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General Interest

Response to Questions Posed by the Food Safety and Inspection Service: Enhancing *Salmonella* Control in Poultry Products

The National Advisory Committee on Microbiological Criteria in Foods (NACMCF) [1]

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Executive summary

Chicken and turkey are major sources of animal protein in the United States. Per capita chicken consumption increased from 77.4 lb. in 2000 to a projected 99.6 lb. in 2023, with processed and cut-up chicken parts comprising the majority of purchases, whereas beef has declined from 67.5 lb. to an estimated 55.8 lb. during the same period (National Chicken Council, 2022). Of the estimated over 1 million cases of foodborne salmonellosis acquired annually in the U.S. (Scallan, Hoekstra, et al., 2011), over 24% are attributed to consumption of chicken and turkey products (Fig. 1) (Interagency Food Safety Analytics Collaboration (IFSAC), 2022; Painter et al., 2013). Although *Salmonella* is killed by adequate cooking, undercooking (e.g., in raw breaded or stuffed chicken products), and cross-contamination with other ready-to-eat foods in the refrigerator or during preparation are contributing factors to transmission of this pathogen. Therefore, FSIS instituted *Salmonella* performance standards for raw poultry carcasses, chicken parts, and raw-comminuted poultry products. Since their implementation, data revealed a steady decline in *Salmonella* prevalence. For example, the prevalence on chicken parts (legs, breast, and wings) decreased 75% over an 8-year period from 2012 to 2020 (Williams et al., 2022). Furthermore, *Salmonella* Typhimurium and Heidelberg infections associated with poultry have declined in the past two decades, likely due to the use of group B *Salmonella* poultry vaccines on broiler farms and other interventions. Notwithstanding this reduction of *Salmonella* prevalence in poultry products, there has not been a concomitant reduction in overall *Salmonella* illnesses (Fig. 2) (Centers for Disease Control and Prevention (CDC), 2021) nor in the proportion attributed to poultry from outbreak data (Fig. 1) (Interagency Food Safety Analytics Collaboration (IFSAC), 2022). Meanwhile, the proportion of illnesses associated with serotypes Enteritidis and Infantis have increased (Williams et al., 2022).

NACMCF reviewed the scientific evidence on *Salmonella* control in the U.S. and abroad, foodborne illness surveillance data, quantitative microbial risk assessments, and microbiological testing of indicator organisms vs. *Salmonella* on poultry throughout the farm-to-fork continuum. Based on this information, this document seeks to provide guidance to FSIS and the poultry industry on what types of microbiological criteria might be used to identify and incentivize effective intervention strategies pre- and postharvest to reduce *Salmonella* in poultry products and thereby prevent human *Salmonella* infections associated with these products.

The infectious dose of *Salmonella* varies widely between serotypes (Teunis et al., 2010). Recent data suggest that most poultry-associated outbreaks in the U.S. involve Enteritidis, Typhimurium, I:4,5,12:i:-, Infantis and Heidelberg, and even fewer serogroups: groups O:4 (formerly group B), O:7 (group C1), and O:9 (group D1). In live poultry, serovars Infantis and Typhimurium are predominant in the Atlantic region, whereas a higher proportion of serovar Schwarzengrund are found in the Southeast, with the exception of the state of Georgia, which has a higher proportion of Kentucky. Vaccination against specific serotypes, such as S. Typhimurium, are common among U.S. broiler breeders, a strategy which has reduced the incidence of contamination of that serotype (Mountainspring & Burleson, 2018). However, vaccine development takes years and lags behind the shifting of predominant serotypes found in flocks. Other U.S. preharvest management practices include competitive exclusion, controlling the quality of feed, biosecurity, moisture control in poultry

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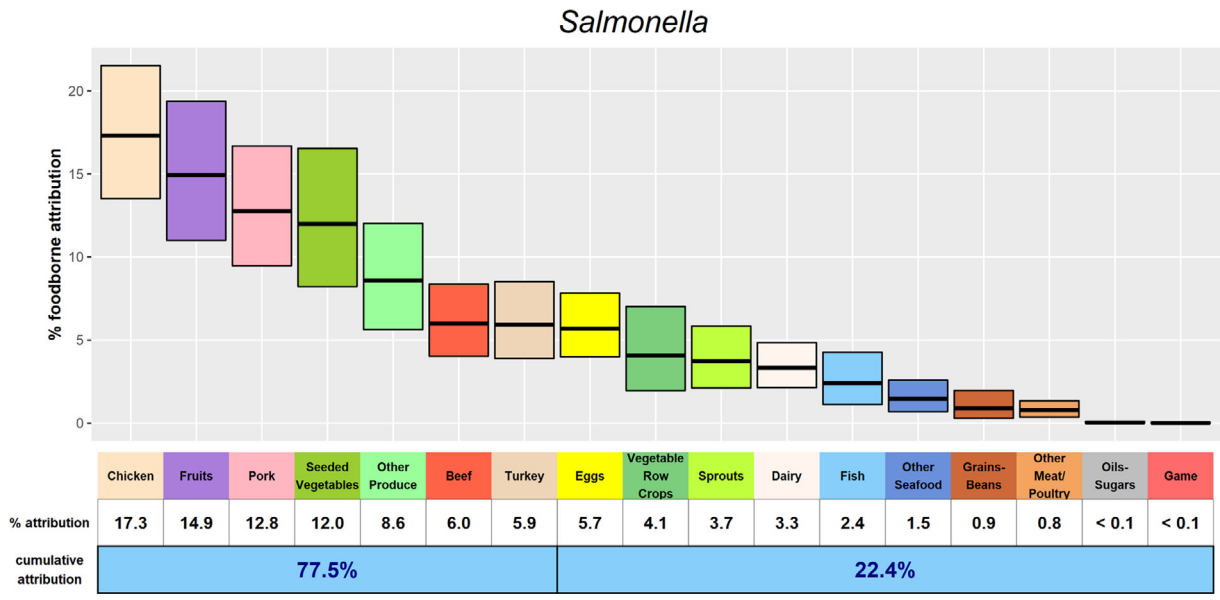

Figure 1. Estimated percentage of foodborne Salmonella illnesses (with 90% credibility intervals) for 2020, in descending order, attributed to each of 17 food categories, based on multiyear outbreak data,[a] United States. (Interagency Food Safety Analytics Collaboration (IFSAC), 2022). [a]Based on a model using outbreak data that gives equal weight to each of the most recent five years of data (2016-2020) and exponentially less weight to each earlier year (1998-2015). Over 75% of illnesses were attributed to seven food categories: Chicken, Fruits, Pork, Seeded Vegetables (such as tomatoes), Other Produce (such as fungi, herbs, nuts, and root vegetables), Beef, and Turkey. The credibility intervals for each of the seven food categories that account for 77.5% of all illnesses overlap with some of the others.

houses, and clean transport coops. Microbial testing (qualitative testing) for total *Salmonella* at breeder or broiler farms can be focused on environmental samples (boot or drag swabs) and cecal testing (Bourassa, 2016); however, sufficiently sensitive tests and specific serotype testing are needed to determine if any changes are required to control *Salmonella* serovars that are most often associated with human illness. One suggestion is that, based on results from microbial testing at farms, highly contaminated birds can be targeted for logistic slaughter (i.e., scheduling their slaughter after less contaminated flocks) or other interventions. However, the value of this approach for scheduling purposes has not yet been realized (Nauta et al., 2009; Rasschaert et al., 2008).

As noted above, the relative number of infections in the U.S. caused by Typhimurium and Heidelberg has declined during the past 20 years (Centers for Disease Control and Prevention (CDC), 2021). This decrease corresponds to the use of a commercial poultry vaccine against *S*. Typhimurium, which may also deliver cross-protection against Heidelberg (Muniz et al., 2017), targeted environmental testing to develop strategic management programs, as well as implementing other intervention strategies. While progress has been made against these two serotypes, overall cases of salmonellosis attributed to poultry remain unchanged with Enteritidis, Typhimurium, Infantis, and I:4,5,12:i:- accounting for 83% of the chicken-associated illnesses. These data suggest that alternate methods for control of *Salmonella* or detection that do not rely solely on serotype may be needed. While vaccines are effective serotype-specific intervention strategies, other methods need development to reduce carriage of *Salmonella* in the bird. Furthermore, attribution data does not specifically identify whether sources of *Salmonella* were whole carcasses, parts, comminuted product, or breaded raw poultry products. More granular data will help determine if all poultry products pose the same risk and allow a targeted management program.

Indicator organisms such as Enterobacteriaceae (Eb) or aerobic plate counts (APCs) have been used by the industry as gauges of process control and to measure microbial reduction on carcasses from slaughter to postchill (Williams et al., 2015). However, some studies have shown that populations of these indicators are not directly correlated to populations of *Salmonella* (Bueno López et al., 2022; De Villena et al., 2022; Sanchez-Plata, 2022). Because of the conflicting and apparent weak correlation between indicators and either the presence or level of *Salmonella* postcarcass wash (Williams et al., 2015; Williams et al., 2017), one approach is to base microbiological criteria on *Salmonella* enumeration. Microbial risk assessments suggest that diverting ground turkey product which tests above a set threshold of *Salmonella* colony forming units (CFU) per gram, compared to current protocols (i.e. not diverting), is expected to remove product from the market that has higher chances of causing infection and may therefore reduce illness (Lambertini et al., 2021). Such a threshold would need to be clearly linked to health-based targets. This concept is currently used by the industry whereby poultry used in breaded and stuffed raw chicken product are enumerated for *Salmonella* by quantitative PCR (qPCR), not by targeting specific serotypes (USDA Food Safety and Inspection Service (FSIS), 2022i). qPCR presents logistical advantages over MPN (most probable number) methods due to its relatively rapid time to detection and, is therefore, more actionable (such as diverting product to high-pressure pasteurization or cooking) during a production day. However, qPCR methods must have high sensitivity to consistently detect *Salmonella* levels at the ranges most often seen in poultry products.

In October 2022, the USDA-FSIS announced that they will be proposing a revised regulatory framework to reduce *Salmonella* infections associated with poultry products (USDA Food Safety and Inspection Service (FSIS), 2022g). The key components include requiring that incoming flocks be tested for *Salmonella* before entering an establishment, that establishments enhance process control monitoring, and that the agency implements an enforceable final product standard.

Current performance standards include all *Salmonella* serotypes, rather than quantification of specific highly pathogenic serotypes. An approach targeting highly pathogenic serotypes could trigger additional mitigating actions and could be more effective in diverting products that have higher infectious potential. The public health benefits of this approach should be evaluated using the quantitative risk assessment approach that incorporates multiple data sources, including data

collected by industry, if available. Independently from the type of MC (microbiological criteria), ensuring that mitigating actions triggered by a MC are implemented is key to its effectiveness.

The Committee's responses to the charge questions are based on the information available at the time of writing. The Committee identified a multitude of data gaps that could affect findings and recommendations to FSIS, including the need for completion of the two quantitative risk assessments for chicken and turkey that are currently in progress (USDA Food Safety and Inspection Service (FSIS), 2022c). These risk assessments will evaluate the public health impact (change in illnesses, hospitalizations, and deaths) achieved by eliminating a proportion of poultry or poultry products that are contaminated with specific levels of Salmonella and/or specific Salmonella subtypes. The risk assessments will also explore the public health impacts of various monitoring and enforcing process control methods, from the rehang to postchill stages. Finally, the risk assessments will address the public health impact of implementing combinations of the aforementioned risk management strategies.

Recommendations:

1. Collect appropriate data to refine food attribution models and determine which form(s) of raw poultry exposure (e.g., consuming processed, parts, whole carcasses, handling live poultry, exposure to poultry manure, etc.) and food handler practices contribute most to salmonellosis associated with chicken and turkey.
2. Expand systematic sampling for *Salmonella* levels, prevalence, and serotypes on poultry preharvest (hatcheries, feed, poultry houses) and FSIS postharvest sampling (slaughter through processing). Prioritize product lines that historically are more frequently contaminated, which are not further processed using a validated lethality step and have been linked to illness such as comminuted poultry products, chicken parts/pieces, and breaded stuffed raw chicken products, to identify *Salmonella* levels and the evolution of predominant serotypes.
3. Incentivize industry to deposit data (anonymized, nonpunitive) on levels of indicator organisms and *Salmonella* prevalence, concentration, and serotypes found at various stages of processing (preharvest through final product) along with practices that may mitigate contamination.
   a) Analyze data to identify alternate process control indicators and to use in risk assessments to update performance standards.
   b) Analyze data to determine how non-*Salmonella* quality indicator sampling (perhaps other than APC) could be established that would provide guidance to companies on how to identify houses that have a higher probability of *Salmonella* contamination and how to modify mitigation strategies accordingly.
4. Frequently (e.g., every 2–3 years) compare the serotypes that are isolated from patients with those isolated from poultry products to determine if intervention strategies used by the industry are effective against all *Salmonella* equally or are selecting for specific serotypes.
5. Develop and validate quantitative testing methods to determine if and how testing and processing adaptations can reduce the likelihood that carcasses and parts with higher levels of *Salmonella* (or serotypes) that are most capable of causing illness are released into commerce.
6. Complete risk assessments for chicken and turkey to assess public health impacts of different risk-based *Salmonella* control strategies, including qualitative and quantitative performance standards, possibly complemented by serotype identification.
7. Upon completion of the risk assessments, consider developing changes to performance standards based on the findings of the risk assessments.
8. Incentivize the industry to develop, validate, and universally implement robust *Salmonella* mitigation programs and qualitative *Salmonella* testing at the breeder, hatchery, grow out, and transport levels. Target conditions in houses, transport crates, and holding areas that harbor and transmit *Salmonella* by universal implementation of known and validated mitigation strategies.
9. Due to extensive data gaps identified by the Committee, the agency should reevaluate this document within 3–5 years after appropriate data have been collected and risk assessments are complete.

Charge from USDA-FSIS to NACMCF

According to the Centers for Disease Control and Prevention (CDC), Salmonella is responsible for approximately 1.35 million cases of foodborne illness each year in the United States. The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) has established qualitative performance standards to limit the occurrence of Salmonella in poultry products (i.e., carcasses, parts, and comminuted products). The goal of these performance standards is to allow FSIS to verify each regulated establishment's control for this pathogen in raw products throughout the slaughter process, and, by meeting these standards, achieve national food safety goals. Over the past 25 years, there have been significant reductions in the proportion of poultry contaminated with Salmonella, but no meaningful reduction in human Salmonella infections attributed to poultry products (Williams et al., 2020). Therefore, FSIS is seeking guidance on how to improve the current system for reducing Salmonella in poultry to better protect public health.

In 2019, the National Advisory Committee on Microbiological Criteria for Food (NACMCF) recommended that FSIS move toward a risk-based disposition of finished raw poultry product, informed by Salmonella levels and serotype (National Advisory Committee on Microbiological Criteria for Foods (NACMCF), 2019). Since then, several studies suggest that setting microbiological criteria (e.g., performance standards) to limit the amount of Salmonella in products and/or to address serotypes more frequently associated with foodborne illness would better protect public health than the current approach. In addition, several studies have demonstrated the feasibility of developing quantitative microbiological criteria based on a change in the concentration of indicator organisms correlated to Salmonella occurrence. These findings, along with new technologies and advances in rapid quantification of pathogens in products, present opportunities for FSIS to enhance the microbiological criteria it establishes to measure industry control of Salmonella in poultry products. FSIS seeks input from the NACMCF on the best options for using quantification and/or particular pathogen characteristics, along with a relevant pathogen indicator, to enhance its microbiological criteria and reduce Salmonella illnesses attributed to poultry products consumed in the U.S.

Background

Salmonella bacteria are a leading cause of foodborne illness. According to CDC estimates, *Salmonella* is responsible for approximately 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths every year in the United States. Using weighted outbreak data from 1998 to 2018, the Interagency Food Safety Analytics Collaboration, a joint effort between CDC, the U.S. Food and Drug Administration, and USDA-FSIS, estimates that over 20% of foodborne salmonellosis are attributed to poultry products (Interagency Food Safety Analytics Collaboration (IFSAC), 2022).

FSIS established limits, referred to as performance standards, on the occurrence of *Salmonella* in poultry products as part of the Pathogen Reduction, Hazard Analysis and Critical Control Point

(PR/HACCP) Systems Final Rule. These standards were designed to improve food safety, reduce the risk of foodborne illness, and enable FSIS to verify process control. Process control is a defined procedure or set of procedures designed by an establishment to provide control of those operating conditions that are necessary for the production of safe, wholesome food. The procedures typically include some means of observing or measuring system performance, analyzing the results generated in order to define a set of control criteria, and taking action when necessary to ensure that the system continues to perform within the control criteria. FSIS has since updated those original performance standards. FSIS relies on qualitative (presence/absence) pathogen sampling data to apply performance standards for *Salmonella* in poultry products. These standards are based on quantitative microbiological risk assessments (Ebel et al., 2012; Williams et al., 2011) and are designed to achieve the national food safety goals. The Healthy People 2020 food safety goal was set at a 25% reduction in foodborne *Salmonella* illnesses, to achieve fewer than 11.5 *Salmonella* infections per 100,000 population per year. In 2020, the case rate for *Salmonella* infection was 13.3 per 100,000 population (Ray et al., 2021). FSIS has proposed performance standards for beef products and intends to propose performance standards for pork products.

Since the implementation of *Salmonella* performance standards for poultry, FSIS has measured a substantively lower occurrence of this pathogen in raw poultry products, but the incidence of human illness associated with the consumption of poultry products has not decreased (Williams et al., 2022). FSIS is interested in developing microbiological criteria (i.e., an alternative type of performance standard(s)) that will result in a substantively reduced level of human illness from *Salmonella* in poultry. FSIS is considering microbiological criteria such as the levels of *Salmonella* in products, and the presence of *Salmonella* serotypes more frequently associated with human illness, rather than the presence/absence of any *Salmonella*. Criteria could be set at various points in the food safety system to better assess industry control over *Salmonella* in these products, for example, prior to establishment interventions and after establishment interventions, to evaluate the effectiveness of an establishment's food safety system in mitigating *Salmonella* in products during the slaughter process.

Several recent studies showed a correlation between indicator organisms and *Salmonella* in poultry. Specifically, a correlation was shown between a change in the quantity of an indicator (i.e., Aerobic Plate Count, APC) from the carcass to finished product and the occurrence of *Salmonella* in beef, pork, and poultry (Williams et al., 2015; Williams et al., 2017). However, the university research on biomapping and the poultry industry's internal testing has evaluated the use of indicator bacteria and found that the populations of indicators offer little to no predictive value for the presence of non-Typhoidal *Salmonella*. Regardless, based on FSIS findings, FSIS is considering developing a quantitative "log-reduction" microbiological criterion (e.g., performance standard) to measure the effectiveness of an establishment's food safety system in controlling *Salmonella* in products. FSIS believes that this type of performance standard would allow for continued monitoring of industry performance in achieving the Healthy People 2030 national food safety goals, thereby improving public health outcomes, while providing better insights into pathogen control throughout the food safety system. As part of this consideration, FSIS would also like to know how the Agency could address *Salmonella* serotypes more frequently associated with human illness, strain characteristics (e.g., virulence factors), and/or the quantity of *Salmonella* in a subset of products tested, prior to and after interventions, when evaluating the industry's control of *Salmonella*.

FSIS is considering the following microbiological criteria to assess industry control:

- The presence of any *Salmonella* or only specific serotypes more likely to cause illness, at preharvest (e.g., as measured at the receiving step in the slaughter process)
- The amount of *Salmonella* and/or presence of serotypes more likely to cause illness, throughout the slaughter process; and
- A relevant indicator for *Salmonella*, throughout the slaughter process

Charge questions

FSIS is seeking guidance on the overarching risk management question: What types of microbiological criteria (e.g., *Salmonella* performance standards) might FSIS use to encourage reductions in *Salmonella* in poultry products so that they are more effective in preventing human *Salmonella* infections associated with these products?

Specific risk management questions posed to NACMCF are as follows:

1. Can we assess the public health impact (e.g., reduction in salmonellosis) of controlling specific *Salmonella* serotypes and/or amount (levels) in poultry products? What types of approaches could be used?
2. What types of microbiological criteria could be established to encourage control of *Salmonella* at preharvest (i.e., in live birds on-farm)?
   Should FSIS consider qualitative microbiological criteria for control of the presence of *Salmonella* in a flock when they are presented for slaughter?
   a) How could FSIS use these criteria to address *Salmonella* serotypes most frequently associated with human illness?
   b) What industry data would provide evidence of control?
3. What types of microbiological criteria could be established for poultry carcasses, parts, and comminuted products prior to applying interventions and after interventions, considering current technology?
   a) Could the quantity of *Salmonella* or quantity of microbiological indicator organisms (e.g., APC) be used? What are the key parameters that need to be considered? What data analysis techniques could be used? How would these criteria be linked to human illness?
   b) How could serotypes frequently associated with human illness be considered in the development of microbiological criteria?
4. How might foodborne illness surveillance data on human *Salmonella* illnesses, data from foodborne outbreaks associated with *Salmonella* in poultry, and data on *Salmonella* serotypes in poultry products be used to identify the *Salmonella* serotypes of greatest public health concern associated with specific poultry products?
   a) Should only the most current data (e.g., 5 years) of foodborne illness surveillance, outbreak, and/or pathogen testing data be used?
   b) Going forward, what methodology and criteria would focus on those *Salmonella* serotypes most frequently associated with human illness and attributable to poultry products?
   c) How frequently should the priority *Salmonella* serotypes associated with poultry be revised considering changes in their occurrence while still ensuring continuity in industry and regulatory testing?
5. There is a documented correlation between a reduction in the quantity of APC between carcasses and finished products and the occurrence of *Salmonella* in finished products for beef, pork, and poultry. How might this information be used to set microbiological criteria to assess process (pathogen) control in poultry?
6. What rapid methods and technologies are available for the quantification of *Salmonella*? How should FSIS make the best use of these methods?
7. Are there particular approaches that would result in selective identification of the serotypes of public health concern? For example, are there approaches to mitigate a potential strain selection bias

introduced by the laboratory method? If needed, what type of research could be conducted to ensure performance characteristics of current laboratory methods (e.g., enrichment, incubation, prescreening) do not result in a biased serotype detection?
8. How should pathogen characteristics derived from whole genome sequencing (e.g., serotype, virulence, antimicrobial resistance) be considered in the development of microbiological criteria?
9. What research is needed to support FSIS' new *Salmonella* strategy in terms of setting microbiological criteria?

Committee responses

Approach by the Committee: The Committee organized the charge questions into three groups: (1) The impact of *Salmonella* and poultry on public health (Q1 and 4); (2) The role of microbial testing at the preharvest and postharvest levels for process control (Q2, 3, and 5); and (3) Methodologies for detection and enumeration of *Salmonella* (Q6, 7, and 8). The working groups considered Q3-a and Q5 on the use of aerobic plate counts as indicators for *Salmonella* contamination similar and, thus, are merged for response. Data gaps for each individual charge question are summarized in response to Q9. The charge did not specifically request an evaluation of the efficacy of various intervention strategies on the farm or postharvest, but the Committee briefly discussed practices that may affect the presence, levels, or serotypes of *Salmonella* throughout the chain.

Q1. Assessing public health impact

Can we assess the public health impact (e.g., reduction in salmonellosis) of controlling specific Salmonella serotypes and/or amount (levels) in poultry products? What types of approaches could be used?

Summary of Question 1 Response Two perspectives are deemed necessary to answer this question: (A) how to ***predict*** the public health impact of hypothetical changes in *Salmonella* control strategies in poultry products ***prior to their implementation***, and (B) how to ***assess*** the effectiveness of the standards in reducing salmonellosis ***once they are implemented***.

Part A: Quantitative microbial risk assessment (QMRA) methods can be used to predict salmonellosis reductions resulting from changes in microbial criteria in poultry products. Incorporating and comparing prevalence, level, and subtype in QMRAs might require the incorporation of emerging Omics methodologies while acknowledging data quality/availability issues.

Part B: Several types of public health surveillance data are available with which to assess the efficacy of standards in reducing salmonellosis. Allowing for some year-to-year variability, case-based serotype- and genotype-based surveillance may reflect important changes within 2–5 years. The goal of reducing poultry-associated salmonellosis by 25% is an important step towards meeting the HHS Healthy People 2030 goal of reducing domestically acquired salmonellosis by 25% by 2030 (U.S. Department of Health and Human Services, 2022).

The objective of microbiological criteria (MC) (i.e., performance standards) is to establish control limits for the contamination of pathogens in foods or food production environments along the supply chain based on a specified measurement method and sampling plan, and ultimately reduce foodborne illnesses. The *Salmonella* Poultry Performance Standards currently employed by USDA FSIS for different poultry products follow a prevalence-based approach, regulating a fraction of maximum allowable positives over the large number of *Salmonella*-positive samples collected and analyzed in a specified time window (USDA Food Safety and Inspection Service (FSIS), 2019a). As an alternative to the current method, controlling specific *Salmonella* serotypes and/or amount (levels) in poultry and in poultry products may potentially further reduce exposure to *Salmonella* through poultry product consumption and therefore mitigate the public health risks. To effectively guide the changes in MC, the associated public health impact needs to be comprehensively evaluated before their implementation, which can be achieved using quantitative microbiological risk assessment (QMRA) approaches.

QMRA approaches have been applied to assess the potential impact of MC in other commodities and hazards in support of risk management decisions, in the U.S. and other countries, for example for *Campylobacter* in poultry (European Food Safety Authority Panel on Biological Hazards, 2011; Nauta et al., 2012; Swart et al., 2013) and *Salmonella* in pork and comminuted beef (USDA Food Safety and Inspection Service (FSIS), 2019b, 2022f). While this existing body of work does not fully account for the current U.S. situation and the range of possible MC to consider, it provides relevant approaches, tools, and application examples for how risk-based models can be used to support the establishment of level/serotype-oriented MC for poultry products. (European Food Safety Authority Panel on Biological Hazards, 2011; Nauta et al., 2012; Swart et al., 2013) and *Salmonella* in pork and comminuted beef (USDA Food Safety and Inspection Service (FSIS), 2019b, 2022f).

Evaluating the public health impact of a MC that controls for prevalence and amount of *Salmonella* on poultry products is feasible using traditional QMRA approaches. QMRAs use a "bottom-up" mechanistic approach to modeling the change in amount of *Salmonella* on poultry products through the production and processing stages relevant to the considered MC, possibly including primary production, slaughter, processing, distribution, through consumer handling and cooking until consumption. Variability and uncertainty in the parameters considered at this stage can be represented. As a result, QMRA models allow estimating the probability distribution of exposure dose (number of *Salmonella* cells ingested) as well as the associated distribution of risk (e.g.., illnesses, hospitalizations, deaths in a population) using a dose–response relationship, if a suitable one is available.

