth, the food must not contain any viable cells in any of five 25-g samples before the food has left the immediate control of the food business operator who produced it. The same product may contain up to 100 CFU per gram by the end of its shelf life, as is the case for RTE foods unable to support the growth of L. monocytogenes. Food business operators manufacturing RTE foods, which may pose an L. monocytogenes risk for public health, are also required to sample the processing areas and equipment for L. monocytogenes as part of their sampling scheme for HACCP validation.

Australia

According to the Australia New Zealand Food Standards Code (FSC [318]), although applicable only in Australia, milk for cheese manufacture is required to be heat treated by pasteurization or subjected to a minimum heat treatment of 62°C for no less than 15 s (thermization) provided that the cheese produced from said milk is subjected to a 90-day holding period at a temperature no less than 2°C or such that the curd is heated to a temperature of no less than 48°C and the cheese or cheese product has a moisture content of less than 36%, after being stored at a temperature of no less than 10°C for a period of no less than 6 months from the date of processing. Additionally, if a cheese is made from unpasteurized milk, the ingredient declaration should include a statement that the milk is unpasteurized. In the case of cheese made from milk other than cow milk, it should also include the common name of the species from which the milk is sourced. Microbiological criteria for cheese as outlined in the Australia New Zealand FSC are listed in Table 5.

Although not in accordance with domestic policy at the time, Australia had allowed the importation of raw milk hard and semihard cheeses from Switzerland for over 30 years. The importation of Swiss cheese was suspended in 1997 following a review of quarantine requirements by the Australian Quarantine and Inspection Service (AQIS) when it was realized that these cheeses were made from raw milk and therefore not in compliance with the FSC (319). Although the National Food Authority advised the AQIS that certain cheeses made in Switzerland, under specific cheesemaking methods, achieved a level of public health and safety equivalent to that of pasteurization back in 1994, a formal application was never submitted, and thus, the FSC was never changed (319).

In response, the Swiss Federal Veterinary Office (SFVO) submitted a request to restore market access.
for Swiss hard and semihard cheeses made from unpasteurized milk. In this application, the risk of foodborne illness due to *E. coli*, *Salmonella* spp., *S. aureus*, *L. monocytogenes*, and *C. jejuni/C. coli* in Swiss hard and semihard cheese was assessed. The microbiological risk assessment of the manufacturing protocols set out in the Swiss Federal Government Ordinances determined that the heating temperatures of milk, continual heating of the curd, and the rapid acidification by the starter cultures as well as the intense brining and long ripening period (90 to 360 days) inactivate pathogenic microorganisms (319). As a result, and based on the knowledge that the SFVO oversees the management of these protocols, the importation of Swiss Emmental, Swiss Sbrinz, and Swiss Gruyère was reinstated (319).

The importation and sale of very hard cooked-curd cheeses (hard grating cheeses) manufactured in Italy from raw milk such as Grana Padano and Parmigiano Reggiano were originally permitted based on compliance with volume 1 of the FSC, which stated that adequate heat treatment of the milk could be tested in terms of alkaline phosphatase destruction. However, at the close of 2002, volume 1 of the FSC was replaced by volume 2, which did not include alkaline phosphatase activity as an adequate measure of pasteurization efficacy, placing these cheeses out of compliance. Therefore, a similar scientific evaluation of the safety of Italian hard grating cheeses with regard to the pathogens of concern as outlined in the Swiss application was conducted. The evaluation concluded that the manufacturing processes for Grana Padano, Parmigiano Reggiano, Romano, Asiago, and Montasio achieved a 5-log10 reduction in bacterial pathogen levels, which is currently used as a benchmark for obtaining microbiologically safe products. The low moisture content (<36%), curd cooking temperatures, and extensive aging were shown to result in the inactivation of milk-borne pathogens. In 2002, volume 2 of the FSC was amended to permit the sale of very hard cheeses (<36% moisture) manufactured from raw milk provided they are aged for a minimum of 6 months at a temperature of at least 10°C (320).

