DAIRY FOODS

Salmonellae, Salmonellosis, and Dairy Foods: A Review

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ABSTRACT

Salmonellae continue to be a major concern for the dairy industry because these bacteria have caused recent outbreaks of illness and have been isolated from various dairy products in the marketplace. Salmonellae are generally not heat resistant and normally grow at 35 to 37°C, but they can grow at much lower temperatures, provided that the incubation time is suitably extended. To minimize problems, foods should be held at or below 2 to 5°C at all times. Both conventional and rapid methods are available to isolate salmonellae from dairy foods and to identify the bacteria. Salmonellae behave differently in different kinds of cheese: they survived in ripening Cheddar cheese for up to 7 mo at 13°C and for 10 mo at 7°C; in cold-pack cheese food for several weeks, depending on the pH and preservative used; and in Domiati cheese 13 to 36 d, depending on the manufacturing process used. When Mozzarella cheese was made, temperatures of stretching and molding (60°C) killed all salmonellae present, but, in cottage cheese, survival of the pathogen depended on the cooking temperature of curd. Spray drying of skim milk killed substantial numbers of salmonellae, but some survivors remained. Butter readily supported growth of salmonellae at room temperature, and neither freezing nor refrigeration for brief periods eliminated salmonellae from butter. Use of appropriate hygienic procedures, e.g., Hazard Analysis Critical Control Point system, during processing should reduce the likelihood of salmonellosis outbreaks associated with dairy foods.

(Key words: salmonellosis, Salmonella, foodborne illness, dairy foods)

Abbreviation key: aw = water activity, BS = bismuth sulfite agar, XLD = xylose lysine deoxycholate agar.

INTRODUCTION

Salmonellae are widespread in the environment and appear in a wide variety of foods and food ingredients, thus posing a great problem to the food industry. The number of human salmonellosis cases per year in the United States is conservatively estimated to be 2 million (51, 122). Food processors are not allowed to sell products containing salmonellae. Such food products, according to the Food, Drug and Cosmetic Act (72), are adulterated because they contain harmful or pathogenic microorganisms.

Contamination of manufactured dairy products by salmonellae has become of increasing concern since 1966, when 11 serovars were isolated from NDM in nine states of the United States (25). Milk and milk products have been implicated in the transmission of human pathogens, including salmonellae (18, 19, 20, 34, 85, 113). Because proper pasteurization kills this pathogen, most milkborne salmonellosis has been associated with raw, heat-treated, or inadequately pasteurized milk or with milk contaminated after pasteurization (14, 34, 40, 85, 110). The massive salmonellosis outbreak involving more than 180,000 persons in the Midwest in 1985 was traced to 2% fat, pasteurized fluid milk (21, 22, 78, 124) that allegedly became contaminated after pasteurization.

Marth (85) reviewed the literature through 1968 on salmonellae and salmonellosis associated with milk and milk products. Hence, it is appropriate again to review the subject so that
dairy industry personnel, public health and regulatory officials, food microbiologists, and academicians have a ready source of information regarding this problem. Furthermore, references cited in this review provide additional details. This review will discuss the following: 1) characteristics of salmonellae, 2) human salmonellosis, 3) outbreaks of human salmonellosis, 4) prevention and control of human salmonellosis, 5) environmental conditions and their effects on growth and survival of salmonellae, 6) isolation and enumeration of salmonellae, 7) salmonellae in milk and dairy products, and 8) control of salmonellae in the dairy factory.

**GENERAL CHARACTERISTICS OF SALMONELLAE**

Salmonellae are short rods, .7 to 1.5 X 2 to 5 μm, conforming to the general definition of the family Enterobacteriaceae. They are Gram-negative (95), usually motile (peritrichous flagella), and facultatively anaerobic. Colonies of salmonellae are generally 2 to 4 mm in diameter. Salmonellae reduce nitrate to nitrite, can produce gas from glucose, can produce hydrogen sulfide on triple-sugar iron agar, are indole-negative, and can utilize citrate as a sole carbon source. Salmonellae usually are lysine-positive and ornithine decarboxylase-positive (Moller's reactions) and urease-negative and do not oxidatively deaminate phenylalanine and tryptophan. Sucrose, salicin, inositol, and amygdalin usually are not utilized by salmonellae. Neither lipase nor deoxyribonuclease is produced. Salmonellae are pathogenic for humans, causing enteric fever, enterocolitis, and septicemia; the bacteria also may infect many other animal species.

Although most salmonellae are motile, non-motile variants may occur (95); *Salmonella gallinarum* or *Salmonella pullorum* always is nonflagellated. Most salmonellae are aero­genic; however, *Salmonella typhi* is an important exception and never produces gas. Anaerogenic variants of normally gas-producing salmonella serovars may occur; this is particularly common with *Salmonella dublin*. Hydrogen sulfide is produced by most salmonellae when grown on suitable media, but a few do not form it (e.g., some strains of *Salmonella choleraesuis* and most strains of *Salmonella paratyphi* A). Lactose is generally not utilized by salmonellae, but many strains of *Salmonella arizonae* have ß-galactosidase activity. About 5% of *Salmonella* strains produce bacteriocins active against *Escherichia coli*, *Shigella*, and *Salmonella* (95).

**Susceptibility to the O1 Phage**

Most serovars in the genus *Salmonella* are susceptible to the O1 phage; this phage is highly specific for *Salmonella* and, thus, is useful for diagnosis. A *Salmonella* phage that attacks only flagellated bacteria has been isolated. Sensitivity to this phage depends on the H antigen. For example, bacteria with antigens of the G complex are resistant (95). The O1 phage test is a simple, rapid, inexpensive, sensitive, and specific procedure to identify salmonellae in the diagnostic laboratory (133).

**Antibiotic Susceptibility**

D'Aoust (35) indicated that patterns of antibiotic resistance (antibiograms) have been used to subgroup *Salmonella* spp. In this method, paper disks impregnated with standard amounts of antibiotic are placed on the surface of Mueller-Hinton agar previously inoculated with the test organism. The zones of growth inhibition are measured following overnight incubation of the plate with the agar medium. Antibiotics frequently used to challenge salmonellae include ampicillin, chloramphenicol, kanamycin, tetracycline, and trimethoprim-sulfamethoxazole.

