

General Interest

Response to Questions Posed by the Food Safety and Inspection Service Regarding *Salmonella* Control Strategies in Poultry†

ADOPTED 7 AUGUST 2018, WASHINGTON, D.C.

NATIONAL ADVISORY COMMITTEE ON MICROBIOLOGICAL CRITERIA FOR FOODS

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MS 18-500: Received 18 October 2018/Accepted 7 December 2018/Published Online 26 March 2019

Key words: Pathogen control; Poultry; *Salmonella*; Virulence

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EXECUTIVE SUMMARY

The U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) considers reducing human foodborne salmonellosis as one of its top priorities. The Agency estimates approximately 360,000 salmonellosis cases are associated with FSIS-regulated products. Consequently, the FSIS released its *Salmonella* Action Plan to protect consumers by making meat, poultry, and egg products safer. Furthermore, the Agency has set new performance standards for poultry products.

Despite these efforts, the Agency believes that the incidence of salmonellosis and prevalence of *Salmonella* contamination on poultry products warrant further action on

the part of food safety agencies, industry, and consumers. Moreover, the FSIS realizes that the focus must be throughout the farm-to-table continuum, and they charged the National Advisory Committee on the Microbiological Criteria for Foods (NACMCF) to address the issue. The NACMCF sought data from literature, subject matter experts, and the industry. Its findings to the specific questions posed by the Agency are as follows:

1. What criteria define *Salmonella* strains that are highly virulent to humans? Are markers serotype specific? What tools are available for continuing to identify the most virulent foodborne salmonellae?

At the present time, there are no defined criteria that distinguish highly virulent *Salmonella* strains from those that are less so.

2. Where does *Salmonella* reside inside and on the surface of poultry, and how do those populations of bacteria contribute to food contamination? Discuss locations, persistence, and resistance to interventions. Discuss the latest information on the ecology of *Salmonella* within or on poultry regarding the gut, cloaca, bone marrow, heart, skin follicles and surfaces, lymphatic system, immune evasion, and other areas. Discuss strategies to mitigate risk factors at these locations.

The majority of carcass contamination is believed to result from leakage of ingesta from the crop during evisceration and aerosolization during picking.

3. Would removing flocks of highly *Salmonella*-contaminated birds entering the slaughter plant reduce foodborne illnesses in humans? What are important considerations for arriving at a threshold level (prevalence or load, e.g., CFU per gram of feces) of *Salmonella* associated with incoming birds that would necessitate additional control steps in the food safety

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† Participating agencies include the U.S. Department of Agriculture, Food Safety and Inspection Service; U.S. Department of Health and Human Services, Food and Drug Administration, and Centers for Disease Control and Prevention; U.S. Department of Commerce, National Marine Fisheries Service; and U.S. Department of Defense, Veterinary Services Activity. This article may be reproduced without prior permission.

system or hazard analysis critical control point (HACCP) plan? What are key considerations or steps for an alternative processing scenario if the threshold level were exceeded?

It is logical to expect that removing highly *Salmonella*-contaminated birds from the slaughter process would result in less human exposure to that source of *Salmonella*, potentially resulting in reduced foodborne illness in humans.

4. What should raw poultry establishments consider when determining the appropriate level of *Salmonella* that would necessitate additional control steps in the food safety system or HACCP plan? What are the factors that affect the threshold level, and at what points of processing should measurements be made?

Because it is currently not possible to establish a science-based threshold, we recommend that process controls be implemented and validated to handle a worst-case level of contamination.

5. As informed by questions 3 and 4, what methods are best suited to measure pathogen levels on animals and in product more rapidly than current tests? What is a sampling scenario that would enable an establishment to test incoming birds and product for a threshold *Salmonella* level and have a result in a timely manner so that processing can proceed as appropriate?

Molecular methods are currently available and are likely to be the basis of more rapid methods in the future. In terms of a threshold, however, it is not practically feasible to implement a sampling scheme to test incoming birds and product for a threshold *Salmonella* level.

6. Considering the farm-to-table continuum for poultry, what are the top three focus points, control measures, or best practices that would be compatible with industry-wide practices and could be addressed or implemented to achieve the highest rate of reduction of *Salmonella* with regard to both foodborne illnesses and product contamination?

All edible poultry products originate at a slaughter establishment, and it is here where most microbial control is currently possible. At this time, the greatest reduction in *Salmonella* can be achieved through continued development, implementation, and monitoring of good manufacturing practices within slaughter establishments.

The NACMCF articulated recommendations to the Agency that focus on risk-based approaches for more effective *Salmonella* control and on research, e.g., prioritized by the USDA National Institutes of Food and Agriculture, on vaccine development, rapid point-of-decision diagnostic assays, and means to reduce transmission and cross-contamination in the live bird and during the slaughter process, respectively.

RECOMMENDATIONS

Although not currently possible, the Committee recommends that the Agency and industry move toward risk-based disposition of finished raw product. This approach would be informed by *Salmonella* level and serotype (or where appropriate, a subtype thereof), and diverted products would be subjected to a validated lethality step (e.g., cooking) or reprocessing.

Concentration—and assays to estimate concentration—and related dose-responses for various demographics are currently poorly defined. It may be possible, however, to arrive at an estimated threshold of level-serotype (or subtype) through modeling.

Such an approach should consider the level of *Salmonella* in terms of direct consumer exposure to raw poultry and of cross-contamination from poultry products that results in secondary consumer exposure.

This approach may take the form of a quantitative microbial risk assessment.

The Agency should request research to better understand mechanisms and sites of cross-contamination of pathogens during processing, packaging, and subsequent distribution in commerce. Examples of data gaps include

- water portioners and the water mist occurring inside the machine or pickers and how best to effect “prevention through design”
- packaging
- retail case

The Agency should encourage development of improved vaccines to better protect against, reduce, and/or eliminate colonization and to provide immunity to flocks.

The Agency should encourage development of quantitative (or semiquantitative) microbiological methods for *Salmonella* analysis.

Ideally, improved diagnostic assays could serve as point-of-care type assays to enable real-time (or near real-time) decision making. Such assays may be specific for *Salmonella* or more broadly for carcass contamination.

Because much uncertainty and disagreement among experts remain over which genetic and environmental aspects contribute to the wide spectrum of *Salmonella* virulence, the Agency should:

- request research to better understand virulence in various animal and cellular model systems and virulence modification by pre- and postslaughter processes (e.g., how exposure to an acid may induce or modulate virulence)

- request research to better understand persistence of *Salmonella* in the environment

The Agency should develop guidance for process control during further processing.

The Agency should request research to further understand the dynamics of *Salmonella* within the bird or in feather follicles. Although much work has been done in the past decades on tissue tropism, new methods have

emerged that may shed additional light on tissues in which *Salmonella* may be harbored.

The Agency should research the mechanisms attributable to host (bird) genetics and microbial community (e.g., competitive exclusion) that increase resistance to *Salmonella* colonization in birds. Further, the Agency should evaluate the feasibility of *Salmonella*-resistant meat birds.

The Agency should work with the U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine to develop an approach to cost effectively and expeditiously approve undefined cultures for use in broiler production.

INTRODUCTION

The USDA FSIS considers reducing *Salmonella* in meat, poultry, and egg products and reducing human foodborne salmonellosis top priorities. The percentage of products regulated by the FSIS that test positive for *Salmonella* has decreased since implementation of the PR-HACCP Rule.

Despite this reduction, the human incidence of salmonellosis reported to the Centers for Disease Control and Prevention (CDC) has not greatly changed over time. After adjusting for cases that do not present to health care providers and those not reported to the CDC, an estimated 1,000,000 domestic salmonellosis cases are attributed to food as a vehicle of exposure (131). Among FSIS-regulated products, the Agency estimates approximately 360,000 salmonellosis cases are associated with consumption of meat, poultry, and egg products. The FSIS is committed to taking steps to prevent *Salmonella*-related illnesses associated with FSIS-regulated products.

In December 2013, FSIS released its *Salmonella* Action Plan (available online at <https://www.fsis.usda.gov/wps/portal/food-safety-education/get-answers/food-safety-fact-sheets/foodborne-illness-and-disease/salmonella/sap>), which outlines the steps the FSIS will take to address *Salmonella* in its regulated products. The comprehensive steps detailed in this plan are geared toward protecting consumers by making meat, poultry, and egg products safer. Key components of the plan include modernizing the poultry slaughter inspection system, enhancing sampling and testing for *Salmonella*, and ensuring that these programs factor in the latest scientific information available and account for emerging trends in foodborne illness. Inspectors will also be empowered with improved tools to pinpoint problems sooner. With more information about a plant's performance history and with better methods for assessing in-plant conditions, inspectors will be better equipped to assess *Salmonella* control in food safety systems in order to help prevent future outbreaks.

In addition, the plan outlines actions FSIS will take to drive innovations that will lower the prevalence of *Salmonella* contamination in FSIS-regulated products, including establishing new or updated performance standards; developing new strategies for inspection and gathering information throughout the full farm-to-table continuum; addressing all potential sources of *Salmonella*; and focusing the Agency's education and outreach tools on *Salmonella*.

Because reducing the number of *Salmonella*-related illnesses is a top priority, the Agency has established new performance standards for chicken parts and ground poultry, which have been expanded to include all types of comminuted chicken and turkey products.

FSIS is working to ensure alignment with the public health objectives outlined in the Healthy People 2020 Initiative (particularly its focus on efforts to reduce foodborne illnesses, such as salmonellosis) and with the Agency's own strategic goals to develop performance standards for *Salmonella*.

Specific charge to the Committee. Incidences of foodborne illness and pathogen contamination on poultry products dictate further action on the part of food safety agencies, industry, and consumers. To achieve the goal of reducing *Salmonella* infections and improving public health, FSIS realizes that the focus must be throughout the farm-to-table continuum and thus seeks the advice of the NACMCF on the following issues.

1. What criteria define *Salmonella* strains that are highly virulent to humans? Are markers serotype specific? What tools are available for continuing to identify the most virulent foodborne salmonellae?
2. Where does *Salmonella* reside inside and on the surface of poultry, and how do those populations of bacteria contribute to food contamination? Discuss locations, persistence, and resistance to interventions. Discuss the latest information on the ecology of *Salmonella* within or on poultry regarding the gut, cloaca, bone marrow, heart, skin follicles and surfaces, lymphatic system, immune evasion, and other areas. Discuss strategies to mitigate risk factors at these locations.
3. Would removing flocks of highly *Salmonella*-contaminated birds entering the slaughter plant reduce foodborne illnesses in humans? What are important considerations for arriving at a threshold level (prevalence or load, e.g., CFU per gram of feces) of *Salmonella* associated with incoming birds that would necessitate additional control steps in the food safety system or HACCP plan? What are key considerations or steps for an alternative processing scenario if the threshold level were exceeded?
4. What should raw poultry establishments consider when determining the appropriate level of *Salmonella* that would necessitate additional control steps in the food safety system or HACCP plan? What are the factors that affect the threshold level, and at what points of processing should measurements be made?
5. As informed by questions 3 and 4, what methods are best suited to measure pathogen levels on animals and in product more rapidly than current tests? What is a sampling scenario that would enable an establishment to test incoming birds for a threshold *Salmonella* level and have a result in a timely manner so that processing can proceed as appropriate?
6. Considering the farm-to-table continuum for poultry, what are the top three focus points, control measures, or best practices that would be compatible with industry-wide practices and could be addressed or implemented to

achieve the highest rate of reduction of *Salmonella* with regard to both foodborne illnesses and product contamination?