QMRAs have been conducted to align MC (i.e., an allowable level) for *Salmonella* prevalence and amount, with some consideration of serotypes of public health concern, in poultry products (postchill) to achieve a desired reduction in foodborne illnesses (e.g., percentage reduction in attributable illnesses) (Ebel and Williams, 2015; Ebel et al., 2012; Lambertini et al., 2019; Lambertini et al., 2021; Oscar, 2021a; Williams et al., 2015). None of the existing QMRAs that evaluate *Salmonella* MC for poultry products consider the public health impact of controlling all three factors discussed here (prevalence, level, and subtype). We discuss elements that should be incorporated into QMRAs to assess all three criteria. Accounting for differences in infectiousness and severity of *Salmonella* subtypes might require the incorporation of emerging omics methodologies while also acknowledging data quality and quantity issues. This will allow for subtype-level differentiation of infectivity (probability of infection, given exposure to the subtype) and virulence (probability of severe illness, given infection from subtype).

Measuring the operational success of MC requires incorporating different sources of existing public health surveillance available for salmonellosis and adjusting for seasonal and cyclical fluctuations to ensure that the efficacy measurement is robust and unbiased. Although roughly 91% of reported salmonellosis is sporadic (cannot be attributed to a recognized outbreak), successful control of *Salmonella* in poultry would be expected to reduce both outbreak-associated cases and sporadic cases (Ray et al., 2021). Current attribution of salmonellosis to different sources is primarily based on outbreak investigations. Emerging source attribution technologies based on Whole Genome Sequencing (WGS) might allow for better estimation of success of the MC in poultry as it would also allow for attribution of sporadic salmonellosis cases.

Q1.A Using quantitative microbial risk assessment (QMRA) methods to predict the public health impact of changes in Microbiological Criteria (MC) in poultry products.

The public health impact of controlling specific *Salmonella* serotypes and/or amount (levels) in poultry and poultry products can be estimated using QMRA. QMRA approaches have been applied to assess the potential impact of MC in other commodities and hazards in support of risk management decisions, in the U.S. and other countries, and can provide useful templates for additional assessments. Examples of recent QMRA studies relevant to the charge question are reviewed and discussed in this section.

MC can be classified into two main categories: (1) MC defined as a threshold (prevalence and/or concentration) of a target organism in the finished product (or other specified production stage), also called food safety criteria, and (2) as a threshold in a target variable associated with processing conditions conducive to the control of the target organism, also called process hygiene criteria. While QMRA can be used to assess the impact of both, most assessments have focused on food safety criteria.

MC can have an impact on public health only if they are associated with risk control actions that keep or bring a process back into control/compliance, and if such actions are enforced. Models used to estimate the impact of a MC must include these risk control actions. For example, in the case of a MC on finished product, a batch of product not meeting the MC may undergo additional validated processing that reduce contamination by a certain number of $\log_{10}$ CFU/g such Log reduction will be accounted for in the model (note: "log" is used in the remainder of the document to mean $\log_{10}$). Risk control actions involving the processing environment can also be modeled, although they are generally more complex to represent in detail. For example, an establishment not meeting a MC may undergo in-depth sanitization, validated within the establishment to bring the MC variable below the MC threshold, or to impart a known reduction. While most commonly product-level MC are paired with product-level risk control measures, and establishment-level MC are paired with establishment-level risk control measures, MC and measures can also be associated differently. For example, an establishment whose product does not meet a MC may undergo in-depth sanitization, possibly in addition to product treatment.

QMRAs have been conducted to support the development or assessment of MC for *Salmonella* prevalence and/or levels, with some consideration of serotypes of public health concern, in poultry products (postchill), including to align a MC with a desired target reduction in attributable illness (Ebel and Williams, 2015; Ebel et al., 2012; Lambertini et al., 2019, 2021; Oscar, 2021a; Williams et al., 2015, 2020) (Table 1).

MC and associated risk management measures can be set for different and multiple stages of the production and processing chain. Risk-based models can be developed to assess the impacts of such MC, by including the production steps downstream of the MC. Hence, to model MC upstream of the finished product, a higher number of variables, as well as their uncertainty and assumptions, would need to be included. Most published studies on MC in postharvest poultry processing have considered MC on the finished product.

QMRAs often treat all *Salmonella* subtypes or serovars equally, for lack of serovar-specific data, in particular dose–response relationships. That is, these models implicitly assume that all serovars have the same probability of infection following an exposure (infectivity) and same probability of a severe illness resulting from an infection (virulence). Infectivity is usually modeled with a dose–response function or curve (Haas et al., 2014), whereas virulence is often modeled as proportions of severe illnesses based on different disease severity outcomes, such as extraintestinal infections, hospitalizations, and/or deaths.

Ebel et al. (Ebel and Williams, 2015; Ebel et al., 2012) provide an approach for establishing a prevalence-reducing MC *Salmonella* in poultry products. This is based on a finding that at lower *Salmonella* prevalence, there is a linear dose–response relationship between prevalence in product and salmonellosis in humans. The advantage

Table 1
Risk assessments of the impact of microbiological criteria (MC) for Salmonella and poultry

Main topic	Country	Reference
Assess effectiveness of qualitative testing results sufficient in predicting a reduction in illnesses in a microbiological food safety risk assessment.	U.S.	(Ebel and Williams, 2015)
Risk-based modelling assessment of the effectiveness of revised performance standards for *Salmonella* contamination of comminuted poultry.	U.S.	(Ebel and Williams, 2020)
Simplified framework for predicting changes in public health from performance standards applied in slaughter establishments.	U.S.	(Ebel et al., 2012)
Risk-based modelling assessment in support of MC for *Salmonella* in poultry.	E.U.	(European Food Safety Authority (EFSA), 2011)
Link between *Salmonella* MC at different stages of the poultry chain.	E.U.	(European Food Safety Authority Panel on Biological Hazards, 2010)
Risk-based modelling assessment of public health effects of raw chicken parts and comminuted chicken and turkey performance standards. (*Salmonella* and *Campylobacter*).	U.S.	(USDA Food Safety and Inspection Service (FSIS), 2015b)
Risk-based modelling assessment of *Salmonella* and *Campylobacter* performance guidance for young chicken and turkeys.	U.S.	(USDA Food Safety and Inspection Service (FSIS), 2011)
Risk-based modelling assessment comparing different prevalence- and concentration-based MC for *Salmonella* in chicken parts (finished product).	U.S.	(Lambertini et al., 2021)
Risk-based modelling assessment of a specific quantitative MC in comminuted turkey (finished product).	U.S.	(Lambertini et al., 2021)
Factors affecting MC for *Salmonella* in raw poultry	U.S.	(Mead et al., 2010)
Assessing *Salmonella* prevalence as indicator of poultry food safety.	U.S.	(Oscar, 2021a)
Risk-based assessment of the effectiveness of performance standards based on APC for *Salmonella* contamination of chicken parts.	U.S.	(Williams et al., 2022)
Additional supporting work on MCs in poultry or *Salmonella* (examples)		
Includes risk-based modelling assessment of performance targets for *Campylobacter* in broiler meat; includes impact of different control options.	E.U.	(European Food Safety Authority Panel on Biological Hazards, 2011)
Risk assessment on performance standards for *Salmonella* in pork.	U.S.	(USDA Food Safety and Inspection Service (FSIS), 2022f)
Public health effects of performance standards for ground beef and beef manufacturing trimmings (*Salmonella*).	U.S.	(USDA Food Safety and Inspection Service (FSIS), 2019b)
Risk-based *Campylobacter* MC in poultry.	E.U.	(Nauta et al., 2012)
Comparison of two MC approaches: microbiological limit, and relative risk limit (*Campylobacter* in broilers).	general	(Nauta et al., 2015)
Risk-based *Campylobacter* MC in poultry.	NL	(Swart et al., 2013)
Assessing impacts of a MC based on indicator organisms, for *Salmonella* (and *E. coli* O157:H7) in beef carcasses.	U.S.	(Williams et al., 2017)

of this QMRA approach is that it simplifies the modeling by reducing the number of parameters and eliminates the uncertainty common to exposure pathways associated with consumer handling and preparation, cross-contamination, and *Salmonella* dose–response. The limitation of this approach is that it assumes the amount of *Salmonella* on contaminated products is independent of its presence, and it treats all serovars as equally infective and virulent.

Lambertini *et al.* (Lambertini et al., 2019, 2021) provided a modeling framework to evaluate the potential relative reduction in public health impact associated with prevalence-based and concentration-based MC for *Salmonella* in poultry products (comminuted turkey and chicken parts), tested at the end of processing. These QMRAs use a "bottom-up" approach to modeling the change in the amount of *Salmonella* on poultry products from the end of the production process, where sampling is assumed to occur, through consumer handling and consumption to estimate exposure. Corrective actions considered include simplified lot-level *Salmonella* reduction measures and establishment-wide hygiene improvements. The comparison of prevalence- and concentration-based MC, as well as a combination of the two, suggests that both approaches can lead to a significant illness burden reduction, when 100% sensitive testing is frequent and when MC are enforced, (i.e., an effective corrective action is implemented upon noncompliance) (Lambertini et al., 2019). These studies identified significant gaps in data on prevalence and concentration levels representative of the range of U.S. processing establishments, concentration variability within and between product lots, conditions during transport and retail, and consumer handling and preparation practices, including transfer coefficients to model cross-contamination in kitchens. Due to the scope of the studies, these two models did not assess the impact of different infectivity or virulence associated with different serovars, assuming that control measures applied at processing, the case of noncompliance, would have a similar impact on all serovars.

In another study, researchers proposed a QMRA model for *Salmonella* control in ground turkey where both the amount of *Salmonella* and hypothetical differences in serovar infectivity were incorporated. For this model, the authors grouped serovars in high and low infectivity groups and used dose–response models from experimental and outbreak data as a proxy for the infectivity in the high and low groups. The model further assumed that all lots with high contamination (>1MPN/g) would be detected and removed using a test with 100% sensitivity and predicted up to 50 million annual salmonellosis cases from ground turkey in the US. Recently, significant issues were reported with this approach (Zagmutt & Pouillot, 2022) that resulted in the retraction of the Sampedro et al. article (Sampedro et al., 2019).

Oscar (Oscar, 2019; Oscar, 2021a; Oscar, 2021b) also used a comparable framework to that of Sampedro *et al.* to contrast enumeration vs. prevalence standards in ground turkey. The approach relied on a series of untested modeling assumptions such as adjusting the dose-responses by serovar that resulted in broad infective doses (ID50) ranging from 1 to 10 log CFU/g.

None of the existing QMRAs that evaluate *Salmonella* MC for poultry products fully consider the public health impact of controlling all three dimensions considered in this question (prevalence, level, and subtype) while also considering realistic scenarios of frequency and sensitivity of product testing and its resulting impact on the contamination of the final product. For example, one scenario that could be investigated is testing finished product batches for both the presence and concentration of the pathogen and contrast it to the less frequent sampling currently done by FSIS. Evaluating the public health impact of a MC that controls for prevalence and amount of *Salmonella* on poultry products is feasible using QMRA approaches. However, evaluating the public health impact of controlling certain *Salmonella* subtypes (i.e., serotypes and genetic factors related to virulence) would require their identification, information on their presence in poultry products, and the ability to predict differences in the probability of illness from foodborne exposure to these *Salmonella* subtypes.

Parameters needed to model MC

Possible inputs and parameters that can be used to model a MC are shown in Table 2 (considering a specific processing stage where a MC is set, i.e., not including all stages needed to assess exposure to consumers). The inclusion of specific parameters depends on the MC options evaluated. It is important to highlight that the number of samples collected in relation to product flow rate plays a key role in the value of information obtained by a testing program and, depending on the context, could make a larger difference than other parameters.

This answer does not include a comprehensive review of available data in relation to QMRA model needs. However, this committee agrees on the importance of adequate data to support risk estimates and to estimate the impact of a MC with sufficient precision. For instance, current data on *Salmonella* prevalence or concentration collected by USDA-FSIS as part of routine monitoring or targeted data collection efforts only partially support QMRA efforts. Presence/absence data are collected infrequently, in particular for smaller establishments. Until recently, concentration data were not routinely collected, the last data collection effort on chicken products, representing only a portion of establishments, was carried out in 2012 (USDA Food Safety and Inspection Service (FSIS), 2012). However, FSIS has launched a 4-month program for collecting paired chicken samples collected at rehang and postchill (USDA Food Safety and Inspection Service (FSIS), 2022h). These samples are tested for *Salmonella* presence, levels (CFU/g), and serotypes. FSIS plans to continue testing chicken carcasses at postchill for *Salmonella* levels, which would make this testing routine. The industry has also been sampling product and processing environments. Combining industry and government data could improve the accuracy of *Salmonella* contamination models.

Including indicator organisms in models

Models to estimate the impact of MC can also include indicator organisms different from the target pathogen, complementing information on the occurrence of the target pathogen, or possibly instead of it. For the inclusion of indicators to be appropriate, a reliable quantitative relationship needs to be available that ties the presence and/or level of the indicator organism to the presence and/or level of the target pathogen. This relationship can be present in the processing environment and/or in the product, depending on the type of MC, and may be general or specific to each establishment. **Question 5** further discusses possible indicators relevant to the control of *Salmonella* in poultry in the U.S. At the time of writing, such indicator/*Salmonella* relationships are not established, although preliminary data exist. When validated data are available, the status of an establishment, based on the selected indicator, can be used in models to estimate the likelihood that the target pathogen is present at an unacceptable level at a certain processing stage, or the likelihood that a risk-relevant event may occur (e.g., cross-contamination). Examples of models and standards, including indicator organisms, exist in other domains. For example, drinking water standards are often based on indicators of fecal contamination, since that is a primary contamination route and the presence of fecal indicators at unacceptable levels indicates a lack of process control (National Research Council, 2004).

Identifying and incorporating serovar virulence into QMRAs

Very recent developments in WGS and bioinformatics have resulted in several potential approaches to use genomics to identify genetic markers of *Salmonella* of public health concern. This information can be used to create groups of isolates or serovars, which can then be used in QMRAs.

Table 2
Key variables to model microbiological criteria (MC) using quantitative microbial risk assessment (QMRA) approaches

Parameter/Variable	Description
Production/processing stage	Stage where samples are collected (may be multiple)
Proportion of establishments	Proportion or number of establishments on which the MC assessment is based. May be 100% or a subset, by type of establishment.
Number of lots/units produced per time unit (e.g., per week)	Production may differ by establishment category.
Lot size	Weight of product in each lot (lbs./lot)
Prevalence of *Salmonella*, by serovars	Number of positives out of total samples tested. If MC selectively targets serovars of concern, results should be provided by serovar.
Concentration distribution, within lots	Parameters needed to describe the distribution of concentration within each lot (CFU/g)
Concentration variability across lots	If applicable; represents variability in concentration across lots. Modeling approach may vary.
Concentration variability across establishments	If applicable; represents variability in contamination for different establishment groups.
Number of lots/units sampled per time unit	Number or proportion of lots sampled per time unit
Lot sampling algorithm	Testing protocol within MC determines which lots are sampled, e.g., systematic, randomized, tier randomized.
Pooled sampling approach (if applicable)	Which samples are pooled, how many and weight of each.
Number of samples per lot	MC parameter. Number of individually analyzed samples (not pooled).
Microbiological criteria (MC) concentration threshold	MC parameter. Multiple thresholds can be established. In the presence/ absence of MC, m is the detection limit of the assay
Number of samples allowed to be above the MC threshold, among the n samples.	If applicable. MC parameter.
Assay sensitivity	Ability of the assay to detect the target, if the target is present. Tied to the rate of false negatives.
Assay specificity	Ability of the assay to exclusively detect the target instead of other organisms. Associated with the rate of false positives.
Assay limit of detection (LOD)	Number of cells in a sample (or per g or mL) that would yield the assay to reliably detect the sample as "positive." See glossary.
Assay limit of quantification (LOQ)	Number of cells in a sample (or per g or mL) above which the assay can reliably and accurately quantify concentration. See glossary.
Batch-level risk management action in case MC is not met (food safety criteria), and associated reduction in target organism in product	E.g., a lot not meeting a MC may undergo additional processing that results in a known log CFU/g reduction. Different tiers of action can be established if there are multiple tiers of MC compliance.
Establishment-level risk management action in case MC is not met (process hygiene criteria), and associated reduction in target organism in product (directly or indirectly)	Quantitative impact of establishment-level corrective action. (e.g., establishment whose product or environmental/ process control samples have not met the MC undergoes in-depth cleaning known to bring the process variable within control, or to or target to a specified level, or yield a specified reduction).
Level of implementation of risk management actions	Proportion of establishments or lots where corrective action is implemented; may be by establishment category.

Karanth et al. (Karanth et al., 2022) provided an exploratory approach to use genomics and machine learning to identify severe *Salmonella* serovars. The authors tested the association between WGS data and severity of *Salmonella*-related health outcomes in the host, indicative of the virulence potential of different strains of *Salmonella*. Sequence reads were collected for 150 *Salmonella* isolates from humans, poultry, and swine sources, categorized as gastrointestinal vs extraintestinal and the pangenome (i.e., all genes from all isolates) used in various statistical models to predict disease presentation. The best model had a predictive accuracy of 76%. As the methodology used the pangenome, their methodology was computationally intensive, which might explain why only 150 isolates were used. The need for intense computation limits its scalability at the present time. Also, disease presentation information was not present for nonhuman isolates (e.g., a swine isolate could not have a human disease presentation unless it was related to a traced foodborne outbreak), so it had to be imputed.

Chen et al., (Chen et al., 2022) used *Salmonella* serovar Saintpaul *(S.* Saintpaul) as a model to identify potentially hypervirulent strains. Single nucleotide polymorphism (SNP) clusters (i.e., groupings of isolates with a low number of point mutations) were queried on NCBI for SNP clusters with either a high proportion of human clinical isolates (HA) or a low proportion of human clinical isolates (NHA). A total of 211 *S*. Saintpaul isolates were combined with 313 *S. enterica* isolates from other serovars to produce a phylogeny of *S.* Saintpaul illustrating the serovar is polyphyletic. Nine SNP clusters from group I (single monophyletic clade) were chosen for further analysis with 5 HA and 4 NHA isolates. Ten isolates were sampled from each SNP cluster to generate an analysis set of 90 contiguous (contig) assemblies. Differential carriage of plasmid, prophage, and individual gene carriage (pangenome-wide associate study) were assessed between the HA and NHA clusters. Seven isolates (4 HA, 3 NHA) were tested for cell invasion and intracellular survival (both in vitro) to quantify phenotypic differences between the SNP clusters. As *S*. Saintpaul is polyphyletic (i.e., same taxonomic grouping, different evolutionary paths), this serovar may not be the best model to test for differences between human and nonhuman isolates.

Recently, Fenske et al., (Fenske et al., 2022) reported a method to group isolates, based on genetic virulence markers and validated the groups against epidemiological data using beef isolates and outbreaks as a model. For this, contig assemblies (*de novo* genome assemblies from short reads) of *Salmonella* isolates from beef, cattle, and humans were collected from FSIS and FDA and surveillance programs (n = 12,337). Putative virulence factors were predicted, representing a virulence gene catalog for each respective isolate. The pairwise similarity between each virulence gene catalog (measure of genomic relatedness between isolates in regions which may impact phenotypic virulence) was estimated using an unsupervised random forest and used in a hierarchical clustering algorithm to group isolates by similarity. Serovar designation was provided posthoc and was not used to generate genomic clusters to control for the impact of polyphyletic serovars. The five groups were externally validated against epidemiological foodborne illness data (overall, and beef attributed) to explore if the genetic-based groups correlated with observed virulence at the serovar level. The isolates in the "high virulence" cluster were most often implicated in foodborne outbreaks of any cause and in beef-associated outbreaks but were less common in beef than all the other four groups combined. They also resulted in a 1.5 times higher incidence of disease and higher hospitalization rates than all other groups combined.

The studies above illustrate the potential of genomic methodologies in the application for QMRAs in poultry where differential MC for serovars are possible, with the Karanth et al. (Karanth et al., 2022) method using the most genes, and the Fenske et al. (Fenske et al., 2022) method being the most scalable as it used only the subset of genes in a virulence catalog and removed all repeated, core *Salmonella* genes.

Using potential genetic serovar or isolate groups to adjust dose–response (DR) models

Only a few DR models exist for specific *Salmonella* serovars. These models have been derived based on animal studies, volunteer feeding trials, and outbreak investigations, so their application to entire populations has resulted in large disconnects between model-predicted illnesses and observed foodborne illnesses recorded in national surveillance systems such as FoodNet (see **Question 4**). For this reason, QMRAs often use adjustment factors to calibrate the model predictions so that they match surveillance data.

A QMRA that is designed to assess MC based on specific target serovars and corresponding levels should thus be able to incorporate dose–response models that apply to the serovars of interest and be able to predict annual salmonellosis cases that are in agreement with surveillance data. Teunis et al. (Teunis et al., 2010) provide a mathematical framework to adjust different dose–response curves by serovars based on outbreak data, where the mean CFU/g of *Salmonella* in the food vehicle, number of exposed individuals, and numbers ill (and/or infected) was available. Although the Teunis framework was developed for individual serovars, this methodology could be expanded to accommodate genomics-based serovar groupings such as those described by Fenske et al. (Fenske et al., 2022). A modification of the Teunis framework, allowing for serovar groupings, would also have to provide a way to map the exposure from poultry to the resulting dose–response, which could be done using calibration methods. Note that these approaches have the advantage of incorporating specific genetic virulence characteristics (Fenske et al., 2022). Such methods can be paired with surveillance-based methods for source attribution- such as those covered in **Question 4**- to provide necessary adjustments to the dose–response curves.

Incorporating quantitative molecular methods into QMRAs

A variety of emerging molecular assays to detect or quantify *Salmonella* are reviewed in **Question 6**. The sensitivity and specificity parameters, and hence the limit of detection (LOD) and limit of quantification (LOQ) of these assays, are key to understand the efficacy of MC standards in reducing foodborne illnesses and should be incorporated in QMRA models. It is crucial that a quantitative assay is sufficiently precise in the range of the MC threshold.

In addition, assay sensitivity impacts sampling plans. Models need to represent the interplay between LOD and LOQ of quantitative assays, the MC threshold, and the number of samples that need to be collected to accurately assess the distribution of *Salmonella* concentration in a lot or other unit. For instance, quantitative methods such as qPCR are generally less sensitive to detect the presence of *Salmonella* than qualitative PCR, i.e., they have a higher LOD. A lower sensitivity would result in the need to collect more samples to determine with high confidence if a quantitative threshold has been exceeded. In addition, the efficiency of the sampling plan can be affected by the variability of the pathogen concentration within and between lots (Mussida et al., 2013). Thus, depending on the MC threshold concentration versus the assay's LOQ quantitative MC may require a higher number of samples per product unit to correctly classify the unit (or a derivative metric of multiple units, such as an establishment). This would be more so for a theoretical standard based on the level of specific serovars, since each serovar would be present in a sample in cell numbers equal to or lower than the cell number of all *Salmonella* spp. serovars summed together. Any model used to determine the impact of an MC and associated sampling plans should account for realistic sensitivity values, and the realistic number of samples that can be feasibly collected.

There is evidence that a large proportion of *Salmonella*-positive poultry product samples harbor low concentrations. For example, in 2012, Nationwide Microbiological Data Collection Program 26% of chicken part samples were positive at screening; these screening-positive samples were analyzed via MPN and 31% of them were found to be below the LOD of the MPN quantitative assay (0.030 MPN/mL in rinsate, corresponding to 0.0066 MPN/g of product); 79% were below 0.30 MPN/mL. Similar results were also observed in previous years (2007–2008) for carcass rinse postchill (USDA Food Safety and Inspection Service (FSIS), 2009a). Numerical examples of the impact of these results are provided under the "Assay Parameters" heading, in the answer to **Question 3.** The practical implication is that if the LOD of an assay were set to detect high levels in chicken products (e.g., 10 CFU/g), its sensitivity would by definition be reduced. As a consequence, a higher number of samples would be required to accurately estimate the proportion of samples contaminated at the lower levels observed in these studies. If only a subset of serovars of concern are included, the number of samples to be tested would have to be increased further.

In conclusion, both in risk models and cost-benefit models, it is important to include sensitivity and specificity parameters of the sampling assay, as well as the sample volume or weight needed to detect positives with a high level of confidence in an individual sample. For currently available assays to support quantitative MC, they have to perform well at all concentrations (low and high) commonly found in poultry meat, as currently established using MPN assays.

Q1.B Assessing the efficacy of microbiological criteria (MC; e.g., standards) in reducing salmonellosis once they are implemented

Q1.B (1). What is the operational definition of success for a MC? Can we devise an operational definition of success of one or more microbiological standards, in terms of reduced health burden? If so, what should or could be measured?

The Healthy People 2030 Food Safety goals agreed upon by FSIS, FDA, and CDC include a specific target of reducing domestically-acquired human salmonellosis by 25% by the year 2030, as measured through FoodNet, and using a baseline period of cases reported in 2016–2018 (U.S. Department of Health and Human Services). If between 25 and 50% of domestically acquired salmonellosis is from poultry (see Q4), then this target is congruent with the FSIS goal under evaluation, of reducing poultry-associated salmonellosis by 25%. Prevention that targets other sources of salmonellosis will also be important to ensure that we reach the overall Healthy People goal. The goal for FSIS pertains to domestically acquired salmonellosis, as the actions taken in the US to control salmonellosis may have little impact on exposures occurring in other countries. According to FoodNet, approximately 10% of salmonellosis in 2017–2019 was associated with foreign travel (Tack et al., 2020).

Q1.B (2). What existing public health surveillance data are available for salmonellosis?