**FOODBORNE SALMONELLOSIS**

To become ill from salmonellae, a person needs to consume food that contains the viable bacteria. The pathogen then enters the digestive tract, grows in the small intestine, and causes inflammation resulting in enterocolitis (64). The symptoms of salmonellosis usually appear about 12 to 36 h after eating contaminated food. A sudden onset of abdominal pain, diarrhea, nausea, vomiting, chills, and fever are common symptoms; dehydration, headache, and prostration also may occur. The severity and duration of symptoms depend on the type of *Salmonella* present, the amount of food (and, hence, of salmonellae) eaten, and the susceptibility of the person involved.
TABLE I. Outbreaks of human salmonellosis associated with milk products and occurring in the United States from 1965 to 1981.

<table>
<thead>
<tr>
<th>Product</th>
<th>Serovar of Salmonella</th>
<th>Number of cases</th>
<th>Year</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td>dublin</td>
<td>14</td>
<td>1981</td>
<td>Washington</td>
<td>(4)</td>
</tr>
<tr>
<td>Raw milk</td>
<td>typhimurium</td>
<td>59</td>
<td>1981</td>
<td>Montana</td>
<td>(41)</td>
</tr>
<tr>
<td>Raw milk, certified</td>
<td>saint paul</td>
<td>1+</td>
<td>1981</td>
<td>California</td>
<td>(15)</td>
</tr>
<tr>
<td>Raw milk</td>
<td>dublin</td>
<td>125</td>
<td>1980-81</td>
<td>Washington</td>
<td>(101)</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>heidelberg</td>
<td>339+</td>
<td>1980</td>
<td>Colorado</td>
<td>(49)</td>
</tr>
<tr>
<td>NDM</td>
<td>agona, typhimurium</td>
<td>1+</td>
<td>1979</td>
<td>Oregon</td>
<td>(35)</td>
</tr>
<tr>
<td>Pasteurized milk</td>
<td>typhimurium</td>
<td>23+</td>
<td>1978</td>
<td>Arizona</td>
<td>(42)</td>
</tr>
<tr>
<td>Raw milk, certified</td>
<td>dublin</td>
<td>33</td>
<td>1977-78</td>
<td>California</td>
<td>(28)</td>
</tr>
<tr>
<td>Human milk</td>
<td>kottbus</td>
<td>7</td>
<td>1977</td>
<td>Maine</td>
<td>(117)</td>
</tr>
<tr>
<td>Raw milk</td>
<td>typhimurium</td>
<td>3</td>
<td>1977</td>
<td>Kentucky</td>
<td>(91)</td>
</tr>
<tr>
<td>Pasteurized milk</td>
<td>newport</td>
<td>43+</td>
<td>1975</td>
<td>Louisiana</td>
<td>(13)</td>
</tr>
<tr>
<td>Raw milk, certified</td>
<td>dublin</td>
<td>44+</td>
<td>1971-75</td>
<td>California</td>
<td>(75, 134)</td>
</tr>
<tr>
<td>Human milk</td>
<td>kottbus</td>
<td>1</td>
<td>1971</td>
<td>Illinois</td>
<td>(77)</td>
</tr>
<tr>
<td>Raw milk</td>
<td>typhimurium</td>
<td>40+</td>
<td>1967</td>
<td>Washington</td>
<td>(52)</td>
</tr>
<tr>
<td>NDM</td>
<td>newbrunswick</td>
<td>29+</td>
<td>1965-66</td>
<td>Nationwide</td>
<td>(24)</td>
</tr>
<tr>
<td>Raw milk</td>
<td>typhimurium</td>
<td>2</td>
<td>1965</td>
<td>Washington</td>
<td>(5)</td>
</tr>
</tbody>
</table>

1Adapted from Bryan (14).

The illness usually lasts from 2 to 6 d, and death is uncommon except in the very young, very old, or persons already weakened by other illness (64). Pathogenicity of foodborne Salmonella has been reviewed recently by D’Aoust (36). A report (33) that fewer than 10 cells of Salmonella typhimurium in Cheddar cheese were infectious emphasizes the importance of constant vigilance and stringent control in food manufacture.

Although salmonellosis commonly results from consumption of foods contaminated with the pathogen, Salmonella heidelberg was transmitted by direct contact from ill calves to a pregnant woman (71). The woman subsequently transmitted the infection at birth to her infant, and then further transmission occurred to other infants in a hospital nursery.

OUTBREAKS OF SALMONELLOSIS ASSOCIATED WITH DAIRY FOODS

Bryan (14) summarized information about some dairy-associated outbreaks of salmonellosis in the United States from 1965 to 1982 (Table 1). Some recent outbreaks will be discussed in the following paragraphs.

In Colorado (49), an outbreak occurred that resulted from cheese contaminated with S. heidelberg. Preliminary findings led to removal of 2087 kg of contaminated cheese from the market. Ultimately, 2830 kg of cheese were responsible for 339 confirmed cases of S. heidelberg infection (49).

Two outbreaks occurred in Canada; one occurred in 1982 and involved Cheddar cheese made from unpasteurized milk. The cheese was contaminated with Salmonella muenster (138). The second occurred in 1984, and the cheese was contaminated with S. typhimurium (40). Raw milk added to pasteurized milk was determined to be the source of Salmonella in this outbreak.

In 1985, between March 22 and mid-April, more than 16,000 culture-confirmed cases of salmonellosis occurred in northern Illinois and surrounding states (7). Investigations by the Illinois Department of Public Health linked the outbreak to 2% pasteurized milk. Salmonella typhimurium was isolated from patients in northern Illinois and from milk in unopened cartons (7, 21). Investigations indicated that the outbreak-related strain of S. typhimurium was heat-sensitive and would not be expected to survive the pasteurization time and temperature conditions in use. The D values at 71.7°C for 18 isolates ranged from .25 to .68 s. An environmental investigation—conducted by the Illinois Department of Health, the FDA, the Centers for Disease Control, Silliker Laborato-
ries Inc., and Jewel (the company involved) personnel on April 2 to 9, 1985—revealed potential cross-connection (skim milk transfer line) through a cluster of valves between the pasteurized skim milk tank and tanks containing condensed skim milk, cream, and raw milk. The factory involved ceased operations when the outbreak occurred and has not reopened.