Committee's approach to answering the charge. The Committee leveraged the expertise of the Committee members and additional experts and the published literature and available results of assays of poultry products to assist in answering the Agency's charge. A subcommittee was formed and further divided into two working groups. Questions 1 and 2 of the charge were addressed by one of the working groups, and questions 3, 4, and 5 were addressed by the other working group. The entire subcommittee addressed question 6. The working groups met in person (three times) and virtually as needed. The working groups also requested the assistance of a number of subject matter experts. One of the face-to-face meetings was held in conjunction with the 2016 International Poultry Processing and Production Expo, allowing working groups to meet directly with industry experts to seek expert information.

RESPONSES OF THE COMMITTEE

Question 1. What criteria define *Salmonella* strains that are highly virulent to humans? Are markers serotype specific? Subquestion: What tools are available for continuing to identify the most virulent foodborne salmonellae?

At the present time, there are no defined criteria that distinguish highly virulent *Salmonella* strains from those that are less so. Much uncertainty exists in terms of distinguishable virulence factors that can explain the spectrum of disease severity. Although molecular methods for serotyping exist, virulence markers for gastroenteritis are not serotype specific. Some markers, such as presence of a *Salmonella* virulence plasmid, are present in a limited number of serotypes (serovars). However, the disease spectrum and public health burden caused by these serotypes vary greatly. No perfect approach exists to identify distinguishable virulence markers. Likely exploring agent-host interactions in animal and, potentially, cell models is a productive approach. However, prior exposure, such as serial culture of gut passage, can influence disease severity.

Material supporting Committee's answer. In the case of *Salmonella*, virulence can be evaluated by the pathogen's ability to colonize or infect the intestine, escape the intestine and invade internal organs, and cause clinical signs related to inflammation of the intestine and/or internal organs, thereby causing gastroenteritis, systemic disease, or death (24). The genetic basis of *Salmonella*'s virulence is explained by the presence of several pathogenicity islands that contain the genes for invasion of the intestine and resistance to killing by white blood cells (56). However, a few serotypes of *Salmonella* also contain a virulence plasmid conferring enhanced ability to attach to the

intestinal cells and to resist killing by normal host defenses (15).

The extent of the disease may be directly related to the infectious dose of the pathogen; however, the susceptibility of individuals to infection and disease significantly varies by age, previous medical history (such as recent antibiotic treatment), current health status, and other factors (70, 71, 90). Feeding studies using healthy human volunteers revealed that gastroenteritis occurs after consumption of a large number of bacteria (10^5 to 10^{10} CFU) (24, 87), but the CDC (35) reported that the incidence rate of salmonellosis is higher in children and the elderly, suggesting that an infectious dose in these groups may be much lower than that in healthy adults (36). Moreover, individuals using proton pump inhibitors (14), those with diabetes (151), immunocompromised individuals, or those receiving immunotherapy (70, 71) are in general more susceptible to infection. *Salmonella* infections in humans mainly result in gastroenteritis; invasive infections such as bacteremia and meningitis occur most commonly in people with weaker immunity, including infants and the elderly, who may have increased risk complications, including death (38). Some serotypes of nontyphoidal *Salmonella* are more likely to escape the gastrointestinal tract and cause systemic disease. These pathogenic *Salmonella* serotypes include Choleraesuis, Dublin, Heidelberg, Oranienburg, Panama, Poona, Rubislaw, Sandiego, and Schwarzengrund (8, 82). Of these, *Salmonella* Heidelberg appears to cause the greatest burden of systemic disease (49).

A few serovars are consistently associated with the greatest incidence of human disease. In 2016 the CDC reported that seven *Salmonella* serotypes were responsible for more than 50% of human disease: Enteritidis, Newport, Typhimurium, Javiana, 1,4,[5],12:i⁻, Infantis, and Muenchen (<https://www.cdc.gov/national-surveillance/pdfs/2016-Salmonella-report-508.pdf>). Forty-one percent of human disease was caused by three *Salmonella* serotypes, Enteritidis (16.8%), Newport (10.1%), and Typhimurium (14.5%), including 1,4,[5],12:i⁻ H2-negative Typhimurium strains), but *Salmonella* serotypes Javiana (5.8%), Infantis (2.7%), and Muenchen (2.6%) contributed a significant percentage of the total. This pattern has remained relatively consistent over time. Globally, two *Salmonella* serotypes, Typhimurium and Enteritidis, dominate in causing disease burden (GRAPHS for visualizing: <https://www.cdc.gov/salmonella/pdf/salmonella-atlas-508c.pdf> <http://www.phac-aspc.gc.ca/cipars-picra/heidelberg/heidelberg-eng.php>). There are currently more than 2,500 serotypes (serovars) of *Salmonella*, defined on the basis of the somatic O (lipopolysaccharide) and flagellar H antigens, according to the Kauffman-White classification. In 2016, the USDA-FSIS (163) reported that 15% of raw meat (broiler, turkey, ground beef) was contaminated with *Salmonella* Enteritidis or *Salmonella* Typhimurium: 3.7% of broiler chicken carcasses were *Salmonella* positive, with *Salmonella* serotypes Kentucky (60.8%), Enteritidis (13.6%), Typhimurium (7.7%), Infantis (6.5%), and Heidelberg (3.4%) being responsible for approximately 92% of the serotypes detected. In contrast, 1.7% of turkey carcasses were *Salmonella* positive, with

Salmonella serotypes Reading (25%), Kentucky (13%), Agona (9%), Hadar (9%), Ouakam (8%), Saintpaul (6%), and Montevideo (6%) comprising approximately 75% of the isolates. All of the prevalent poultry serotypes have been associated with laboratory-confirmed human cases from 2003 to 2013, including *Salmonella* serotypes Montevideo (11,377), Saintpaul (9,420), Agona (5,072), Hadar (2,857), Kentucky (984), Reading (619), and Ouakam (10). Although not all of these cases resulted from poultry products, it is clear that these serotypes are potentially pathogenic for humans. Indeed, it is unclear whether there is any serotype that is not pathogenic for highly susceptible humans.

Human challenge studies were performed with *Salmonella* serotypes Typhi, Typhimurium, Anatum, Pullorum, Meleagridis, Sofia, Bovismorbificans, Newport, Derby, and Bareilly, confirming that a broad array of serotypes can cause human disease. The NACMCF Committee investigated whether highly virulent *Salmonella* strains harbor unique genes or markers that differentiate them from less virulent *Salmonella* strains. For example, *Salmonella* Typhi isolates possess pathogenicity islands that confer specific virulence properties causing typhoid fever in humans. *Salmonella* serovars Enteritidis, Choleraesuis, Dublin, and Typhimurium contain a virulence plasmid that has been shown to be important in the typhoid fever mouse model. Although invasive disease may be more common with virulence plasmid-containing isolates, the majority of *Salmonella* serotypes that cause gastroenteritis in humans do not possess this virulence plasmid, and many large outbreaks of human salmonellosis have been caused by serotypes that do not contain the plasmid. Therefore, we could not find evidence in the literature for any high-virulence determinant per se that was correlated with human foodborne disease.

Salmonella Enteritidis is a good model for investigating the genetic basis of high virulence. *Salmonella* Enteritidis isolates represent a closely related population of strains in which some strains are actual clones of each other (40). These strains can vary significantly in virulence properties, including biofilm formation, motility, and invasion, because gene expression is affected by many factors (135). Hypervirulent strains of *Salmonella* serovars Choleraesuis and Bovismorbificans have been shown to evolve in response to environmental conditions resulting in changes in global gene regulation but may quickly revert to normal virulence (65). These findings indicate that expression of hypervirulence may not be predictable and is not easily assayed in a laboratory test. Additionally, an isolate may be linked to a severe outbreak with a particular food source once and have low impact at another time, highlighting again that severity of *Salmonella* infection depends not only on the expression of virulence attributes but also on the immunological status of the infected individual, environmental factors experienced by the isolate, and the host response. Pulse-Net and whole genome sequencing data, useful in outbreak identification and tracebacks, are insufficient to predict high virulence. Although food attributes such as high fat or protein content can increase

the infectivity of *Salmonella* by offering protection during transit in the host gastrointestinal tract (24, 118), the highly virulent isolates of *Salmonella* cannot be correlated with particular food types.

The ability of *Salmonella* to cause gastroenteritis has been attributed to its ability to invade epithelial cells of the gastrointestinal system resulting in mucosal inflammation and diarrhea (24, 41). In vitro monolayer cell cultures, which can be prepared from many tissue types and species of animals, only look at the ability of *Salmonella* to invade cells (90, 152). Although this invasion ability does not reveal the full virulence arsenal of the pathogen, *Salmonella* Kentucky appears less invasive than do the serotypes commonly associated with foodborne illness, which may explain why this serotype is less prevalent in humans than in poultry (39, 77). Nevertheless, there are no data suggesting that the highly virulent *Salmonella* serotypes are more tissue culture invasive than their less virulent counterparts. We questioned whether the severity of the infection in humans was correlated with specific responses in other animal hosts, either part of the natural transmission mode or in animal models in the laboratory. Only *Salmonella* Typhi and *Salmonella* Paratyphi A are human restricted and not observed in nonhuman hosts, but most *Salmonella* isolates pertinent to clinical medicine are also capable of asymptomatic colonization and/or persistence in other animal species (asymptomatic carriers causing subclinical infections) including food animal sources. For example, *Salmonella* Kentucky is the most predominant serotype isolated from U.S. poultry products, yet it has a low impact on human illness and has not been associated with any large foodborne outbreaks in the United States (35, 133). Several animal models have been developed for *Salmonella* infection, but few are able to capture both the enteric (typhoid) fever syndrome and gastroenteritis (69). In addition, the mechanisms determining which type of disease is caused by which serotype in which host are still poorly understood. For example, *Salmonella* Typhimurium causes enterocolitis in calves, and the animals can succumb to dehydration. In newly hatched chicks, this serotype will cause systemic disease and diarrhea, whereas older chickens are asymptomatic carriers. In immunocompetent humans, this serotype causes localized self-limiting gastroenteritis, but bloodstream infections and systemic disease may develop in immunocompromised individuals. In susceptible mouse strains, *Salmonella* Typhimurium will cause a systemic typhoid fever-like disease but no diarrhea. *Salmonella* serovars that lack host specificity, such as Typhimurium and Enteritidis, tend to be more frequently associated with disease in young animals than in adults. These results suggest that these serovars are not adapted to cope with a fully mature immune system. On the other hand, host-specific serovars have acquired the ability to breach defense mechanisms in adults. Moreover, host-adapted *Salmonella* serovars produce more serious disease than do non-host-adapted serotypes (5, 15).