Several types of public health surveillance data are available to help track salmonellosis (see Table 3). Basic case surveillance in the US is based on reporting to CDC of cases of salmonellosis through state epidemiology departments, and the serotypes and strains isolated from patients' specimens through public health laboratories. Routine serotyping in those laboratories began in 1963 with reporting to CDC (Brachman and JB, 1965). Among the approximately 2,600 serotypes described (Centers for Disease Control and Prevention (CDC), 2011), 883 were reported to the National *Salmonella* Surveillance System to have been isolated from humans during 2006 through 2016 (Centers for Disease Control and Prevention (CDC), 2018). The top 20 serotypes accounted

Table 3
U.S. Sources of Salmonella surveillance information

Case-based surveillance	
National *Salmonella* Surveillance System	Case-based reporting from state and local health department epidemiology offices to CDC. This includes serotype for the great majority. Summarized in 2016 46,623 cases were reported (of which 39,980 were serotyped) (Centers for Disease Control and Prevention (CDC), 2018).
PulseNet	Individual clinical isolates of *Salmonella* are sequenced in local and state public health laboratories and reported to CDC's PulseNet database. They are summarized in BEAM Dashboard, starting with data from 2017. Between 2017 and 2019 a mean of 41,278 *Salmonella* isolates were reported to PulseNet (Centers for Disease Control and Prevention (CDC), 2022a). CDC and state health departments test human isolates, FSIS and FDA test nonhuman strains.
National Notifiable Disease Surveillance System (NNDSS)	This is information reported to CDC about diagnosed salmonellosis cases and other notifiable diseases that does not include serotype. It is summarized annually on the NNDSS website (Centers for Disease Control and Prevention (CDC), 2022e)
Outbreak-based surveillance	
Foodborne Disease Outbreak Surveillance System (FDOSS)	Foodborne Disease Outbreak Surveillance System (FDOSS)(Centers for Disease Control and Prevention (CDC), 2022b), as reported to the National Outbreak Reporting System (NORS)(Centers for Disease Control and Prevention (CDC), 2022f) by local and state health departments and investigating offices at CDC. Summarized on NORS Dashboard website (Centers for Disease Control and Prevention (CDC), 2022f). Between 2016 and 2019, an annual mean of 143 foodborne salmonellosis outbreaks were reported.
Specialized surveillance	
FoodNet	Part of the CDC's Emerging Infections Network, and supported by FSIS and FDA, 10 state and large county group sites report all diagnosed infections of seven types of bacteria, in addition to Cyclospora, including salmonellosis, since 1996. Since 2014, a subset of patients has been interviewed about a standardized set of exposures ("Case Exposure Ascertainment") and these data are linked with corresponding PulseNet, NARMS, and outbreak data. FoodNet data are summarized in annual and periodic publications and at FoodNet Fast (Centers for Disease Control and Prevention (CDC), 2021). Between 2016 and 2019, a mean of 8472 salmonellosis cases were reported to FoodNet, of which 10% were travel-associated, 7% were outbreak associated, and 10% were diagnosed by culture-independent methods alone (Centers for Disease Control and Prevention (CDC), 2021).
National Antimicrobial Resistance Monitoring System (NARMS)	A tri-agency collaboration since 1997. CDC measures the antimicrobial susceptibility of a sample of 1 out of 20 *Salmonella* isolates from humans, referred by state public health labs. FDA tests retail samples of meat and poultry for *Salmonella* and other organisms, and FSIS tests samples gathered during inspection (poultry cecal samples since 2013). Human NARMS is summarized on the NARMS NOW website (Centers for Disease Control and Prevention (CDC), 2020). Between 2016 and 2019, NARMS CDC tested an annual mean of 2543 *Salmonella* strains per year. Results for testing by FDA and FSIS are also available on respective websites (U.S. Food and Drug Administration (FDA), 2022; USDA Food Safety and Inspection Service (FSIS), 2022e).

for 80.7% of isolates with a reported serotype. The same five serotypes have persisted as the most common causes of human illness for the last decade (Enteritidis, Newport, Typhimurium, Javiana, I:4,5,12:i:-) (Collins, 2022). In 1996, state public health laboratories began conducting molecular subtyping, using pulsed-field gel electrophoresis (PFGE) patterns to identify clusters of *Salmonella* with similar patterns and submitting the subtyping information to CDC through PulseNet, the national network for molecular subtyping which, this became routine for *Salmonella* isolates in all states by the early 2000s (Gerner-Smidt et al., 2006; Swaminathan et al., 2006). Routine subtyping has greatly improved detection and investigation of dispersed outbreaks, and source attribution. In mid-2019, PulseNet switched to genome sequencing for subtyping *Salmonell*a (multilocus core genome sequence typing, cgMLST), which offers substantially greater precision in measuring relatedness than did PFGE. Whole-genome sequencing (WGS) data allow the systematic prediction of serotype and antibiotic resistance profile from sequence data (McDermott et al., 2016; Zhang et al., 2019). PulseNet, which includes all state and some large city health department public health laboratories, and both FSIS and FDA as members, previously used PFGE and now used WGS to characterize regulatory isolates as well. The sequences of approximately 40,000 *Salmonella* isolates from humans are reported to PulseNet each year (Bacteria, Enterics, Amoeba, and Mycotics (BEAM) Dashboard)) (Centers for Disease Control and Prevention (CDC), 2022a).Table 4.

FoodNet. More specialized surveillance includes FoodNet, CDC's sentinel site surveillance system, supported by FSIS and FDA, which collects standardized information on all laboratory-diagnosed infections with *Salmonella* and seven other pathogens in a panel of 10 states or multicounty areas, encompassing 15% of the US population (Henao et al., 2010). Human salmonellosis incidence of laboratory-diagnosed cases reported to FoodNet has not decreased since the 1990 s (Fig. 2). Approximately 8,500 *Salmonella* infections are reported to FoodNet sites each year, and are summarized on the public-facing website FoodNet Fast (Centers for Disease Control and Prevention (CDC), 2021). Data on long-term health sequelae are not collected. Travel history data are collected; if 10% of those infections are travel-associated, FoodNet gathers reports of approximately 7,200 domestically-acquired cases per year. Standardized case exposure data have been collected in FoodNet since 2014 that are now being linked to PulseNet genomic data for future analysis.

NARMS. The National Antimicrobial Resistance Monitoring System (NARMS) is another tri-agency surveillance collaboration. Since 1997, as part of NARMS, CDC has determined antibiotic resistance profiles of *Salmonella* by phenotypic methods, based on 1 in 20 isolates from *Salmonella* from human infections, referred by state public health departments (Centers for Disease Control and Prevention (CDC), 2020, 2022d). As part of NARMS, these human samples are tested in parallel with isolates collected from various meats at slaughter by FSIS (USDA Food Safety and Inspection Service (FSIS), 2022e), and from retail food samples of meat and poultry collected by FDA (U.S. Food and Drug Administration (FDA), 2022a, 2022b). CDC PulseNet now provides resistance predictions obtained by WGS to NARMS.

Data on *Salmonella* serotypes in poultry products. FSIS participates in PulseNet and NARMS, submitting characterization of *Salmonell*a isolates obtained at slaughter from cecal samples (samples from individual animals before extensive processing), as well as from carcass rinsates, cultures of poultry parts and comminuted poultry. The FDA tests samples of chicken breasts obtained at retail as part of NARMS, and also sequences the *Salmonella* they isolate, entering the results into the NCBI database, and PulseNet. Noteworthy for its absence from NARMS is the systematic sampling and characterization of *Salmonella* from poultry at earlier stages of production, such broiler breeder flocks, or grandparent flocks, and from feeds or feedstuffs.

Reports of foodborne outbreaks associated with *Salmonella* in poultry. Possible outbreaks of cases that appear to be related can be detected because they occur in a local group with an obvious place or other exposure in common, or because a dispersed group or cluster of cases is identified that have the same molecular or genomic subtype. Epidemiological investigations of these possible outbreaks may identify a common source, confirming them as outbreaks. Investigated out-

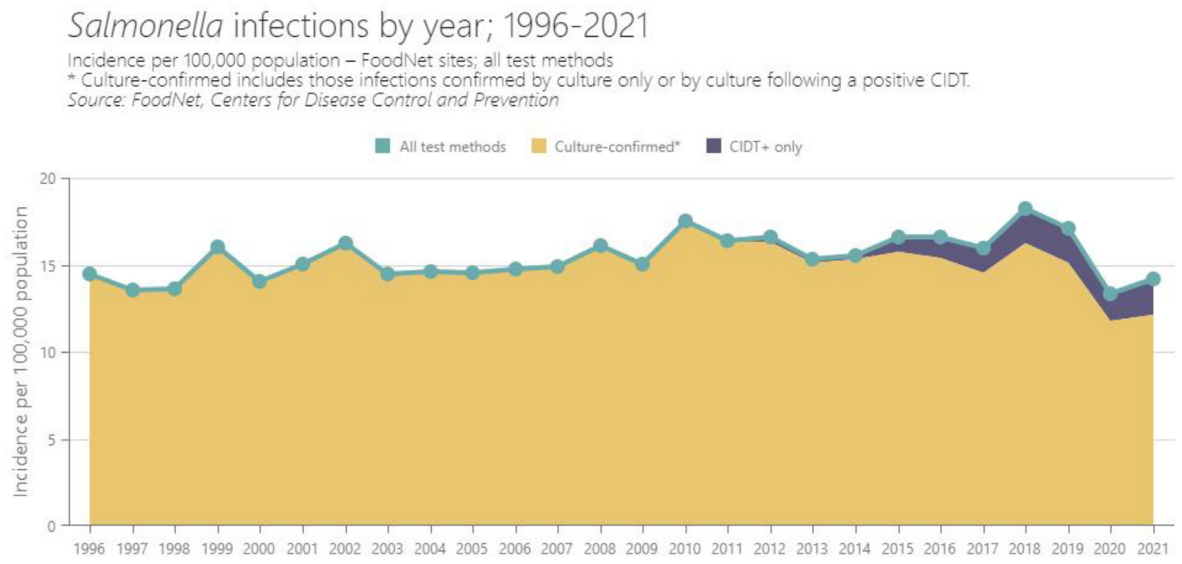

Figure 2. Trends in salmonellosis incidence in the U.S., as detected by culture-independent diagnostic test (CIDT) only vs. CIDT plus culture confirmation. (Centers for Disease Control and Prevention (CDC), 2021).

breaks are reported by public health departments and the CDC to CDC's Foodborne Disease Outbreak Surveillance System (FDOSS) using the National Outbreak Reporting System (NORS) platform (Wikswo et al., 2022). FDOSS collects details on implicated food types, characteristics of illnesses, and location of exposure (Centers for Disease Control and Prevention (CDC), 2022b). To aid in source attribution, CDC applies the categories of implicated foods developed by the Interagency Food Safety Analytics Collaboration (IFSAC) (composed of experts from the FDA, CDC, and USDA-FSIS) to the free text fields reported through NORS (Richardson et al., 2017). Approximately 150 foodborne salmonellosis outbreaks were reported to FDOSS in NORS annually between 2013 and 2019 (NORS Dashboard) (Centers for Disease Control and Prevention (CDC), 2022f). This system distinguishes outbreaks by route of exposure, allowing to differentiate foodborne (and waterborne) outbreak-associated cases from those resulting from animal contact. It also includes outbreak-associated cases resulting from environmental, person-to-person, and indeterminate/unknown exposures.

Q1.B (3). What can be done to improve the power and discrimination of current foodborne illness datasets?

Successful control of a major source of salmonellosis would be expected to reduce both outbreak-associated cases and sporadic cases. The impact on human domestic salmonellosis can be tracked using case-surveillance data. Most reported cases are not part of identified outbreaks: Food Net has reported that in 2017–2019, 91% of reported salmonellosis was not part of recognized outbreaks (Ray et al., 2021).

There are challenges to surveillance data collection and opportunities for improvement. The utility of case surveillance for source attribution and trend tracking is increased by FoodNet interviews, particularly by the more detailed Case Exposure Ascertainment interviews, which began to be collected in 2014, when they can be linked to NGS (next generation sequencing) of isolates. Streamlined data gathering and reporting for FoodNet may make those data available and analyzed more quickly.

The COVID19 pandemic of 2020–2022 affected *Salmonella* surveillance, as it did many other public health efforts. Reported cases of salmonellosis were 22% lower in 2020 than in the preceding 3 years (Ray et al., 2021). Contributing factors likely included the steep decline in international travel, changes in where and what people ate, and changes in health care-seeking behavior as some avoided direct contact with the medical facilities. These effects may not be permanent after the pandemic is resolved, and it may be prudent to exclude data from the years 2020 and 2021 in future assessments of longer-term trends.

Surveillance based on serotyping and genetic subtyping currently depends on having the actual isolate (i.e., bacterial cells cultured from the original sample) in hand, so that serotype and antimicrobial resistance can be predicted from sequence data. The growing use of culture-independent diagnostic tests (CIDTs) in clinical laboratories means that doctor and patient may more quickly learn the cause of the illness, though without antibiotic resistance determination. However, unless an isolate can be obtained, public health will not have a sequence for tracking, outbreak detection, or source attribution. The current solution is to encourage following up a CIDT positive test with a "reflex" culture of the specimen that was positive in the CID test to retrieve an isolate, which can then be characterized and sequenced (Association of Public Health Laboratories (APHL), 2022). A longer-term solution is to develop and deploy more advanced metagenomic methods to retrieve the *Salmonella* genome from the specimen that is positive by CIDT (Association of Public Health Laboratories (APHL),

Table 4
Salmonella poultry performance standards (USDA Food Safety and Inspection Service (FSIS), 2019a)

Product	Performance Standard[a]	Maximum Acceptable Percent Positive	Minimum Number of Samples to Assess Process Control[b]
Broiler Carcasses	5 of 51	9.8%	11
Turkey Carcasses	4 of 56	7.1%	14
Comminuted Chicken	13 of 52	25.0%	10
Comminuted Turkey	7 of 52	13.5%	10
Chicken Parts	8 of 52	15.4%	10

[a] The performance standard is represented as a fraction of maximum allowable positives over the target number of samples collected and analyzed in a 52-week window.
[b] FSIS must analyze at least this number of samples in a single 52-week window in order to categorize an establishment for the standard listed.

2022). A pilot test of one such approach began in two states in 2021 (personal communication, Heather Carleton, CDC). Recently, a high-throughput quantitative PCR assay was validated that is able to identify 40 Salmonella serotypes and distinguish different genomic lineages and polyphyletic profiles (Cadel-Six et al., 2022). Once a metagenomic replacement for culture can be developed and implemented across the 50 states, it will bridge current PulseNet surveillance to a culture-independent future for public health. Until this as achieved, state health departments will need to obtain isolates from reflex cultures and to sequence those isolates.

Q1.B (4). What analyses of datasets can help inform progress towards the FSIS goal?

Annual summaries of the frequency of *Salmonella* infections by serotype have been part of national surveillance reports for many years. Reducing the lag in publishing them would make them more immediately useful, for example by predicting them from PulseNet and providing them as provisional data through the BEAM Dashboard (Centers for Disease Control and Prevention (CDC), 2022a). Annual FoodNet surveillance summaries are produced the following year, and the data in FoodNet Fast are also updated at the same time. These case surveillance data are robust enough to permit annual assessments of progress in reducing the frequency of the most common serotypes in human infections, using established statistical approaches to detect significant differences from previous years. Based on these data, attribution models (see next section and Question 4) can estimate the proportion of cases attributable to poultry or subcategories thereof.

Serotype data on nonhuman *Salmonella* isolates collected from poultry sources as part of NARMS and in the regulatory sampling of USDA could be summarized each year and compared with trends in human infections. A time trend model that smoothed surveillance data across multiple years, adjusting for seasonal and cyclical differences, could be helpful so that the estimation of success is not biased by the fluctuations in epidemiological data in any given year. A possible approach has been proposed by Williams et al. (Williams et al., 2021), who combined FoodNet and FSIS sampling data and penalized cubic B-spline regression methods (Powell, 2016; Powell et al., 2018) to compare trends in *Campylobacter* on chicken meat and campylobacteriosis cases in humans. USDA's Office of Risk Assessment and Cost Benefit Analysis has provided a preliminary analysis of data collected from 1996-2021 (M. Powell, USDA, personal communication, Docket FSIS-2022–0031) using similar methodology to compare trends in *Salmonella* serovar presence in chicken meat against trends in serovars causing *Salmonella* infections in humans. The preliminary analysis suggests that cases of salmonellosis due to serotypes associated with chicken have gradually decreased similar to the decrease in *Salmonella* in chicken products. Nonchicken serotypes have increased during the same period. FDOSS outbreak data may also be summarized at regular intervals, likely annually, once the pressure of the COVID pandemic on state and local public health departments allows them to report outbreak investigations more quickly to NORS.

Other regular analyses that would be helpful would include: 1) Outbreak data such as the annual number of outbreaks and outbreak-associated illnesses associated with chicken, with turkey, and with all poultry combined; 2) More detailed data routinely provided on the type of chicken product that was the source, (e.g., parts, whole, comminuted, frozen breaded, frozen breaded stuffed, etc.); 3) Annual ranking, by frequency, of serotypes by assessment of the burden of outbreak illnesses transmitted by poultry, and whether that burden is decreasing, stable or increasing; 4) Poultry sampling data, such as the annual ranking of serotypes obtained from FSIS poultry sampling overall, from raw poultry products destined for restaurants or institutions separately from those destined for grocery stores; and 5) Annual ranking of serotypes obtained from NARMS FDA retail chicken samples.

In addition, CDC could produce an updated estimate of the overall health burden of salmonellosis and other foodborne infections at more frequent intervals (last estimated in 2011) (Scallan, Griffin et al., 2011; Scallan, Hoekstra et al., 2011). This would depend on conducting more frequent, ideally annual FoodNet population surveys to assess current care-seeking and laboratory specimen submission behaviors.

Q1.B (5). Available source attribution methodology and how it can be used to sufficiently discriminate poultry-caused illnesses from both outbreak and sporadic salmonellosis cases.

This question is answered in Q4.

Q1.B (6). Are year-to-year changes, trends, and lags captured in the existing foodborne datasets and source attribution?

Year-to-year changes are captured in case-based surveillance with large numbers reported each year in each of the major systems, allowing for robust comparisons. As noted above, some smoothing across several years may be useful to limit the impact of single large events. PulseNet data in particular are analyzed continuously to look for clusters of related cases and may be used in the future to produce regular updates of sequenced-based source attribution estimates. (See answer to Q4 for more discussion on this topic.)

The annual burden of microbiological foodborne illness, published by the CDC in 2011, which updated the previous 1999 estimates (Mead et al., 1999), still serves as the best available general estimates of the health burden of foodborne diseases in the United States, by pathogen. The reported incidence of infections with four priority bacterial pathogens (*E. coli* O157, *Listeria monocytogenes*, *Salmonella*, and *Campylobacter*) has changed little in the last decade (Centers for Disease Control and Prevention (CDC), 2021), and thus the health burden may also be presumed to have changed little. Should the incidence of these and other infections decrease substantially, the burden estimates will become increasingly dated. Although the process of revising these burden estimates and providing uncertainty ranges has begun, it can take several years to complete (personal communication, B. Bruce, CDC). Therefore, some lag in identifying current disease incidence is likely to persist and needs to be considered by FSIS in evaluating the success of implementing new MC.

Foodborne illness surveillance and monitoring systems only detect a fraction of all cases that occur, and most reported cases do not have a known source. Also, infrequent large events can have a marked effect on the case count in a given year. This adds statistical "noise" to the numerical signal needed to detect temporal changes in foodborne salmonellosis trends and its main food sources. Using FoodNet as an example, Ebel et al. (Ebel et al., 2017) estimated a 4% chance of detecting a true 10% change in salmonellosis 1 year after a risk reduction change has taken effect. In a different scenario, a sustained reduction of 30% in salmonellosis for 4 years still resulted in only approximately 50% chance of detecting such change. In a separate study, a similar calculation was used to show that a 25% reduction in salmonellosis cases from comminuted chicken would not be detected by FoodNet alone, as this annual reduction would be smaller than the expected year-to-year variability of the surveillance system (Ebel and Williams, 2020; Ebel et al., 2017). In the future, serotype prediction based on PulseNet sequencing, and swifter analysis and presentation of preliminary National PulseNet surveillance data (e.g., on the BEAM dashboard) may increase the sensitivity of national public health surveillance to detect changes of this magnitude in a shorter time frame.

Because of the statistical power limitations of individual *Salmonella* surveillance and monitoring systems, for assessments of changes (or stability) in the incidence of salmonellosis, this committee advises the use of data from more than one system. For example, although FoodNet utilizes high-quality data, it comes from only 15% of the population, while other *Salmonella* surveillance systems are nationwide in scope. We also advise the use of genomic data and models for both incidence and source attribution, as described in answers to questions 1 and 4.

Because the data in FDOSS/NORS are more limited, they need to be combined across several years to define and analyze trends for specific food categories. Currently, the IFSAC outbreak-based source attribution model estimates (described in **Question 4**) are updated annually, although the data are smoothed over several years.

Q1.B (7). What new approaches are being developed and could provide more accurate/precise burden data in the future?

The microbiological foodborne illness burden estimates, developed by the CDC, depend on accounting for the sequential steps in the diagnosis and reporting of specific infections. These include assessment of the frequency of acute gastrointestinal illness in the population, of health care-seeking behavior, of the frequency with which diagnostic tests are ordered and performed, and of the frequency of reporting. Future estimates could be improved by reassessing these probabilities as practices change, evaluating the effects of variation in clinical severity on the likelihood of hospitalization, and could also incorporate estimates of the burden of long-term health sequelae.

Q4: Identifying serotypes of greatest public health impact

A) ***How might foodborne illness surveillance data on human Salmonella illnesses, data from foodborne outbreaks associated with Salmonella in poultry, and data on Salmonella serotypes in poultry products be used to identify the Salmonella serotypes of greatest public health concern associated with specific poultry products?***

B) ***Should only the most current data (e.g., 5 years) of foodborne illness surveillance outbreak and or pathogen/testing be used?***

C) ***Going forward, what methodology and criteria would focus on those Salmonella serotypes most frequently associated with human illness and attributable to poultry products?***

D) ***How frequently should the priority Salmonella serotypes associated with poultry be revised considering changes in their occurrence while still ensuring continuity in industry and regulatory testing?***

Summary of Question 4 Response

Several approaches have been used to attribute human salmonellosis to specific foods and sources. These include case-control studies, analysis of reported foodborne outbreaks, and most recently, source attribution based on whole genome sequence genotyping. Attribution based on outbreak data and on genotype, both, give the greatest weight to data from the most recent years. Like attribution based on reported outbreaks, genotype-based attribution indicates that poultry is the leading source of human salmonellosis. It also indicates that a small number of serotypes account for most poultry-associated salmonellosis in recent years, led by Enteritidis, Typhimurium, I:4,5,12:i:-, Infantis, and Heidelberg, and even fewer serogroups: groups O:4 (formerly group B), O:7 (group C1), and O:9 (group D1). The effectiveness of a prevention strategy that includes serogroup or serotype targets should be evaluated annually with case-based surveillance; changes in the attribution model would likely take several years to observe. The most current data should be used for all analyses, but the number of years to be included depends on the rate at which new information is added. Combining data from several recent years in a trend model can smooth out the effects of single annual fluctuations, such as large outbreaks. It is anticipated that targeted intervention strategies in the industry will affect predominant serotypes found to be associated with human illness. Subsequently, as other serotypes become predominant in illness, this in turn will affect industry responses.

Before answering the charge question on the predominant serotypes associated with poultry-related salmonellosis, we considered several of the serotype-focused interventions used by the industry for live birds. These control programs (described below) have resulted in various levels of success, but ultimately affect and help explain changes in predominant serotypes responsible for foodborne illness attributed to poultry products (also see response to Question 2).

Effect of serotype-targeted interventions to reduce Salmonella in the live bird populations

- In the 1990 s, the United Kingdom experienced a rapid increase in *Salmonella* Enteritidis (SE) infections related to both eggs and poultry meat, much of it caused by SE phage type 4; at the peak, SE comprised 70% of salmonellosis (Lane et al., 2014; O'Brien, 2013). With a voluntary industry effort based on vaccination and other flock-based measures, first targeting layer flocks, then including broiler breeders, the number of SE phage type 4 infections reported in the UK dropped dramatically, from ~18,000 in 1997 to 459 in 2010, and the overall number of *Salmonella* infections reported annually fell from ~33,000 in 1997 to ~9,000 in 2010 (European Food Safety Authority Panel on Biological Hazards, 2019; Lane et al., 2014; O'Brien, 2013) (Fig. 3). No other serotype emerged to "replace" SE.
- The United States has experienced a profound decline in the incidence of infections caused by Typhimurium and Heidelberg over the last 20 years (Centers for Disease Control and Prevention (CDC), 2021) (see Fig. 4). The reasons for these declines have not been well documented, but they were corresponding to when a commercial poultry vaccine against Typhimurium became available. Highly publicized recalls in 2013 of retail chicken from a West Coast producer whose products were implicated in an outbreak of Heidelberg infections may have accelerated wider vaccine use (Gieraltowski et al., 2016). Typhimurium vaccines in poultry appear to decrease colonization and infection not only of Typhimurium but of other serotypes that share the same O group antigens, such as Heidelberg, as both share antigenic O formula: II:4,5,12:i:- (Crouch et al., 2020; Muniz et al., 2017). Some producers may have also implemented targeted environmental testing with a focus on breeder flocks and increased other prevention measures as well. With a mix of strategies, by 2019, Typhimurium had dropped from its long-standing position as the most common serotype isolated from humans as late as 2007, to third most common. The incidence of Typhimurium infections decreased by 70%, from 3.7 per 100,000 in 1999 to 1.3 in 2019 (Centers for Disease Control and Prevention (CDC), 2021). Heidelberg had dropped from the 4th most common serotype isolated from humans in the 1990 s to the 24th most common in 2019. The incidence decreased by 92%, from 1.07 per 100,000 in 1999 to 0.08 in 2019 (Centers for Disease Control and Prevention (CDC), 2021).
- In 2003, the European Union (EU) issued regulations for breeder flocks requiring stringent control measures for specific serotypes that were, based on comparing serotypes found in food animal reservoirs with those most commonly causing human infections. They chose Enteritidis, Typhimurium (including the monophasic variant I:4,5,12:i:-), Hadar, Virchow, and Infantis as targets for prevention (European Food Safety Authority Panel on Biological Hazards, 2019). This was based on their frequency in human infections, their transmissibility through food, evidence of recent increase or spread, and increases in resistance to treatment. In 2019, based on changes in prevalence (World Health Organization (WHO), 2022b), the suggested group of serotypes to target was updated to Enteritidis, Typhimurium (including the monophasic variant), Infantis, and another to be determined – perhaps Heidelberg or Kentucky (European Food Safety Authority Panel on Biological Hazards, 2019). Updates to the list may vary by country (Leati et al., 2021). Serotyping is carried out via culture methods (White-Kauffmann-Le Minor scheme) (European Commission, 2005).

Q4.A(1) and (2); and Q4.C. How might foodborne illness surveillance data on human *Salmonella* illnesses, data from foodborne outbreaks associated with *Salmonella* in poultry, and data on *Salmonella* serotypes in poultry products be used to identify the *Salmonella* serotypes of greatest public health concern associated with specific poultry products? What methodology and criteria would focus on those *Salmonella* serotypes most frequently associated with human illness and attributable to poultry products?

***Question 4.A(1): Surveillance data on human illnesses caused by Salmonella*:** Several surveillance and monitoring systems provide data on individual *Salmonella* infections, on outbreak events, on antimicrobial resistance in human and nonhuman isolates, and on frequency in meat and poultry (see response to Q1 Part 2b and Table 3).

Question 4.A(2): How can [these data] be used to identify the Salmonella serotypes of greatest public health concern associated with specific poultry products?* and *Question 4.C: What methodology and criteria would focus on those Salmonella serotypes most frequently associated with human illness and attributable to poultry products.

The relevant serotypes of greatest public health concern are those that are common causes of reported human illness, are present in poultry, and are transmitted through foods. CDC surveillance provides data on the frequency of diagnosed illness caused by each serotype. Human illness reporting tends to be skewed towards more severe illnesses as milder cases are less likely to be diagnosed or reported, so models accounting for this are used to estimate the true number of illnesses in burden studies (Scallan, Hoekstra et al., 2011; Scallan et al., 2021). If the use of culture-independent diagnostic tests increases without an increase in associated reflex culturing, a greater proportion of reported infections will lack serotype data (Ray et al., 2021). FSIS data from slaughterhouses and retail surveys can confirm the presence and frequency of serotypes in raw poultry.

