Chapter 7

Salmonella

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Clinical pathologists in France in the early 19th century first identified a typhoid bacillus that was the causative agent for typhoid fever. This agent was eventually identified as Salmonella. In the United States, Salmon and Smith (1885) first isolated Bacillus cholerae-suis, now known as Salmonella cholera-suis, from swine with “hog cholera” (Le Minor, 1981). Theobald Smith, a researcher under Daniel E. Salmon in the USDA’s Bureau of Animal Industry, was the first American to identify Salmonella as a separate strain or genus. Although Smith actually identified the bacteria, Salmon’s name as administrator was listed first on the research paper, so the new bacterium was named for Salmon. In this chapter, the sections are intended to give the reader a basic understanding of the characteristics and illness, along with the sources and incidence of Salmonella in the environment and food commodities. Factors affecting survival in food processing operations, use of interventions, and discriminative detection methods are also discussed.

TYPE OF ILLNESS AND CHARACTERISTICS OF THE ORGANISM

Salmonellosis is a human infection caused by Salmonella bacteria. The establishment of a human Salmonella infection rests on the ability of the organism to attach (colonization) and enter (invasion) intestinal columnar epithelial cells (enterocytes) and specialized M cells overlying Peyer’s patches (Ponka et al., 1995). The majority of persons infected with Salmonella develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment. However, in some persons the diarrhea may be so severe that the patient needs to be hospitalized. In these patients, the Salmonella infection may spread from the intestines to the bloodstream and then to other body sites and can cause death, unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness. In rare instances, chronic conditions, such as reactive arthritis, Reiter’s syndrome, and ankylosing spondylitis, have resulted from Salmonella infections in patients. Bacterial prerequisites for the onset of these chronic diseases include the ability of the bacterial strain to infect mucosal surfaces, the presence of outer membrane lipopolysaccharides, and a propensity to invade host cells (Hakalehto et al., 2007; Burslem et al., 1990; Sackett et al., 1993). Voetsch et al. (2004) determined that the average confirmed annual human illness in the United States caused by nontyphoidal Salmonella infections was approximately 15,000 hospitalizations and 400 deaths. However, approximately 1.4 million cases of illness have been estimated to occur in the United States annually. The annual cost associated with salmonellosis has been estimated to be several billion dollars (Voetsch et al., 2004).

Salmonella spp. are facultatively anaerobic gram-negative non-spore-forming rods belonging to the family Enterobacteriaceae. Salmonella spp. are capable of adapting to extreme environmental conditions. The majority of Salmonella spp. are motile by peritrichous flagella. However, nonflagellated variants, such as S. enterica serovar Pullorum and S. enterica serovar Gallinarum, and nonmotile Salmonella strains resulting from dysfunctional flagella do occur. Salmonella spp. have the ability to metabolize nutrients by...
the respiratory and fermentative pathways. The optimal temperature of growth is between 35 and 40°C. However, dependent on the Salmonella strain and the type of food matrix, the range of growth can occur between 2 and 54°C. Furthermore, Salmonella strains have an optimum pH for sustained growth between 6.5 and 7.5.

The nomenclature of Salmonella has changed significantly over time and has progressed through a succession of taxonomical schemes based on biochemical and serological characteristics, principles of numerical taxonomy, and DNA homology. The Centers for Disease Control and Prevention (CDC) has adopted as its official nomenclature the scheme in which the genus Salmonella contains two species, each of which contains multiple serotypes (Brenner et al., 2000). The two species are S. enterica and S. bongori. S. enterica is divided into six subspecies, each referred to by a Roman numeral and a name (I, S. enterica subsp. enterica; II, S. enterica subsp. salamae; III, S. enterica subsp. arizonae; IIIb, S. enterica subsp. diarizonae; IV, S. enterica subsp. houtenae; and VI, S. enterica subsp. indica). S. enterica subspecies are differentiated biochemically and through genomic relatedness. A total of approximately 2,450 serotypes have been identified, with the largest number of these in the S. enterica subsp. enterica group (D’Aoust, 2000).

Salmonella strains vary in their pathogenic abilities. S. enterica subspecies are considered significant etiological agents for human food-borne-related illnesses. However, it is important to remember that public health agencies in the United States consider all species of Salmonella important to public health. Therefore, isolation procedures should recover all serotypes of Salmonella.

