RESPONSE TO QUESTIONS POSED BY THE UNITED STATES DEPARTMENT OF AGRICULTURE, FOOD SAFETY AND INSPECTION SERVICE

Adopted DDMMMYYYY, Washington, DC

NATIONAL ADVISORY COMMITTEE ON MICROBIOLOGICAL CRITERIA FOR FOODS

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1.0 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 result from 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:

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

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

FSIS Question 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, the heart, skin follicles/skin surfaces, lymphatic system, immune evasion, and other? Discuss strategies to mitigate risk factors at these locations.

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

FSIS Question 3: Would removing flocks of highly *Salmonella*-contaminated birds entering the slaughter plant reduce foodborne illnesses in humans? What are important considerations to arriving at a threshold level (prevalence or load: e.g. CFU/gm 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/steps for an alternative processing scenario if the threshold level is exceeded?

NACMCF Answer: 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.

FSIS Question 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?

NACMCF Answer: As 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.

FSIS 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? 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?

NACMCF Answer: Molecular based 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.

FSIS 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, which could be addressed or implemented to achieve the highest rate of reduction of *Salmonella* with regard to both foodborne illnesses and on product?

NACMCF Answer: 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 GMPs within slaughter establishments.

The NACMCF articulated recommendations to the Agency that focus on risk based approaches for more effective *Salmonella* control, and to request 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.

2.0 RECOMMENDATIONS

2.1. While it is 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* concentration and serotype (or where appropriate, a subtype thereof) and diverted products be sent to a validated lethality step (e.g., cooking) or reprocessing.

- Concentration – and assays to estimate concentration – and related dose-response for various demographics are currently poorly defined. It may be possible, however, to arrive at an estimated threshold of concentration-serotype (or subtype) through modeling.
- Such an approach should include considerations of infectious dose on poultry and that resulting from cross-contamination resulting in secondary consumer exposure.
- This approach may take the form of a quantitative microbial risk assessment.

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

- Water portioners due to the water mist occurring inside the machine or pickers and how best to effect "prevention through design"
- Packaging
- Retail case

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

2.4. The Agency should encourage development of quantitative (or semi-quantitative) microbiological methods for *Salmonella*.

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

2.5. Because much uncertainty and disagreement among experts remain over what 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, as well as virulence modification by pre- and post-slaughter processes (*e.g.*, how exposure to an acid may induce or modulate virulence).
- Request research to better understand persistence in the environment of *Salmonella*.

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

2.7. The Agency should request research to further understand the dynamics of *Salmonella* within the bird or in feather follicles. While much work has been done of tissue tropism in the

past decades, new methods have emerged that may shed additional light on the tissue in which *Salmonella* may be harbored.

2.8. The Agency should research into 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.

2.9. The Agency should work with FDA Center for Veterinary Medicine to develop an approach to cost-effectively and expeditiously approve undefined cultures for use in broiler production.

3.0 INTRODUCTION

The U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (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 CDC has not greatly changed over time. After adjusting for cases that do not present to healthcare and those not reported to the CDC, an estimated 1 million domestic salmonellosis cases are attributed to food as a vehicle of exposure (Scallan et al. 2011). Among FSIS-regulated products, the Agency estimates approximately 360,000 salmonellosis cases are from meat, poultry, and egg products. The FSIS is committed to taking steps to prevent *Salmonella*-related illnesses associated with FSIS products.

In December 2013, FSIS released its *Salmonella* Action Plan that outlines the steps it will take to address *Salmonella* in FSIS regulated products. The comprehensive steps detailed in this plan are geared towards protecting consumers by making meat, poultry, and egg products safer. Key components of the plan include modernizing the poultry slaughter inspection system, enhancing *Salmonella* sampling and testing, 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 has 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 like *Salmonella*), as well with the Agency's own strategic goals to develop performance standards for *Salmonella*.

3.1. 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 improve public health, FSIS realizes that the focus must be throughout the farm-to-table continuum and thus seeks the advice of the National Advisory Committee on the Microbiological Criteria for Foods (NACMCF) on the following issues.

1. What criteria define *Salmonella* 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, the heart, skin follicles/skin surfaces, lymphatic system, immune evasion, and other? 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 to arriving at a threshold level (prevalence or load: e.g. CFU/gm 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/steps for an alternative processing scenario if the threshold level is 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, which could be addressed or implemented to achieve the highest rate of reduction of *Salmonella* with regard to both foodborne illnesses and on product?

3.2. COMMITTEE'S APPROACH TO ANSWERING THE CHARGE

The Committee leveraged the expertise of the Committee members, additional experts and published literature and available results of assays of poultry products to assist in answering the agency's charge. A sub-committee was formed and further divided into 2 working group. Items 1 and 2 of the charge addressed by one of the working groups, and items 3, 4, and 5 were addressed by the other working group. The entire sub-committee addressed item 6. The working groups met in person (3 times) and virtually as needed. The working groups also requested 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 face to face with industry experts to seek expert information.

4.0. RESPONSES OF THE COMMITTEE

4.1. QUESTION 1. What criteria define *Salmonella* that are highly virulent to humans? Are markers serotype specific? Sub-question: What tools are available for continuing to identify the most virulent foodborne salmonellae?

ANSWER

At the present time, there are no defined criteria that distinguish highly virulent *Salmonella* from those that are less so. Much uncertainty exists in terms of distinguishable virulence factors that can explain the spectrum of disease severity. While 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

Virulence can be defined as the ability of a pathogen to cause disease in a host. In the case of *Salmonella*, this characteristic can be evaluated by the pathogen's ability to colonize or infect the intestine, escape the intestine and invade to infect internal organs, cause clinical signs related to inflammation of the intestine and/or internal organs thereby causing gastroenteritis, systemic disease or death (Blaser and Newman 1982). 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 resisting killing by white blood cells (Galan 2001). However, a few serotypes of *Salmonella* also contain a virulence plasmid conferring enhanced ability to attach to the intestinal cells and enhanced ability to resist killing by normal host defenses (Baumler et al. 1998).

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 (Lax et al. 1995; Hohmann 2001; Hsu et al. 2003). Feeding studies using healthy human volunteers revealed that gastroenteritis occurs after consumption of a large numbers of bacteria (10^5-10^{10}) (Blaser and Newman 1982; Kothary and Babu 2001) but the Centers for Disease Control and Prevention (CDC) have reported that the incidence rate of salmonellosis is higher in children and the elderly suggesting that an infectious dose may be much lower than in healthy adults (CDC FoodNet Annual Report 2012). Moreover, individuals using proton pump inhibitors (Banatvala et al. 1999), with diabetes (Telzak et al. 1991), immunocompromised or receiving immunotherapy (Hohmann 2001; Hsu et al. 2003) 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 (Chen et al. 2012). Some serotypes of nontyphoidal *Salmonella* are more likely to escape the gastrointestinal tract and cause systemic disease. These pathogenic serotypes include *S*. Choleraesuis, Dublin, Heidelberg, Oranienburg, Panama, Poona, Rubislaw, Sandiego, and Schwarzengrund (Jones et al. 2008; Angelo et al. 2016). Of these, Heidelberg appears to cause the greatest burden of systemic disease (Dutil et al. 2010) [Ref Canadian data: http://www.phac-aspc.gc.ca/cipars-picra/heidelberg/heidelberg-eng.php].