One way to quantify the importance of poultry as a source of human infection has been through case-control studies of sporadic infections. For example, a FoodNet study conducted in 1996–1997 identified eating chicken, and specifically chicken prepared outside the home, as a specific risk factor for serotype Enteritidis (SE) infections (Kimura et al., 2004). This study attributed 27% of SE infections to that source; before this study, SE infections had been attributed

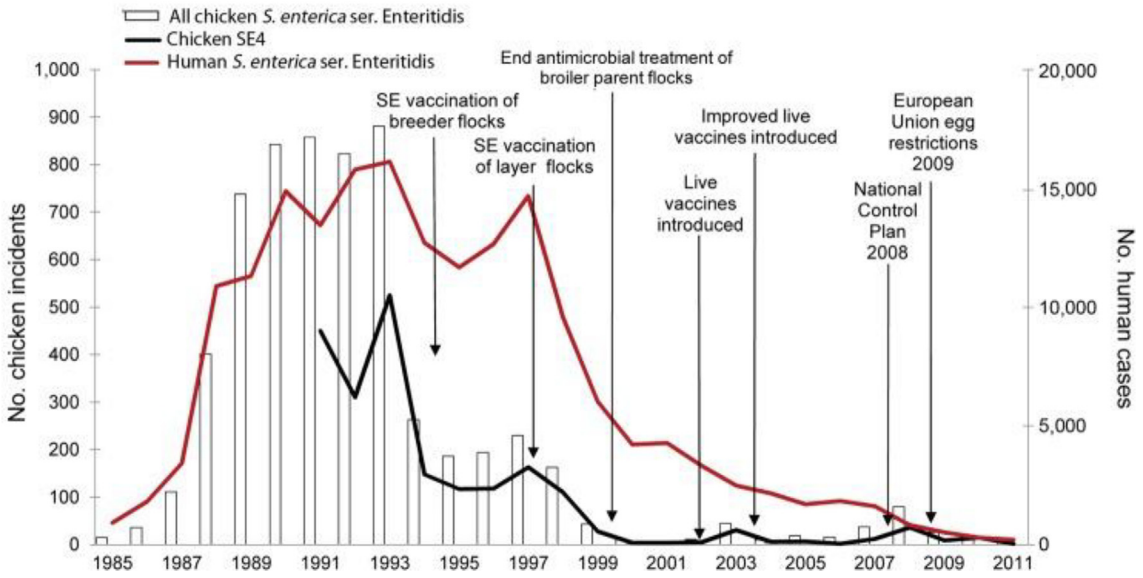

Figure 3. Trends in the reporting of incidents of Salmonella enterica in chickens in Great Britain versus laboratory reporting of human S. enterica serovar Enteritidis infection, England and Wales, 1985–2011, S. enterica serovar Enteritidis phage type 4 (SE4). Figure shows the most recent trends from the UK, showing a systematic decline in S. Enteritidis postintervention (Lane et al., 2014).

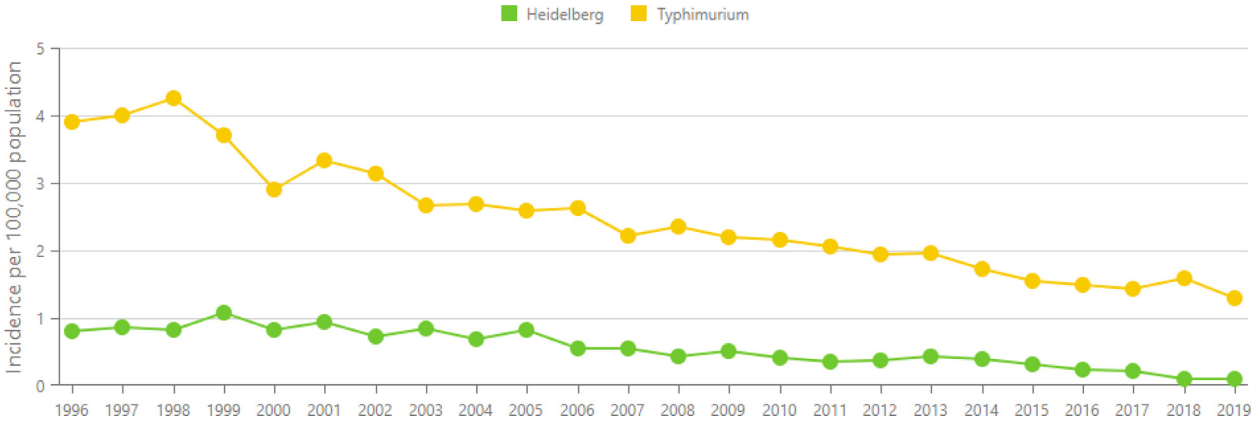

Figure 4. Trends in salmonellosis incidence associated with S. Typhimurium and S. Heidelberg in the U.S. Salmonella infections by year: 1966-2019. Incidence per 100,000 population – FoodNet sites; cultured confirmed (Centers for Disease Control and Prevention (CDC) (2022c)).

mostly to eggs. However, a parallel study of Typhimurium infections did not identify a specific source, although it did clarify the importance of preceding antibiotic exposure in increasing host susceptibility (Glynn et al., 2004). Such broad studies of sporadic cases can provide important insights and help to determine the overall burden of illness. Case-control studies attribute disease to the point of consumption, in contrast to some other methods of attribution which can include attribution to reservoir as well, thereby capturing cross-contamination of foods by an upstream source. Moreover, conducting case-control studies requires considerable time to design, execute, and analyze, is expensive, and has often yielded sparse new information (Fullerton et al., 2012). Thus, FoodNet has conducted few case-control studies in recent years. However, improved methods and data could make case-control analyses an important component in the assessment of major sources in the future. Nearest neighbors matched analysis is a statistical method that matches a number of each case's characteristics with those of controls. Method provides a stronger comparison group for each variable under analysis, with calculation of odds ratios and population-attributable risks (Cui et al., 2022), and is being explored as a less resource-intensive approach to case-control studies. These methods may be applied to cases from FoodNet's case exposure ascertainment data, using the recent FoodNet Population Survey, conducted during 2018–2019 as a source for control data. The future possibility of an ongoing FoodNet Population Survey to provide timely data from control persons about exposures paired with matching questions posed to cases could provide more timely estimates of the major sources and their changes over time.

Outbreak investigations of foodborne salmonellosis can provide direct evidence that foodborne transmission of a particular serotype occurs. Outbreak data in FDOSS can provide information by year on food categories that have been reported as sources of particular serotypes. Detailed analysis of FDOSS data also can indicate changes over time in the serotypes and broad categories of poultry products linked to outbreaks, (e.g., parts, whole chickens, and frozen meals). These data can help assess the importance of various serotypes attributed to particular foods. For example, in the mid-1980 s, CDC epidemiologists noted that although few SE outbreaks were attributed directly to shell eggs, most of the foods implicated contained eggs; they proposed that eggs were the source of the global increase in SE infections (St. Louis et al., 1988).

For the past decade, IFSAC has annually updated the results of an attribution model that estimates the percentage of all salmonellosis that can be attributed to 17 food source categories (Interagency Food Safety Analytics Collaboration (IFSAC), 2022). The model uses outbreak data reported to FDOSS; it gives less weight to outbreaks that occurred more than 5 years earlier (back to 1998) and accounts for other factors, such as whether the outbreak was very large or occurred in multiple states (Batz et al., 2021; Richardson et al., 2017). The estimates for poultry were 10% for chicken and 7% for turkey in 2012 (17% collectively), and 17.3% for chicken and 5.9% for turkey (23.2% collectively) as of 2020 (Interagency Food Safety Analytics Collaboration (IFSAC), 2015, 2022). These estimates are based on vehicles identified in foodborne outbreaks, and do not capture the original source when *Salmonella* in raw products such as poultry cross-contaminates other foods, which are then the source of an outbreak. They currently provide the best ongoing estimates of the sources of domestically acquired salmonellosis in the U.S. These IFSAC estimates do not include serotype because data on individual serotypes are sparse; however, a pilot analysis of data on SE, the most common cause of salmonellosis, has been attempted (CDC unpublished data).

Source attribution can also be predicted by comparing isolates from humans with isolates from a variety of animal and other sources. In Denmark, this was done using multilocus variable number tandem repeat typing (de Knegt et al., 2016). In the United States, a current IFSAC effort is using a genome sequence-based approach for source attribution. This approach may address some limitations of outbreak vehicle-based source attribution, as it can directly assess sporadic cases, and is likely to reflect an original source or reservoir before cross-contamination, if those sources are represented in the nonhuman isolates. Led by CDC's Analytics team, this IFSAC study has compared isolates from humans with those from a variety of foods and animals (Rose et al., 2022). The model was initially trained using the nonhuman isolates to identify the alleles that best differentiate these strains by animal and food source. Then human isolates were evaluated in the trained model. The initial assessment used nonhuman isolates collected over the past two decades, and human isolates collected 2014——2017 in FoodNet sites after excluding those known to be associated with foreign travel. The model predicts likely food category sources for these infections. Overall, 26% of infections were predicted to have a poultry source (Fig. 1). Restricting analysis to the subset of isolates with $>$ 50% probability of attribution to a single food source, 46% of such infections were predicted to be due to chicken and 3% to turkey. As the IFSAC study using WGS aims at identifying original source or reservoir (e.g., hatchery or grower level), the immediate source of exposure (e.g., consuming poultry meat or handling live bird) is not included in the estimates. For example, the total attribution of cases to poultry does not differentiate between sporadic salmonellosis cases resulting from chicken consumption versus cases from handling live chickens (Centers for Disease Control and Prevention (CDC), 2022g). Future sporadic case-control studies could address this gap.

The model was also able to predict food sources for the most common serotypes: Chicken was the predicted source for 86% of SE infections, 66% of Typhimurium, 55% of Infantis, 53% of Heidelberg, 29% of I:4,5,12:i:-, and $<$1% of Newport and Javiana infections. Turkey was the predicted source for 9% of Heidelberg infections and $\leq$1% of infections caused by the other serotypes listed above for chicken. These percentages can be applied to estimates of the incidence or number of illnesses caused by each serotype to obtain an estimate of chicken-associated illnesses caused by each serotype or a ranking of the most important serotypes. Doing this results in an estimate that the serotypes that cause the most chicken-associated illnesses are, in order, SE, Typhimurium, Infantis, and I:4,5,12:i:-; together these four account for an estimated 70.4% of chicken- and turkey-associated illnesses. Although a large percentage of Heidelberg infections are attributed to chicken, that serotype has now become uncommon, so it results in relatively few attributed illnesses. Retaining Heidelberg as a serotype of concern may be justified by concern that it could increase if targeted control measures already in place are lifted.

Some of the poultry-associated serotypes identified by the IFSAC WGS source attribution model share serogroup-specific O-antigens, relevant to vaccine choice: Typhimurium, I:4,5,12:i:- and Heidelberg are part of serogroup 4, (previously known as Group B), SE is part of serogroup 9 (Group D), and Infantis is part of serogroup 7 (Group C1) (Centers for Disease Control and Prevention (CDC), 2011). In the future, the serotypes chosen as targets for control could also include other poultry-associated serotypes with important antimicrobial resistance, or likelihood of hospitalization as additional criteria.

One can also compare the frequency of serotypes in different food animal reservoirs with those that cause human infections. An advanced version of this method has been used in Europe to develop a *Salmonella* source attribution model across 24 countries (de Knegt et al., 2015). Because animals can be reservoirs of nontyphoidal *Salmonella*, one would expect a change in the presence of a serotype in poultry to precede a change in the same direction in illnesses caused by that serotype in humans. Moreover, one would expect the changes in serotype frequency in poultry and food to be detected from the variety of sampling programs done pre- and postharvest, such as FSIS' cecal samples for testing in the NARMS program, and carcass samples for regulatory purposes. Measures of illness in humans may lag behind animal and food sampling because of the time it takes for a new strain

to spread though many flocks, the time it takes for animals to be raised and slaughtered, the resulting food distributed and because most chicken is cooked sufficiently to kill *Salmonella*. Therefore, assessing the frequency of serotypes in live poultry and poultry at slaughter would help to monitor those serotypes most likely to cause illness now or in the near future. Similarly, routine sampling of poultry feed and feed ingredients could be relevant because this is a documented way that serotypes enter the food supply, where even low levels of contamination could be important because pathogens can multiply in moist environments on farms and because animals consume large volumes of feed. The routine practice of choosing one isolate to represent the *Salmonella* population present in a sample may mean that important serotypes present at lower frequency may be missed (Siceloff et al., 2022). In the future, it may be helpful to adopt metagenomic methods able to detect and characterize multiple serotypes that may be present in a sample.

A complementary approach to the source attribution methods described here also involves identifying serovars of concern using genomics. These emerging methods are reviewed on the answer to question #1. By directly targeting profiles of virulence genes or the pangenome, these methods can provide an early warning of possible serovars or indeed isolate-level pathogen subpopulations that might be responsible for an increased risk of salmonellosis burden. Since these methods can be regularly updated using all relevant *Salmonella* isolates submitted to NCBI, they could be combined with other foodborne surveillance and monitoring systems to provide a more rapid response to evolving changes in *Salmonella* infectivity and virulence. This would address some of the statistical power and resulting time lag limitations described earlier for the *Salmonella* surveillance and monitoring systems.

Q4.B Should only the most current data (e.g., 5 years) of foodborne illness surveillance outbreak and or pathogen/testing be used?

The most current data should be used for all analyses, but the number of years to be included depends on the rate at which new information is added regarding the spectrum of serotypes responsible for illness changes. Combining data from several recent years in a trend model can smooth out the effects of single annual fluctuations, such as large outbreaks. When data from the most recent 3–5 years do not provide sufficient sensitivity to detect change, data from the most recent years could be combined with discounted data from preceding years; such analyses can still be updated each year with new data, as is done in the IFSAC annual source attribution report. PulseNet collects data from approximately 40,000 isolates each year; FoodNet has data on about 7,200 domestically–acquired illnesses each year, as the surveillance area covers 15% of the U.S. population, while FDOSS collects about 16 poultry–associated outbreaks each year. For poultry samples, many isolates are obtained from carcasses, fewer from ceca and other sources, resulting in thousands of sequenced isolates submitted to FDA's GenomeTrakr. Data from food samples are more limited; they include isolates from the NARMS retail food program and isolates submitted to NCBI by state agriculture departments.

There is also an opportunity to study the results from 2020 and 2021, when the COVID19 pandemic disrupted many aspects of the food supply. Whether there were serotype- or food source-specific effects remains to be examined in detail. If there were, then future estimations may need to consider excluding those years from more general models of attribution.

Q4.C How frequently should the priority Salmonella serotypes associated with poultry be revised considering changes in their occurrence while still ensuring continuity in industry and regulatory testing?

Ideally, a serotype, serogroup, or subtype-based control strategy would be reevaluated at annual intervals for evidence of the effectiveness of control methods, and to provide a timely alert for the need for possible changes in control measures based on a decline, or lack of decline, in illnesses, or emergence of other serotypes. It would seem prudent to retain targets even if those serotypes become rare, as halting the control measures that led to their decrease may provide an opportunity for a rebound.

The frequency of reported infections with targeted serotypes or subtypes can be reviewed annually using case surveillance data. Outbreak-based attribution can also be analyzed annually, but the sparsity of data will limit the sensitivity for detecting change quickly, particularly at the serotype level. In the last decade, approximately 16 chicken- or turkey- associated outbreaks of salmonellosis were reported each year. To detect meaningful change at the level of a common serotype for one food category, at least 5 years or ~80 such outbreaks may be needed to provide reasonable sensitivity to detect a change. Successful prevention, that reduces the number of outbreaks, could stretch this time span further.

Ideally, source attribution analyses based on the IFSAC WGS model would be repeated annually so progress can be measured by the estimated overall number of illnesses that can be attributed to chicken as well as the number caused by targeted serotypes, and other serotypes emerging as sources. This method depends on two analytic datasets, one of nonhuman isolate sequences, and the other of human isolate sequences. The dataset of nonhuman isolate sequences may increase at a rate that would allow the training data analysis to be repeated at regular intervals, (e.g., 1–3 years), and data representing older sources are minimized or excluded. More strain sequences could be added from other sources, (e.g., the APHIS NAHMS studies or state agricultural databases). Sequences of *Salmonella* strains isolated from humans are now being added to the PulseNet database at the rate of ~40,000 each year, of which ~7,200 are in FoodNet sites. Once appropriate sequencing workflows and automation can be established, it will likely be possible to repeat the WGS-based IFSAC source attribution predictions at annual intervals.

Subtype-specific prevention strategies will likely need to be modified as the incidence of infection with specific serotype or subtype targets change. It would be appropriate to include a regular assessment of the need for modification of targets, ideally annually, as part of a regulation, as well as guidance on likely methods to be used and a mechanism to evaluate the role of improved methods and data sources in making assessments. For example, reevaluating the prevention strategy could be indicated if: (1) no change was observed in the incidence of infections caused by a serotype (as tracked in FoodNet surveillance) at the end of at least two years of full implementation (or other time window indicated by a power analysis, accounting for the expected rate of reduction) compared with a chosen baseline, such as the prepandemic one (2016–2018) used to assess progress towards the Healthy People 2030 Goal, and (2) the WGS-based IFSAC model and possibly other analyses indicate no significant change in the percentage of infections caused by that serotype attributed to chicken. A statistically significant or major (e.g., ≥15% increase or decrease) change in illnesses attributed to chicken overall or in a targeted serotype, serogroup, or subtype, should merit reassessment of the prevention strategy. A method should be clearly defined for the target metrics (e.g., confidence metric, p value, % change) required changes in guidance or policy based on the results of these assessments. Because of data limitations, reassessment may also be deemed appropriate to protect public health, even if the changes do not reach formal statistical significance, for example, due to applicable lessons learned from other contexts or advances in research.

Q2: Microbial criteria at preharvest level

What types of microbiological criteria could be established to encourage control of Salmonella at preharvest (i.e., in live birds on-farm)? Should FSIS consider qualitative microbiological criteria for control of the presence of Salmonella in a flock when they are presented for slaughter? How could FSIS use these criteria to address Salmonella serotypes most frequently associated with human illness? What industry data would provide evidence of control?

Summary of Question 2 Response FSIS does not have jurisdiction to mandate testing on the farm level. However, because of microbial criteria set for postharvest poultry, testing of incoming flocks and their environment may assist in processors in achieving target reduction of *Salmonella* prevalence and levels coming into their facilities. Before deciding whether qualitative vs. quantitative data is best for determining control of *Salmonella* in a flock, substantially more data (including industry and university research data) should be analyzed to ascertain, the correlation between *Salmonella* prevalence, levels, and serovars, either in flocks presented at slaughter or on farm shortly before presenting for slaughter. Power of detection and enumeration needs to be sufficient to provide evidence on both process control and long-term association of contamination trends in flocks with farm features and farm management measures (including any potential associations with indicator organisms), (i.e., the real-world cost-effectiveness of on-farm control measures).

In considering possible MC or testing programs to encourage *Salmonella* control preharvest, the following conclusions emerged:

- U.S. poultry producers voluntarily implement several *Salmonella* control practices on farms and use qualitative testing to monitor the effectiveness of management in breeder flocks, hatchery, grow out, and transport. This approach has resulted in the lower prevalence on chilled carcasses, but not resulted in fewer illnesses associated with poultry.
- Multiple routes of contamination have been observed, and no single control measure has been found to be effective in controlling *Salmonella* on farms. Hence, farms should be incentivized to apply a rigorous multiple-barriers approach that combines a suite of best practices, including testing at multiple points (such as feed, litter, etc.) to inform farm management operations.
- Qualitative *Salmonella* testing of breeder flocks is recommended to avoid using contaminated eggs for hatching.
- Vaccines are likely the only serovar-specific control strategy at preharvest but will not eliminate all *Salmonella* from flocks. Other farm-based measures (see Management Practices for Controlling *Salmonella* at preharvest in this document), while not directly serovar-specific, will similarly reduce but may not eliminate all *Salmonella*.
- Feed treatments (e.g., heat), preventing recontamination, and qualitative testing for the presence of *Salmonella* in feed are recommended to control this important entry route.
- Sampling protocols and assays exist to test on-farm environmental samples (e.g., litter, feed, water, dust) for both detection (qualitative assays) and enumeration (quantitative assays). While quantitative data theoretically could support a more accurate assessment of risk and mitigating actions, little published research exists to assess the additional value of enumerating *Salmonella* in environmental samples compared to presence/absence, or what levels of *Salmonella* should trigger corrective actions. In designing sampling protocols, frequency, and number of samples need to account for currently achievable sensitivity.
- Testing the farm environment (litter, feed, water) for *Salmonella* 1–2 weeks prior to slaughter could help identify highly contaminated flocks and inform immediate management actions (e.g., processing schedules, transport, remediation of houses, or other mitigation strategies on the flock).
- Testing of *Salmonella* in birds at receiving for slaughter could also inform future control actions and process control to be implemented on farms or at processing. These tests could be complemented by testing for a subset of high-concern serovars. However, complex serovar occurrence patterns at different stages are likely to complicate interpretation and actionability.
- More research is needed to quantify the impact of preharvest control measures and combinations thereof on patterns of *Salmonella* levels, which would also improve the ability of risk models to estimate impacts and, hence, the cost-effectiveness of *Salmonella* control actions.
- Assays to detect specific serovars of concern exist but are currently cumbersome. New molecular methods to rapidly identify (or quantify) multiple serovars are promising but require additional validation.

Q2.A Should FSIS consider qualitative microbiological criteria for control of the presence of *Salmonella* in a flock when they are presented for slaughter?

To determine qualitative MC that are meaningful to assess the presence of *Salmonella* in flocks presented at slaughter, one should consider the existing data regarding testing to verify *Salmonella* control on the farm. The Joint FAO/WHO Expert Meeting on Microbial Risk Assessment (JEMRA) recently reviewed data on control measures for *Salmonella* in the broiler production chain (World Health Organization (WHO), 2022a). The panel concluded that no single control measure is sufficient to control contamination prevalence or level in poultry meat. Rather, utilizing multiple strategies, such as those outlined below as best practices for the poultry industry, is most effective to ultimately reduce the carriage of *Salmonella* through to the food supply. Testing for *Salmonella* in breeder flocks and eggs intended for broilers is recommended to exclude eggs from infected flocks being transported for hatching.

Current US practices include testing for the presence of *Salmonella* in hatcheries, as well as at breeder and broiler houses, to inform voluntary management actions by the company (see Fig. 5 for description of US commercial broiler production and (The Poultry Site, 2011; USDA Animal and Plant Health Inspection Service (APHIS), 2017)). The overall compliance with the USDA FSIS performance standard during processing by the poultry industry is high and further reduction in prevalence may need an incentive for the industry to increase the testing of live birds before processing. A proposed requirement to test flocks before entering the processing plant by FSIS USDA would increase available information to base risk management decisions on, but it is unclear what the impact would be, or what incentives the industry would respond to.

Testing the flocks for the presence/absence of *Salmonella* prior to scheduling for slaughter is a tool with limited documented impact (Nauta et al., 2009; Rasschaert et al., 2008). Testing for specific serotyping could help target vaccine development and focus their use where needed but focusing solely on the currently highly prevalent serotypes may miss shifts in the strains that cause illness. In addition, the fact that serovars detected at processing or in the product might show different patterns from those most commonly observed on farms complicates the use of serovar-specific on-farm testing information to inform risk control in the product. Thus, the industry should consider

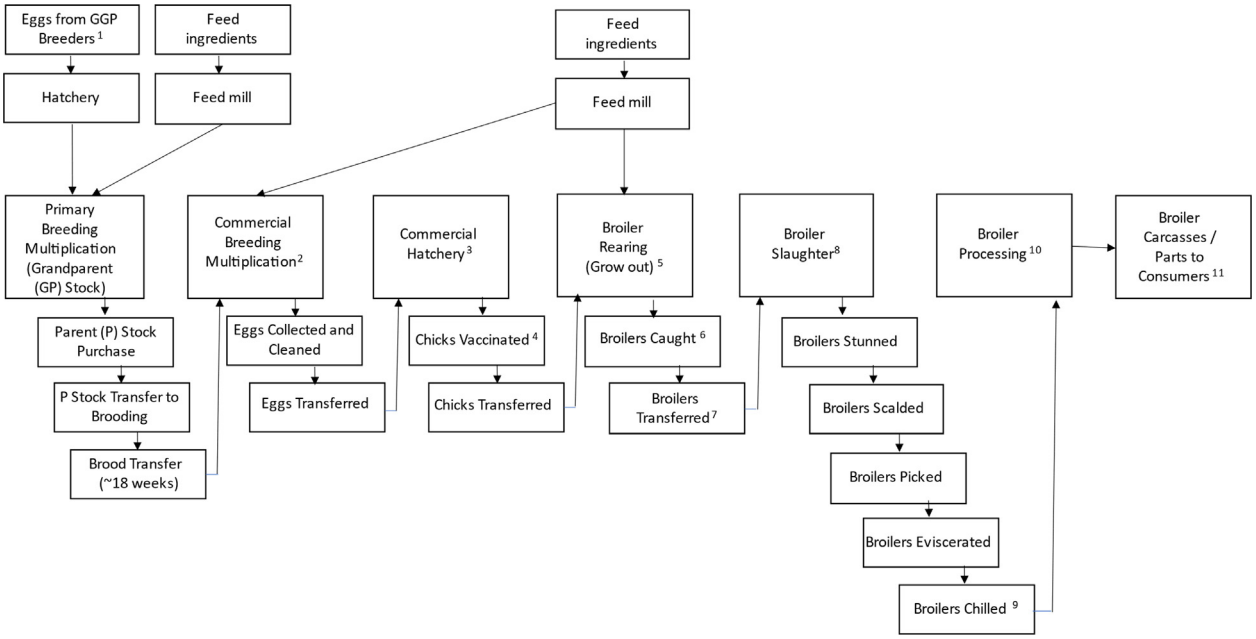

Figure 5. Commercial broiler production in the USA. Assumption 1M birds/week complex (many complexes are 2.5 – 4 x larger).

utilizing testing approaches that test overall *Salmonella* as a means to measure the effectiveness of different interventions at preharvest, but in combination with serovar-specific monitoring to determine if the interventions select for or exclude virulent strains.

What have we been testing, and what could be tested?

In the U.S., there are currently no required industry-wide routine on-farm quality indicator sampling plans or sampling techniques. Hatchery sampling most often relies on testing settle plates (open plates for a given period of time for air monitoring, such as *Salmonella* and dust) within hatcheries and/or testing of paper tray liners, placed under newly hatched chicks, for the presence of *Salmonella*. For primary breeders, breeders, or broilers, *Salmonella* testing is most often done with boot socks (socks worn over shoes as the technician walks around the broiler house) or drag swabs (filter swabs moistened and dragged around the house) (Bourassa, 2016; Buhr et al., 2007). Some facilities have implemented environmental sampling programs where rodents, flies, beetles, and soil around houses are sampled for *Salmonella*. The results of these tests may be used to inform (voluntary) management actions by individual establishments. A range of Salmonella control actions that have been or could be implemented in the U.S. context are summarized in the section "Management Practices for Controlling *Salmonella* at preharvest" (Question 2). This is in contrast to the approach used in Sweden, where a flock is destroyed if *Salmonella* is detected, and all litter is removed and composted for at least 6 months to prevent contamination of the surrounding environments (Appendix B**, Case Study**). This approach is equivalent to requiring negative tests for admittance of a flock to a slaughterhouse.