The outbreak-related *S. typhimurium* was characterized by analysis of antimicrobial resistance patterns. It was resistant to tetracycline, ampicillin, carbenicillin, streptomycin, and sulfisoxazole but was sensitive to chloramphenicol, trimethoprim-sulfamethoxazole, cephalothin, gentamicin, nalidixic acid, kanamycin, and trimethoprim (7). The FDA tested the outbreak strain with additional antimicrobials and found that it also was resistant to sulfadiazine, penicillin, and triple sulfa (7).

In 1989, Mozzarella cheese and shredded cheese products were identified by epidemiological evidence as the vehicles of infection in an outbreak of salmonellosis in Minnesota and Wisconsin (9, 43). The serotypes involved were identified as *Salmonella javiana* and *Salmonella oranienburg*. These serotypes have rarely been associated with dairy products (9, 43). This outbreak suggests that cheese could be a more common source of *Salmonella* than previously recognized.

Both *S. javiana* and *S. oranienburg* were isolated from 2 (3%) of 68 453.5-g blocks of Mozzarella cheese distributed in Minnesota. The most probable number of *Salmonella* in these samples was 36 and 4.3/100 g, respectively (9). It is estimated that 1500 to 15,000 outbreak-associated cases of salmonellosis may have occurred in Minnesota. The outbreak was recognized because of an increased incidence of infection with *S. javiana* in Minnesota. The widespread nature of the outbreak was confirmed by the increased incidence of infection with *S. javiana* that occurred simultaneously in Wisconsin and North Dakota (9). A likely source of *Salmonella* in this outbreak appears to be either contamination of finished product through its handling by an infected production worker or environmental contamination of the finished product within the factory. The factory where the cheese was made was not operating when the outbreak occurred and when the investigation was completed. Thus, it was impossible to know what might have happened at the factory.

Recently, the Communicable Disease Surveillance Centre (London, England) received reports of three family outbreaks of *Salmonella enteritidis* PT4 associated with consumption of homemade ice cream or sorbet made with fresh eggs (10); seven people were affected. *Salmonella enteritidis*, which probably came from the eggs, was isolated from the ice cream or sorbet in all three outbreaks (10).

**CONTROL AND PREVENTION OF HUMAN SALMONELLOSIS**

Gravani (64) indicated that prevention of salmonellosis can be achieved when management and employees work together 1) to prevent contamination: keep raw foods away from processed foods; avoid cross contamination; practice good personal hygiene; wash hands thoroughly after going to the toilet and after handling raw food; thoroughly clean and sanitize all equipment, utensils, and food contact surfaces; and avoid recontamination of heated products; 2) to inhibit microbial growth: hold foods below 7°C or above 60°C, do not allow foods to remain at room temperature for a long time, and cool foods quickly; and 3) to kill microorganisms: salmonellae are not very heat resistant and can easily be destroyed by proper heat treatments. When the nature of these bacteria is understood and the principles of good sanitation are followed, salmonellosis can be prevented.

Recently, Perdigon et al. (107) studied prevention of gastrointestinal infection using milk fermented with *Lactobacillus casei* and *Lactobacillus acidophilus*. Such milk could be consumed and, thus, used as a simple immunobiological method to prevent gastrointestinal infections in infants and to control enteric disorders in others. However, it is necessary to study in more detail this fermented milk product, which could be used to prevent or to treat enteric infections, thus reducing the use of antibiotics.

Silliker (118) suggested that the salmonellosis problem could be controlled if the use of *Salmonella*-contaminated feeds were discouraged and if the growth of the organism were stopped in those animals who become infected in spite of our prevention efforts. A
combination of the Scandinavian program of controlling salmonellae in livestock and the Canadian promise to educate tomorrow's homemaker may provide the means for reducing the magnitude of this significant public health problem (118). The Scandinavian countries have intensive programs for control of Salmonella in domestic animals, especially on poultry. Those countries are essentially "closed shops": the movement of animals is limited through extensive border controls.

ENVIRONMENTAL CONDITIONS AND THEIR EFFECTS ON SALMONELLAE

Heat Treatments

The sensitivity of salmonellae to moist heat is widely accepted, and milk pasteurization treatment is sufficient to kill even exceedingly large numbers of Salmonella cells (60). D'Aoust et al. (38) studied thermal inactivation of Salmonella species in fluid milk. They (38) heated inoculated milk at 60 to 74°C using a pilot-scale plate pasteurizer unit. The mean and minimum residence times of milk in the holding tube of the pasteurizer were 17.6 and 16.2 s, respectively. D'Aoust et al. (38) determined the thermal resistance of Salmonella senftenberg 775W, of S. muenster previously isolated from raw fluid milk, and of two mixtures each consisting of 10 Salmonella strains isolated from human or nonhuman sources. They (38) found that heating at 63°C produced a 4 log$_{10}$ or greater reduction in the number of viable Salmonella, including the heat-resistant S. senftenberg 775W, and that heating at 60°C caused a minimum 2 log$_{10}$ decrease. Those findings warrant some caution in the use of subpasteurizing temperatures for thermal processing of fluid milk, a practice that prevails in some segments of the cheese industry.

Survival of salmonellae during heating is a function of composition of the heating medium rather than just the a$_w$ of the environment. For example, when Salmonella montevideo was heated at 57.2°C in solutions of sucrose, glycerol, fructose, or sorbitol, all at a$_w$ 0.96 and pH 6.9 ± 1, the D values were 16.5, 1.2, 1.3, and 5.5 min, respectively (60).

Ng et al. (100) tested the heat resistance of several serotypes of Salmonella in trypticase soy broth supplemented with 2% (wt/vol) yeast extract. They reported D values of .9 to 1.3 min at 57°C for most of the serotypes tested. Two serotypes, Salmonella blockley 2004 and S. senftenberg 775W, had D values of 5.8 and 31.0 min, respectively.

Rubin (115) reported a direct correlation between the increase in casein concentration and length of survival of salmonellae in yogurt whey. This suggested that casein exerts a protective effect on S. typhimurium in acid dairy products and that the degree of protection depends on the casein concentration, the form of the casein molecule, and the pH.