A few serovars are consistently associated with the greatest incidence of human disease. In 2013 the Centers for Disease Control reported that 10 serotypes were responsible for more than 50% of human disease, Enteritidis, Typhimurium, Newport, 1,4,[5],12:i-, Javiana, Heidelberg, Infantis, SaintPaul, Muenchen, Montevideo. (https://www.cdc.gov/nationalsurveillance/pdfs/salmonella-annual-report-2013-508c.pdf). This phenomenon remains relatively consistent over time. Globally, two serotypes dominate, Typhimurium and Enteritidis, 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. Thirty-three percent of human disease was caused by two serotypes, Enteritidis (15.1%) and Typhimurium (18.1 % including 1,4,[5],12:i:- H2 negative Typhimurium strains) but Newport (8.3%), Javiana (5%), Heidelberg (3.1%) and Infantis (2.9%) contributed a significant percentage of the total. In 2014, the USDA-FSIS reported that 15% of raw meat (broiler, turkey, ground beef) were contaminated with Enteritidis or Typhimurium: 3.7% of broiler chicken carcasses were *Salmonella*-positive with 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 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 Montevideo (11,377), SaintPaul (9,420), Agona (5,072), Hadar (2,857), Kentucky (984), Reading (619), and Ouakam (10). While not all of these cases resulted from poultry products, it is clear that the majority of these serotypes are potentially pathogenic for humans.

Human challenge studies were performed with Typhi, Typhimurium, Anatum, Pullorum, Meleagridis, Sofia, Bovis-morbificans, Newport, Derby, and Bareilly confirming that a broad array of serotypes can cause human disease. The NACMCF investigated whether highly virulent *Salmonella* harbor unique genes or markers that differentiate them from less virulent *Salmonella.* For example, *S.* Typhi isolates possess pathogenicity islands that confer specific virulence properties causing typhoid fever in humans. *Salmonella* serovar Enteritidis,

Choleraesuis, Dublin and Typhimurium contain a virulence plasmid, which has been shown to be important in the typhoid fever mouse model. While 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 are 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 correlated with human foodborne disease.

S. Enteritidis is a good model for investigating the genetic basis of high virulence. Enteritidis isolates represent a closely related population of strains in which some are actual clones of each other (Cho et al. 2007). They can vary significantly in virulence properties, including biofilm formation, motility, and invasion because gene expression is affected by many factors (Shomer et al. 2016). Hypervirulent strains of 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 (Heithoff et al. 2012). 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 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 improve the infectivity of *Salmonella* by offering protection during transit in the host gastrointestinal tract (Blaser and Newman 1982; Podolak et al. 2010), 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 (Blaser and Newman 1982; Coburn et al. 2007). *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 (Lax et al. 1995; Tenor et al. 2004). Although this does not reveal the full virulence arsenal of the pathogen, *S.* Kentucky appears less invasive than the serotypes commonly associated with foodborne illness, which may explain why it is less prevalent in humans than its prevalence in poultry (Joerger et al. 2009; Cheng et al. 2015). Nevertheless, there is no data suggesting that the highly virulent *Salmonella* are more tissue culture invasive than less virulent counterparts. We questioned whether the severity of the infection in humans was correlated to specific responses in other animal hosts, either part of the natural transmission mode or in animal models in the laboratory. Only *S.* Typhi and Paratyphi A are human restricted 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, *S.* Kentucky is the most predominant serotype isolated from U.S. poultry products, yet it has a low impact on human illness and it has not been associated with a large foodborne outbreak in the U.S. (CDC 2012; Shah et al. 2017). Several animal models have been developed for *Salmonella* infection but few are able to capture both the enteric (typhoid) fever syndrome and the gastroenteritis (Higginson et al. 2016). In addition, the mechanisms determining which type of disease is caused by which serotype in which host are still poorly understood. For example, serovar Typhimurium causes enterocolitis in calves and the animals can succumb to dehydration. In newly hatched chicks, it will cause systemic disease and diarrhea, whereas older chickens are asymptomatic carriers. In immunocompetent humans, it causes localized self-limiting gastroenteritis but bloodstream infections and systemic disease may develop in immunocompromised individuals. In susceptible mouse strains, it 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 they 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 non-host adapted serotypes (Baumler et al. 1998; Almeida et al. 2013).

Host-to-host transmission is a key phase of a 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 superspreaders) 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. 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 *S.* Typhimurium, the gastrointestinal microflora played a large role in keeping the mice infected at low level, 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 (Lawley et al. 2008; Gopinath et al. 2012). To the best of our knowledge, a supershedder phenotype has yet to be observed in human patients, but, with the rise of antibiotic resistance in *Salmonella,* treatment could have more impact on the microbiota than on the antibiotic resistant pathogen. Since *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, have persisted for more than one week, or invasive disease is suspected (Switaj et al. 2015). In the absence of *Salmonella* confirmation, a fluoroquinolone like 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. If *Salmonella* has been confirmed, severe cases could also receive the macrolide azithromycin or the third-generation cephalosporin ceftriaxone, a class of β lactam antibiotics (Switaj et al. 2015). Intuitively, while antimicrobial treatment can be life-saving, antimicrobial resistance may contribute to bacteremia, treatment failure, and poor clinical outcomes (Krueger et al. 2014; Taylor et al. 2015). Although not considered virulence genes *sensu stricto*, it is undeniable that presence of genes conferring resistance to fluoroquinolones, macrolides and/or cephalosporin in *Salmonella* constitutes a risk to vulnerable populations, especially in serotypes recognized as invasive nontyphoidal *Salmonella* (Angelo et al. 2016).

Tools to assist virulence identification:

A core constellation of virulence genes in *S. 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 and different combinations of genes and gene expression are likely responsible for human disease under variable host immune responses and environmental conditions. In addition, to be a successful foodborne pathogen, additional virulence factors that permit survival in the animal host and the environment may also play important roles in the ecological fitness of the foodborne salmonellae. For example, Addwebi *et al* (Addwebi et al. 2014), hypothesized that *S. enterica* serovar Enteritidis uses both common *S. enterica* virulence factors and *S.* Enteritidis-specific virulence factors 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 characterization 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 the 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 pathogenic potential of isolates and guide regulatory action. Although imperfect, similar molecular risk ranking strategies enabled characterization of Shiga-toxin producing *E. coli* (STEC) (Bugarel et al. 2011; Franz et al. 2015).

