Quantitative Risk Assessment for *Salmonella* in Raw Turkey and Raw Turkey Products

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Glossary[1]

Foodborne Illness Source Attribution	Identification of which foods are the most important sources of selected major foodborne illnesses.
Colony Forming Units (cfu)	Colony forming units (cfu) is an estimation of the number of viable microbial cells in a sample. They are typically expressed as a rate per unit of volume or mass such as cfu per gram (cfu/g) or cfu per milliliter (cfu/mL).
Dose-Response Assessment	The determination of the relationship between the magnitude of exposure (dose) to a microbiological organism and the severity and/or frequency of associated adverse health effects (response).
Exposure Assessment	The qualitative and/or quantitative evaluation of the likely intake of a microbial hazard via specific foods. It provides an estimate of the likelihood and level of the hazard in a specified portion of that food. The exposure assessment may also identify the frequency and amount of food consumed in a given period for a given (sub)population and may combine the information to estimate the population exposure to a microbiological hazard. The exposure assessment details the various steps of the farm-to-fork pathway so that the effect of pertinent steps/processes, or changes to them can be assessed.
Indicator Organism	Indicator organisms, such as Enterobacteriaceae (EB) or aerobic counts (AC), have been used as gauges of process control and to measure the microbial reduction from carcasses at slaughter to post-chill.
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.
Hazard Characterization	The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with the hazard. For the purpose of microbiological risk assessment, it provides a description of the adverse effects that may result from ingestion of a hazard, whether that is a microorganism or its toxin. This should include a dose–response relationship where possible. Those health effects include, for example, diarrheal illnesses, hospitalizations, and deaths. In the context of MRA are usually considered to be acute, rather than chronic, health effects. This component may include identification of

[1] The definitions compiled in this glossary are largely consistent with those adopted in the 2023 NACMCF response to FSIS questions on *Salmonella* in poultry (National Advisory Committee on Microbiological Criteria for Foods (NACMCF). (2023). *Response to questions posed by the Food Safety and Inspection Service: Enhancing Salmonella control in poultry products*. https://www.fsis.usda.gov/sites/default/files/media_file/documents/NACMCF%20Salmonella-Poultry17Mar2023.pdf).

	different adverse effects, including sequalae and their likelihood, for different subpopulations, such as neonates or immunocompromised people.
Hazard Identification	The identification of biological agents capable of causing adverse health effects and which may be present in a particular food or group of foods. It is a qualitative process intended to identify microbial hazards (infectious agents or toxins produced by microorganisms) of concern in food.
Limit of detection (LOD)	LOD is the lowest level of microbial cells that can be reliably detected using a standard test.
Limit of quantification/ quantitation (LOQ)	LOQ is the lowest level of microbial cells that can be quantified based on predefined goals 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.
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.
Pathogen Reduction; Hazard Analysis and Critical Control Point (PR/HACCP)	The PR/HACCP rule, fully implemented in 1996, was designed to reduce the occurrence and numbers of pathogenic microorganisms, harmful bacterial, on meat and poultry products, reduce the incidence of foodborne illness associated with the consumption of meat and poultry products, and provide a new framework for modernization of the current system of meat and poultry inspection.
Public Health Information System (PHIS)	The Public Health Information System (PHIS), a dynamic, comprehensive data analytic system, was launched as part of FSIS' effort to collect, consolidate, and analyze data in order to improve public health.
Post-chill	Post-chill refers to the point in the process where the turkey carcasses exit the chiller after all slaughter interventions have taken place but before entering coolers or proceeding to further processing.
Prevalence	The frequency of a disease in a population at a particular time point. Often expressed as a proportion or percentage.
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.
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.
Receiving	The point in the slaughter process where poultry arrive at the establishment in transport cages, are unloaded, and are hung on shackles before stunning and bleeding.
Rehang	Rehang refers to the location in the process after the hock cutter and prior to evisceration
Risk Assessment	A decision-support tool to provide risk managers with a rational and objective picture of what is known about a health risk and its causes at a particular point in time (FAO/WHO 2021). A scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization.
Risk Characterization	The process of determining the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment.
Serocluster	Genomics classified group of *Salmonella* serotypes, grouped based on *virulence* similarities (see **Appendix A** for more detail)
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. 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.
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.

Executive Summary

Introduction

The United States Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) protects the public's health by ensuring that meat, poultry, and egg products are safe, wholesome, and properly labeled. The Agency is committed to reducing foodborne infections associated with FSIS-regulated products, in particular *Salmonella* illnesses attributable to poultry. *Salmonella* on poultry remains a significant food safety concern in the United States (U.S.). The U.S. Centers for Disease Control and Prevention (CDC) estimates *Salmonella* infection is responsible for over 1 million illnesses, 26,500 hospitalizations, and 420 deaths in the U.S. every year (Scallan, 2011). Of these illnesses, an estimated 66 percent are from food (Beshearse et al., 2021), with over 17 percent from eating chicken and almost 6 percent from eating turkey (IFSAC, 2022). This imposes an estimated $3.7 billion economic burden in a typical year. Almost 90 percent of this burden, $3.3 billion, is due to deaths; 8 percent, $294 million, is due to hospitalization; and the remaining 2 percent is due to non-hospitalized cases (Hoffmann, 2021).

Reducing foodborne illness from *Salmonella* in poultry remains a public health priority. On October 17, 2022, FSIS proposed a regulatory framework to control *Salmonella* in poultry products and more effectively reduce foodborne *Salmonella* infections linked to these products (USDA, 2021; FSIS, 2022b). Central to this effort is this quantitative microbial risk assessment (QMRA) to provide information to address risk management questions about the predicted public health impact of controlling the prevalence, levels[2], and/or specific serotypes of *Salmonella* on turkey presented for slaughter, on turkey carcasses throughout the slaughter process, and/or in final turkey products. This QMRA was refined in response to external peer review (available here) provided in accordance with Office of Management and Budget (OMB) information quality guidelines[3] and in response to interagency review through the Interagency Risk Assessment Consortium.

Risk Management Questions

This risk assessment addresses the following risk management questions:

1. What is the public health impact (change in illnesses, hospitalizations, and deaths) achieved by eliminating at receiving a proportion of turkey contaminated with specific levels of *Salmonella* and/or specific *Salmonella* subtypes?
2. What is the public health impact (change in illnesses, hospitalizations, and deaths) achieved by eliminating final product contaminated with specific levels of *Salmonella* and/or specific *Salmonella* subtypes?
3. What is the public health impact of monitoring/enforcing process control from rehang to post-chill? Monitoring could include analytes such as *Enterobacteriaceae*, Aerobic Count, or other indicator organisms, analysis could include presence/absence or levels and the monitoring could also include variability of actual result versus expected result, log reduction, absolute sample result, or other individual establishment specific criteria.
4. What is the public health impact of implementing combinations of the risk management options listed above?

[2] The terms "level" and "concentration" are used interchangeably throughout this document.
[3] EO 12866 and Modernizing Regulatory Review

Structure and Scope

This is a quantitative probabilistic food safety risk assessment. It examines the relationship between the amount of *Salmonella* (hereafter referred to as the 'level' or 'concentration' of *Salmonella*) and the presence of certain *Salmonella* serotypes on turkey received for slaughter and/or on turkey products (i.e., turkey carcasses, parts, and comminuted turkey) and the probability of foodborne illness. It also examines the relationship between changes in microbiological indicator organisms (i.e., aerobic count) on turkey carcasses from rehang to post-chill and changes in foodborne illnesses.

This risk assessment contains the traditional four components identified in the Codex *Principles and Guidelines for the Conduct of Microbiological Risk Assessment CAC/GL-30* (FAO/WHO, 1999) and in the Food and Agricultural Organization of the United Nations and World Health Organization *Microbial risk assessment – Guidance for food* (FAO/WHO, 2021):

- hazard identification,
- exposure assessment,
- hazard characterization, and
- risk characterization.

Where possible, these four components are referenced in relation to specific sections of the risk assessment; however, these components were developed for a traditional mechanistic risk modeling approach; and this risk assessment does not directly utilize that approach, given the broad scope of the risk management questions. Therefore, this document is organized according to the risk management questions provided above.

The hazard identification identifies the *Salmonella* associated with foodborne illness from consuming turkey. This risk assessment leveraged FSIS' *Risk Profile for Pathogenic Salmonella Subtypes in Poultry* (available here) to identify *Salmonella* serotypes in turkey linked to foodborne illness. This independently peer-reviewed risk profile provided a comprehensive review of the scientific literature and foodborne illness data to identify certain *Salmonella* serotypes in poultry linked to foodborne illness. FSIS expanded on this work through a Cooperative Agreement (FSIS-02152022) with the University of Maryland's Joint Institute for Food Safety and Applied Nutrition (UMD-JIFSAN), in partnership with EpiX Analytics to differentiate *Salmonella* serotypes by virulence using advanced bioinformatics (i.e., machine learning) to evaluate genomic data.

When considering final product standards and receiving guideline risk management options (also identified in this risk assessment as 'scenarios'), the exposure assessment provides a characterization of the amount of *Salmonella* consumers are exposed to from each turkey serving. This exposure assessment characterizes current *Salmonella* contamination levels (colony forming units(cfu) per gram (cfu/g)) on carcasses at the rehang and post-chill slaughter steps and in final turkey products (i.e., turkey carcasses, turkey parts and comminuted turkey products). Special consideration is given to the proportion of higher virulence *Salmonella serotypes* in each product. National Health and Nutrition Examination Survey (NHANES) U.S. dietary data on the product serving size is used to estimate the amount of *Salmonella* consumers are exposed to in a serving of each product. The hazard characterization utilizes a peer-reviewed beta-Poisson *Salmonella* dose-response relationship (Teunis, 2022; Teunis, 2010) modified to take into consideration differences in the virulence of *Salmonella* serotypes based on genomic data (**Appendix A**). This dose-response relationship estimates the probability of illness given a consumer exposure to a specified amount (dose) of *Salmonella* in a serving of turkey.

Finally, the risk characterization component integrates outputs of the exposure assessment and hazard characterization components to provide risk estimates of the probability of salmonellosis per serving from consumption of each type of turkey product. The model is applied to current epidemiological foodborne salmonellosis cases attributed to each type of turkey to assess the number of illnesses prevented by various risk management options (Interagency Food Safety Analytics Collaboration, 2022).

The risk assessment model for turkey serves as a decision-support tool used to evaluate the public health impact of risk management options for the control of *Salmonella* on turkey products. The risk assessment model parameters were adjusted to evaluate final product standard options and explore receiving guideline scenarios based on the available data.

Separate consideration was given to Risk Management Question #3 regarding process control monitoring. Process control is assessed by FSIS in terms of indicator organisms and, as such, cannot be directly tied to *Salmonella* levels and serotypes. The main model outlined here is thus not appropriate for evaluation of the process control risk management options—and the public health impact of those options—in this risk assessment. Nonetheless, the same slaughter and processing paradigm can be analyzed and the connection between *Salmonella* prevalence and levels can be made (**Appendix C**), making this analysis a key part of the *Salmonella* control framework under consideration in this risk assessment.

Conceptual Model

Figure 1 illustrates the conceptual model for the general approach used in this risk assessment to evaluate the public health benefits of the risk management options above. The three-component model: (1) slaughter and processing, (2) growth and die off, and (3) public health outcomes, is sufficiently flexible to describe the U.S. turkey industry and targeted enough to answer the specific risk management questions.

Scenarios (that is, options) for receiving guidelines, process control monitoring, and final product standards serve as inputs to the overall model with estimates of the annual illnesses prevented as the output for each scenario, whenever appropriate. Given the limited current data available for turkey products, results in terms of annual illnesses prevented can only be appropriately assessed in final product standards for comminuted turkey.

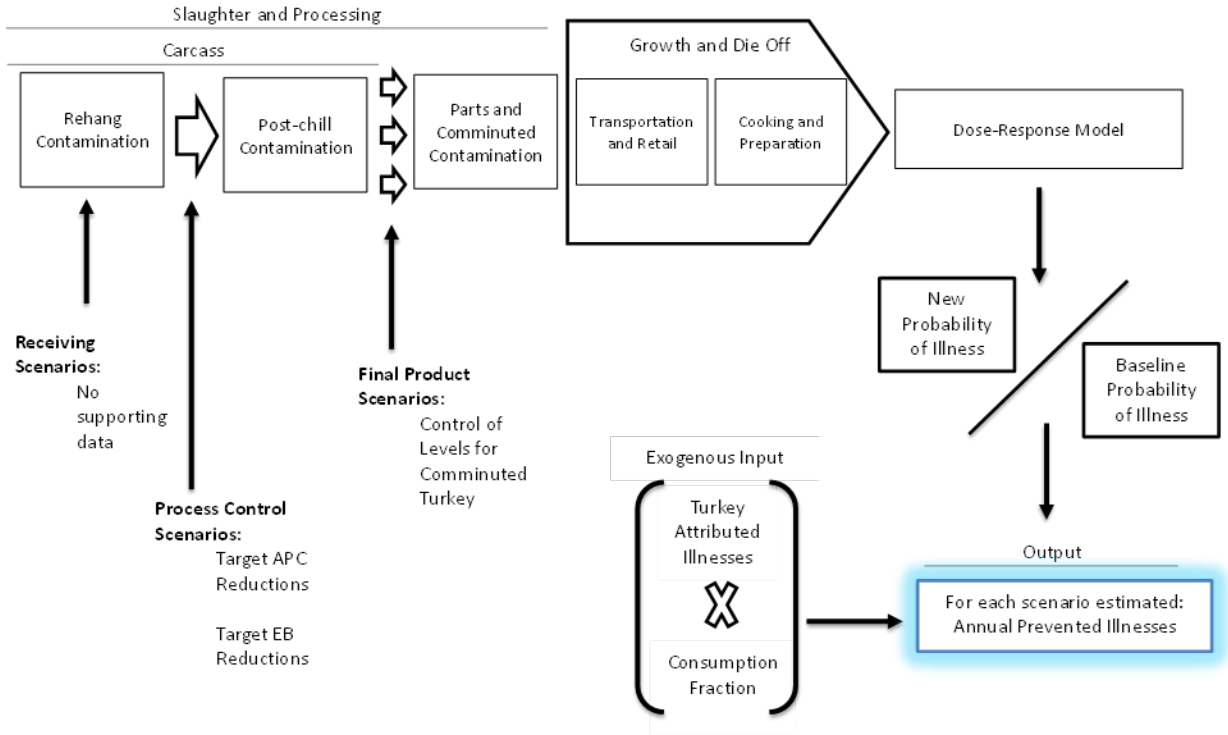

Figure 1: The conceptual risk assessment model.

Data

This risk assessment utilized a combination of FSIS pathogen sampling data, consumption data, and human illness data. The FSIS data used in the risk assessment is presented by turkey product type:

Turkey Carcasses

- Establishment-level FSIS Pathogen Reduction; Hazard Analysis and Critical Control Point (PR/HACCP) turkey carcass post-chill *Salmonella* verification program sample results from 2016 through 2021
- FSIS establishment-level *Salmonella* data from FSIS' young turkey microbiological baseline study from August 2008 through August 2009

Comminuted Turkey

- Establishment-level FSIS PR/HACCP comminuted turkey samples from the *Salmonella* verification program results from 2016 through 2021
- FSIS establishment-level *Salmonella* data from raw ground turkey study from January through March and September through November 1995

Turkey Parts

- No consistent/current industry-wide data is readily available

Human illness estimates used in this risk assessment come from the Centers for Disease Control and Prevention (CDC), including sporadic foodborne illness data from the Foodborne Diseases Active Surveillance Network

(FoodNet) and foodborne illness outbreak data from the National Outbreak Reporting System (NORS)(CDC, 2021a). The Interagency Food Safety Analytics Collaboration (IFSAC) foodborne illness source attribution estimates were also used. Data on the consumption of turkey in the U.S. were obtained from the National Health and Nutrition Examination Survey (NHANES).

Key Findings:

Key findings from this risk assessment are presented below. First, an overview of the descriptive data analysis conducted is presented (including a description of *Salmonella* risk per serving of turkey product), followed by estimates of the public health impacts of various scenarios for final product standards (Risk Management Question #2), receiving (Risk Management Question #1), and process control (Risk Management Question #3).

Data Description

Prevalence

Salmonella prevalence estimates for all existing turkey sampling is presented in Table 1 and described below by turkey product:

Turkey Carcasses

The prevalence of *Salmonella in* FSIS PR/HACCP sampling in turkey carcasses at post-chill has been below 1% since 2016, which is equivalent to less than 20 positives each year out of 2,000 samples across the industry. Further, there historically have been very few *Salmonella*-positive detections in turkey carcasses. Hence, many establishments consistently meet (i.e., pass) current FSIS performance standards based on prevalence (4 out of 56 as the maximum allowable positive samples or approximately 7.1%). Those establishments that sporadically fail to meet the standard are typically lower volume producers. However, some higher volume establishments approach the threshold for allowable percentage of *Salmonella*-positive samples on occasion. However, it is likely that the low rate of *Salmonella* recovery from turkey carcasses is a function of sponge sampling and rinsate limitations than truly indicative of the true *Salmonella* prevalence in turkey. Conclusions that make use of this data should be used judiciously.

The 2008-2009 FSIS young turkey carcass microbiological baseline study, which included both rehang and post-chill sampling points, did estimate prevalence; with 0.1023% prevalence at rehang and 0.0187% at post-chill, representing an 8% reduction in *Salmonella* prevalence during the slaughter process. However, FSIS rehang sampling data has not been routinely collected since the 2008-2009 microbiological baseline, preventing an accurate, up-to-date assessment of prevalence at that slaughter step.

FSIS does not currently enumerate *Salmonella* in turkey carcass samples.

Turkey Parts

FSIS does not currently assess the prevalence of *Salmonella* on turkey parts. Further, FSIS does not, and has not historically, had a sampling program for turkey parts. Therefore, it was not possible in this risk assessment to assess any of the risk management questions for turkey parts. FSIS continues to engage the turkey industry in conversations regarding data sharing. This data gap also presents an opportunity of future data collection and research efforts.

Comminuted Turkey

FSIS established a *Salmonella* performance standard for comminuted turkey in 2016 and the Agency also maintains a *Salmonella* sampling program for comminuted turkey. In current sampling, the national prevalence of *Salmonella* in comminuted turkey fluctuates between 14% and 25%. Prevalence peaked in 2018 but has since dropped to 18% by 2021. In regard to FSIS' current performance standard (7 out of 52 as the maximum allowable positive samples or approximately 13.5%), analyses identified a mixture of establishment sizes not meeting the standard, with the majority of large establishments not passing the FSIS comminuted turkey performance standard in a 52-week period (FSIS, 2023b). **Table 1** provides current estimates for *Salmonella* prevalence statistics at rehang and post-chill.

Table 1: Estimated prevalence by commodity and sampling location.

Commodity	Data Set	Sample Location	Year	*Salmonella* Prevalence	Standard Deviation	95% confidence interval
Turkey Carcass	Baseline	Rehang	2008-09	0.1023	0.000083	(0.0851, 0.1208)
		Post-chill	2008-09	0.0187	0.000023	(0.0106, 0.0292)
	PR/HACCP	Post-chill	2021	0.0028	0.000001	(0.0011, 0.0053)
Comminuted Turkey	PR/HACCP	Post-chill	2021	0.1763	0.00051	(0.1343, 0.2227)

Enumeration

Enumeration data is limited to comminuted turkey samples. In particular, in PR/HACCP samples, a portion of detected samples were further tested for *Salmonella* levels using the most probable number (MPN) estimation method with a limit of detection (LOD) of 0.3 MPN/g (FSIS, 2022a). Samples collected in 2020 were not used in this risk assessment because the MPN analysis was not consistently performed. The compiled comminuted turkey dataset consisted of 1,178 samples, of which 157 were positive on the *Salmonella* screening test. The mean and standard deviation of the lognormal concentration distribution of the population derived from the data were estimated as $\mu = -4.857$ and $\sigma = 2.333$, respectively.

Data analyses indicate that current and past chicken product samples have low estimated *Salmonella* levels at post-chill (<1cfu/g) (see **Table 2**), 84.26% of comminuted turkey products produced in the U.S. having a *Salmonella* level below 1cfu/g. It is rare for consumers to be exposed to a serving from comminuted turkey product that has at least 10 cfu of *Salmonella* per gram.

Table 2: The estimated amount of comminuted turkey product per *Salmonella* threshold level. Further details provided in Chapter 3). The level of detection (LOD) of the quantitative polymerase chain reaction (qPCR) enumeration technology used by FSIS at present is 10 cfu/unit.

	Comminuted Turkey
Tests *Salmonella* Negative	84.26%
Tests *Salmonella* Positive	15.74%
Tests *Salmonella* Positive and ≥1 cfu/mL or /g	12%
Tests *Salmonella* Positive and ≥10 cfu/mL or /g	4%
Tests *Salmonella* Positive and ≥100 cfu/mL or /g	1%

Serotypes

There were 49 different serotypes found in comminuted turkey products, as compared to only 19 serotypes isolated on turkey carcasses in the PR/HACCP program. Reading and Hadar ranked as the top two serotypes in both carcasses and comminuted, comprising more than 30% of the serotype samples for each commodity. Hadar was also observed most often in the turkey microbiological baseline studies and appeared in the top ten CDC FoodNet annual summary from 2020. Although Enteritidis was most frequently associated with human salmonellosis (according to 2020 FoodNet annual summary (CDC, 2022a)), it is rarely observed (or detected) on turkey carcasses or in comminuted turkey products (**Table 19**). Serotype information is not available for turkey parts due to the lack of routine FSIS sampling of this product.

Salmonella serotypes were also categorized into two clusters derived from a machine learning algorithm using virulence factors to estimate the genetic similarity between serovars (**Table 3**). A full description of the methods used by EpiX Analytics is provided in **Appendix A**. The higher virulence cluster 1, of which the most frequent serotypes appearing in comminuted turkey samples are Typhimurium, Hadar and Muenchen, has been identified as the higher virulence grouping of serotypes, as compared to the lower virulence cluster 2. For both turkey carcasses and comminuted turkey, where data available, serotypes were split between seroclusters with approximate average of 0.3 in cluster 1 and 0.7 in cluster 2 using data from 2016 through 2021.

Table 3: Summary of the five most frequent serotypes in turkey by cluster. Note, all serotypes in each cluster are considered to be equally virulent for the purpose of this analysis.

Higher virulence Serotypes	Lower virulence Serotypes
Hadar	Reading
I 4,[5],12:i:-	Infantis
Typhimurium	Schwarzengrund
Muenchen	Uganda
Saintpaul	Agona

The higher virulence serotypes which appear most frequently in FSIS comminuted turkey samples (Hadar, Typhimurium, and Muenchen) are referred to as "serotypes of public health significance" in this risk assessment. The portion of FSIS PR/HACCP *Salmonella* positive samples that are sequenced as a serotype of public health significance is 25% for comminuted turkey product.

Risk per Serving Description

Two virulence-adjusted dose-response models based on the above serotype clusters were used to answer the risk management questions. These models provides a description of risk of illness per serving for poultry products, that can be informative to risk managers.

The scenarios in **Table 4** describe the initial level of FSIS-sampled product in a lot of raw comminuted turkey at the end of production for lots that fail different threshold levels. The risk of illness from consuming a serving of comminuted turkey product varies based on both the amount and virulence of the *Salmonella* that remain after cooking. Additionally, risk from a serving considers the serocluster. The average initial level for failing lots is Table 14 is the average lot level of a lot that tests at or above the initial level, i.e., the conditional expected value. The average dose consumed is a practical description of the range of doses consumed (after transportation and cooking) which is the expected value after applying the attenuation distribution to the average lot level. As previously assumed, the attenuation distribution was calibrated to raw chicken products due to the lack of data appropriately describing all raw turkey products.

These illustrative calculations differ from the probability of illness estimates that are outputs from the simulation in **Chapter 5**, which factors in the full distribution of initial contamination values above the threshold. Following multiplication of the average initial level by the attenuation distribution, we calculate the average dose per serving and integrate each dose-response function across the resulting distribution to calculate probabilities of illness per serving. We also predict the likelihood that lots will fail the different level thresholds. The results illustrate that the average initial contamination levels of failing lots and the average doses per serving increases as the initial level thresholds increase, but in a non-linear pattern.

As has been discussed, the majority of exposures consumers face are to doses of *Salmonella* below the limit of detection of the FSIS *Salmonella* assay (0.03cfu/mL for parts and carcasses; 0.003 cfu/g for comminuted products). Furthermore, a serving that tests at or above 10cfu/g and has a serotype of public health significance is likelier to cause illness than an average serving. Comparison to the baseline probability of illness for comminuted turkey (see **Table 4**), suggests the probability of illness from servings at or 10cfu/g with a serotype

of public health significance is 1,400-folder higher than the average.

Table 4*:* Risk of illness per serving of turkey product based on the initial level of *Salmonella* in FSIS-sampled products.

Measurement	Initial threshold level (cfu/g)				
	0.003	**0.033**	**1**	**10**	**100**
Average level (cfu/g) for failing lots	163	348	1,373	4,249	15,479
Average dose consumed* (cfu/serving) for average failing lot	26	55	218	673	2,453
Probability that dose ≥ 1 cfu/serving in failing lots*	7.2%	9.9%	16.5%	23.6%	33.6%
Probability of illness per million servings* given the average initial level, higher virulence	8,008	11,655	21,844	34,896	56,597
Probability of illness per million servings* given the average initial level, lower virulence	1,583	2,354	4,597	7,608	12,874
Likelihood of consumer exposure to raw product (at or above initial threshold level)	15.7%	7.4%	1.9%	0.60%	0.16%

*Attenuation distribution based on raw chicken products

Indicator Organisms

Multipoint sampling (e.g., sampling at both rehang and post-chill, paired by flock) is required for the evaluation of process control, and thus was not assessed for either turkey parts or comminuted turkey. For turkey carcasses, the log reduction and presence/absence of two indicator organisms, namely aerobic count (AC) and *Enterobacteriaceae* (EB), were measured on turkey carcasses at different stages of the slaughter process during the 2008-2009 FSIS young turkey microbiological baseline study.

In that study, both AC and EB were detected in the majority of rehang samples (> 95%). An average reduction of roughly 2 logs via process control interactions were achieved for both AC and EB. At post-chill, AC was consistently detected and quantifiable whereas EB was not. To that end, analysis of the EB data yielded a drop in presence (quantifiable and above the LOD) from rehang to post-chill by nearly half.

Results of Scenario Analysis

Final Product Standards

An overview of the total illnesses prevented for the final product standard scenarios, by turkey products, is presented in **Table 5**. As discussed above, FSIS does not currently have any data on turkey parts, so it was not possible to assess the risk management questions for this product type. Similarly, lack of comprehensive enumerated data on turkey carcasses prevented a robust assessment on final product scenarios.

A major assumption of this modeling approach is that consumer demand for raw turkey products will continually be met by the industry, and so every lot removed (as a result of a new standard) will ultimately be replaced by another average lot. While this approach differs from other modeling approaches described in the academic literature, FSIS believes this approach represents a more realistic assessment of the current turkey industry and, therefore, the identified public health benefits.

Table 5: Summary of illnesses prevented for final product standard scenarios.

Scenarios		Comminuted Turkey
		Illnesses Prevented
Level	100 cfu/gram	1,400 (8%)
	10 cfu/gram	2,100 (12%)
	1 cfu/gram	2,550 (14.2%)
	0.1 cfu/gram	2,700 (15%)
	Screening Level (0.003 cfu/g)	2,500 (14%)
Serotype	Cluster 1 (Higher Virulence) Serotype Diversion	NA

Level-Based Final Product Standards

Turkey Carcasses

As stated above, FSIS does not currently enumerate Agency PR/HACCP turkey carcass samples. This fact, combined with the very low prevalence rates of *Salmonella*, made a reliable estimate of the current level distribution of *Salmonella* on post-chill carcasses infeasible. Additionally, in the FSIS 2008-2009 young turkey carcass microbiological baseline study, less than 5% of rehang samples and less than 1% of post-chill samples were quantifiable. The lack of ample data limits FSIS' ability to assess a level threshold-based performance standard for turkey carcasses.