Sublethal heat injury of S. typhimurium has been associated with degradation of ribosomal RNA and DNA (62, 63, 79, 126, 127), reduced enzyme activities (128), changes in transport kinetics (108), and lipid content (109, 129). D'Aoust (30) studied the efficacy of 32 additives to Levine eosin-methylene blue salts medium for recovery of S. typhimurium that was injured by sublethal heat. In order of decreasing effectiveness, lactate, mannitol, and $\alpha$-glycerophosphate mediated 90% or more recovery of injured cells; similar levels of

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recovery were obtained on eosin-methylene blue salts medium supplemented with 1% (wt/vol) tryptic soy broth, protease peptone, on plate count agar.

**pH**

The optimal pH for growth of salmonellae is generally accepted to be between 6.5 and 7.5. Although the organisms proliferate rapidly at those pH, they also can grow readily in more acidic environments. The minimal pH at which salmonellae can initiate and sustain growth is not well defined and varies depending on the serotype, the temperature of incubation, and the nature and composition of the growth medium (23). Minimal pH at which salmonellae initiated growth under optimal laboratory conditions were 4.05 when broth was adjusted with hydrochloric or citric acid, 4.10 with tartaric acid, 4.20 with gluconic acid, 4.40 with lactic acid, 4.60 with succinic acid, 4.70 with glutaric acid, 5.10 with adipic or pimelic acid, 5.40 with acetic acid, and 5.50 with propionic acid (23). The growth-limiting pH depends on several factors, of which the most important is the acid molecule itself. Other factors, such as temperature, oxygen supply, and level of inoculum, also are important.

The antimicrobial action of organic acids is well known. These acids exert their effect through their undissociated molecules (47). Thus, activity of the acids is dependent on pH, which determines the degree of dissociation. At low pH, the proportion of undissociated molecules is greater than at pH approaching neutrality. Of the food-type organic acids, acetic acid is the most detrimental to bacteria, particularly when tests are conducted on laboratory media. Park et al. (106) found that, when added to cold-pack cheese food, lactic acid was somewhat more effective in inactivating *S. typhimurium* than was acetic acid. The reverse was true for *Listeria monocytogenes* (116). It is unclear whether reactions between acetic acid and cheese food ingredients have diminished the effects of the acid on *S. typhimurium.*

**Sodium Chloride**

Growth of *S. heidelberg*, *S. typhimurium*, and *Salmonella derby* in shake cultures of nutrient broth containing 0 to 8% NaCl was determined at 8, 12, 22, and 37°C (87). In addition, growth of *S. heidelberg* in 0 to 9% NaCl at 39, 41, 43, and 45°C also was tested (87). At 8°C, *S. heidelberg* grew with 1 and 2% added NaCl, *S. typhimurium* grew with 1% added NaCl, and *S. derby* failed to grow when 1 or 2% NaCl was added to the medium.

When incubated at 12°C, all three serotypes grew in the medium with 0 to 4% NaCl. At 22°C, NaCl tolerance increased from 1 to a maximum of 5 to 8%. When incubated at 37°C, the organisms increased in numbers in up to 7 to 8% NaCl. Low concentrations of NaCl stimulated growth of salmonellae at some temperatures.

For practical purposes, foods with low NaCl content may not support growth of salmonellae at low temperatures. However, if storage temperatures are raised, these bacteria may be able to grow even when NaCl is present (87). Governmental researchers (8) discovered that apparent anomaly during studies designed to examine the interaction of pH, temperature, atmosphere, and NaCl content on growth of *S. typhimurium* in a culture medium of glucose and mineral salts. They (8) noted that growth decreased at higher NaCl concentrations under aerobic conditions and that anaerobic incubation repressed the amount of growth relative to that under aerobic conditions. Anaerobiosis provided protection against the effects of high NaCl concentrations. *Salmonella* growth was measured at NaCl concentrations ranging from 0 to 5%, in 1% increments.

**Low Temperature**

Salmonellae grow best at 35 to 37°C, but they can grow at much lower temperatures. Minimum growth temperatures were obtained experimentally when salmonellae were incubated at 1.1 to 12.5°C on the surface of agar in a temperature-gradient incubator. The minimum temperatures allowing slight growth of *S. heidelberg*, *S. derby*, *S. typhimurium*, *Salmonella aertrycke*, *S. montevideo*, *Salmonella newport*, and *Salmonella thompson* were 5.5, 6.1, 6.1, 5.5, 6.5, and 6.4°C, respectively (86). The length of incubation influences the minimum temperature at which salmonellae initiate growth. Thus, *S. heidelberg* initiated growth at 7.5°C when incubation was 5 d and
at 5.9°C when incubation was 12, 19, 26, or 34 d. *Salmonella typhimurium* initiated growth at 7.5°C when incubation was for 5 or 12 d and at 6.7°C when incubation was for 19, 26, or 34 d. Values for *S. derby* were 8.3°C (5 or 12 d of incubation) and 7.5°C (19, 26, or 34 d of incubation) (86).

It is important to recognize that growth of salmonellae may occur at temperatures below 6°C after a relatively long time. Thus, good temperature control is important when foods are to be refrigerated for an extended period. In such instances, food temperatures should be below 5°C at all times (86).

Airoldi and Zottola (2) studied growth and survival of two strains of *S. typhimurium* inoculated into optimal, minimal, and nutrient-deficient media and incubated at 7°C. They (2) found that *S. typhimurium* survived in all media for extended periods; one strain survived for more than 1 yr at 7°C in 1% peptone water and tryptic soy broth. Zottola and Smith (140) indicated that *S. typhimurium* survived for 9 d in sweet water at 1°C and for 14 d in a mixture of 30% propylene glycol and 70% water at −1°C. This mixture is used as a cooling liquid in dairy factories and could be a source of microorganisms in finished dairy products. The aw of the 30:70 mixture was determined to be .885 (140), which is below the level at which salmonellae will grow. The same authors (140) showed that the minimal aw for growth of salmonellae is .90 and that growth is independent of the solute used to control aw.

Stanfield et al. (121) applied cultures of salmonellae (two strains of *S. typhimurium* and one strain of *S. tennessee*) to the exterior surfaces of waxed cardboard or plastic milk containers. Contamination sites were sampled with premoistened cotton swabs during 14 d of refrigeration. A cell suspension of salmonellae was heat shocked at 48°C for 30 min (102, 126) and also was used in this study. Heat-stressed cells of all three *Salmonella* strains survived for up to 2 d (waxed containers) and 4 d (plastic containers). Unstressed cells of all three *Salmonella* strains survived up to 14 d on both types of containers (121).