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. In the case that the isolates were 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 poultry may not be evident in mammalian disease models.

4.2. 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 and heart, skin follicles/skin surfaces, lymphatic system, immune evasion and other? Discuss strategies to mitigate risk factors at these locations.

ANSWER

Subsequent to infection, *Salmonella* can invade deep tissues, such as livers of broilers and this 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 from the crop during evisceration and aerosolization during picking. Several pre-slaughter strategies to reduce the burden of *Salmonella* in flocks entering slaughter establishments have been shown to be effective, and data demonstrating a correlation of flock-status of *Salmonella* with pre- and post-chill contamination have been reported (Amerah et al. 2012; Alali and Hofacre 2016). However, correlation of pre-slaughter status and finished product contamination with *Salmonella* is not certain in commercial settings.

MATERIAL SUPPORTING COMMITTEE'S ANSWER

Pre-harvest 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* involved, poultry colonization may be asymptomatic. Regardless, if birds destined for slaughter harbor *Salmonella* with pathogenic potential to humans either in their bodies or on their surface, they 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 (Cox et al. 1996a; Bailey et al. 2001; Liljebjelke et al. 2005; Alali and Hofacre 2016)). 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 (Byrd et al. 1998; Liljebjelke et al. 2005).

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. It 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* (Curtiss and Hassan 1996; Bailey et al. 2007; Volkova et al. 2010; Berghaus et al. 2011).

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 (2011 ACPV workshop). Bacterins stimulate higher levels of serum antibody (compared to live vaccines) in parents, thus maternal antibody is transferred to the progeny, which may reduce colonization (Bailey et al. 2007). Treating the chicks with a live vaccine, after passively transferred maternal immunity has waned

can enhance subsequent resistance to colonization (Bailey et al. 2007). 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 US CDC review (Crump et al. 2002) suggested that because of an increased incidence of *S. enterica* serotype Agona in animal feed, there was a concurrent in human illnesses attributed to this serotype with as many as one million additional illnesses occurring. To control *Salmonella* and other pathogens in feed, feed manufacturing facilities must identify the microbial growth niches and reducing conditions that lead to growth (Jones 2011). The three categories that must be addressed are, i) prevent the introduction of *Salmonella,* ii) reduce the multiplication of the organism, and iii) procedures to kill the bacteria. Killing *Salmonella* may involve thermal processing (pelleting) or chemical addition.

Pelleting has been reported to reduce *Salmonella* from 50 to 93% and rely mainly on steam to kill the bacteria (Hacking et al. 1978; Jones et al. 1991; Blackman et al. 1992; Veldman et al. 1995; Jones and Richardson 2004; Jones 2011). Pelleting adds steam to the feed during the conditioning process. Care should be taken in the cleaning of the equipment because the moisture can provide an avenue for *Salmonella* growth (Jones 2008). 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* than fed a non-pelleted diet (Wong et al. 2004). However, it may be dependent on the coarseness of grain. Coarse grain produces more volatile fatty acids that will inhibit the growth of *Salmonella* versus fine ground grain (Reid et al. 1996; Reid et al. 1998; Reid and Hillman 1999; Silvi et al. 1999).

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 (Furuta et al. 1980; Ha et al. 2000; Ricke et al. 2005). Preventing *Salmonella* contamination of the feed must include obtaining uncontaminated feed ingredients, strict biosecurity, and sanitation. Since plant-based and animal proteins have been previously identified as risk for *Salmonella* status of birds, consideration of this possibility should be taken into account in feed formulation and preparation (EFSA 2008; Jones 2011).

Genetic resistance to *Salmonella*

An unutilized approach for *Salmonella* control is to breed birds that are more resistant to *Salmonella* infections by natural selection (Calenge et al. 2011; Calenge and Beaumont 2012).

Considering that the genetics of the majority of the commercial poultry lines produced in the world are controlled by two to 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 the post-genomic analyses facilitates the identification of markers or genes controlling a measurable phenotype and the ability to select for them naturally (Calenge and Beaumont 2012; Thanh-Son et al. 2012). Resistance to early *Salmonella* intestinal colonization has been mainly studied by investigating genomic regions controlling intestinal colonization (Malek et al. 2004) or by studying innate immunity from increased expression of pro-inflammatory cytokines and chemokines (Beal et al. 2006; Wigley et al. 2006). Interestingly, the same inbred lines show increased resistance to *Campylobacter* colonization at hatch (Boyd et al. 2005). 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 (Swaggerty et al. 2009; Swaggerty et al. 2011; Swaggerty et al. 2014). 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, its implementation 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 (Cox et al. 1996b; Kallapura et al. 2014a; Kallapura et al. 2014b). Some *Salmonella* serovars colonizing chickens have broad host ranges (e.g., Typhimurium, Enteritidis, Kentucky, Heidelberg) while others are host specific and cause illness in the birds (e.g., Pullorum, Gallinarum) (Foley et al. 2013). Manipulation of the intestinal microflora, diet, and host immunity has been the basis for a number of pre-harvest intervention strategies (Alali and Hofacre 2016). Examples include administering a competitive exclusion product at day-of-hatch and inclusion of probiotics and/or prebiotics in the feed to reduce colonization through growout (Totton et al. 2012; Kerr et al. 2013). While 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.

<u>Probiotics, including competitive exclusion</u>

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 (Nurmi and Rantala 1973). Some probiotics/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 reduction of foodborne pathogens. Competitive exclusion studies with undefined culture led to the development of various commercial products (Schneitz 2010). CE treatments have to be applied at the earliest opportunity since they are not effective as a treatment for *Salmonella*-positive chicks. Generally, protective microbiota are delivered by spray application, just prior to leaving 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 microflora. Alternatively, eggs can be injected during incubation, a few days before hatching, but some embryos may die in the process (Mead 2000). Field evaluations have shown that CE treatments, combined to stringent hygienic measures on the farm, can lead to substantial reduction in the contamination of chickens on the farm and of carcasses at slaughter (Stavric and D'Aoust 1993).

Despite encouraging efficacy data, several countries, including the U.S., 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 bird(s). Therefore efforts have focused on the identification of key protective elements in undefined cultures with a view towards the development of a product of defined bacterial composition.

The most common type of defined probiotic (also known as direct-fed microbial) for poultry includes single-strain or combinations of lactic acid bacteria (LAB), *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 microflora in chickens and turkeys, providing increased resistance to infection by enteric bacterial pathogens, including *S.* Heidelberg, as early as 1 hour following the administration of a probiotic (Higgins et al. 2007; Higgins et al. 2008; Menconi et al. 2011). The most acclaimed effect for some probiotics is their positive influence of 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 levels and number of T cells (Stanley et al. 2014). There is evidence to support the theory that multistrain and/or multispecies probiotic supplementation is more effective than a single strain. In other words, *Salmonella* species can be inhibited by a mixed culture of *L. crispatus* and *Clostridium lactatifermentans, Bacillus subtilis,* and *Enterococcus faecium* (Stanley et al. 2013).