That said, examination of foodborne illness data from the CDC—and related IFSAC estimates of *Salmonella* attribution to turkey—indicates that a not insignificant number of *Salmonella* illnesses in the population are attributed to turkey. Therefore, it follows logically that some *Salmonella* must exist on turkey carcasses, but it perhaps is not being identified adequately through FSIS' current sampling techniques. FSIS is presently assessing the viability of its current *Salmonella* sampling program for turkey carcasses.

Turkey Parts

As stated previously, FSIS does not currently, nor has historically, collected enumeration data on turkey parts, which makes the development of a level-based performance standard infeasible at present.

Comminuted Turkey

Unlike turkey carcasses and parts, it is possible to address the final product standard risk management question for comminuted turkey. A comminuted turkey performance standard that diverts test-positive lots based on a

level threshold of 1cfu/15g is the most effective risk management option, with 2,700 illnesses prevented annually, which equates to slightly over 15% of the approximately 18,000 comminuted turkey illnesses estimated to occur annually. A comminuted turkey performance standard that diverts test-positive lots based on a level threshold (at the current LOD, or screening level) of 0.033 cfu/g is a similarly effective risk management option, with 2,500 illnesses prevented annually, which equates to 14% of the approximately 18,000 comminuted turkey illness that occur annually. These estimates do not fully take into account fluctuations in *Salmonella* levels across establishments and time, but repeated simulations converge toward the average. **Table 6** estimates the number of annual illnesses prevented based on level thresholds (i.e., microbial criteria) in final product comminuted turkey with the 95% credible intervals.

As is good practice, risk assessment model's sensitivity to inputs was analyzed. All the major model inputs, including the mean of the attenuation multiplier, estimates for the *Salmonella* serotype mixture in product lots, the initial contamination distributions, and the choice of dose-response model, were systematically analyzed (see section **5.6**) and results were used to develop a robust uncertainty analysis. This uncertainty analysis was conducted for the major threshold scenarios under consideration by the risk managers. The illnesses prevented estimates with the 95% credible intervals are summarized in **Table 6**.

Results suggest substantial overlap in the 95 percent credibility intervals across progressively higher level thresholds. Overlapping credible intervals suggest that differences in the most likely effectiveness between different level thresholds may not be meaningful.

Table 6: Estimated annual illnesses prevented under final product standards for *Salmonella* threshold levels of interest in comminuted turkey.

***Salmonella* threshold level**	**Annual illnesses prevented,** most likely (95% credible interval)
0.03 cfu/g	2500 (700 - 4900)
1 cfu/g	2300 (600 - 4800)
10 cfu/g	2000 (500 - 4300)
100 cfu/g	1400 (200 - 3500)

Serotype-Based Final Product Standards

As stated previously, the number of positive *Salmonella* samples at post-chill were very limited. This, combined with lack of robust sampling at rehang, made it infeasible to estimate the public health impact of performance standards that focus on serotype for all turkey products, as the underlying mixture of serotypes in a lot (i.e., within a flock or day of production) could not be validated. Theoretical mixtures could be evaluated, but it is not advisable for making risk management decisions. Furthermore, a serotype-based approach would target a

subset of the failing lots from the level-based final standard defined at the screening level.

Receiving

As the receiving step only occurs in slaughter establishments, scenarios for this risk management question could only potentially be assessed for turkey carcasses. As stated previously, FSIS does not have regulatory discretion in the pre-harvest environment, nor does it routinely collect data on the nature of flocks that are presented for slaughter—where FSIS jurisdiction begins. Specifically, FSIS does not have data on the *Salmonella* serotypes present on live birds, nor the *Salmonella* contamination levels. Further, FSIS does not have robust, generalizable data on the types of pre-harvest interventions, such as vaccination, employed by the live bird industry.

Given the lack of robust data, it was not possible to estimate the public health impact of performance standards at receiving that address either *Salmonella* levels or serotype. That said, attempts were made to develop a hypothetical model (**Appendix B**) of cluster distributions based on limited data from turkey carcasses at rehang, that would allow for the development of performance standards at receiving. This hypothetical model does demonstrate that focusing on reducing the cluster 1 distribution at receiving could have an impact on reducing illnesses. However, it is not advisable at this time to make risk management decisions based on this cursory model, though further exploration of this approach warrants future consideration.

Process Control

As process control (rehang to post-chill) only occurs in slaughter establishments, scenarios for this risk management question were only assessed for turkey carcasses. Process control analyses were conducted using rehang and post-chill samples. Analyses of the 2008 FSIS microbiological baseline data indicates that the turkey industry was consistently identifying AC and EB in the majority of rehang samples (>95%), and simultaneously, achieving a large reduction in AC and EB. Given the further decrease in *Salmonella* across the turkey industry since this study and the lack of current data, it is assumed that establishments are still achieving 1-2 log reductions in these indicator organisms. This finding, however, demonstrates that any new performance standards that rely on changes in process control would be limited in its ability to reduce the overall burden of *Salmonella* illnesses from turkey.

Historically it has been the case that indicator organisms are not strongly correlated with the presence of *Salmonella* at post-chill or a log reduction in EB (Williams, 2015). This makes achieving decreases in turkey associated salmonellosis with a standard that targets indicator organisms a challenge. Furthermore, as a result of these weak relationships, it follows that the correlation between AC or EB and *Salmonella* serotypes or level is also weak. Therefore, it was not possible to assess the risk management question regarding the public health impact (illnesses, hospitalizations, and deaths) of monitoring/enforcing process control from rehang to post-chill in the same manner as it was estimated for final product standards, which incorporate *Salmonella* contamination distributions.

Consequently, this analysis instead focused on assessing the potential of three process control performance standards to achieve the 25% Healthy People 2030 (HP2030) illness reduction targets for *Salmonella* (HHS, 2020).

Three process control standards were investigated on the available FSIS microbiological baseline data from 2008-2009:

1. an AC-reduction standard that sets a minimum threshold for the average change in log10 AC between

rehang and post-chill,

2. an AC-elimination standard that sets a minimum fraction of post-chill samples where AC is not observed with the current assay (i.e., samples below the LOD identifying presence/absence), and

3. an EB-elimination standard that sets a minimum fraction of post-chill samples where EB is not observed with the current assay (i.e., samples below the LOD identifying presence/absence)

Scenarios were run assuming underperforming establishments adjust their practices toward meeting a level of control according to the indicator organism metrics listed above from rehang to post-chill. That is, by setting a log reduction or presence fraction target/guideline, the overall prevalence that results from that change can be assessed.

Utilizing the 2008-2009 microbiological baseline data, mandatory standards of an AC-reduction (requiring 1log10 reduction), AC-elimination (requiring less than 0.20 samples with AC detections), or EB-elimination standard (requiring less than 0.45 samples with EB detections) would achieve the targeted Healthy People 2030 25% reduction in S*almonella* prevalence; from approximately 2% to 1.5%.

If these standards were voluntary, higher standards (i.e., larger AC log reduction or smaller fraction of AC or EB presence at post-chill) would be required to achieve the 25% reduction in prevalence (which amounts to a prevalence less than 1.5%). However, this is not a huge benefit compared to the mandatory standard, which reduces the prevalence to approximately 1.75%.

That said, the elimination standards require only 1 sample at post-chill to implement, rather than 2 samples to test a reduction in AC, so implementation of an elimination standard of AC or EB is more practical from a logistical and cost perspective. It is important to note that an underlying assumption for these elimination standards necessitate that the indicator organisms remain consistently present (and at high levels) on incoming carcasses.

Further complicating the analysis, the prevalence of *Salmonella* on turkey carcasses has dropped substantially, with a current rate of less than 1%. Turkey carcass establishments, therefore, are likely still achieving (or even exceeding) these indicator targets, leaving little room for improvement in the industry. Further, regardless of how many samples are required (1 for EB v. 2 for AC), the expected cost of these efforts should be considered carefully in light of the minimal impact on public health.

Nevertheless**,** indicator organisms were readily measured and quantified compared to *Salmonella* levels at both sampling locations. Future analyses would require more current information to validate appropriate targets for AC and EB.

Conclusions

This quantitative risk assessment examines the relationship, where feasible, between the level of *Salmonella* and/or presence of certain *Salmonella* serotypes on turkey received for slaughter and on turkey products (i.e., carcasses, turkey parts, and comminuted turkey) and the probability of foodborne illness. It also examines the relationship between changes in microbiological indicator organisms (i.e., AC) on turkey carcasses from rehang to post-chill and changes in foodborne illnesses.

The overall lack of certain turkey data, as compared to chicken sampling data, does limit the ability to assess the public health benefit of some risk management options for mitigating salmonellosis from turkey carcasses and parts. That said, data was available to evaluate the public health benefit of controlling *Salmonella* in comminuted turkey products and evaluating process control on turkey carcasses.

Microbial Profile of Turkey Products

- *Salmonella* is infrequently detected on turkey carcasses at post-chill resulting in a consistently low annual prevalence (<1%), and carcass samples are currently not enumerated to determine levels.
    - The state of *Salmonella* on turkey parts could not be properly assessed without any industry-wide sampling data.
- Data analyses indicate that current and past turkey product samples have low *Salmonella* levels at the final product stage (<1cfu/unit), with 84.26% of comminuted turkey products produced in the U.S. having a *Salmonella* level below 1 cfu/g.
    - It is unusual for consumers to be exposed to a serving from comminuted turkey product that has more than 10 cfu of *Salmonella* per gram.
- The most frequent higher virulence *Salmonella* serotypes in turkey products, based on genomic and outbreak data are: Hadar, Typhimurium, Muenchen. Together these serotypes make up 25% of *Salmonella* positive comminuted turkey product samples.

Final Product Standards

- Final product standards could only be explored for threshold levels in comminuted turkey; however, the model could be adapted to other products should data become available.
- Risk Per Annum: Of the annual 42,669 U.S. foodborne salmonellosis cases attributed to consuming turkey products, it is estimated that 42% (17,921) are from comminuted turkey consumption.
- Risk Per Serving*:* The per serving risk posed by a comminuted turkey product containing *Salmonella* levels at or above 10 cfu per gram and containing a serotype of public health significance is 1,400-folder higher than the average.
- Public Health Impact: Approximately 2,100 illnesses per year would be prevented as a direct result of not allowing comminuted turkey product exceeding 10 cfu/g into commerce based on FSIS' verification sampling program.
- Estimating Public Health Benefits*:* The public health benefits estimated from establishing enforceable final product standards is limited to the direct benefits of FSIS' current level of verification sampling and testing of turkey products (e.g., 5 samples per month).
- Additional Benefits: Public health benefits are anticipated from industry response to enforceable final product standards, but information on industry behavior is needed to quantify these potential benefits.

Preharvest Control

- It was not possible to estimate the public health impact of performance standards at receiving that address either *Salmonella* levels or serotype.

- Attempts were made to develop a hypothetical model (**Appendix B**) of cluster distributions based on limited data from turkey carcasses at rehang, that would allow for the development of performance standards at receiving.

Process Control

- Model development for process control monitoring was robust, but limited *Salmonella* recovery at the end of production mean few of the results are actionable.

- It is assumed that turkey carcass establishments are achieving a 1-2log AC reduction between rehang and post-chill, as was the case in the 2008-2009 turkey carcass microbiological baseline data.

- Voluntary and mandatory targets for AC-reduction from rehang to post-chill, AC-elimination at post-chill, and EB-elimination at post-chill were successfully modeled, and showed modest potential for public health benefit, though more robust, current data is necessary for definite conclusions.

- Indicator organisms were readily measured and quantified compared to *Salmonella* levels at both sampling locations.

Cross-Cutting Issues

- The methods to answer these risk management questions were developed in this work and the concurrent *FSIS' Quantitative Risk Assessment for Salmonella in Raw Chicken and Raw Chicken Products* (available here) but turkey data limitations could not be overcome at this time.

- Certain data limitations were circumvented by the use of the best available poultry data, i.e., chicken. This was only done when there was a good practical and analytical basis to do so.

  Complete descriptions of these instances are provided in this document. They include the use of an attenuation distribution calibrated to chicken data and development of dose-response models using *Salmonella* serotype data from both chicken and turkey.

- Combination of Scenarios: It was not possible to model the effects of multiple risk management options in sequence (Risk Management Question #4).

  - Combining final product standards with process control scenarios was not possible because the correlation between indicator organisms and *Salmonella* does not extend down to the resolution of *Salmonella* levels and serotypes.

  - However, it is reasonable to expect that industry risk-managers respond to a final product standard by taking actions to improve process control or reduce live-bird contamination, thus reducing the likelihood that their products would fail such standards. These actions, while not modeled in this risk assessment given the paucity of data, would likely greatly enhance the number of illnesses reduced.

- Data Sharing: Sharing data is useful for enhancing the characterization of *Salmonella* levels and benefits of controlling this pathogen in final poultry products. FSIS supports continued efforts to work collaboratively with our industry and other stakeholders to share data. Industry data has the potential to enhance the findings presented here and reduce uncertainty in the scenarios modeled in this risk assessment

Chapter 1 Introduction

The United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) protects the public's health by ensuring that meat, poultry, and egg products are safe, wholesome, and properly labeled. The Agency is committed to reducing foodborne infections associated with FSIS-regulated products, in particular *Salmonella* illnesses attributable to poultry.

Salmonella in poultry remains a significant food safety concern in the U.S. Centers for Disease Control and Prevention (CDC) estimates *Salmonella* infection is responsible for over 1 million infections, 26,500 hospitalizations, and 420 deaths in the U.S. every year (CDC, 2022b). Of these illnesses, approximately 66% are attributed to food (Beshearse, 2021) with nearly 6% of *Salmonella* illnesses attributed to turkey (IFSAC, 2022). All salmonellosis cases impose an estimated $3.7 billion in economic burden in a typical year. Almost 90 percent of this burden, $3.3 billion, is due to deaths; 8 percent, $294 million, is due to hospitalization; and the remaining 2 percent is due to non-hospitalized cases (Hoffmann, 2021).

On October 17, 2022, FSIS announced that it is mobilizing a stronger, and more comprehensive effort to reduce *Salmonella* illnesses associated with poultry products by establishing an enhanced food safety framework to address *Salmonella* contamination on poultry (FSIS, 2022b). Central to this effort is the conduct of this quantitative microbial risk assessment (QMRA) to provide information on the predicted public health benefit of controlling the prevalence, levels, and/or specific serotypes of *Salmonella* in turkey presented for slaughter, throughout the slaughter process, and/or in final turkey products.

This risk assessment addresses the following risk management questions:

1. What is the public health impact (change in illnesses, hospitalizations, and deaths) achieved by eliminating at receiving a proportion of turkey contaminated with specific levels of *Salmonella* and/or specific *Salmonella* subtypes?

2. What is the public health impact (change in illnesses, hospitalizations, and deaths) achieved by eliminating final product contaminated with specific levels of *Salmonella* and/or specific *Salmonella* subtypes?

3. What is the public health impact of monitoring/enforcing process control from re-hang to post-chill? Monitoring could include analytes such as *Enterobacteriaceae*, Aerobic Count, or other indicator organisms, analysis could include presence/absence or levels and the monitoring could also include variability of actual result versus expected result, log reduction, absolute sample result, or other individual establishment specific criteria.

4. What is the public health impact of implementing combinations of the risk management options listed above?

This risk assessment addresses these questions in a mathematical model simulating *Salmonella* in turkey from the re-hang step during the slaughter process to consumption. Included in this report is information on the risk assessment model (approach, parameters, equations), the data considered and ultimately used, underlying assumptions informed by data and science, and characterization of the relative reduction in the risk per product serving and annual number of attributable illnesses with estimates of the certainty of these predictions.

Concurrently, FSIS developed the *Quantitative Risk Assessment for Salmonella in Raw Chicken and Raw Chicken Products* (available here) .Throughout this document it will be referred to as the Chicken Risk Assessment.

1.1 Policy Context

Pathogen reduction performance standards have been applied to meat and poultry slaughter establishments since the inception of the Pathogen Reduction; Hazard Analysis and Critical Control Point (PR/HACCP) rule (FSIS, 1996). Under a performance standard—which are two-class attribute sampling plans—each establishment is subjected to a series of sampling occasions. FSIS uses these *Salmonella* sampling results to assess establishment performance during a reference period of one completed 52-week moving window based on a 3-category system. Establishments at or below half of the performance standard over the previous moving window are placed in Category 1, those that meet the standard in that period are placed in Category 2, and those that fail the standard in the previous moving window are placed in Category 3. FSIS posts on its website the category status of individual establishments for pathogen reduction performance standards for *Salmonella* in young chicken carcasses, young turkey carcasses, raw chicken parts, and not-ready-to-eat (NRTE) comminuted chicken and turkey products, based on FSIS verification sampling results. Public dissemination of establishment categorization has been shown to serve as a market-based incentive to encourage establishments to reduce *Salmonella* contamination in failing establishments (Ollinger, 2020).

Recognizing the need for standards, FSIS began sampling and testing NRTE comminuted chicken and turkey products on June 1, 2013, and a *Salmonella* performance standard for raw comminuted turkey in 2016.

Analyses of the effectiveness of current FSIS *Salmonella* performance standards indicates there has been an overall reduction in the occurrence of *Salmonella* on meat and poultry products (Williams, 2022a).

1.2 Purpose and Scope

The overall purpose of this risk assessment is to assess—at different points in the turkey slaughter process—the public health benefits of various risk management options.

This risk assessment was modeled to evaluate their potential effect on the entire U.S. turkey industry, rather than any particular slaughter and processing establishment or subset of establishments. As much as possible, these risk management options are evaluated for the three main turkey products (carcasses, parts, and comminuted turkey).

Table 7 summarizes a basic description of these product categories and some features that are important to this risk assessment; further details are in **Appendix B**.

Each risk management question addresses different areas of the turkey slaughter process, receiving, process control, and final product. It was not possible to model the public health impact for each turkey product for each of these areas. Specifically, the first risk management question only applies to carcasses because receiving is the initial step of the slaughter process. Assessment of process control—Risk Management Question 3—requires two points of sampling to adequately model changes to the industry. As such, process control is only assessed for carcasses, and is not directly examined for parts and comminuted product. Similarly, assessment of the public health benefit of elimination of product with specific *Salmonella* subtypes is only possible with two points of sampling to adequately model changes to the industry. As such, the impact of serotype is only assessed for carcasses, but not for parts or comminuted products.

Table 7: Description of turkey products. These model assumptions are based the current FSIS sampling frequency, product definitions, and laboratory methodology (FSIS, 2021; FSIS, 2022a).

Turkey Product	Definition	Lot Size	FSIS Sampling Unit
Carcass	Young turkey carcasses	1 flock (~22,000 birds)	Rinsate (30 mL)
Parts	Legs, Breasts, Wings	NA	NA
Comminuted	Ground Turkey	1 day of production	Ground (325 g)

Analysis of all risk management scenarios in this risk assessment is predicated on the assumption that the industry will maintain the overall pathogen reductions that have been achieved in the past.

1.3 Conceptual Model

Figure 2 illustrates the conceptual model used to determine the public health benefits of the risk management options above. The three-component model: (1) slaughter and processing, (2) growth and die off, and (3) public health outcomes, is sufficiently flexible to describe the U.S. turkey industry and targeted enough to answer the specific risk management questions.

Scenarios (that is, options) for receiving guidelines, process control monitoring, and final product standards serve as inputs to the overall model, and an estimate for annual illnesses prevented is the output for each scenario, whenever appropriate. For process control standards, illnesses prevented could not be estimated due to the weak correlation between indicator organisms and pathogens.

To provide support for this risk assessment, FSIS entered into a Cooperative Agreement in with the University of Maryland's Joint Institute for Food Safety and Applied Nutrition (UMD-JIFSAN), in partnership with EpiX Analytics. With UMD-JIFSAN, FSIS gained a partner to assist in the obtaining of industry data in a confidential and secure manner. EpiX Analytics provided expertise in dose-response modeling. With that expertise, EpiX Analytics developed two dose-response models that describe the probability of illnesses given ingestion of a *Salmonella* concentration dose: one model for higher virulence serotypes and one for lower virulence serotypes. EpiX Analytics' approach uses whole genome sequencing (WGS) data to cluster serotypes (i.e., serocluster) based on virulence gene markers and then estimates the difference in infectivity between these two seroclusters based on epidemiological data for foodborne illnesses associated with *Salmonella* from Centers for Disease Control and Prevention (CDC) National Outbreak Reporting System (NORS) and CDC Foodborne Diseases Active Surveillance Network (FoodNet). The EpiX Analytics' dose-response model is based on earlier work (Teunis, 2022; Teunis, 2010).

Confidence in Effect

To ensure confidence in the results of this risk assessment—and for these results to serve as scientific support for regulatory rulemaking—it is imperative that the public health impact of any change in FSIS' current approach can be attributed specifically to the adoption of a given risk management option, rather than by chance alone. Therefore, a key feature of this risk assessment is the determination which of these scenarios are improving public health outcome by diverting product lots with higher-than-average risk, rather than by random chance.

1.4 Report Organization

This report begins with a description of the current status of *Salmonella* contamination across the U.S. turkey industry. We begin with a description of the public health context of *Salmonella* and its serotypes and introduce

a novel virulence-gene informed clustering of these serotypes accordingly; additional details of the *Salmonella* serotype hazard identification are summarized in **Appendix A**[4]. In the Microbial Profile chapter, *Salmonella* prevalence and concentration distributions are provided for turkey product categories (carcasses, parts, comminuted) where data is available. The full details of the methods and data used in these descriptions are in Appendices B and C. **Chapter 4** describes the baseline exposure assessment and ends with a descriptive example of risk per serving.

The rest of this report is an analysis of the predicted public health impact of the risk management scenarios as summarized in the risk management questions. These scenarios fall into three categories: final product standards (**Chapter 5**), receiving guidelines (**Chapter 6**), and process control monitoring (**Chapter 7**). When possible, analysis of each scenario category begins with an introduction describing the interpretation of the risk management being answered and a summary of key modeling assumptions, followed by a description of the modeling method used and, finally, presentation and discussion of results.

Chapter 5, final product standards, contains the majority of the risk assessment model development including the hazard characterization (i.e., dose-response model). In **Chapter 6**, receiving guidelines are considered using much the same approach, however, data limitations restricted what could be evaluated to rehang sampling. In **Chapter 7**, process control monitoring is analyzed and has separate risk characterization, as this risk management question is best evaluated using indicator organism data. Data limitations that prevent evaluation of certain scenarios are characterized throughout.

The report concludes with a summary of overall results and recommendations for data needs based on limitations identified in the conduct of this risk assessment.

Theory and data analysis details are provided in the Appendices. Full descriptions of data used in this risk assessment are available in **Appendix B** and data used in the dose-response development in **Appendix A**. Details of the population description methodology are available in **Appendix C.**

1.5 Model Approach

The first goal of this risk assessment is to define a probabilistic model that explains the current state of pathogen contamination in the U.S. turkey carcass population at the rehang and post-chill locations of the slaughter process. For all risk management scenarios, FSIS considered the guidance it received from the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) related *to Enhancing Salmonella Control in Poultry Products* referred to as the NACMCF 2023 response hereafter(NACMCF, 2023).

For the receiving scenarios assessed within the 'slaughter and processing' component of the model, FSIS utilized Agency rehang sampling data. This data source was selected as FSIS does not routinely collect data on live birds because the Agency does not have regulatory discretion in the poultry preharvest environment. Efforts are ongoing to enhance FSIS data with industry-supplied data through FSIS' Cooperative Agreement with the UMD-

[4] To provide support for this risk assessment, FSIS entered into a Cooperative Agreement (FSIS-02152022) with the University of Maryland's Joint Institute for Food Safety and Applied Nutrition (UMD-JIFSAN), in partnership with EpiX Analytics. With UMD-JIFSAN, FSIS gained a partner to help obtain industry data in a confidential and secure manner. EpiX Analytics provided expertise in dose-response modeling. As a part of this Agreement, EpiX Analytics used genomics to classify serovars into groups (clusters) based on virulence similarities and developed dose-response models for the serovar clusters. Details of the EpiX Analytics methodology are provided below in the report entitled "*Using genomics to identify nontyphoidal Salmonella serovars of concern and estimating dose-response models amenable to risk assessments in poultry."*

JIFSAN, as described previously. Further, the scientific community has not established a standard sampling method for *Salmonella* on live birds. FSIS has partnered with the USDA Agricultural Research Service to attempt to mitigate some of these data gaps (FSIS, 2023a).

For the process control scenarios assessed within the 'slaughter and processing' component of the model, the impact of a reduction in indicator organisms between rehang and post-chill on end-point *Salmonella* prevalence was assessed. Given the weak correlation between indicator organisms and pathogen prevalence (Ebel, 2015), and that the dose-response model constructed for this risk assessment is *Salmonella* serotype informed and level-based, the public health impact from the process control scenarios are assessed separately.

For the final product scenarios assessed within the 'slaughter and processing' component of the model, a probabilistic model for *Salmonella* contamination at post-chill describes potential human exposure from meals derived from whole turkey carcasses. Data collected at the end of the grinding process are used to describe potential consumer exposure to *Salmonella* from fabricated comminuted turkey. The effect of various risk management options can then be assessed by adjusting the parameters of these population models in accordance with the anticipated effect of different risk management options.

We summarize the effects of the myriad pathways contaminated product may follow from the end of processing through commerce and preparation using an attenuation distribution. This attenuation distribution aims to capture the variability associated with mixing, partitioning, growth, cooking and serving size processes between production and consumption of comminuted turkey. As a starting point due to lack of complete data to develop and support a more refined model, a lognormal attenuation distribution ($\mu = -5.00log10, \sigma = 1.91log10$) that was calibrated previously for chicken using a single distribution for *Salmonella* contamination, a general *Salmonella* dose-response function from WHO-FAO (FAO/WHO, 2000) and a prior estimate for total *Salmonella* illnesses attributed to chicken were considered (Ebel, 2015). The log10 mean of this distribution (-5log10) is consistent with a scenario where a raw chicken product is properly handled to avoid growth of *Salmonella*, then subjected to cooking to achieve a minimum internal temperature of 165 ºF (the FSIS recommended final cooking temperature for poultry), which the Agency has determined will deliver at least a 7-log reduction of *Salmonella (*FSIS*, 2017)*, and consumed in a serving size of 100 g (+2log10).

Lacking alternative estimates, this default attenuation distribution is used across analyses of chicken products (carcasses, parts, comminuted chicken) as well as comminuted turkey (which is generally handled/consumed in a similar manner as comminuted chicken products). Consumer phase models have been assessed given the broad nature of food handling practices across products (Nauta, 2011; Neves, 2018) and their impact on the relative risk to public health. In particular, (Nauta, 2011) demonstrated that the effect of different consumer phase models between products for Campylobacter is generally small when considering implementation of a variety of control measures. However, the authors observed the largest differences in estimated risk when measures were aimed at removing highly contaminated product (i.e., shortening the tail of the contamination distribution rather than reducing the mean). It is inherently difficult to accurately and consistently model changes between the last post-chill product sample and the dose at consumption. Nevertheless, FSIS conducted a sensitivity analysis to explore the effects of alternative attenuation distribution parameters on the estimated effectiveness of risk management options.

Further, it is assumed that this attenuation distribution can be used across *Salmonella* serotypes and in the derivation of the dose-response functions for different *Salmonella* serotype clusters (**Appendix A**).

The results of this risk assessment are likely different from other recent poultry risk assessments (Lambertini, 2019; Lambertini, 2021; Oscar, 2021). These risk assessments are viewed as more akin to attribution studies that calculate the effect of removing all product that has a specific risk characteristic (e.g., specific serotypes or

levels above a specified threshold value). The removal of all servings with the specified risk characteristic would require the testing of all servings, so the previously published estimates are seen as aspirational upper bounds for the number of illnesses that could be prevented. Much of the focus of this study will focus on how different risk management options would aid FSIS' ability to correctly identify product with a specified risk characteristic and then ensure that affected product is either rendered safe for human consumption or removed from commerce. Thus, the goal of the risk assessment can be restated as determining what fraction of all illnesses associated with product having the risk characteristic will be prevented because of actions taken as a direct consequence of FSIS testing and enforcement of standards.

In order to estimate the direct impacts of a new FSIS regulation, this risk assessment for *Salmonella* in turkey pays particular attention to modeling the sampling process and testing methods in a manner consistent with how the regulation is to be implemented in practice. This will include consideration of sampling frequency, sample unit size (e.g., lbs.), testing methods (detection or enumeration), and measurement uncertainty.