**SOURCES AND INCIDENCE IN THE ENVIRONMENT AND FOODS**

The natural habitat of Salmonella is the gastrointestinal tract of animals. However, it is ubiquitous and has been isolated from numerous other sources, such as seafood, fruits, and vegetables. These sources usually become exposed to Salmonella by either direct or indirect contact with contaminated fecal matter. Historically though, Salmonella has been thought of as primarily a pathogen of poultry meat, eggs, swine, and other farm animals. USDA, Food Safety and Inspection Service (FSIS) hazard analysis critical control point (HACCP) samplings confirm this association of Salmonella with raw meat and egg products (Table 1). Broiler chickens (11.4%), turkeys (20.3%), and ground chicken (43.0%) had the highest incidence among the FSIS-regulated meat products in 2006. Because chicken is the meat product with the highest frequency of Salmonella-positive samples, many people assume that the serotypes most frequently associated with human illness would be the serotypes most commonly found on poultry. The Salmonella incidence data from the CDC for humans and from the National Antimicrobial Resistance Monitoring System for farm animals are relatively consistent from year to year. As seen in the 2004 data (Table 2), S. enterica serovar Typhimurium, S. enterica serovar Enteritidis, and S. enterica serovar Newport are the most frequently isolated Salmonella serovars from human salmonellosis cases. However, S. enterica serovar Kentucky, S. serovar Typhimurium, and S. enterica serovar Heidelberg are the serotypes most frequently associated with poultry. The biggest change in poultry isolations in recent years is the rise in the incidence of S. serovar Kentucky and the increase in the incidence of S. serovar Enteritidis in broiler chickens.

Of particular concern since the mid-1990s has been a pandemic associated with S. serovar Enteritidis associated with raw or lightly cooked shell eggs.

**Table 1. Percent positive Salmonella tests in FSIS HACCP verification samples—2006**

<table>
<thead>
<tr>
<th>Product</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broilers</td>
<td>11.4</td>
</tr>
<tr>
<td>Market hogs</td>
<td>4.0</td>
</tr>
<tr>
<td>Cows/bulls</td>
<td>0.8</td>
</tr>
<tr>
<td>Steers/heifers</td>
<td>0.3</td>
</tr>
<tr>
<td>Ground beef</td>
<td>2.0</td>
</tr>
<tr>
<td>Ground chicken</td>
<td>45.0</td>
</tr>
<tr>
<td>Ground turkey</td>
<td>20.3</td>
</tr>
<tr>
<td>Turkeys</td>
<td>7.1</td>
</tr>
</tbody>
</table>


**Table 2. Salmonella enterica serotypes in humans versus chickens (slaughter), 2004**

<table>
<thead>
<tr>
<th>Rank</th>
<th>Human Serovar</th>
<th>%</th>
<th>Chicken Serovar</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Typhimurium</td>
<td>19.2</td>
<td>Kentucky</td>
<td>44.4</td>
</tr>
<tr>
<td>2</td>
<td>Enteritidis</td>
<td>14.1</td>
<td>Typhimurium</td>
<td>13.4</td>
</tr>
<tr>
<td>3</td>
<td>Newport</td>
<td>9.3</td>
<td>Heidelberg</td>
<td>13.1</td>
</tr>
<tr>
<td>4</td>
<td>Javiana</td>
<td>5.0</td>
<td>Enteritidis</td>
<td>6.6</td>
</tr>
<tr>
<td>5</td>
<td>Heidelberg</td>
<td>4.9</td>
<td>Schwarzengrund</td>
<td>2.8</td>
</tr>
<tr>
<td>6</td>
<td>Montevideo</td>
<td>2.4</td>
<td>Montevideo</td>
<td>2.3</td>
</tr>
<tr>
<td>7</td>
<td>1,4,[5],12:i:-</td>
<td>2.1</td>
<td>Thompson</td>
<td>1.7</td>
</tr>
<tr>
<td>8</td>
<td>Muenchen</td>
<td>2.1</td>
<td>Mbandaka</td>
<td>1.5</td>
</tr>
<tr>
<td>9</td>
<td>Saintpaul</td>
<td>1.9</td>
<td>Infantis</td>
<td>1.5</td>
</tr>
<tr>
<td>10</td>
<td>Braenderup</td>
<td>1.9</td>
<td>1,4,[5],12:i:-</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*Values are percentages of total isolates positive for the serotypes.*
The overall incidence of serovar Enteritidis contamination of eggs from commercial flocks in the United States has been estimated at around 0.005% (Ebel and Schlosser, 2000). Like most other paratyphoid (non-host-adapted) *Salmonella* serotypes, serovar Enteritidis is usually introduced to chickens via the gastrointestinal tract. Of particular concern is that after invasion through mucosal epithelial cells serovar Enteritidis can be disseminated systemically to a wide array of internal organs, including reproductive tissues (Gast and Beard, 1990; Humphrey et al., 1993). However, studies have also shown that several other serotypes can also be recovered from internal organs and tissues (Bailey et al., 2003; Cox et al., 2006, 2007a). By colonizing the ovary (the site of yolk maturation and release) and the oviduct (the site of albumen secretion around the descending yolk), serovar Enteritidis appears to gain access to the contents of eggs (Miyamoto et al., 1997; De Buck et al., 2004). The prevalence of serovar Enteritidis in egg layers and the incidence of serovar Enteritidis in human illnesses peaked in the late 1990s in the United States. Egg quality assurance programs have been sponsored by the industry, states, and the federal government, and their effectiveness in influencing the epidemiology of serovar Enteritidis in the United States is documented by Mumma et al. (2004).

