Chapter 1
Salmonella in Poultry Meat Production

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1.1 *Salmonella*: A Major Foodborne Pathogen in Poultry

Foodborne illness caused by various pathogens represents a major public health concern that results in significant loss to the U.S. economy (Marder et al. 2017; Scharff 2012). In 2016, the Centers for Disease Control and Prevention (CDC) Foodborne Diseases Active Surveillance Network (FoodNet) identified 24,029 infections, 5512 hospitalizations, and 98 deaths caused by pathogens such as *Campylobacter*, *Cryptosporidium*, *Cyclospora*, *Listeria*, *Salmonella*, Shiga toxin-producing *E. coli*, *Shigella*, *Vibrio*, and *Yersinia* (Marder et al. 2017). Among the bacterial pathogens associated with foodborne illness, non-typhoidal *Salmonella* (NTS) caused the second largest number of confirmed and culture-independent diagnostic test (CIDT)-positive infections (8172 cases) in the USA, second only to *Campylobacter* that caused 8547 illness cases (Marder et al. 2017).

Salmonella is a major foodborne pathogen implicated in outbreaks causing human illness for over a century (Bean and Griffin 1990; CDC 2000, 2013; Chalker and Blaser 1988). The organism is historically considered as the causative agent of the "meat poisoning" outbreak reported in Germany in 1888 and was first isolated by A. Gärtner, naming it as *Bacillus enteritidis*. In the USA, salmonellosis was designated as a notifiable disease in 1943, and since then, a steady increase in the reported incidence of *Salmonella* has been noted (Angulo and Swerdlow 1999; Tauxe et al. 1989). Since the mid-1980s, the pathogen gained tremendous importance due to its association with foodborne illnesses worldwide (Rodrigue et al. 1990; Tirado and Schmidt 2001). Currently, many serotypes of *Salmonella* are prevalent, and others are emerging as health threats to humans who contract the infection

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by consuming *Salmonella*-contaminated food products, the major animal-derived foods being poultry meat and eggs.

Salmonella is a Gram-negative, non-spore-forming, and motile bacillus belonging to the *Enterobacteriaceae* family. It is a facultative anaerobe that grows between 8 °C and 45 °C and at a pH range of 4–8. The pathogen is broadly classified into typhoidal and NTS based on host adaptability and infectious nature. The NTS has a wide range of vertebrate hosts, whereas host range of typhoidal *Salmonella* is limited to humans (Feasey et al. 2012; Winter et al. 2010).

Since chickens serve as natural hosts for many NTS serovars, the pathogens are frequently isolated from poultry and poultry products, with meat and shell eggs being the most commonly implicated vehicles in outbreaks. Most serovars of *Salmonella* colonize almost every part of the chicken intestinal tract, with highest predilection potential noticed in the paired blind sacs at the hind end of the tract called the ceca. Once colonized, the pathogen can be excreted through the feces without chickens showing any obvious clinical signs of infection. This eventually leads to the horizontal transmission of infection to other healthy birds and flocks, contamination of carcasses during slaughter, contamination of eggs with feces, and the retrograde transmission of infection via the transovarian route by major serovars such as *S*. Enteritidis and *S*. Heidelberg (Gantois et al. 2008; De Reu et al. 2006).

Although zoonotic in nature, NTS often causes self-limiting gastroenteritis in healthy humans. However, the infection process is more severe in immunocompromised individuals, children, and older adults, and the infectious dose can be low. The incubation period of the disease typically ranges 12–72 h with the illness lasting for 2–7 days. Patients usually recover within a week without any antibiotic treatment except in cases of severe diarrhea, where intravenous fluid therapy is warranted (Feasey et al. 2012). However, the severe illness caused by antibiotic-resistant strains of *Salmonella* may result in longer periods of stay in the hospital (Lee et al. 1994). The infection often proceeds to bacteremia and invasive form in immunocompromised individuals (Antunes et al. 2016; Chen et al. 2013). The fecal excretion of the pathogen from infected humans leads to the transmission of the pathogen among different vertebrate hosts (Dhanoa and Fatt 2009).

1.2 *Salmonella* in Poultry Production

1.2.1 S. *Pullorum and* S. *Gallinarum*

Diarrheal diseases have been a serious problem in poultry rearing/production systems that resulted in significant economic loss to the producers/industry, historically. *Salmonella* serovars such as *S.* Gallinarum and *S.* Pullorum were commonly isolated from poultry intestinal contents, droppings, and internal organs ever since poultry rearing was considered a financial enterprise (Stafseth and Mallmann

1928). The industry was aware of the importance of hygienic practices in poultry production to avoid diarrheal diseases from their valuable flocks. During that time, the major focus of the poultry sector was selection of superior breeds for improved egg production. Poor hatchability and smaller eggs were significant concerns, and the market trend was more toward producing eggs with superior hatchability. To aid this process, selective breeding and progeny testing were made common practices.