1.6 Introductory Tables and Figures

Given the length of the document and the complexity of the model development, FSIS has provided some introductory summary tables and figures to aid the reader. As previously discussed, **Figure 2** is a schematic representation of the risk assessment model. **Table 8** outlines the required information and assumptions used in each of the three scenario analyses. **Table 9** summarizes the interpretation of the risk management questions and which scenarios were successfully implemented. **Table 10** contains the model parameters and variables from the final product standard analysis.

Figure 2: Conceptual risk assessment model.

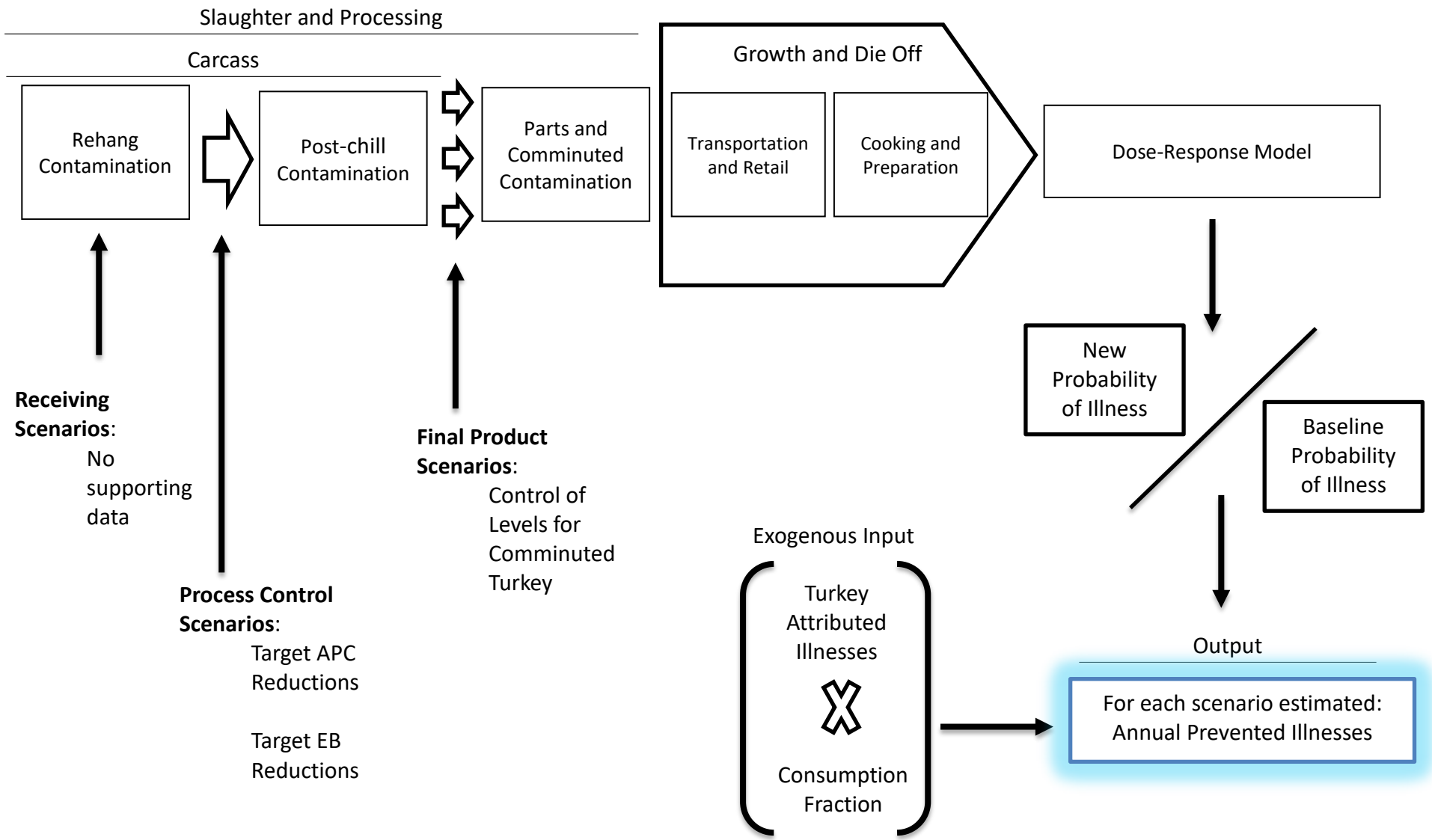

Table 8: Risk assessment information and assumptions.

Information Needed	Assumption Used	Supporting Data or Information
***Salmonella* in Poultry Baseline Profile**		
Salmonella Microbial Profile	FSIS data are representative of turkey slaughter and processing establishments under FSIS jurisdiction	FSIS establishment level PR/HACCP and Microbial Baseline data (see **Appendix B**)
	All flocks contain some *Salmonella*. This risk assessment does not assume that all birds have *Salmonella*.	Analysis of FSIS 2022 Exploratory Chicken Carcass Data with two samples per flock – see FSIS' Chicken Risk Assessment (available here) (Cox, 2020; Obe, 2023; Rasamsetti, 2023; Thompson, 2018)
	Multiple *Salmonella* strains are present in flocks.	
	Salmonella serotypes can be clustered into two groups based on virulence gene markers.	See **Chapter 2** for the FSIS summary of the EpiX Analytics report in **Appendix A**. For transparency, FSIS has developed a separate description of the EpiX Analytics Serotype Clustering that clarifies the approach taken by EpiX Analytics (**Bioinformatics Supplemental Materials** available here).
	Epidemiological data can be used to categorize overall serocluster virulence: higher virulence serocluster (labeled C1) and lower virulence serocluster (labeled C2).	See **Appendix A** and attached FSIS Guide to the EpiX Analytics Serotype Clustering. For enhanced transparency in compliance with the Office of Management and Budget information quality guidelines, FSIS has developed a separate description to the EpiX Analytics Serotype clustering that clarifies the bioinformatics approach taken by EpiX Analytics (available here).
	Flocks contain a dominant serocluster.	Contingency table analysis of FSIS 2022 Exploratory Data in FSIS' Chicken Risk Assessment (available here) subsection 3.6
Production Volume Data		FSIS establishment-level annual production volume data
Estimated number of human *Salmonella* illnesses	42,669 turkey-associated *Salmonella* illnesses per year.	This value is calculated as the product of the total number of CDC FoodNet cases per year (7,600), the share of these cases that are foodborne (66 percent) and of domestic origin (89 percent), the under-diagnosis multiplier for

attributable to turkey consumption		*Salmonella* (24.3)(Ebel, 2012b) and dividing by the FoodNet catchment area (15 percent). The portion attributed to turkey is based on IFSAC estimates (5.9).
Final Product Standards		
Determination of lot concentration	An accurate method will be used to determine the threshold status of tested lots.	This information need was highlighted in the NACMCF 2023 final report (NACMCF, 2023) and methods for evaluating enumeration data for accuracy are presented in the FSIS' chicken risk assessment (available here).
FSIS adulterant testing procedure and frequency	FSIS sampling and lab methodology for carcasses and comminuted product continue as currently utilized.	FSIS Microbiological Lab Guidebook (FSIS, 2022a) and Sampling Instructions (FSIS, 2021)
	FSIS will continue current sampling frequency.	Small establishments: 24 samples/year Large establishments: 60 samples/year
	Lot size will remain as currently used by industry in PR/HACCP documentation.	Carcasses: 1 flock Comminuted: 1 day of production
Fate of diverted product	Consumer demand for raw turkey products will be met by the industry; hence, every removed lot will be replaced by another lot in the aggregate.	This assumption is considered reasonable because of turkey industry practices, see **Chapter 5** for more detail.
	There is no public health benefit from diverting random lots.	The removed lot and the lot replacing it will both be of the same average risk.
Salmonella growth and die-off after slaughter and processing.	The effect of mixing, transportation, storage, cross contamination, cooking and handling is described by an attenuation distribution.	A lognormal attenuation distribution ($\mu = -5.00 \log 10$, $\sigma = 1.91 \log 10$) was calibrated previously for chicken using a single distribution for *Salmonella* contamination, a general *Salmonella* dose-response function from WHO-FAO and a prior estimate for total *Salmonella* illnesses attributed to chicken (Ebel, 2015).
	The chicken attenuation distribution is applicable to turkey products.	The attenuation multiplier based on chicken data is a reasonable preliminary approach to simulate exposure, particularly in comminuted turkey products.

Consumption of higher doses of *Salmonella* is associated with a higher probability of illness.	Dose-response relationship used in QMRA.	Teunis' dose-response model using outbreak data is the primary underpinning of this theory as applied in this document (Teunis, 2022; Teunis, 2010; Teunis, 2008).
Receiving Guidelines		
Contamination at Flock Receiving	Rehang data is descriptive of incoming contamination.	The only available data source of near incoming contamination is the FSIS Microbial Baseline data collected at rehang. However, limited test-positive turkey carcass data inhibits modeling.
Process Control		
Utility of indicator organisms to monitor process control		The weak correlation between post-chill *Salmonella* prevalence and aerobic count (AC) reductions from rehang to post-chill are analyzed.
Illness Reduction and Prevalence	A reduction in pathogen prevalence results in a proportional reduction in illnesses.	(FSIS, 2015)

Table 9: Interpretation of risk management questions and table of scenarios.

Risk Management Question	Scenario Description	Product	Range of Scenarios	Public Health Metric
What is the public health impact (change in illnesses, hospitalizations, and deaths) achieved by eliminating at receiving a proportion of chicken contaminated with specific levels of Salmonella and/or specific Salmonella subtypes?	No receiving scenarios could be implemented in turkey products.	None	None	None
What is the public health impact of monitoring/enforcing process control from rehang to post-chill? Monitoring could include analytes such as Enterobacteriaceae, Aerobic Count, or other indicator organisms, analysis could include presence/absence or levels and the monitoring could also include variability of actual result versus expected result, log reduction, absolute sample result, or other individual establishment specific criteria.	Compliance with a target log reduction in AC from rehang to post-chill.	Turkey Carcasses	Mandatory: 1log10 reduction of AC Voluntary: 2.3log10 reduction of AC	The Healthy People 2030 target of a 25% salmonellosis reduction.
	Compliance with a target AC elimination from rehang to post-chill	Turkey Carcasses	Mandatory: At least 20% of post-chill samples have no AC Voluntary: At least 30% of post-chill samples have no AC	The Healthy People 2030 target of a 25% salmonellosis reduction.
	Compliance with a target EB elimination from rehang to post-chill.	Turkey Carcasses	Mandatory: At least 45% of post-chill samples have no EB Voluntary: At least 80% of post-chill samples have no EB	The Healthy People 2030 target of a 25% salmonellosis reduction.
What is the public health impact (change in illnesses, hospitalizations, and deaths) achieved by eliminating final product contaminated with specific levels of Salmonella and/or specific Salmonella subtypes?	Levels-Based Threshold Standards: Lots are diverted if a regulatory sample tests above a predetermined level (cfu/g or cfu/mL).	Comminuted Turkey	Standards at concentrations from 1cfu/2,600g to 100cfu/g were simulated for comminuted turkey product.	Annual illnesses prevented. Resolution does not extend to deaths and hospitalizations.

	The terms level and concentration are used interchangeably throughout the document.			
	Serotype Standard: Lots are diverted if a regulatory sample tests positive for a serotype of higher virulence	None	*Serotype standards could not be modeled for any turkey commodity.* Insufficient serotyping at multiple points in the slaughter process to ensure reliable serotype distribution within a lot – turkey carcasses have limited test positives and comminuted turkey are only tested at final product	None
What is the public health impact of implementing combinations of the risk management options listed above?	No combinations of scenarios could be implemented.	None	None	None

Table 10: Table of model parameters and variables for final product standard guideline estimates.

Description	Parameter/ variable	Value/model	Units
log10 mean final product concentration	μ	-4.857	cfu/g
log10 std dev final product concentration	σ_x	2.333	cfu/g
conversion of rinse sample conc per mL to conc per gram	g	$\log10\left(\frac{400}{0.14}\right)+\log10\left(\frac{1}{4\times454}\right)=0.1968$	mL/g
log10 mean initial final production concentration in grams	μ_x	μ	cfu/g
log10 mean of attenuation distribution	μ_a	-5	g/serving
log10 std dev of attenuation distribution	σ_a	1.91	g/serving
concentration threshold	T	policy input	cfu/g
fraction of lots that pass	ω	$\Phi\left(\frac{T-\mu}{\sigma_x}\right)$	proportion
dose distribution at consumption	d	$10^{Normal\left(\mu_x+\mu_a,\sqrt{\sigma_x^2+\sigma_a^2}\right)}$	cfu/serving
dose-response functions for clusters 1 and 2	R_1, R_2	polynomials	proportion
probability of illness from cluster 1 or 2	$P\left(ill \mid C1/C2\right)$	$\int R_{1/2}\left(d\right)f\left(d\right)\partial d$	proportion per serving
dose distribution given that lot passes	$d_{x\le T}$	$10^{TruncNormal\left(T,\mu,\sigma_x\right)}\times10^{Normal\left(\mu_a+g,\sigma_a\right)}$	cfu/serving
proportion of *Salmonella* from cluster 1	c	0.3	proportion

probability of illness among passing lots from C1 *Salmonella*	$P(ill \mid pass, C1)$	$\int R_1(d_{x \le T}) h(d_{x \le T}) \partial d_{x \le T}$	proportion per serving
prob of illness among passing lots from C2 *Salmonella*	$P(ill \mid pass, C2)$	$\int R_2(d_{x \le T}) h(d_{x \le T}) \partial d_{x \le T}$	proportion per serving
probability of illness among all passing lots	$P(ill \mid pass)$	$c \times P(ill \mid pass, C1) + (1-c) \times P(ill \mid pass, C2)$	proportion per serving
probability of illness among failing lots from C1 *Salmonella*	$P(ill \mid fail, C1)$	$\frac{\left[P(ill \mid C1) - \omega \times P(ill \mid pass, C1)\right]}{(1-\omega)}$	proportion per serving
probability of illness among failing lots from C2 *Salmonella*	$P(ill \mid fail, C2)$	$\frac{\left[P(ill \mid C2) - \omega \times P(ill \mid pass, C2)\right]}{(1-\omega)}$	proportion per serving
probability of illness among all failing lots	$P(ill \mid fail)$	$c \times P(ill \mid fail, C1) + (1-c) \times P(ill \mid fail, C2)$	proportion per serving
baseline probability of illness across all servings	$P_{baseline}(ill)$	$\omega \times P(ill \mid pass) + (1-\omega) \times P(ill \mid fail) = c \times P(ill \mid C1) + (1-c) \times P(ill \mid C2)$	proportion per serving
total lots produced per year	L	8120	lots
total lots tested per year	n	1355	lots
share of failing lots that are diverted	α	$\frac{n}{L}$	proportion
number of illnesses before policy	λ_{ill}	17,921	illnesses/yr
new prob of illness after policy	$P_{new}(ill)$	$\omega \times P(ill \mid pass) + (1-\omega)\left[\alpha \times P_{baseline}(ill) + (1-\alpha) \times P(ill \mid fail)\right]$	proportion per serving
illnesses prevented by policy	I_{avoid}	$\left(1 - \frac{P_{new}(ill)}{P_{baseline}(ill)}\right) \lambda_{ill}$	illnesses/yr

Chapter 2 Identifying *Salmonella* of Greatest Concern

2.1 Public Health Context

This risk assessment leveraged FSIS' *Risk Profile for Pathogenic Salmonella Subtypes in Poultry* (available here), referred to as FSIS' *Salmonella* Risk Profile, to identify *Salmonella* serotypes in chicken and turkey (individually and in aggregate) linked to foodborne illness. This independently peer-reviewed risk profile provided a comprehensive review of the scientific literature and foodborne illness data to identify certain *Salmonella* serotypes in poultry linked to foodborne illness. The genus *Salmonella* is classified on biochemical reactions, surface protein antigen profiles and DNA sequence. Currently, there are 2 recognized species, *enterica* and *bongori,* in which there are about 2,500 serotypes. The Kauffman-White Scheme was the original typing scheme used to describe serotypes, based on somatic (O) antigens, capsular Vi antigens, flagellar (H) antigens and lipopolysaccharides (Yan, 2004).

Salmonella subtypes are a group of *Salmonella* organisms with the same attributes. *Salmonella* serotypes are a subtype defined by a combination of O- and H- antigens (*i.e.*, a serogroup is a subtype with the shared attribute of O- and H- antigens) (Bauer, 2014). Thirty-two *Salmonella* subtypes (28 serotypes and 4 serogroups) can be attributed to human salmonellosis from consuming chicken and turkey products.

Evidence suggests that exposure to *Salmonella* subtypes of concern can cause severe or debilitating human health outcomes, including acute gastroenteritis, bacteremia (bacteria in the blood), and focal infections (persistent infection of an organ or region) resulting in hospitalization or chronic disease lasting beyond one year. The domestic foodborne hospitalization rate for *Salmonella* is about 2% and the fatality rate is about 0.04% for all *Salmonella* (Scallan, 2011). Antibiotic-resistant *Salmonella* infections pose a risk of treatment failure in the case of invasive disease and have been associated with severe outcomes.

2.2 Clustering Serotypes by Virulence Gene Markers

As part of the FSIS' Cooperative Agreement with University of Maryland, EpiX Analytics categorized *Salmonella* serotypes into clusters derived from a machine learning algorithm using *Enterobacteriaceae* virulence factors.

High resolution genomic analyses have recently evolved as a result of the development of new computationally intensive approaches (Chen, 2022; Karanth, 2022; Njage, 2019; Wheeler, 2018) to assess a number of *Salmonella* strains or subtypes and the underlying genetic variability. To describe a broader range of serotypes, EpiX Analytics employed a genomics-based approach to group *Salmonella* serotypes based on a virulence gene profile of 193 *Enterobacteriaceae* virulence factors. To that end, seroclusters (i.e., groups of serotypes) could be constructed based on genetic similarities, and subsequently, validated via epidemiological characteristics. The resulting seroclusters could then be considered to develop refined dose-response model relationships. In the following sections, key components of the methods are summarized. Additional details and results of the EpiX Analytics methodology are provided below in **Appendix A** their report entitled "*Using genomics to identify nontyphoidal Salmonella serovars of concern and estimating dose-response models amenable to risk*

assessments in poultry[5]*."*

In conducting these analyses, FSIS worked closely with EpiX Analytics to routinely review data, analyses, and findings. EpiX Analytics also routinely consulted with FSIS to make analytic decisions to move the dose-response model towards completion. One key decision needed to complete this analysis was the selection of how many seroclusters should be utilized in the dose-response model. For FSIS, this decision was largely driven by the limited number of unique serotypes observed at post-chill, which is in turn a function of the small number of serotypes that account for nearly all *Salmonella*-positive poultry samples (Shah, 2017; Williams, 2022b). A discussion of FSIS' decision to use two seroclusters is described in the following section. Further, given FSIS' focus in recent years on *Salmonella* Infantis, the Agency conducted additional analyses to support the decision to include Infantis in cluster 2, as described below.

FSIS has also developed *Bioinformatics Supplemental Materials* (available here) to further describe the genomics-based clustering to ensure the transparency requirements of the Information Quality Act (Section 515 of Public Law 106-554), which is required for risk assessments used to inform rulemaking, such as this *Salmonella* in turkey risk assessment, are met. In addition to describing the EpiX Analytics approach, this appendix outlines general best practices in bioinformatics, contextualizes the approach used by EpiX Analytics within the discipline, and provides limitations of the approach and future directions that are of interest to FSIS.

Clustering approach

Clustering methods may group *Salmonella* serotypes in a variety of manners; however, in the approach performed by EpiX Analytics, the clusters were driven by the presence/absence of virulence factors (VFs) that are informative for clustering *Salmonella* serotypes into defined groupings. This clustering method relied on genes lost or gained in the isolate data curation (i.e., predicting open reading frames and gene annotation of isolate assemblies) as opposed to phylogenetic similarity measured by single nucleotide polymorphisms (SNPs), core genes, or O-antigen genes. Moreover, clustering was agnostic to the biological function or role of individual virulence factors as well as point mutations or insertions/deletions of genes that can modify gene function resulting in public health risk as illustrated by the emergence of *Salmonella* Reading (Miller, 2020). Nonetheless, further analysis into the biological function of each virulence gene can be utilized in future iterations and enhance virulence clustering models and classification schemes.

Over 40,000 pre-assembled isolates from human and animal sources (poultry and beef/bovine) in the U.S. and virulence factors from the *Enterobacteriaceae* family were compiled from public databases. VFs from the Enterobacteriaceae family were considered as these are more peripheral markers that may correspond to pathogenesis and affords the opportunity to include VFs commonly passed through horizontal gene transmission, while also providing the ability to find differences between serovars that the core genome would not uncover. Each pre-assembled *Salmonella* isolate was subsequently annotated to determine the virulence gene profile (presence/absence). Virulence genes that were present in the majority of isolates (>95%) as well as limited gene presentation (i.e., <10 total isolates) were removed from further analysis. Hence, 193 genes available for the clustering analysis included 57 *Salmonella* VFs, 94 E. coli VFs, 10 Shigella VFs, and 32 Yersinia VFs. The full list of these virulence factors

[5] Description of the previous iteration of EpiX Analytic's serotype clustering are available at Fenske, G. J., Pouzou, J. G., Pouillot, R., Taylor, D. D., Costard, S., & Zagmutt, F. J. (2023). The genomic and epidemiological virulence patterns of Salmonella enterica serovars in the United States. *PLoS One*, *18*(12), e0294624. https://doi.org/10.1371/journal.pone.0294624

along with additional descriptive characteristics are provided in FSIS' *Bioinformatics Supplemental Materials* (available here).

The genetic similarity between all isolates was then estimated via an unsupervised random forest (URF) approach balancing computational expense and performance. To that end, 10,000 trees were simulated with 60 features (virulence factors) randomly selected as candidates for each split to maintain an appropriate level of efficiency without loss in the predictive power of the algorithm. Following the unsupervised random forest simulation, an isolate proximity matrix was estimated by averaging the distance between terminal nodes for each isolate across all trees. This result would imply the isolate relatedness; i.e., isolates in the same terminal nodes are more similar to each other. It is important to note that this averaging across trees can potentially overestimate the low values and underestimate the high values. Finally, the isolates were grouped into *k* clusters using hierarchical clustering (Ward's method) and non-parametric bootstrapping to assess the stability (Jaccard stability and serotype switching). Results of cluster assignment based on how the majority of isolates classified (i.e., best cluster) are depicted in **Table 11**. (Risk multiplier section below).

Table 11: Best cluster assignment for 42 *Salmonella* serotypes resulting from EpiX Analytics' analysis. The default labelling of the clusters (1, 2, 3, …) are determined by algorithm, however, results for k=2, 3, and 4 also exhibit decreasing associated risk as the numeric label increases in each k scenario.

Serotype	2 Clusters	3 Clusters	4 Clusters
Muenchen	1	1	1
I 4,[5],12:i:-	1	1	1
Typhimurium	1	1	1
Newport	1	1	1
Berta	1	1	1
Enteritidis	1	1	1
Litchfield	1	1	1
Saintpaul	1	1	1
Dublin	1	1	1
I 4,[5],12:b:-	1	1	1
Blockley	1	1	1
Hadar	1	1	1
Kentucky	2	3	4
Infantis	2	2	3
Schwarzengrund	2	2	2
Montevideo	2	2	2
Reading	2	2	2
Heidelberg	2	2	2
Anatum	2	2	2
Javiana	2	2	2
Cerro	2	2	2
Thompson	2	2	2
Braenderup	2	2	2
Agona	2	2	2

Senftenberg	2	2	2
Uganda	2	2	2
Mbandaka	2	2	2
Mississippi	2	2	2
Muenster	2	2	2
Johannesburg	2	2	2
Meleagridis	2	2	2
Oranienburg	2	2	2
Bareilly	2	2	2
Give	2	2	2
Lubbock	2	2	2
Brandenburg	2	2	2
Albany	2	2	2
Norwich	2	2	2
Alachua	2	2	2
Panama	2	2	2
Kiambu	2	2	2
Poona	2	2	2

Comparison of clustering results

To test whether other genomics-based clustering methods produced similar groupings, FSIS compared the k=4 cluster results from the most abundant serovars in the risk assessment with clusters obtained using reference-free SNPs, referred to as the "Timme cluster" (Timme, 2013), core genome approach, the "Worley cluster" (Worley, 2018), and O-antigen groupings (Grimont) in **Table 12.** Additionally, the broad grouping of lower virulence cluster 2 serotypes were further discretized in these approaches. Using the O-antigen grouping classified serotypes from both clusters into different groupings: D1 included Dublin and Enteritidis from cluster 1 as well as Javiana from cluster 2; C2-C3 contained Hadar, Muenchen, and Newport from cluster 1 and Kentucky from cluster 3; B included I 4,[5],12:i:- and Typhimurium from cluster 1 and Heidelberg, Reading and Schwarzengrund from cluster 2.

Risk multiplier

The clusters were validated by linking them to epidemiological data (i.e., documented outbreaks attributed to poultry sources (CDC, 2021a) with consideration of prevalence in animal sources from FSIS poultry sampling programs). In this sense, the relative risk estimate is skewed towards strains to which a poultry consumer is likely to be exposed. FSIS constructed

There were 1,616 outbreaks initially considered based on data obtained from CDC NORS between 2009 and 2020. Within these outbreaks, 216 unique serotype-outbreak combinations were identified and filtered down to outbreaks that were likely attributed to poultry based on IFSAC classification and text mining of food ingredients associated with the outbreak origin sources. Strains with higher association to poultry-attributed outbreaks typically grouped together in cluster 1 for all *k* clustering scenarios.

FSIS conducted a preliminary exploratory analysis to illustrates the temporal dynamic of outbreaks for cluster 1 (C1) serotypes (**Figure 4**) and cluster 2 (C2) serotypes (**Figure 5**). Cluster 1 serotypes consistently comprised the dominating proportion of poultry-attributed outbreaks since 2013 with the majority of outbreaks associated with Enteritidis. Two of the twelve cluster 1 serotypes (Litchfield and Dublin) were not associated with any poultry-attributed outbreaks. On the other hand, cluster 2 serotypes, Infantis and Reading, outbreaks have been increasingly observed more recently, but not to the same extent (i.e., number of outbreaks).

Table 12: Comparing seroclusters developed under different approaches with EpiX Analytics' k=4 cluster scenario.