The combined effects of pH and temperature on three serotypes of salmonellae, including *S. heidelberg* ATCC 8326, *S. typhimurium* ATCC 6994, and *S. derby* ATCC 6966, were investigated by Matches and Liston (88), who concluded that three of the more common serotypes of *Salmonella* spp. that cause food-borne illness grew on the surface of agar only over a narrow pH range between 6 and 8 at temperatures close to 5°C. Generally, foods held at temperatures below 5°C do not support growth of *Salmonella* spp. However, D'Aoust (37) recently reported that salmonellae proliferated at 2°C in fresh meats within 6 d and in shell eggs at 4°C within 10 d.

### Isolation and Enumeration of Salmonellae

Procedures to isolate salmonellae from foods are time-consuming and require a minimum of 4 to 5 d to obtain presumptive evidence of salmonellae contamination (3, 68, 73). Because damaged cells may account for a substantial proportion of the bacterial population in processed foods, the food microbiologist must choose the most appropriate methods for detecting damaged as well as uninjured cells (6).

The procedures for detecting salmonellae generally include preenrichment of food samples in a nonselective broth medium, enrichment in a selective broth medium, isolation of colonies presumed to be salmonellae on differential plating media, biochemical screening, and serological confirmation of isolates presumed to be salmonellae (31). Preenrichment in a nonselective broth medium provides for the uninhibited growth of indigenous bacterial flora and the resuscitation and proliferation of stressed or injured salmonellae to detectable levels (84). Generally enrichment of food samples in either selective or nonselective broths is superior to direct plating, especially when small numbers of salmonellae are anticipated (57). D'Aoust (32) reviewed the effectiveness of different preenrichment and selective enrichment conditions for isolation of salmonellae from foods.

Preenrichment in nonselective media increases recovery of salmonellae from products exposed to extreme environmental conditions during processing or storage (27, 45, 56). D'Aoust and Maishment (39) studied the efficacy of Clausen, EE(Mossel), Eugon, GN, Ter-gitol 7, lactose, and nutrient broths as salmonellae preenrichment media, using 165 food samples with an incident contamination level.
that ranged from 1.5 to 460 salmonellae/100 g. Replicate food samples (100 g) were preenriched in each of the seven media (900 ml) for 6 and 24 h at 35°C; various amounts (10, 1.0, and .1 ml) of preenriched cultures were selectively enriched in tetrathionate brilliant green (43°C) and selenite cystine (35°C) broths and then plated on bismuth sulfite (BS) and brilliant green sulfa agars. Good recovery was obtained with all media (95 to 100%) except EE (Mossel) (74%) after 24 h of incubation. Use of 1.0 ml rather than 10 ml of preenrichment transfer volume proved to be completely reliable and reduced the cost of analysis. Reconstitution (wet blending) of a larger amount of sample (dried milk) and then testing the equivalent of 100 g of solids increased the chances for salmonellae detection. Rehydration incubation for some time, e.g., 1 h, and then dilution to 1:10 solids to liquid ratio seemed to be beneficial (111). Additional information on factors that affect recovery of *Salmonella* spp. from foods has been provided by Andrews (6).

**Enrichment**

Selective enrichment inhibits growth of nonsalmonellae and facilitates isolation of salmonellae on differential agar media (48). Tetrathionate and selenite cystine broths, selenite brilliant green (16, 17), and modified Rappaport (132) enrichment media have been used in food analysis. Several formulations of tetrathionate broth, which differ in composition and selectivity, are currently in use (31). These include the Mueller tetrathionate broth as modified by Kauffman and the Mueller-Kauffman tetrathionate broth widely used in Europe (44, 131).

Wilson et al. (137) compared methods to isolate salmonellae from skim milk, 2% fat milk, whole milk, and buttermilk. Those authors (137) evaluated lactose broth, lactose broth plus brilliant green dye, buffered peptone water, and each milk type plus brilliant green dye as preenrichment broths. Incubation at 35 and 43°C was evaluated for use at the preenrichment stage. Recovery of salmonellae was determined after selective enrichment in selenite cystine, tetrathionate, and Rappaport-Vassiliadis broths. Results indicated that fluid milk should be examined for salmonellae by being preenriched in lactose broth, subcultured to selenite cystine and tetrathionate broths, and streaked to selective agars with 35°C as the incubation temperature throughout the analysis. However, selective enrichment at 41 to 43°C may be superior to that at 35°C (31, 54, 84).

Recently, the performances of Rappaport-Vassiliadis medium and tetrathionate brilliant green broth were compared for detection of salmonellae in pasteurized fluid whole milk that was artificially contaminated with *S. typhimurium*. The Rappaport-Vassiliadis medium was more sensitive and more selective than the tetrathionate brilliant green broth (130).

**Plating**

Various selective and differential agents are employed in plating media for isolation of salmonellae. Factors important in effectiveness of plating media are ability to support growth of salmonellae, inhibition of interfering bacteria, differentiation of salmonellae from other bacteria, stability, and reproducibility. Common serotypes of salmonellae can be confirmed with a few simple biochemical tests. More extensive biochemical tests are needed to differentiate atypical salmonellae from other bacteria (97).

Many plating media have been described, and numerous modifications have been proposed, as outlined by several authors (26, 35, 48, 97). Differential agar media range from only slightly selective, such as MacConkey agar, through moderately selective and differential, including Salmonella-Shigella, desoxycholate citrate, Hektoen enteric, and xylose lysine desoxycholate (XLD) agars, to highly selective and differential, such as BS, brilliant green, and brilliant green sulfa agars (48).

**Brilliant Green and Brilliant Green Sulfa Agars**

The brilliant green and brilliant green sulfa agars are basically peptone and yeast extract media with brilliant green dye to suppress Gram-positive organisms and coliforms. Sulfapyridine in brilliant green sulfa agar suppresses *Proteus* spreading to a greater extent than that of salmonellae. The selective and differential system depends on two carboxy-
drates, lactose and sucrose, and on an acid indicator system utilizing phenol red. Salmonellae are unable to utilize lactose or sucrose and their metabolism of peptone results in alkaline end products; therefore, because phenol red produces a red color at alkaline pH, salmonellae colonies are red. Sugar fermenters, such as coliforms, form yellow colonies because their production of acid causes phenol red to turn yellow (48). However, biotypes of salmonellae that can utilize lactose or sucrose do occur, and then the effectiveness of the media is reduced.