Despite the widespread perception of meats and eggs as the predominate source of *Salmonella*, an analysis of the 23 *Salmonella* outbreaks in which the etiology of the causative food was known, reveals that only 5 outbreaks were definitively linked to poultry meat of eggs. The remaining 18 outbreaks were associated with nonmeat products, including multiple outbreaks associated with tomatoes, cantaloupe, and raw milk (http://www.about-salmonella.com, 2007). The largest outbreaks in 2006 were associated with tomatoes and peanut butter and in 2007 with pot pies (http://cdc.gov). While meat and poultry must always be considered potential sources of *Salmonella*, the recent and numerous outbreaks associated with other vectors, primarily produce, suggest that public health, regulatory, and food production industries must be aware of the almost ubiquitous presence of *Salmonella*.

**INTRINSIC AND EXTRINSIC FACTORS THAT AFFECT SURVIVAL AND GROWTH IN FOOD PRODUCTS AND CONTRIBUTE TO OUTBREAKS**

Although the potential growth of food-borne *Salmonella* is of primary importance in safety assessments, the propensity of these pathogens to persist in hostile environments further heightens public health concerns and the need to control this pathogen in the food chain (Humphrey, 2004). This significantly contributes to outbreaks occurring from contaminated food products, whether the products are from animal origin or another source. Intrinsic factors that affect survival and growth of *Salmonella* in food products include, but are not limited to, acidity, pH, moisture content, nutrient content, competitive microflora, and natural/added antimicrobials in the food.

The growth of *Salmonella* in acidic environments has been demonstrated to be significantly more robust if the bacterium has been preconditioned by previous exposure to lower pH conditions (Huhtanen, 1975). Acid stress can also trigger enhanced bacterial resistance to other adverse environmental conditions, as demonstrated by the growth of S. serovar Typhimurium at pH 5.8, leading to an increased thermal resistance at 50°C, an enhanced tolerance to high osmotic stress (2.5 M NaCl) ascribed to the induced synthesis of the Omp C outer membrane proteins, a greater surface hydrophobicity, and an increased resistance to the antibacterial lactoperoxidase system and surface-active agents, such as crystal violet and polymyxin B (Leyer and Johnson, 1993). The presence of *Salmonella* spp. that have increased acid tolerances in foods heightens the level of public health hazard because this could minimize the antimicrobial action of gastric acidity (pH 2.5) and promote the survival of *Salmonella* within the digestive system of humans (D’Aoust, 1991a).

High salt concentrations have long been recognized for their ability to extend the shelf-life of foods by inhibiting the growth of endogenous microflora (Pohl et al., 1993). This bacteriostatic effect results from a dramatic decrease in water activity ($a_w$). *Salmonella* growth is inhibited in the presence of 3 to 4% NaCl; however, salt tolerance increases with increasing temperature in the range of 10 to 30°C (D’Aoust, 1991b). *Salmonella* will not grow in foods with an $a_w$ value of <0.93. The $a_w$ in fresh meat, poultry, and fish ranges between 0.99 and 1.00 and in eggs, natural cheeses, fresh fruits, and vegetables from 0.95 to 1.00. *Salmonella* has been shown to proliferate at pH values ranging from 4.5 to 9.5, even though the optimum pH for growth is 6.5 to 7.5. Meat and poultry have a pH range of 5.1 to 6.4. Fish and shellfish have a pH range of 5.5 to 7.0. Most fresh fruits have a pH range of 1.8 to 6.7, and vegetables have a pH range of 3.8 to 7.3.

Extrinsic factors that affect survival and growth of *Salmonella* in food products include, but are not limited to, temperature, storage conditions, and packaging/atmosphere. *Salmonella* can readily adapt to extreme environmental conditions. Heat is used widely in food manufacturing processes to control...
the bacterial quality and safety of end products. *Salmonella* has the ability to acquire greater heat resistance following exposure to sublethal temperatures. The rapid adaptation of the organism to rising temperatures in the microenvironment with a level of enhanced thermotolerance is quite distinct from that described in conventional time-temperature curves of thermal lethality. This adaptive response uncovers potentially serious implications for the safety of thermal processes that expose or maintain food products at marginally lethal temperatures.