Overall, sampling and laboratory testing methods exist that are technically feasible to use on farms and which could be used to assess the contamination status of either flock or farm (Garcia et al., 2011; New Zealand Food Safety, 2022; U.S. Food and Drug Administration (FDA), 2008; USDA Animal Plant Health Inspection Service (APHIS), 2019). The most meaningful matrices that can be practically sampled on farms include litter (fecal matter from a flock mixed with litter material), dust deposited on surfaces, feed, and water. Feed and water can be sampled directly. Environmental dust deposited on surfaces can be collected using swabs. Environmental sampling protocol guidelines exist for layers, although not for broiler chickens (U.S. Food and Drug Administration (FDA), 2008). All these matrices can be analyzed using either qualitative or quantitative assays. Cloacal swabs, blood, or organ samples from individual birds can be collected if health issues are suspected with the flock, but are impractical for routine testing, can pose animal welfare issues, and in the case of cloacal swabs, are less sensitive than other approaches (Garcia et al., 2011).

The boot sock method is a simple and relatively inexpensive method to collect litter samples. Samples can be analyzed using culture-based or molecular assays in either qualitative or quantitative fashion. While this sampling approach has sensitivity limitations, in a recent study using IMS-PCR and artificially inoculated litter, the boot sock method was found to have a *Salmonella* LOD of 10, 1, 0.1 CFU/g using enrichment steps of 0, 4–6, and 8 h respectively, with good agreement between IMS-PCR results and culture methods (Hyeon et al., 2019). ISODS (Intermittently stepped-on drag swabs) were also found to have similar detection power as boot socks (as % detected), and higher than drag swabs or direct sampling of litter or feces (Buhr et al., 2007).

In terms of laboratory assays, qualitative (presence/absence) assays include culture-based enrichments, qualitative PCR, and antibody tests (see response to Question 6). Quantitative culture-independent molecular assays that can be used to test on-farm matrices (e.g., litter, dust, feed, water, etc.) are reviewed in **Question 6** (Appendix B Table B2 for approved quantitative methods, and Table B3 for select examples methods under development but which have not yet been validated as time of writing of this document). Quantitative culture-based assays are primarily MPN approaches (USDA Food Safety and Inspection Service (FSIS), 2014b). Qualitative (presence/absence) assays include culture-based enrichments, qualitative PCR, and antibody tests. Many of the qualitative or semiquantitative (i.e., above/below a specified concentration threshold) assays are approved by NPIP, AFNOR, or AOAC (USDA Food Safety and Inspection Service (FSIS), 2022b).

Q2.B How could FSIS use microbiological criteria to address *Salmonella* serotypes most frequently associated with human illness?

There are multiple entry points for *Salmonella* during production that can lead to contaminated carcasses at slaughter (Fig. 5), and it can be important to distinguish the serovars present between different

stages to identify entry routes and enact appropriate controls where they can be most effective. Serovar occurrence patterns vary by region. In an analysis of data from 2016 – 2020, Siceloff (Siceloff et al., 2022) found regional differences including higher proportions of serovars Infantis and Typhimurium in the Atlantic region (states including CT, DE, MA, MD, ME, NH, NJ, NY, PA, RI, VT, VA, and WV) and higher proportion of serovar Schwarzengrund in the Southeast (states including AL, GA, FL, KY, MS, NC, SC, and TN), with the exception of the state of Georgia, which has a higher proportion of Kentucky. In addition, serovar occurrence patterns vary through the production and processing chain (Fig. 6). For example, for broilers produced in Georgia, serovar Kentucky was the most common serovar in breeders (68%) during production but not at processing (Siceloff et al., 2022). CRISPR-SeroSeq performed on these breeder samples showed that 32% of samples contained multiple serovars, with up to 11 serovars found in a single flock (Siceloff et al., 2022). Hence, potential MC set at different stages of the production chain should consider which serotypes are most abundant at that stage, and whether that stage is a critical control point for a serotype.

Potential microbiological criteria at preharvest

Potential MC applied at preharvest discussed in this section include:

- *Salmonella* testing of the farm environment (e.g., litter, feces, water, dust (Arnold et al., 2009; Bourassa, 2016; Buhr et al., 2007; Mueller-Doblies et al., 2009)) to assess contamination of a specific flock and/or the farm; tests can be qualitative (or as additional risk assessment data and assays become available, quantitative), complemented by serovar identification to assess the presence of high-concern serovars; this test, if sufficiently sensitive, could inform both processing actions for the flock as well as farm management actions.
- Indicator testing of the farm environment, if an association with *Salmonella* presence or levels is demonstrated, with similar purpose as above.
- *Salmonella* testing of a flock presented at slaughter, to verify on-farm process control and provide feedback regarding farm management actions (custom processing of a flock tested at receiving would be unfeasible under common operations).
- *Salmonella* testing of breeder flocks (qualitative, possibly quantitative), to control vertical transmission to broiler flocks.

MC at any stage rely on the availability of a sampling and testing protocol with adequate sensitivity and specificity compared to thresholds of public health importance. In addition, the achievable sensitivity of a testing protocol needs to be matched by adequate sampling frequency or sample numbers to achieve a reasonable detection power, so that results can meaningfully inform management actions associated with the MC, as discussed in **Question 1**.

While there are currently no routine sampling programs being utilized industry-wide, sampling methods and laboratory assays (qualitative and quantitative) that could support preharvest testing or MC exist. For instance, hypothetically, a validated method (qualitative, semiquantitative, or quantitative, see **Question 6**) might be used to test flocks one to two weeks before processing, to inform risk management at processing (as well as during transport, and on farm (e.g., to prepare for the next flock). For example, additional risk-reduction measures could be implemented on higher-risk flocks, or flocks from high-risk farms, when received for processing (see **Question 3**) (USDA Food Safety and Inspection Service (FSIS), 2021a). For this purpose, testing for generic *Salmonella* is expected to provide the most cost-effective and actionable information, although complementary identification of serotypes of concern could allow for a more accurate estimate of risk from a flock and, hence, of the degree of *Salmonella* reduction necessary at processing, if processing conditions allow for such flexibility. Further analyses would be needed to define what

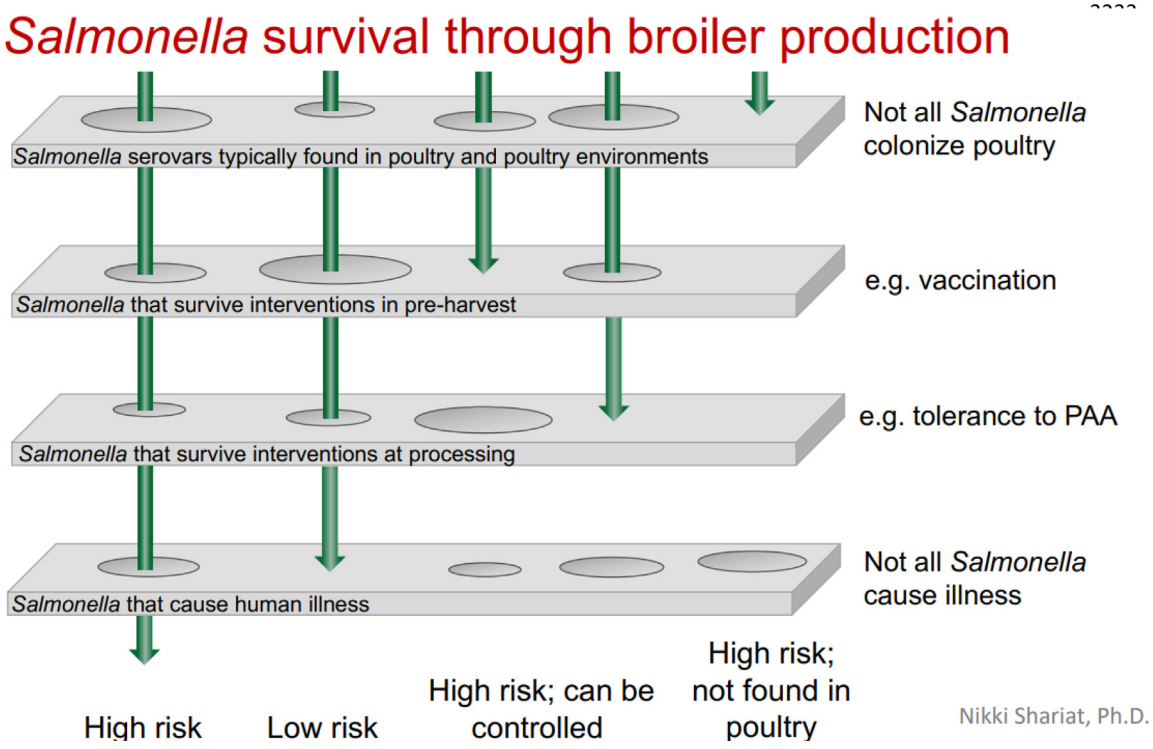

Figure 6. Salmonella survival through broiler production (Shariat, 2022).

"high-risk flock" (or "high-risk farm") means operationally in relation to farm process control and risk management actions, and in relation to (increased) risk mitigation measures needed during processing.

On-farm flock testing results could also potentially be used to reduce the risk of cross-contamination across flocks during processing, e.g., by scheduling more heavily contaminated flocks at the end of a processing day or potentially on days with other contaminated flocks. However, it should be noted that many factors need to be considered if scheduled days for processing flocks are changed. Some of these factors include, (1) trucks may need to be filled from multiple houses, (2) it could be costly for trucks to visit multiple houses or farms in a single day, and (3) growers are paid based on weight from a house/farm. Furthermore, studies suggest that changes in processing schedules to account for higher or lower flock contamination (so-called "logistical slaughtering") result in a weak correlation with pathogen reduction in finished meat samples (Nauta et al., 2009; Rasschaert et al., 2008). One hypothesis for the lack of correlation is possible cross-contamination with inadequately sanitized transport crates or slaughter equipment.

The FSIS does not have jurisdiction at the farm level and cannot require testing. However, per FSIS 2022 proposed framework to reduce *Salmonella*, processing facilities may be required to test incoming flocks for *Salmonella* at receiving (USDA Food Safety and Inspection Service (FSIS), 2022g). Operationally, testing would most feasibly be completed on the farm prior to transport, and data shared with the processing facility. While testing on farm would be informative, coordination across agencies (FSIS and APHIS) would be needed to implement an MC of regulatory relevance.

Salmonella spp. and/or serotype data collected at flock receiving (and potentially at other early processing stages) may be used to provide feedback and create an incentive for targeted control mechanisms applied on farm during live production. For example, as an illustrative scenario where a flock is tested for *Salmonella* levels at receiving, the producers whose flocks are, over a set time window, performing worse than the industry average or worse than a specified threshold for the prevalence or levels of *Salmonella* (or select serovars) could be subjected to additional preventive or remedial actions, or be subjected to financial disincentives from the processor. Independently from potential regulatory actions, the information would provide feedback to producer farms about their process control or the need for increased control actions. For this purpose, *Salmonella* testing on a flock at or shortly after receiving, possibly complemented by serotyping if not already carried out in previous preharvest stages, could provide valuable information to focus on-farm measures.

Conversely, testing at flock receiving is unlikely to have a sufficiently fast time-to-results to inform processing actions on that flock; testing on farm may provide more timely and actionable information on the contamination status of a flock, albeit possibly less accurate. We recommend comparing the value of information vs. cost of testing flocks on farm vs. at presenting for slaughter, using statistical modeling approaches.

More research is needed to determine if qualitative and/or quantitative testing on farm and resulting changes in processing (see also **Question 3**) would significantly affect *Salmonella* prevalence and levels on carcasses and in finished products. In addition, research is needed to determine if testing for non*Salmonella* indicator(s) could provide guidance to companies on which houses have a higher probability of *Salmonella* contamination or level of contamination. At this time, there is no clear correlation between non-*Salmonella* populations (i.e., indicator microorganisms) that can be used to predict which flocks are likely to be highly contaminated; testing specifically for *Salmonella* appears to be the best strategy (see also **Question 5**).

Industry data could be leveraged to better understand how a MC at flock receiving, as well as testing on farms, could be used to inform management actions on farms, and whether enumeration of APC (or other indicator organisms) could predict the likelihood of *Salmonella* occurrence or its levels.

Testing breeders, feed, and the farm environment could provide information on specific *Salmonella* entry or spread routes known to be associated with broiler farm contamination. For example, feed testing implemented in Sweden, and subsequent exclusion of positive lots, has contributed to the reduction of *Salmonella* (National Veterinary Institute (SVA), 2020). Breeder flocks have been found to often be the likely source of *Salmonella* in broilers (European Food Safety Authority Panel on Biological Hazards, 2019). For the purpose of controlling contamination from these routes, qualitative or quantitative testing for generic *Salmonella* –carried out before feed or eggs/chicks reach the next stage- is likely to provide sufficient information to inform process control on farm. Some serovars can be closely associated with some routes, (e.g., *S*. Enteritidis was back-traced from broilers to breeders more often than other serovars, in some studies) (Byrd et al., 1998; Kim et al., 2007). In these cases, testing and controlling for relevant serovars of concern in a stage/route (upstream of the grow-out farm) could be more cost-effective than testing for generic *Salmonella*. However, in general, a serovar does not consistently map to one source only, and hence serovar testing on farm is unlikely to be cost-effective for on-farm process control.

Management practices for controlling Salmonella at preharvest

Any MC, as well as other approaches to risk management, need to be associated with effective risk control measures. MC can verify that control measures are effective and can trigger additional actions in case the MC is not met. In this section, we provide a brief summary of available *Salmonella* control actions that could be used in conjunction with a MC, or on their own, while, in the next section, we discuss serovar-specific MC and control strategies.

Several strategies to control *Salmonella* at multiple preharvest stages exist and have been implemented. Currently, in the U.S., these measures are voluntary; many are recommended as best practices (Buhr, 2022; The Poultry Site, 2011; USDA Animal and Plant Health Inspection Service (APHIS), 2017; USDA Food Safety and Inspection Service (FSIS), 2021a; World Health Organization (WHO), 2022a). Evidence of effectiveness varies by measure, and it is recognized that no single measure will control *Salmonella*. While high animal welfare practices (e.g., housing, transport conditions, etc.) have been reported by Italian researchers to be associated with lower carriage of *Salmonella* in skin and cecal content samples, levels of *Campylobacter* were higher in that group (Iannetti et al., 2020). Therefore, practices should be evaluated comprehensively across hazards as to not trade one contamination issue for another. A nonexhaustive list of preventive control measures at different preharvest stages and potential testing points include:

Breeders:

- Cull visibly soiled eggs that may be contaminated with *Salmonella*-containing feces (Fulnechek, 2022)
- *Salmonella*-free chicks
- Competitive exclusion treatments, prebiotics, probiotics
- Vaccination program (including serotypes from Groups B, C, and D) (Fulnechek, 2022)
- Biosecurity (Cunningham and Fairchild, 2009)
- Rodent and insect control program
- Footbaths / movement of workers
- Testing for *Salmonella* in chicks could be used to verify process control
- Qualitative or quantitative testing in breeder flocks can be used to prevent eggs from contaminated flocks reaching the hatchery

Feed:

- Testing for *Salmonella* in feed could verify that feed does not introduce *Salmonella* on farms
- Control quality of ingredients
- Allow sufficient time in conditioner to give time/temperature/-moisture for *Salmonella* inactivation
- Prevent postpelleting (processing) recontamination. Pay particular attention to cooling area to avoid conditions favorable to *Salmonella* growth
- Clean and sanitize feeders between flocks

Hatchery:

- Cleaning/sanitation programs
- Control air movement in hatchery
- Chemical disinfection program in hatch cabinets during the hatch period
- Do not reuse tray liners
- Competitive exclusion, probiotics
- Testing for *Salmonella* in chicks could verify process control and inform *Salmonella* control actions at the hatchery and/or on a chick flock (e.g., not shipping contaminated chick flocks to grow-out farms)

Grow-out (broilers):

- Only *Salmonella*-free chicks
- Competitive exclusion treatments, prebiotics, probiotics
- Implement biosecurity and hygiene plans
- Litter treatments prior to flock placement and reapplication as needed
- Moisture control (no leaking nipple drinkers)
- Proper working ventilation system (reduce stress on birds – litter amendments if necessary)
- Rodent and insect control program
- Limit movement of workers / visitors
- Testing of flock or flock environment for *Salmonella* before transport to slaughter could determine the contamination level of a specific flock and inform risk mitigation actions at slaughter and processing

Transport:

- Proper feed and water withdrawal time
- Clean and disinfect transport coops to reduce cross-contamination from previously transported flocks that were heavily contaminated to uncolonized flocks
- To the extent possible, limit time in transport cages
- Testing a flock at receiving or at slaughter, summarized by farm over a time window, could provide feedback to producer farms and inform farm management actions. However, test results on individual flocks received are unlikely to be useful to inform management actions for that flock at processing (unless, in the future, a real-time test is developed and used)

Response when *Salmonella* is detected in the preharvest environment (The Poultry Site, 2011)

- Determine the origin of the contamination
- Dispose of poultry litter/feces and other potentially contaminated farm waste in a safe manner
- Enhance cleaning and disinfection of buildings, surfaces, and equipment
- Allow adequate time between flocks if previous flocks were *Salmonella*-positive
- Before restocking the facility, a bacteriological examination should be carried out

As summarized above, a range of *Salmonella* control strategies are or can be implemented at preharvest. A Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) has reviewed evidence on the effectiveness of *Salmonella* control measures in poultry, both on farms and during processing, although findings of the review are not yet available at the time of finalizing this report (World Health Organization (WHO), 2022a). Sweden instituted a rigorous *Salmonella* control program to reduce the prevalence of *Salmonella* on birds grown and processed in Sweden (European Food Safety Authority (EFSA), 2012) (see Appendix B Case Study for details and comparison with U.S. practices). Some practices used to reduce *Salmonella* on U.S. farms, such as biosecurity and control of feed to exclude *Salmonella,* are also incorporated in the Sweden program. The Sweden model further relies on microbial testing for the presence of *Salmonella* in cecal droppings or cecum. If any serotype of *Salmonella* is isolated, the flock is destroyed (Wierup et al., 1995). Because the method of control depends on the eradication of *Salmonella*-positive breeders or broilers, replication of this system in U.S. operations has been, thus far, considered controversial and potentially too costly to implement, while some advocates suggest that a rigorous cost/benefit analysis be conducted. While the economic burden associated with poultry-related salmonellosis has been estimated (Scharff, 2020), a cost assessment of implementing an eradication model, compared to other approaches, has not been carried out for the U.S.

Serotype-Specific control strategy and MC

Vaccines can target a specific serotype or serogroup and are likely the only serotype-specific intervention strategies. Almost all broiler breeders in the U.S. are vaccinated against some serovars of *Salmonella* (Mountainspring and Burleson, 2018). Vaccines can be live deletion mutant strains that are derived from a specific serovar of *Salmonella*, (i.e., *S.* Typhimurium) or autogenous killed-cell vaccines against a cocktail of strains found in a local area.

Commercially available vaccines have been developed for *Salmonella* in the poultry industry. For food safety purposes, they are meant to reduce infective pressure in a flock. Vaccines are commonly administered in-ovo, stimulating the innate and adaptive immune responses for zoonotic poultry diseases and may be administered post-hatch as a finely-aerosolized spray or eye drops, administered the day of placement in broiler houses. *Salmonella* vaccinations of broilers are generally considered impractical as the bird's immune system is not fully competent until the birds are a week or older and handling of birds in the broiler house is problematic. Although autogenous vaccines may require less time for development, they require multiple injections, are labor-intensive to administer, and may be impractical on the large production scale production found in the U.S. Furthermore, they must be remade with newly isolated *Salmonella* from the flock regularly.

Vaccines have likely contributed to a significant reduction in *S.* Enteritidis in eggs and in *S.* Typhimurium for the broiler industry; they may have contributed to the reduction in *S.* Typhimurium compared to other serotypes (Dorea et al., 2010; Hurst et al., 2023; Young et al., 2007). The vaccine industry is currently developing new vaccines against additional serotypes of *Salmonella*. Safety and efficacy requirements of commercial vaccine development take three or more years to develop. Therefore, changes in target serovars – if implemented-should take into account vaccine development timelines.

Serovar-specific control strategies, such as vaccines, could be paired with generic *Salmonella* MC to monitor the cumulative effectiveness of a suite of on-farm control measures. While serovar-specific MC should be considered, serovar shifts over time, as well as inconsistent occurrence patterns between preharvest and processing (viz., there is initial evidence that shifts in the relative serovar abun-

dance, going from breeders to carcasses and products (Siceloff et al., 2022), may complicate the interpretation of test results and risk control decisions). A recent study by the European Food Safety Authority (EFSA) pointed out the expected higher effectiveness of an all-*Salmonella* target for breeding hens, compared to a target including five high-concern serovars (European Food Safety Authority Panel on Biological Hazards, 2019). On the other hand, an additional serovar-specific MC at preharvest could allow focusing resources on strains of higher concern (i.e., be more risk-based) using more targeted measures (primarily vaccines, targeting known sources of specific serovars). Combinations of all-*Salmonella* and serovar-specific MC, at preharvest or throughout the preharvest-processing chain in multiple-barrier fashion, could offer cost-effective trade-offs. However, since vaccines alone do not eliminate a *Salmonella* strain (Buhr, 2022; Young et al., 2007), and a suite of control measures are needed, quantitative *Salmonella* testing is likely to offer the most actionable information.

Several examples of serovar-specific control efforts implemented at a national level, including MC implemented as part of these efforts, are summarized in **Question 1** (part 2), showing various degrees of success.

Q2.C What industry data would provide evidence of control?

Testing for *Salmonella* in birds at receiving for slaughter could inform process control to be implemented on farms or at processing. These tests could be complemented by testing for a subset of high-concern serovars. However, complex serovar occurrence patterns at different stages are likely to complicate interpretation and actionability. More research is needed to quantify the impact of preharvest control measures and combinations thereof on patterns of *Salmonella* levels, which would also improve the ability of risk models to estimate impacts and hence the cost-effectiveness of *Salmonella* control actions.

The Working Group for Questions 3 and 5 felt that Questions 3 and 5 were related, and in fact Question 5 may be a subpart of Question 3. The consensus was to develop a knowledge base of the information currently available and use that to further develop an answer to these questions.

Q3: Microbial criteria on carcasses, parts, comminuted poultry

What types of microbiological criteria could be established for poultry carcasses, parts, and comminuted products prior to applying interventions and after interventions, considering current technology?

A. **Could the quantity of *Salmonella* or quantity of microbiological indicator organisms (e.g., APC) be used? What are the key parameters that need to be considered? What data analysis techniques could be used? How would these criteria be linked to human illness?**
B. **How could serotypes frequently associated with human illness be considered in the development of microbiological criteria?**

Summary of Question 3 Response While indicator organisms may be useful for process control to verify that an intervention step has been applied (such as an antimicrobial carcass rinse), several unpublished studies (university and industry personal communications) suggest a very weak correlation between the concentration of indicator organisms (e.g., APC and Enterobacteriaceae) and presence or level of *Salmonella* postchill. Rather, enumeration of *Salmonella* (not serotype-specific) with a set limit of quantification (limit to be determined pending further research) could be used to identify highly contaminated lots to be diverted for further processing. Removing materials that are highly contaminated is expected to reduce consumer exposure. This may be even more important for comminuted, breaded, stuffed chicken products, injected poultry, parts and pieces, and similar products with a high degree of handling that can disseminate *Salmonella* through commingling of contaminated poultry meat. The lack of serovar-specific enumeration techniques limits the options to quantitation of generic *Salmonella* to be used in combination with serotyping isolates. Tracking changes in predominant serotypes from incoming birds to finished product, trends over time (e.g., 3–5 years), and comparison with clinical isolates associated with poultry will help direct improvement in preharvest control, understand harborage points in the birds and environment, and develop processing intervention strategies.

Q3.A(1) Use of quantification of *Salmonella* vs. indicator organisms as microbial criteria in poultry before and after applying interventions.

Salmonella prevalence (qualitative) testing does not differentiate between carcasses with low levels of *Salmonella* vs. those harboring high levels that are more likely to cause illness (McEntire et al., 2014; Sanchez-Plata, 2022; Siemens, 2022). Risk assessments suggest that while the prevalence of *Salmonella*, which is currently used in the FSIS performance standards for poultry, affects the risk of salmonellosis, neither prevalence alone nor total bacteria populations (e.g., APC; discussed in detail in **Question 5** response) are correlated with the risk of salmonellosis (Lambertini et al., 2019; Oscar, 2021a). Rather, prevalence could be used in conjunction with *Salmonella* enumeration or semiquantitative testing. In the context of current strategies to control *Salmonella* during processing, quantification of *Salmonella* as a performance standard may have a role in identifying product batches harboring higher pathogen levels, which could be diverted for alternate processing. Further refinement of standards may be possible as additional data and interventions become available. It is recognized that several postproduction factors could lead to an increase in *Salmonella* levels in the product before consumption; hence, low but nonzero levels at product packaging could still pose a nonnegligible risk.

Currently, the FSIS Pathogen Reduction Performance Standards do not require that poultry processors conduct pathogen testing. Rather, monitoring compliance with the Pathogen Reduction regulatory requirements is conducted by FSIS. Statistically demonstrable performance that is worse than a set standard (or, in the future, not meeting a selected Key Performance Indicator- KPI (USDA Food Safety and Inspection Service (FSIS), 2022d)) could trigger a Comprehensive Food Safety Assessment by FSIS (or other measures to be established). Establishments that fail to maintain the required documentation, including documented efforts to improve performance, would be subjected to the Rules of Practice at 9 CFR, Section 500 (Code of Federal Regulations (CFR), 2022), including, as appropriate, the Suspension of Inspection. There is some evidence that public posting of establishments' performance may have contributed to prevalence reduction across the industry (Ollinger and Bovay, 2020).

Potential MC approaches applicable to postharvest poultry processing

In poultry processing establishments, data on *Salmonella* occurrence and/or levels could be collected at the following multiple in-process steps (Fig. 5): stunned/slaughtered/"feather on" carcasses, postpicked carcasses, carcasses at rehang, postevisceration/prechill carcasses (both on-line processed carcasses and off-line reprocessed carcasses and parts), postchill carcasses and parts (off-line processed parts), and postprocessing (immediately prior to packaging) finished products (e.g., cut portions, deboned portions, giblets, and comminuted product). Such monitoring data could be used in several ways to inform process control and risk management actions and could be used for performance standards in lieu of the current *Salmonella* prevalence model.

When choosing MC, the feasibility and usefulness of serovar-specific approaches should be considered. For example, the lack of enumeration techniques for specific serovars limits the option of serovar-specific MC to qualitative ones. Several options for MC during processing could be considered, including:

- Prevalence-based (qualitative) MC on the pathogen in the finished product
- Concentration-based (quantitative or semiquantitative) MC on the pathogen in the finished product
- Concentration-based (quantitative) MC on the pathogen during processing (e.g., at one stage, or reduction between two or more stages)
- Concentration-based (quantitative) MC on an indicator organism during processing (e.g., at one stage, or reduction between two or more stages)
- A combination, (e.g., a quantitative indicator MC at processing and a quantitative or qualitative pathogen MC in the finished product)
- A combination, (e.g., of a qualitative MC on selected serovars and a quantitative MC on all *Salmonella)*.