Although methods such as sanitation, immunization, and elimination of carriers and birds that showed signs of disease were practiced to control fowl pox and pullorum disease in hopes of saving the production strains (Hutt 1938), numerous outbreaks of *S.* Pullorum were reported in poultry in the early 1900s, and the carrier status of chicken for pullorum disease had been established. *S.* Pullorum emerged as a significant pathogen in poultry production. The egg-borne transmission of the disease was reported in 1909, and the septicemic nature of the pathogen was first reported in 1913. The young birds were mainly susceptible to *S.* Pullorum, and the disease was known as "fatal septicemia of young chicks" or "bacillary white diarrhea" or "pullorum disease" (Bullis 1977; Tittsler 1930). The bacterium was isolated from the liver, heart, lungs, and ovaries. A severe economic loss was reported due to the loss of egg production and mortality. The serum agglutination test and pullorum test were commonly employed to detect the disease in the flock (Tittsler 1930).

Commercial hatcheries became the source of infection, and the use of disinfectants was practiced in hatcheries. The dedicated incubators and use of formaldehyde for fumigation of eggs to control *S.* Pullorum became a common practice (Bullis 1977; Bushnell and Payne 1932). Responding to the situation, the National Poultry Improvement Plan (NPIP) was introduced in the USA in 1935 to control the pullorum disease. As a part of the NPIP, screening tests such as whole blood tests, tube agglutination tests, and rapid serum tests were used to detect *S.* Pullorum in the poultry flocks to eradicate and limit the disease. Based on the test results, the flocks were categorized into pullorum-tested flocks, pullorum-passed flocks, and pullorum-cleaned flocks (Bullis 1977).

However, *S.* Gallinarum caused fowl typhoid in adult chickens and was recognized as early as 1888. The tests that were used to screen pullorum disease were also used to detect *S.* Gallinarum. With the introduction of NPIP, the establishment of pullorum-free flocks also resulted in reduced incidence of fowl typhoid. In addition, the breeds such as White Leghorn were inherently resistant to these diseases, and the rearing of breeds resistant to infection became a common mitigation practice. Later, in 1954, screening of *S.* Gallinarum was also included as a part of NPIP. Moreover, antibiotics were used in poultry production to control these pathogens, resulting in tremendous improvement (Bullis 1977). Pullorum disease and fowl typhoid have been currently eradicated from the commercial flocks of developed countries such as the USA and Canada (Shivaprasad 2000). Although non-zoonotic, these pathogens still cause major economic problems in developing countries since they are highly adapted to poultry (Barrow and Freitas Neto 2011).

1.2.2 *Non-typhoidal* Salmonella

Though the eradication of *S.* Pullorum and *S*. Gallinarum could be achieved, the emergence of NTS resulted in significant safety concerns over the production of poultry meat and eggs for human consumption. The NTS *Salmonella* serovars caused 28% illness associated with foodborne outbreaks during 1973–1987 (Bean and Griffin 1990). On a later time-frame, 40% *Salmonella*-associated foodborne illness were reported in the USA from 1993–1998 (CDC 2000). The proportion of foodborne salmonellosis by poultry meat and eggs increased significantly from 1993 to 1998 compared to that occurred in the preceding decade. In the following decade (1998–2008), NTS *Salmonella* contributed 18% of the total illness associated with foodborne outbreaks in the USA (CDC 2013), underscoring a constant presence of NTS as the etiological agent in those outbreaks. *Salmonella* remains a major foodborne bacterial pathogen in the USA over a period of 50 or more years (Bean and Griffin 1990; CDC 2000, 2013; Chalker and Blaser 1988).

Two major epidemiological events that occurred in relation to the *Salmonella* serovars in the previous century were the emergence of *S*. Enteritidis as a major pathogen in poultry and the emergence of antibiotic-resistant strains of *Salmonella* (Rabsch et al. 2001). Although the poultry-adapted serovars of *Salmonella* such as *S.* Pullorum and *S*. Gallinarum were eradicated from commercial flocks in the USA by 1950, this successful event, however, created an environmental niche to be occupied by *S*. Enteritidis which was abundant in the rodent population. Since *S*. Gallinarum possessed cross-immunity against *S*. Enteritidis infection, it is reasonable to believe that the eradication of one resulted in the emergence of the other. In addition, higher bird density and vertical integration of poultry production system also facilitated the transmission of *S*. Enteritidis among poultry flocks (Foley et al. 2008, 2011).