*Salmonella* can actively grow within a wide temperature range and has also exhibited psychrotrophic properties, as reflected in the ability to grow in foods stored at 2°C to 4°C (D’Aoust, 1994). In addition, exposing cells to low temperatures can increase the ability of some *Salmonella* spp. to grow and survive at refrigeration temperatures (Alpuche-Aranda et al., 1994; D’Aoust, 1991b). It has been shown that the composition of the freezing menstruum, the kinetics of the freezing process, the physiological state of *Salmonella*, and the serovar-specific responses to extreme temperatures determine the fate of *Salmonella* during freezer storage of foods (Corry, 1976). This has raised concerns for the efficacy of chill temperatures in ensuring food safety. Widespread refrigerated storage of foods packaged under vacuum or modified atmosphere to prolong shelf-life has also been utilized. Gaseous mixtures consisting of 60 to 80% (vol/vol) CO₂ with varying proportions of N₂ and/or O₂ have been found to inhibit the growth of aerobic spoilage microorganisms, such as *Pseudomonas* spp., without promoting the growth of *Salmonella* spp. (D’Aoust, 1994). A more in-depth review of stress responses and the ability of *Salmonella* to survive in foods can be found in the work of Humphrey (2004).

### FOOD PROCESSING OPERATIONS THAT INFLUENCE THE NUMBERS, SPREAD, OR CHARACTERISTICS

The ecology and behavior of *Salmonella* in foods of animal origin have been studied extensively. However, the behavior of *Salmonella* within other types of commodities, such as fruits and vegetables, is not fully elucidated (Beuchet, 2002). The occurrence of *Salmonella* in food products which are capable of harboring the organism, but traditionally not considered to harbor the organism, presents serious problems to food safety.

With regard to meat products, specifically poultry, the problem with eliminating *Salmonella* is complicated by the fact that these organisms are natural commensal microflora of the digestive tract and usually present in very high numbers. The prevalence of *Salmonella* in preharvest poultry is higher than any of the other meat commodities; therefore, reducing/eliminating the organism at the processing plant becomes a challenge. Once the birds reach market age, prior to catching, the feed is withdrawn to allow clearance of the gastrointestinal tract. This process may increase *Salmonella* incidence in the crop due to consumption of litter and fecal matter. The increase of the pH and decrease in lactic acid within the crop may actually be the cause of *Salmonella* increase within the crop or a combination of the two (Corrier et al., 1999; Hinton et al., 2000; Smith and Berrang, 2006). During transportation to the processing plant, cross-contamination from transport coops can occur. Once at the processing facility, the birds enter the killing stage, and cross-contamination from the neck-cutting knife can occur (Mead et al., 1994). Scalding, defeathering, and evisceration also cause cross-contamination between carcasses. Immersion chilling can be a source of cross-contamination if no antimicrobials are used; but with the addition of chlorine or other antimicrobials, the chiller has been shown to greatly reduce *Salmonella* and other microorganisms on the carcass. Air chilling is also utilized and is effective in reducing *Salmonella* cross-contamination but is less effective in reducing total bacterial load because there is no washing effect, as is seen in immersion chilling. However, it is important to point out that, as previously discussed, rapid exposure to decreased temperatures can increase the ability of some *Salmonella* spp. to grow and survive at refrigeration temperatures. This could be of particular concern if the *Salmonella* spp. become entrapped and/or attached within feather follicles, which could provide protection from interventions utilized in the chill tanks.

The emergence of antimicrobial resistance in *Salmonella* which can be traced to food-producing animals has generated much controversy and attention. The use of antimicrobial agents in production and processing facilities has contributed to the emergence and persistence of resistant strains. The meat animal and aquaculture industries are believed to contribute to increased *Salmonella*-resistant bacteria (Threlfall, 2002; Angulo et al., 2000). Furthermore, utilization of antibiotics at subtherapeutic levels as growth promoters could also be contributing to resistance (Antunes et al., 2003).

Fruit and vegetable contamination can come from the use of noncomposted animal manure, untreated sewage or irrigation water, or animals directly or indirectly in the fields. Increases in geographic distribution have also caused changes in the handling and storage practices for these commodities. This in turn could possibly influence the ability
of *Salmonella* to survive in these products. Cross-contamination of the products could occur during the collection, washing, or packaging steps within the processing chain. Sanitizers utilized to decontaminate the surface of these products lack the ability to infiltrate the cracks and intercellular spaces; therefore, *Salmonella* can survive and proliferate on the product (Mahmoud and Linton, 2008; Takeuchi and Frank, 2000; Zhuang and Beuchat, 1996).