BS Agar

Several procedures recommended by AOAC (11), FDA (50), and National Academy of Science (99) for salmonellae isolation include BS agar as one of the plating media. Edwards and Ewing (46) reported that salmonellae usually grow well on BS agar and that it is a good medium for their isolation, including isolation of rare serotypes of salmonellae that rapidly ferment lactose. Most salmonellae appear as black colonies on this medium because of hydrogen sulfide production (48). D'Aoust (32) has reviewed enrichment and plating conditions that are needed to make this medium effective for detecting salmonellae in foods.

Desoxycholate-Citrate Lactose Sucrose Agar

Desoxycholate-citrate lactose sucrose agar is based on peptone and meat extract and utilizes sodium desoxycholate for inhibitory action against Gram-positive organisms and coliforms. The medium also contains carbohydrates that, if used, result in acid production and a change of neutral red from colorless to red. Normally, salmonellae colonies are colorless on this medium (48).

MacConkey Agar

MacConkey agar is a simple, peptone-based medium that has small amounts of bile salts and crystal violet to inhibit Gram-positive organisms. Lactose is incorporated as a fermentable carbohydrate, and neutral red reacts in acidic conditions, but, because salmonellae commonly do not ferment lactose, colonies are uncolored and transparent (48). However, lactose-positive salmonellae produce a coliform-type reaction on this medium. MacConkey agar is commonly used to purify isolates thought to be salmonellae. Use of this agar to isolate salmonellae from foods is uncommon.

XLD and Hektoen Enteric Agars

Isenberg et al. (74) found the performance of XLD and Hektoen Enteric agars to be very similar: both media readily permitted recovery of salmonellae. Rollender et al. (114) claimed the superiority of XLD agar and mentioned that salmonellae identification was greatly facilitated by distinctive morphological appearance after growth on this medium.

Rapid Methods

The absence of salmonellae in processed milk should be ascertained before marketing (70); this requires a rapid method, e.g., that of Hirsh and Martin (69). Their procedure uses the Salmonella-specific bacteriophage, Felix-O1 (65, 83, 133). This bacteriophage increases in number when salmonellae are present, and this increase can be detected by HPLC techniques. Hirsh and Martin (70) reported an adaptation of this method to detection of Salmonella spp. in milk. Fewer than 5 salmonellae/ml of milk were detected within 24 h of sample collection.

A technique using enzyme-labeled antibodies was developed as a method to detect salmonellae in food samples. The total analysis time is 48 h (76). An enzyme immunoassay also was developed to detect salmonellae in foods. Indirect test protocols were developed for use with microtitration plates or Gilford microcuvettes (94).

Thompson (125) reviewed the current status of immunofluorescent methodology for salmonellae. Hartman and Minnich (67) reviewed information on automated methods for rapid identification of salmonellae in foods.

SALMONELLA IN MILK AND MILK PRODUCTS

Fluid Milk

Low fat (2%) milk from a single milk processing factory in Illinois was identified as the
vehicle for transmission of salmonellae in a large foodborne outbreak, although the milk had been pasteurized. The pasteurization process may have been inadequate, or the milk may have become contaminated after pasteurization (7, 22). McManus and Lanier (92) reported that *Salmonella* spp. were isolated from 32 (4.7%) of 678 samples of raw milk collected from bulk tank trucks of milk suppliers in Wisconsin, Michigan, and Illinois. Isolation of salmonellae and campylobacters was recently reviewed by Fricker (54).

Subramanian and Marth (123) studied multiplication of *S. typhimurium* in sterile skim milk with and without added hydrochloric, lactic, and citric acids. They (123) inoculated cells of *S. typhimurium* into samples of sterile skim milk (approximately 10^3 cells/ml), which were then acidified with hydrochloric, lactic, or citric acids and incubated at 22 or 37°C. Acids were added at 2-h intervals in uniform amounts sufficient to reduce the pH from 6.7 to 4.0 (hydrochloric acid), 4.25 (lactic acid), or 4.48 (citric acid). Samples taken at 4-h intervals were plated on plate count agar, and plates were incubated at 37°C for 24 h. At 37°C, maximum numbers (10^9 cells/ml) appeared after 16 h of incubation. Citric acid was most inhibitory and was followed in order by lactic and hydrochloric acids. At 22°C, the highest numbers appeared in all milk samples after 16 h of incubation. Again, citric acid proved to be the most inhibitory, followed in order by lactic and hydrochloric acids.

Park and Marth (103) reported that salmonellae grew in skim milk containing antibiotics (penicillin, streptomycin, and tetracycline). Thus, salmonellae could grow even when certain antibiotics in milk retarded or prevented growth of and acid production by lactic acid bacteria. Behavior of *S. typhimurium* in skim milk during fermentation by lactic acid bacteria also was studied by Park and Marth (104). They (104) inoculated skim milk with *S. typhimurium* (approximately 10^3/ml) and with *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis*, *Streptococcus salivarius* ssp. *thermophilus*, *Lactobacillus delbruekii* ssp. *bulgaricus*, mixtures of the last two, a mixture of *Lactobacillus helveticus* and *Strep.

**Yogurt**

Fermented dairy products are bactericidal to both pathogens and spoilage microorganisms (58, 135); yogurt is more effective than other cultured milks (29, 59, 96, 120). Egyptian yogurt was artificially infected with *S. typhi*, *S. typhimurium*, *S. dublin*, *S. oranienburg*, *Salmonella oslo*, *S. paratyphi* A, and *S. paratyphi* B (1). The artificially infected yogurts were held at -1 and 4°C, and at room temperature (24°C). *Salmonella typhi* was most sensitive to yogurt, but *S. typhimurium* survived the longest at the different storage temperatures used. *Salmonella typhimurium* survived 68, 23, and 19 d at freezing, refrigeration, and room temperature, respectively. In contrast, *S. typhi* survived 30, 16, and 11 d under the same conditions.