1.2.3 S. *Enteritidis: A Major Serovar*

S. Enteritidis is the most genetically homogenous serotype of all *Salmonella* (Porwollik et al. 2005). Although limited in genomic diversity, the field isolates of the serotype vary in their capabilities to form biofilms, growth characteristics, production of high molecular mass lipopolysaccharides, and survival within the egg albumen (Clavijo et al. 2006; Jain and Chen 2007; Yim et al. 2010). In chickens, the pathogen varies in its virulence potential to cause mortality or to colonize the intestinal tract and invade the spleen and liver (Gast and Benson 1995, 1996). On-farm investigations indicate that once chickens are exposed to the pathogen, the entire flock can become colonized rapidly (Berrang et al. 2009; Foley et al. 2008). This could be attributed to the ability of the pathogen to proliferate in the gastrointestinal tract of chicken (Poppe 2000) and the multitude of sources in farms contributing to pathogen spread in birds.

S. Enteritidis is invasive in both young and adult chickens (Shah et al. 2011). Young chickens develop systemic disease with varying degrees of mortality (Duchet-Suchaux et al. 1995; Velge et al. 2005). The affected chicks may show all or some signs such as anorexia, depression, ruffled feathers, huddling together in groups, reluctance to move, drowsiness, dehydration, white diarrhea, stained and pasted vents, and stunted growth (McIlroy et al. 1989). However, adult chickens, once colonized with the pathogen, may remain as asymptomatic carriers, shedding the pathogen to the environment continuously or intermittently (Golden et al. 2008; Velge et al. 2005). Chickens infected with high doses of *S.* Enteritidis can subsequently develop clinical salmonellosis with high mortality, whereas infection with low doses will result in clinically healthy carrier birds (Desmidt et al. 1997; Gast and Benson 1995; Van Immerseel et al. 2004a, b). Currently, improvement in the vaccination strategies and the development of targeted interventions to control *S.* Enteritidis in/on eggs and meat have tremendously improved the situation. However, the emergence of other NTS serovars, such as *S.* Heidelberg, *S.* Oranienburg, *S.* Infantis, *S.* Hadar, *S.* Kentucky, and others, have raised serious concerns for the industry (Dutil et al. 2010; Foley et al. 2011; Wong et al. 2014; CDC, 2016; Hindermann et al., 2017).

1.2.4 Antibiotic-Resistant Salmonella

The development of antibiotic resistance in NTS serovars, including the most prevalent serovars such as *S.* Heidelberg and *S.* Kentucky, is an increasing concern for the U.S. poultry industry (Dutil et al. 2010; Foley et al. 2011; White et al. 2001). For example, the outbreak isolates of *S.* Heidelberg in the recent foodborne outbreaks were resistant to many clinically relevant drugs such as streptomycin, ampicillin, gentamicin, tetracycline, sulfamethoxazole, chloramphenicol, and trimethoprim-sulfamethoxazole. In addition, the isolates were resistant to the drug of choice to treat human salmonellosis—ceftriaxone (Medeiros et al. 2011; Foley et al. 2011; Hoffmann et al. 2014). Ceftriaxone-resistant *S.* Heidelberg was isolated from the retail meat sold in the USA (White et al. 2001) and Canada (Dutil et al. 2010). The resistant genes are encoded on plasmids in *S.* Heidelberg. *S.* Kentucky also possesses plasmids that encode genes for antibiotic resistance, resistance to disinfectants, iron acquisition, and bacteriocin production that enhance the survival of the pathogen in poultry flocks (Han et al. 2012).

Isolation of antibiotic-resistant strains of the *Salmonella* is not restricted to the U.S. poultry market. Jørgensen et al. (2002) reported that 70% of *Salmonella* isolated from 241 whole carcasses collected from retail stores in England were resistant to least one antibiotic, and 46% were resistant to more than one antibiotic. In a Portugal study, Antunes et al. (2003) detected 10 different serotypes of *Salmonella* from 60% of chicken samples, of which 50% were resistant to nalidixic acid and enrofloxacin. In a U.S. study, Cui et al. (2005) reported that all *S.* Typhimurium isolates obtained from retail chicken were resistant to more than five antimicrobials,

whereas those isolated from organic chicken were resistant to more than 17 antimicrobials. Out of the 569 samples positive for *Salmonella* (N = 4745), Roy et al. (2002) reported 92 samples collected from various environmental sources had isolates having resistance to erythromycin, lincomycin, and penicillin antibiotics, whereas all were susceptible to sarafloxacin and ceftiofur. In a different study, Parveen et al. (2007) found high levels of *Salmonella* from pre- and post-chilled poultry carcasses and water samples collected at the entrance of the chiller. Among the serovars isolated, 79.8% were resistant to at least one antibiotic, whereas 53.4% were resistant to more than one antibiotic, including tetracycline, ampicillin, amoxicillin-clavulanic acid, ceftiofur, streptomycin, and sulfisoxazole.