***Salmonella* Serotype**	**URF or SRF**	**EpiX Analytics k=4**				**reference-free SNPs (Timme 2013) N=156**						**Core Genome (Worley 2018) N=445**		**O-antigen (WHO Formulary)**					
	N > 500	C1	C2	C3	C4	B1	B2	B3	B4	A1	A2	B	A	E1	C1	K	D1	C2-C3	B
Enteritidis	5510	x									x		x				x		
Typhimurium	3421	x									x		x						x
Newport	2740	x									x		x					x	
I 4,[5],12:i:-	987	x									x		x						x
Dublin	697	x									x		x				x		
Saintpaul	612	x									x		x						x
Muenchen	607	x									x		x					x	
Hadar	558	x									x							x	
Schwarzengrund	1528		x						x			x							x
Reading	1299		x									x							x
Javiana	971		x				x					x					x		
Heidelberg	728		x								x		x						x
Anatum	673		x								x		x	x					
Cerro	591		x								x		x			x			
Thompson	549		x								x		x		x				
Braenderup	525		x								x		x		x				
Infantis	5604			x		x							x		x				
Kentucky	6413				x					x			x					x	

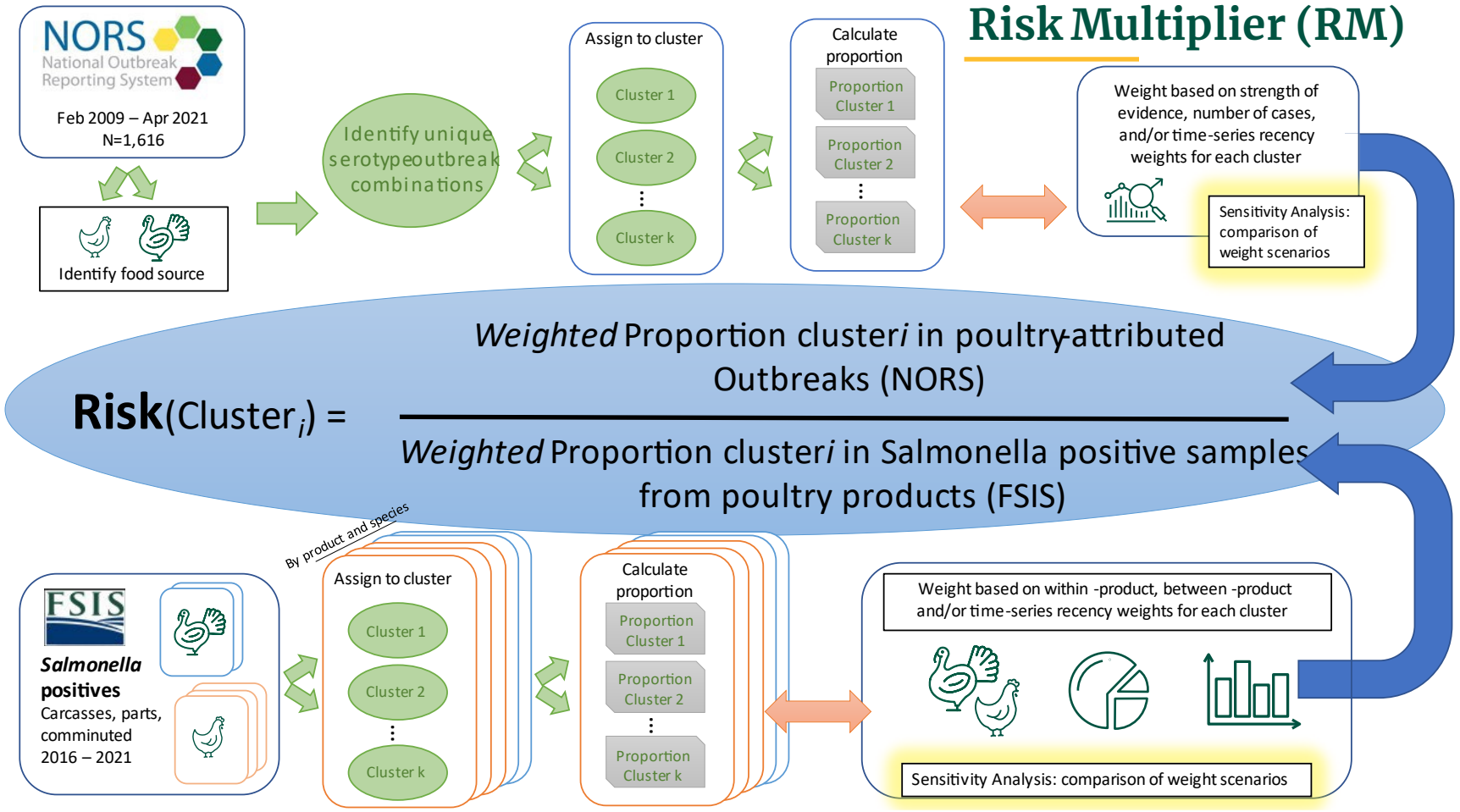

Figure 3: FSIS diagram of risk multiplier estimation for each serocluster.

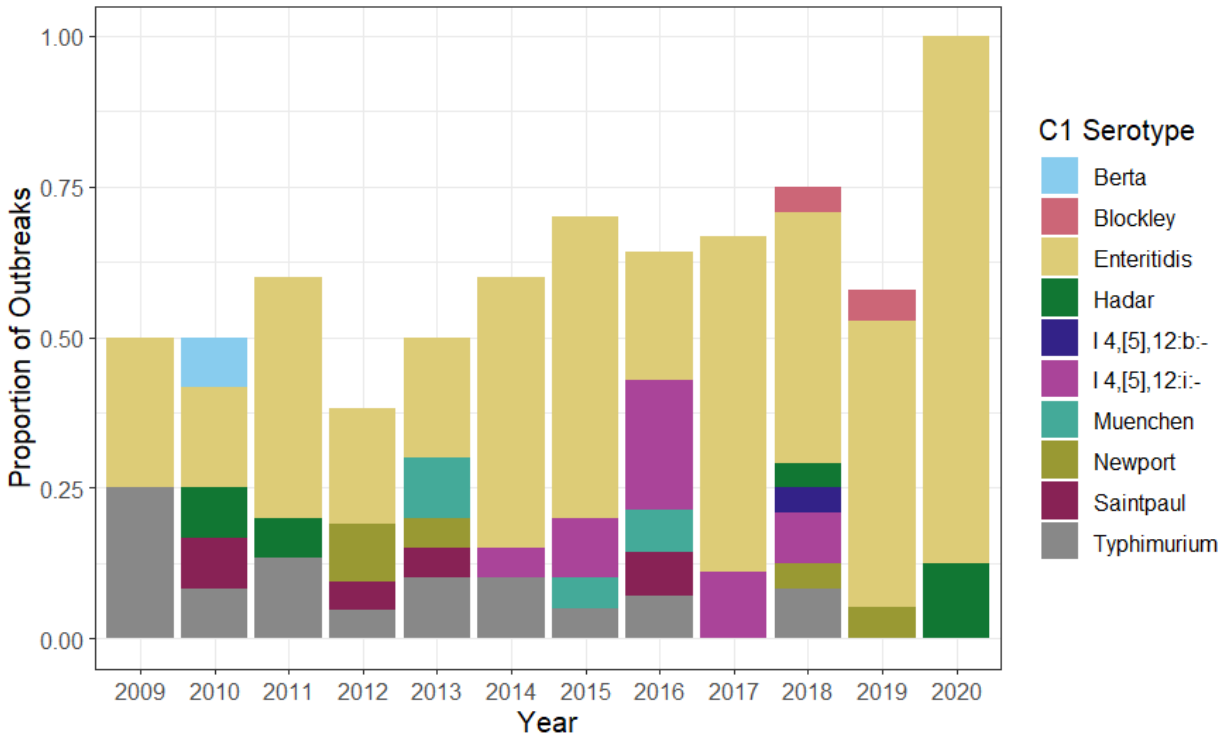

Figure 4: Composition of cluster 1 poultry-attributed outbreaks across time.

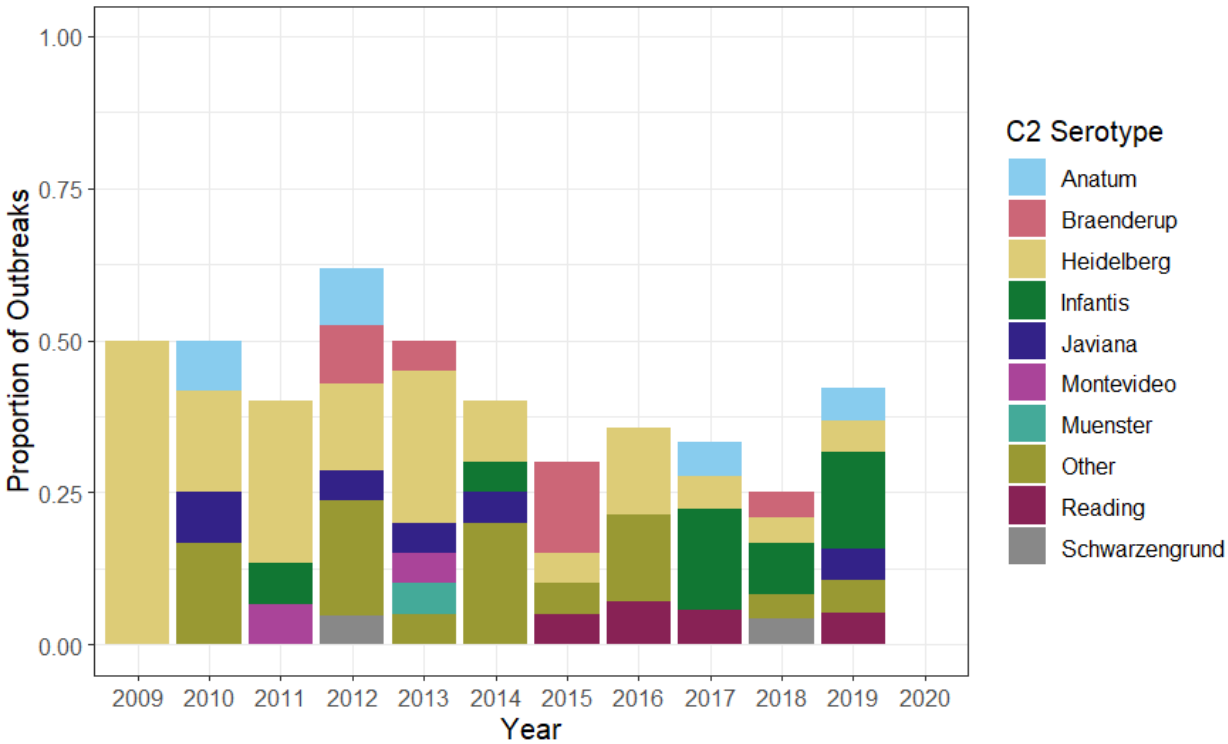

Figure 5: Composition of cluster 2 poultry-attributed outbreaks across time.

To elucidate the impact of more contemporary strains, additional transformations of the outbreak

proportion should be considered (e.g., time-series component, number of primary cases, severity/strength of evidence). Of particular interest is a time-series component, as recent outbreaks are more representative of the current status of foodborne illness compared to more historical data. Following the approach described in (Batz, 2021), a recency weighting was used to capture the time-series component and provide one factor to encapsulate the shifting dynamic of outbreaks. Poultry-associated outbreaks older than 5 years (i.e., prior to 2017) were subjected to an exponential decay function with a decay parameter defined as 5/7 (0.7142).

Figure 6 illustrates the increasingly dominant picture of poultry-attributed outbreaks associated with cluster 1 serotypes compared to the broad range of cluster 2 serotypes as well as how the weights shift across time. Although there was a relative balance of cluster 1 and cluster 2 poultry-attributed outbreaks prior to 2015, these have less influence or weight on the risk multiplier estimation. Moreover, EpiX Analytics considered several additional factors to estimate the proportion in outbreaks component of the risk multiplier (**Table 13**); the complete derivation is described in **Appendix A**. Nonetheless, these epidemiological dynamics highlight the notion that risk multipliers must be continuously assessed to describe risk to public health.

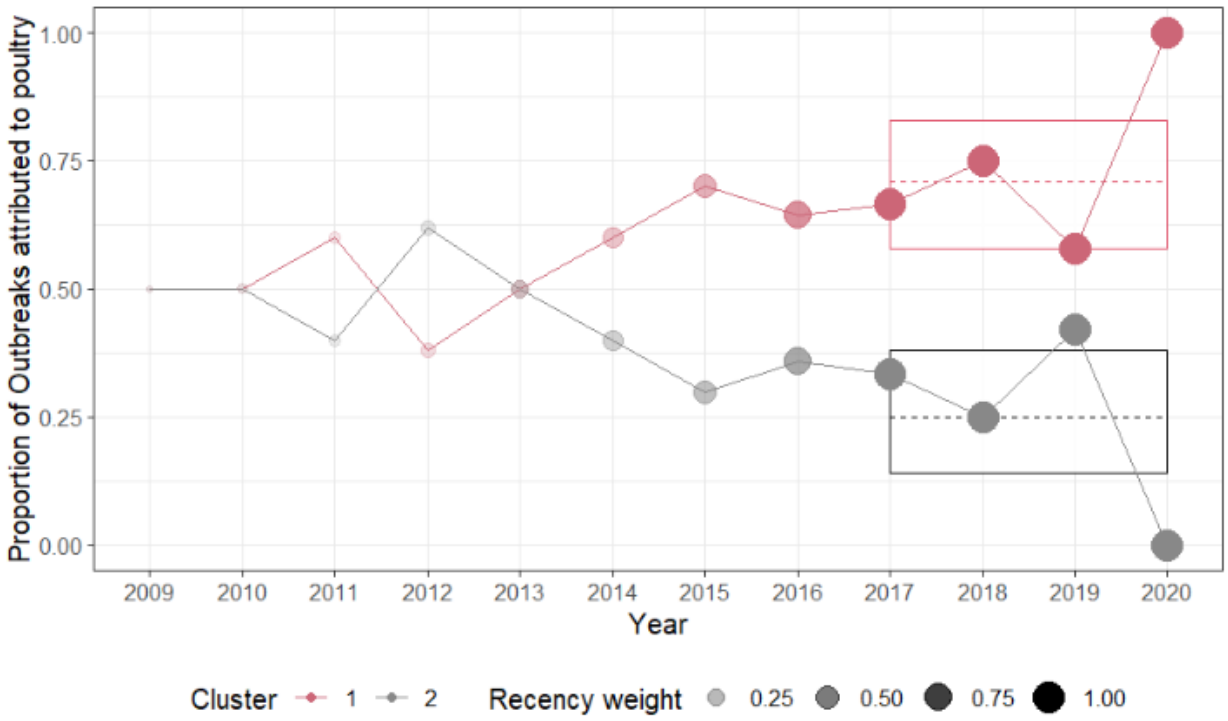

Figure 6: Time-series weighting scenario on the overall poultry-attributed outbreak proportion with the risk multiplier numerator estimation (dashed line) including confidence bounds (rectangles) overlaid on biased recent timeframe (2017-2020).

The risk multiplier denominator is derived from 2016-2021 FSIS regulatory sampling programs with consideration of product (i.e., chicken or turkey) and commodity (e.g., carcass, parts, comminuted). That is, to estimate the current status of *Salmonella* in poultry by cluster, annual production volumes were used to determine within-product and -commodity weights as well as between-product and -commodity weights based on general product availability and consumption rates, and a time-series (i.e., exponential

decay) function (Batz, 2021) was applied to emphasize recent data compared to historical (prior to 2017, as previously considered). In particular, the following weights were incorporated: 83% chicken parts, 6% comminuted chicken, 11% chicken carcasses, 75% turkey carcasses, 25% comminuted turkey, and an overarching 5/1 chicken to turkey ratio. These weights dictate a commanding influence of chicken parts, followed by turkey and chicken carcasses, and finally, comminuted product across time. Additional details on the product type break down by cluster are described in Chapter 3.

The majority of *Salmonella* in poultry detected belongs to cluster 2, however, the majority of *Salmonella* in poultry-attributed outbreaks stem from cluster 1 serotypes (**Table 13**). Cluster 1 had a relative risk of 2.1 (95% CI 1.7-2.5) whereas cluster 2 was approximated as 0.38 (95% CI 0.21-0.58). Isolates that could not be assigned to a cluster are also presented.

Table 13: Risk multiplier estimation including the 95% confidence interval for k=2 seroclusters derived by EpiX Analytics.

	Cluster 1	**Cluster 2**	**Not Assigned**
Proportion in outbreaks (numerator)	0.71 [0.58; 0.83]	0.25 [0.14; 0.38]	0.039 [0.012; 0.081]
Proportion in poultry (denominator)	0.33 [0.31, 0.35]	0.66 [0.64; 0.68]	0.010 [0.006; 0.017]
Risk multiplier	**2.1 [1.7; 2.5]**	**0.38 [0.21; 0.58]**	**3.9 [1.1; 9.1]**

A description of the risk multiplier calculation is in EpiX Analytics' report, **Appendix A**.

Sensitivity analysis

Different scenarios were considered in the derivation of the serocluster risk multipliers. The baseline case (presented in **Table 13**) considers various weights to evaluate/balance contributions from chicken and turkey outbreak characteristics including a mixed-effects model, a time-series component to differentiate recent information from historical data, and proportional cluster attribution rates. **Table 14** summarizes the different modeling scenarios assessed by EpiX Analytics during risk multiplier calculations for comparison with the baseline. It is readily observed that the associated risk was mostly consistent in each model except in the cases: (1) only using turkey data or (2) removing the time-series. Using turkey data only unnecessarily constrains the information feeding into the model. Out of the 216 unique serotype-outbreak combinations, only 44 were definitively attributed to turkey with a majority occurring prior to 2017. At the same time, within-product weights to estimate the proportion in poultry (i.e., denominator) considers 75% turkey carcasses to 25% comminuted turkey.

As described throughout this report, turkey carcass data is limited (roughly <10 detections annually), and thus, serotype proportions fluctuate dramatically. Additional data is required to appropriately refine the weighting scenario to consider turkey alone under this approach. In the case where the time-series is removed, the risk multiplier becomes heavily biased to historical data and does not accurately represent the changing dynamic of serocluster risk to public health.

Table 14: Risk multiplier sensitivity analysis of select scenarios considered by EpiX Analytics.

	Cluster 1	**Cluster 2**
Baseline*	2.1 [1.7; 2.5]	0.38 [0.21; 0.58]
Outbreak counts transformation	2.0 [1.6; 2.4]	0.39 [0.24; 0.55]
Estimated Primary cases transformation	2.0 [1.3; 2.5]	0.51 [0.22; 0.84]
No recency weighting	1.8 [1.4; 2.1]	0.53 [0.36; 0.70]
Recency weight starting to decrease after 1 year	2.4 [1.8; 2.9]	0.32 [0.14; 0.58]
Turkey only	1.7 [0.77; 3.0]	0.65 [0.22; 1.10]
Chicken only	2.2 [1.8; 2.6]	0.32 [0.16; 0.52]
Do not weight different products	2.4 [1.9; 2.9]	0.36 [0.21; 0.56]
Outbreaks Definitively or Probably attributed to poultry	2.1 [1.8; 2.4]	0.39 [0.24; 0.55]
Use best Cluster	2.1 [1.7; 2.5]	0.38 [0.21; 0.59]

The Choice of Two Seroclusters

When considering the number of clusters to use, the serotypes in the higher virulence cluster 1 (e.g., Enteritidis and Typhimurium) remained the same across the choices of 2, 3 or 4 clusters. Serotypes in this cluster have an estimated relative risk of 2.1 (i.e., the risk of illness is 2.1 times higher than the probability of illness, prior to knowing that the strain belonged to cluster 1). In the 2-cluster model, the relative risk for the lower virulence cluster 2 is 0.38. This leads to a large difference in the probability of illnesses between the clusters, which is estimated to be 2.1/0.38=5.5. Adding a third cluster did not change the serotypes in cluster 1 but did divide cluster 2 into two lower virulence clusters, with the lowest virulence cluster 3 consisting primarily of Kentucky. Adding a fourth cluster (**Table 15**) resulted in a new cluster consisting of Infantis alone. The relative risk for the Infantis cluster was 0.31, so the influence of Infantis would be less in the 4-cluster model than the 2-cluster model. While the relative risk for the cluster consisting of Kentucky in either the 3- or 4-cluster models is low, a conservative assumption is to include Kentucky with the other lower virulence serotypes. This choice is justified by noting that the majority of Kentucky isolates from U.S. poultry samples are of the Group 1 variety. Nevertheless, the more virulent Kentucky Group 2 (Soltys, 2021) has been recently isolated from chicken samples in the U.S. (Thompson, 2018), so these findings should be revisited periodically to determine if *Salmonella* Kentucky maintains its lower virulence status.

Additionally, Infantis is not currently considered a *Salmonella* subtype of concern linked to illness from consuming turkey (see Table 10 of FSIS' *Risk Profile for Pathogenic Salmonella Subtypes in Poultry* (available here). FSIS also conducted a sub-analysis to compare the changes in salmonellosis cases reported to FoodNet, with the change in the proportion of *Salmonella* positive samples in chicken carcasses whose serotype was Infantis in the Chicken Risk Assessment (available here).

Table 15: Dose-response model multipliers for 2, 3, and 4 *Salmonella* seroclusters.

	Multipliers for k = 4 (Estimate [bootstrap 95% CI])			
	Cluster 1	**Cluster 2**	**Cluster 3 (Infantis)**	**Cluster 4 (Kentucky)**
Multiplier	2.1 [1.7;2.5]	0.81 [0.44;1.30]	0.31 [0.0095;0.89]	0.01 [0.000;0.094]

Dose-response models

Dose-response models, developed by EpiX Analytics, were approximated for the k=2 serocluster result. The higher virulence cluster 1 dose-response model was estimated using outbreak data and employing a beta-Poisson model of infection for a given dose as derived in (Teunis, 2010; Teunis, 2008). The risk multipliers (**Table 13**) were then used to scale the relative risk of illness from exposures to each cluster. That is, cluster 1 dose-response was developed using data from the literature on Enteritidis and Typhimurium (two primary serotypes in cluster 1) and scaled a second dose-response model for the lower virulence cluster 2 based on the risk multiplier ratios. Finally, a polynomial regression was fit to the dose-response functions for swift implementation in the risk assessment model.

The polynomial approximation of the dose-response models was used to estimate some useful illness doses. For higher virulence cluster 1 *Salmonella* serotypes, the ID50, the dose at which 50% of individuals in an exposed population will experience symptomatic illness, is approximately 2000 cfu. For lower virulence cluster 2 *Salmonella* serotypes, the ID50 is not attained, with at most 40% of an exposed population becoming ill at doses higher than 1 billion cfu. There is a 1 in 100 hundred probability of illness at 1 cfu of higher virulence *Salmonella* per serving. While for the lower virulence serotypes, the dose response model estimates a 0.002 probability of illness at 1 cfu *Salmonella* per serving. For comparison, the FAO/WHO *Salmonella* dose-response model estimated a 13 percent chance of becoming ill if ingesting an average dose of 100 organisms (FAO/WHO, 2002). Even at the level of 1 organism ingested, there was still a non-zero chance of illness (0.25%).

Further details of the virulence-adjusted dose-response models development are described in EpiX Analytics' report, **Appendix A**.

2.3 Serotypes of Public Health Significance

The higher virulence serotypes which appear most frequently in FSIS comminuted turkey samples (Hadar, Typhimurium, and Muenchen) are summarized in **Table 16** and referred to as "serotypes of public health significance" in this risk assessment. The portion of FSIS PR/HACCP *Salmonella* positive samples that are sequenced as a serotype of public health significance is 25% for comminuted turkey product.

Table 16: Higher virulence *Salmonella* serotypes in FSIS PR/HACCP poultry sampling. An X indicates the serotype is among the top 10 FSIS serotypes for that product. the average percent of *Salmonella* positive samples that are higher virulence or top 3 higher virulence positive are also included.

Higher Virulence Serotypes	**Comminuted Turkey** 2016-2021 N=1,219
Berta	
Blockley	
Dublin	
Enteritidis	
Hadar	X
I 4,[5],12:b:-	
I 4,[5],12:i:-	X
Litchfield	
Muenchen	X
Newport	
Saintpaul	
Typhimurium	X
Top 3 higher virulence serotypes	23%
All higher virulence serotypes	33%

Chapter 3 Microbial Profile

The first goal of the risk assessment is to define a probabilistic model that explains the current state of pathogen contamination in the U.S. turkey population. The effect of various risk management options can then be assessed by adjusting the parameters that describe the population in accordance with the anticipated effect of different risk management options.

The microbial data used in the *Salmonella* model is summarized in **Table 17**. Full descriptions of data used in this risk assessment are available in **Appendix B** with detail of the population description methodology in **Appendix C**.

Table 17: Description of main sources of data used in the risk assessment.

Product	Data	Limitations
Carcass Microbial Data	• Establishment-level FSIS PR/HACCP turkey carcass post-chill samples from the *Salmonella* verification program. results from 2016-2021 (post-chill) • FSIS establishment-level *Salmonella* data from young turkey microbiological baseline study from 2008 through 2009 (post-chill and rehang).	• *Salmonella* recover methods are limited in turkey carcasses. • No recent enumeration data is available. • FSIS has not historical sampled at preharvest and no pre-evisceration data is available.
Parts Microbial Data	• None available.	• FSIS has never sampled turkey parts through regulatory sampling or exploratory sampling. • There are no standard methods for *Salmonella* collection from turkey parts.
Comminuted Microbial Data	• Establishment-level FSIS PR/HACCP comminuted turkey samples from the *Salmonella* verification program results from 2016 through 2021 (*Salmonella* Prevalence, Serotype). • FSIS establishment-level *Salmonella* data from comminuted turkey microbiological baseline study from 1995.	• No sampling data available prior to post-chill final products (i.e., up-stream (preharvest) to analyze process control for this product).

From the *Salmonella* testing data, it is possible to estimate the prevalence of test-positive carcasses, the prevalence of carcasses with a specific serotype or belonging to a virulence cluster, and an industry-wide distribution of *Salmonella* colony forming units per milliliter in the assay. These estimates are made for both *Salmonella* on rehang and post-chill carcass.

Turkey establishments typically employ a multifaceted systems-based approach to minimize pathogen contamination derived from PR/HACCP principles. However, current *Salmonella* contamination in the turkey industry is more limited than chicken contamination data, as described in the Chicken Risk Assessment (available here). As such, this risk assessment is limited to assessing the situation at post-chill, with minimal available information regarding enumeration. Historical data from previous FSIS turkey microbiological baseline studies are considered as a reasonable starting point for rehang conditions and comparative analysis.

3.1 *Salmonella* Prevalence

Historically, there have been very few *Salmonella* positive detections in turkey carcasses, and hence, many establishments consistently meet (i.e., pass) current FSIS performance standards based on prevalence (**Figure 7**). Those that sporadically fail to meet the standard are typically lower volume producers; however, some higher volume establishments approach the threshold on occasion—see the next section **Production Volume** for a description of turkey production volume. However, it is likely that the low rate of *Salmonella* recovery from turkey carcasses is a function of sponge sampling and rinsate limitations than truly indicative of the true *Salmonella* prevalence in turkey and such conclusions should be applied judiciously.

The national prevalence of *Salmonella* at post-chill (**Table 18**) was calculated using a design-based paradigm incorporating production volume weights. Details of the methods are described in **Appendix C,** including additional industry prevalence estimates from recent years (2016-2021). The national post-chill prevalence for *Salmonella* on turkey carcasses has consistently remained very low. The current sampling data lacks information at or prior to rehang; as an alternative, the 2008-2009 FSIS young turkey carcass microbiological baseline study was evaluated, which included both rehang and post-chill sampling points. In this study, the rehang and post-chill prevalence was estimated at 0.1023% and 0.0187%, respectively, representing an 8% reduction in *Salmonella* prevalence during the slaughter process. In the interim years, rehang has not been robustly sampled, preventing an accurate assessment of prevalence, while the post-chill prevalence on carcasses has dropped below 1% since 2016. This is equivalent to less than 20 positives each year out of 2,000 samples across the industry.

Comminuted turkey, on the other hand, presents a different dynamic (**Figure 8**). Here, we observe a mixture of establishments by production volume not meeting the performance standard, with the majority of larger (i.e., higher production volume) establishments not passing the FSIS comminuted turkey performance standard in a 52-week period. In addition, more variability in the national prevalence in comminuted turkey has been observed recently, fluctuating between 0.14% and 0.25%. Prevalence peaked in 2018 but has since dropped down to 0.18% in 2021.