**Cheese**

*Cheddar and Colby Cheeses.* White and Custer (136) studied survival of salmonellae in Cheddar cheese. *Salmonella newport*, *Salmonella newbrunswick*, and *Salmonella infantis* were singly added to cheese milk to evaluate their behavior in Cheddar cheese made from the milk and to determine survival times in cheese stored at 4.5 or 10°C. Of 48 lots of cheese inoculated with salmonellae, detectable numbers of these pathogens appeared in 16 lots aged for 9 mo at 4.5°C and in 6 lots aged for 9 mo at 10°C. This indicates that salmonellae, when initially present in large numbers (approximately 10^3/ml of cheese milk), can survive for a long time in Cheddar cheese of high pH (5.9). The same authors (136) indicated that studying behavioral patterns of pathogens in cheese would give the processor some guidelines to follow in the analysis of routine manufacturing procedures to ensure avoidance of any practice that might render cheese susceptible to contamination by salmonellae.
Park et al. (105) reported that salmonellae survived in ripening Cheddar cheese for up to 7 mo at 13°C and for 10 mo at 7°C. They noted that growth of these pathogens during early stages of ripening and the subsequent, extended survival of salmonellae were probably, at least in part, the result of high moisture (average 43 ± 2%) content and high pH (5.75 after overnight pressing) of the cheese. The high pH resulted from use of a slow acid-producing starter culture.

In contrast, Geopfert et al. (61), working in the same laboratory, reported that S. typhimurium grew rapidly during the manufacture of stirred-curd Cheddar cheese until NaCl was added to the curd. They observed that 10 to 12 wk of ripening at 13°C or 14 to 16 wk at 7.5°C were required before viable salmonellae in cheese became essentially undetectable.

Factors affecting growth and survival of salmonellae in the experimental manufacture of 7 lots of Colby and 65 lots of Cheddar cheese were studied by Hargrove et al. (66). They indicated that the rate and amount of acid produced during cheese making, the pH of cheese, and the type and size of starter inoculum all were important in suppressing growth and survival of salmonellae. Salt, moisture, chemical additives, and pasteurization of the milk (before artificial contamination) had little or no effect on the salmonellae. Addition of large numbers of Propionibacterium and Leuconostoc cells seemed to favor survival of salmonellae. Lactobacilli and enterococci tested had no effect on the salmonellae.

D’Aoust et al. (40) found that concentrations of S. typhimurium PT10 in Cheddar cheese, which was implicated in a major Canadian foodborne illness outbreak, ranged from .36 to 9.3/100 g. Such slight contamination likely accounted for uneven distribution of the organism among subsamples of the individual lots of cheese. Results further indicate that S. typhimurium survived for up to 8 mo in Cheddar stored at 5°C, which again points to the inadequacy (for consumer protection) of current regulations requiring 60-d storage (at ≥1.7°C) of cheese manufactured from raw or heat-treated (subpasteurization) milk before sale.

Mozzarella Cheese. Eckner et al. (43) examined a patient isolate of S. javiana implicated in an outbreak of salmonellosis in Minnesota for its behavior when subjected to Mozzarella manufacturing conditions. Mozzarella-type cheese was made from milk inoculated with S. javiana, which survived and grew through the acid-ripening phase. However, the temperature (60°C) attained in the cheese mass during stretching and molding killed all salmonellae present. No cheese in subsequent process steps was positive for Salmonella.

Cold-Pack Cheese Food. Park et al. (106) made 14 lots of cold-pack cheese food that were inoculated with S. typhimurium. Cheese food was then stored at 4.4 and 12.8°C. Number of salmonellae decreased rapidly during wk 1 of storage regardless of temperature or composition of the product. Viable salmonellae could not be recovered after 3 wk at 12.8°C or after 5 wk at 4.4°C from cheese food adjusted to pH 5.0 with lactic acid and fortified with .24% potassium sorbate. Substituting sodium propionate for sorbate resulted in 14 and 16 wk of survival by salmonellae when cheese food was held at 12.8 and 4.4°C, respectively. Partial or complete replacement of lactic acid by acetic acid added to cheese food containing sorbate was accompanied by somewhat longer survival (4 to 5 wk at 12.8°C and 6 to 9 wk at 4.4°C) of salmonellae than when only lactic acid was used. Elimination of added acid from the cheese food resulted in survival of salmonellae for 6 wk (at 12.8°C) and 7 wk (at 4.4°C) when potassium sorbate was present, for 16 wk (at 12.8°C) and 19 wk (at 4.4°C) when sodium propionate was used, and in excess of 27 wk at either temperature when no preservative was used.

Cottage Cheese. The effects of cooking temperatures ranging from 43.3 to 54.4°C on the fate of salmonellae during manufacture of cottage cheese were determined by McDonough et al. (90). Salmonellae survived at 43.3 and 46.1°C in all trials and at 48.9°C in one of five trials. None survived at 51.7°C. Creamed cottage was inoculated with salmonellae, stored at 4.4°C, and analyzed periodically. No marked decrease in numbers was observed in cheese during storage (90).

Domiat Cheese. Domiati cheese was manufactured from pasteurized buffalo milk with 5 and 10% added NaCl. Cheese milk was artificially contaminated with S. typhi at 104 to 106 cfu/ml. The resultant cheese was pickled for 39 d at room temperature (98). Naguib et al. (98)
reported that S. typhi survived in Domiati cheese for 15 to 36 days, depending on the manufacturing process. Initial numbers in the range of $10^4$ to $10^6$ cfu/ml did not affect longevity of S. typhi in the cheese. When cheese milk contained 5% added NaCl, the average survival time of S. typhi in Domiati cheese was 34 days; it was 16 days when the milk contained 10% added NaCl. Thus, it is recommended that pasteurized milk be used when Domiati cheese is made with a relatively low NaCl concentration (98).

**NDM**

Licari and Potter (82) inoculated NDM with S. typhimurium and S. thompson and then stored it at 25 to 55°C for up to 8 weeks. A reduction in Salmonella population of three or more log cycles was obtained in 4 to 8 weeks at 45 and 55°C. Salmonella thompson was more resistant to heat during storage than was S. typhimurium. Storage at 35 or 25°C reduced Salmonella numbers considerably in 4 to 8 weeks, and such storage would be expected to affect the numbers in contaminated commercial powders. However, such storage did not make heavily contaminated powders Salmonella-negative in 8 weeks and would not substitute for good sanitation in the production of NDM.