In 2004, France submitted an application to amend the FSC once again to allow for the sale of Roquefort cheese, a traditional French blue cheese made from raw sheep milk. Roquefort maintains protected designation of origin status and can therefore be produced only in a specific region of France. As with previous applications, a scientific evaluation of product safety regarding pathogens of concern was conducted, with the addition of *Brucella melitensis* and *Coxiella burnetii*. In contrast to the hard and very hard cheese varieties of previous applications, pathogen control for Roquefort is rooted in the implementation of an effective HACCP plan. These strategies rely heavily on microbiological testing (including final product) and the use of standard operating procedures and good manufacturing practices controlled by the Confederation of Roquefort Producers (CRP). Although the manufacturing process for Roquefort is not fully bactericidal, the rapid acidification of milk and/or curd (decrease in pH from 6.5 to <5.0 within 6 to 8 h and then to pH 4.8 within 24 h), the reduction in aw (∼0.92), and the 90-day aging period

### TABLE 5 Microbiological criteria for cheese as outlined in the Australia New Zealand FSC (318)

<table>
<thead>
<tr>
<th>Microorganism or toxin</th>
<th>Food</th>
<th>Sampling plan</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n  c</td>
<td>m     M</td>
</tr>
<tr>
<td><strong>Salmonella</strong></td>
<td>Soft and semisoft cheese (moisture content &gt; 39%) with pH of &gt;5.0</td>
<td>5  0</td>
<td>Absence in 25 g</td>
</tr>
<tr>
<td></td>
<td>All raw milk cheese (cheese made from milk not pasteurized or thermized)</td>
<td>5  0</td>
<td>Absence in 25 g</td>
</tr>
<tr>
<td><strong>Listeria monocytogenes</strong></td>
<td>Soft and semisoft cheese (moisture content &gt; 39%) with pH of &gt;5.0</td>
<td>5  0</td>
<td>Absence in 25 g</td>
</tr>
<tr>
<td></td>
<td>All raw milk cheese (cheese made from milk not pasteurized or thermized)</td>
<td>5  0</td>
<td>Absence in 25 g</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>All cheese</td>
<td>5  1</td>
<td>10 CFU/g</td>
</tr>
<tr>
<td><strong>Campylobacter</strong></td>
<td>Raw milk unripened cheeses (moisture content &gt; 50%) with pH of &gt;5.0</td>
<td>5  0</td>
<td>Absence in 25 g</td>
</tr>
</tbody>
</table>
aid in reducing potential hazards and therefore must be monitored (321). Furthermore, the unpasteurized milk used for cheese production must be tested and demonstrated to have no detectable levels of \( L. \) monocytogenes in 25 ml of milk per tanker. The final step prior to approval of this application involved an onsite verification audit to verify the integrity of the regulatory control implemented by the CRP as enforced by the French government, including the inspection of facilities along the entire supply chain and examination of HACCP plans and documentation as well as the results of routine monitoring and testing. The sheep dairy farms supplying milk to Roquefort producers, operating under the supervision of the Departmental Veterinary Services Directorates and the CRP while in compliance with the standards outlined in the Roquefort Decree, keep extensive records on animal health, including veterinary treatments, breeding activity, and transport to and from the farm, allowing for the traceability of final product back to the raw milk supply (321).

Based on the information provided by the French government, various challenge studies, a review by Food Science Australia, and a review of the scientific literature, it was concluded that any pathogens, if present, “would be unlikely to survive or proliferate during the manufacture of Roquefort cheese” and that “the consumption of Roquefort cheese poses a low risk to public health and safety.” This conclusion was supported by the finding that there have been no reported food-borne outbreaks attributed to the consumption of Roquefort (321). Although the changes in processing requirements for cheese and cheese products outlined in standard 4.2.4 of the FSC did not originally apply to New Zealand, as of 2007 New Zealand opened its borders to the direct importation of the aforementioned raw milk cheeses from countries other than Australia (322).

In late 2009, the New Zealand Food Safety Authority (NZFSA) introduced new legislation [Animal Products (Raw Milk Products Specifications) Notice 2009] that allows for certain raw milk cheeses to be produced in and imported into New Zealand. This notice set additional requirements for the production and processing of raw milk to be used in the manufacture of raw milk products intended for human consumption and for the production and processing of said raw milk products. In addition to food safety criteria as outlined in Table 6, the raw milk product specification notice sets an aerobic plate count limit of 300,000 CFU/ml of raw milk immediately prior to the commencement of manufacture (323). A supplemental code of practice provides additional technical information and base criteria for producers and processors of raw milk and raw milk products. The code is not mandatory unless it is included within the operator’s risk management program required for dairy processors. The code sets out additional measures for such a program and provides microbiological acceptance criteria for raw milk and monitoring criteria for raw milk at the start of manufacture as shown in Tables 7 and 8, respectively (324).