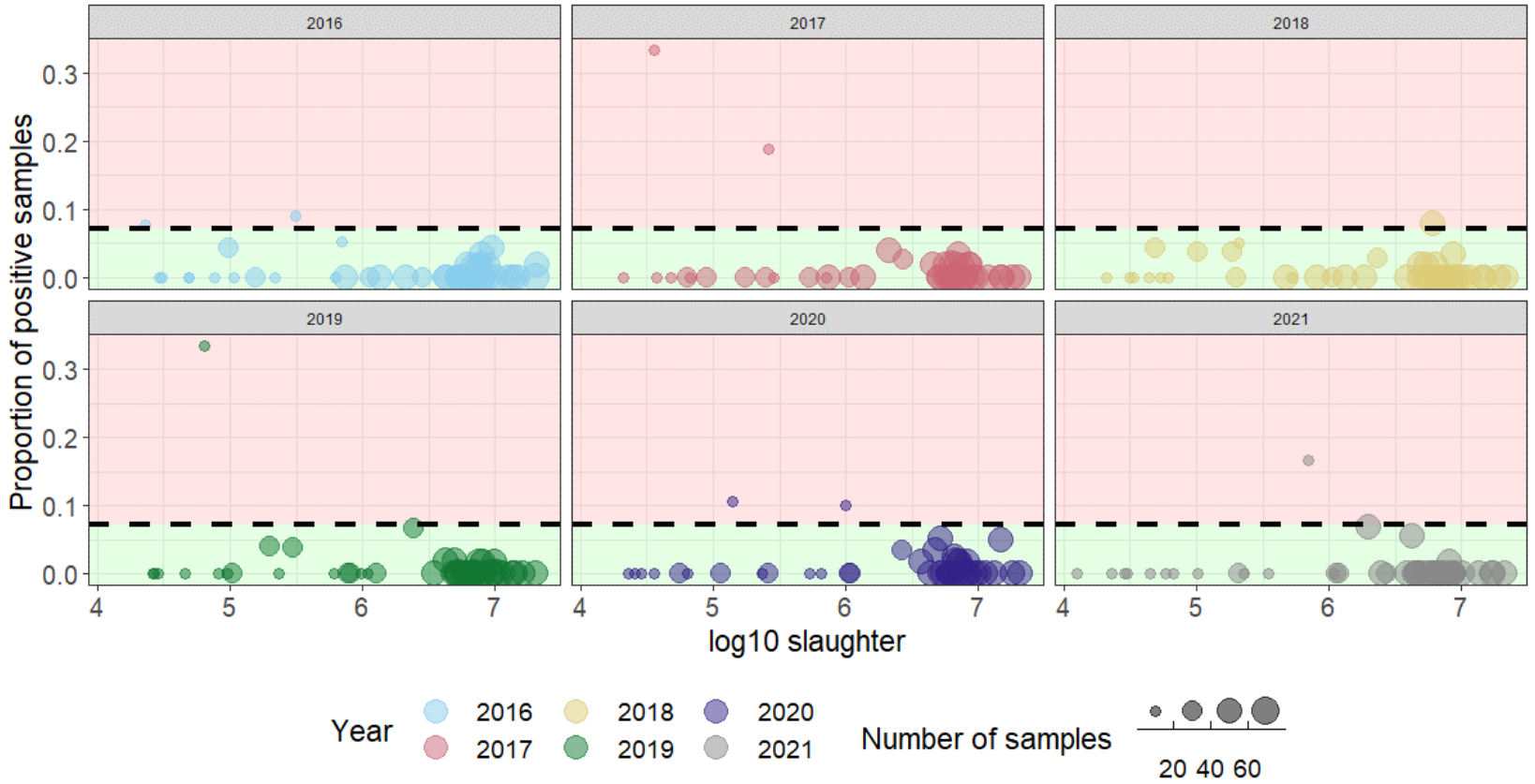

Figure 7: Annual proportion *Salmonella* positive on turkey carcasses by establishment and year. Each point represents a different establishment and year. The horizontal dashed line indicates the performance standard threshold (4 of 56 or 7.1%). Note this does not consider the minimum number of samples (n = 14) required to assess process control.

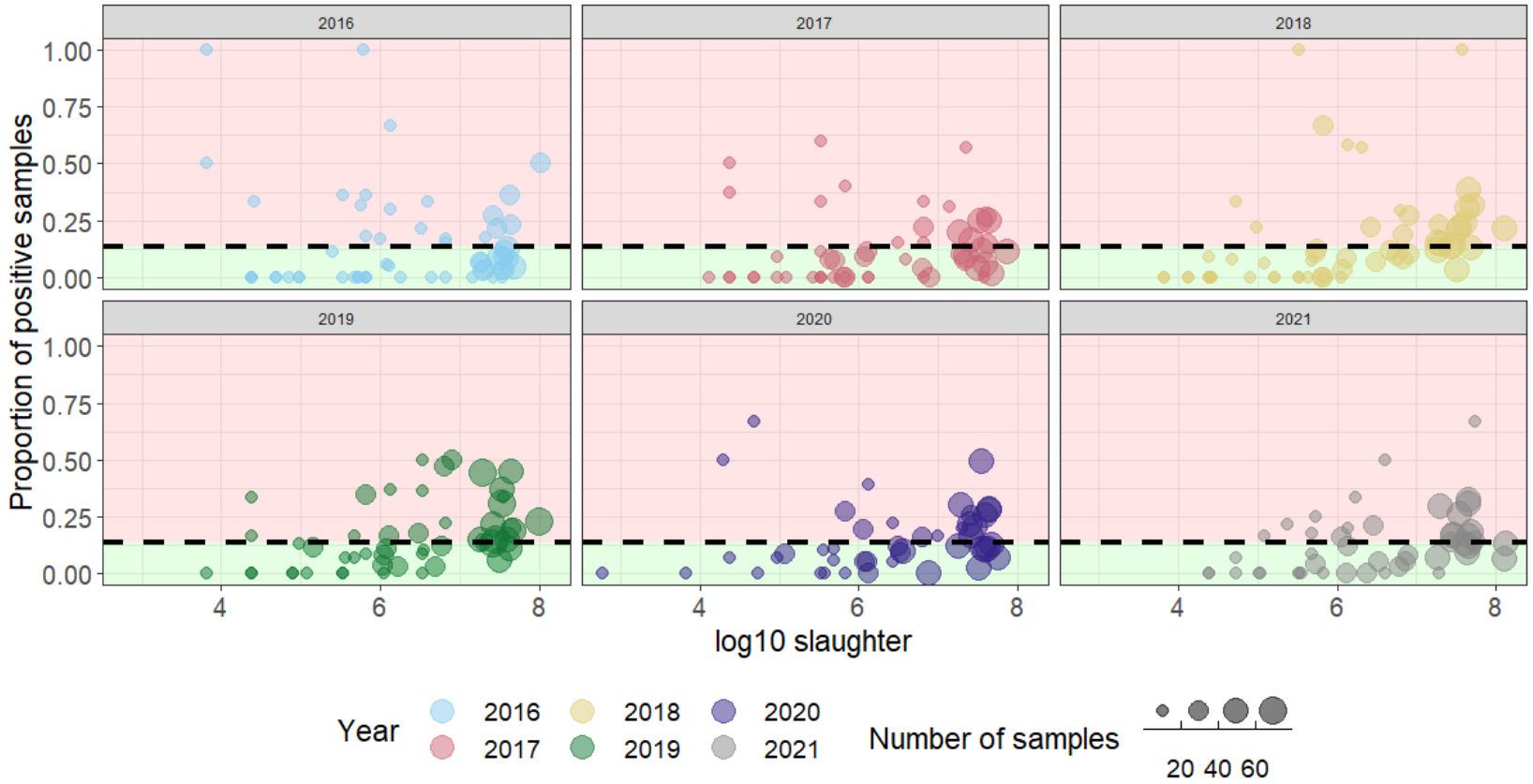

Figure 8: Annual proportion positive for *Salmonella* in comminuted turkey by establishment and year. Each point represents a different establishment and year. The horizontal dashed line indicates the performance standard threshold (7 of 52 or 13.5%). Note that this does not consider the minimum number of samples (10) required to assess process control.

Table 18: Estimated volume-weighted prevalence by product type and sampling location.

Commodity	Sample Location	Year	*Salmonella* Prevalence ($\hat{P}$)	Standard Deviation ($\sqrt{var\left[\hat{P}\right]}$)	95% confidence interval
Turkey Carcass	Rehang	2008-09	0.1023	0.000083	(0.0851, 0.1208)
	Post-chill	2008-09	0.0187	0.000023	(0.0106, 0.0292)
	Post-chill	2021	0.0028	0.000001	(0.0011, 0.0053)
Comminuted Turkey	Post-chill	2021	0.1763	0.00051	(0.1343, 0.2227)

Production Volume

The FSIS PR/HACCP program tracks the occurrence of microbial pathogens to analyze the average prevalence for each establishment over 52-week moving windows for performance standards. The concentration of *Salmonella* was estimated using a presence/absence screening test. All samples were selected randomly at a post-chill location using a sponge sample for carcasses and a 2-lb final product sample for comminuted turkey (of which 325 grams is tested by FSIS laboratories). Inspectors take sponge samples of small areas (i.e., 5cm x 10cm) of the back and of the thigh (FSIS, 2021) to create a composite that will determine the status of the carcass.

FSIS collects carcass samples from high volume establishments (e.g., >10 million birds processed) on a weekly basis whereas smaller establishments are sampled less often. In particular, as of late 2019, FSIS schedules sampling at establishments producing turkey carcasses based on daily production; FSIS schedules at least two samples per month for establishments producing between 1,001 and 250,000 lbs. per day, and 5 samples per month to those establishments producing over 250,000 lbs. per day. This yields approximately 24 samples annually in small establishments and upwards of 50 to 60 samples in high volume establishments. On average, large establishments are subject to at least 52 samples per year. This bimodal sampling distribution is highlighted by the split in the annual sampling frequency per establishment (**Figure 9**).

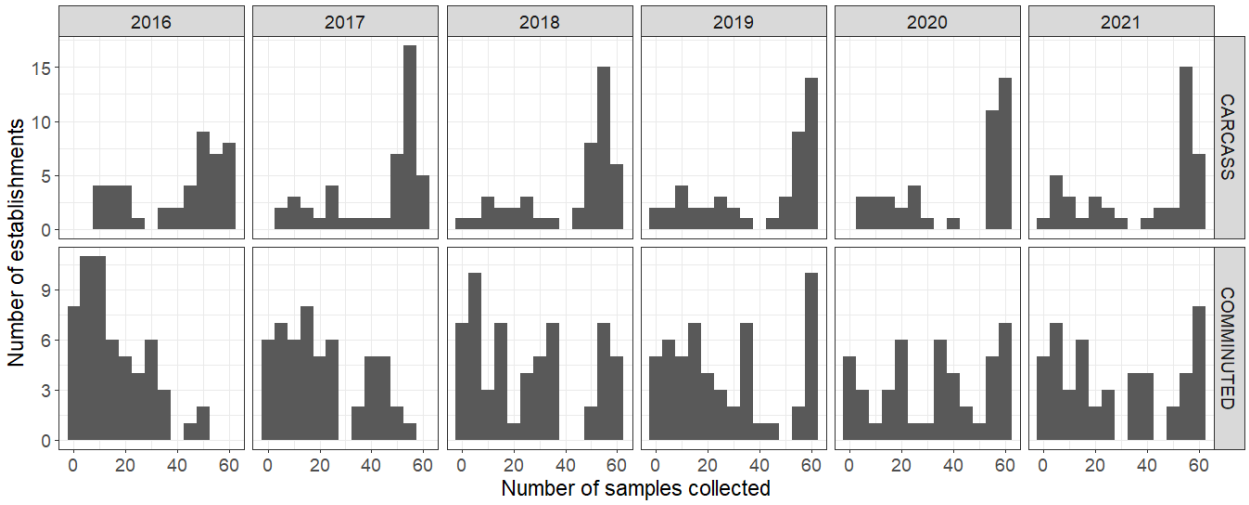

Figure 9: Sampling distribution in each establishment by year.

3.2 *Salmonella* Levels

No turkey carcass samples have been enumerated recently in the PR/HACCP program, and few *Salmonella* positives were observed. Hence, a reliable estimate for the current concentration distribution of *Salmonella* on carcasses is not feasible. Additionally, in the FSIS young turkey carcass microbiological baseline study, less than 5% of rehang samples and less than 1% of post-chill samples were quantifiable. This lack of ample data limits the robustness of scenario analysis of *Salmonella* levels on turkey carcasses.

In comminuted turkey PR/HACCP samples, a portion of detected samples were further tested for *Salmonella* levels using the most probable number (MPN) estimation method with a limit of detection (LOD) of 0.3 MPN/g. One sample was not analyzed and that missing result was addressed by using imputation by simple random sampling (van Buuren, 2011). Samples collected in 2020 were not used in this risk assessment because the MPN analysis was not consistently performed. The compiled comminuted turkey dataset consisted of 1,178 samples, of which 157 were positive on the screening test.

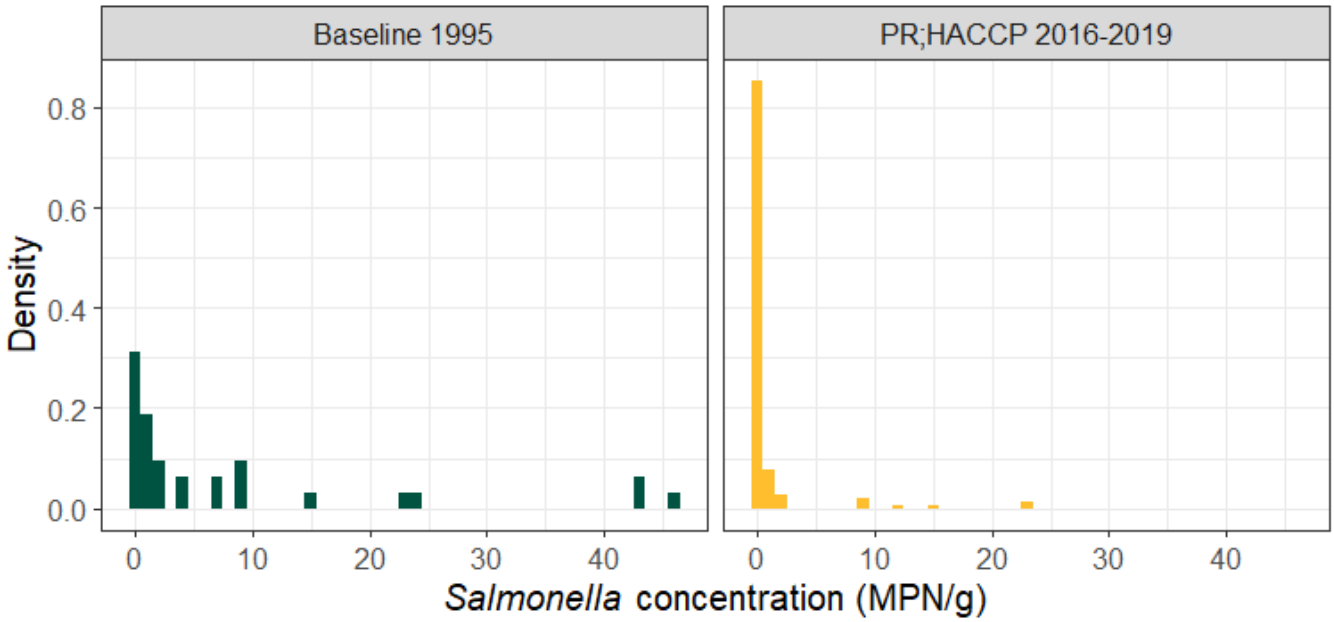

Figure 10: *Salmonella* concentration in comminuted turkey from enumerated samples compared to microbiological baseline study. One sample with a concentration of 240 MPN/g in the PR/HACCP program was removed for easier visualization of the side-by-side comparison.

The quantifiable comminuted turkey samples were based on five dilutions (10, 1, 0.1, 0.01, and 0.001) with three replicates per dilution. Left-censored samples (<0.003 MPN/g) were set to 1/325 since the original 325g sample screen *Salmonella* positive. The distribution of *Salmonella* concentration in comminuted turkey is illustrated in **Figure 10** alongside the FSIS raw ground turkey microbiological baseline study from 1995. This presents a preliminary finding of generally lower levels being observed in comminuted turkey, as compared to 28 years ago. The parameter values for the lognormal concentration distribution of the population derived from the comminuted turkey data are $\mu = -4.857$ and $\sigma = 2.333$, and the distribution is illustrated in **Figure 11**. The implied prevalence for comminuted turkey, derived from the cumulative distribution of the lognormal evaluated at the LOD=1/325, was

0.157, which is relatively similar to the prevalence estimates for calendar year 2021 (0.1763, 95% CI (0.1343, 0.2227)). Furthermore, a Monte Carlo simulation using a Poisson-lognormal with intensity randomly sampled from the derived lognormal concentration distribution (**Figure 11**) yields a 19% probability of at least one viable *Salmonellae* at post-chill in comminuted turkey product.

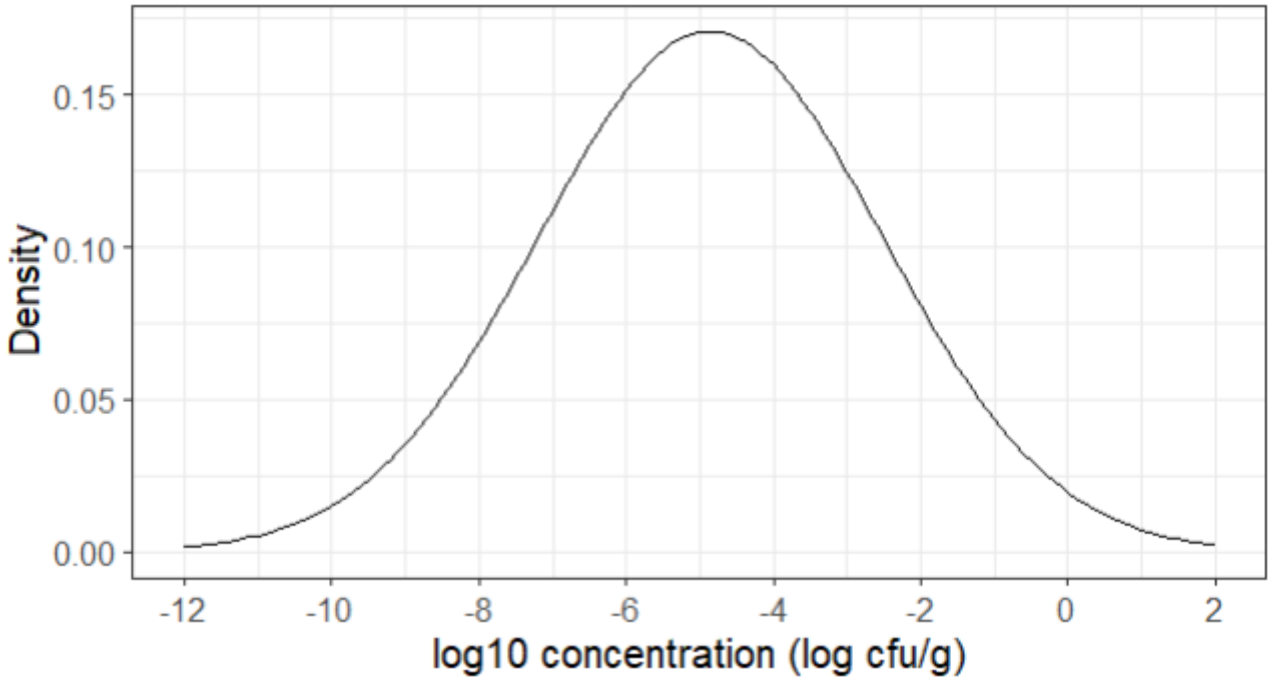

Figure 11: Fitted lognormal distribution of *Salmonella* concentration in comminuted turkey.

3.3 *Salmonella* Serotypes

In samples that were confirmed positive for *Salmonella*, WGS was used to identify serotypes. **Table 19** highlights the top serotypes found by commodity from FSIS PR/HACCP sampling over a six-year period alongside historical microbiological baseline sampling programs. There were 49 different serotypes found in comminuted turkey products, as compared to only 19 serotypes isolated on turkey carcasses in the PR/HACCP program. Serovars Reading and Hadar ranked as the top two in both carcasses and comminuted, comprising more than 30% of the serotype samples for each commodity. Serovar Hadar was also observed most often in the turkey microbiological baseline studies and appeared in the top ten CDC FoodNet annual summary from 2020.

Table 19: Top serotypes detected in post-chill sampling programs compared to the top serotypes in CDC FoodNet's 2020 annual summary. Total counts of each serotype are also indicated. Highlighted serotypes are those that fall into the "higher virulence" serotype cluster.

Rank	Carcass		Comminuted		CDC FoodNet 2020
	2016-21 PR/HACCP (N=80)	**2008-09 Baseline** (N=24)	**2016-21 PR/HACCP** (N=1,219)	**1995 Baseline** (N=97)	
1	Reading (23)	Hadar (13)	Reading (278)	Hadar (20)	Enteritidis
2	Hadar (11)	Heidelberg (2)	Hadar (125)	Reading (10)	Newport
3	Agona (7)	Albany (2)	Infantis (93)	Muenster (9)	Javiana
4	Infantis (6)	Senftenberg (1)	Schwarzengrund (91)	Saintpaul (8)	Typhimurium
5	I 4,[5],12:i:- (5)	Saintpaul (1)	Typhimurium (75)	Heidelberg (8)	I 4,[5],12:i:-
6	Schwarzengrund (5)	Muenchen (1)	Uganda (71)	Typhimurium (6)	Hadar
7	Muenchen (4)	Agona (1)	Agona (66)	Schwarzengrund (5)	Infantis
8	Senftenberg (4)	Berta (1)	Senftenberg (60)	Senftenberg (5)	Muenchen
9	Typhimurium (3)	412:d:- (1)	Muenchen (55)	Brandenburg (5)	Saintpaul
10	Uganda (3)	4512:r:- (1)	I 4,[5],12:i:- (49)	Agona (4)	Mississippi

**Shaded cells indicate cluster 1 serotypes*

The distribution of serotypes fluctuates widely as there were few positives in turkey carcass across time (**Figure 12**), whereas comminuted turkey has relatively similar serotype proportions annually (**Figure 13**). Other top *Salmonella* serotypes from the CDC FoodNet summary include I 4,[5],12:i:-, Infantis, and Typhimurium, which are on an upward trend in the proportion of detections in turkey over the last few years. Although Enteritidis was most frequently associated with human salmonellosis in 2020, it is rarely observed (or detected) on turkey carcasses or in comminuted turkey products.

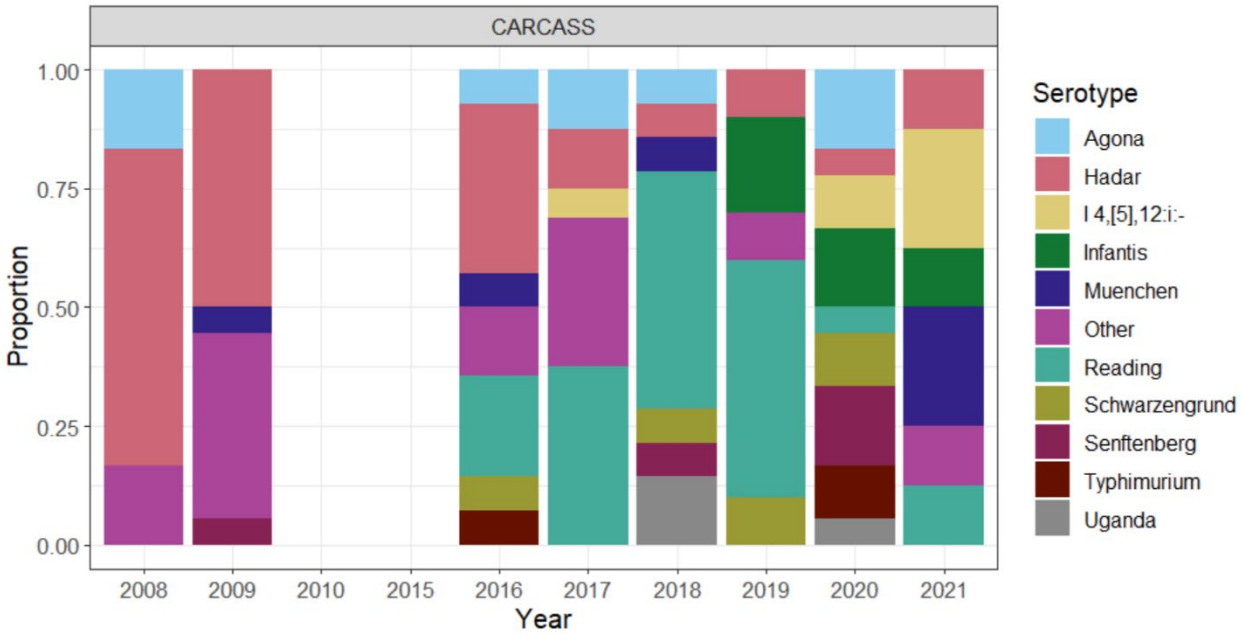

Figure 12: *Salmonella* serotypes detected on turkey carcasses across time.

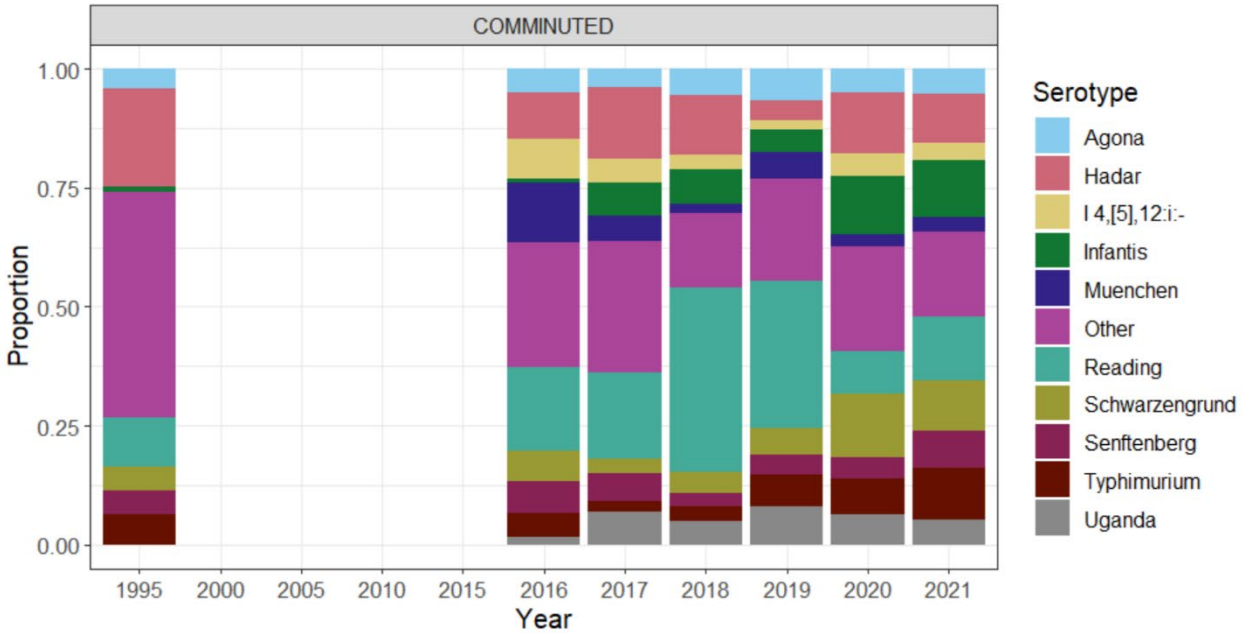

Figure 13: *Salmonella* serotypes detected in comminuted turkey across time.

Seroclusters based on virulence

As part of the Cooperative Agreement between FSIS and EpiX Analytics, *Salmonella* serotypes were categorized into two clusters derived from a machine learning algorithm using 193 virulence factors from the Enterobacteriaceae family. Two stable clusters were constructed and denoted either higher or lower virulence based on epidemiological characteristics (described in section **2.2**). For EpiX Analytics description of the method see (**Appendix A**) and the FSIS' *Bioinformatics Supplemental Materials* (available here).

Table 20 provides the overarching breakdown of serotypes by cluster (i.e., serocluster composition), and the most frequent serotypes detected in FSIS 2016-2021 PR/HACCP turkey samples for each cluster are summarized in **Table 20**. In particular, higher virulence cluster 1 (referred to as C1 or cluster 1), which includes Typhimurium, Hadar, Muenchen, and I 4,[5],12:i:-, has been identified as the higher virulence grouping of serotypes compared to lower virulence cluster 2 (referred to as C2 or cluster 2) which includes key *Salmonella* serotypes for poultry: Reading and Infantis. Analysis of MPN and quantitative polymerase chain reaction (qPCR) poultry enumeration data (results not shown) did not indicate a significant difference in concentration of *Salmonella* by cluster.

Table 20: Summary of the most common serovars found in turkey samples or associated to turkey-related outbreaks by cluster.

"Higher virulence" Cluster 1	"**Lower virulence" Cluster 2**
Hadar	Reading
I 4,[5],12:i:-	Infantis
Typhimurium	Schwarzengrund
Muenchen	Uganda
Saintpaul	Agona

Although *Salmonella* positives in turkey carcasses are rare, the dominant cluster occurrence has switched since the 2008-2009 microbiological baseline, where serotypes from cluster 2 are now more often identified (**Figure 14**). Nevertheless, the average annual proportion of cluster 1 and cluster 2 is 0.326 and 0.674, respectively. For comminuted turkey, cluster 2 has been commonly observed in positive samples, yet approximately 60 detections of cluster 1 occur every year, illustrating a consistent presence of cluster 1 serovars in comminuted final products (**Figure 15**). Within cluster 1, Hadar, I 4,[5],12:i:-, and Typhimurium average 48 of those detections (~80%) each year since 2019 in comminuted turkey and comprise 80% of all cluster 1 detections on turkey carcasses since 2019. In FSIS PR/HACCP sampling over the 2016-2021 period, the cluster 1 proportion amounts to a similar annual average proportion in comminuted turkey at roughly 0.309.