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 (Van Immerseel et al. 2002; Sohail et al. 2012). Examples include fructo-oligsaccharides and mannan-oligosaccharide (MOS) that have been shown to reduce the abundance of *S.* Enteritidis in cecal contents of experimentally infected chickens (Fernandez et al. 2002). Also, there has been some success in reducing *Salmonella* infection in broilers by incorporating the yeast cell wall products, e.g., *Saccharomyces boulardii*, in the feed (Line et al. 1997).

Bird Health and Raising

Newly hatched chicks are typically colonized by *Salmonella* quickly since their gut has limited microflora and may be susceptible. NACMCF (1997) reviewed existing literature in their development of a generic Hazard Analysis and Critical Control Point (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 (Alali and Hofacre 2016). (Hofacre, personal communication. 1/26/16).

The health and treatment of birds through the grow-out phase is a key factor affecting carriage of *Salmonella*. The International Commission on Microbiological Criteria for Foods (ICMSF 2005) reports 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 contaminated, *Salmonella* can be transmitted readily among birds. A Canadian study linked prevalence (50% overall) of *Salmonella* in 81 flocks to various risk factors obtained 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. They suggested that this was a possible measurement of the quality of biosecurity by the producer. They found no correlation of *Salmonella* prevalence with pest control programs, downtime, manure disposal or sanitation (Arsenault et al. 2007).

Typically, broilers are harvested at approximately 47-65 days of age after being grown under very controlled conditions to ensure a uniform size of the bird. Uniformity of bird size can help with process controls, making gut contents are less likely to be spilled during the slaughter process, as the equipment can be set very precisely to accommodate the expected size of birds (Scott Stillwell, personal communication 1/26/16).

Chemical litter treatments

If acidity is reduced below about pH 5, conditions are unfavorable for *Salmonella* and other potential pathogens (Corrier et al. 1999a; Corrier et al. 1999b). To achieve this, chemical treatment 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 include aluminum

sulfate (Moore and Miller 1994), ferrous sulfate (Huff et al. 1984), phosphoric acid (Reece et al. 1979), sodium bisulfate (Moore et al. 1996), and acetic acid (Parkhurst et al. 1974).

Moore et al. (Moore et al. 1996) evaluated several chemical treatments for ammonia utilization and phosphorus solubility and found that aluminum sulfate was best at reducing ammonia volatilization, followed by phosphoric acid, ferrous sulfate, sodium bisulfate, and calcium-ferrous-sulfate. All treatments significantly reduced litter pH when compared to the control litter. Aluminum sulfate was most effective in 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 house was higher compared to the other treatments. In another study, sodium bisulfate was shown to be effective in controlling *Salmonella, Clostridium,* and *Pasteurella* in litter (Terzich 1997). Furthermore, the application of this product was effective in 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 post-harvest agricultural commodities, especially poultry. A cocktail of bacteriophages was able to reduce *S.* Enteritidis about 1 log CFU/cm^2 on samples of chicken skin experimentally contaminated with 1×10^5 CFU/cm^2 *S.* Enteritidis (Hungaro et al. 2013). More than one log reduction of *S.* Typhimurium and *S.* Enteritidis were 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 (Spricigo et al. 2013). Recently, bacteriophage was used to reduce approximately 1 log CFU/g of *Salmonella* in ground chicken (Grant et al. 2017; Yeh et al. 2017). However, oral bacteriophage administration has demonstrated various levels of efficacy in reducing the colonization of *Salmonella* in the gastrointestinal tract of chickens (Sklar and Joerger 2001; Toro et al. 2005; Atterbury et al. 2007; Hurley et al. 2008; Lim et al. 2012). These data suggest that bacteriophages might serve as an alternative agent to reduce *Salmonella* contamination.

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 (Humphrey et al. 1993; Ramirez et al. 1997; Corrier et al. 1999b). 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

timeframe longer than 12 hours may result in thinning of the gut wall and liquefying of any ingested food that can result in leakage during evisceration (Warriss et al. 2004). These changes can occur with any stress to the bird or the gastrointestinal environment. These stressors can include 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 re-acidify the crop using inorganic or organic acids (Byrd et al. 2001; Wolfenden et al. 2007). These studies suggest that incorporation of some organic acids in the drinking water during pre-transport 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 litter pH increases in the poultry house, the number of bacteria, including pathogens, tends to increase. As water activity (a_w) and pH of litter decreases, the number of bacteria decreases with an optimal a_w of 0.84 or less and an optimal pH of 4 or less (Payne et al. 2007). One way to control moisture with in a house is to construct a well-ventilated poultry house that minimizes the sweating that may occur. 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 parameters. 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 has been found in up to 66% of air samples (Gast et al. 2004).

Biosecurity

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

Seasonality

A characteristic of cooler months is periods of less available natural light that 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. (Volkova et al. 2010). They found that longer relative duration of reduced lights during the grow-out period was associated with reduced detection of *Salmonella* on the exterior of birds one week before harvest and on the broiler carcasses at the post-chilling point of processing. They suggested that starting reduced lighting for ≥18 hours per day later in the grow-out period was associated with decreased detection of *Salmonella* on the exterior of broilers arriving for processing and in the post-harvest drag swabs of litter from the grow-out house.

A study by FSIS scientists (Linville et al. 2016) 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 may be due to physiological stress on poultry during the grow-out period, as well as the effect of weather on the movements of vectors, including rodents and migrating birds.

Slaughter control of *Salmonella*

Flock scheduling

If 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 particularly highly contaminated with *Salmonella*, these birds might be scheduled in this scenario to be processed towards 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* (Mead et al. 2010). 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/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 (Salehi et al. 2016). In addition, most *Salmonella* serovars infecting chickens can disseminate systemically, at least transiently, including to the liver (Roy et al. 2001). 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 (Alali et al. 2013; Alali and Hofacre 2016). 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 (Kim et al. 1996). Systemic contamination of extra-intestinal tissues, such as the liver, spleen and gall bladder, can occur with some serotypes. A salmonellosis outbreak (CDC 2012) was linked with the consumption of chicken livers contaminated with *S.* 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 (Lillard 1986a; b; Kim et al. 1996). 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 (Lillard 1986a) 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. Ineffectiveness of rinsing to remove bacteria from broiler carcasses has been demonstrated (Lillard 1988). Aerobic bacteria and Enterobacteriaceae were detected via rinsing, stomaching and blending of broiler carcass skin and, while a gradual reduction occurred after 10 rinses, 10^5 aerobes and 10^4 *Enterobacteriaceae* could still be detected after 40 rinses. Kim et al (Kim et al. 1996) used confocal scanning laser microscopy to show that most *Salmonella* cells attached to the flat portion of the skin surface washed off easily, while *Salmonella* cells remaining were located in crevices and entrapped in feather follicles, even after rinsing. Unattached floating *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/consistent detection in 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 if *Salmonella* are entrapped or tightly bound in crevices or feather follicles. Singh et al. (Singh et al. 2015) compared the ability of swabbing, stomaching and grinding to detect a range of bacteria including mesophilic aerobic bacteria (MAB), *E. coli* and coliforms. Less than 35% of MAB appeared to be loosely associated with the skin of the broiler and therefore detectable by swabbing or stomaching, while greater than 65% of MAB 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 notes only a 1.4% positive rate for whole carcasses, but a 22.1% positive rate for chicken parts, 29.3% positive rate for ground and other comminuted chicken (not mechanically separated), and 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/absence in order to better understand what is happening to concentrations of *Salmonella* (McEntire et al. 2014). While 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) (McEntire et al. 2014).