At the time of this writing, Food Standards Australia New Zealand (FSANZ) was in the process of assessing requirements for the domestic production of raw milk products in Australia (proposal P1007), as New Zealand has its own arrangements for regulating primary production businesses. According to the 2nd Assessment Report published in August of 2011, FSANZ, in consultation with the Standards Development Committee, limited the scope of the current proposal (P1007) to products for which the properties and/or processing factors eliminate pathogens that may have been present in the raw milk (category 1) (325). FSANZ will develop a separate new proposal to progress the technical work to provide the regulatory framework for products for which the properties and/or processing factors may allow the survival of pathogens that may have been present in the raw milk but do not support the growth of these pathogens (category 2). Regulatory changes to permit category 2 products are more involved and will need to be supported with additional technical work to develop implementation materials. This approach means

### Table 6: Microbiological criteria for raw milk cheese in New Zealand as outlined in Animal Products (Raw Milk Products Specifications) Notice 2009 (323)

<table>
<thead>
<tr>
<th>Microorganism or toxin</th>
<th>Food</th>
<th>Sampling plan</th>
<th>Limits (m, M)</th>
<th>Stage where the criterion applies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salmonella</strong></td>
<td>All raw milk cheese</td>
<td>5</td>
<td>Absence in 25 g</td>
<td>While under the control of the manufacturing processor</td>
</tr>
<tr>
<td><strong>Listeria monocytogenes</strong></td>
<td>All raw milk cheese</td>
<td>5</td>
<td>Absence in 25 g</td>
<td></td>
</tr>
<tr>
<td><strong>Staphylococcal enterotoxins</strong></td>
<td>All raw milk cheese</td>
<td>5</td>
<td>Absence in 25 g</td>
<td></td>
</tr>
</tbody>
</table>
that the further technical work needing progress on the product and performance criteria for category 2 products (and supporting guidance) will not slow down permissions for category 1 products. Production of cheeses for which the intrinsic characteristics and/or processing factors are likely to allow the survival of pathogens that may have been present in the raw milk and may support the growth of these pathogens (category 3) will not be allowed, as such products present a high level of public health and safety risk that cannot be reduced sufficiently (325).

Canada

In 1945, following the aforementioned outbreaks of food-borne illness linked to cheese in North America, Canada passed regulations requiring that all hard-pressed cheese (from raw or pasteurized milk) be aged 90 days or longer. The regulations further specified that during the initial 10 days of aging, cheeses must be held at a temperature no less than 58°F, then at 45°F or greater for the remaining duration of aging (304). The Food and Drug Regulations (FDR) were amended in 1952, no longer requiring cheese made from pasteurized milk to be held or marked with the date of manufacture. Pasteurized milk cheese could now be sold at any age desired by the manufacturer. Cheese not made from pasteurized milk must be date stamped and held under the conditions specified in the regulations (326). These standards, similar to those of the United States, permit the sale of raw milk cheese provided the cheese has been stored at a temperature of at least 2°C for a minimum 60 days. Microbiological criteria were also established such that no person shall sell cheese made from an unpasteurized source if the cheese contains more than 500 E. coli organisms/gram of cheese or more than 1,000 S. aureus organisms/gram of cheese. In 1996, Health Canada (HC) proposed that all cheeses offered for sale be made from pasteurized milk or meet specific conditions considered equivalent to pasteurization (327). This proposal was met with great opposition from cheesemakers, notably in the province of Quebec. Following extensive consultations, HC withdrew this proposed amendment based on information provided by the Scientific Expert Advisory Committee that HC created to review the submissions made by industry, consumers, and stakeholders during the consultation period (328). In response to the findings, the committee agreed that raw milk soft and semisoft cheeses pose a higher risk than do hard cheeses made from raw milk. Following a risk assessment conducted by the Food Directorate, it