*Salmonella* has been recovered from numerous other types of products. Interventions are usually not aimed against *Salmonella* in these types of processing facilities. Therefore, the *Salmonella* presence increases the likelihood of the resulting product being contaminated and a possible illness or outbreak occurring from consumption of the product. An example is *Salmonella* in dry-roasted cocoa beans which are utilized to produce chocolate products. Thermal inactivation of *Salmonella* in molten chocolate is difficult because the time-temperature conditions required to effectively eliminate the pathogen in this product would likely result in an organoleptically unacceptable product. The more troubling concern is that *Salmonella* has the ability to survive for many years in the finished product when stored at ambient temperature (D’Aoust, 1984). In 2007, *Salmonella* was isolated from peanut butter, which has never been considered a product in which *Salmonella* would be of concern. It was found that the contamination of the product was due to cross-contamination from environmental sources, not from the ingredients themselves. Therefore, from a processing view, no matter the product being processed, it is important to remember that *Salmonella* and other pathogens could potentially contaminate the products; therefore, adequate standard operating procedures, sanitation standard operating procedures, good agricultural practices, HACCP, or other practices should be in place to prevent, it is hoped, pathogenic organisms from adulterating the food product.

**RECENT ADVANCES IN BIOLOGICAL, CHEMICAL, AND PHYSICAL INTERVENTIONS TO GUARD AGAINST THE PATHOGEN**

Probably more work has been done to control *Salmonella* in chicken production and processing than in any other food. A comprehensive review of these efforts is discussed below.

Evidence of the role that on-farm interventions can play in effectively helping to control *Salmonella* can be seen in Sweden and Denmark, where on-farm control programs have significantly suppressed *Salmonella* in broiler chicken production. Sweden’s and Denmark’s programs were initiated in about 1990 and 1995, respectively. In both programs, extensive testing of feed, the environment, and processed chickens was conducted. No *Salmonella*-positive feed was permitted, and all breeder birds that tested positive for *Salmonella* were eradicated. In Sweden, if any *Salmonella*-positive flocks are identified, they must be killed and properly disposed of. In Denmark, the initial phases of the program are as in Sweden, but *Salmonella*-positive grow-out broilers are processed separately and can be sold to the consumer. Initially the cost of implementing the programs in both countries was paid for by the government, but now the programs are paid for through industry checkoffs. Final economic analysis for a similar program in the United States is ongoing, but it will likely not be economically feasible to implement this same program in the United States. However, alternative methods of achieving similar results may be possible.

The European experience has confirmed that the best way to control *Salmonella* in food systems is to control the pathogen on the farm and to prevent it from ever entering the processing plant. The size and competitive nature of the U.S. poultry industry make implementation of new *Salmonella* intervention technologies that would significantly increase costs of production a challenge, unless there is a concomitant decision by the entire industry to implement the technology. There are several technologies that are currently being researched and in some cases used by the U.S. poultry industry which offer the opportunity to significantly reduce *Salmonella*. Research and anecdotal evidence suggest that the use of live and killed-cell vaccines in breeders, hatch cabinet disinfection, competitive exclusion treatments in breeders and broilers, and extensive biosecurity in breeder and broiler operations should yield beneficial results, without the extensive costs of eradication programs.

Interviews with U.S. poultry producers suggest that as much as 50% of the industry is now using killed-cell vaccination or a combination of killed-cell and live vaccines to help reduce *Salmonella* in broiler breeders and their progeny. Killed vaccines can be more universal in nature, consisting of strains of *S. serovar Typhimurium* and *S. serovar Enteritidis* and some other strain, such as *S. serovar Heidelberg* or *S. serovar Kentucky*. In other cases, the killed vaccines are a collection of autogenous strains associated with a particular company or production complex. Live vaccines are often deletion mutant strains of *S. serovar Typhimurium* and are used to boost immune response and will not persist in the birds or the environment for long periods of time.
There is a paucity of peer-reviewed research concerning the long-term effectiveness of these programs when implemented in large-scale commercial operations; however, anecdotal responses from many in the poultry industry suggest that after 1 or 2 years, the level of *Salmonella* can be reduced by 20 to 50% from prevaccination levels.