Host-to-host transmission is a key phase of the life cycle of a pathogen, and strains that persist longer in a host increase the ability of the pathogen to spread. The

mechanisms of bird-to-bird transmission in commercial houses is not completely certain, and surveillance methods generally focus on group-level status. It is, however, possible that the concept of supershedders (or super-spreaders) is relevant to within-house transmission of *Salmonella*. Animals that shed pathogens at high concentrations (albeit poorly defined concentrations) are sometimes termed supershedders, and in some settings, they constitute the main reservoirs of transmission, accounting for at least 80% of the total *Escherichia coli* O157:H7, for example, shed in the environment (145). Nevertheless, development of the supershedder phenotype is not inherent to special attributes in bacterial pathogens and has been linked to the host instead. In a mouse animal model, persistently infected with *Salmonella* Typhimurium, the gastrointestinal microbiota played a large role in keeping the mice infected at low levels, but alterations in the intestinal microbiota by antibiotic use led to the production of supershedder mice with severe colitis. This highlights the importance of the host microbiota in protecting from acute *Salmonella* infection and in the establishment of the supershedder state (58, 89). To the best of our knowledge, a supershedder phenotype has yet to be observed in human patients, but with the likely presence of antibiotic resistance in *Salmonella*, treatment could have more impact on the microbiota than on the antibiotic-resistant pathogen. Because *Salmonella* infections are self-limited in healthy patients, full recovery occurs without the use of antibiotics. Consequently, antibiotic therapy is usually not indicated unless symptoms are severe or have persisted for more than 1 week or invasive disease is suspected (149). In the absence of *Salmonella* confirmation, a fluoroquinolone, such as ciprofloxacin (or trimethoprim-sulfamethoxazole in children) is generally recommended to shorten the duration of symptoms and prevent bacteremia in older adults, newborns, and immunocompromised patients. When *Salmonella* has been confirmed, severe cases could also receive the macrolide azithromycin or the third-generation cephalosporin ceftriaxone, a class of β -lactam antibiotics (149). Although antimicrobial treatment can be lifesaving, antimicrobial resistance may contribute to bacteremia, treatment failure, and poor clinical outcomes (88, 150). Although not considered virulence genes *sensu stricto*, genes conferring resistance to fluoroquinolones, macrolides, and/or cephalosporins in *Salmonella* undeniably constitute a risk to vulnerable populations, especially in *Salmonella* serotypes recognized as invasive and nontyphoidal (8).

Tools to assist virulence identification. A core constellation of virulence genes in *Salmonella enterica* that are necessary to cause severe human illness has not been defined. Following the epidemiological disease triad theory, the manifestation of diseases caused is a result of the interactions of the host, the environment, and the organism. A large number of diverse combinations of genes and gene expression are likely responsible for human disease under variable host immune responses and environmental conditions. In addition, a successful foodborne pathogen must have additional virulence factors that permit survival in the

animal host, and the environment may also play an important role in the ecological fitness of foodborne salmonellae. For example, Addwebi et al. (1) hypothesized that *S. enterica* serovar Enteritidis uses both common *S. enterica* virulence factors (genes or genomic islands common to most other *Salmonella* serovars: SEN0803, SEN0034, SEN2278, and SEN3503) along with eight genes previously known to contribute to enteric infection (*hilA*, *pipA*, *fliH*, *fliJ*, *csgB*, *spvR*, and *rfbMN*) and *Salmonella* Enteritidis-specific virulence factors (transposon insertions *pegD* and SEN1393 in newly identified *Salmonella* Enteritidis-specific genes) in the colonization of chicks.

Due to the important roles of the host and the environment in disease, predicting *Salmonella* pathogenicity based on serotyping alone or in combination with other phenotypic or genetic characterizations poses considerable challenges. Moreover, because of the genetic plasticity of the bacterial genome, *Salmonella* serotypes do not remain stable. The loss or acquisition of genes through horizontal gene transfer, or even mutations in single nucleotides, can result in a change in serotype or in virulence.

Nevertheless, subtyping methods based on phenotypes and genotyping have proven to be invaluable tools for retrospectively identifying epidemic clones of *Salmonella* and subsequently tracking their dissemination throughout human and animal populations. The growing application of next-generation sequencing, gene expression, and agent-host interaction in agriculture, food safety, and public health, when coupled with epidemiological and experimental data, holds great promise to better understand *Salmonella* virulence factors essential for severe human disease. This information could then be used in a prospective manner to rank the pathogenic potential of isolates and guide regulatory action. Although imperfect, similar molecular risk ranking strategies enabled characterization of Shiga toxin-producing *E. coli* (28, 54).

In summary, caution should be used when interpreting genotypic comparison data because differences in virulence may be a result of similar genotypes with differential expression of genes. Tools that assess gene expression may provide approaches for analysis and identification of such subtle differences contributing to virulence, further complicated by the difficulty in linking genotype to virulence. When isolates are obtained from clinical specimens, virulence can be assumed. However, the potential virulence in humans of isolates obtained from animals, food, and the environment is unknown. In vitro and in vivo animal models for disease are imperfect. Factors critical for virulence in tissue culture or in a mouse model may not be important in human infection. Likewise, factors critical for colonization or virulence in poultry may not be evident in mammalian disease models.

Question 2. Where does *Salmonella* reside inside and on the surface of poultry, and how do those populations of bacteria contribute to food contamination? Sub-questions: Discuss locations, persistence, and resistance to interventions. Discuss the latest information on the ecology of *Salmonella* within or on poultry

regarding the gut, cloaca, bone marrow, heart, skin follicles and surfaces, lymphatic system, immune evasion, and other areas. Discuss strategies to mitigate risk factors at these locations

Subsequent to colonization, *Salmonella* can invade deep tissues, such as livers, of broilers, which may represent a food safety threat. In addition, *Salmonella* may be present within feather follicles and on the surface of broilers when they enter the slaughter establishment. Despite these potential sources of *Salmonella*, the majority of carcass contamination is believed to result from leakage of ingesta during crop removal and from feces during evisceration, as well as aerosolization during picking. Several preslaughter strategies to reduce the burden of *Salmonella* in flocks entering slaughter establishments have been shown to be effective, and data demonstrating a correlation between flock status of *Salmonella* and pre- and postchill contamination have been reported (2, 6). However, correlation between preslaughter status and finished product contamination with *Salmonella* is not certain in commercial settings.

Material Supporting Committee's Answer

Preharvest sources of *Salmonella* in poultry. Poultry are susceptible to colonization by a wide variety of *Salmonella* serotypes, most of which are potential pathogens for humans. Depending upon the serotype and virulence profile of the *Salmonella* strain involved, poultry colonization may be asymptomatic and is highly variable between flocks. Regardless, when birds destined for slaughter harbor either in their bodies or on their surfaces *Salmonella* with pathogenic potential to humans, these birds pose a threat to the safety of the food supply. Meat birds can acquire *Salmonella* from infected flockmates or from the environment. However, many studies have shown that parent flocks are commonly the source of contamination (2, 12, 45, 91). Control measures for *Salmonella* in poultry can be classified as those that target (i) exposure and colonization within an individual animal, (ii) transmission between parent flocks and progeny, and (iii) transmission between birds within a flock (31, 91).

The likelihood of *Salmonella* carriage among poultry is governed by the interaction of the host, bacterial strain, and environment, notably the innate and acquired immunity of the bird that modulates the ability of the organism to disseminate systemically within the bird, the expression of virulence factors of the organism, the dose and frequency of exposure, the microbiota, and the interaction of these factors.

Breeder Level Intervention Strategies

Vaccination and genetic resistance to *Salmonella* vaccination. *Salmonella* vaccination is one tool in a multifaceted approach to overall *Salmonella* reduction and/or elimination of specific *Salmonella* serotypes. Vaccination aims to reduce the susceptibility of individual birds to infection, the horizontal transmission of infection within

flocks, the pathogen load in poultry house environments (and therefore the likelihood of transmission to subsequent flocks), the vertical transmission of infection to progeny of breeding flocks, and the frequency of product contamination and disease transmission to consumers. The most effective strategy is to focus on vaccination of breeder flocks and reduce vertical transmission of *Salmonella* (11, 18, 48, 172).

Salmonella vaccination programs can include a live attenuated vaccine and/or a killed vaccine (bacterin). The initial vaccination is followed by the administration of a multivalent bacterin consisting of the serotypes that have been found in breeders (7). Bacterins stimulate higher concentrations (compared with live vaccines) of serum antibodies in the parents; thus, maternal antibody is transferred to the progeny, which may reduce colonization (11). Treating the chicks with a live vaccine after passively transferred maternal immunity has waned can enhance subsequent resistance to colonization (11). Although vaccines can be protective and limit horizontal transmission of infection within flocks, they must be given multiple times to all birds in each flock and therefore have a recurrent cost.

Feed contamination. *Salmonella* control on the farm also requires preventing contamination of the feed. A CDC review (47) suggested that because of an increased incidence of *S. enterica* serotype Agona in animal feed, there were concurrent human illnesses attributed to this serotype with as many as 1 million additional illnesses occurring. To control *Salmonella* and other pathogens in feed, feed manufacturing facilities must identify the microbial growth niches and reduce conditions that lead to growth (79). The three categories that must be addressed are (i) prevent the introduction of *Salmonella*, (ii) reduce the multiplication of the organism, and (iii) implement procedures to kill the bacteria. Killing *Salmonella* may involve thermal processing (pelleting) or chemical additives.

Pelleting has been reported to reduce *Salmonella* from 50 to 93% and relies mainly on steam to kill the bacteria (23, 62, 79–81, 170). Pelleting adds steam to the feed during the conditioning process. Care should be taken in the cleaning of the equipment because moisture can enhance conditions for *Salmonella* growth (78). Pelleting may not always be the answer for controlling *Salmonella*. In some instances, animals fed a pelleted feed were twice as likely to become seropositive for *Salmonella* as those fed a non-pelleted diet (179). However, rates of positive status may be dependent on the coarseness of the grain. Coarse grain produces more volatile fatty acids that will inhibit the growth of *Salmonella* than does finely ground grain (123–125, 137).

In addition to pelleting of the feed, chemicals can be added to feed to reduce *Salmonella*. These chemicals include blends of organic acids (formic and propionic acids) and formaldehyde (55, 61, 126). Preventing *Salmonella* contamination of the feed must include obtaining uncontaminated feed ingredients, strict biosecurity, and sanitation. Because plant-based and animal proteins have been previously identified as risks for *Salmonella* status of birds,

consideration of this possibility should be taken into account in feed formulation and preparation (50, 79).

Genetic resistance to *Salmonella*. An unutilized approach for *Salmonella* control is to use natural selection to breed birds that are more resistant to *Salmonella* infections (33, 34). Considering that the genetics of the majority of the commercial poultry lines produced in the world are controlled by two or three companies, there is potential to select for increased innate immune robustness resulting in the ability to resist infection by a wide spectrum of pathogens. This attribute must be balanced with the expression of other commercially important phenotypes that impact the economics of production.