1.2.5 Salmonella *Serotypes in Poultry Meat Products*

It is well evidenced by the literature that poultry meat plays a major role in causing *Salmonella*-associated foodborne outbreaks since the 1950s (Bean and Griffin 1990; CDC 2000, 2013; Chalker and Blaser 1988). Poultry meat is the cheapest source of protein, and a large majority of U.S. population likes to have it in their diet (NCC 2017). Since poultry are the natural reservoirs of *Salmonella*, unhygienic processing and abused storage conditions of poultry meat can contribute to the incidence of salmonellosis in humans (CDC 2013).

Poultry meat, including the whole carcass, cut-up parts, and processed meats, are significant sources of several *Salmonella* serotypes that can cause disease in humans. In an early Canadian study, *Salmonella* was detected from 73.7% turkey carcasses and 38.2% chicken carcasses (Lammerding et al. 1988). Later, Logue et al. (2003) studied the incidence of *Salmonella* in two turkey processing plants in the Midwestern USA. Surface swabs were collected from poultry carcasses pre-chill and post-chill. Samples were also collected from the chill water. The overall incidence of *Salmonella* was found to be 16.7% after enrichment, and more positive samples were observed in pre-chill than post-chill. Major serotypes recovered were *S.* Senftenberg, *S.* Agona, *S.* Heidelberg, and *S.* Hadar. Jørgensen et al. (2002) studied the prevalence of *Salmonella* in 241 whole raw chicken samples purchased from retail shops in the UK at two different winter seasons of 1998/1999 and 1999/2000. The study found that *Salmonella* were present in 25% of the chicken samples. Among these, 19% of *Salmonella* was detected from both inside and outside of the chicken packages. The predominant serotypes detected were *S.* Indiana, *S.* Enteritidis, and *S.* Hadar (Jørgensen et al. 2002). Roy et al. (2002) detected *Salmonella* in 569 samples (11.99%) among 4745 samples collected from poultry liver and yolk sac, chicken ground meat, rinse water from spent hens and broilers, hatchery fluff, and drag samples from poultry environment during 1999/2000 in the Pacific Northwest. Out of the 97 positive samples serotyped, *S.* Heidelberg (25.77%), *S.* Kentucky (21.64%), *S.* Montevideo (11.34%), *S.* Hadar (5.15%), and *S.* Enteritidis (5.15%) were the major serotypes isolated. Likewise, the incidence of *Salmonella* in several poultry products obtained from a local butcher shop in Belgium revealed

that 60% of the samples were contaminated with *Salmonella* consisting of ten different serotypes. The most prominent serotypes isolated in the study were *S.* Enteritidis and *S.* Hadar (Antunes et al. 2003). In a study conducted in Spain to isolate *Salmonella* from 198 samples of chicken meat for sale in retail outlets, it was reported that the pathogen was isolated from 35.83% of the samples where the predominant serovars were *S.* Enteritidis (47.88%), *S.* Hadar (25.35%), and serotype 4, 12: b:-(II) (19.71%) (Domınguez et al. 2002). In yet another study conducted in Maryland, USA, Cui et al. (2005) reported 61% of organic and 44% of conventional chickens were contaminated with *Salmonella*. Between the years 2002 and 2006, *Salmonella* was isolated from 59.7% ground turkey, 36.9% chicken breast, and 3.4% pork chops among retail meat outlets in the USA (Zhao et al. 2008).