Outside of preventing *Salmonella* from ever entering the breeder or grow-out farms and being an effective method for preventing *Salmonella* in processed broilers, the development and use of undefined competitive exclusion (CE) cultures probably have been the most effective interventions (Bailey et al., 2000). In 1972, Esko Nurmi and coworkers were the first to publish on the use of CE cultures to help control *Salmonella* in broiler chickens. Nurmi took intestinal material from healthy adult birds and fed the material back to newly hatched chicks, making them, in a very short time, much more resistant to becoming colonized with *Salmonella* than chicks of the same age with no added microflora. Several commercial products were eventually made using the concepts initiated by Nurmi. These products are used in many countries around the world today, but not in the United States (at least not legally with pathogen claims) because they were comprised of many strains of bacteria (undefined culture), some of which were not or could not be identified. The FDA has taken jurisdiction over these types of products and handles them as they do all “drugs,” which has made getting approval more problematic. Many people have made “probiotic” or defined CE products (in which all the component bacteria are known), but to this point no products have been demonstrated to be as effective as the undefined CE products. There was one product, Preempt, which was a continuous-flow defined culture comprised of 21 specific strains of bacteria, which gained FDA approval and was used briefly by some companies in the United States in the late 1990s or early 2000s. Preempt never proved to be particularly effective in reducing *Salmonella*, and there were production problems in maintaining the balance of organisms in the continuous flow. Therefore, Preempt is no longer commercially available.

The other area of “on-farm” pathogen control falls under the general category of biosecurity. For the purposes of this discussion, cleaning and disinfection, rodent control, and control of movement of people and their vehicles (anything entering the property or facilities) are linked under this category. Cleaning and disinfection programs vary widely. In Scandinavia, where all of the houses are built on concrete floors and out of materials designed to handle the harsh winters, the houses are cleaned down to the concrete and disinfected with chemical washes by a contract cleaner between every flock. In the United States, some houses have concrete floors, but many have dirt floors, and most are not built to be completely washed and cleaned between flocks. Growers within companies will either clean out the houses between flocks or rear broilers on what is called “built-up” litter, a process in which wood pine (or some other product) shavings are put down on top of the old bedding material between every flock. This process can often continue for 1 or 2 years (5 to 10 flocks) before cleanout. Several factors, such as company policy, flock health, availability of bedding material, and effective disposal of the litter, play into this decision. Rodent control has been shown to be critical for control of *S. enteritidis* in egg-laying flocks and likely plays a role in the control of all types of *Salmonella* in broiler grow-out. Most U.S. companies have a policy on rodent control, but implementation of the program is left up to the individual grower. Effective programs are available for those that choose to follow them.

Biosecurity varies widely in the poultry industry. Most biosecurity programs are implemented primarily to control and prevent movement of viruses and other potential disease-causing organisms which will affect the health and production of the animals. Help in controlling the movement of bacterial pathogens associated with food safety is a side benefit of these biosecurity programs. At the broiler breeder level, entry into and exit from the facilities always involve foot baths and can also include shower-in for entry into the facility. At the broiler grow-out level, biosecurity generally involves restricting access to the facilities to those authorized and the use of dedicated or disposable footwear and footbaths with disinfectants, such as quaternary ammonium compounds or iodine compounds. A few companies also have installed disinfection stations that will spray the tires and undercarriages of trucks and other vehicles entering and leaving the grow-out facilities.

In the integrated poultry production operations, the hatchery may be the most critical area for *Salmonella* control for two reasons. First, the newly hatched chick is more susceptible to colonization by *Salmonella* than older birds. As few as 1 to 10 cells can colonize a day-of-hatch chick compared to the more than 1,000 cells needed to colonize an older chicken (Cox et al., 1990a). Second, because there are often between 10,000 and 40,000 hatching eggs in a single hatch cabinet, even if only one or a few eggs are contaminated with *Salmonella*, when these eggs hatch they can spread *Salmonella* to many other chicks in the hatch cabinet. These chicks can then become colonized with *Salmonella* and subsequently spread the *Salmonella* to other chicks during
transport or in the grow-out house. An association with *Salmonella* serotypes recovered from the hatchery can be found on the final processed carcass (Bailey et al., 2002). Many studies have shown the need to properly clean eggs and to disinfect between hatch groups (Bailey et al., 1994; Cox et al., 1990b, 1991, 1997, 2007b). Several chemicals, including hydrogen peroxide or properly controlled formalin, can effectively prevent the spread of *Salmonella* during the actual hatch period (last 2 or 3 days in the hatch cabinet).

Since the implementation of the HACCP pathogen reduction plan in 1996 (http://www.fsis.usda.gov/oa/background/finalrul.htm), the primary means of attempting to control *Salmonella* in poultry has been the use of chemical disinfection in the processing plant. The primary reason for this approach is to try to emulate the pasteurization of milk, in which a terminal treatment for pathogens is applied at the end of processing, just before the product goes to the consumer.