Licari and Potter (81) also studied survival of S. typhimurium, S. thompson, S. tennessee, and Salmonella kentucky inoculated into concentrated skim milk (approximately $10^7$ cfu/100 g), which was then spray-dried under various conditions. Although spray drying at commercial temperatures killed substantial numbers of salmonellae in skim milk, in no instance did it yield salmonellae-free powder (81). The same authors (80) studied quantitative recovery of Salmonella added to NDM and reported that the most probable number assay is suitable for investigations of Salmonella survival during spray drying and further handling of NDM.

McDonough and Hargrove (89) studied heat resistance of salmonellae in concentrated milk. They inoculated concentrated milk (50% solids) with $10^5$ to $10^7$ salmonellae/g. Survival of all species tested (S. senftenberg 775W, S. typhimurium TM1, and S. newbrunswick 1608) was greater in concentrated than in fluid milk (10% solids). Salmonella senftenberg survived at 65.5°C for 6 minutes in the concentrated milk, but there was no survival in samples of fluid milk treated for 3 minutes at this temperature.

Miller et al. (93) studied survival of salmonellae and E. coli during the spray drying of various food products. They (93) employed two inlet-outlet air temperature combinations throughout the study. The first, 225 ± 2°C inlet and 93 ± 2°C outlet, resulted in NDM containing 3 ± 0.2% moisture when a concentrate containing 40% solids was dried and 3.4 to 3.7 ± 0.2% moisture when the concentrate contained 20% solids. The second combination, 165 ± 2°C inlet and 67 ± 2°C outlet air temperature, resulted in NDM containing 6.0 ± 0.2% moisture regardless of whether concentrates containing 20 or 40% solids were fed to the dryer.

Two strains of salmonellae were inoculated (approximately $10^7$ cfu/g) into the concentrates for spray drying. Destruction of the test organisms was greater when conditions were adjusted to produce the low moisture product regardless of the total solids level of the concentrate fed to the dryer. Results also indicate that several factors are important in determining to what extent enteric microorganisms survive the spray-drying process (93). First, product temperature during the drying process is significant; high product temperature and microbial cell destruction are closely related. Second, particle density or particle crust diameter influences the cell destruction rate. Thinner particle crusts increase the number of cells killed by heat. Strain variation with regard to sensitivity to spray drying is considerable. High fat content in the product being dried appears to enhance the destruction of enteric bacteria, quite possibly, because of longer retention of thermal energy in the lipid material. No single parameter governs the behavior of salmonellae during the drying process.

**Butter**

Smis et al. (119) made three small experimental lots of butter from commercial cream contaminated with Salmonella typhimurium var. copenhagen. Both cream and wash water were contaminated, and resulting butter contained $10^3$ to $10^5$ salmonellae/g. Contaminated butter was held for 10 weeks at 25, 4.4, 0, -17.8, and -23.3°C. Numbers of salmonellae
increased at 25°C and decreased at ≤4.4°C. The most appreciable decrease in viable salmonellae was at −17.8 or −23.3°C in unsalted butter followed in order by lightly salted (1.7% NaCl) butter and moderately salted (2.2% NaCl) butter. Furthermore, Smis et al. (119) reported that NaCl content (1 to 4%), as usually employed by the butter industry, is not appreciably bactericidal to *S. typhimurium* var. *copenhagen*. Butter readily supports growth of salmonellae at room temperature (24°C), but refrigeration or freezing for brief periods does not eliminate salmonellae from butter. Zagaevski (139) reported that salmonellae remained viable for up to 9 mo in butter.

**CONTROLLING SALMONELLA IN THE DAIRY FACTORY**

Salmonellae are widespread in the environment and, hence, can enter the dairy factory from various sources. Ayres (12) listed a large number of sources, including insects, birds, rodents, pets, cattle, water and ice, raw milk, other raw foods of animal origin, and raw fruits and vegetables. Humans sometimes can be a source of salmonellae. Dairy factories can control the entrance of insects, birds, rodents, pets, and, to some extent, infected humans. However, because the factory converts raw milk into finished dairy products, it cannot prevent entrance of raw milk, which can contain salmonellae. According to results of an FDA survey (92), 4.7% of 678 samples of raw milk taken from bulk tank truck loads of Midwestern milk contained salmonellae. This suggests that raw milk must be handled carefully to prevent contamination of the premises and of finished products by salmonellae.

Recently, Ryser and Marth (116) reviewed information on *L. monocytogenes* and listeriosis related to milk, milk products, and dairy ingredients. Much of what they reported about control of *L. monocytogenes* in dairy factories also is applicable to control salmonellae. Ryser and Marth (116) suggested that all raw milk be filtered and subsequently clarified or separated by centrifugation to remove extraneous matter and somatic cells (i.e., leukocytes) before pasteurization. Special care also should be used in cleaning and sanitizing separators, clarifiers, and the surrounding area.

Proper pasteurization is the only commercially practical means by which all pathogens that do not form spores in raw milk, including salmonellae, can be inactivated. Thus, pasteurization equipment must be designed, installed, maintained, and operated properly (116). Obviously, treatment at greater heat, as in UHT milk, also inactivate salmonellae. Also, care should be taken during filling and packaging operations. Product extruder heads are particularly prone to contamination and, therefore, should be sanitized frequently during filling operations. Cheese makers should prepare cheese from pasteurized milk whenever possible.

Franco (53) suggested that food processors should use the Hazard Analysis Critical Control Point system to ensure the safety of processed foods. The system serves to identify points during production at which a product may be subject to contamination with pathogens or spoilage bacteria and also points where these unwanted microorganisms can grow. Ultimately, implementation of the Hazard Analysis Critical Control Point system can serve 1) to minimize cross-contamination of finished products and maintain utensils and other equipment in a sanitary condition, 2) to implement effective rodent and insect control programs, 3) to minimize nonessential traffic in food processing areas, 4) to educate employees so that they follow proper personal hygiene practices, and 5) to ensure that technically competent managers are committed to the principles of food hygiene.

Additionally, the consumer 1) can be conscious of basic sanitation and health practice, especially hand washing, and the use of clean equipment and cutting boards; 2) can use proper methods in storing, preparing, and serving food; and 3) can be aware of potential microbial contaminants of raw foods (53).

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