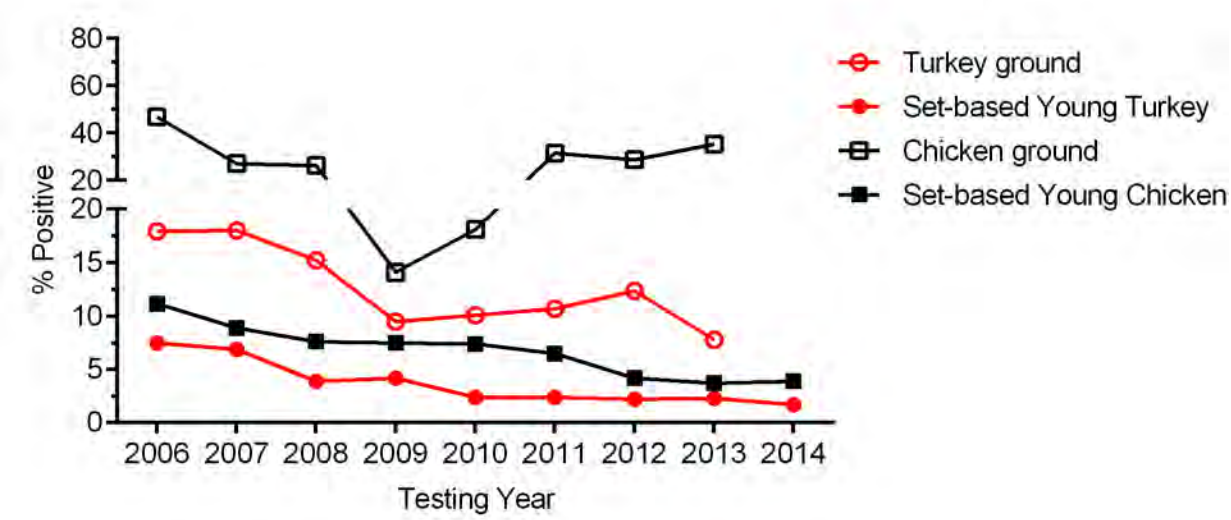
Quantities of *Salmonella* on a range of products through FSIS quarterly testing are discussed below. It should be considered that, with the exception of ground product, this data was obtained

using swab and rinse sampling and, therefore, the possibility of false negatives where tightly associated *Salmonella* were not detected may exist. More recently, there has been concern regarding false negatives due to residual chemical interventions on carcasses. This led to the recent incorporation of neutralized buffered peptone water in FSIS detection procedures (USDA-FSIS 2016).

Salmonella in final product

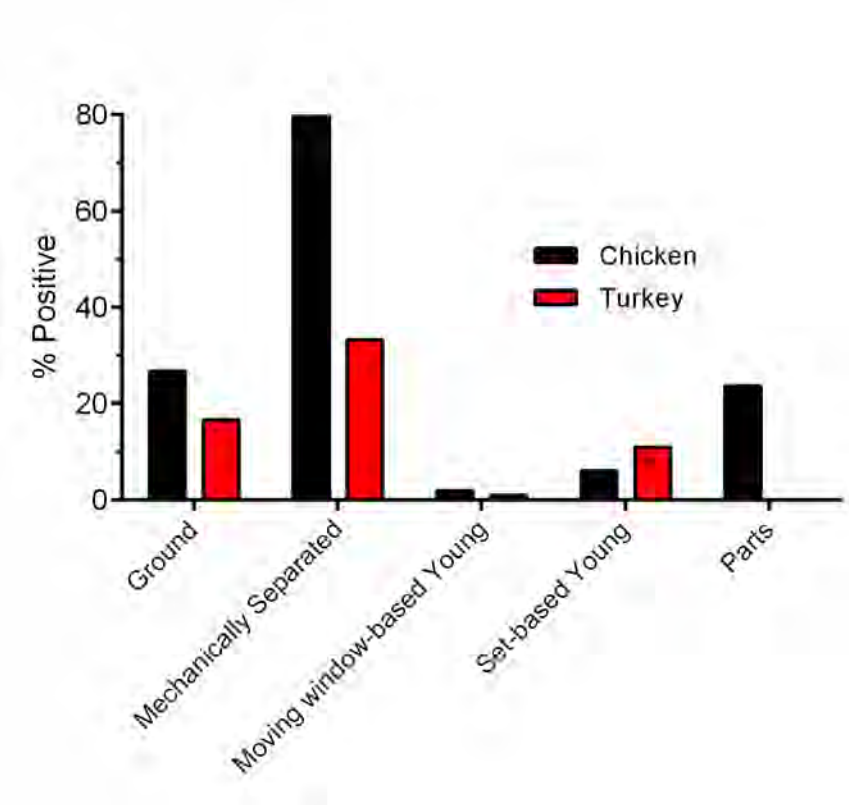
As part of the HACCP implementation plan, FSIS continually tests poultry production facilities for *Salmonella* and requires all poultry plants to develop and implement a system of preventive controls for *Salmonella*. Quarterly testing by FSIS between 2006 and 2014 on ground and set-based chicken and turkey demonstrated that the type of product, as well as the kind of poultry, differs in terms of *Salmonella* positivity rate. Chicken products, whether ground or set-based, are more likely to contain *Salmonella* than turkey, while ground meats of either species are more likely to be contaminated (Figure 1). Again, chicken products are more likely to be *Salmonella* positive (Figure 2) but mechanically separated chicken was more likely to be positive than ground chicken or turkey (86.4% versus 26.7% respectively). FSIS Category Posting for *Salmonella* verification sampling is available online at: https://www.fsis.usda.gov/wps/portal/fsis/topics/data-collection-and-reports/microbiology/salmonella-verification-testing-program/salmonella-verification-testing-program. These data must be interpreted cautiously, as 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 the ground turkey positive rate ranged from 192 samples collected in the 1st quarter with 15.1% positive to 0 samples collected in the 3rd and 4th quarter. 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 conclusions. Sarlin et al. (Sarlin et al. 1998) reported that swabs, typically used for sampling turkeys, were less effective ($P < 0.05$) for *Salmonella* detection than either skin or carcass *rinse* samples typically used to sample chickens.