To implement the MC approaches under different scenarios as aforementioned, regular samplings at different stages of the poultry supply chain are required. Although no sampling plan can warrant the absolute absence of *Salmonella*, sampling on a regular basis at targeted points, following well-designed protocols, can be used to measure the deviation of contamination from baseline, determine the needs of corrective plans, and verify the effectiveness of implemented safety management practices and corrective actions. Simulation results conducted by EFSA showed that an increased number of samples tested for *Salmonella* is associated with a reduced possibility of produced batches being rejected (European Food Safety Authority Panel on Biological Hazards, 2010).

If a population-based *Salmonella* MC is established, once the MC threshold population is defined, it is necessary to identify with sufficient accuracy, whether a sample is above or below the threshold (i.e., a semiquantitative approach) in order to prove that the MC has been met. This approach is already used by some companies to assess their processes (Siemens, 2022). At the same time, the full quantification information provides additional information that can be used for trend and root cause analyses in support of process control protocols. The tradeoffs of test sensitivity and quantitative target are discussed in **Question 1**.

For the purposes of evaluating the microbiological reductions achieved during processing, as well as plant hygiene and process control (including biomapping), metrics based on indicator microorganisms such as *Enterobacteriaceae* (EB) or Aerobic Plate Count (APC) could be tested (Bueno López et al., 2022; De Villena et al., 2022; Sanchez-Plata, 2022). The data deriving from such testing are relatively inexpensive and, given that there is no need for a pathogenic positive control culture, the testing may be conducted using simple, standard microbiological methods at onsite laboratories – avoiding the need for expensive and time-consuming sample shipment to centralized biosafety level-2 microbiological testing laboratories. However, at this time, data on the association between metrics based on levels of indicator organisms and presence or levels of *Salmonella* in poultry during processing are still scarce and inconclusive (see **Question 5**).

MC combining *Salmonella* thresholds (or thresholds in selected serotypes) and indicator microorganism thresholds are possible and could provide complementary information on both process control and public health risk. For example, more frequent indicator testing combined with relatively less frequent *Salmonella* testing might provide cost-effective information to assess whether additional risk control measures are necessary, in a timely fashion. Although more evidence is needed to estimate the effectiveness of such approaches, QMRAs based on existing evidence to derive different scenarios of efficacy could inform the public health impact of such approaches and inform more targeted data collection efforts.

Examples of Canadian and EU Microbial Criteria for *Salmonella* in finished poultry products, and *Salmonella* control programs:

- In Canada, a surge in *Salmonella* Enteritidis (SE) infections occurred in 2016–2018, and many outbreaks were linked to raw breaded processed poultry products, including chicken nuggets, chicken strips, and chicken tenders (personal communication, Kate Thomas, Canadian Food Inspection Agency; (Hurst et al., 2023)). The outbreaks prompted regulatory changes requiring that such products either be cooked before being sold or tested and shown to be *Salmonella*-free; the rules were finalized in early 2019 (Canadian Food Inspection Agency (CFIA), 2019). SE cases reported in Canada fell by more than 50%, from ~13 per 100,000 in 2017 to 5.8 per 100,000 in 2019 (Canadian Food Inspection Agency (CFIA), 2019).
- The EU adopted a similar approach for finished poultry products to what was adopted for flocks. The EU regulation (European Commission, 2003) was set in 2003 to take effect in December 2011 targeting the absence of *Salmonella* spp. in carcasses and fresh poultry meat (broilers, laying hens, turkeys). Since by December 2011 most poultry flocks would have been out of compliance from a target $<=1\%$ flock-level prevalence, the EU created a new regulation (European Commission, 2011), also starting in December 2011, where only *S*. Typhimurium and *S*. Enteritidis would have needed to be absent from fresh poultry meat and carcasses, based on an EFSA report citing those two serovars as causing approximately 80% of all salmonellosis in the EU (European Food Safety Authority (EFSA), 2010). Serotyping is carried out via culture methods (White-Kauffmann-Le Minor scheme) (European Commission, 2005). In contrast, poultry meat preparations intended to be eaten cooked (e.g., salted raw poultry meat) were regulated under a different law (European Commission, 2005) requiring the absence of all *Salmonella* in 25 grams and remained unmodified. Despite these measures, no consistent decline has been observed in human salmonellosis in the EU as a whole (Fig. 7).

One possible approach relevant to MC – as well as to other risk management frameworks- involves process control (Halim Lim et al., 2017; Lim et al., 2014; Lim et al., 2015), where the standard to be met is phrased as the consistent maintenance of a microbial (or other) parameter at (or below, if appropriate) a specified level over time, within a specified tolerance. A detailed description of statistical process control

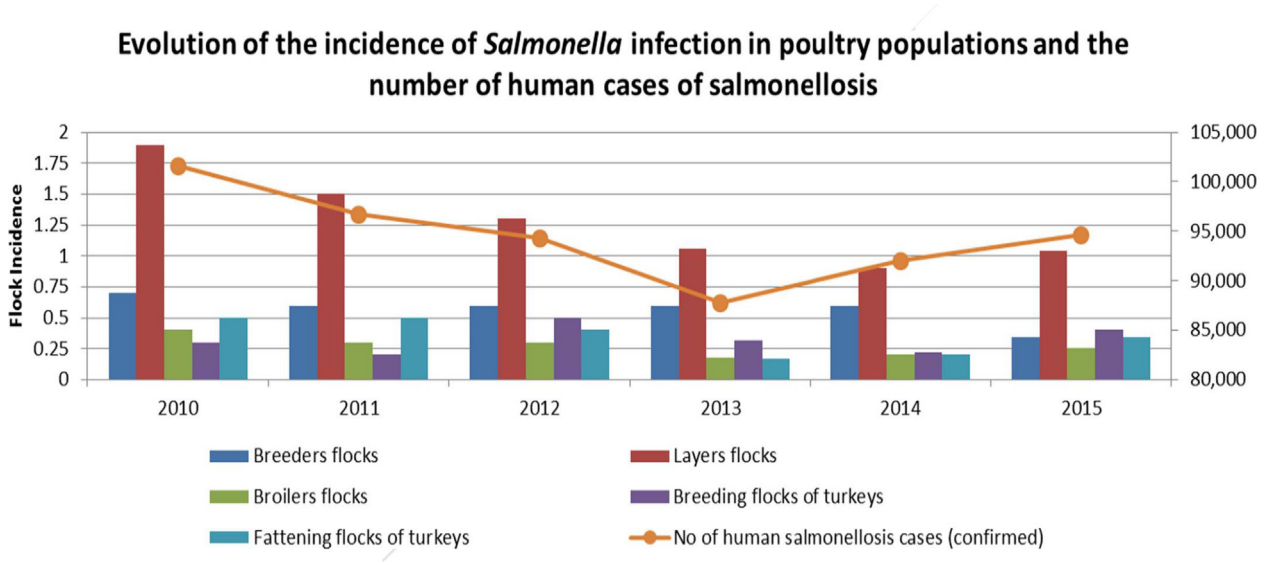

Figure 7. Evaluation of the incidence of Salmonella infection in poultry populations and the number of reported human cases of salmonellosis (European Food Safety Authority Panel on Biological Hazards, 2019) Figure shows recent trends in EU member states, highlighting no clear decline in salmonellosis postintervention. The intervention implemented in 2011 consists of MC prescribing the absence of S. Typhimurium or Enteritidis in 25 g fresh poultry meat, and for all Salmonella in poultry meat preparations (intended to be eaten cooked).

in the context of food processing, including poultry, can be found in a previous NACMCF report (National Advisory Committee on Microbiological Criteria for Foods (NACMCF), 2018). Data from each monitoring location would be statistically analyzed to assess process trends over time and space, (e.g., utilizing Statistical Process Control Charts (e.g., Xi & MR charts)). Other relevant measures may be recorded, such as source of the flock being slaughtered, season of the year, line speed, temperature of scalding, and any relevant metrics of interventions being employed. Statically significant trends departing from SPC parameters (i.e., "out of control" signals) could trigger a corrective action plan to restore the target performance level or to capture the improved performance towards such target. Corrective actions could be informed by root cause analyses or longer-term trend analyses, which could identify factors associated with deviations from target metrics, possibly including occurrence/levels of the pathogen to control. Deviation from process control could also trigger risk control actions in the case that product had been processed while not in-control, for example, possibly including in the future treatments of proven effectiveness. Documented *Salmonella* abatement technologies applicable to raw poultry products exist, such as high-pressure processing or of irradiation (Silva et al., 2018) or peracetic acid (Cano et al., 2021).

A process being in statistical control is not equivalent to the process meeting microbiological specifications (National Advisory Committee on Microbiological Criteria for Foods (NACMCF), 2018). For process control metrics to be part of a MC, a quantitative relationship to *Salmonella* presence or concentration would be needed. That is, this approach would require defining an acceptable risk-based target level of the adopted metric. A process control metric could be assessed at a specific processing stage (similarly to a MC), but could also be evaluated over longer time spans, hence, capturing longer-term processes including the impact of corrective actions that take longer times to be implemented or take effect. Quantitative microbiological analyses would provide substantially improved data to assess the status of process control. Documenting corrective actions would be needed to both demonstrate that such action was taken, and to build further evidence of its effectiveness in real settings. A process control approach involves extensive data collection and analysis, and, hence, could be resource-intensive. However, advances in management and rapid analysis of large datasets could increase feasibility. Issues of equity across large and small establishments should be considered.

Q3.A(2) What are the key parameters that need to be considered in developing MC?

Parameters of the sampling and testing protocol can impact the value of the resulting information, for instance, the point of sampling, the frequency of sampling in relation to product flow rate, sample volume or weight, assay sensitivity and specificity, and how test results are analyzed (e.g., summarized over which time frame or moving window, or by-product flow rate). Key parameters may vary depending on the scope of a MC, (e.g., characterizing the performance of an establishment ("process hygiene criteria") vs. assessing a batch of product ("food safety criteria")). A summary of MC parameters impacting risk, and hence also QMRA estimates of MC impacts, are outlined in **Question 1**. Selected aspects are illustrated below.

Assay parameters. If considering molecular quantification methods for *Salmonella,* instead of culture-based methods such as MPN, the assays should be able to provide consistent, sensitive quantification at low bacterial loads (e.g., under 1 CFU/g, depending on which MC threshold would result in the target illness reduction) in order to capture the majority of the bacterial concentrations observed postchill. For example, in the 2007–2008 poultry baseline study (USDA Food Safety and Inspection Service (FSIS), 2009a), FSIS collected 3,275 samples postchill, and 267 (8.15%) were positive. From those positive samples, the most common level was 0.036 CFU/g, while only 18 samples (6.7%) exhibited concentrations above 1 CFU/g. Extrapolating those figures to the current *Salmonella* prevalence in poultry of roughly 4.5% and assuming that bacterial concentrations have remained the same (it is likely lower, since prevalence has declined since), if 10,000 poultry samples were taken annually, over 93% of the 450 expected positive samples would have concentrations under 1 CFU/g. In contrast, an average of 2–3 samples a month would have loads above that threshold. This stresses the importance of quantification methods reliable at low concentrations, in particular, in the range of considered MC thresholds. The different performances of quantitative tests are further discussed in **Question 6**.

The point above is also true for serotype-specific MC, which would also require high specificity to the considered serotype. In addition,

biases in the detection and quantification of different serotypes may be introduced by different assays, as discussed in **Question 7**.

Metrics demonstrating effective process control. Key parameters include the absolute quantitative microbiological performance or other Key Performance Indicator (KPI) demonstrating sequential microbiological reduction (as measured by the indicator microorganisms, as the expected low quantitative levels of *Salmonella* spp. results may not result in a statistically meaningful reduction between sequential processing steps) through each step of the process. Process control could be demonstrated by statistical stability demonstrating consistent microbiological reductions through the process and/or indications of statistical trends that are evaluated via root cause analyses to drive continuous process improvement. A quantitative threshold in *Salmonella* levels at a specific stage (e.g., finished product) would also be needed to assess whether the process consistently results in sufficient bacterial reduction to protect public health, based on risk targets (while still in the context of continuous improvement).

Q3.A(3). How would these criteria be linked to human illness?

Any MC and associated risk control measures should be risk-based, that is linked to human illness burden targets, and/or associated *Salmonella* levels in finished products. As discussed in **Question 1**, if MC result in a sustained reduction of human salmonellosis, the combination of measures implemented to control *Salmonella* would be reflected in epidemiological data. However, given the year-to-year variability in salmonellosis reporting, a consistent reduction in salmonellosis might still take several years to be evident in the epidemiological data. QMRA models can be used to estimate the impact of individual measures and help determine the extent of testing and corrective actions necessary to reduce the burden by a target amount. For example, QMRA models indicate that reduced concentrations of *Salmonella* on consumer-ready poultry products are likely to yield a reduction in human cases of salmonellosis (see **Question 1**).

Through process control and associated continuous improvement methodologies, the poultry industry could be required to demonstrate processes are consistently capable of meeting the quantitative microbiological performance standards/MC combined with efforts to continuously improve process performance and capability.

Qualitative vs. quantitative MC. Qualitative MC are based on presence/absence (detection/nondetection) of the pathogen in a sample and are thus represented in terms of the proportion of positives out of the total samples (i.e., prevalence). Quantitative MC, instead, are based on the concentration of the pathogen found in a sample. But both prevalence and concentration metrics stem from the same underlying distribution of concentration, i.e., cell numbers per unit weight in the product. Hence, prevalence and concentration MC are not completely separated approaches. As described in **Question 1** and under *Assay parameters* in this chapter, *Salmonella* concentrations in U.S. poultry are low, with only 6.7% of samples above 1 CFU/g postchill in the 2007–2008 poultry baseline study performed by FSIS (USDA Food Safety and Inspection Service (FSIS), 2009a). So, in order to implement a quantitative MC, assays should be able to characterize all concentrations found in poultry products, and at the very least at and above a candidate MC threshold of public health significance. Complexity is added by variability across lots, flocks, establishments, regions, etc., which is not well characterized. Some authors have proposed that a linear relationship between prevalence and illness incidence exists for *Salmonella* at low concentrations (Ebel and Williams, 2015; Ebel et al., 2012), which would make it sufficient to use prevalence in low concentration settings. However, the current qualitative performance standards and subsequent prevalence reduction in poultry meat and products have not resulted in a corresponding decrease in human salmonellosis from poultry in the U.S. Current evidence supports that a quantitative MC on finished product, if set at an appropriately protective threshold and using a rapid test, could identify product batches that require additional processing before being sent to retail (in addition to informing process control). More evidence would be needed to support a quantitative MC upstream during processing, (e.g., carcasses at rehang, which would allow for more time to adapt processing operations based on test results). Evidence relevant to MC options, including levels of indicator organisms, is discussed in **Question 5**.

It is possible that a quantitative MC for selected or all serovars, in combination with a qualitative MC targeting select serovars of high public health concern, might result in a more marked reduction in human salmonellosis, if a stricter MC were to be applied for serovars of high concern compared to current qualitative performance standards. The efficacy of such MC options and specific MC thresholds should be evaluated using QMRAs, as further described in Question 1. Relevant target metrics would be the concentration of generic *Salmonella* in a sample of set weight, and the frequency of detection of high-concern serovars. Even more important than the distinction between qualitative and quantitative targets is the implementation of risk control measures associated with a MC, either voluntarily or via enforcement. Question 1 provides a brief review of risk-based studies that illustrate how both prevalence-based and concentration-based MC, associated with effective control actions, can potentially reduce illness burden, if control actions are implemented.

Q3.B. How could serotypes frequently associated with human illness be considered in the development of microbiological criteria?

Considerations outlined above, including both MC on finished products as well as MC approaches at establishment level that adopt a process control approach, are also applicable to serovar-specific MC.

Adopting a process control approach, the poultry processing industry could potentially be required to assess the serotype of each *Salmonella* spp. sample positive result against an FSIS-maintained list of serotypes of public health concern and maintain failure rate statistics or other KPIs from tracking trends in the frequency of detection of those serotypes. To provide a hypothetical example, a process control metric/KPI could be required to track and trend the frequency of isolation of serotypes of public health concern, reported per standard production unit of measure (e.g., per 1,000 birds slaughtered). Such KPI could be calculated annually by FSIS from accumulated establishment-specific data for each establishment size (as defined in the PR-HACCP regulations). Frequency of sampling is a key parameter to obtain adequate detection power and actionable information and should be included in assessments of MC impact (see Q1). Continuing with this example, all statistically significant trends above a threshold, signaling recurring loss of process control, could trigger a Comprehensive Food Safety Assessment by FSIS, or other measures to be established. Establishments that fail to maintain the required documentation, demonstrating efforts to improve performance and reduce the frequency of the target *Salmonella* serotypes, could be subjected to the Rules of Practice at 9 CFR, Section 500, including, as appropriate, the Suspension of Inspection. Trends signaling loss of process control could also be evaluated (voluntarily or not) using root cause analysis tools to determine the cause, to facilitate efforts to ensure the reduced frequency of detection is sustainably maintained in the future, hence driving continuous process improvement efficiently. In assessing the potential impact of different MC, the effectiveness of establishment-level corrective actions (e.g., a FSIS Food Safety Assessment) should be compared to the effectiveness of risk control measures demonstrated to reduce *Salmonella* (or the target serovars) on the product, as well as combinations of establishment-level and product batch-level approaches.

The answer to **Question 4** provides a reasoned review of the factors and available data and approaches to consider in vetting *Salmonella* serovars of priority public health interest linked to poultry consumption, for the potential design of a serovar-specific MC.

Q5: Use of indicator organisms to evaluate efficacy of microbial reduction steps

There is a documented correlation between a reduction in the quantity of APC between carcasses and finished products and the occurrence of *Salmonella* in finished products for beef, pork, and poultry. How might this information be used to set microbiological criteria to assess process (pathogen) control in poultry?

The objective of microbiological performance standards or MC is to control the contamination of pathogens in foods along the supply chain, and ultimately mitigate associated public health risks. From this perspective, a pathogen-based performance standard provides the most relevant information on which standard to base risk management actions. However, its implementation in practice can be laborious, highly resource-demanding, and sometimes problematic; for instance, considering the very large sample size needed for an acceptable statistical power to correctly classify a food production establishment into either high or low contamination groups, in particular, when the rate of occurrence of contaminated food units is low (Williams et al., 2015). Hence, it is worthwhile to explore methods to supplement the pathogen-based system for the development and implementation of microbiological performance standard by regulatory agencies and the food industry in a more feasible and effective manner.

Employing indicator organisms to reflect the microbiological status in water and foods can be traced back to more than one century ago. Since then, their use has become widespread in microbiological testing programs employed by regulatory agencies and the industry. Albeit, the most common use is to evaluate the microbiological quality of food and predict the product shelf life, indicator organisms are frequently used as safety indicators to determine the presence of foodborne pathogens, identify the insufficiency in meeting GMP requirements, and assess the integrity of process control. The long-established usage of indicators suggests the potential of an indicator-based performance standard in foodborne pathogen control, representing an analytical substitute for the detection of the target pathogen. Indicator organisms do not directly represent the specific target pathogen but are used to suggest the possible presence of a specific organism or source of contamination, or the occurrence of poor hygiene conditions.

While there are no identified organisms that directly reflect the presence or absence of *Salmonella* in poultry (USDA Food Safety and Inspection Service (FSIS), 2021a), several indicators have been used for foods in specific applications for ongoing verification of process control, including aerobic plate count (APC), coliform group, generic *E. coli*, and *Enterobacteriaceae*. These commonly tested nonpathogenic bacteria can be considered as the candidates to evaluate their suitability in the use of indicator-based performance standard for *Salmonella* control, for several reasons. These indicators naturally share ecological association with *Salmonella*, such as their common enteric origin and similarity in taxonomic classification. USDA FSIS uses *E. coli* as the major determinant for the development of process control verification criteria in meat and poultry (9 CFR 310.25). Nationwide, FSIS conducted the baseline sampling of the four indicator bacteria through Young Chicken Survey (USDA Food Safety and Inspection Service (FSIS), 2009a) and Young Turkey Survey (USDA Food Safety and Inspection Service (FSIS), 2009b) to inform the establishment of statistical process control limits and procedures. In addition, a substantial amount of data is generated by poultry industry and/or academic researchers. Federally inspected establishments may collect samples on a routine basis at more processing steps and a higher frequency in comparison to the regulatory sampling programs (De Villena et al., 2022). With the increasing availability of extensive nationally representative data, there is an opportunity to combine and analyze these multisource datasets to address the important question of whether an alternative performance standard built upon indicator organisms can be effective in process control improvement and food safety protection against *Salmonella*.

When considering candidate indicator organisms, several attributes should be considered. Based on the International Commission on Microbiological Specifications for Foods (ICMSF), the following attributes are suggested for the selection of a safety indicator suitable for the use in an indicator-based performance standard (International Commission on Microbiological Specifications for Foods (ICMSF), 2018; Tortorello, 2019):

- Characteristics of the indicator can be stably identified over time and through food production and processing steps.
- The presence or level of the indicator organism indicates (e.g., semiquantitatively or quantitatively) the degree of the potential for contamination, or lack of process control. Testing results provide actionable feedback for process control.
- The detection and/or quantification of the indicator should be easy, rapid, inexpensive, sensitive, reliable, and safe for an in-establishment use.
- The responses of the indicator to the intrinsic properties of foods and extrinsic conditions of the food production and processing environment should behave in a similar but slightly more conservative manner in comparison to the target pathogen (e.g., inactivation, growth, and survival kinetics). For example, growth of the indicator should be slightly faster, while survival of the indicator should be greater in response to the food safety hurdles.
- Quantitative/qualitative correlation between the level of the indicator and the level of target pathogen can be established.

The usefulness of an indicator depends on the purpose of its testing. The reliability of an indicator to predict the presence or amount of a target pathogen, needed in a risk-based MC, is related to the strength of the quantitative correlation between the presence or amount of the two. In many cases, there may not be a strong statistical correlation between the presence or amount of an indicator and the presence or amount of a specific target organism. However, this does not rule out the usefulness of the indicator for the industry as a method of monitoring process control, where indicator trends and patterns can highlight deviations from normal or target conditions and inform adjustments, (e.g., in terms of plant hygiene, achieved microbial reduction, or presence of a deviation to investigate) (Erkman and Bozoglu, 2016). Trends in the levels of the indicator organism may be associated with other process variables, which may ultimately contribute to the presence of the target pathogen. This is an area where biomapping research and industry data on *Salmonella* levels could provide valuable insights.

Mesophilic aerobic populations may be useful to indicate the degree of process control, with the understanding that there are many variables, which may affect the outcome. Mesophilic aerobic bacterial populations, commonly referred to as Aerobic Plate Count (APC), are simply a reflection of the bacteria present in a sample which will grow under the prescribed conditions of plating media, incubation temperature and incubation time. As such, the initial composition of the microbiota in the sample has a significant effect on the outcome of the analyses and the APC in no way reflects the “total” microbial population in the sample.

There is limited and inconclusive evidence on the correlation between indicator organisms and *Salmonella* at specific stages during poultry processing. For instance, one microbiological survey of generic *E. coli*, *Salmonella,* and *Campylobacter* in 20 poultry processing plants suggested a correlation between lower levels of *E. coli* and that of the two pathogens (Altekruse et al., 2009). While one set of FSIS data lacked a meaningful correlation between generic *E. coli* (GEC) and *Salmonella* (Williams and Ebel, 2014). In addition, unpublished data provided by the poultry industry and university researchers suggests that indicator bacteria have very limited predictive value for *Salmonella* prevalence [unpublished industry data, personal communications, A. Siemens, Cargill; R. Kalinowski, Tyson; E. Moorman, Butterball, and ref. (Sanchez-Plata, 2022).].

Approaches to *Salmonella* control based on the concentration of indicator organisms have been investigated or applied for other prod-

ucts. For example, the Agricultural Marketing Service (AMS) analyzes beef samples as part of procurement requirements for federal nutrition assistance programs (Vial et al., 2019, 2020). This program tests samples at two points during processing (boneless beef trims, and the finished product, i.e., ground beef) for Aerobic Plate Count (APC), Total Coliforms, and Generic E. coli (GEC), as well as for the presence of *Salmonella* and STEC and/or *E. coli* O157:H7. Any lot or sublot exceeding critical limits (i.e., MC), including specified indicator concentrations and presence of *Salmonella* in 325 g of product, are identified and diverted from federal nutrition assistance programs (Vial et al., 2019, 2020). AMS beef data from 2015 to 2018 suggest trends could be investigated in the context of other MC that combine target pathogen(s) and indicators. Overall, exceeding of indicator critical limits was not significantly correlated with the presence of either *Salmonella* or pathogenic *E. coli* (Vial et al., 2019, 2020). Based on preliminary findings, the correlation between indicator concentration and *Salmonella* prevalence also appeared weak [personal communication, D. Doersher, USDA-Agriculture Marketing Service and (Vial et al., 2020)]. However, some trends emerged. For example, the correlation between any of the three indicators was found to be higher in pathogen-positive samples, and indicator levels were higher on some days of the week.

The predictive value of indicator patterns and time trends in providing process control and pathogen occurrence information should also be considered, in addition to indicator-*Salmonella* association at a specific point. A promising example is APC reduction between two process stages. Currently, many poultry establishments are sampling poultry carcasses at the rehang step and after chilling using a whole carcass rinse (USDA Food Safety and Inspection Service (FSIS), 1996). Based on retrospective analyses of regulatory data, one study found a weak but nonnegligible correlation between *Salmonella* prevalence and the $\log_{10}$ difference in APC levels from rehang to postchill carcass samples (correlation coefficient of -0.4), i.e. the higher the reduction in APC, the lower the prevalence of *Salmonella* (Williams et al., 2015). The "true" correlation of this relationship may vary from establishment to establishment, but the change in population may be useful as a means of monitoring overall process control and application of antimicrobial treatments. If these findings were confirmed by additional data, ideally including *Salmonella* levels, a MC based on a minimum APC reduction between two processing stages, quantifiable via existing assays, might be a viable MC candidate metric. An assessment of the potential performance of a similar MC was carried out for U.S. beef carcasses (Williams et al., 2017). Conversely, one study compared two groups of establishments implementing two different intervention approaches. Both groups had significant reduction in APC and EB levels throughout processing. However, differences in indicator reduction between the two groups did not correspond to different *Salmonella* levels in the product, suggesting a lack of or weak correlation that warrants further analysis (De Villena et al., 2022).