On 23 December 1999, the Food Safety and Inspection Service (FSIS) published in the *Federal Register* a final rule, “Food ingredients and sources of radiation listed or approved for use in the production of meat and poultry products.” In January 2000, FDA and FSIS entered into a memorandum of understanding that outlines the procedures that are followed by FDA and FSIS regarding the joint review of requests for the use of food ingredients and sources of radiation in meat and poultry products. Except in certain circumstances, FDA will now list in its regulations (21 CFR) food additives and sources of radiation that are safe and suitable for use in the production of meat or poultry products. Approved chemicals with application for addition to meat and poultry production are listed (http://www.fsis.usda.gov/OPPDE/radad/FSISDirectives/7120.1.htm). Chemicals used in poultry processing to eliminate or reduce *Salmonella* are listed in Table 3.

In 2006, a survey of intervention treatments at five processing plant locations (prescalder brushes, online reprocessing [OLR], chiller treatments, chiller pH control [acidified], and postchill treatments) was responded to by eight integrated companies with a total of 100 processing plants. Chemical applications could be applied by spray with or without scrubber brushes or as dips. More intervention efforts were

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Use</th>
<th>Suggested mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidified sodium chlorite</td>
<td>Spray of dip solution: 500–1,200 ppm sodium chlorite and any GRAS acid to achieve pH 2.3–2.9; prechiller or chiller solution: 50–150 ppm sodium chlorite and any GRAS acid to achieve pH 2.8–3.2</td>
<td>Broad-spectrum germicides: oxchlorous compounds act by breaking bonds on cell membrane surfaces</td>
</tr>
<tr>
<td>Cetylpyridinium chloride</td>
<td>Not to exceed 0.3 g/lb of poultry, propylene glycol concentration 1.5 times that of cetylpyridium chloride</td>
<td>Hydrophilic portion reacts with the cell membrane, resulting in the leakage of the cellular components, leading to cell death</td>
</tr>
<tr>
<td>Chlorine-sodium hypochlorite</td>
<td>20–50 ppm in chill water; also used in processing water at 10–30 ppm</td>
<td>Oxidation of cell components, resulting in cell death</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>Not to exceed 3 ppm in poultry process water contacting whole fresh carcass</td>
<td>Oxidation of the cellular membrane and cellular constituents at high concentrations; it breaks the cell wall</td>
</tr>
<tr>
<td>Ozone</td>
<td>Antimicrobial agent as stated in 21 CFR 170.3(o)(2), used in gaseous or aqueous phase</td>
<td>Direct molecular reaction and indirect reactions involving free radicals, oxidation of cell membrane</td>
</tr>
<tr>
<td>Peroxyacetic acid</td>
<td>Maximum concentration of 220 ppm peroxyacetic acid, 110 ppm hydrogen peroxide, and 13 ppm 1-hydroxyethylidene-1, 1-diphosphoric acid</td>
<td>Strong oxidation of cell membrane and other cell components, resulting in cell death</td>
</tr>
<tr>
<td>Trisodium phosphate</td>
<td>8–12% solution in conjunction with a water spray containing 20 ppm chlorine; solution can be applied by spraying or dipping chilled or prechilled carcasses for up to 15 s</td>
<td>Disruption of cell membrane causing leakage of intracellular fluid; details of the antimicrobial mechanism have not been completely elucidated</td>
</tr>
</tbody>
</table>
made in the chiller treatments (93%) and in OLR (86%) than with the scalders (18%) or postchill dips (12%). Chiller treatments were overwhelmingly chlorine (hypochlorous acid) followed by peracetic acid and chlorine dioxide. The key to effective chlorine treatments in the chiller is to maintain chiller water at or near pH 7.0. Depending on the area of the country where the plant is located or whether trisodium phosphate is being used in the plant, the chill water may need to be acidified. Ninety plants used carbon dioxide to control pH in the chill tank compared to three plants that used citric acid. Chemical treatments used for OLR were far more diversified. Sodium chlorite (33%) and trisodium phosphate (24%) were used most frequently. Only 12 plants used a postchill treatment, and 8 of these used sodium chlorite. Food safety managers at the processing plants felt strongly that multiple hurdles will have to be used for successful reduction of *Salmonella* and that none of the interventions will work without attention to the entire process.

As seen in this recent industry survey, chemical treatments in the processing plant are the principal method that companies are currently using to control *Salmonella* and indirectly *Campylobacter* in chicken production and processing. There are potentially some concerns that may arise from this extensive use of chemicals during processing. The first would be direct health concerns that some of the chemicals may complex with the proteins in the meat and potentially be a human health concern. The second is the perception of many consumers that the use of chemicals is bad for them (note the rapid interest in all things organic), and finally, there are export issues with many countries that do not allow the use of any chemicals during processing.