The availability of the chicken and turkey genome sequences coupled with genomic analyses facilitate the identification of markers or genes controlling a measurable phenotype and the ability to select for them naturally (33, 156). Resistance to early *Salmonella* intestinal colonization has been mainly studied by investigating genomic regions controlling intestinal colonization (98) or by studying innate immunity from increased expression of proinflammatory cytokines and chemokines (16, 177). Interestingly, the same inbred poultry lines show increased resistance to *Campylobacter* colonization at hatching (26). Whether the variation in the innate response to particular pathogens is due to genetic traits that can be exploited in commercial breeding flocks is yet unknown (146–148). The previous studies highlight the potential for breeding resistance to pathogens; however, the genetics of innate immunity have been shown to elicit a feed conversion cost. Therefore, implementation of these selective breeding programs may be a challenge at the commercial level.

Chicks and Growout: Developing Beneficial Microbiota in Chickens That Will Provide Protection from Pathogens

Day-of-hatch chicks are very susceptible to colonization with *Salmonella* by multiple routes of exposure (46, 83, 84). Some *Salmonella* serovars colonizing chickens have broad host ranges (e.g., serovars Typhimurium, Enteritidis, Kentucky, and Heidelberg), whereas others are host specific and cause illness in the birds (e.g., serovars Pullorum and Gallinarum) (53). Manipulation of the intestinal microbiota, diet, and host immunity has been the basis for a number of preharvest intervention strategies (2). Examples include administering a competitive exclusion product at the day of hatch and inclusion of probiotics and/or prebiotics in the feed to reduce colonization through growout (85, 155). Although older birds may clear the infection over time, broiler chickens are harvested at a relatively early age, while they are still shedding *Salmonella*. Therefore, it is essential to prevent the initial colonization of *Salmonella* to limit horizontal transmission in the broiler house.

Probiotics, including competitive exclusion. Competitive exclusion (CE) is a term that has been used to describe the protective effect of the natural or native bacterial flora of the intestine in limiting the colonization of

some bacterial pathogens (112). Some probiotics and direct-fed microbials have also been shown to reduce *Salmonella* colonization and provide a valuable tool for the poultry industry in combating the occurrence of intestinal disease and in reduction of foodborne pathogens. CE studies with undefined cultures led to the development of various commercial products (132). CE treatments have to be applied at the earliest opportunity because they are not effective as a treatment for *Salmonella*-positive chicks. Generally, protective microbiota are delivered by spray application just before chicks leave the hatchery, with subsequent administration in the drinking water on the farm. If it is necessary to chlorinate the water supply on the farm, the chlorine must be inactivated before the water is used for CE treatment to avoid any adverse effect on the protective microbiota. Alternatively, eggs can be injected during incubation a few days before hatching, but some embryos may die in the process (103). Field evaluations have shown that CE treatments, combined with stringent hygienic measures on the farm, can lead to substantial reduction in the contamination of chickens on the farm and of carcasses at slaughter (144).

Despite encouraging efficacy data, several countries, including the United States, prohibit the application of undefined cultures to birds due to concerns of the possible transmission of human and/or avian pathogens that may be present in the source materials from donor birds. Therefore, efforts have focused on the identification of key protective elements in undefined cultures with a view toward the development of a product of defined bacterial composition.

The most common type of defined probiotic (also known as a direct-fed microbial) for poultry includes single strains or combinations of lactic acid bacteria (13), *Bacillus*, other intestinal bacteria, and yeast. Despite promising results from laboratory studies, these products have varying efficacy in commercial poultry production. In some studies, some probiotics have been shown in both laboratory and field studies to accelerate the development of normal microbiota in chickens and turkeys, providing increased resistance to infection by enteric bacterial pathogens, including *Salmonella* Heidelberg, as early as 1 h following the administration of a probiotic (67, 68, 106). The most acclaimed effect for some probiotics is their positive influence on the immune system by influencing the existing microbiota as they pass through the gastrointestinal tract. Different strains of *Lactobacillus* can improve chicken immunity by increasing serum cytokine concentrations and the number of T cells (143).

Prebiotics. Prebiotics are non- or partially digestible feed ingredients that beneficially affect the host by selectively stimulating the proliferation and activity of one or a few bacteria (141, 169). Examples include fructo-oligosaccharides and mannan oligosaccharide, which have been shown to reduce the abundance of *Salmonella* Enteritidis in cecal contents of experimentally infected chickens (52). Also, there has been some success in reducing *Salmonella* infection in broilers by incorporating

yeast cell wall products, e.g., *Saccharomyces boulardii*, in the feed (96).

Bird Health and Raising

Newly hatched chicks are typically colonized by *Salmonella* quickly because their gut has limited microbiota and may be susceptible. NACMCF (110) reviewed existing literature in their development of a generic HACCP plan for broiler slaughter and processing. Potential sources of *Salmonella* are numerous and can include water, feed, litter, the hatchery, bird movement, vehicles, fomites, insects, rodents, and wildlife (2).

The health and treatment of birds through the growout phase is a key factor affecting carriage of *Salmonella*. The International Commission on Microbiological Specifications for Foods (76) reported that the general health status of a flock and incidence of various poultry-specific diseases can impact the potential for *Salmonella* colonization of poultry as well as the levels on the carcasses after processing. Once introduced, *Salmonella* can be transmitted readily among birds. A Canadian study linked prevalence (50% overall) of *Salmonella* in 81 flocks to various risk factors identified via a survey questionnaire. Among many risk factor studies, only the failure to permanently lock the chicken house was significantly associated with *Salmonella* colonization at slaughter. The authors suggested that this failure was a possible measurement of the quality of biosecurity by the producer. They found no correlation of *Salmonella* prevalence with pest control programs, down time, manure disposal, or sanitation (9).

Typically, broilers are harvested at approximately 47 to 65 days of age after being grown under very controlled conditions to ensure a uniform size. Uniformity of bird size can help with process controls, e.g., making gut contents less likely to be spilled during the slaughter process, because the equipment can be set very precisely to accommodate the expected size of the birds.

Chemical litter treatments. When pH is reduced below about 5, conditions are unfavorable for *Salmonella* and other potential pathogens (43, 44). To achieve this reduction, chemicals can be added to the litter to lower the pH and reduce ammonia production. Such treatments must be cost effective and safe for farm workers. Several chemical additives have been used to decrease the pH of poultry litter. Examples of these chemicals are aluminum sulfate (108), ferrous sulfate (72), phosphoric acid (122), sodium bisulfate (107), and acetic acid (116).

Moore et al. (107) evaluated several chemical treatments for ammonia utilization and phosphorus solubility and found that aluminum sulfate was best for reducing ammonia volatilization, followed by phosphoric acid, ferrous sulfate, sodium bisulfate, and calcium-ferrous-sulfate. All treatments significantly reduced litter pH when compared with the control litter. Aluminum sulfate was most effective for controlling both ammonia volatilization and phosphorus solubility. These data suggest that aluminum sulfate has some possible environmental benefits by reducing phosphorus runoff into groundwater; however, the

initial cost per treatment of the chicken house was higher compared with the other treatments. In another study, sodium bisulfate was shown to be effective for controlling *Salmonella*, *Clostridium*, and *Pasteurella* in litter (153). Furthermore, the application of this product was effective for litter acidification and extended the life of insecticides for the control of darkling beetles.

Bacteriophage. Bacteriophages are viruses that are specific obligate bacterial parasites and usually possess high specificity for one bacterial species. There has been a recent resurgence of interest with bacteriophage therapy. Recent studies demonstrate the ability of bacteriophages to reduce pathogens on pre- and postharvest agricultural commodities, especially poultry. A cocktail of bacteriophages was able to reduce *Salmonella* Enteritidis by about 1 log CFU/cm² on samples of chicken skin experimentally contaminated with 1×10^5 CFU/cm² (74). A more than 1-log reduction of *Salmonella* Typhimurium and *Salmonella* Enteritidis was also measured in chicken breasts dipped for 5 min in a solution containing the bacteriophage cocktail and then refrigerated at 4°C for 7 days (142). Recently, bacteriophage was used to reduce approximately 1 log CFU/g *Salmonella* in ground chicken (59, 182). However, oral bacteriophage administration has demonstrated various levels of efficacy for reducing the colonization of *Salmonella* in the gastrointestinal tract of chickens (10, 75, 95, 139, 154). These data suggest that bacteriophages might serve as an alternative agent to reduce *Salmonella* contamination.

Poultry house management. Feed withdrawal has been shown to change the microenvironment in the chicken crop by reducing the number of lactobacilli, decreasing the concentration of volatile fatty acids, and increasing crop pH (44, 73, 120). With these changes that occur during withdrawal, the crop microenvironment has the potential to increase the expression of invasion genes of pathogenic bacteria required for intestinal invasion. A time frame longer than 12 h may result in thinning of the gut wall and liquefying of any ingested food, which can increase leakage during evisceration (174). Such changes may also result from other stress to the bird or the gastrointestinal environment, such as feed deprivation, water deprivation, feed ingredient changes, vaccinations, and disease. One way to reverse the increasing crop pH due to feed withdrawal would be to reacidify the crop using inorganic or organic acids (32, 178). These studies suggest that incorporation of some organic acids in the drinking water during pretransport feed withdrawal may reduce *Salmonella* contamination of crops and broiler carcasses at processing.

Moisture in the litter environment of a poultry house can also be of concern. As the litter moisture and pH increase in the poultry house, the number of bacteria, including pathogens, tends to increase. As water activity (a_w) and pH of the litter decrease, the number of bacteria decreases, with an optimal a_w of 0.84 or less and an optimal pH of 4 or less (117). One way to control moisture is to construct a well-ventilated poultry house that minimizes sweating. Most new poultry houses utilize tunnel ventilation

that keeps the air flowing to remove heat, dust, moisture, and ammonia. Poultry producers utilize automated ventilation systems to minimize the stress that occurs to the birds due to these factors. Care must be taken to assure that these tunnel-ventilated houses move enough air to prevent dust and aerosolized *Salmonella* from being spread from bird to bird. *Salmonella*-positive birds can spread *Salmonella* via aerosols, and *Salmonella* has been found in up to 66% of air samples (57).

Biosecurity. Good biosecurity principles are recommended for the exclusion of important disease-causing agents (e.g., highly pathogenic avian influenza) and vermin. Although biosecurity practices are not designed specifically for *Salmonella* and efficacy for controlling *Salmonella* is lacking, these practices are nonetheless recommended.

Seasonality. A characteristic of cooler months is less available natural light, which may be associated with lower *Salmonella* prevalence. A model study of the effects of reduced lighting on *Salmonella* status of the flock was reported by Volkova et al. (172). They found that longer relative duration of reduced lighting during the growout period was associated with reduced detection of *Salmonella* on the exterior of birds 1 week before harvest and on the broiler carcasses at the postchilling point of processing. These authors suggested that reducing lighting to less than 18 h per day later in the growout period was associated with decreased detection of *Salmonella* on the exterior of broilers arriving for processing and in the postharvest drag swabs of litter from the growout house.

A study by FSIS scientists (97) related *Salmonella* prevalence on poultry carcasses to weather factors, including temperature extremes and precipitation. Generally, higher prevalence was observed after such events. It was suggested that this association may be due to physiological stress on poultry during the growout period as well as the effect of weather on the movements of vectors, including rodents and migrating birds.