Frozen chicken nuggets, strips, and eggs were the main poultry foods implicated in the causation of human *S.* Heidelberg infections in Canada (Currie et al. 2005). Bohaychuk et al. (2006) detected *Salmonella* in 30% of raw chicken legs and meat and poultry products collected from a retail market in Alberta, Canada. In a Portugal study, Antunes et al. (2003) found *Salmonella* in 60 samples of poultry products obtained from local shops and canteens and detected ten different serotypes of *Salmonella* in 60% of samples and identified *S.* Enteritidis and *S.* Hadar as more prevalent. Jackson et al. (2013) studied the link between different *Salmonella* serotypes and various foods, including poultry, by analyzing outbreaks that occurred between 1998 and 2008. The study found that eggs and poultry meat were vehicles in more than 80% cases of *Salmonella* outbreaks caused by *S.* Enteritidis, *S.* Heidelberg, and *S.* Hadar. In another epidemiological study, Chittick et al. (2006) analyzed the national foodborne outbreak data from 1973 to 2001 and found that among 6633 outbreaks of known etiology, 184 (3%) were contributed by *S.* Heidelberg. Among these, 3 outbreaks were due to egg consumption, 17 cases were related to consumption of foods prepared using eggs, 25 cases were related to poultry, and 8 cases were due to consumption of food containing both poultry and eggs.

Foley et al. (2008) had observed that serovars *S.* Senftenberg and *S.* Hadar have become more prevalent in poultry, compared to *S.* Enteritidis, and *S.* Typhimurium. *S.* Heidelberg was reported to be more isolated from clinical cases and suggested to be more virulent than other serovars. The study concluded that among the top ten serovars of *Salmonella* associated with human infections, the majority were from swine and poultry, including *S.* Heidelberg. In a different study, Parveen et al. (2007) reported high *Salmonella* contamination in processed poultry products. In this study, 480 pre-chill and post-chill poultry carcasses and the chill water from entry and exit point were enriched and analyzed using an automated BAX system and culture methods to detect *Salmonella*. Approximately, 88.4% of pre-chill and 84.1% post-chill carcasses were found to be positive for the pathogen. In addition, 92% of the samples collected from entry points were found to be positive for *Salmonella*, whereas none were identified at the exit point. The predominant serotypes isolated were *S.* Kentucky (59.5%) and *S.* Typhimurium (17.8%) (Parveen et al. 2007). In yet another study, Lestari et al. (2009) studied the prevalence of *Salmonella* isolated from 141 conventionally raised and 53 organically raised chicken carcasses from 27 retail stores located in Baton Rouge, Louisiana. Recovery rates were similar.

Twenty-two percent of the conventionally raised chicken was found to be positive for *Salmonella*, whereas 20.8% organic chicken was found to be positive for *Salmonella*. Out of the eight serotypes isolated, predominant ones were *S.* Kentucky, *S.* Hadar, and *S.* Enteritidis (Lestari et al. 2009).

1.3 *Salmonella* in Vertically Integrated Production Systems

1.3.1 Breeders

Salmonella has multiple routes of entry in a poultry production system. Once the pathogen is introduced in poultry, the infected birds act as a constant source of infection through horizontal and vertical transmission of the pathogen in large poultry grow-out houses. *Salmonella* colonizes the reproductive organs such as ovary and oviduct, and during egg formation, the pathogen may enter internal contents such as the vitelline membrane and albumen (Gast et al. 2004, 2007; Heyndrickx et al. 2002). Subsequently, the chicks hatching from the contaminated eggs will serve as a source of infection to the flock. This is the common process involved in vertical transmission (Cason et al. 1994; Cox et al. 2000; Gast 1994). Therefore, the breeder stocks harboring *Salmonella* in the vertically integrated system have an imperative role in the prevalence and persistence of *Salmonella* in broiler meat production.

The constant presence of *Salmonella* in the poultry houses is mainly due to the vertical transmission of the pathogen from breeder flocks and horizontal transmission occurring in the housing facilities. *Salmonella* testing conducted in processing facilities of seven consecutive flocks of two vertically integrated broiler production systems in Georgia revealed a high prevalence *Salmonella* serovars such as *S.* Typhimurium, *S.* Montevideo, *S.* Kentucky, and *S.* Enteritidis. In addition, the carcass isolates of *S.* Enteritidis and *S.* Typhimurium showed indistinguishable PFGE patterns with the serovars isolated from the breeder flocks indicating the likelihood of *Salmonella* originating from the breeder flocks, subsequently contaminating the carcasses (Liljebjelke et al. 2005). Another retrospective study conducted by Crespo et al. (2004) also reported the continuum of *S*. Arizona from breeder flocks to eggs and meat.