885 Figure 1. FSIS *Salmonella* Sampling Results: Poultry Carcasses and Ground Poultry, 2006-
886 2014.



887

888 Figure 2. FSIS *Salmonella* Sampling Results: Comparison of *Salmonella* Positive Rates in
889 Chicken and Turkey by Sampling Project.



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

ANSWER

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 of 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 (Heyndrickx et al. 2002). Volkova et al. (Volkova et al. 2009) showed that the best predictors of post-chill broiler carcass contamination (positive or negative) with *Salmonella*, was the frequency of litter contamination on day one and the day of harvest. In another study, however, flock-level concentration of *Salmonella* was associated with concentration of *Salmonella* on pre- and post-chill carcasses (Berghaus et al. 2013). Even if it was 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 post-harvest controls and conditions that might impact the level of *Salmonella* (Mead et al. 2010). 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 if all salmonellae should be considered or if 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, 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 multi-hurdle pathogen reduction approach to management of *Salmonella* is needed to reduce prevalence and presumably reduce illnesses, though data that definitively show this are limited (WHO 2002). 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 (Russell 2002) and Liljebjelke (Liljebjelke et al. 2005) 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 provide 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 (GAPs) at the farm, as mentioned in the response to Question 2.

b. What are key considerations/steps for an alternative processing scenario if the threshold level is 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 expected load of *Salmonella* in incoming birds. Use of historical data might 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 if process controls should be reassessed and validated to address predicted risks.

4.4. 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?

ANSWER

As 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 since loss of control at any single step can negate the others. To best assess this, 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. Carcass mapping 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 (USDA-FSIS 2015c).

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.*, pre-requisite 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) data
- Carcass mapping
- Finished product testing
- Environmental monitoring data
- Sanitation effectiveness monitoring data
- 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 multi-hurdle approach for reduction of *Salmonella* during harvest are the initial bacterial load on birds at live receiving and minimizing 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 hanging area (Bilgili 2004).

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 (White et al. 1997; Hahn 2014). Recommendations for transport considerations are contained in the FSIS

DRAFT FSIS Compliance Guideline for Controlling Salmonella *and* Campylobacter *in Raw Poultry*.

Salmonella-contaminated neck skins have also been linked to fecally soiled cages (Heyndrickx et al. 2002), so it is recommended that transport crates and trucks be rinsed and soaked, washed and sanitized (Mead et al. 1994; Corry et al. 2002). 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 a soak tank with brushes (Allen et al. 2008). 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 an EPA registered product, following labeled directions. Effective sanitation with chemicals was shown to result in a 3- to 5-$\log_{10}$ reduction of aerobic plate count (APC), *Enterobacteriaceae*, and *Campylobacter*, and effective use of sanitation can also reduce levels of *Salmonella* (Allen et al. 2008). Cages should be allowed to completely dry between uses; a time period of up to 48 hours has been suggested as beneficial (Berrang and Northcutt 2005).

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 (Harbaugh et al. 2006).

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. 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. (Slavik et al. 1995) found that scalding at 60°C was significantly more effective than lower scald temperatures; the higher temperature achieved a reduction of *Salmonella* counts by 0.3-0.5 log more 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 (Buhr et al. 2014). 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 scalder; however, they should be maintained to prevent additional cross contamination (Pacholewicz et al. 2016).

Defeathering

Feather-picking or plucking machines are equipped with rubber "fingers" that help remove feathers from the carcass. Plucker fingers are regularly contaminated by 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 (Bolder 2007). 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 (Valnegri et al. 2010).

Evisceration

Evisceration and dressing is a critical stage 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 (NACMCF 1997; FAO/WHO 2009; USDA-FSIS 2015b). 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* (FAO/WHO 2009).

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 (Bilgili et al. 2002; Russell 2005). 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 (Wideman et al. 2016). While age of 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 (Yang et al. 2001). Controlling flow rate, flow direction, and cleanliness of the chiller will mitigate much of the organic material that builds up with use (Russell 2009). 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 (Mead et al. 2000). Overall, chilling to 4ºC or lower will inhibit *Salmonella* growth.

Interventions

Several chemical interventions can be applied to poultry products during processing and further processing. Options include chlorine compounds, cetylpyridinium chloride, ozonated water, peroxyacetic acid (PAA) and other organic acids, and trisodium phosphate, among other compounds approved for use. Some processing aids are more effective for specific applications (eg, 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 antimicrobial may provide more surface area contact than spray application, especially in further processing. If used during pre-chill, some interventions may require a rinse step in order to prevent residual processing aid from negatively impacting the pH of the chill water (Buncic and Sofos 2012). A listing of FSIS-approved chemicals for use in meat, poultry and processed egg products is available (USDA-FSIS 2017a).

There are also non-chemical interventions, such as high pressure pasteurization, that can effectively address *Salmonella* contamination (Silva and Gibbs 2012). 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 (Bernard 2012).

Sanitation

Slaughterhouse establishments should also consider the sanitation at their facility, including equipment design, sanitary, and hygienic conditions. Maintaining sanitation during operations and thorough cleaning and sanitation of product contact surfaces at least once daily is critical to addressing opportunities for cross-contamination with *Salmonella*. Non-chemical options may include the use of steam and ultrasound to disinfect surfaces, providing those surfaces do not have high levels of debris (Musavian et al. 2015). Product build up, such as fat and tissue, prevent both chemical and non-chemical 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 sanitation 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 (Yiannas 2008).

Other

Other measures necessary for the control of *Salmonella* at establishments include control of humidity, aerosols and condensation, positive appropriate air flow, control of cross-

contamination, and pH. These infrastructural 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 to relate to the hurdles they have in place and how they handle variability in data.

These measurements could be qualitative, such as fecal contamination or processing defect, or they can be quantitative such as sanitizer concentrations, pH, or temperature of scald and chill water. FSIS in 9CFR 381.94(a)(2)(iii)(A) requires at a minimum that samples be collected pre-chill and post-chill at a frequency of once per 22,000 birds and be tested for indicator organisms. Detailed information on sampling protocol design is recommended by USDA-FSIS (USDA-FSIS 2015a).

4.5. 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?

ANSWER

Molecular based 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 allow for detection of low levels in approximately 24 hours. Recently, developments in semi-quantitative methods have demonstrated that threshold results might be achieved in as few as 8 hours. In addition, the movement from traditional serotyping to genetic based testing should allow rapid determination of serotypes that have a great public health impact. While nucleic acid based 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. draws a similar conclusion (Park et al. 2014).