Slaughter Control of *Salmonella*: Flock Scheduling

When a facility is biomapping and tracking *Salmonella*, farms that are likely to be positive may be identified. If a flock came from a farm that was highly contaminated with *Salmonella*, these birds could be scheduled in this scenario to be processed toward the end of the slaughter shift to prevent cross-contamination to subsequent houses of birds. This approach obviously takes a lot of coordination and communication in addition to assuming the company knows which farms (if any) are positive for *Salmonella* (102). The logistics of scheduling in modern U.S. complexes are prohibitive and quantitative, and real-time diagnostic assays are not yet available.

***Salmonella* on the Final Product: Presence or Absence, Levels, and Detection Challenges**

The majority of *Salmonella* contaminating finished poultry products are presumed to originate from fecal

contamination derived from the feathers, skin, or ruptured intestinal or cloacal contents (129). In addition, most *Salmonella* serovars infecting chickens can disseminate systemically, at least transiently, including to the liver (127). The presence of *Salmonella* in livers and bone marrow may also cause a small amount (0.8%) of contamination in the processing of ground product (1, 2). Attachment of *Salmonella* of fecal origin to the skin or within feather follicles is believed to contribute to contamination of end product, especially during the chill step (86).

Systemic contamination of extraintestinal tissues, such as the liver, spleen, and gall bladder, can occur with some serotypes. A salmonellosis outbreak (35) was linked with the consumption of chicken livers contaminated with *Salmonella* Heidelberg.

Transient versus resident bacteria. When discussing the presence of *Salmonella* on raw poultry skin, it has long been established that there are two different populations of bacteria that must be considered (86, 92, 93). The transient population is generally described as “loosely” attached and easily rinsed off the skin surface. The greater challenge for processing purposes is the resident population that is entrapped in crevices and feather follicles and therefore not only more difficult to remove but also protected from interventions. Lillard (92) found that *Salmonella* appeared to be transferred from a surface film to skin during prolonged (60-min) water immersion and suggested that preventing formation of a surface film by altering surface tension may decrease contamination during immersion. The ineffectiveness of rinsing for removing bacteria from broiler carcasses has been demonstrated (94). Aerobic bacteria and *Enterobacteriaceae* were detected via rinsing, stomaching, and blending of broiler carcass skin, and although a gradual reduction occurred after 10 rinses, 10^5 CFU of aerobes and 10^4 CFU of *Enterobacteriaceae* could still be detected after 40 rinses. Kim et al. (86) used confocal scanning laser microscopy to show that most *Salmonella* cells attached to the flat portion of the skin surface washed off easily, but the *Salmonella* cells remaining were located in crevices and entrapped in feather follicles, even after rinsing. Unattached *Salmonella* cells appeared to be floating in entrapped water in the follicle. The presence of resident or tightly associated *Salmonella* on carcasses presents challenges to both effective processing interventions and proper and consistent detection in the final product.

Detection methodology. The presence of the resident population of bacteria, in particular, poses a challenge to consistent and effective detection of *Salmonella* on carcasses. Generally, rinse and swab methods will recover only weakly attached bacteria, potentially giving false-negative results when *Salmonella* cells are entrapped or tightly bound in crevices or feather follicles. Singh et al. (138) compared the ability of swabbing, stomaching, and grinding to detect a range of bacteria, including mesophilic aerobic bacteria (134), *E. coli*, and coliforms. Less than 35% of the mesophilic aerobic bacteria appeared to be loosely associated with the skin of the broiler and therefore

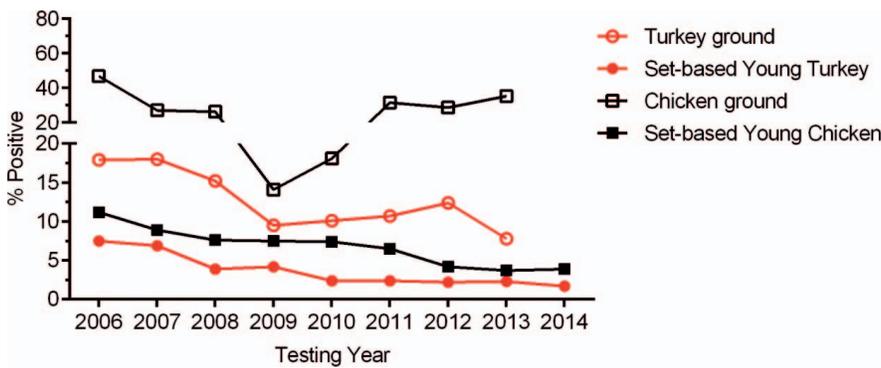


FIGURE 1. FSIS *Salmonella* sampling results: poultry carcasses and ground poultry, 2006 through 2014.

detectable by swabbing or stomaching, whereas greater than 65% of the mesophilic aerobic bacteria appeared to be tightly associated and were only recovered by grinding.

The 2015 FSIS quarterly report for quarter 3 *Salmonella* testing of selected raw meat and poultry products (161) notes only a 1.4% positive rate for whole carcasses but a 22.1% positive rate for chicken parts, a 29.3% positive rate for ground and other comminuted chicken (not mechanically separated), and a 72.7% positive rate with limited exploratory sampling of mechanically separated chicken.

There is a growing body of data that indicates testing for *Salmonella* on the final product should be quantitative rather than presence or absence in order to better understand what is happening to levels of *Salmonella* (101). Although historically quantitation has been achieved by utilization of most-probable-number (MPN) techniques, current practice in the industry includes using molecular methods to identify samples that exceed a specific limit or threshold (i.e., development of a microbiological limit) (101).

Incidence of *Salmonella* on a range of products identified through FSIS quarterly testing are discussed below. It should be considered that, with the exception of

ground product, these data were obtained using swab and rinse sampling; therefore, the possibility of false negatives where tightly associated *Salmonella* cells were not detected may exist. More recently, there has been concern regarding false negatives due to residual chemicals on carcasses. This concern led to the recent incorporation of neutralized buffered peptone water in FSIS detection procedures (162).

Salmonella in final product. As part of the HACCP implementation plan, FSIS continually tests poultry production facilities for *Salmonella* and requires all poultry plants to develop and implement a system of preventive controls for *Salmonella* (157). For “moving window” evaluation, FSIS continuously samples (up to five times per month) poultry establishments producing young chicken and turkey carcasses and raw chicken parts so that it can closely monitor an establishment’s process control performance over time. FSIS uses these test results to assess establishment performance during a reference period of 13 completed 52-week moving windows. Each week, a new window is completed, and a lagging window drops out of the 13-window frame (167). Quarterly testing by FSIS between 2006 and 2014 on ground product and sample sets of chicken and turkey demonstrated that the type of product as well as the kind of poultry differ in terms of *Salmonella* positivity rate. Chicken products, whether ground or set based, are more likely to contain *Salmonella* than are turkey products, whereas ground meats of either species are more likely to be contaminated (Fig. 1). Again, chicken products are more likely to be *Salmonella* positive (Fig. 2), but mechanically separated chicken was more likely to be positive than ground chicken or turkey (86 versus 24 and 18%, respectively). These data must be interpreted cautiously, because sampling was not consistent between the products throughout the years, and sampling methods are often different for turkeys versus chickens. For example, in 2014 ground meats were not tested at all, and in 2013 for ground turkey, 192 samples were collected in the first quarter, with 15.1% positive, but 0 samples were collected in the third and fourth quarters. In many cases where there were high positive rates, the minimal numbers of samples collected potentially makes the positive rate artificially high. Power calculations should be conducted to determine the minimal number of samples required for testing per product type and location to make statistically significant

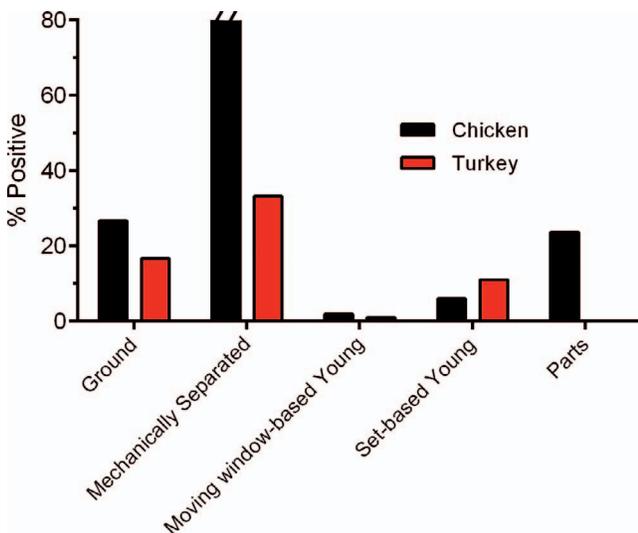


FIGURE 2. FSIS *Salmonella* sampling results: comparison of *Salmonella* positive rates in chicken and turkey by sampling project.

conclusions. Sarlin et al. (130) reported that swabs, typically used for sampling turkeys, were less effective ($P < 0.05$) for *Salmonella* detection than were either skin or carcass rinse samples typically used to sample chickens.

Question 3. Would removing flocks of highly *Salmonella*-contaminated birds entering the slaughter plant reduce foodborne illnesses in humans? What are important considerations for arriving at a threshold level (prevalence or load, e.g., CFU per gram of feces) of *Salmonella* associated with incoming birds that would necessitate additional control steps in the food safety system or HACCP plan?

It is logical to expect that removing highly *Salmonella*-contaminated birds from the slaughter process would result in less human exposure to that source of *Salmonella*, potentially resulting in reduced foodborne illness in humans. Given uncertainty in this approach, however, process controls should be validated to address a worst-case scenario for contamination of incoming birds and should be continually operating at that level to address the potential risk from highly contaminated birds.

Material Supporting Committee's Answer

Studies have reported a great deal of variability in *Salmonella* prevalence, not only between flocks but within flocks and between houses on the same farm, which further complicates the ability to identify contaminated birds as they arrive at the processing plant. Strong agreement does not exist in the published literature regarding the predictive ability of farm sampling and subsequent *Salmonella* contamination on the neck skin at the end of processing (66). Volkova et al. (171) showed that the best predictors of postchill broiler carcass contamination (positive or negative) with *Salmonella* was the frequency of litter contamination on day 1 and the day of harvest. In another study, however, the flock-wide *Salmonella* level was associated with the level of *Salmonella* on pre- and postchill carcasses (17). Even if it were possible to determine levels of *Salmonella* in flocks several days before being transported to the slaughter plant, it is questionable whether the identification of clean or contaminated flocks would still hold true upon delivery to the plant. Birds have been shown to shed *Salmonella* at varying frequencies and times, and those incidents appear to be unpredictable, making it difficult to identify an appropriate time to sample. Rather than establishing lot- or flock-specific thresholds, *Salmonella* management programs should be based on historical trend analyses of specific farms and transportation supplying birds to the slaughter process. It is important to note that the establishment of a threshold level in incoming birds requires a holistic approach considering both pre- and postharvest controls and conditions that might impact the level of *Salmonella* (102). Sampling birds immediately before entering the slaughter process would be ideal, but detection technology does not currently exist to provide the rapid detection needed for this scenario. In addition, the staging of feed and water withdrawal prior to transport to

the slaughter plant necessitates *Salmonella* contamination information being gathered and acted upon within a few hours.