1.3.2 Hatchery

In a vertically integrated broiler production system, hatcheries could be reservoirs of the pathogen, and the serovars of *Salmonella* present in processing environment are often traced back to hatcheries. Hatcheries harboring *Salmonella* could contaminate the eggs and eventually lead to the colonization in chicks (Bailey et al. 1994). *Salmonella* serovars can survive as an endemic population in hatcheries and can act

as a source of infection to the subsequent flocks (Bailey et al. 2002). *Salmonella* colonization in day-old chicks is of critical importance since the chicks are susceptible to the low infectious dose of *Salmonella.* In addition, less microbial diversity and an unstable gut microbiome will make the flocks susceptible to *Salmonella* (Oakley et al. 2014).

1.3.3 Farmed and Wild Animals, Rodents, and Other Vectors

Salmonella has wide host range and is distributed all over the environment. Domestic animals such as cattle, small ruminants, and pigs harbor NTS and act as a source of infection, especially in organic production or free-range settings (Davies and Wray 1996; Hoelzer et al. 2011). Wild birds such as raptors, vultures, crows, and gulls also serve as potential carriers of *Salmonella.* In addition, domestic pigeons, passerines, colonial water birds, finches, and house sparrows carry *Salmonella* in their intestines (Tizard 2004). *Salmonella* has been isolated from a wide variety of wild animals including squirrels, raccoons, foxes, mink, tigers, wild boars, rhinoceroses, seals, hedgehogs, and white-tailed deers. The transmission of *Salmonella* happens when infringement of wild and captive animals occurs (Hoelzer et al. 2011).

The carrier status of rodents for *Salmonella* serovars such as *S.* Typhimurium and *S.* Enteritidis often warrants pest control programs in poultry farms. It could be the direct transmission of the pathogen from the birds to the pests or vice versa (Wales et al. 2007). The rodents amplify the pathogen load in the environment and transmit those to the food animals, especially in the organic production system. Then the pathogen constantly circulates in the food chain (Meerburg and Kijlstra 2007). *Salmonella* prevalence in the farm premises due to rodents was estimated at 5.2% (Skov et al. 2008). Studies also revealed the genotypic and serological similarity between samples isolated from rodents and chicks (Liebana et al. 2003).

1.3.4 Human Traffic and Related Activities

Movement of people in and out of the farms is a major *Salmonella* introduction process in a poultry farm. *Salmonella* can be introduced into the farm through cages, feeders, drinkers, clothes, and boots (Wales et al. 2007). The movement of employees between different farms and contact with different species of animals are also potential threats to the safety. Therefore, proper physical barriers, disinfection procedures, dedicated clothes, and boots could be useful to reduce the introduction of the pathogens into the flock (Newell and Fearnley 2003).

The crates used for transportation of birds to the farms and processing plants carry *Salmonella. Salmonella* survives on crates even after washing them using quaternary ammonium compounds with an exposure time of 10 or 20 s. The flocks that were previously *Salmonella*-negative became positive from the contamination of the crates

(Slader et al. 2002). Therefore, the movement of portable equipment, including the transport crates could be an immediate source of *Salmonella* infection to the processing facility or poultry farms (Heyndrickx et al. 2002; Slader et al. 2002).

1.3.5 Feed, Litter, and Water

Contaminated feed is one of the main sources for contraction of *Salmonella* infection by poultry. Most of the time, the traditional techniques would not allow the recovery of a low level of *Salmonella* from the feed, although *Salmonella* numbers as low as ten cells can colonize in day-old chicks (Maciorowski et al. 2006; Park et al. 2011). Also, less than one *Salmonella* per gram feed is sufficient to cause colonization in 1- to 7-day-old chicks (Schleifer et al. 1984).

Salmonella survives in poultry feed in a strain-dependent manner. Most of the virulence genes are downregulated during its survival in a low water activity environment such as poultry feed (Andino et al. 2014). *Salmonella* serovars such as *S.* Typhimurium can persist in feed for months and act as a source of infection to the chicks or adult chickens. *S.* Typhimurium survives in feed for 40 days, 16 months, or 18 months at 38 °C, 25 °C, and 11 °C, respectively (Williams and Benson 1978). Therefore, hurdle technologies and intervention strategies are recommended during feed manufacturing, transportation, and storage (Maciorowski et al. 2004)

Salmonella persists in poultry litter and acts as a major source for intestinal colonization by the pathogen in chicks (Fanelli et al. 1970). Similar to the survivability in feed, serovars such as *S.* Typhimurium survives in the litter for months and acts as a source of infection to the chicks or broiler chickens. *S.* Typhimurium survives in the litter for 13 days at 38 °C and 18 months at 25 °C or 11 °C (Williams and Benson 1978).