**DISCRIMINATIVE DETECTION METHODS FOR CONFIRMATION AND TRACE-BACK OF CONTAMINATED PRODUCTS**

Isolation and identification of *Salmonella* by traditional cultural methods requires a series of steps for isolation and identification, which takes 4 to 6 days to complete. To reduce the screening time, rapid methods (i.e., miniaturized biochemical kits, antibody/DNA-based tests, and modifications to conventional tests) are utilized to detect the presence of *Salmonella* in samples (McMeekin, 2003). With most of the rapid methods commercially available, screening time for a *Salmonella* presence in the sample can be performed in as few as 2 days. The 2-day limitation is due mainly to the samples having to be enriched prior to testing to increase sensitivity and specificity (Feng, 1997). However, a positive result through utilization of rapid methods still needs to be confirmed by traditional cultural methods (Feng, 1996). A more detailed review of rapid methods can be found in the work of Feng (1996, 1997) and McMeekin (2003).

Traditional cultural isolation from samples involves an overnight preenrichment step utilizing a nonselective enrichment, such as buffered peptone water or lactose broth. After preenrichment, the sample is then transferred to selective enrichments, such as tetraionate, Rappaport-Vassiliadis, and selenite cystine broths, and incubated for an additional 24 hours. After enrichment, the samples are then plated onto selective agar plates, such as brilliant green sulfa, bismuth sulphite, xylose lysine deoxycholate, XLT4, modified lysine iron, and Hektoen enteric agars, and incubated for 24 h. In addition, chromogenic plating media can be utilized (Schonenbrucher et al., 2008). After incubation, presumptive positive samples are then subjected to biochemical screening on triple sugar iron (TSI) and lysine iron agars, which involves another day of incubation. Briefly, *Salmonella* spp. are oxidase and catalase negative, generally produce hydrogen sulfide and decarboxylate lysine, and do not hydrolyze urea. Many of the traits listed above traditionally have formed the basis for the presumptive biochemical identification of *Salmonella* isolates (Cox and Mercuri, 1976). Typical *Salmonella* isolates will produce acid and gas from glucose in TSI agar medium and will not utilize lactose or sucrose in TSI or in differential plating media, such as brilliant green, xylose lysine deoxycholate, and Hektoen enteric agars. *Salmonella* spp. produce an alkaline reaction from the decarboxylation of lysine to cadaverine in lysine (LI) agar, generate hydrogen sulfide gas in TSI and LI media, and fail to hydrolyze urea. The biochemical classification of food-borne and clinical *Salmonella* isolates is generally coupled with serological confirmation evaluating the somatic (O) lipopolysaccharides on the bacterial outer membrane, flagellum (H) antigens on the peritrichous flagella, and the capsular (Vi) antigen.

The significant number of illnesses due to *Salmonella* has made epidemiological trace-back to the cause of infection a very valuable tool to correct problems and reduce the incidence of food-borne outbreaks. Phenotyping and genotyping methods exist in order to evaluate *Salmonella* strains in research and from a public health standpoint. Serotyping, phage typing, biotyping, and R typing are examples of phenotyping methods. The enormous number of *Salmonella* serotypes, the various sources from which *Salmonella* can be isolated, and genetic differences that occur within a single serotype mean that it is not sufficient to carry out only serotyping in order to accurately determine the source of infection
CONCLUDING REMARKS

Historically, Salmonella has been thought of as a problem associated almost exclusively with meat and poultry products. Advances in methods to genetically characterize Salmonella have greatly assisted epidemiologists in their ability to accurately determine the source and spread of Salmonella. Recent epidemiological studies have clearly demonstrated that if it were ever true that meat and poultry were the primary sources of outbreaks, that may no longer be the case. Outbreaks associated with tomatoes, cantaloupes, peanut butter, and other produce suggest that vigilance and interventions from the farm all the way through food processing must be maintained at all times in order to ensure the production of safe and wholesome foods without Salmonella.

It is clear that the problem of Salmonella in the global food chain and its current and projected repercussions for human health are causes for concern. Numerous studies have suggested that antimicrobial resistance among bacteria is on the rise, and this has led to changes in control and treatment strategies. Increased understanding of Salmonella at the molecular level will possibly lead to better intervention strategies, real-time screening methods, and a dramatic reduction of Salmonella in certain food products. However, with the increase in globalization, efforts to control Salmonella will continue to be a significant issue well into the future as new products are continually introduced into the food arena.

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