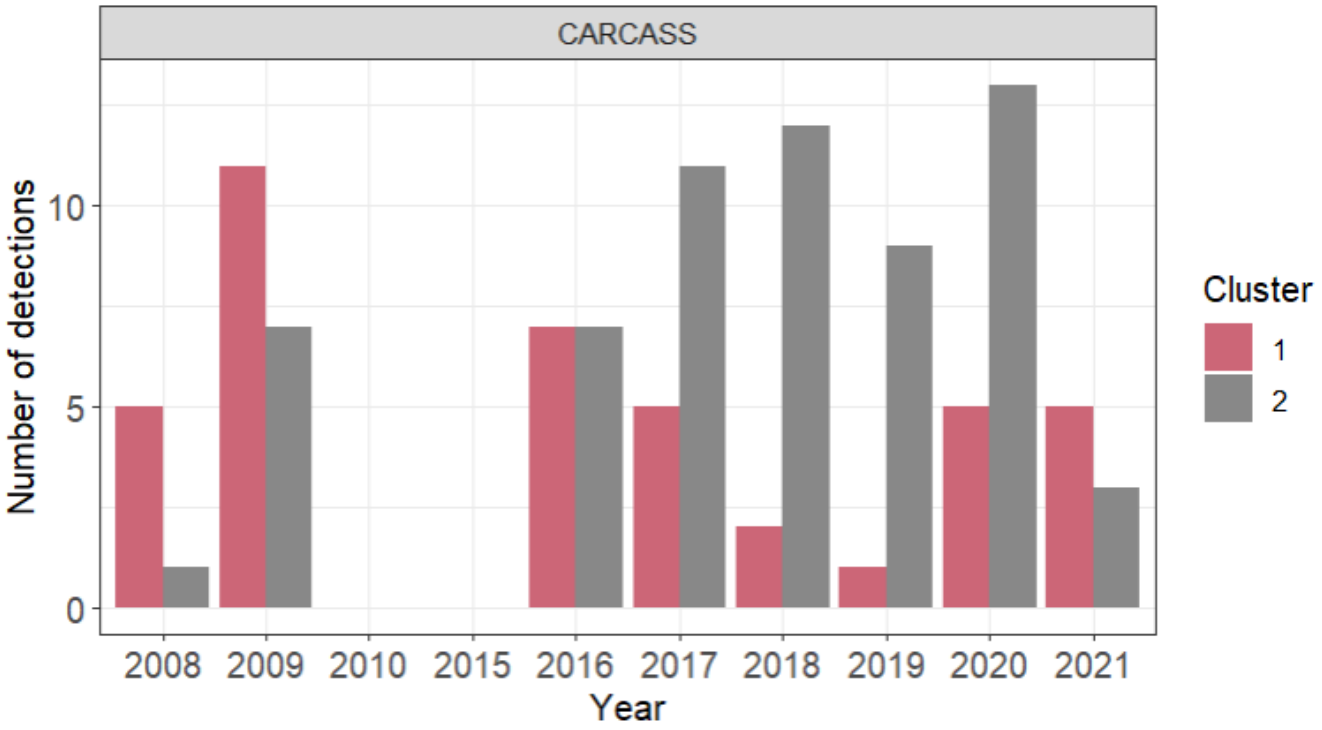

Figure 14: *Salmonella* serotype clusters from turkey carcasses across time.

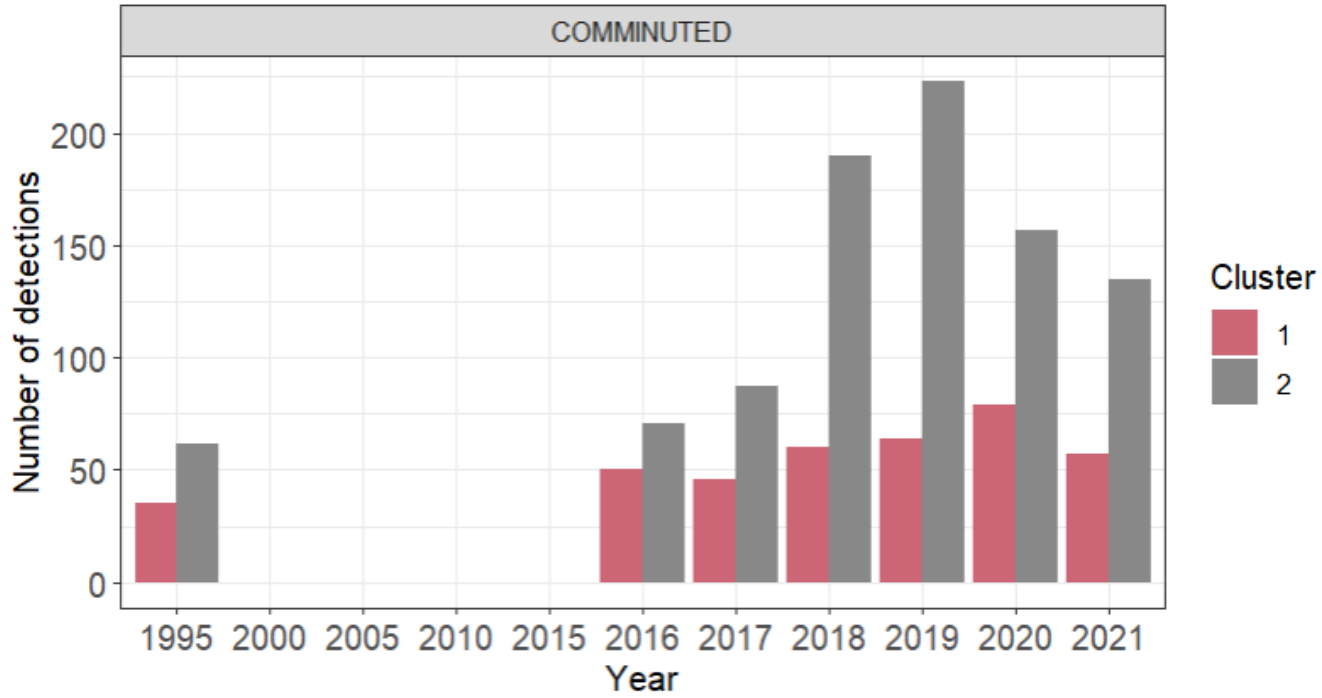

Figure 15: *Salmonella* serotype clusters in comminuted turkey across time.

Serocluster risk estimation

Epidemiological data was considered to estimate the associated risk for each serocluster. EpiX Analytics defined risk in terms of a 'risk multiplier', which is the ratio for each cluster of the proportion of poultry-attributed outbreaks to the proportion of poultry samples. Therefore, the previous section describing the distribution of seroclusters in turkey product samples would influence the denominator of the risk ratio. To account for the fluctuation across time, a recency weight (Batz, 2021) was incorporated as a time-series decay component to offset more historical trends compared to recent conditions. Additionally, the associated risk multiplier for each serocluster is prescribed under several scenarios that adjust the timescale of the recency parameter and evaluates modeling combinations of poultry weights (i.e., within and between chicken and turkey products).

3.4 Indicator organisms

Indicator organisms were measured at rehang and post-chill in FSIS' 2008-2009 young turkey microbiological baseline study.

Aerobic count (AC) and *Enterobacteriaceae (*EB) distributions at rehang and post-chill are summarized in **Figure 16** and **Figure 17**, respectively. There were 1,438 quantifiable samples for AC at rehang (a 99.7% detection rate) with a mean of 3.20log10. At post-chill, the number of quantifiable samples for AC dropped to 1,269 (88% rate) with a mean of 1.14log10. That is, an average reduction of approximately 2 logs via process control interactions in terms of AC. EB was also largely quantifiable at rehang (1,394 samples or approximately 97%) with a mean of 1.68log10. At post-chill, only 523 samples were quantifiable for EB, yielding a mean of -0.59log10. Similar to AC, EB also presented a nearly 2 log reduction was achieved during process control in the turkey carcass microbiological baseline study. Initially, samples below the (LOD = 1.2 cfu/cm^2) were assumed to be half of the LOD and samples that exceeded the upper limit of the diagnostic range were doubled. However, ultimately, a maximum likelihood function was used to fit the lognormal distributions with consideration of censored data.

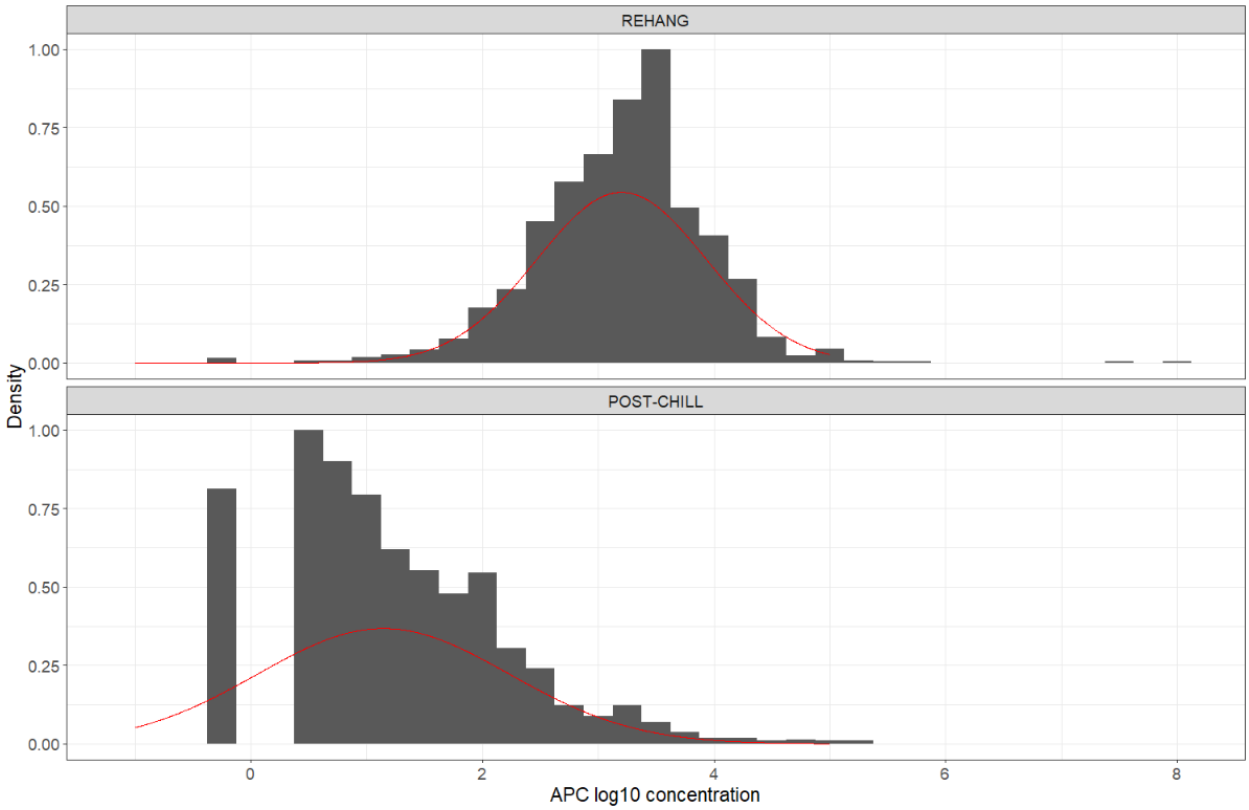

Figure 16: Log-transformed AC distribution at rehang and post-chill.

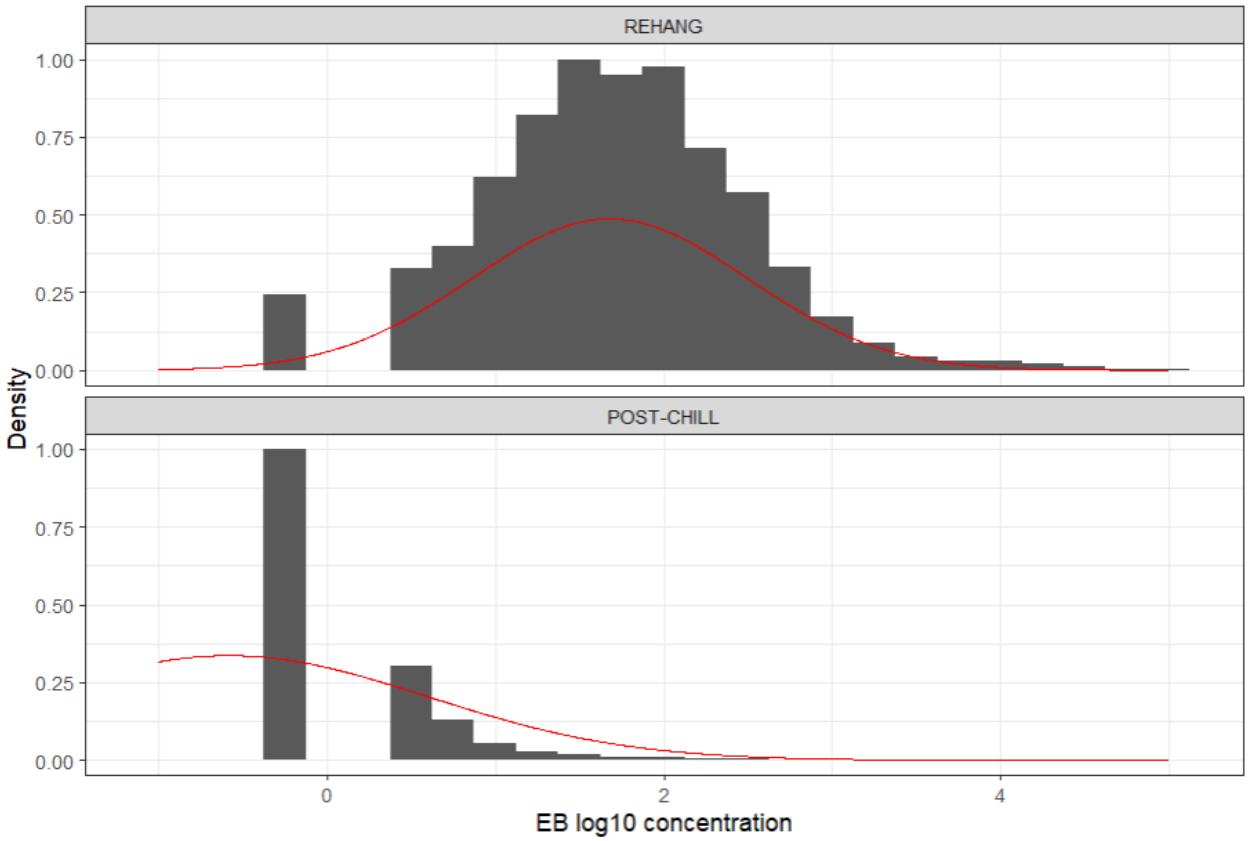

Figure 17: Log-transformed EB distribution at rehang and post-chill.

Chapter 4 Baseline Exposure Assessment

4.1 Foodborne Illness Surveillance

Foodborne illness surveillance in the U.S. relies on a broad network of local and state health departments and CDC. The CDC Foodborne Diseases Active Surveillance Network (FoodNet) conducts surveillance for nine laboratory-diagnosed infections, including, *Salmonella*, identified by culture or culture-independent diagnostic test for bacterial pathogens of samples from patients. The network was established in July 1995 and is a collaborative program among CDC, 10 state health departments, FSIS, and the Food and Drug Administration (FDA). The surveillance area includes 15% of the U.S. population (48 million persons). Personnel at each FoodNet site collect information about cases of infection and share that information with CDC through FoodNet's database.

The CDC National Outbreak Reporting System (NORS) includes data on illnesses resulting from contact with animals, environmental contamination, spread by person-to-person, waterborne transmission, and other enteric illness outbreaks. CDC also maintains the Foodborne Disease Outbreak Surveillance System (FDOSS) for collecting and reporting data about foodborne disease outbreaks in the U.S. In FDOSS, outbreaks are defined as the occurrence of >2 cases of a similar illness resulting from the ingestion of a common food (Gould et al., 2013). NORS data provide detailed food items considered as vehicles of the outbreaks and are more reliable to determine the causative contaminated food vehicles. Each of the implicated food vehicles has been grouped into one of 17 broad commodity classes (J. A. Painter et al., 2013; Richardson et al., 2017)

NORS data described 1,616 *Salmonella* outbreaks from 2009 to 2020. Using the Interagency Food Safety Analytics Collaboration's (IFSAC) food categorization field (CAFC) there were 106 outbreaks attributed to chicken, 34 attributed to turkey, and 231 attributed to multiple sources that may include poultry products. Since 2015, turkey outbreaks included *Salmonella* serotypes Anatum, Enteritidis, Hadar, I 4,[5],12:i:-, Infantis, Newport, Reading, and Schwarzengrund. The largest recent multi-state outbreak associated to turkey (in the NORS dataset) was an outbreak of Reading starting in 2017 (NORS, 2022). Most recently, *Salmonella* Hadar infections in humans increased in 2020 and 2021, with many infections linked to a multistate outbreak of *S.* Hadar in ground turkey (CDC, 2021b).

Foodborne illness outbreaks attributed to *Salmonella*-contaminated foods provide the most robust data source available for the attribution of illnesses to different commodities due to the large number of outbreaks, relative to the other foodborne bacterial pathogens (IFSAC, 2019), the occurrence of *Salmonella* outbreaks across all 17 commodity classes (Painter, 2009; Richardson, 2017), and the general similarity between the characteristics of sporadic cases identified through laboratory surveillance and outbreak cases (Ebel, 2016).

Foodborne illness source attribution is the process of identifying which foods are the most important sources of selected major foodborne illnesses. The Interagency Food Safety Analytics Collaboration (IFSAC) produces annual estimates for *Salmonella*, among other pathogens. The implicated foods were divided into 17 categories for the analysis, and the method gives the greatest weight to the most recent five years of outbreak data (2016–2020). In 2020, 5.9% of *Salmonella* illnesses were attributed to turkey.

4.2 Turkey Consumption

Data on the consumption of turkey in the U.S. were obtained from the National Health and Nutrition Examination Survey (NHANES) U.S. dietary data. The NHANES program suspended field operations in March 2020 due to the coronavirus disease 2019 (COVID-19) pandemic. As a result, data collection for the NHANES 2019-2020 cycle was not completed and the collected data are not nationally representative. Therefore, data collected from 2019 to March 2020 were combined with data from the NHANES 2017-2018 cycle to form a nationally representative sample of NHANES 2017-March 2020 pre-pandemic data.

Table 21 contains mean serving sizes for three turkey categories: overall turkey, turkey parts, and comminuted turkey. Additional details are described in **Appendix B**.

Table 21: Meaning serving size of turkey product.

Turkey Product	Mean Serving Size (grams)
Turkey (overall)	79.7
Parts	56.6
Comminuted	83.5

4.3 Empirical Baseline Probability of Illness

Surveillance systems and surveys provide vital information about the burden of foodborne illness in the U.S., but they do not capture *every* illness. Because only a fraction of illnesses are diagnosed and reported, periodic assessments of the total burden of illness are required. CDC developed an approach to estimate the total number of foodborne illnesses from *Salmonella* and other priority pathogens (Scallan, 2011). This approach utilizes data from CDC FoodNet and other surveillance databases and corrects for underreporting and under-diagnosis. The adjusted number is multiplied by the proportion of illnesses acquired in the U.S. (that is, not during international travel) and the proportion transmitted by food to yield an estimated number of illnesses that are domestically acquired and foodborne (Beshearse, 2021).

In recent years, CDC has worked to develop updated estimates of the burden of foodborne illness. As a part of this effort, new analyses have been conducted to revisit the multiplier used by CDC to determine the percent of *Salmonella* illnesses that are foodborne in nature. In Scallan (2011), an estimate of 94% was utilized, which was derived from based on FoodNet case-control study of sporadic illness and on outbreaks reported to the CDC from 1996-2006 (Mermin, 2004; Scallan, 2011). More recently, CDC conducted a structured expert judgement (SEJ) (Beshearse, 2021) to revisit the estimate of percent foodborne for *Salmonella* and many other pathogens. In this SEJ, the authors looked holistically at multiple pathways, including foodborne, waterborne, person-to-person, and animal contact. Based on this work, the authors determined that the percent of all *Salmonella* that were foodborne in nature was 66%. As such, this risk assessment utilizes this 66% foodborne estimates in its calculations of the total number of *Salmonella* illnesses prevented from the various risk management options.

It is estimated there are 42,669 turkey-associated *Salmonella* illnesses per year based on latest IFSAC attribution rate (0.059). This value is calculated as the product of total FoodNet cases per year (7,600),

the share of these cases that are foodborne (66 percent) and of domestic-origin (89 percent), the under-diagnosis multiplier for *Salmonella* (24.3)(Ebel, 2012b) and then divided by the FoodNet population coverage (15 percent). The total cases are subsequently allocated by commodity using NHANES consumption statistics. In particular, that 0.42 of all turkey-associated *Salmonella* illnesses result from exposure to comminuted (ground) turkey products, which is approximately 17,921(Lambertini, 2021).

Using FSIS data, the total number of turkey carcasses produced in 2021 was nearly 220 million, with more than 10% exported (USDA-ERS, 2023). Using USDA-ERS estimates for retail boneless turkey available for consumption in the U.S. (~18.3 pounds/carcass produced) and NHANES estimates for the average serving size for turkey (79.7 g), we estimate 21 billion servings of turkey—in all its forms—are consumed in the U.S. each year. The ratio of total turkey illnesses to total turkey servings (2 per million) provides an empirical estimate of the risk of illness per serving (Hsi, 2015).

4.4 Descriptive Estimates of Risk per Serving

Adequately answering the risk management charge questions necessitated the use of the virulence-adjusted dose-response model; the development of which is outlined in **Appendix A** and Chapter 2. This model provides a description of risk of illness per serving for poultry products, beyond the empirical estimate described above.

The scenarios in **Table 22** describe the initial concentration of FSIS-sampled product in a lot of raw comminuted turkey at the end of production for lots that fail different *Salmonella* concentration thresholds. The risk of illness from consuming a serving of comminuted turkey product varies based on both the amount and virulence of the *Salmonella* that remain after cooking. In particular, risk from a serving considers virulence by serocluster. The average initial concentration for failing lots in Table 14 is the average concentration of a lot that tests at or above the initial concentration, i.e., the conditional expected value. The average dose consumed is a practical description of the range of doses consumed (after transportation and cooking) which is the expected value after applying the attenuation distribution to the average lot concentration. As previously assumed, the attenuation distribution was calibrated to raw chicken products due to the lack of data appropriately encapsulating all raw turkey products.

These illustrative calculations differ from the probability of illness estimates that are outputs from the simulation in **Chapter 5**, which factors in the full distribution of initial contamination values above the threshold. As has been discussed, the majority of exposures consumers face are to doses of *Salmonella* below the limit of detection of the FSIS *Salmonella* assay (0.03cfu/mL for parts and carcasses; 0.003 cfu/g for comminuted products). While the probability of illness per serving estimates give some description of the degree to which risk increases with dose, it is of more interest that the sizable lognormal attenuation distribution ($\mu = -5.00log10, \sigma = 1.91log10$) applied to the raw comminuted turkey concentration (a distribution with relatively fat tails) results in high average consumed doses.

Following multiplication of the average initial concentration by the attenuation distribution, we calculate the average dose per serving and integrate each dose-response function across the resulting distribution to calculate probabilities of illness per serving. We also predict the likelihood that lots will fail the different concentration thresholds. The results illustrate that the average initial contamination concentrations of failing lots and the average doses per serving increases as the initial concentration thresholds increase, but in a non-linear pattern.

In contrast, the ratio of average dose per serving to average initial contamination concentration is the same for each concentration threshold. This ratio, 0.16, is the expected value of the attenuation distribution ($e^{-5\times\ln(10)+0.5\times(1.91\times\ln(10))^2}$) that modifies the initial contamination value to account for the effects of mixing, partitioning, growth, attenuation (e.g., cooking) and serving size between production and consumption. It is notable that this expected value of the attenuation distribution represents the 98th percentile (approximately) of that extremely skewed distribution. For comparison, the median, 95th, 99th and 99.9th percentiles of the attenuation distribution are 0.00001 (i.e., 10^{-5}), 0.014, 0.28, and 8, respectively.

Furthermore, the probabilities of illness increase with the concentration threshold standard. The increase is not linear because the average initial contamination above the threshold is not changing in a linear pattern and the dose-response functions are non-linear (particularly at doses above 1 cfu/serving).

Table 22*:* Risk of illness per serving of turkey product based on the initial concentration of *Salmonella* in FSIS-sampled products.

Measurement	Initial concentration threshold (cfu/g)				
	0.003	0.033	1	10	100
Average initial concentration (cfu/g) for failing lots	163	348	1,373	4,249	15,479
Average dose consumed* (cfu/serving) for average failing lot	26	55	218	673	2,453
Probability that dose ≥ 1 cfu/serving in failing lots*	7.2%	9.9%	16.5%	23.6%	33.6%
Probability of illness per million servings* given the average initial concentration, higher virulence	8,008	11,655	21,844	34,896	56,597
Probability of illness per million servings* given the average initial concentration, lower virulence	1,583	2,354	4,597	7,608	12,874
Likelihood of consumer exposure to raw product (at or above initial concentration threshold)	15.7%	7.4%	1.9%	0.60%	0.16%

*Attenuation distribution based on raw chicken products

Chapter 5 Final Product Standards

The second risk management questions states:

> *What is the public health impact (change in illnesses, hospitalizations, and deaths) achieved by eliminating final product contaminated with specific levels of Salmonella and/or specific Salmonella subtypes?*

We address this risk management question by considering the direct public health effects of a test-and-hold final product standard with the removal of any sampled contaminated lots surpassing a microbial criteria threshold (i.e., specific levels of *Salmonella*). A major assumption of this modeling approach is that consumer demand for raw turkey products will continually be met by the industry, and so every lot removed (as a result of the standard) will ultimately be replaced by another average lot.

While this approach differs from other modeling approaches described in the scientific literature, FSIS believes this approach represents a more realistic assessment of the current turkey industry and FSIS inspection practices and, therefore, the identified public health benefits. Industry action has indicated the capacity to shift product deemed to be adulterated into cooked product streams. In particular, following the 2011 *Salmonella* outbreak in ground turkey outbreak, the turkey industry re-examined and enhanced food safety efforts, as observed in an increase in diversion of contaminated lots to cooked products (Cargill, 2015).

A key driver for the model approach outlined below are the two dose-response *Salmonella* models developed by EpiX Analytics for this risk assessment (**Appendix A**). *Salmonella* serotypes were categorized into two clusters based on a gene-based approach assessing underlying genetic similarity, and subsequently, validated and fit to separate dose-response models via epidemiological data. As a result, a higher and lower virulence serocluster was identified which required consideration of *Salmonella* levels and serotypes in the following scenarios.

All public health outcome predictions presented in this chapter are based on a determination of pass/fail status of each lot using a test with high accuracy, and the testing method used for risk management option implementation should be considered when evaluating the results below.

5.1 Sampling methods

When an establishment is sampled, FSIS inspectors randomly select a single product (i.e., carcass or comminuted product) at the post-chill location. For carcasses, inspectors take a sponge sample of small areas (i.e., 5cm x 10cm) of the back and of the thigh to create a composite that will determine the pathogen status of the carcass. Sponge sampling likely underestimates the prevalence (and ultimately the levels) of *Salmonella* on turkey carcasses. For comminuted, a 325g sample of 2-lb final product is taken. Current FSIS qualitative testing for *Salmonella* has a LOD of 1cfu/325g.

Further details of the sample collection methodology and laboratory techniques used to assess the sample are described elsewhere (FSIS, 2021; FSIS, 2022a).