When attempting to establish a threshold *Salmonella* level to identify highly contaminated flocks or birds, it must also be determined whether all salmonellae should be considered or the focus should be on only those serotypes or genotypes that are considered to be of public health significance. The criteria that make *Salmonella* highly virulent to humans, the mechanisms of pathogenesis, the host response, and virulence factors were discussed in the response to question 1.

In light of the above barriers to establishment of a specific threshold for poultry at receipt at the processing facility, utilization of historical preharvest trend analyses and biomapping may provide more useful information for validation of a poultry processing system designed to deliver a quantifiable reduction of *Salmonella* on processed birds. This approach could be used by the processor to determine when additional or more effective process controls may be needed.

In the absence of being able to identify flocks with high *Salmonella* contamination before slaughter, it is necessary to provide an in-plant process that can deliver sufficient validated *Salmonella* reduction, regardless of the incoming contamination level. A holistic multihurdle pathogen reduction approach to management of *Salmonella* is needed to reduce prevalence and presumably reduce illnesses, although data that definitively show this are limited (180). It should be recognized that the production of poultry is a continuum, and the potential for the introduction of pathogens at any point should be considered. Russell (128) and Liljebjelke et al. (91) recommended that a focus on pathogen reduction should extend through all stages of breeding, hatching, growout, transportation, and processing. Verification of process control through establishment of serovar-level performance standards of the finished product(s) might have a more realistic impact on public health than establishment of threshold levels at receipt of live birds.

While process controls are important, failure to apply proper practices on the farm can increase the risk of heavy *Salmonella* contamination in birds delivered to the slaughter facility. The introduction of heavily contaminated birds to the slaughter plant can be minimized through the application of good agricultural practices at the farm, as mentioned in the response to question 2.

Question 3a. What are key considerations or steps for an alternative processing scenario if the threshold level were exceeded?

At present, data do not exist that enable development of a microbial threshold for *Salmonella* in incoming birds. As such, slaughter establishments need to validate their HACCP programs to achieve microbial process control to reduce or eliminate the expected load of *Salmonella* in incoming birds. Historical data might be used to build statistical models to predict the potential for elevated levels of *Salmonella* from a particular farm. In addition, monitoring of external factors, such as weather or

seasonality, may help indicate the possibility of a higher than normal contamination level. These factors could alert the processor of the potential for increased risk. Historical knowledge of process controls and plant capability can be used by a processor to determine whether process controls should be reassessed and validated to address predicted risks.

Question 4. What should raw poultry establishments consider when determining the appropriate level of *Salmonella* (“threshold”) that would necessitate additional control steps in the food safety system or HACCP plan?

Because it is currently not possible to establish a science-based threshold, we recommend that process controls be implemented and validated to handle a worst-case level of contamination. Many things can affect outcome because loss of control at any single step can negate the others. To best assess controls, each establishment needs to look at the whole food safety system from breeder farm through processing so it is not overwhelmed by the incoming load. Evaluation of prevalence and concentration of *Salmonella* on carcasses and parts throughout the production process unique to each facility can help to identify pathogen reduction at each step in the process. Once these are defined, the controls at the various points across the whole system need to be validated. This must be done for each establishment because of the individual differences in equipment and processes (160).

Material Supporting Committee’s Answer

One must know the capabilities of the unit operations and overall process controls and the efficacy of the supporting control programs (i.e., prerequisite programs). Historical and real-time data on food safety controls from the farm to production that might indicate a need to examine control steps include

- Good Agricultural Practices (GAP)
- carcass mapping
- finished product testing
- environmental monitoring
- sanitation effectiveness monitoring
- auditing results assessing sanitary design

What Are the Factors That Affect the Threshold Level?

On-farm factors as described above need to be considered and mapped appropriately. This section addresses considerations for controlling or preventing *Salmonella* throughout the processing environment. The challenges to any multihurdle approach for reduction of *Salmonella* during harvest are the initial bacterial load on birds at live receiving and the external contamination from live receiving through chill. Consideration needs to be given to unloading the birds to minimize stress, movement, and therefore possible cross-contamination through to the hanging area (21).

Transportation. In the poultry industry, transportation includes loading, transport, and delivery of the birds to the processor. Current practice is to accomplish this within a window of time designed to minimize external contamination of the birds and stay within the maximum feed withdrawal time (63, 175). Recommendations for transport considerations are contained in the *Draft FSIS Compliance Guideline for Controlling Salmonella and Campylobacter in Raw Poultry* (159).

Salmonella-contaminated neck skins have also been linked to fecally soiled cages (66), so it is recommended that transport crates and trucks be rinsed, soaked, washed, and sanitized (45, 105). Proper sanitary design of transport cages enables effective cleaning and sanitation between loads of poultry and limits accumulation of contaminants in niches that can ultimately form biofilms that are more difficult to remove. Ideally, sanitation should be done in an area separate from where processing occurs. Additional best practices for sanitation include periodic wash water replacement and enhanced crate washing systems such as a soak tank with brushes (4). Cages and transport containers need to be effectively cleaned with detergent to remove organic matter prior to the sanitizing step.

Following cleaning, cages and transport containers need to be sanitized using a product registered by the Environmental Protection Agency, following the label directions. Effective sanitation with chemicals was shown to result in a 3- to 5-log reduction of aerobic plate counts, *Enterobacteriaceae*, and *Campylobacter*, and effective use of sanitation can also reduce levels of *Salmonella* (4). Cages should be allowed to completely dry between uses; a time period of up to 48 h has been suggested as beneficial (20).

Cooling sheds. During the summer, misting and fanning of birds are often employed as way to help keep birds cool as an approach to protect animal welfare. Providing too much moisture, however, can increase the spread of bacteria and may have a negative effect on the ability of birds to dissipate heat as well (64).

Scalding. Scalding the birds not only assists in the removal of feathers but also removal of some debris from the carcass; however, pathogens may survive scalding. Consistent temperature of the scald water and minimizing cross-contamination during scalding and subsequent feather picking are keys to the success of this hurdle. In practice, scald conditions are variable in terms of times, temperatures, size of birds, and use of chemicals. Slavik et al. (140) found that scalding at 60°C was significantly more effective than scalding at lower temperatures; the higher temperature achieved a 0.3- to 0.5-log greater reduction of *Salmonella* counts than scalding at 52 or 56°C. This step may also be one of the first stages at which approved chemical interventions can aid in reducing cross-contamination. Using a series of scald tanks; applying agitation, counter-current flow, overflow, and water replacement; and adding interventions to the scald water to control pH are viable methods to reduce cross-contamination during defeathering (29). Additionally, in scalding steps, options exist for steam

and traditional scalders. Brushes have also been used to remove dirt and debris from the feathers prior to entering the scalding; however, brushes should be maintained to prevent additional cross-contamination (114).

Defeathering. Feather-picking or -plucking machines are equipped with rubber “fingers” that help remove feathers from the carcass. Plucker fingers are regularly contaminated because of their close contact with the carcass, and washing pluckers during operation is not only essential to prevent buildup of debris but also to help prevent attachment of microbes. In addition, plucker fingers require regular replacement, so proper maintenance is important (25). Alternatively, in a process that has been applied to ducks and turkeys, dry slaughter and evisceration using paraffin can accomplish the process without introducing water and associated aerosols to reduce cross-contamination without the addition of chemical interventions (168).

Evisceration. The evisceration and dressing stage is critical for controlling fecal contamination in the processing environment. Steps to prevent the rupturing of viscera as well as decontamination efforts to address any incidental viscera leakage are needed to prevent and control *Salmonella* contamination of the carcass. Slaughtering birds consistent in size for which the automated equipment is tailored can prevent the rupturing of viscera and resulting cross-contamination (42, 110, 159). Additionally, regular cleaning steps to prevent debris buildup on equipment are necessary to prevent cross-contamination, in particular as the viscera are removed. The dressing after evisceration should include high levels of employee hygiene and aseptic techniques for washing and trimming carcasses.

Reprocessing. Clear reprocessing plans for carcasses that are dropped, soiled, or otherwise damaged must exist and may include additional chemical interventions for those carcasses. Online and offline reprocessing should involve thorough washing to remove visible contamination both inside and outside the carcass. A chemical intervention spray or dip following this wash can result in greater than 2-log reduction of *Salmonella* (42).

Chilling. Two primary chill systems (air and immersion) each have advantages and disadvantages in food safety that vary by implementation. Immersion can introduce cross-contamination between carcasses by virtue of the bird-to-bird contact. However, with agitation and/or chemical intervention, the overall contamination of the carcasses is still reduced (22, 128). Chemical interventions used in the primary chiller and dip tank provide effective antimicrobial action when coupled with regular cleaning of tanks and regular addition of fresh water to mitigate the impact of organic material buildup (176). Although age of the scalding water may not significantly impact efficacy, chill water used for immersion can shift composition significantly over time, reducing the effect of added antimicrobials through reaction with compounds in the chill water (181).

Controlling flow rate, flow direction, and cleanliness of the chiller will mitigate the effect of the organic material that builds up with use (128). In air-based chilling, birds are spaced to reduce cross-contamination. However, if spray is introduced into the air chiller, microbial aerosols may contribute to cross-contamination (104). Overall, chilling to 4°C or lower will inhibit *Salmonella* growth.

Interventions. Several chemical interventions can be applied to poultry products during processing. Options include chlorine compounds, cetylpyridinium chloride, ozonated water, peroxyacetic and other organic acids, and trisodium phosphate, among other compounds approved for use. Some processing aids are more effective for specific applications (e.g., trisodium phosphate in air versus immersion); these interventions should be carefully matched to the setup of the individual processor. Some considerations for the use of antimicrobials include concentration and application method (spray, dip, etc.). Immersion in an antimicrobial agent may provide more surface area contact than spray application, especially in further processing. If used during prechill, some interventions may require a rinse step in order to prevent any residual processing aid from negatively impacting the pH of the chill water (30). A listing of FSIS-approved chemicals for use in meat, poultry, and processed egg products is available (164).

There are also nonchemical interventions, such as high-pressure pasteurization, that can effectively address *Salmonella* contamination (136). Establishments should consider practical aspects when determining which interventions they will implement. In addition, establishments should consider at which steps in the process to apply interventions to most effectively address *Salmonella* contamination. Establishments can obtain this information through carcass or process mapping (i.e., by performing *Salmonella* sampling and testing at points throughout the process) from the point of incoming birds to finished product. Through mapping and monitoring at multiple points in the processing environment, the establishment can make informed decisions on the adequacy of hurdles in place and where alterations are needed (19).