<table>
<thead>
<tr>
<th>Microorganism or toxin</th>
<th>Food</th>
<th>Minimum frequency</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine somatic cell count</td>
<td>Farm dairy bulk milk tank at time of collection or acceptance</td>
<td>1 test per wk per farm</td>
<td>400,000 cells/ml</td>
</tr>
<tr>
<td>Other somatic cell count</td>
<td>Farm dairy bulk milk tank at time of collection or acceptance</td>
<td>1 test per wk per farm</td>
<td>1,000,000 cells/ml</td>
</tr>
<tr>
<td>Aerobic plate count</td>
<td>Farm dairy bulk milk tank at time of collection or acceptance</td>
<td>1 test per wk per farm</td>
<td>100,000 CFU/ml</td>
</tr>
<tr>
<td>E. coli</td>
<td>Farm dairy bulk milk tank at time of collection or acceptance</td>
<td>1 test per wk per farm</td>
<td>100 CFU/ml</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Farm dairy bulk milk tank at time of collection or acceptance</td>
<td>According to operator’s program</td>
<td>Set by the operator</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microorganism or toxin</th>
<th>Food</th>
<th>Minimum frequency</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic plate count</td>
<td>Representative sample of each bulk raw milk silo taken at the time of use</td>
<td>Each raw milk silo at the time of manufacture</td>
<td>300,000 CFU/ml</td>
</tr>
<tr>
<td>E. coli</td>
<td>Representative sample of each bulk raw milk silo taken upon receipt once contents are mixed</td>
<td>Each raw milk silo</td>
<td>100 CFU/ml</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Farm dairy bulk milk tank at time of collection or acceptance</td>
<td>According to operator’s program</td>
<td>Set by the operator (absence in 1 ml unless evidence to support an alternative)</td>
</tr>
</tbody>
</table>
was recognized that the extended storage of these soft and semisoft cheese varieties may allow for the growth of food-borne pathogens such as *L. monocytogenes* and may result in a spoiled and unpalatable product. Furthermore, the FDR’s current microbiological criteria for cheese made from unpasteurized milk are outdated and do not reflect the risk posed by more recently identified food-borne pathogens such as *L. monocytogenes* and *E. coli* O157:H7. Acknowledging that *L. monocytogenes* represents a serious risk postprocessing, HC sought to develop a policy to replace the 60-day aging requirement for soft and semisoft cheeses. The new policy would focus on strengthening production criteria and end product testing coupled with stringent raw milk production when the milk is intended for the manufacture of raw milk soft and semisoft cheeses. The implementation of a new code of practice would emphasize improved process controls and strengthen product testing to achieve safety equivalent to that provided by pasteurized products. HC has identified several elements that could be incorporated into an updated policy for soft and semisoft cheeses made from unpasteurized milk, including establishing microbiological criteria for milk used in the production of unpasteurized soft and semisoft cheese, updated microbiological criteria for cheese, and required record keeping supporting enforcement. The policy may also include an education campaign for consumers and mandatory labeling requirements for cheese. Such changes in policy are driven not only by inequity in trade and market access presented as a result of differing provincial regulations but also by international trade. Since 2001, the Canadian Food Inspection Agency has permitted imports from France of soft and semisoft cheese varieties made from unpasteurized milk, including establishing microbiological criteria for milk used in the production of unpasteurized soft and semisoft cheese, and required record keeping supporting enforcement. The policy may also include an education campaign for consumers and mandatory labeling requirements for cheese. Such changes in policy are driven not only by inequity in trade and market access presented as a result of differing provincial regulations but also by international trade. Since 2001, the Canadian Food Inspection Agency has permitted imports from France of soft and semisoft cheese varieties made from unpasteurized milk, including establishing microbiological criteria for milk used in the production of unpasteurized soft and semisoft cheese, and required record keeping supporting enforcement. The policy may also include an education campaign for consumers and mandatory labeling requirements for cheese. Such changes in policy are driven not only by inequity in trade and market access presented as a result of differing provincial regulations but also by international trade. Since 2001, the Canadian Food Inspection Agency has permitted imports from France of soft and semisoft cheese varieties made from unpasteurized milk, including establishing microbiological criteria for milk used in the production of unpasteurized soft and semisoft cheese, and required record keeping supporting enforcement.

HC has also recently updated the *Policy on Listerial Monocytogenes in Ready-to-Eat Foods* (329), which, like the Codex Alimentarius Commission and EU, proposes microbiological criteria for the verification and control of *L. monocytogenes* in RTE foods with a view towards protecting public health while ensuring fair practices in food trade. The updated policy includes end product compliance criteria similar to the international Codex Alimentarius Commission standards and states that environmental monitoring programs should be included in all plants used for the production of RTE foods. Priority is placed on foods which can support the growth of *L. monocytogenes* (e.g., surface-ripened soft cheese), while lower priority is placed on products in which the organism cannot grow or has limited growth potential whereby levels do not exceed 100 CFU/g throughout the stated shelf life (329).