5.2 Re-Hang to Post-Chill Serotype Concordance Analysis

Of the 1,442 paired samples in the FSIS turkey carcass microbiological baseline, 144 samples were confirmed positive for *Salmonella* (and serotyped) at rehang, compared to only 24 samples at post-chill

(**Figure 18**). At rehang, the frequency of clusters 1 and 2 are generally split evenly, with a portion of the establishments in this study observing a more dominant cluster presence. At post-chill, however, cluster 1 appears twice as often as cluster 2, even in the limited post-chill observations, which is further pronounced in high volume establishments.

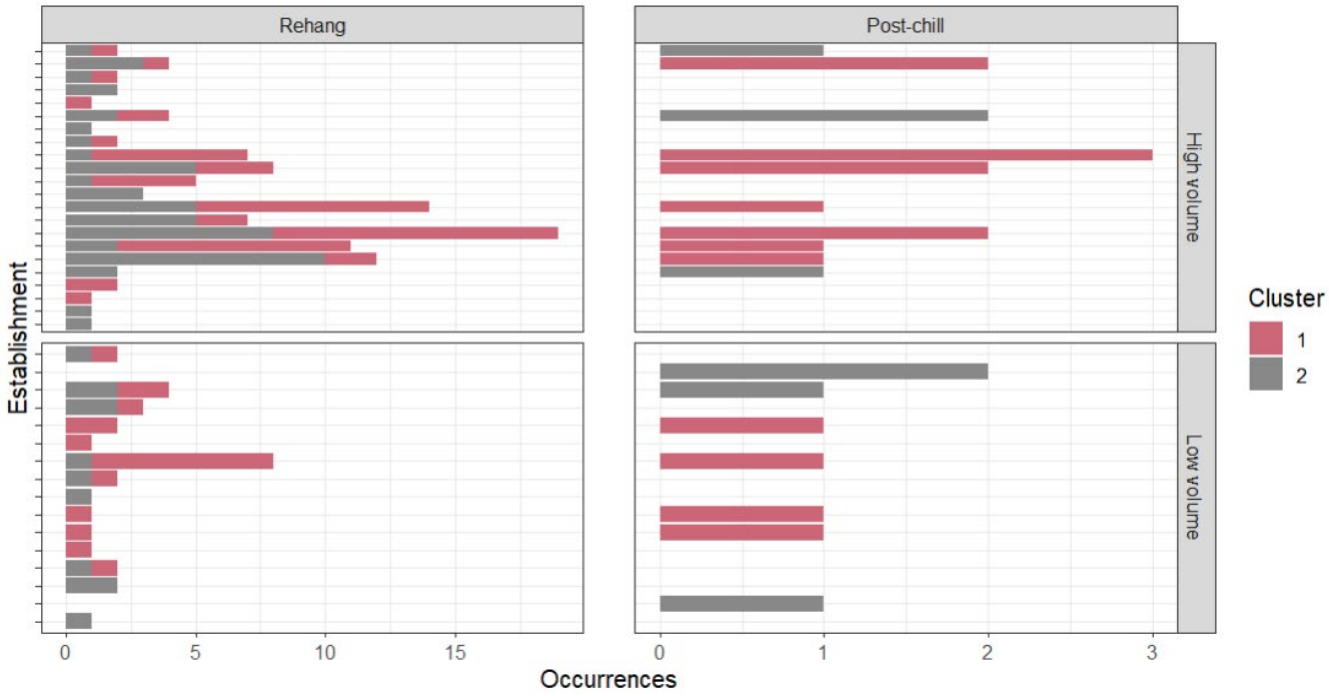

Figure 18: Serotype cluster occurrences on turkey carcasses at rehang versus post-chill by establishment and production volume.

Another factor likely to affect pathogen occurrence is establishment ownership, with some establishments being owned and operated by a single corporation. Pathogen control programs for these establishments are likely coordinated across all establishments operated by the corporation, so it is reasonable to expect that there are no significant differences in performance for the collection of establishments owned by a single corporation. Grouping establishments by corporation provides an opportunity to investigate the overarching patterns of *Salmonella* on turkey entering and leaving establishments following similar intervention processes. Small establishments are aggregated together here and labelled "Other" for direct comparison with larger corporations (**Figure 19**). The proportions of clusters at rehang illustrates the variability of incoming flock mixtures at the corporation level.

Salmonella serotype detection is generally limited to the most abundant serovars due to the current sampling techniques employed. However, recent studies have demonstrated multiple *Salmonella* serotypes can be present within the same flock or a single sample (Cox, 2020, Rasamsetti, 2023, Thompson, 2018,Obe, 2023). Although current turkey sampling programs lack refined, higher resolution serotyping to uncover the exact nature of serotype mixtures within a sample or flock, the assumption of multiple serotypes present in a flock holds. Under this assumption, if a particular serotype is in high abundance within a flock/lot, then the results of *Salmonella* positive samples at two points in the slaughter process (i.e., rehang and post-chill) should regularly agree. If a variety of serovars is present within a flock/lot, as observed in chicken carcasses (Thompson, 2018), then the rehang sample can become a poor predictor of the serotype identified at post-chill. Subsequently, the assumed distribution

mixture of serotypes in that flock/lot. In the young turkey microbiological baseline study, in particular, there was a single rehang sample (1 out of 144 positives) that was identified to contain multiple serotypes. Surprisingly, the paired post-chill sample (i.e., from the same flock) was negative for *Salmonella*.

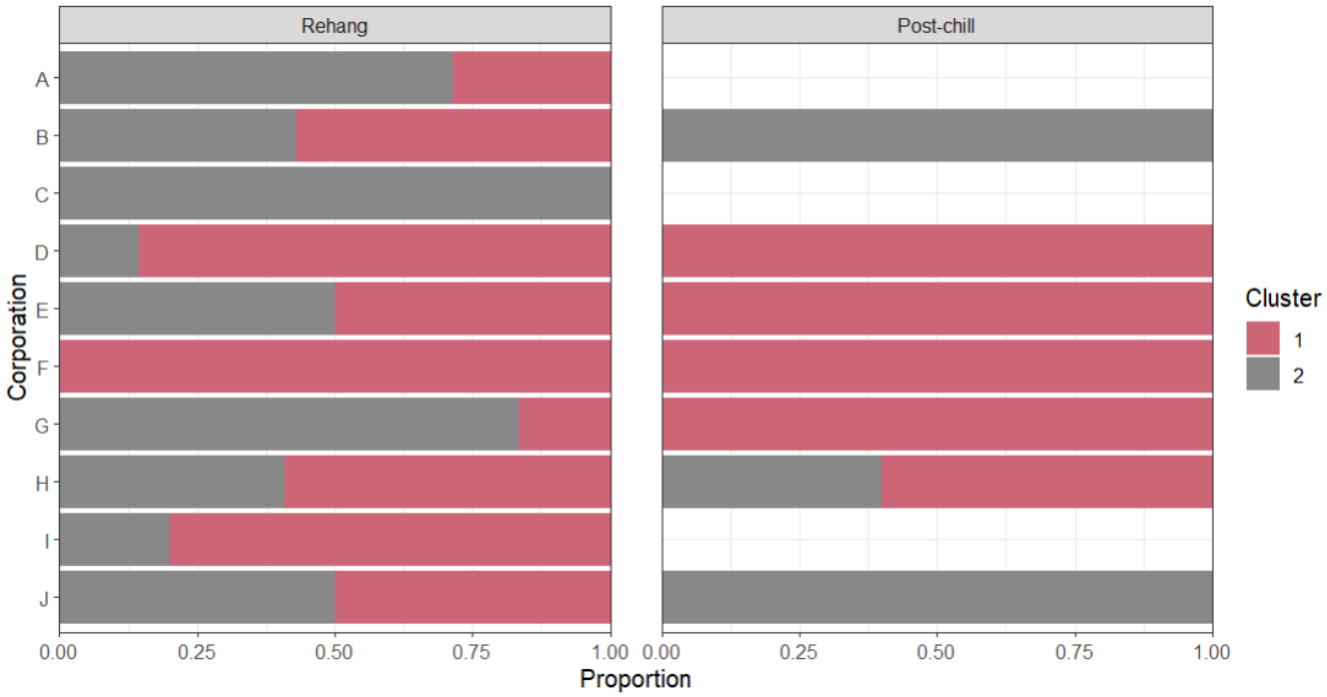

Figure 19: Serotype cluster ratio of turkey carcasses at rehang versus post-chill by corporation.

There were 8 paired samples (<1%) that tested positive at both sampling locations. Six of these paired samples (75%) were in concordance; that is, the serotype identified from the rehang and post-chill sample matched. The paired serotypes and clusters are provided in **Figure 20** and **Table 23**. In this limited dataset, Hadar, from the higher virulence cluster 1, was detected most often at rehang and post-chill. Six paired samples identified a cluster 1 serotype (Hadar and Saintpaul) at the post-chill location, although two observed a cluster 2 serotype at rehang. These two paired samples support the theory that multiple serovars are indeed present in some sampled lots. However, he homogeneity in this dataset cannot be validated without additional paired observations.

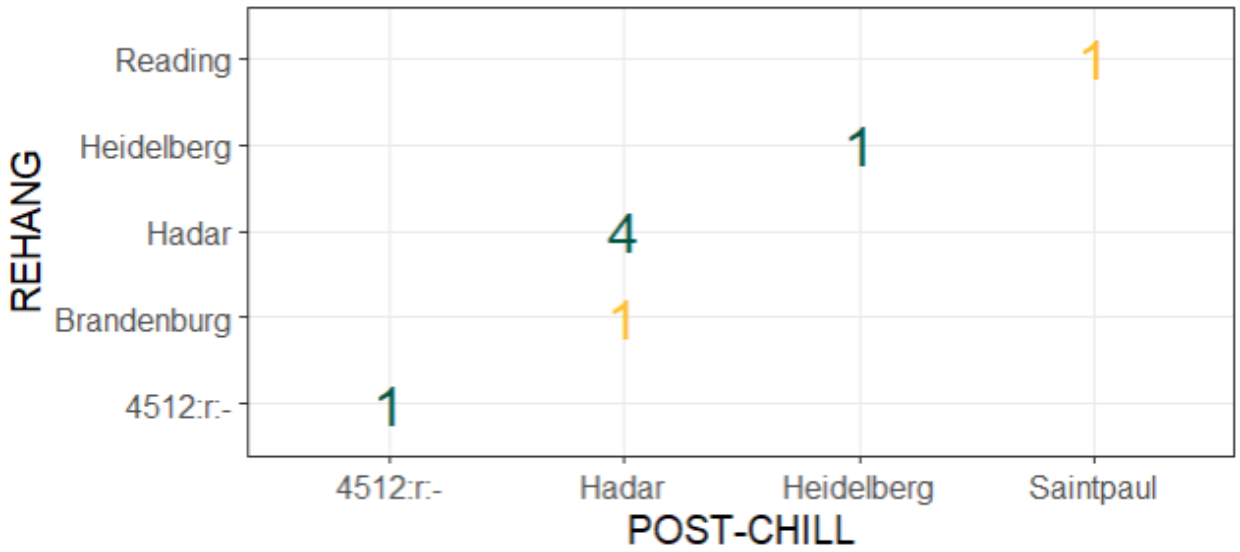

Figure 20: Concordance of serotype pairs at rehang and post-chill in turkey carcasses (2008-2009).

Table 23: Contingency table for rehang versus post-chill by clusters.

Sample Location	**Post-chill**		
Rehang	**Cluster**	**1**	**2**
	1	4	0
	2	2	2

As a result, it is assumed that the average proportion of cluster 1 versus cluster 2 in any lot is approximately 0.3 and 0.7, respectively, as observed in PR/HACCP *Salmonella*-positive post-chill data.

5.3 Modeling Approach

Hazard Characterization for Final Product Standards: Dose-Response Model

Two clusters of serotypes were defined (see Chapter 2) and, further, dose-response functions were developed for each cluster. The first cluster consists, generally, of the more virulent *Salmonella* serotypes; we call this grouping C1. The second cluster consists, generally, of the less virulent serotypes, although some serotypes commonly observed among human illnesses (e.g., Heidelberg, Infantis) are included in this grouping called C2.

A simplifying assumption was adopted due to the lack of complete data in terms of *Salmonella* contamination across all raw turkey products. The role and contribution from turkey carcasses and turkey parts is highly uncertain. On the other hand, robust data on the *Salmonella* contamination of raw chicken products provides a reliable starting point. Moreover, capturing the complete poultry picture considers between- and within-product production and consumption rates. In that sense, noting that poultry production is roughly split 5/1 between chicken and turkey (FSIS, 2011), the weight or influence on defining the overarching distribution is biased towards chicken. Additionally, without valid distributions for all turkey products (i.e., carcasses and parts) and clear consumption rates (as carcasses and parts have historically been combined in consumption estimates), appropriate aggregation of turkey products into the *Salmonella* distribution across all of poultry is severely limited, and ultimately, highly uncertain.

Therefore, a lognormal distribution (*Log10Normal*(-3.037117, 1.279985)) was used to reflect the initial contamination of *Salmonella* in a mixture of raw poultry products. **Figure 21** highlights the differences between the assumed distribution and those derived for various poultry products. It is clear that the comminuted turkey distribution is highly variable, and the average level of contamination falls below the majority of chicken products. Hence, under the current approach, the method will likely overestimate the risk associated to comminuted turkey final products.

Nonetheless, an alternative dose-response model as well as a model simplification are explored to address the sensitivity of this assumption on results related to turkey risk and illnesses.

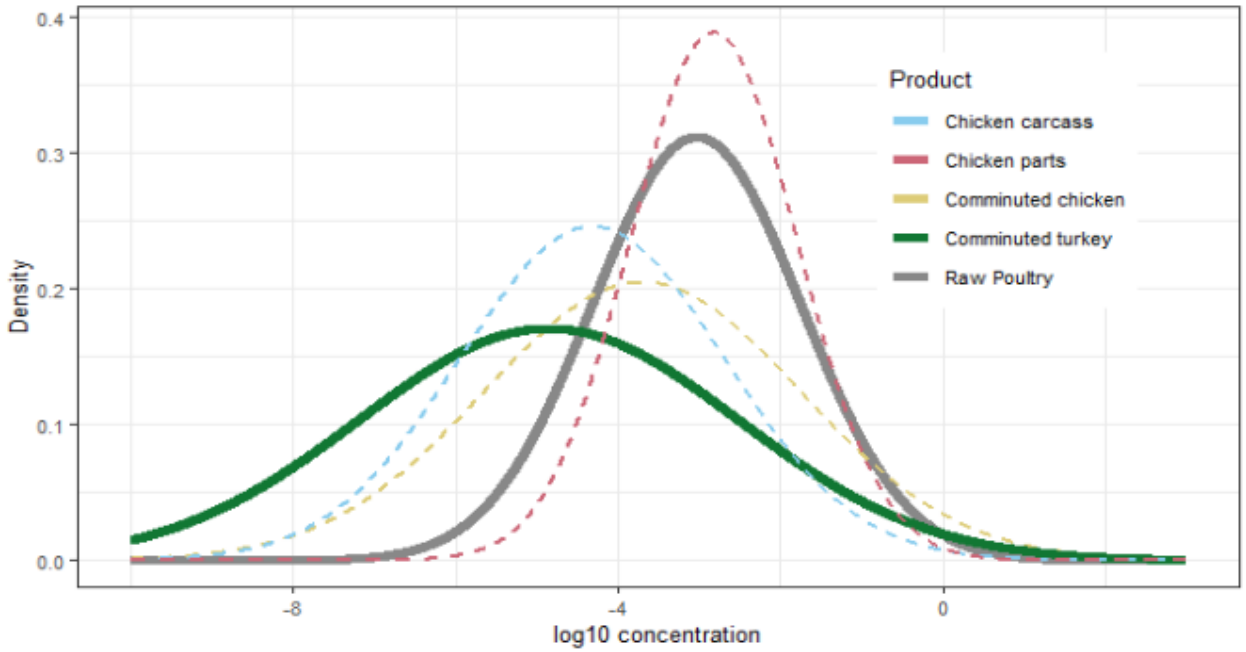

Figure 21: Concentration distribution comparison between comminuted turkey and comminuted chicken and the overarching *Salmonella* contamination distribution in raw poultry products.

An attenuation distribution that considers all the effects of partitioning, mixing, growth, and attenuation that typically occurs between poultry production and consumption was defined as $Log10Normal\left(-5,1.91\right)$ (Ebel, 2015). Noting that the dose-response and attenuation relationship can only be applied to modeling final product scenarios in comminuted turkey, the relationship posited for comminuted chicken is assumed here as well for comminuted turkey. Furthermore,(Nauta, 2011) have demonstrated that the effect of different consumer phase models between products is generally small. Together the initial contamination distribution and attenuation distribution constitute an (log10) exposure distribution.

The derivation of the EpiX Analytics-developed dose-response function parameters for clusters 1 and 2 depends on maintaining the following relationship:

$$\frac{\int R_1(d)h(d)\partial d}{\int R_2(d)h(d)\partial d} = \frac{RR_1}{RR_2}.$$

The left side of this equation is a ratio of the outputs of integrating the dose-response functions for cluster 1 ($R_1(d)$) and cluster 2 ($R_2(d)$) across the exposure distribution, $h(d)$. The outputs of these integrals can be interpreted as the overall probability of illness per serving given the dose-response function. Importantly, this assumes that exposure distributions do not differ between the seroclusters.

The right side of this equation is a ratio of relative risk variables, termed risk multipliers. The numerator of this ratio RR_1 is the expected increased probability of illness given exposure to C1 serotypes. This is estimated as the ratio of the fraction of outbreak illnesses attributed to C1 serotypes (e.g., 71 percent) to the fraction of poultry isolates determined to be C1 serotypes (e.g., 33 percent); this latter term serves as a proxy for the relative exposure probability. For example, the equation, $RR_1 = 2.15$ implies that a C1 exposure increases risk of illness 2.1 times some baseline risk. Similar reasoning for the denominator RR_2 concludes that the fraction of outbreak illnesses attributed to C2 serotypes (e.g., 25 percent) as compared to the fraction of poultry isolates determined to be C2 serotypes (e.g., 66 percent) is 0.38, or that a C2 exposure reduces risk of illness $1/0.38 = 2.65$ times some baseline risk. As explained in **Appendix A,** substantial uncertainty attends the estimation of these relative risk terms.

The ratio $\frac{RR_1}{RR_2} = \frac{2.15}{0.38} = 5.66$ indicates that the probability of illness per serving from C1 exposures is 5.66 times larger than the probability of illness per serving from C2 exposures. Therefore, the parameters for the two dose-response function must be selected to maintain this relative probability of illness.

The parameters of $R_1(d)$ and $R_2(d)$ are estimated using numerical techniques based on a simplifying assumption that, although the mean of the beta distribution underlying their beta-poisson dose-response model differs between C1 and C2, the sum of those beta parameters must be equal. Given the complexity of this model – which uses a 2F1 hypergeometric confluent function of the second kind – the calculation of each integral is simplified using a polynomial expression such that, for example,

$$\int R_1(d)\,h(d)\,\partial d \approx \sum_d coef1_1 \times \ln(d_i + 1) + coef2_1 \times \ln(d_i + 1)^2 + \ldots + coef9_1 \times \ln(d_i + 1)^9 .$$

Although the dose-response functions are developed based on an exposure distribution that encompasses all exposures to all poultry products, this risk assessment is concerned with distinguishing between the probability of illness from exposures to units that pass or fail standards imposed by FSIS on individual forms of the poultry products (e.g., carcasses, parts, or comminuted). Therefore, the dose-response functions are applied as described below.

Concentration-based criteria

To estimate the number of illnesses prevented given a proposed concentration-based risk management option, two scenarios are compared: (1) a baseline scenario (i.e., the "before" scenario) without any additional intervention reacting to the concentration-based criteria and (2) a new scenario (i.e., the "after" scenario) where product units or lots not meeting the concentration-based criteria are subjected to a mitigation measure (i.e., diverted and replaced). Let ω be the fraction of units that pass the pre-determined concentration criteria. Then, the baseline probability of illness is given by the risk characterization:

$$P_{baseline}(ill) = \omega \times P(ill \mid pass) + (1-\omega) \times P(ill \mid fail)$$

where $P(ill \mid pass)$ and $P(ill \mid fail)$ are the conditional probabilities of illness given the unit passes or fails the concentration-based criteria. Next, let α be the fraction of tested units that fail the proposed concentration criteria which are ultimately replaced with random, untested units. Then, the new probability of illness is

$$P_{new}(ill) = \omega \times P(ill \mid pass) + (1-\omega)\left[\alpha \times P_{baseline}(ill) + (1-\alpha) \times P(ill \mid fail)\right]$$

Therefore, to estimate the number of illnesses prevented, the following model can be used(Ebel, 2015; Ebel, 2012a; Williams, 2011):

$$I_{avoided} = Poisson\left(\left(1 - \frac{P_{new}(ill)}{P_{baseline}(ill)}\right)\lambda_{ill}\right)$$

where λ_{ill} indicates the annual rate of illnesses prior to the proposed policy option. **Figure 22** illustrates the steps of the modeling process.

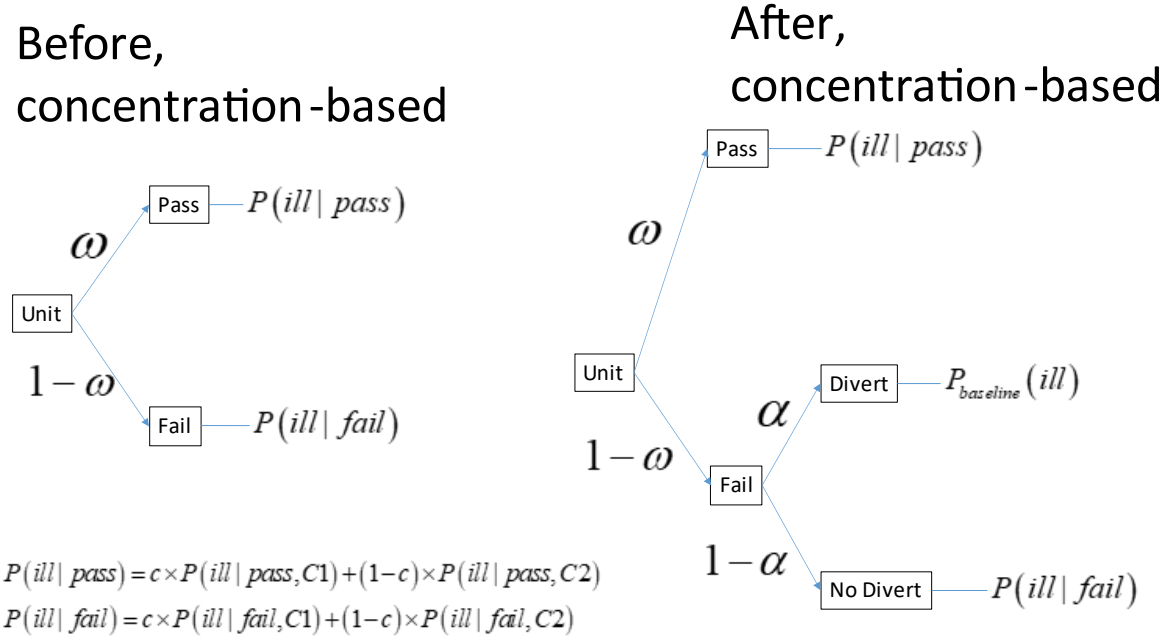

Figure 22: Schematic depiction of the possible pathways which product moves before and after implementation of a concentration-based diversion strategy.

Dose-response modeling

EpiX Analytics developed dose-response functions for two seroclusters, C1 and C2 (**Appendix A**). In

turkey commodities, serocluster C1 proportion is roughly 0.3.

For units that pass (or fail) the concentration criteria, the probability of illness is further discretized by the proportion of *Salmonella* that is in C1 versus C2,

$$P(ill \mid pass) = c \times P(ill \mid pass, C1) + (1-c) \times P(ill \mid pass, C2)$$
$$P(ill \mid fail) = c \times P(ill \mid fail, C1) + (1-c) \times P(ill \mid fail, C2)$$

where c is the proportion of *Salmonella* that is in C1 (default is 0.3). Subsequently, one of two dose-response functions (i.e., R(d) for C1 or C2) are implemented. In assessing exposure, we begin with an initial level $x \sim lognormal(\mu_x, \sigma_x)$ and an attenuation factor $a \sim lognormal(\mu_a, \sigma_a)$. Then the dose at consumption (i.e., exposure distribution) is $d = x \times a$.

A failing unit is defined as having an initial concentration greater than or equal to some threshold concentration T (i.e., $x \geq T$). To determine the probability of illness per serving (i.e., risk characterization) for a passing or failing unit, conditioned on cluster *j*, we solve the following:

$$P(ill \mid pass, Cj) = \int_{x<T} R_j(d) h(d) \partial d$$
$$P(ill \mid fail, Cj) = \int_{x \geq T} R_j(d) h(d) \partial d$$

Below outlines the model procedure:

1. For a particular product, solve $P_{baseline}(ill) = c \times \int R_1(d) h(d) \partial d + (1-c) \times \int R_2(d) h(d) \partial d$.

   In other words, the probability of illness per serving across all exposures is the weighted average of the probability of illness per serving across all exposures to C1 and C2. This can be accomplished using numerical integration because the exposure distribution, in log10, is simply the sum of two normal distributions (i.e., initial contamination and attenuation).

2. Using Monte Carlo simulation, sample from a truncated form of initial concentrations, x, where its minimum is defined as negative infinity and its maximum is $\log_{10}(T)$. Multiply the vector of initial concentrations less than the concentration criteria threshold by a vector of samples from the attenuation distribution to simulate exposure doses from passing units.

3. Use the simulated exposures for passing units to estimate $P(ill \mid pass) = c \times P(ill \mid pass, C1) + (1-c) \times P(ill \mid pass, C2)$.

4. Use the components of previous steps to solve for the probabilities of illness for failing exposures. For example, $\int R_1(d) h(d) \partial d = \omega \times P(ill \mid pass, C1) + (1-\omega) \times P(ill \mid fail, C1)$; i.e., the probability of illness from all C1 exposures is the weighted average of passing and failing exposures. Such an expression can be solved for $P(ill \mid fail, C1/C2)$.

5. The fraction of units passing, ω, is determined as the cumulative probability that $x < T$.

6. The parameter α is the fraction of failing units that are diverted and replaced by random units. If L is the number of units produced per year (e.g., production lots) and n is the total number of failing units tested per year, then $\alpha = \frac{n}{L}$ (i.e., if all units are tested, then all failing units will be diverted).

Serotype-based criteria

A serotype-based option requires assumptions regarding the distribution of serotypes within a lot based on a single sample. As described in section 3.2, there is limited paired data from turkey carcass sampling to accurately inform/represent the underlying distribution of seroclusters within a lot. Similarly, the absence of paired sampling data for comminuted turkey challenges theoretical comparisons of carcasses tested at rehang and final comminuted product samples.

Model approximation

Model simplifications in the dose-response function and the attenuation effect between production and consumption are considered to potentially approximate the reduction in illnesses prevented.

Recall that the proportional reduction in illnesses is given by,

$$1 - \frac{P_{new}(ill)}{P_{baseline}(ill)}$$

and the ratio

$$\frac{P_{new}(ill)}{P_{baseline}(ill)} = \frac{\int R(d_{new}) h(d_{new}) \partial d}{\int R(d_{baseline}) h(d_{baseline}) \partial d}$$

describes the relative probability of illness per serving after a policy effect (new) to before (baseline). Ultimately, an effective policy must change the exposure distribution such that, after integrating across a dose-response function, it reduces the probability of illness per serving relative to that probability before the policy.

If the dose-response relationship is approximately linear ($R(d) \approx \gamma d$), then we are left with a ratio of average doses per serving ($\frac{P_{new}(ill)}{P_{baseline}(ill)} \approx \frac{\bar{d}_{new}}{\bar{d}_{baseline}}$) (Williams, 2011). Given $d = x \times a$, where x is the initial contamination concentration random variable and a is an attenuation random variable that is independent from x and does not change after the policy, then the final simplification is a ratio of the average initial contamination concentrations,

$$\frac{P_{new}(ill)}{P_{baseline}(ill)} \approx \frac{E[x_{new}]E[a]}{E[x_{baseline}]E[a]} \approx \frac{\bar{x}_{new}}{\bar{x}_{baseline}}$$

If these approximations are reasonable, then the general effect of the concentration standards can be estimated without considering the specific dose-response functions or attenuation between production

and consumption. A similar conclusion with respect to so-called “prevalence-based” standards was reported previously (Ebel, 2015).