Another possible approach to indicator-based metrics is provided by the 2014 Modernization of Poultry Slaughter Inspection Rule (USDA Food Safety and Inspection Service (FSIS), 2014a). Rule provisions include voluntary testing for indicators at prechill (rehang) and postchill and encourage the use of the reduction in indicator levels between these two stages as a metric supporting process control, in addition to biomapping (USDA Food Safety and Inspection Service (FSIS), 2015a). Analysis of industry data on indicators collected as part of this program, combined with regulatory and voluntary testing for *Salmonella*, could contribute insights into the effectiveness of indicator-based metrics in predicting potential pathogen contamination, and in supporting process control. Moreover, indicator-based metrics should be considered together with nonmicrobiological process metrics or online inspection endpoints, such as the presence of visible fecal matter on a carcass, or off-line inspection procedures, such as scheduled-and-performed procedures and observed and recorded noncompliances (USDA Food Safety and Inspection Service (FSIS), 2015a, 2017). If this data is used to enhance process control, then the results could be beneficial in lowering the prevalence of nontyphoidal *Salmonella* in raw poultry.

Evidence is insufficient on the association of indicators with specific *Salmonella* serotypes. Data from the poultry industry has shown a quantifiable reduction in *Salmonella* concentration during processing, from hot rehang to the subsequent processing stages to the finished product (chicken parts), consistent with the decline in *Salmonella* prevalence along the process (Beers, 2022; Krombeen, 2022). These data illustrate that, while quantification at the low concentrations observed in finished product can be problematic, quantifications at earlier processing stages for the purposes of a process control target/MC is feasible, as an alternative to process control metrics based on indicator organisms.

In conclusion, within the limitations and evidence gaps described above, APC of other indicators measured at a specific processing stage are unlikely to have a satisfactory predictive value for the presence, level, or virulence of nontyphoidal *Salmonella*. That is, *Salmonella* may be present in samples which contain low, intermediate, and high populations of indicator bacteria. However, a change in APC from an early sampling point on the slaughter line to a final sampling point on the processing line, as well as absolute levels at the final point, may provide useful information about the effectiveness of the process in maintaining hygienic conditions. As such, APC trends may be useful to enhance process control protocols, which in turn could be beneficial for controlling *Salmonella*. Further analyses would be necessary to determine whether it could be beneficial to more explicitly include indicator trends as additional MC metrics. In the future, once *Salmonella* prevalence and/or levels are low enough that testing assays may not be able to reliably detect its presence, (i.e., the proportion of nondetects increases), the value of information from indicator organisms to indicate departures from process control may increase.

Additional considerations on how indicator organisms can be included in MC are provided in **Question 3**. Additional consideration on how indicator organisms can be included in QMRA models to estimate the impact of MC is provided in **Question 1**.

Q6: Methods for quantification of Salmonella

What rapid methods and technologies are available for the quantification of *Salmonella*? How should FSIS make the best use of these methods?

Quantitative MC may be used in the processing plant under FSIS jurisdiction as either a product-release criteria or as a method to measure effectiveness of interventions. Rapid molecular quantitative methods might also be used by the farm to establish levels of *Salmonella* present, both as a potential hazard entering the plant and potentially also to evaluate interventions on the farm. There are two commercially available, AOAC-validated methods for the rapid molecular quantification of *Salmonella* at the time of publication, in addition to traditional cultural approaches such as MPN or plate counts on selective or chromogenic agars. One method includes an enrichment step, while the other does not (Appendix B Table B2). Enrichment means that the sample is kept for a defined time at conditions that favor *Salmonella* growth, if present, to increase the sensitivity of the assay. In currently available assays, a nonselective followed by a selective enrichment allows detection and quantification of *Salmonella* at concentrations as low as 1 CFU/g or mL. However, significant biases due to variability in growth rate and competitive background microbiota are associated with enrichment (see **Question 7**). Not including the enrichment step eliminates most of these biases, but only achieves a higher limit of quantification of about 10 CFU/g or mL.

Q6.A Background: Traditional culture methods for enumeration

Cultural-based methods have historically been used to determine the concentration of microorganisms in a sample (i.e., for bacteria, cells per unit of volume or weight). The population is enumerated or estimated by either using agar plate count or Most Probable Number

(MPN) methods. MPN is utilized when organisms are at lower levels (i.e., <10 CFU/g or mL) or when an enrichment is needed to address resuscitation, possibly from bacterial injury. In the food processing chain, *Salmonella* is subjected to various unfavorable environmental conditions (i.e., heat, sanitizers, storage conditions, etc.) that, if not resulting in an inactivation, can lead to the formation of injured, stressed, or viable but not culturable cells, which can negatively impact enumeration (Du et al., 2007; Petran et al., 2015; Salive et al., 2020).

MPN analysis requires multiple test portions to be analyzed from the same sample, requiring significant resources of time and supplies (media and laboratory disposables) and associated costs. Time-to-result is dependent upon the detection system used for determining the presence of *Salmonella*. After incubation, a reference cultural method or a rapid detection system can be used to determine positive growth. The USDA Microbiology Laboratory Guidebook presents a dilution scheme for MPNs in Appendix 2.05 (USDA Food Safety and Inspection Service (FSIS), 2014b), with analyses following the method in Chapter 4.12 for the detection of *Salmonella* (USDA Food Safety and Inspection Service (FSIS), 2021b). This method describes both the cultural approach and molecular detection system (USDA Food Safety and Inspection Service (FSIS), 2022a). Using a rapid detection system, following the enrichment of multiple samples for MPNs, shortens the time-to-result (USDA Food Safety and Inspection Service (FSIS), 2014b, 2021b).

Plating for enumeration requires selective media, including chromogenic media, to allow for the identification of typical *Salmonella* isolates in the presence of high levels of background microbiota as what would be found in raw poultry. While plating for enumeration is relatively easy from a laboratory operations perspective, in comparison with MPNs, there are challenges to this method due to the inability to recover injured cells or detect fastidious strains or serotypes that may be inhibited by selective additives. As a result, plating methods can underestimate levels of viable *Salmonella* that pose a risk to public health (Du et al., 2007; Li et al., 2014; Oliver, 2010; Reissbrodt et al., 2000; Salive et al., 2020). These limitations can be mitigated by using fluorescent-based methods (Cho and Kim, 1999; Reissbrodt et al., 1996) and non–culture-based enumeration techniques (Ahmad et al., 2017; Ou et al., 2021).

Q6.B. Available rapid methods and technologies

In recent years, methods have been developed using quantitative PCR (qPCR) for enumerating *Salmonella* in poultry. The cycle threshold (CT) value, which is inversely related to the amount of *Salmonella* in the sample, is used to quantify the number of colony-forming units (CFUs) (AOAC International, 2021). Many of these methods require that the sample is enriched in specified media for a prescribed time and temperature (See Appendix B Tables B2 and B3). One method that has been developed without enrichment uses centrifugation of the sample to concentrate cells (bioMerieux Gene-Up Quant *Salmonella*). Two of these methods, Hygiena's SalQuant™ and bioMérieux's GENE-UP® QUANT *Salmonella*, have been validated by AOAC Performance Tested Method certification for enumeration of *Salmonella* in 2021 and 2022, respectively as presented in Appendix B Table B6.

Other enumeration assays that have been developed or are expected to be commercialized with varying levels of evaluations from internal data or collaborations with service laboratories or poultry producers are presented in Appendix B Table B7. These methods include dd-Check *Salmonella*, iQ-Check *Salmonella*, SalLimits, Molecular Detection Assay 2- Salmonella Quantification, and SureCount *Salmonella* Species. Enumeration of *Salmonella* has been studied by using digital droplet PCR (ddPCR) that uses partitioning live encapsulated bacterial cells (Bio-Rad). This approach can be achieved without enrichment. Other methods are being developed including Loop-mediated isothermal amplification (LAMP, see Appendix B Table B3). ThermoFisher developed a multiplex PCR method for enumeration of *S.* Typhimurium and *S.* Enteritidis that is in the process of being validated at the time of this writing.

Q6.C. How can USDA FSIS use these methods

Appendix B Table B6 shows two currently validated enumeration methods commercially available and their specifications (as of the time of adoption of this document). These two methods, Hygiena's SalQuant™ and bioMérieux's GENE-UP Quant Salmonella, have AOAC Performance Tested Method (PTM) approval being evaluated for robustness and tested in an independent laboratory according to guidelines for selective matrices (AOAC International, 2019). The methods were compared to the USDA FSIS MLG MPN method as presented in Chapter 4.12 (USDA Food Safety and Inspection Service (FSIS), 2022a) and according to Appendix 2.05 (USDA Food Safety and Inspection Service (FSIS), 2014b). Since these are enumeration methods, the quantitative validation guidelines were used for the comparison (AOAC International, 2019). The limits of quantification, as shown in Appendix B Table B2, present both the claim based on the target levels as defined in the guideline and the actual limits from the validation study. It should be noted that, as part of AOAC's PTM, concentrations as low as 37 CFU/mL in poultry carcass rinse were tested (AOAC International, 2021, 2022a). Given that most concentrations observed in poultry products are lower than this limit, additional validation may be warranted before new assays are adopted for poultry testing.

USDA FSIS can consider the validation status of each method, the impact of bias during the test methods, the workflow when selecting *Salmonella* enumeration methods, and the ability of the methods to detect the pathogen concentrations commonly observed in poultry. An enumeration assay should also be considered in the broader context of the sampling protocol, including number and frequency of samples collected in relation to product flow rate. Assay parameters relevant to MC are also discussed in **Question 1** (Table 2).

As indicated in the responses above, quantitative methods could be used to establish levels of *Salmonella* leaving the farm, both as a potential hazard entering the plant and also as a method to evaluate interventions on the farm. However, insufficient data is currently available to define actionable limits. Quantitative methods could also be used in the processing plant as either a product-release criteria, (USDA Food Safety and Inspection Service (FSIS), 2022i) or as a method to measure the effectiveness of interventions.

Q7: Methods for selective identification of serotypes/biases

Are there particular approaches that would result in selective identification of the serotypes of public health concern? Is there strain selection bias introduced by laboratory methods? And if so, what strategies can be used to mitigate this bias?

a. **For example, are there approaches to mitigate a potential strain selection bias introduced by the laboratory method?**
b. **If needed, what type of research could be conducted to ensure performance characteristics of current laboratory methods (e.g., enrichment, incubation, prescreening) do not result in a biased serotype detection?**

Summary of Question 7 Response

Laboratory methods used for detection, enumeration, and isolation can be biased toward specific *Salmonella* strains depending on media type, temperature, and incubation time. While the intent of preenrichment is to support growth of all *Salmonella* equally, differences in growth characteristics of *Salmonella* and background microbiota may result in a greater proportion of strains that are more robust. Further bias is introduced if an insufficient number of colonies are chosen for identification, which results in nondetected serotypes within a mixed culture. There is no single procedure to mitigate bias. A single media type or no enrichment mitigates bias by allowing a wider variety of *Salmonella* to be recovered that may be affected by selective

components. On the other hand, multiple selective enrichments or selective plating media often lead to the recovery of a narrower variety of *Salmonella* strains and serotypes. At the time of this publication, detection and quantification methods are under development that, when successful, will offer the possibility of simultaneous detection and quantification of multiple serotypes in the same sample.

Culture-based quantitative methods, as well as enrichments carried out prior to molecular assays, provide an estimate of the number of organisms in a food, relying on the medium employed and the time and temperature of incubation (Petran et al., 2015). This is also the case for qualitative methods for determining presence or absence. Both testing approaches depend upon the conditions for growth and whether they are optimal for the target organism or group of organisms. Failure of the target organism or group of organisms to be detected (or enumerated) can be attributed to intrinsic or extrinsic factors (e.g., time, temperature, media) influencing growth. For the purposes of this response, the focus will remain on *Salmonella* and strain selection bias.

It is well-known that sampling, method choice, and enrichment practices have inherent biases due to a variety of reasons. These biases impact the detection of *Salmonella* and the selective identification of serotypes. Some examples include:

- Whole carcass rinse preenrichment released more *Salmonella* cells than standard rinse aliquot preenrichment. However, false negatives were observed at times and the approach failed to detect all serotypes present; nonetheless, it was found to be the best method to determine true prevalence by Cox et al. (2019). Preenrichment bias impacts all serovars, although it is possible that different serovars are impacted to different degrees.
- Nonselective media recipe may affect the detection of *Salmonella*. Williams et al. (Williams et al., 2022) reported that the proportion of chicken carcass samples that tested positive for *Salmonella* increased from approximately 0.02 to almost 0.06 when the rinse changed from BPW to BPW with a neutralizing agent. This was not the case for detection of *Salmonella* in chicken parts though. Media formulation impacts all serovars. Although it is possible that different serovars are impacted to different degrees depending on their individual growth characteristics, such as generation time under otherwise ideal conditions, utilization of nutrients, and ability to equally compete with other microbes in the enrichment.
- Selective media may affect the growth of *Salmonella*. It was reported that *S.* Enteritidis is selected in Tetrathionate (TT) broth and *S.* Schwarzengrund is selected in Rappaport-Vassiliadis (RV) broth (Cox et al., 2019). The shift in serotypes throughout the enrichment time limits the ability to identify *Salmonella* by culture methods. The use of multiple media recipes (i.e., selective, nonselective broths and agar plates) mitigates the bias by accounting for variation in sensitivity to selective ingredients and atypical biochemical profiles that affect growth on the media.
- Approaches for confirming presumptive *Salmonella* may only require sampling 1 typical colony from a selective plate, to be confirmed (USDA Food Safety and Inspection Service (FSIS), 2022a). Colonies from other selective plates may not be selected if the initial colony confirms as *Salmonella*. Even when a method requires up to 12 colonies to be confirmed across three selective plates representing two selective enrichments (USDA Food Safety and Inspection Service (FSIS), 2022a), routine laboratory practice may only identify 1–2 colonies. It only requires one isolate to confirm and report a sample as positive for *Salmonella* saving on labor and supplies (Cox et al., 2019; O'Bryan et al., 2022).
- The shift in serotypes throughout the enrichment time limits the ability to identify *Salmonella* serovars by culture-based approaches. This shift over time may be due to different growth kinetics of different serovars in selective media, or possibly due to competitive inhibition. Cox et al. (2019) using clustered regularly interspaced short palindromic repeats (CRISPR) analyses to differentiate individual serovars demonstrated that the *Salmonella* serovar profile shifted when original isolates from poultry carcasses were compared with the serovar profile after enrichment.
- This last example of CRISPR demonstrates one approach to mitigating bias, although this approach is not readily available for routine testing in laboratories.
- There are many methods currently and historically available that can differentiate serotypes that are commonly associated with disease. Example platforms include PCR-based approaches (e.g., TaqMan, MLST), WGS, and classic serotyping. However, serotypes are typically identified after selective enrichment for *Salmonella*, which introduces selection bias, as discussed above (USDA Food Safety and Inspection Service (FSIS), 2022a).
- Bacterial cells, including *Salmonella*, could enter a distinct state called the viable but nonculturable (VBNC) state, which is potentially able to be resuscitated. This could impact the accurate detection for *Salmonella* in the VBNC state, hindering enumeration and underestimation of the viable pathogen (Reissbrodt et al., 2000)

A current study characterizing *Salmonella* is being conducted at USDA Agriculture Research Service (ARS) that will investigate a novel molecular assay for the detection of select *Salmonella* serovars in raw meat enrichments that would identify Highly Pathogenic *Salmonella* (HPS) preventing the bias of serotyping from an isolate [(Harhay, 2020) and https://www.ars.usda.gov/research/project/?accnNo=437924)]. The work has potential to be applied to poultry samples for culture isolation using *Salmonella*-specific immunomagnetic separation (IMS) to determine prevalence, direct plating enumeration of *Salmonella* present within an enrichment to examine the limit of detection, the HPS molecular detection assay, and the Neogen (legacy 3 M) *Salmonella* Molecular detection assay for comparison to the FSIS approved molecular detection method. This study will also characterize all *Salmonella* isolated for determination of serotype and antibiotic resistance (https://www.ars.usda.gov/research/project/?accnNo=437924), which is an example of how both identification and detection can be achieved.

Methods for qualitative detection of multiple targets are commercially available (Appendix B Table B3), but at the time of adoption of this report not all have completed AOAC approval process and refinement of applications. FSIS list five AOAC-validated rapid tests for *Salmonella* serotypes or serogroup (iQ-Check *S.* Enteritidis, GENE-UP *S.* Enteritidis & *S.* Typhimurium (SEST), RapidCheck SELECT *Salmonella* Enteritidis Test System & RapidChek CONFIRM *Salmonella* Enteritidis Immunomagnetic Separation (IMS) Kit, Thermo Scientific RapidFinder *Salmonella* species, Typhimurium and Enteritidis Multiplex PCR Kit, Reveal 2.0 Group D1 *Salmonella*) (USDA Food Safety and Inspection Service (FSIS), 2022b). These methods have been validated for raw poultry product, and environmental samples (e.g., raw chicken breast, chicken nuggets, boot and drag swabs, stainless steel, shell eggs, chicken carcass rinsates, poultry feed, etc.) and could be employed as a screen to determine which samples do not contain the target serotype (USDA Food Safety and Inspection Service (FSIS), 2022b).

As serotypes of concern change over time, multiplex PCR assays can target specific serotypes and others are expected to be developed. For example, ANSES (the French Agency for Food, Environmental and Occupational Health & Safety) has recently developed and validated a high-throughput qPCR assay able to identify 40 *Salmonella* serotypes, and distinguish different genomic lineages and polyphyletic profiles (Cadel-Six et al., 2022). This PCR platform can be used either on complex matrices (e.g., foods, carcasses washes, etc.) for rapid screening, or with pure isolates as a confirmation step (personal communication, P. Fach, ANSES). However, at the time of this writing, it is not yet commercially available.

After detection and isolation of *Salmonella*, commercial *Salmonella* serotype identification methods can be utilized. Two include:

- The Neogen NeoSeek *Salmonella* serotyping is a technology that utilizes a targeted amplicon sequencing approach to identify the serotype(s) of *Salmonella* present in a sample. Samples may be submitted as isolates or enrichment broths. If a broth is submitted, up to three *Salmonella* serotypes can be identified if present. Briefly, sample processing involves extraction and purification of DNA, amplification of targets using multiplex PCR, and sequencing on an Illumina MiSeq. Serotypes are called using proprietary software to analyze sequencing results, including MLST (Multilocus sequence typing) targets. This service is not AOAC validated. (https://www.neogen.com/categories/bacterial-sequencing/neoseek-salmonella-serotyping/?recommendationId=2128188658911; Accessed October 29, 2022).
- Check & Trace *Salmonella* (CTS) is a rapid genetic test based on a microarray platform to identify *Salmonella* serotypes (Diep et al., 2019). Each position on the microarray represents a specific DNA marker associated with a unique *Salmonella* target sequence. Spots only become visible if the DNA markers exactly match the corresponding DNA sequences of the *Salmonella* isolate. The combination of present and absent spots yields a pattern. The database includes top serotypes from outbreaks, although is limited and is periodically updated. This method is AOAC Performance Tested Method (PTM) validated (AOAC International, 2022b)

Research is needed to address the items listed above to mitigate bias in *Salmonella* detection and specifically changes in the serotypes of concern due to virulence. A list of select research needs is reported in Question 9.

Q8: Role of whole genome sequencing in developing microbial criteria

How should pathogen characteristics derived from whole genome sequencing (e.g., serotype, virulence, antimicrobial resistance) be considered in the development of microbiological criteria?

There is insufficient information on the presence and absence of genes correlated with pathogenicity and virulence to make recommendations, at the time of writing this document.

Q8.A Recent WGS-based advancements in *Salmonella* characterization

Numerous studies support that Whole Genome Sequencing (WGS) is a critical element in establishing microbiological criteria for *Salmonella.* WGS can define serotype, predict antimicrobial resistance profiles, and offer considerable insight into virulence capacity of an isolate as thoroughly reviewed in Cheng et al., 2019 (Cheng et al., 2019). The greatest value of WGS is at the convergence of serotype, epidemiological, and phenotypic data to differentiate *Salmonella* with high public health relevance from *Salmonella* of limited public health relevance. Some serotypes in the U.S. (e.g., *S.* Cerro and *S.* Kentucky) (Centers for Disease Control and Prevention (CDC), 2018) are much less likely to cause severe, invasive human disease than others (e.g., *S.* Choleraesuis, *S.* Dublin (Jones et al., 2008)). However, *S.* Kentucky has also been identified as a highly invasive strain, notably in Africa (Igomu, 2020).

WGS can be used to differentiate hypo- and hypervirulent serovars and clades by leveraging data beyond serotyping. The presence or absence of virulence genes can and has been defined by WGS. Thus, WGS can contribute to identifying and weighting relevant strain characteristics, including virulence, which inform best targets for control resulting in the greatest public health benefit. WGS studies found that *S.* Cerro isolates have a premature stop-codon in *sopA* (Kovac et al., 2017; Rodriguez-Rivera et al., 2014), which contributes to host cell entry (Raffatellu et al., 2005). *S.* Kentucky is the most commonly isolated serovar from broiler chickens in the United States (USDA Food Safety and Inspection Service (FSIS), 2014c), yet constitutes 0.1% of reported human salmonellosis cases (Centers for Disease Control and Prevention (CDC), 2018). These *S.* Kentucky isolates lack certain virulence genes (e.g., *grvA, sseI, sopE*, and *sodCI* (Beutlich et al., 2011; Cheng et al., 2019) and other metabolism-associated genes (Tasmin et al., 2017) that may reduce its ability to cause severe disease.

Recent phylogenetic analyses have determined that commonly isolated serovars (viz., Newport, Montevideo, Kentucky, Paratyphi B, Derby, Nchanga, Cerro, Bareilly, Stanleyville, Dusseldorf, Livingstone, etc.) are polyphyletic (Cao et al., 2013; den Bakker et al., 2011; Sévellec et al., 2018; Timme et al., 2013; Worley et al., 2018; Yoshida et al., 2016). This underscores that serotype alone cannot define the relative virulence potential of a strain. There are also important clinical implications of polyphyletic *Salmonella* serovars whereas clades of the same serovar differ in the virulence factors that they encode (Miller and Wiedmann, 2016). One example of this is *Salmonella* Kentucky where two distinct clades have been defined by WGS. (Chen et al., 2020).

Q8.B. Current limits to WG sequencing to characterize pathogen virulence

There are several limitations to WGS to characterize pathogen virulence. First, there is a need to expand the public databases (e.g., NCBI) of sequenced isolates associated with illness to determine if eliminating a given serotype by intervention strategies implemented by the poultry industry will simply give rise to another serotype. Research on polyphyletic serovars will offer more insight.

Numerous virulence factors have been identified that contribute to *Salmonella* pathogenicity (Daigle, 2008; Sabbagh et al., 2010). The interactions of these factors and the resulting strain virulence and pathogenicity have not been completely elucidated, but single genes and pathogenicity islands have been identified as key virulence traits. However, there is currently no agreed-upon definition of virulence genes presence/absence profile that can reliably predict the severity of disease.

Host-interactions (e.g., environmental conditions) further complicate the ability to predict virulence. For example, in a study examining the pathogenicity islands (SPI) from *Salmonella* isolated from different environments that included warm-blooded mammals (i.e., porcine, bovine, equine, and avian), environment, and human clinical isolates, it was observed that SPI-1, SPI-3, and SPI-5 had genetic variation across the 13 *Salmonella* serovars tested, while SPI-2 and SPI-4 were well conserved across the same serovars examined.

There is emerging potential for WGS data to be incorporated into quantitative microbial risk assessments (QMRA), but it is currently limited by a lack of standardization in assembly, processing, and integration of WGS and metadata (Chen et al., 2020). The application of WGS to further inform QMRAs and selectively assess serovars of concern is further discussed in Question 1.

Nevertheless, WGS subtyping adds an additional level of discriminatory power that can be used to aid in epidemiologic investigation traceback studies. With continued WGS, a better understanding of important virulence traits and their association with foodborne illness outbreaks may be possible.

Q9: Research needs

What research is needed to support FSIS' new Salmonella strategy in terms of setting microbiological criteria?

Questions 1 and 4 data gaps

- Update estimates of the health burden of salmonellosis and other foodborne diseases at regular intervals
- Analysis of CEA FoodNet data linked with PulseNet to provide the next generation of attribution.
- With appropriate anonymization, add sequences of *Salmonella* isolates from live animal sampling from poultry labs, USDA Animal Plant Health Inspection Service (APHIS) field studies, and the National Veterinary Services Laboratories (NVSL) to national databases, as well as from sampling of feed and feedstuffs.

- Conduct a risk assessment that determines the public health impacts of different risk-based *Salmonella* control strategies to enhance our ability to select and implement risk-based *Salmonella* control strategies. This will require substantially more data to inform which strategy will have the largest public health impact. For example, it remains unknown whether a reduction in *Salmonella* levels in food or a more targeted reduction in specific serotypes or subtypes would result in fewer cases of salmonellosis.
- Complete the *Salmonella* quantitative risk assessments for chicken and poultry to answer risk management questions. https://www.fsis.usda.gov/inspection/inspection-programs/inspection-poultry-products/reducing-Salmonella-poultry/Salmonella-1
- Assess the role of antimicrobial use for *Salmonella* control at preharvest (and its change as a result of implementing a MC and associated *Salmonella* control measures) in the burden of poultry-attributable human salmonellosis, and, more widely, to the spread of antimicrobial-resistant *Salmonella* and genes.
- Assess health impacts associated with long-term health sequelae of *Salmonella* infection, and account for such burden and costs in a cost-benefit analysis.
- Collect quantitative information needed for QMRAs, including *Salmonella* prevalence, levels, and subtypes of concern on poultry within a product lot, between product lots, product form (ground vs. parts, etc.), and between slaughter and processing establishments.
- Refine attribution models that differentiate illness associated with different products, specifically with comminuted, parts, or whole carcasses to focus resources for intervention on highest risk poultry products.
- Identification of those *Salmonella* serotypes or subtypes that pose the greatest concern (e.g., most virulence) and their presence in specific poultry products.
- Dose-response data and fitted relationships for *Salmonella* serotypes of concern.
- Evaluate the frequency of consumer handling and type of preparation practices for specific types of poultry products that contribute to the potential for cross-contamination with other foods or undercooking.
- Conduct studies on transfer coefficients for transfer of the amount of *Salmonella* to other foods and food contact surfaces during consumer preparation of poultry products as part of meals.
- Study human behavior as a route of transmission during normal consumer preparation of poultry products.
- Develop a public awareness campaign to educate consumers on proper handling, preparation, cooking, and storage practices for carcasses, parts, and ground poultry products.
- Increase the number of *Salmonella* isolates sequenced from produce and other nonmeat/poultry sources to strengthen the IFSAC Source attribution model.
- Expand NARMS or other sampling programs to include animal feeds and feed ingredients, as well as poultry-based pet foods.
- Examine the results from 2020 and 2021, when the COVID19 pandemic disrupted many aspects of the food supply. If there were food source-specific effects, then future estimations may need to consider excluding those years from more general models of attribution.
- When metagenomic models are available, assess the presence of even low numbers of relevant serotypes in samples from farm or slaughterhouses.

Question 2 data gaps

- Continue research to determine if non-*Salmonella* quality indicator organism sampling could be implemented, that would provide guidance to companies on which houses have a higher probability of *Salmonella* contamination.
- Develop a cost-benefit assessment for the U.S., accounting for both intervention implementation costs and cost of illness, for various degrees of standard stringency including qualitative nondetection of *Salmonella* in flocks.
- Identify industry practices that alter the presence and variability of *Salmonella* serovars between lots of product.
- Eliminate conditions in house that encourage *Salmonella.*
- Develop cultures for competitive exclusion that are capable of controlling more than a single serotype.