Poultry drinking water can be contaminated with feed, litter, droppings, or dust carrying *Salmonella.* The residual organic contamination reduces the free available chlorine (FAC) in the water and changes the pH of the water which in turn reduces the efficacy of chlorination (Poppe et al. 1986). *Salmonella* at a level of 4–5 log CFU/ml has been recovered from the poultry drinking water. The main source of *Salmonella* contamination to the poultry drinking water is from the *Salmonella* attached to the trough drinkers and plastic bell drinkers (Renwick et al. 1992). Nipple drinkers are less likely to be contaminated with *Salmonella* because of their closed nature (Poppe et al. 1986). *Salmonella* forms biofilms in pipes and drinkers and acts as a persistent source of infection to the poultry (Poppe et al. 1986).

1.3.6 Aerosols

Salmonella survives in the aerosols, dust particles, and droplets. *Salmonella* persists in the dust particles for years and serves as a constant source for pathogen colonization (Davies and Wray 1996). The pathogen is often found in air inlets or fans and can be a recontamination source (Higgins et al. 1982). Studies conducted in a

controlled environment by regulating the air flow of the cabinet between challenged and non-challenged birds revealed that *Salmonella* could be transmitted from infected to non-infected birds via aerosols. Also, 33% of the non-challenged birds became infected with *S.* Enteritidis. Moreover, *S.* Enteritidis was isolated from the feathers of 77% non-challenged birds (Gast et al. 1998).

Aerosolizing of *S.* Enteritidis causes systemic infections through nasal and conjunctival routes (Baskerville et al. 1992; Humphrey et al. 1992) and elicits varying degrees of immune response in a dose-dependent manner. A low infectious dose of 10^3 CFU *S.* Enteritidis can cause lung infection and systemic infection in the liver, spleen, kidney, ovary, and oviduct in 2-day-old chicks. Also, the pathogen can be excreted through feces for 28 days (Cooper et al. 1996).

Currently different antibacterial interventions are practiced at the farm level to control NTS serovars. The interventions include prebiotics, probiotics, organic acids, short-chain fatty acids, vaccines, bacteriophages, and essential oils and are being supplemented through feed or drinking water (Atterbury et al. 2007; Callaway et al. 2008; Donalson et al. 2007, 2008; Higgins et al. 2008; Van Immerseel et al. 2006; Kollanoor Johny et al. 2009, 2012; Nair et al. 2016; Patterson and Burkholder 2003; Tellez et al. 2012; Zhang-Barber et al. 1999). These interventions will be explained in detail in a following chapter.

1.3.7 Processing Environment

Salmonella-colonized flocks excrete the pathogen through the feces that transmit the infection to the other birds in the flock, contaminating the poultry farm. The sharing of the common equipment also causes the introduction of the pathogen to the processing facility (Heyndrickx et al. 2002). Among the different stages of poultry processing, scalding, picking, evisceration, and chilling reduce the total microbial load on the carcass. In addition, cross contamination of carcasses is possible during these stages, if a single carcass is contaminated with *Salmonella* (Heyndrickx et al. 2002). Therefore, these are considered as critical operations in poultry processing in terms of reducing the prevalence of *Salmonella* on poultry carcasses (Svobodová et al. 2012). However, other steps of poultry processing are also important. For example, inappropriate stunning causes wing flapping and quivering which lead to soiling of the carcass with feces and transfer of *Salmonella* from inside to the outside of the body (Gregory 2005). Therefore, poultry processing is considered as a complicated and delicate procedure where a breach in the hygiene and sanitation affects public health that ultimately leads to billions worth product recalls in the industry.

1.3.7.1 Scalding

Scalding is the process in which broiler carcass is immersed at 59–64 °C for 30–75 s (hard scald) or 51–54 °C for 90–120 s (soft scald) to loosen up the skin for facilitating further picking (FSIS 2015). This is the first step in poultry processing where

the carcasses are immersed in water, and there is a high possibility of cross contamination with pathogens, including *Salmonella* (Carrasco et al. 2012; Russell 2008). A study conducted by Nde et al. (2007) revealed that *Salmonella* survived the scalding process, and the same isolates were identified before and after defeathering and from the rubber fingers of the defeathering equipment. *Salmonella* can be attached to the skin during the scalding process, evades the action of common antimicrobial agents, and acts as a source of infection in the subsequent stages of processing (Kim et al. 1996; Lillard 1990; Nchez et al. 2002; Yang et al. 2001). Also, a higher concentration of antimicrobial agent is necessary to kill *Salmonella* if it is attached to the skin surfaces (Yang et al. 2001).