In 2008, the Canadian province of Quebec introduced new rules permitting the manufacture and sale of cheeses from raw, unpasteurized milk without mandatory aging (330). Briefly, the regulation describes specific requirements for facility design, construction, and layout, including the barn, milking parlor, and bulk tank room. Dairy producers must have the health of their herds tested monthly by a veterinary surgeon in a program specified within the regulation. Milk must be utilized within 24 h of collection, and manufacturers are required to test this milk monthly for the presence of *L. monocytogenes* and *S. aureus* and every 3 months for the presence of *Salmonella* spp. Milk must be free of *L. monocytogenes* and contain ≤2,000 CFU/ml of *S. aureus*. Manufacturers not utilizing municipal water systems are required to test water once per month. Water must be free of fecal coliforms and *E. coli* and

### TABLE 9 Microbiological criteria for cheese in Quebec as outlined in *Règlement laitiers et succédanés de produits laitiers* (330)

<table>
<thead>
<tr>
<th>Microorganism or toxin</th>
<th>Food</th>
<th>Sampling plan</th>
<th>Limits (CFU/g)</th>
<th>Stage where the criterion applies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>c</td>
<td>m</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>All raw milk cheese</td>
<td>5</td>
<td>2</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>Fresh pasteurized cheese</td>
<td>5</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>All raw milk cheese</td>
<td>5</td>
<td>2</td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td>Fresh pasteurized cheese</td>
<td>5</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Fresh lactic cheese, ≥50% moisture</td>
<td>5</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Coliforms</td>
<td>Fresh lactic cheese, ≥50% moisture</td>
<td>5</td>
<td>2</td>
<td>50</td>
</tr>
</tbody>
</table>
contain ≤10 CFU/ml of total coliforms. If water does not pass, all products potentially contaminated must be removed from the market.

Although cheeses do not need to be aged for a specified amount of time, they must still be aged at a temperature ≥2°C. As with milk, cheeses must be tested monthly for the presence of L. monocytogenes and S. aureus and every 3 months for the presence of Salmonella spp. and must be free of both Salmonella and L. monocytogenes. Cheeses must also contain less than 500 CFU/g of E. coli and less than 1,000 CFU/g of S. aureus. If any cheese exceeds the limits for E. coli or S. aureus, the manufacturer must have the necessary number of samples of those cheeses analyzed to ensure that the standards set out in the sampling plan outlined in Table 9, which also includes the standards for pasteurized milk cheeses, are met.

**Sampling Plans**

Many regulations discussed here employ what are referred to as two- and three-class sampling plans. In these plans, a “lot” is defined as a quantity of food or food units produced and handled under uniform conditions and “sample” is a number of food units that resembles the microbiological characteristics of the lot. Two-class plans use only one microbiological criterion to decide if a sample unit is acceptable or not, where “n” is the number of sampling units from the lot and “c” is the maximum number of sample units that are allowed that exceed a specified microbiological criterion “m.” The threshold value, “m,” is the maximum number or level of the specified organism (CFU/gram or milliliter) beyond which the level of contamination is hazardous or unacceptable. If presence of an organism is acceptable, then c > 0, whereas c = 0 in cases where presence is unacceptable. Three-class plans are used to designate acceptable, marginally acceptable (satisfactory), and unacceptable food, where “m” represents the lower limit while “M” marks the upper limit or maximum number of the specified organism. In the three-class plans, a lot is often accepted when all samples are <m and when <c of n samples are >m but <M. A lot will be rejected when >c of n samples are >m and <M or if any sample is >M.

**DISCLAIMER**

The information provided here is for informational purposes only and may not be used as a substitute for legal advice regarding food safety laws in any jurisdiction. The U.S. government restricts how raw milk may be used in cheese, and some state and local laws restrict the sale and use of raw milk and raw milk dairy products. Regulations are also subject to change at any time and do so often.

**REFERENCES**


Microbiological Quality and Safety Issues in Cheesemaking


Microbiological Quality and Safety Issues in Cheesemaking


D’Amico


Microbiological Quality and Safety Issues in Cheesemaking