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 acid based method (USDA-FSIS 2017c). 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 in the culture method requires approximately 3 days, while the nucleic acid based test only requires only 24 to 30 hrs.

There currently is not a USDA method for enumeration of *Salmonella* in carcass rinses. Use of most probable number (MPN) techniques and direct plating on selective agar are the most

common methods for enumeration of *Salmonella* in poultry samples (Brichta-Harhay et al. 2008). 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. Polymerase chain reaction (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 nucleic acid based rapid methods that have gone through a recognized validation program. FSIS publishes and routinely updates a list of validated methods (USDA-FSIS 2017b). 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 included technologies to concentrate target cells through strategies such as magnetic capture and filtration (Mandal et al. 2011).

Real-time PCR methods have been shown to be able to quantify the level of the target pathogens in a sample (Malorny et al. 2008; Oscar 2014). These methods are more rapid than conventional methods, but also may require more technical expertise and relatively expensive equipment (Park et al. 2014). Another more rapid approach to enumeration includes the MPN dilution plan and the use of a PCR assay for detection (Malorny et al. 2008).

If risk assessment data are available to show a threshold level that can help to protect public health, semi-quantitative methods may play an important role in setting performance standards. Semi-quantitative methods have been developed to allow for the rapid determination of levels that are above a selected threshold (Wales et al. 2006). A study by Chaney et al. (Chaney 2015) showed that inoculated levels of *Salmonella* in ground turkey above 1 cfu/g could be detected within 8 hours. 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-Le Minor scheme based on the O and H antigens. The method takes about 3 days to complete. Alternative molecular based serotyping is also available (Guard et al. 2012; Pulido-Landínez et al. 2013). While 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 (WGS) has been able to provide even greater level of specificity for differentiating strains. The access to rapid, low cost methods to get WGS data has opened an opportunity to potentially replace traditional serotyping methods (Allard et al. 2012). The use of more detailed genetic testing methodology provides significantly more information than traditional serotyping and in a much shorter time (Ranieri et al. 2013).

To determine if new methods can achieve the desired result, validation of the method is required. There are recognized procedures for the validation of microbiological methods (Feldsine et al. 2002) (AOAC, 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 and FDA or recognized organizations such as AOAC International (https://www.aoac.org/), AFNOR (http://www.afnor.org/en/), and ISO (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.

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?

ANSWER

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: (1) establishing a threshold *Salmonella* level requires further studies and (2) rapid microbiological testing methods that would allow evaluation of *Salmonella* levels and prevalence 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/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 a statistical process control (SPC) monitoring via microbiological testing (NACMCF 2015) 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 (on finished product) (USDA-FSIS 2015b), which may maximize the frequency of *Salmonella*-negative finished product. In this context, SPC 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. SPC 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 (USDA-FSIS 2015b).

If 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 if the test results obtained through their sampling are above the process limits they have 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 (USDA-FSIS 2015b) 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* (USDA-FSIS 2015b).

Note: Scheduled slaughter and processing and ongoing verification testing programs are not a substitute for pre- and post-harvest interventions to control *Salmonella*. While 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, as in-coming birds or poultry products, provides useful information about microbiological quality, safety, sanitation, and the effectiveness and extent of process control. While 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 (NACMCF 2015).

When a microbiological sampling program is properly designed and implemented, it can provide valuable 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 (USDA-FSIS 2015b). There are a number of factors that need to be considered when designing a sampling plan at the farm level and also 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*/indicators of process control)
- Sampling units (*e.g.*, flocks/houses at pre-harvest; carcasses/parts at (post-harvest)
- Sampling scheme (*e.g.*, random, systematic, cluster)
- Microbiologically independent lotting practices
- Sampling locations (*e.g.*, flocks/houses at pre-harvest; post chill/packaged product at post-harvest) where samples will be collected
- Sample collection procedures
- Pre-harvest: boot swabs, drag swabs, litter samples, cloacal swabs
- Post-harvest: product (*e.g.*,: post-chill carcass, parts, ground product etc.)
- Procedures for ensuring sample integrity
- Microbiological testing method for sample analysis (*e.g.*, qualitative, semi-quantitative, quantitative)
- Microbiological laboratory performing the analysis
- Method for evaluating test results (*e.g.*, p-chart, incident chart, x-bar charts)
- Actions taken based on the test results

In a previous report by NACMCF (NACMCF 2015), Appendices B through H, K and L detail various methods available for charting test results and identifying exceptions suspect for assignable causes. For results that are binary (*e.g.*, positive/negative) with very low frequency of positives, 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, average and range charts can be used.

4.6. 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, which could be addressed or implemented to achieve the highest rate of reduction of *Salmonella* with regard to both foodborne illnesses and on product?

ANSWER

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 GMPs 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 pre-operation environmental monitoring programs that include assessment of appropriate indicator organisms.
- Implementating 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. For example:
    - Biocontrols (e.g., bacteriophage or plant-based antimicrobial products)
    - Novel chemicals and application methods
    - Irradiation and other 'cold sterilization' approaches
- Applying GMP cold chain management.
- Developing appropriate microbial-verification sampling schemes and then use of data to monitor and improve process control.
- FSIS should work with the industry to focus establishment improvements on the most frequently reported Noncompliance Records (NRs; Figures 3, 4 and 5) that are related to public health.
- Publishing individual establishment performance standard category status.

Figure 3. FSIS Poultry Non-Compliance Records Represented Proportionally by cited regulation(s), 2015-2016.

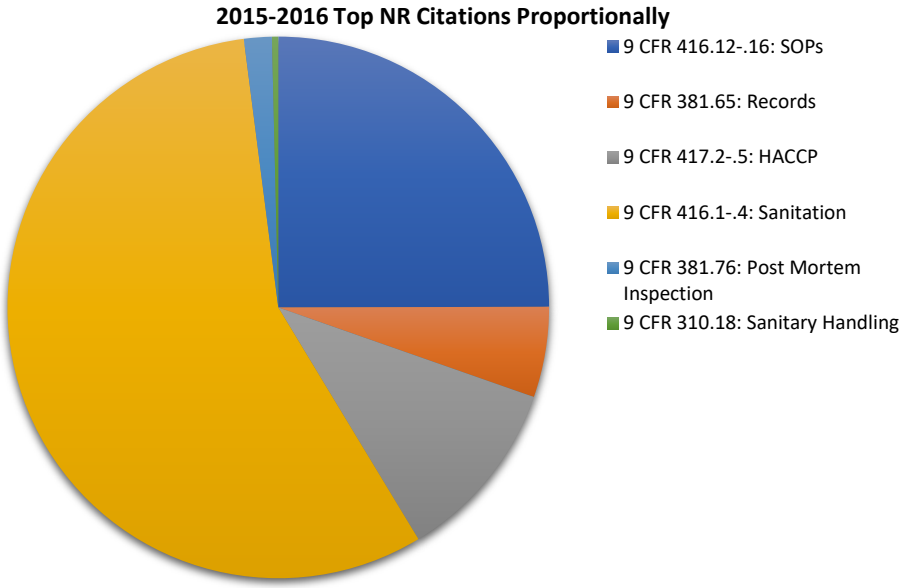


Figure 4. Proportion of Poultry Non-Compliances citing regulations of particular public health concern ("PHR regs"), 2015-2016 FSIS non-compliance Data.