1.3.7.2 Defeathering

Defeathering is another step in poultry processing where the possibility of cross contamination is high if contaminated water is used along with improper disinfection of rubber picking fingers (Nde et al. 2007). *Salmonella* that are attached to the skin on a carcass cross-contaminates other carcasses. A high prevalence of 47% and 63% *Salmonella* before and after defeathering, respectively, was noticed in this study (Nde et al. 2007). Other studies also reported significantly high *Salmonella*-positive carcasses after defeathering (71%) compared to that of pre-defeathering (21%) in the conventional defeathering method (Clouser et al. 1995a, b). Rubber fingers/picking fingers can cause peristaltic movements which also lead to the expulsion of feces (Berrang et al. 2001). Since the picking fingers are not changed between the carcasses, there is a high likelihood of carcass cross contamination (Nde et al. 2007). Therefore, sanitation using appropriate disinfectants is recommended during the defeathering process.

1.3.7.3 Evisceration

Evisceration is a critical step in poultry processing where an effective application of antimicrobial agents is recommended to prevent contamination of carcasses with intestinal contents. A faulty evisceration can lead to contamination of carcasses with fecal material and intestinal contents. Therefore, proper feed withdrawal before slaughtering, antimicrobial rinses such as chlorine, proper maintenance of evisceration machinery, and removal of ceca and crop without tear are recommended (FSIS 2015).

1.3.7.4 Chilling

Poultry carcasses are immersed in cold water during the chilling process to reduce the carcass temperature to 40 °F (4.4 °C) or below within 4–8 h of slaughtering to prevent the growth of pathogenic bacteria (FSIS 2014). The carcasses leaving the chillers often carry *Salmonella* (Lillard 1990; Nchez et al. 2002). Under natural

conditions, the carcasses exiting the chillers contains 1–30 CFU *Salmonella* per carcass (Waldroup 1996). The possibility of cross contamination is high in chillers compared to other steps in processing. Lillard (1990) showed 37% incidence of *Salmonella* on carcasses exiting the chillers, whereas in all other stages of processing the incidence was 10–20%.

In addition, Lillard (1990) reported that immersion chilling has washing effects and reduces the aerobic *Enterobacteriaceae* members. However, the incidence of *Salmonella* on post-chill carcasses was high indicating cross contamination of carcasses in chilling tanks and converting *Salmonella*-negative carcasses to positive. The same study observed a 15% and 28% increase in the incidence of *Salmonella* on post-chill carcasses compared to the pre-chill carcasses. The chilling process alone had no effect on reducing the pathogen numbers (Yang et al. 2001). The chilling process and associated water uptake also aid pathogen attachment on the skin since the process exposes deep channels and crevices on the skin (Kim et al. 1996). Along with these, aging of chilling water and increase in organic load in water reduce the efficacy of common antimicrobial agents, including chlorine and pose a significant threat for carcass contamination (Kim et al. 1996; Lillard 1990; Nagel et al. 2013; Nchez et al. 2002; Yang et al. 2001).

Currently, different antimicrobial interventions including chlorine, organic acids, essential oils, sodium hypochlorite, acetic acid, trisodium phosphate, sodium metabisulfite, and per acetic acid are applied or studied to control/eliminate NTS in poultry processing (Bucher et al. 2012; Burt 2004; Milillo and Ricke 2010; Nagel et al. 2013; Nair et al. 2014, 2015; Tamblyn et al. 1997; Tamblyn and Conner 1997; Venkitanarayanan et al. 2013). Among the different antimicrobial agents, USDA-approved safe and suitable antimicrobial agents for the application of meat, poultry, and egg products are described in the FSIS Directive (FSIS 2017). Those interventions will be dealt in detail in the subsequent chapters.

1.4 Conclusions

Intensive production of poultry in a vertically integrated system and the high consumption rate and demand for poultry meat in the USA make poultry meat a potentially important vehicle for foodborne outbreaks. Live poultry and poultry meat are commonly encountered in human salmonellosis as epidemiological links between them are understood. *Salmonella* colonization in the gastrointestinal tract of poultry and the excretion of the pathogen through droppings result in environmental contamination and contamination of poultry carcasses during processing. In a vertically integrated production system, *Salmonella* that are potentially present in the breeder flocks can be found on poultry carcasses if the intervention strategies are not effective to control the pathogen during production and processing steps. The persistence of *Salmonella* is often worsened by horizontal transmission of the pathogen by different carriers in and out of the farm and processing facilities. Therefore, vector control programs, proper biosafety measures, accurate disinfection, and intervention strategies are necessary to control *Salmonella* in poultry production systems.

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