Question 3 data gaps

- Conduct research to determine if qualitative and/or quantitative testing and processing scheduling would significantly affect the number of *Salmonella*-positive processed carcasses.
- Increase the FSIS sampling frequency and sequencing of *Salmonella* isolates from different types of poultry, including comminuted poultry products, mechanically separated meat, breaded raw stuffed chicken, and other highly processed products.
- Expand training and knowledge for plant personnel in statistical process control and Continuous Improvement methodologies.
- Continue research to determine if qualitative and/or quantitative testing and resulting changes in processing scheduling would significantly affect the number of positive processed *Salmonella* carcasses.
- Develop predictive microbiology models (i.e., growth, survival, and thermal inactivation) and/or biomapping studies, characterizing the responses of *Salmonella* prevalence, concentration, and subtypes to operation/handling conditions during processing and post-processing steps.
- Collect evidence on public health impacts of preharvest vs postharvest MC (or an integrated combination). (Q2 and Q3)
- Collect evidence on incentives and BCA on preharvest vs. postharvest MC (or an integrated combination). (Q2 and Q3)

Question 5 data gaps:

- Provide incentive for industry to deposit data (anonymized, non-punitive) on the presence or concentration of indicator organisms (such as APC or others), and the presence/prevalence or concentration of *Salmonella* (possibly by serotype), in the finished product and/or at critical processing stages in the establishment. Use pooled data to develop a comprehensive analysis of quantitative relationship between indicators and *Salmonella.*
- Develop trend analyses and root cause analyses quantitatively linking indicator levels (or other process control metrics, such as variability, trends, or changes between processing stages) to the presence or concentration of *Salmonella* in the finished product and/or at critical processing stages in the establishment.

Question 6 data gaps

- Develop detection and enumeration methods that are based on virulence rather than serotypes that are changing over time.
- Develop enumeration methods that are more laboratory friendly, considering operational workflow.
- Develop a probabilistic evaluation of the accuracy of qPCR methods at the concentrations found in poultry and poultry products.
- Estimates of sensitivity and specificity of quantitative *Salmonella* assays.

Question 7 data gaps

- Develop and evaluate methods that reduce or eliminate the preenrichment step of methods. Investigate the power of whole genome sequencing to accomplishment this.

- Evaluate media recipes, specifically with relevant serotypes from poultry and competition, within the matrices of concerns.
- Determine the significance of atypical phenotypical and biochemical strains of *Salmonella* that routine laboratories may be down-selecting during analyses.
- Evaluate enrichment conditions impacting the growth of serovars of concern (e.g., time, temperature, media formulation, detection).
- Develop metrics to evaluate and mitigate bias in detection, isolation, and identification methods.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Glossary

Term	Definition
Colony-forming Units (CFUs)	CFUs is an estimation of the number of culturable microbial cells in a sample. CFUs are typically expressed as a rate per unit of volume or mass such as CFU/g or CFU/mL.
Fit-for-purpose	Demonstration (validation) that a specific method delivers expected results in a specific matrix or conditions, in relation to the purpose of the information/results.
Infectivity	The ability of an organism to cause infection. In risk assessments, this is incorporated as the probability of human infection following oral exposure to any amount of *Salmonella*. This probability can vary depending on pathogen factors such as the serovar or subtype, and host susceptibility.
Limit of Detection (LoD)	LoD is the lowest concentration of microbial cells (CFU per g or per mL) that can be reliably detected using a standard test.
Limit of Quantification (LoQ)	LoQ is the lowest concentration of microbial cells (CFU per g or per mL) that can be quantified based on predefined goals for of confidence in the estimation. LoQ is typically higher than the LoD as estimating a numerical value requires more information than requiring a positive/negative result.
Key Performance Indicators (KPI)	Critical (key) indicators of progress toward an intended result or goal. Per FSIS: "The KPI will measure the percent reduction in raw poultry samples contaminated with *Salmonella* serotypes commonly associated with human illness as a percentage of all samples analyzed for all types of *Salmonella* contamination. The samples considered in the KPI are those collected from products subject to a performance standard (i.e., from chicken parts, chicken and turkey carcasses, and comminuted chicken and turkey)."(USDA Food Safety and Inspection Service (FSIS), 2022d)
Mean Time Between Failure (MTBF)	Mean period of time between two "failures", (i.e., the inverse of the frequency of failure, where failure is defined as an episode of departure

Glossary (*continued*)

Term	Definition
	from a set target or standard (which may or may not have regulatory relevance)).
Microbiological criteria (MC)	Also called performance standards, MC defines the acceptability of a product based on presence (absence) or number of microorganisms per unit of the product. It can also be based on a biomarker of the microorganism, such as toxins, metabolites, or genetic components. The product unit can be defined as a lot, volume, mass, or area.
Omics	Characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism or organisms. Includes disciplines such as genomics, proteomics, metabolomics, metagenomics, phenomics and transcriptomics.
Pathogenicity	The ability of an organism to cause disease. In risk assessments, this is usually modeled as the probability of clinical disease given infection. This probability can vary depending on pathogen factors such as the serovar or subtype, and host susceptibility.
Performance standards	The establishment of system standards, targets, and goals to improve public health practices. Performance standards set the results to be achieved but not the specific means used to achieve those results.
Performance Test Methods (PTM)	An AOAC program that provides a third-party review and certification for proprietary test method performance.
Poultry	Domestic food producing birds, specifically chickens (*Gallus gallus domesticus*) and turkey (*Meleagris gallopavo domesticus*).
Quantitative microbial risk assessment (QMRA)	Quantitative microbial risk assessment is a mathematical modeling approach used to estimate the risk of infection and/or illness when a population is exposed to microorganisms from a variety of sources, in this case in foods. QMRA estimates can be used to predict the potential reduction (increase) in foodborne illnesses resulting from the implementation of strategies to mitigate foodborne pathogens in foods.
Serotype	See serovar definition.
Serovar	The term serovar is used to distinguish groups within a *Salmonella* species that share distinctive surface structures, namely the O surface antigen and the H antigen that is part of the flagella *(7)*. Consequently, serovars represent phenotypical differences between individual bacteria belonging to a *Salmonella* species, and do not necessarily represent evolutionary differences as elucidated in the *Salmonella* genome. Note that in this report, the term serovar and serotype are used interchangeably.
Subtype	*Salmonella* subtype is a term used to distinguish differences within a serovar (serotype), such as defined using whole genome sequencing (WGS), pulsed-field gel electrophoresis (PFGE) or multi-locus sequence typing (MLST). Subtyping provides a more detailed characterization of heterogeneity between *Salmonella* bacteria than serovar groupings as it is based on genetic differences.
Test sensitivity	The probability that a test performed on a contaminated sample will yield a positive result. For qualitative (positive/negative) test results. This probability is affected by the limit of detection of the test; whereas, for quantitative test results, the probability is affected by the limit of quantification.
Viable but not culturable bacteria (VNCB)	Bacteria that are in the state of exceptionally low metabolic activity and do not divide but are alive and have the ability to become culturable once resuscitated.
Virulence	The ability of an organism to cause severe illness. In risk assessments, this is usually modeled as the probability of severe illness given infection. Virulence in bacteria is mediated by genes often called “virulence factors.” Both pathogen and host factors contribute to whether disease occurs and to disease severity.

Appendix B. Supporting resources for questions 1–7

(See Figs. B1–B7)

Appendix B Q2-Case Study: Sweden’s Salmonella Control Program and comparison with U.S. production practices

The Swedish Board of Agriculture (SBA) began a *Salmonella* control program for poultry in 1970 whereby broiler producers participating in the program were required to meet SBA’s requirements and agree to governmental monitoring of all aspects of broiler production. All flocks that tested positive for *Salmonella* are destroyed and houses are remediated. For those broiler producers participating in SBA’s initial program, the government paid the costs related to destroying the infected flock(s). The concept of control in the Sweden program is that if broilers are never exposed to *Salmonella,* then they cannot become colonized and subsequently they will not be contaminated after processing. Therefore, no chemical treatments are used in the processing

plant due to excluding *Salmonella* positive birds from entering the plant.

In 1984, Sweden made *Salmonella* testing compulsory 10–14 days before slaughter, using a boot-sock method. The government also stopped payments to broiler producers for infected flocks, with private insurers paying 90% of the losses if the producer was a participant in Sweden's SBA *Salmonella* control program. As a result of the on-farm *Salmonella* control program, the European Food Safety Authority reported in 2010 that less than 0.1% of meat samples collected at the slaughterhouse or cutting plant were positive for *Salmonella* (European Food Safety Authority (EFSA), 2012, National Veterinary Institute (SVA), 2021). The latest report also shows very low prevalence (0.27% in chicken meat at production stage; 0% in turkey meat at production stage) (National Veterinary Institute (SVA), 2020). It should be noted, in contrast with other programs, that vaccines are not used in Sweden. Beyond controls used at the farm level, Sweden does not allow immersion cooling in order to reduce the potential for cross contamination at the processing level.

Aside from the mandatory aspect of testing and destroying positive flocks, other aspects of Sweden's control program for *Salmonella* free broiler production are used in the U.S. to reduce *Salmonella* in poultry, poultry feed and the poultry environment, but on a voluntary basis. The programs do not specifically address *Campylobacter*, which remains a predominant foodborne bacterial pathogen in the EU and the U.S.

The Sweden case study is also useful to illustrate not only options and potential impact of MC and multiple-barrier *Salmonella* control measures, but also adopting a food system approach, the crucial role of a strong enabling environment (e.g., insurance, government program, etc.) to support a feasible and sustainable implementation. Although the sizes of individual houses are similar between the U.S. and Sweden, the number of farms and birds are significantly greater in the U.S. (Table B1). This results in unique challenges in applying the Swedish model to U.S. operations in terms of sustainability during any implementation step. However, advocates of the program suggest that the U.S. also has a strong enabling environment that could be leveraged.

Sweden's Five Principles of *Salmonella*-Free Broiler Production (Lindblad, 2007)

1) Start with *Salmonella*-free day-old chickens
2) Rear chicks in a *Salmonella*-free environment
3) Provide feed and water that is free from *Salmonella*
4) Regularly monitor and test for *Salmonella* in the whole production chain
5) Take immediate action whenever *Salmonella* is detected

To implement these principles, Sweden adopted the following practices and regulations (Roberts and Lindblad, 2018):

- All imported birds are quarantined until it is determined that they are *Salmonella* free.
- All feed is tested for *Salmonella* and has strict regulations regarding fertilizing, harvesting, transportation and storage.
- All breeder and broiler houses must be free of rodents and wild birds, and the houses must have an "ante room" for personal hygiene control -- some ante rooms include showers.
- Most of Sweden broiler houses have cement floors, not dirt floors, to improve cleaning and sanitation.
- When a flock is moved out of a house, all the litter must be removed within 24 hours. The broiler house must then be cleaned and sanitized and must sit idle for a minimum of 2 weeks.
- If *Salmonella* is found, the flock is destroyed, and all the litter is removed and composted for at least 6 months to prevent contamination of the surrounding environment(s).
- Twice a year, a state veterinarian visits each facility and takes environmental samples to test for *Salmonella.* Broiler houses and feed mills must be *Salmonella* free to operate.
- Sweden also requires "neck skin" samples to be collected twice a day every day (after carcass air chilling) in broiler slaughterhouses.
- When *Salmonella* is detected, a veterinarian is appointed by the SBA to investigate.
- Whenever *Salmonella* is verified, regardless of prevalence or serotype, the flock is destroyed.

Benefits/Cost Analysis (BCA). In Sweden, a BCA compared Sweden's Salmonella regulations to the less-rigorous controls in Denmark and the Netherlands. Sundstrom et al. (Sundstrom et al., 2014) found the expected increase in human salmonellosis cases and the associated increase in reactive arthritis and irritable bowel syndrome (IBS) to be more costly than any reduction in Salmonella-control costs in those countries. Evaluating the costs and benefits of Salmonella-free broilers in the United States involves tallying up the externalities of broiler production that is currently borne by the U.S. public. Externalities are defined as costs such as the acute and long-term health costs, productivity losses, and pain and suffering due to illness from eating or working with Salmonella-contaminated foods. Scharff (Scharff, 2020) estimates that salmonellosis costs for the U.S. are $5–16 billion annually, with poultry estimated to be responsible for 23% of salmonellosis cases (Painter et al., 2013).

In addition to medical costs and loss of life, *Salmonella* in poultry contributes to environmental losses and potential of spread of antibiotic resistant *Salmonella* strains. For example, the nutrient-rich runoff from broiler facilities has caused algae blooms and "dead zones" in U.S. bays and oceans – the Chesapeake Bay contamination is extremely notable in causing losses to both the fishing and crabbing industries (US Environmental Protection Agency (EPA), 2016). Another concern is the spread of multi-drug resistant bacteria. The impact of adding antibiotics to animal feed can be significant. For example, Armbruster and Roberts, (2018) suggest that 60–80% of tetracycline used in food animals is excreted in feces or urine, ending in animal waste, which, in turn, can be applied as a farm fertilizer and lead to the development of more antibiotic resistant bacterial strains (Armbruster and Roberts, 2018).

(See Table B1, Table B2, Table B3).

Table B1
Comparison of broiler production in Sweden and the United States

Feature	Sweden (Lindblad, 2007)	United States (MacDonald, 2014)	Comments
Genetics	Ross and Cobb 99% (fast) 4.3 lbs. in 33 days Feed Conversion = 1.6 7 grow outs/year	Fast growing birds 6.13 lbs. in 49.5 days Feed Conversion = 1.9 5.5 grow outs/yr	Sweden harvests birds earlier.
Grow out house size	Aver. Size 15,780 sq. ft. Aver. 4.4 houses/farm 100 broiler growers	Aver. size 18,618 sq. ft. Aver. 4 houses/farm 233,770 poultry farms including broilers, eggs, and turkeys (National Agriculture Statistics Service, 2015)	U.S. slaughters 162 million broilers/week vs. Sweden 90 million/yr ERS estimates 33 billion pounds of chicken produced in the United States in 2016 (Haley, 2017)
Housing and cleanout between flocks	Enclosed with sanitary perimeters, as hygiene barrier, with change of footwear and coveralls 100% total cleanout and sanitation between flocks	Enclosed, fan ventilation 77% not cleaned out and sanitized between flocks, 56% no change of clothing, 17 days between flocks	U.S. has no *Salmonella* control regulations in housing design or cleanout regulations between flocks
Litter disposal	All litter removed between flocks and sold as fertilizer to farmers. But if test *Salmonella* positive, on-farm composting of litter.	33% of litter sold for use by other farms in 2011	U.S. has no *Salmonella* requirements for litter. U.S. contamination of soil and water with excess nutrients and pathogens (US Environmental Protection Agency (EPA), 2016)
Feed	Farm-grown wheat. Purchased feed must be *Salmonella*-negative Fishmeal is not allowed in feed.	Provided by contractor-mostly corn and soybeans. No regulations prohibiting *Salmonella* in feed.	U.S. has no regulations on *Salmonella* in feed. Sweden allows no fishmeal in feed because of sustainability concerns.
Antibiotic use in feed	Not allowed.	Antibiotics important in human health being phased out of animal feed by FDA.	U.S. antibiotics used on-farm add to antibiotic resistance of human pathogens.
Vaccination	Not used	Vaccinations against select serogroups used by more than 90% of broiler houses (Mountainspring and Burleson, 2018)	Vaccine development lags behind shifting of predominant serotypes found in U.S. broiler populations.
On-farm *Salmonella* tests	Negative *Salmonella* test in grandparent breeding stock for broilers, hatchery, breeders, feed. Flock tested before slaughter: must be *Salmonella*-negative *to* get into slaughterhouse	No on-farm regulations for *Salmonella* control.	U.S. has no on-farm regulations for *Salmonella* control and no on-farm tests required but conducted on a voluntary basis. On-farm control highest likelihood of controlling *Salmonella* (Pew Charitable Trusts and the Center for Science in the Public Interest, 2014).
Slaughterhouse *Salmonella* tests	Neck skin tests daily for *Salmonella*, gas kill, blast air chill (no immersion chill), no chlorine or other chemicals allowed. If test is *Salmonella*-positive, flock is depopulated.	Performance standard allows 10% *Salmonella* rate in whole carcass rinse test (USDA Food Safety and Inspection Service (FSIS), 2019a).	U.S. does not test daily; allows 10% *Salmonella*-positive tests (USDA Food Safety and Inspection Service (FSIS), 2019a). Chlorine, water absorption and U.S. kill method has potential to reduce flavor & tenderness (Smart Chicken organizational communication at https://www.smartchicken.com/ourstory)

Table B2
Summary of Approved Methods for Molecular Quantitation of Salmonella in Poultry Products (see individual suppler websites for updates)

	bioMérieux	Hygiena
Website for updated information	https://www.biomerieux-industry.com/en-us/products/gene-up-quant-Salmonella	https://www.hygiena.com/food-safety-solutions/pathogen-detection/bax-system-salquant/
Product Name	GENE-UP® QUANT Salmonella	SalQuant™
Technology/ platform	qPCRNonenrichment concentration	qPCR Enrichment
Approvals	AOAC Research Institute (RI) Performance Tested Method (PTM) #061801 GENE-UP QUANT Salmonella is an application of the EnviroPro Sal (PTM #061801) (AOAC International, 2022a)	AOAC RI PTM #081201, (AOAC International, 2021) Official Methods of Analysis submitted in October 2022 (AOAC International, 2022a)
Applicable matrices and test portion sizes	AOAC (AOAC International, 2022a) Chicken carcass rinsates (40 mL) (USDA Food Safety and Inspection Service (FSIS), 2021b) Raw ground turkey (100 g) Microtally (40 mL taken from 200 mL enrichment volume) Internal studies: boot swabs, poultry organs, intestinal tract (personal communication, bioMérieux)	Validated matrix extensions at AOAC in 2021 – comminuted chicken (325 g) and Turkey (325 g) (AOAC International, 2021) Validated matrix extensions at AOAC in 2022 – poultry rinsates (30 mL) (AOAC International, 2021) Internal studies: boot swabs, dust swabs, feet swabs, cloacal swabs, poultry pads (cardboard and straw), feed, trailer drag swabs, cecal tonsils, crop, lungs, liver, spleen, low-level rinsates, chicken pieces, poultry carcass swabs, breaded stuffed raw chicken products
Time to result (TTR)	TTR = <4h	TTR = 7.5 – 9.5 h
LOQ Limit of Quantification Claim (actual validation study)	Nonenrichment: 10 CFU/g or mL (AOAC PTM study 99 CFU/g or mL).	1 CFU/mL 6h rinsate enrichment (AOAC PTM study results present as low as 10 CFU/g or mL). 1 CFU/g 8h comminuted ground enrichment (AOAC PTM study results present as low as 10 CFU/g or mL).
Range of quantification	10 – 10,000,000 CFU/g or mL.	1–10,000 CFU/g or mL.
Accuracy	Within 0.5 log CFU when compared to MPN result	Within 0.5 log CFU when compared to MPN result.
References	User guide available from vendor upon request AOAC RI # 061,801 (AOAC International, 2022a). Poster Presentation (Mills et al., 2022).	User guide https://cdn.brandfolder.io/KA71VJV5/at/gxkwprpcrs9n8kmwhsrcg5/BAX-System-Q7-Users-Guide.pdf AOAC RI #081201 (AOAC International, 2021) Quantification Guidebook Poster Presentation (Stephens et al., 2021; Weller et al., 2021)
Advantages	Nonenrichment reduces bias by eliminating culture-based enrichment, concentrating cells initially and provides faster time to result. <4h same shift procedure Universal workflow and data analysis algorithm Proven compatibility with primary production samples (on farm, boot, internal work).	Universal workflow is utilized with RT *Salmonella* Assay that was AOAC Official Method of Analysis (OMA) approved in 2012 (Wallace et al. (2014)). https://cdn.brandfolder.io/KA71VJV5/at/v5rwkqjq8cbsqhk3s8hqkn/ins-bax-q7-assay-salmonella-rt.pdf Prevalence sample and quantitative sample come from same sample enrichment. The quantitative sample has a shorter enrichment time and keeps incubating for the prevalence sample at a later time. Large dynamic range (1–10,000 CFU/g or mL) or 5 log range. Regulatory sample size validated.
Limitations	Procedure is not the same as the standard qualitative method for presence/absence. Sample prep method requires centrifugation. Data to show the capability distinguishing live versus dead cells to be provided.	Individual curve per matrix requires validation when adding a new matrix (i.e., there are 20 curves today). Culture based bias from the impact of natural microbiota and determination of lag and log phase for each strain.

Table B3
Examples[1] of Methods in Development for Molecular Quantification of Salmonella (check individual supplier websites for updates on validation and approvals)

	Bio-Rad	Bio-Rad	Hygiena	Neogen (Legacy 3 M)	Thermo Fisher
Website for updated information	https://www.bio-rad.com/en-us/category/digital-pcr-food-applications?ID=PU4PWZRT8IG9	https://www.bio-rad.com/en-us/product/iq-check-Salmonella-ii-pcr-detection-kit?ID=d23decd6-2349-4e87-9087-3fe30ec6d3be	https://www.hygiena.com/food-safety-solutions/pathogen-detection/bax-system-pcr-assays-for-salmonella/	https://www.3 m.com/3M/en_US/p/d/v000228752/ for molecular detection qualitative assay	https://www.thermofisher.com/order/catalog/product/PT0100A
Product Name	dd-Check Salmonella[a]	iQ-Check Salmonella	SalLimits[a]	3 M™ Molecular Detection Assay 2- Salmonella Quantification[a]	SureCount Salmonella Species, Typhimurium and Enteritidis Multiplex PCR Assay[a]
Technology/ platform	ddPCR	qPCR	RT PCR threshold testing (positive or negative at 10 cells)	Loop-mediated isothermal amplification (LAMP) and bioluminescence, along with cloud-based software	qPCR
Approvals	Internal studies supporting the use of the method	Internal studies supporting the use of the method AOAC 081,904 (AOAC International, 2022c)	Internal studies supporting the use of the method	In development for quantification using the qualitative molecular detection assay kit platform	Internal studies supporting and submission for approval at AOAC in process as of October 2022
Applicable matrices	Internal work by collaborating with outside service laboratory and poultry producers: Poultry rinsates (30 mL) Ground poultry Microtally	Internal work by collaborating with outside service laboratory and poultry producers:): Ground turkey (325 g) Poultry rinsate (30 mL)	Internal studies by collaborating with outside service laboratory and poultry producers: Comminuted chicken (325 g) Turkey (325 g) Poultry rinsates (30 mL)	Internal data: poultry rinse (30 mL) raw poultry (325 g) primary production (i.e., boot swabs/socks, others in development)	5 matrices in the current study for quantitation (data submitted to AOAC in October 2022) (Hughes et al., 2022; Leak et al., 2022) fresh raw ground turkey (325 g) chicken piece rinse (30 mL) 30 mL poultry carcass rinse
Time to result (TTR)	TTR = 5.5–6 h	TTR = 4.5–5 h	TTR = 4–8 h at LOD10	Total = 7.5–8 h nBPW rinses at LOQ 1 CFU/mL	TTR = 8 h
LOQ	Poultry Rinse 1 CFU/mL	Table showing in Bio-Rad's technical note (need reference) based on strain/serotype and matrix 10 CFU/g	LOD10 based on threshold 4 h rinsate enrichment 6 h comminuted ground enrichment [a]Validated at LOD1 for 6 h and 8 h, respectively	LOQ may vary by matrix. LOQ for poultry rinse: 1–10 CFU per original 30 mL rinse sample	The LOQs for finished goods: 1 CFU per 30 mL rinse from chicken parts or carcasses; 1 CFU per 30 mL homogenized ground meat sample, including turkey, pork, and beef. Environmental 30 CFU per 30 mL
Range of quantification	1 – 1,000,000 CFU/mL	1 CFU/mL or 10 CFU/mL limit	Threshold above 10 CFU/g (mL), qualitative result <10 = negative at this LOD >10 = positive	1–2000 CFU / 30 mL rinse	Finished products: 1 to 10,000 CFU per sample
Accuracy	Twofold difference (personal communication, BioRad)	Technical note	Not applicable due to threshold reporting	Within 0.5 log, CFU (est.) compared to MPN result	Within 1 log, 99.8% agreement Within 0.5 log 80% agreement
References	https://www.bio-rad.com/en-us/category/digital-pcr-food-applications?ID=PU4PWZRT8IG9	User guide upon request Technical note and internal research upon request	User guide Quantification Guidebook Peer-reviewed publications available from Hygiena	Available at upon commercialization	Instruction-For-Use AOAC-PTM study (Stephenson et al., 2022)
Advantages	Nonenrichment reduces bias by eliminating culture-based enrichment and partitioning of intact bacterium cells initially and provides faster time to result Endpoint PCR when the droplet lights up (positive) No enrichment (addressing bias and different strains) Shortened time to result in 6 h Savings in time and labor for creating std	Universal workflow with same *Salmonella* assay Extensive research with a variety of strains addressing growth rate bias Sample sizes up to 375 g, including regulatory sizes Shortened time to results in 4.5 – 5 h, total Same assay as qualitative	Universal workflow with RT *Salmonella* Assay Shorter enrichment time compared to SalQuant (faster) Regulatory sample size validated	Universal workflow with same *Salmonella* assay Technology utilized is recognized and utilized by USDA in the MLG 4.12 Shorter enrichment time compared to qualitative assay Can maintain enrichments for prevalence Calculations are automatically provided with the MDS Software (planned version 2.8.0.0 or later)	Simultaneous detection, quantitation, and differentiation of the three target analytes, (i.e., *Salmonella* spp., *S.* Enteritidis, *S.* Typhimurium) Universal workflow with same *Salmonella* assay with nonproprietary media that runs with the Applied Biosystems QuantStudio 5, maximizing multiplexing Calculations are all automatically managed with the RapidFinder Analysis Software (V2.0 or later) 5 log range of quantitation LOQ at 1 CFU for finished product samples

(*continued on next page*)

Table B3 (*continued*)

	Bio-Rad	Bio-Rad	Hygiena	Neogen (Legacy 3 M)	Thermo Fisher
	curve Sample sizes up to 375 g				Validated with nonproprietary media for all matrices
Limitations	Working on improvement of the throughput allowing more samples to be analyzed Hands-on technician time not optimized and making some improvements with the filter and centrifuge steps Additional instrumentation needed as compared to qPCR	Individual curve per matrix requiring validation when adding a new matrix Impact of microbiota due to enrichment to be considered	Look at SalQuant list and use related	Implementation process to verify method and application as expected (i.e., impact of background microbiota, degree of injury) Offered in Q3 (to be determined) Eight-hour enrichment puts time constraints on completing test in a single work shift	Defining finished product vs. environment samples in the process QS5 new

[a] Include emerging technologies.

[1] This table includes examples of methods that were identified by the Committee as being under development at the time of writing the document. This table may not include methods developed after November 2022, nor should inclusion on this table be considered as an endorsement by NACMCF or FSIS, without validation and AOAC approval.

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