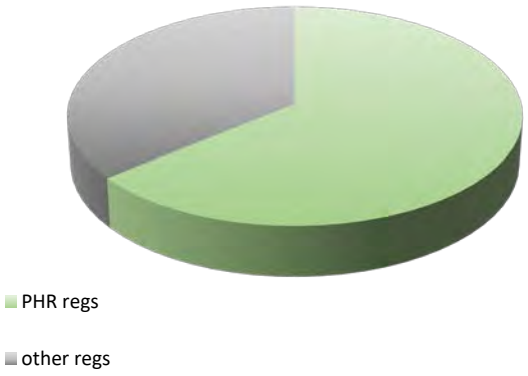
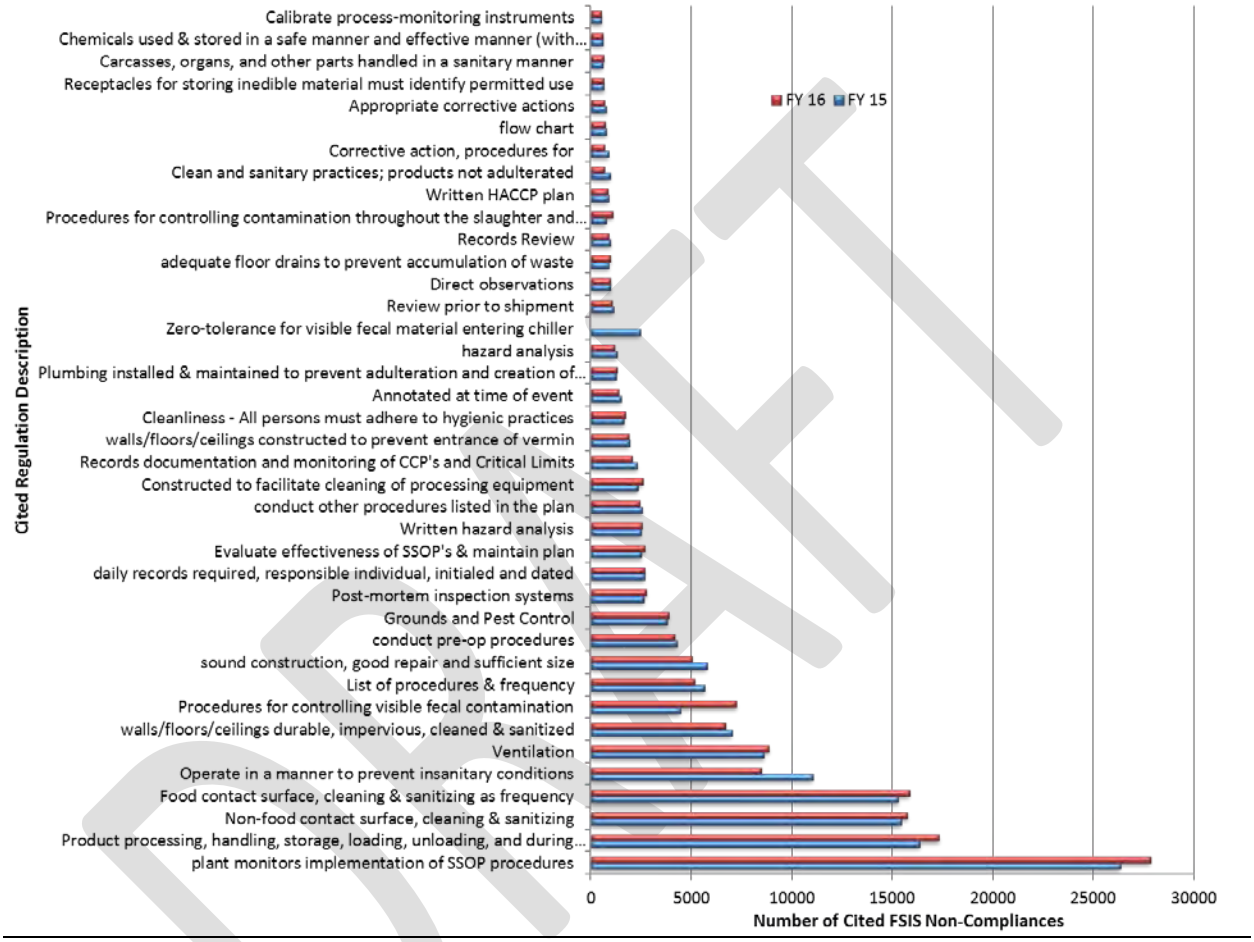


Figure 5. Detailed view of non-compliance records, FSIS 2015-2016; no 2016 data for "Zero-tolerance for visible fecal material entering chiller"; Federal Register Docket No: 2014-18526, Page 49,634: 381.65(f) replaced 381.65(e) for controlling visible fecal contamination.



Answer 2. Due to differences in allowed in-plant slaughter interventions, scale of operations, and live-bird house design, producers in the European Union 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 prevention or elimination of colonization with *Salmonella*, should be an effective control to reduce *Salmonella* in finished product and contribute to improvements in public health. The Committee recommends:

- USDA and industry work with sources of breeders to establish a *Salmonella* control program in parent flocks. This should be targeted to those serotypes of greatest public health burden (*S.* Enteritidis, Typhimurium, and Heidelberg).
- Identify genetic lines with increased resistance to infection and colonization.
- Implement effective pre-slaughter controls within contract growers.
    - Continue to innovate and develop new pre-slaughter interventions and management strategies with increased effectiveness (including house design, litter management, and products applied to birds).
- Develop farm-level surveillance (e.g., of the environment or birds) to aid detection and control of serotypes of public-health consequence.
- Evaluate live haul (i.e., catching, loading in crates, transportation on trucks, and unloading) and develop 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 on which to target control, focus should be on those serotypes that cause the greatest public-health concern (*S.* Enteritidis, Typhimurium, and Heidelberg).
- As whole genome sequencing libraries increase in size, and as they 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 and 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 (poultry, packaging, testing, etc.), 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/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 innovation design to improve packaging (materials, systems to minimize cross-contamination, etc.) and equipment (*e.g.*, cooking equipment that allows improved process control).
- Understand post-packaging contamination of the packaging material.
- Research to fill data gaps of cross-contamination in display cases and delis.

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