Sanitation. Slaughterhouse establishments should also consider the sanitation at their facility, including equipment sanitary design and hygienic conditions. Maintaining sanitation during operations and thorough cleaning and sanitizing of product contact surfaces at least once daily is critical to addressing opportunities for cross-contamination with *Salmonella*. Nonchemical options may include the use of steam and ultrasound to disinfect surfaces, providing those surfaces do not have high amounts of debris (109). Product buildup, such as fat and tissue, prevents both chemical and nonchemical sanitizers from reaching product contact surfaces. Using antimicrobial interventions does not replace the need to minimize product buildup during operations. Written and validated cleaning and sanitizing programs using technologies and operations appropriate for the plant and equipment are necessary to maintain sanitary

conditions at the establishment. In order to be effective, these programs must be implemented and supported by well-trained personnel within a food safety culture (183).

Other. Other measures necessary for the control of *Salmonella* at establishments include control of humidity, aerosols, and condensation; positive appropriate air flow; and control of cross-contamination and pH. These infra-structural controls can reduce and control environmental contamination in the processing facility.

At What Points of Processing Should Measurements Be Made?

Measurements should initially be made throughout the process to validate process controls and subsequently to monitor and verify these process controls and to drive continuous improvement. A prudent establishment collects data related to the hurdles they have in place and how they handle variability.

These measurements could be qualitative, such as fecal contamination or processing defects, or they can be quantitative such as sanitizer concentrations, pH, or temperatures of scald and chill water. FSIS (158) requires at a minimum that samples be collected prechill and postchill at a frequency of once per 22,000 birds and be tested for indicator organisms. Detailed information on sampling protocol design is provided by the FSIS (158).

Question 5. As informed by questions 3 and 4, what methods are best suited to measure pathogen levels on animals and in product more rapidly than current tests?

Molecular methods are currently available and are likely to be the basis of more rapid methods in the future. The current state of detection methods for *Salmonella* in poultry products allows for detection of low concentrations in approximately 24 h. Recently, developments in semi-quantitative methods have demonstrated that threshold results might be achieved in as few as 8 h. In addition, the movement from traditional serotyping to genetic testing should allow rapid determination of serotypes that have a great public health impact. Although nucleic acid tests appear to be well suited for more rapid testing, innovation through new technologies and improvements to existing technologies should not be discounted. An extensive review of this subject by Park et al. (115) draws a similar conclusion.

Material Supporting Committee's Answer

The detection and quantification of *Salmonella* must rely on microbiological methods that can accurately and effectively achieve the desired results. The current reference method used by USDA-FSIS to detect the presence or absence of *Salmonella* in raw poultry and environmental samples includes both a phenotype-based method and a nucleic acids method (166). Both methods rely on an enrichment step to allow the detection of low levels of *Salmonella* in a sample. The time to achieve a negative

result with the culture method is approximately 3 days, whereas the nucleic acids test requires only 24 to 30 h.

There currently is not a USDA method for enumeration of *Salmonella* in carcass rinses. MPN techniques and direct plating on selective agar are the most common methods for enumeration of *Salmonella* in poultry samples (27). The time to a result for both MPN and direct plating methods varies from 1 to 2 days. It is not practical for processors to make real-time decisions based on pathogen testing when results take longer than a few hours.

The on-going advancement of methods based on molecular detection provides an enhanced basis for rapid detection and can potentially provide both qualitative and quantitative results. PCR methods and other amplification methods based on detecting specific sequences of DNA or RNA have moved to the forefront of technologies used for routine testing for pathogens. These methods offer speed and ease of use for laboratories.

There are numerous qualitative rapid nucleic acids methods that have gone through a recognized validation program. FSIS publishes and routinely updates a list of validated methods (165). The rate-limiting step for these methods is typically the time for enrichment of the sample. To significantly decrease the time to result for the detection of *Salmonella*, continued research is needed to identify improvements to the enrichment step. Current approaches for this task include technologies to concentrate target cells through strategies such as magnetic capture and filtration (100).

Real-time PCR methods have been shown to be able to quantify the level of the target pathogens in a sample (99, 113). These methods are more rapid than conventional methods but also may require more technical expertise and relatively expensive equipment (115). Another more rapid approach to enumeration includes the MPN dilution plan and the use of a PCR assay for detection (99).

If risk assessment results were available to show a threshold level that can help to protect public health, semiquantitative methods could play an important role in setting performance standards. Semiquantitative methods have been developed to allow for the rapid determination of levels that are above a selected threshold (173). A study by Chaney et al. (37) showed that inoculated levels of *Salmonella* in ground turkey above 1 CFU/g could be detected within 8 h. It is likely that methods that can achieve the desired result within one operational shift might have the potential to serve a role in making process scheduling decisions to control the entry of potentially highly contaminated birds into the facility.

Cultures from positive samples can be further tested to determine the serovar and/or the genetic type. This information can be important for investigating public health issues. As noted in response to question 1, there are more than 2,500 serovars of *Salmonella*. Determining the serovar for *Salmonella* is done using the Kauffman-White scheme based on the O and H antigens. The method takes about 3 days to complete. Alternative molecular serotyping is also available (60, 119). Although serotyping has been done for many years, public health investigators now rely on more specific genetic tests when doing investigations.

More recently, whole genome sequencing has been able to provide even greater level of specificity for differentiating strains. The access to rapid, low-cost methods to get whole genome sequencing data has opened an opportunity to potentially replace traditional serotyping methods (3). The use of more detailed genetic testing methodology provides significantly more information than traditional serotyping and in a much shorter time (121).

To determine whether new methods can achieve the desired result, validation of the method is required. There are recognized procedures for the validation of microbiological methods (51) (e.g., AOAC International and FSIS). These procedures provide a robust set of criteria for comparing methods and ensuring some level of equivalency between methods that may operate using fundamentally different technologies. It may also be important to show the method has been validated by a regulatory agency such as USDA or FDA or by recognized organizations such as AOAC International (<https://www.aoc.org/>), AFNOR (French Standardization Association; <http://www.afnor.org/en/>), and International Organization for Standardization (<https://www.iso.org/home.html>). External certification can provide assurance that rigorous standards were followed when validating a new method against recognized, established methods.

Question 5b. What is a sampling scenario that would enable an establishment to test incoming birds and product for a threshold *Salmonella* level and have a result in a timely manner so that processing can proceed as appropriate?

It is not currently practically feasible to implement a sampling scheme to test incoming birds and product for a threshold *Salmonella* level. Providing a timely result on incoming birds or product for a threshold *Salmonella* level such that an establishment can design processing as appropriate is not currently practical for two reasons: (i) establishing a threshold *Salmonella* level requires further studies and (ii) rapid microbiological testing methods that would allow evaluation of *Salmonella* prevalence and concentration on incoming live birds and poultry products are evolving and currently have a limited use by industry.

Material Supporting Committee's Answer

Challenges in implementing a scheme for incoming live birds and product are attributed to factors such as identifying independent microbiological lots at the farm level, processing plant logistics, transportation schedule, and hold and release procedures pending testing results, which may generate complex issues in the supply chain. Nevertheless, it is important for establishments to evaluate and validate process capability and monitor the extent of control within a manufacturing process.

A more feasible approach is to develop statistical process control monitoring via microbiological testing (111) at the farm level with the goal of validating process controls in anticipation of expected contamination levels combined with an establishment's ongoing verification testing (of finished product) (159), which may maximize the frequency

of *Salmonella*-negative finished product. In this context, statistical process control monitoring refers to performing statistical trend analysis of microbiological test results from samples collected at the farm level utilizing various sampling collection methods (e.g., drag swabs, litter samples, boot swabs, and cloacal swabs). Validation of process controls provides assurance that process interventions are sufficient to control expected levels of pathogen contamination. Statistical process control can also provide establishments with reasonable assurance that their HACCP system is functioning as designed and that they are likely to meet applicable performance standards (159).

When the establishment determines that trends in test results indicate a loss of process control, the establishment should take action to investigate the cause. An establishment should describe the actions it will take when the test results obtained through its sampling are above the process limits it has set. This description should include what the action will be, who will take the action, how the outcome of this action will be documented, and how it will be verified. Establishments should use the information provided in draft FSIS guidelines (159) to improve management practices and to assist in investigating when there is a loss of process control. When an establishment makes validated changes in process interventions, process control should improve. As a result, establishments should be able to produce raw poultry products that have less contamination with *Salmonella*. For more details please refer to sections VII and VIII in the *Draft FSIS Compliance Guideline for Controlling on Salmonella and Campylobacter in Raw Poultry* (159).

Scheduled slaughter and processing, and ongoing verification testing programs, are not substitutes for pre- and postharvest interventions to control *Salmonella*. Although the objective of scheduled slaughter is to prevent transfer of pathogens from positive flocks to negative ones during slaughter or processing, the objective of ongoing verification testing is to verify that the establishment's validated preventive measures are continuing to adequately function.

Designing a Sampling Program

Strategic microbiological testing of foods (e.g., incoming birds or poultry products) provides useful information about microbiological quality, safety, sanitation, and the effectiveness and extent of process control. Although it is rarely possible to use microbiological testing of foods to ensure safety and wholesomeness, it is possible to design strategic sampling schemes and select appropriate target organisms (*Salmonella* and/or indicators) and assays that can aid in the management and control of suppliers. Testing data can be used to help assess manufacturing and monitoring systems such as HACCP and preventive control programs. This section addresses how to design a microbiological sampling program and is intended to provide guidance for poultry establishments in evaluating their microbiological data and the extent to which their manufacturing process is in control (111).

When a microbiological sampling program is properly designed and implemented, it can provide valuable

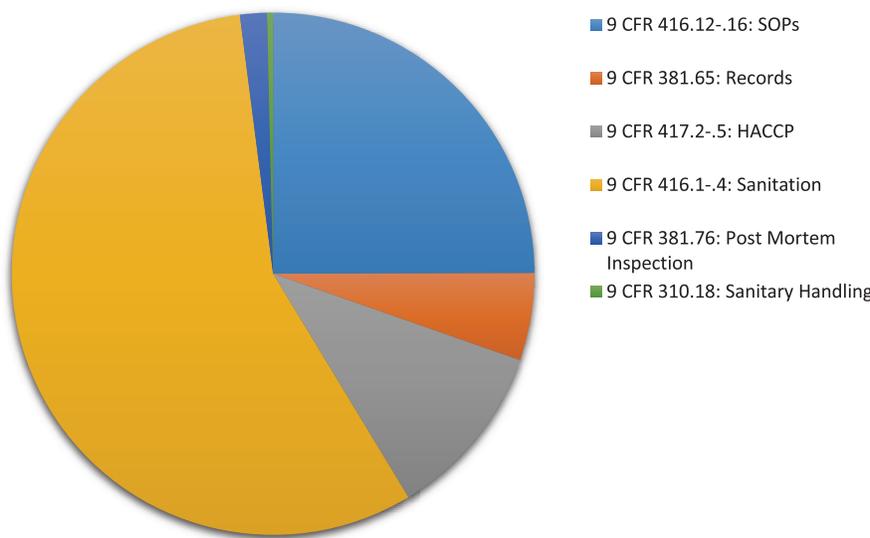


FIGURE 3. FSIS poultry noncompliance records represented proportionally by cited regulation(s), 2015 to 2016.

information about an establishment’s process control. When not properly designed and implemented, the test results can provide inaccurate and unreliable information that may not represent the establishment’s actual process control (159). There are a number of factors that need to be considered when designing a sampling plan at the farm level and at the processing level. Sample collection and analysis involves multiple steps, all of which should be successfully performed and documented to maintain the identity and integrity of the sample. A well-designed microbiological sampling program should clearly define the

- intended purpose of the testing program
- organisms of concern that will be the target of testing (e.g., *Salmonella* and/or indicators of process control)
- sampling units (e.g., flocks or houses at preharvest; carcasses or parts at postharvest)
- sampling scheme (e.g., random, systematic, or cluster)
- microbiologically independent lotting practices
- sampling locations (e.g., flocks or houses at preharvest; postchill or packaged product at postharvest)
- sample collection procedures
 - preharvest: boot swabs, drag swabs, litter samples, and cloacal swabs
 - postharvest: product (e.g., postchill carcass, parts, or ground product)
- procedures for ensuring sample integrity
- microbiological testing method for sample analysis (e.g., qualitative, semiquantitative, or quantitative)
- microbiological laboratory performing the analysis
- method for evaluating test results (e.g., p-chart, incident chart, or x-bar charts)
- actions taken based on the test results

In a previous report by NACMCF (111), appendices B through H, K, and L detailed various methods available for charting test results and identifying exceptions suspect for assignable causes. For results that are binary (e.g., positive or negative) with very low frequency of positive results, a g-chart based on mean time between events is recommended. For high-frequency binary results, a p-chart based on

proportions is recommended. For quantitative results, mean and range charts can be used.

Question 6. Considering the farm-to-table continuum for poultry, what are the top three focus points, control measures, or best practices that would be compatible with industry-wide practices and could be addressed or implemented to achieve the highest rate of reduction of *Salmonella* with regard to both foodborne illnesses and product contamination?

The subcommittee has identified four answers to this question, and they are presented in no specific order of priority.

Answer 1. All edible poultry products originate at a slaughter establishment, and it is here where most microbial control is currently possible. At this time, the greatest reduction in *Salmonella* can be achieved through continued development, implementation, and monitoring of good manufacturing practices within slaughter establishments. Various aspects of effective process control include

- accomplishing prerequisite programs associated with cleaning the plant and maintaining equipment and the facility
- verifying effectiveness of sanitation processes through comprehensive preoperation environmental monitoring programs that include assessment of appropriate indicator organisms
- implementing a consistent sanitary dressing program to prevent contamination with ingesta and feces (and therefore enteric pathogens) throughout the slaughter process as part of the slaughter HACCP system and meet zero tolerance requirements for feces on poultry carcasses entering the chilling system
- using validated interventions and processing aids at targeted sites for efficient reduction of pathogens
- continuing to promote, innovate, and improve microbial interventions and processing aids such as

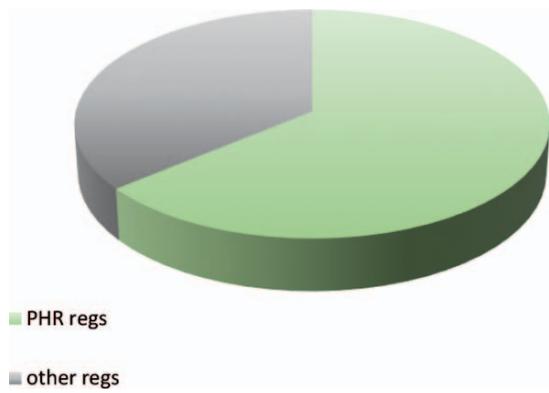


FIGURE 4. Proportion of poultry noncompliances citing regulations of particular public health concern (PHR regs), 2015 to 2016.

- biocontrols (e.g., bacteriophage or plant-based antimicrobial products)
- novel chemicals and application methods
- irradiation and other cold sterilization approaches

applying good manufacturing practices for cold chain management
 developing appropriate microbial-verification sampling schemes and then using the data to monitor and improve process control
 prioritizing establishment improvements based on the most frequently reported noncompliance records (NRs; Figs. 3 through 5) related to public health (FSIS should work with the industry to assist this focus)
 publishing individual establishment performance standard category status

Answer 2. Due to differences in allowed in-plant slaughter interventions, scale of operations, and live-bird house design, producers in the European Union (EU) have focused food safety efforts on farm-level *Salmonella* control. The results from the EU indicate that effective and targeted control of *Salmonella* on farms can reduce *Salmonella* entering slaughter establishments on birds and on resultant raw poultry products. As such, reduced prevalence on farm, or where possible, and prevention or elimination of colonization with *Salmonella* should be effective for reducing *Salmonella* in finished product and

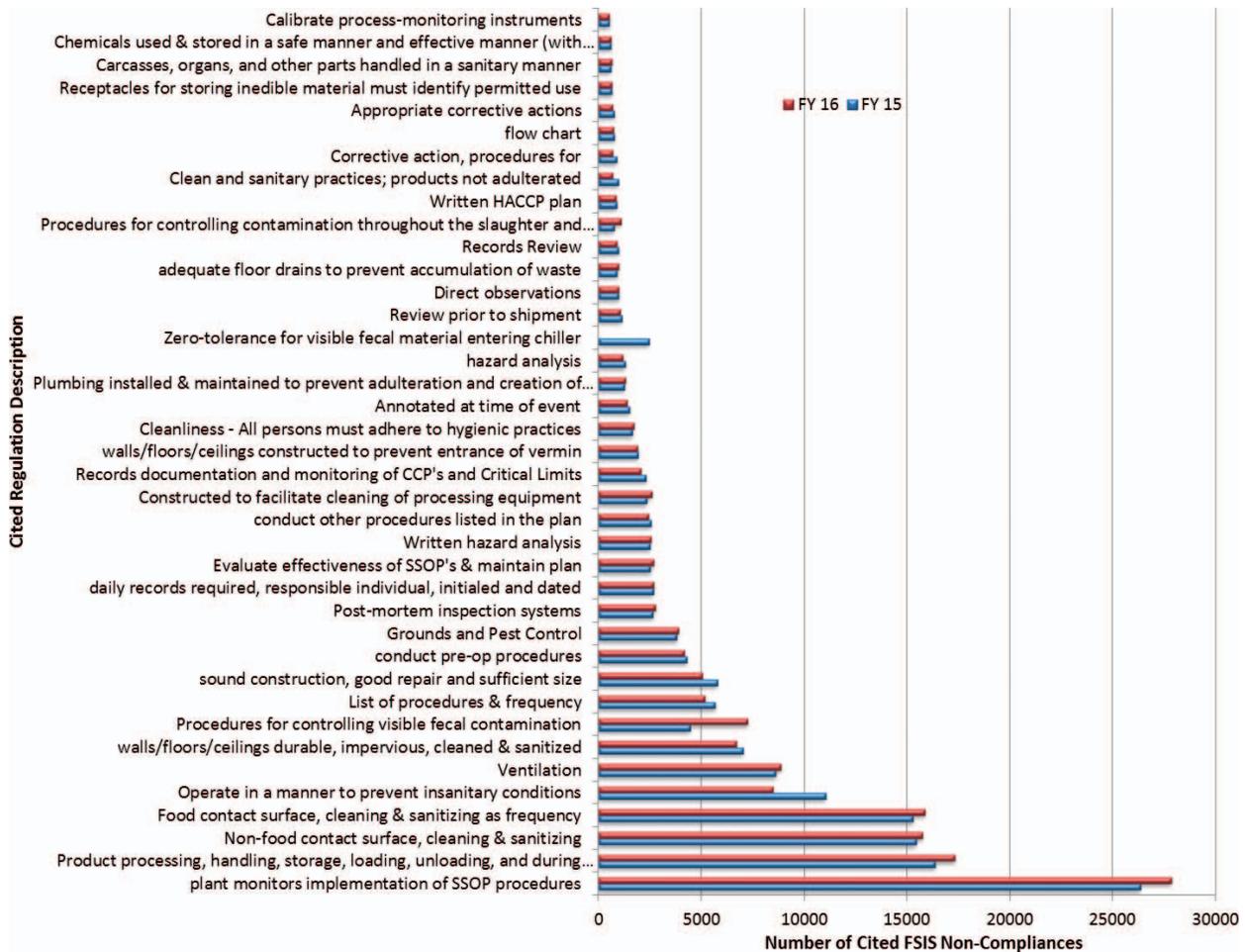


FIGURE 5. Detailed view of noncompliance records, FSIS, fiscal years (FY) 2015 and 2016. No 2016 data are available for “Zero-tolerance for visible fecal material entering chiller”; 21 August 2014 Federal Register document 2014-18526, p. 49,634: 381.65(f) replaced 381.65(e) for controlling visible fecal contamination (<https://www.federalregister.gov/documents/2014/08/21/2014-18526/modernization-of-poultry-slaughter-inspection>).

contributing to improvements in public health. The Committee recommends:

working with breeders to establish a *Salmonella* control program in parent flocks targeted to those serotypes of greatest public health burden (serotypes Enteritidis, Typhimurium, and Heidelberg)
 identifying genetic lines with increased resistance to infection and colonization
 implementing effective preslaughter controls within contract growers, i.e., continuing to innovate and develop new preslaughter interventions and management strategies with increased effectiveness (including house design, litter management, and products applied to birds) and developing farm-level surveillance (e.g., of the environment or birds) to aid detection and control of serotypes of public health consequence.
 evaluating live haul (i.e., catching, loading in crates, transportation on trucks, and unloading) and developing best practices to enhance animal welfare and minimize shedding and cross-contamination

Answer 3. Identify and develop approaches that exclude serotypes of greatest public health concern from raw poultry products.

In the absence of clearly defined virulence markers for targeting control, control should focus on *Salmonella* serotypes that cause the greatest public health concern (serotypes Enteritidis, Typhimurium, and Heidelberg). As whole genome sequencing libraries increase in size and are linked to human health outcome data, it may be possible to more closely associate suspected virulence factors with health outcomes. A more detailed assessment of genetic factors associated with human virulence for poultry-associated serotypes of *Salmonella* is recommended.

This approach will require collaboration—and coordinated efforts—of slaughter establishments, broiler growers, owners of parent flocks, the Agency, diagnostic assay companies, and allied industries that produce technologies that might target these serotypes during any stage of production.

Answer 4. Promote greater collaboration among industry (e.g., poultry, packaging, and testing), the Agency, customers, and consumers to decrease the opportunity for cross-contamination and consumer exposure after raw poultry leaves slaughter establishments.

Develop new educational approaches based on sound and valid social science and behavioral research to identify barriers to food preparers adopting “best behaviors.”

Target food preparers to aid in safe handling practices to decrease cross-contamination and reduce consumer exposure to foodborne pathogens.

Encourage innovative design to improve packaging (e.g., materials and systems to minimize cross-contamination) and equipment (e.g., cooking equipment that allows improved process control).

Understand postpackaging contamination of the packaging material.

Conduct research to fill data gaps of cross-contamination in display cases and delis.

ACKNOWLEDGMENTS

NACMCF acknowledges the contribution of Mr. Jude Smedra (FSIS) for critical review and formatting this report for publication. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture and other participating agencies.

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