5.4 Results

It is estimated there are 42,669 *Salmonella* illnesses from consuming turkey products per year in the U.S. based on latest IFSAC attribution rate (0.059). This value is calculated as the product of total CDC FoodNet cases per year (7,600), the share of these cases that are foodborne (66 percent) and of domestic-origin (89 percent), the under-diagnosis multiplier for *Salmonella* (24.3)(Ebel, 2012b) and then divided by the CDC FoodNet population coverage (15 percent). The total cases are subsequently allocated by commodity using NHANES consumption statistics. In particular, that 0.42 of all turkey-associated *Salmonella* illnesses result from exposure to comminuted turkey products, which is approximately 17,921(Lambertini, 2021).

Several characteristics of the turkey industry are summarized by sector using 2021 FSIS data in **Table 24** (full model variables and parameterization presented previously in **Table 10**). Of note, the unit size and as a consequence the total number of units produced by each industry are affected by risk management decisions. For this analysis, FSIS defines a flock (approx. 22,000 birds) or an entire day of production as susceptible to diversion for the carcass and comminuted industries, respectively. FSIS aimed to collect about one sample per week from larger establishments, while smaller establishments were sampled less frequently. This strategy allows for roughly 17% of units to be sampled in a given year.

Table 24: Relevant parameters for the assessed products’ industries are shown.

Description	Parameter	Value
No. lots/yr	L	8,120
No. samples/yr	n	1,355
Sampling proportion/yr	n / L	0.17
Concentration distribution	$lognormal\left(\mu_x, \sigma_x\right)$	$lognormal\left(-4.857, 2.333\right)$
Cluster 1 proportion	c	0.3
Illnesses/yr.	λ_{ill}	17,921

Concentration-based standard at LOD

First, consider a risk management option that implements a concentration-based threshold for comminuted turkey that diverts any lot testing above the LOD (1cfu/325g). Point estimates and pass/fail results from 10 million Monte Carlo iterations are provided in **Table 25**. The probability of illness per serving among lots that pass the LOD standard is generally three orders of magnitude lower than the corresponding probability for failing lots. Additionally, the probability of illness per serving from C1 is only one order of magnitude greater than that probability from C2. Together, the scenario results in a predicted point estimate of approximately 2,500 illnesses prevented per year.

Table 25: Final product standard results using LOD concentration threshold for comminuted turkey.

Description	Parameter	Value
LOD concentration threshold	T	1cfu/325g (LOD)
Fraction of lots compliant	ω	0.84
Probability of illness per serving from passing lots	$P(ill \mid pass)$	$1.57\text{x}10^{-7}$
Cluster 1	$P(ill \mid pass, C1)$	$3.84\text{x}10^{-7}$
Cluster 2	$P(ill \mid pass, C2)$	$6.00\text{x}10^{-8}$
Probability of illness per serving from failing lots	$P(ill \mid fail)$	0.0002
Cluster 1	$P(ill \mid fail, C1)$	0.0004
Cluster 2	$P(ill \mid fail, C2)$	$7.09\text{x}10^{-5}$
Baseline probability of illness per serving	$P_{baseline}(ill)$	$2.52\text{x}10^{-5}$
New probability of illness per serving	$P_{new}(ill)$	$2.16\text{x}10^{-5}$
Fraction of illnesses prevented	$1-\frac{P_{new}(ill)}{P_{baseline}(ill)}$	0.14
Illnesses prevented per year	$\left(1-\frac{P_{new}(ill)}{P_{baseline}(ill)}\right)\times\lambda_{ill}$	2,500

The share of all lots that are diverted (i.e., $(1-\omega)\alpha$) is about 2.6%, but the result of this diversion is an overall reduction in illnesses of 14%. This effectiveness can be derived directly as the product of the fraction of lots diverted times the proportional difference in probability of illness per serving between the failing lots and the baseline probability of illness:

$$(1-\omega)\times\alpha\times\left(\frac{P(ill \mid fail)-P_{baseline}(ill)}{P_{baseline}(ill)}\right)=0.14$$

Additionally, in the baseline, passing lots contribute about 1% of the total probability of illness while failing lots contribute about 99% $\left(\frac{\omega\times P(ill \mid pass)}{P_{baseline}(ill)}\approx 0.01\right)$. We can also determine that C1 contributes about 70% of the total probability of illness among passing or failing lots, while C2 contributes about 30% $\left(\frac{c\times P(ill \mid pass, C1)}{P(ill \mid pass)}\approx 0.7\right)$.

Alternative concentration-based standards

Increasing the concentration threshold necessarily increases the fraction of units passing (ω). This directly corresponds to increases in the probability of illness among both passing units and failing units

(by including higher doses among passing and by removing lower doses among failing). However, the overall baseline probability of illness should remain the same, since the exposure distribution is not affected by changing the concentration threshold (before any risk management effects). Moreover, this occurs because the rate of increase in the probability of illness among passing units is smaller than the rate of increase in the probability of illness among failing units as the concentration threshold increases. These relative effects can be appreciated by recognizing that an increased concentration threshold admits into the passing population some higher risk units, but these units represent a small share of all passing units (so, the average for passing units does not increase much). In contrast, removing those same units from the failing population of units has a greater effect on the average for failing units.

Simulating a range of concentration-based thresholds (from 1cfu/2600g (-3.41 log10) to 100cfu/g (2 log10)), we can analyze the effects on *Salmonella* illness reductions where failing lots are diverted and replaced by an average lot (i.e., baseline risk) or a passing lot. **Figure 23** illustrates the number of comminuted turkey related illnesses that would be prevented by each diversion-replacement scenario. The results suggest that a concentration threshold above the current LOD might generate a larger reduction in illnesses, especially when a random, untested lot is considered for replacement.

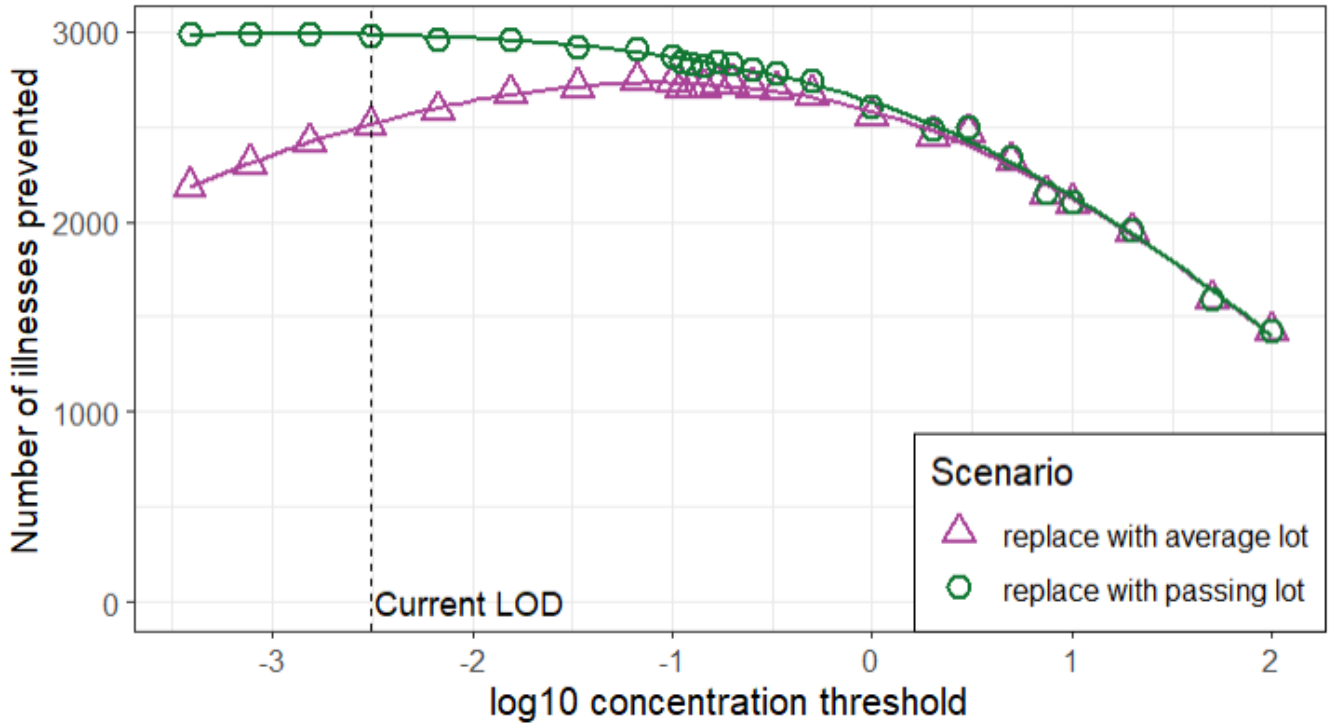

Figure 23: Number of illnesses prevented under different concentration thresholds for lot diversion and replacement scenario for comminuted turkey products. Vertical dashed line indicates current LOD of 1/325 g.

In scenarios with average lots replacing diverted lots, the predicted number of illnesses prevented increases to more than 2,700 when the concentration threshold is set to 1cfu/30g (-1.5log10), and peaks around 2,740 illnesses prevented under a concentration threshold of 1cfu/15g (-1.2log10). This increase in concentration threshold eliminates diverting lots with less than average risk, and as a result, improves the effectiveness of diversion. At a concentration threshold of 10cfu/g and 100cfu/g, the number of illnesses prevented is about 2,100 (an 11.7 percent reduction) and 1,400 (an 8.0 percent reduction),

respectively.

For the alternative scenario where passing lots replace diverted lots, the maximum illnesses prevented approaches 3,000 (a 16.7 percent reduction) as the concentration threshold is reduced toward 1cfu/2600g. At the LOD concentration threshold of 1cfu/325g, the predicted illnesses prevented is nearly the same rate (16.6 percent reduction).

It is important to note that when the concentration threshold is reduced, more lots become eligible for diversion, but the additional lots necessarily represent a lower risk of illness than the other failing lots. At some concentration threshold, we begin diverting lots whose risk of illness was actually lower than the average risk across all lots, and the effect is to moderate the overall reduction in illnesses. At an extremely low concentration thresholds, the concept is apparent because we fail every lot (whose average risk is equivalent to the population's average) and simply replace those lots with others of equivalent risk; which does not prevent any illnesses.

Comparison to model approximation approach

First, using log10 parameters, the average initial concentration for comminuted turkey is given by,

$$\bar{x}_{baseline} = e^{-4.857\times\ln(10)+0.5*(\ln(10)*2.333)^2} = 25.666\,\text{cfu/g}.$$

The conditional expected value for lots that are below a concentration threshold (T) can be calculated by

$$E\left[x \mid x \le T\right] = \frac{\bar{x}_{baseline} \times \Phi\left(\frac{\ln(T) - \mu - \sigma^2}{\sigma}\right)}{\omega}$$

where μ and σ are in natural log units, $\Phi(\ )$ is the cumulative probability from a standard Normal distribution, and ω is fraction of all lots that are below concentration threshold T. On the other hand, the conditional expected value for lots above any threshold T follows

$$E\left[x \mid x > T\right] = \frac{\bar{x}_{baseline} - \omega \times E\left[x \mid x \le T\right]}{1-\omega}$$

Consider a concentration threshold at the LOD for comminuted turkey (i.e., 1cfu/325g), for example, one can readily observe $E\left[x \mid x \le T\right] = 0.0003\,\text{cfu/g}$ and $E\left[x \mid x > T\right] = 17.386\,\text{cfu/g}$. Using these values, we can calculate our simplified replacement for

$$P_{new}(ill) \approx \bar{x}_{new} \approx \omega \times E\left[x \mid x \le T\right] + (1-\omega)\left[\alpha \times \bar{x}_{baseline} + (1-\alpha) \times E\left[x \mid x > T\right]\right] \approx 3.913$$

Therefore, the approximate reduction in illness is about 16.98% (where α is the fraction of non-compliant establishments diverted as defined previously). This compares with 17% reported above for this same scenario using the full model. Across a full range of concentration thresholds, the approximation for the proportional reduction in illnesses tends to be similar or somewhat larger than that estimated using the full model (**Figure 24**). The differences become more pronounced as the concentration threshold increases above 1cfu/15g. Moreover, these differences suggest that the assumption of linearity in the dose-response function (applicable to the approximation) becomes less appropriate as effects of the diversion policies are applicable to larger dose concentrations. As shown previously, using a linear approximation overestimates the probability of illness as dose increases

(Williams, 2011).

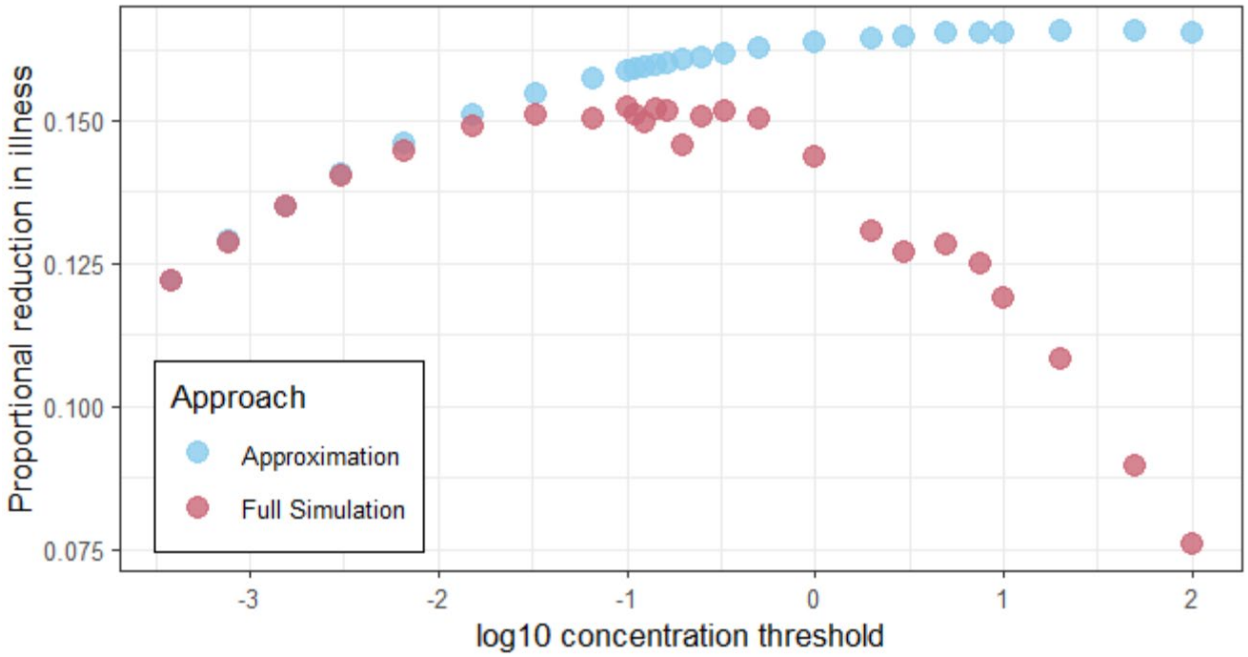

Figure 24: Model comparison of approximation method and full simulation on the reduction in illnesses across a range of concentration thresholds for comminuted turkey. Approximation approach considers only changes in the mean initial contamination concentration.

This analysis suggests that results estimated from a simplified model that only considers changes to the initial contamination distribution are comparable to estimates from a full model that simulates a) the full range of initial contaminations by passing and failing status, b) the modification of these initial contamination levels by an attenuation distribution, and c) separate estimates of probability of illness given dose for two virulence clusters via dose-response functions. Such findings support the general idea that both the attenuation and dose-response functions have limited influence on the full model's estimates; i.e., the full model's results are not highly influenced by either attenuation or dose-response relationships. Nevertheless, application of attenuation and dose-response models are necessary for improved accuracy in estimates as the threshold increases

5.5 Lots Diverted

At the request of FSIS risk managers, the risk assessment model and other data analyses contained in this document were used to develop the following estimates of annual lots diverted for the threshold scenarios under consideration.

Table 26 contains estimates for the main threshold scenarios run in the risk assessment, along with serotype-based diversion scenarios. Although the model could not be used to estimate the public health benefit of the latter, the virulence informed dose-response model did inform which higher virulence serotypes are of greater public health concern. The top three (i.e.,Top 3) most prevalent higher virulence serotypes in turkey are Hadar, Typhimurium, and Muenchen. Microbial profile data was used to estimate the following lot descriptions.

Table 26: Annual lots diverted estimates for main final product standard scenarios.

Product	Scenarios (cfu/g)	Lots Diverted	Total Lots	Percent of Lots Diverted	Pounds of Product Diverted	Total Pounds Production	Percent of Total Weight Diverted
Comminuted Turkey	0.003	213	8,120	2.6%	60,594,828	2,310,000,000	2.6%
	0.003 + Top 3	53		0.7%	15,077,586		0.7%
	1	25		0.3%	7,112,069		0.3%
	1 + Top 3	6		0.07%	1,706,897		0.07%
	10	8		0.10%	2,275,862		0.1%
	10 +Top 3	2		0.02%	568,966		0.02%
	100	2		0.02%	568,966		0.02%
	100 + Top 3	1		0.01%	284,483		0.01%

5.6 Sensitivity *analysis*

To conduct sensitivity analysis, we change individual model inputs – while holding others at their baseline values – and explore changes to the proportional public health effectiveness across a range of concentration thresholds.

Serocluster proportion

First, we examine the effects of assuming the share of organisms in cluster 1 are 0% or 100% versus the estimated baseline value of 30% in comminuted turkey (**Figure 25**).

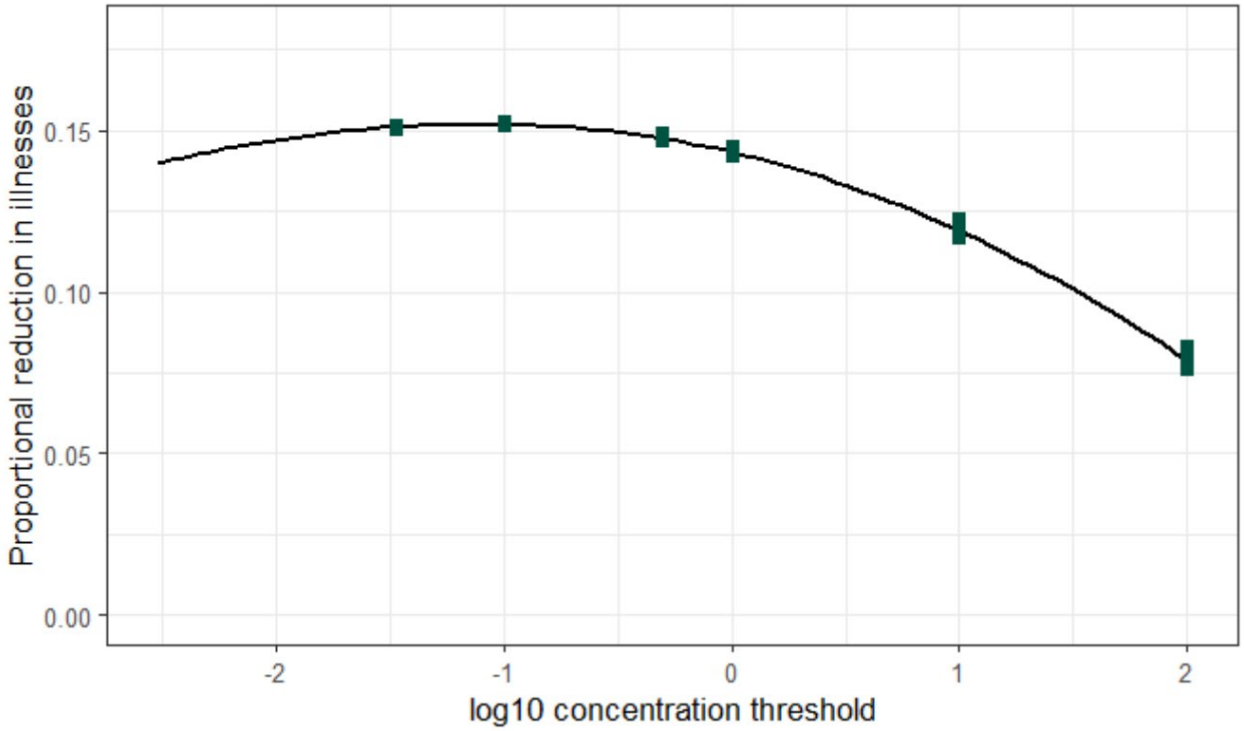

Figure 25: Analyzing the effect of serocluster proportion in comminuted turkey lots on the reduction in illnesses.

Increasing or decreasing the probability of cluster 1 organisms results in very little change in the proportional reduction in illnesses across the range of concentration thresholds. Although the probability of illness per serving is somewhat larger when 100% of organisms are assumed to be C1, the public health effectiveness is slightly lower for all concentration thresholds because the dose-response function for C1 is more non-linear than that for C2. Consequently, the opposite behavior is evident when we assume 0% of organisms are C1; the probability of illness per serving is somewhat smaller but the public health effect is slightly greater across the range of concentration thresholds.

Attenuation

Second, we examine the effects of increasing or decreasing the degree of attenuation between initial contamination and consumption by increasing or decreasing the negative mean of the log10 distribution

from its default of -5 log10s.

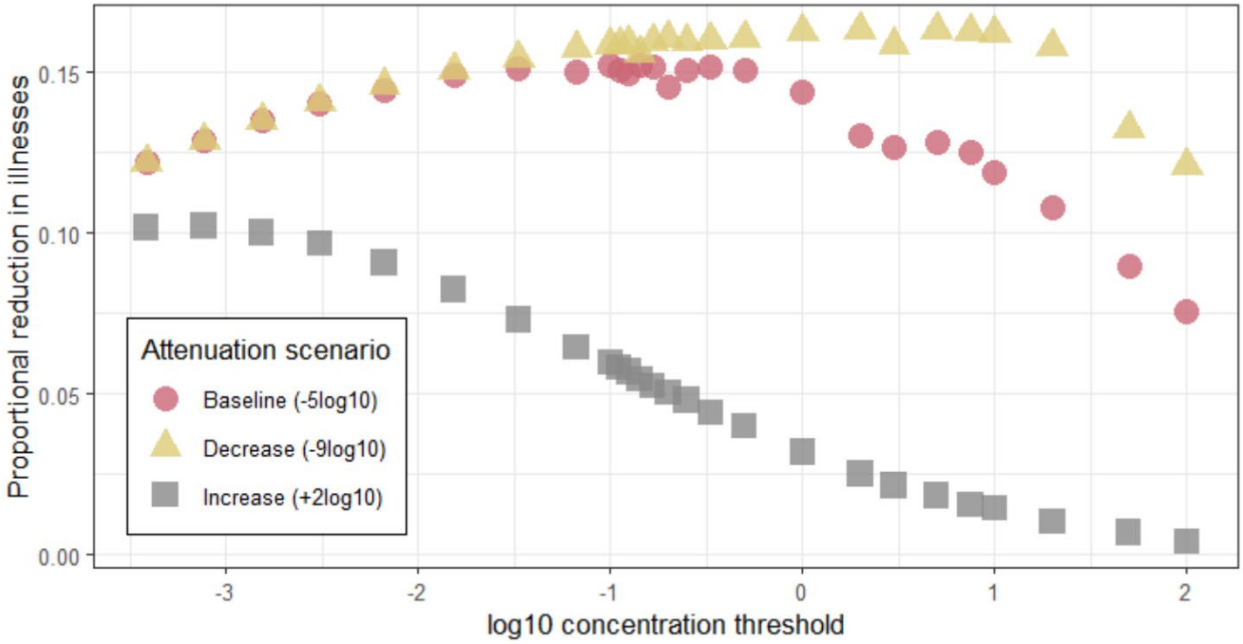

Figure 26: Analyzing the effect of changing the mean attenuation on the reduction of illnesses.

Changing the mean of the attenuation distribution to -9 log10 has the effect of substantially reducing the magnitude of the doses consumed. The predicted proportional reduction in illnesses for higher concentration thresholds approaches the effect calculated using the linear approximation (**Figure 26**) where both the attenuation variable and the dose-response functions are ignored. Such a finding demonstrates that more attenuation of the initial contamination distribution generates low doses where the assumption of a linear dose-response relationship is most appropriate. Although this change in the attenuation distribution generates baseline probability of illness per serving estimates that are too low – therefore, inconsistent with empirical expectations – its approach to the linear approximation suggests that the approximation may represent an upper bound for the effectiveness of *Salmonella* concentration thresholds.

Changing the mean of the attenuation distribution to +2 log10 models the consumption of doses that are essentially unaltered from the initial contamination levels. For example, if the default -5 log10 is thought to represent a -7 log10 average reduction combined with a +2 log10 serving size, then this change only considers the serving size adjustment to the initial contamination. Generally, this change results in a lowered public health effectiveness – relative to the baseline model – across the range of concentration thresholds considered. It also illustrates the progressively important influence of the non-linear dose-response functions on moderating the effect of increasing concentration thresholds. Although this change in the attenuation distribution generates baseline probability of illness per serving estimates that are too high – therefore, inconsistent with empirical expectations – it may represent a lower boundary of the effectiveness of concentration thresholds.

Initial contamination distribution

We also examine the effects of increasing or decreasing the mean or standard deviation of the log10

initial contamination distribution by increasing or decreasing these parameters by 1 log10 unit. Such changes are well beyond the magnitude of uncertainty about the fitted parameters of the initial contamination distribution, but the general effect of changing these parameters is easier to observe by exaggerating the change (**Figure 27**).

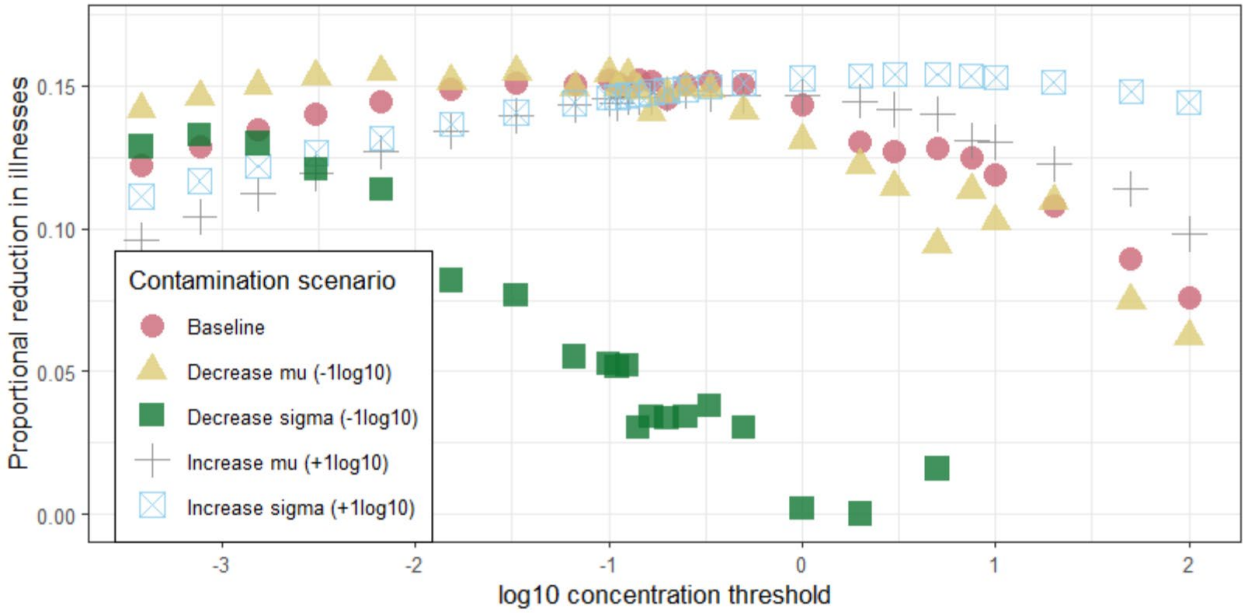

Figure 27: Analyzing the effect of initial contamination distribution adjustments on the reduction in illnesses.

Changing the mean of the initial contamination distribution (mu) shifts this distribution to higher or lower concentrations in log10 values. Increasing this mean by 1 log10 (from -4.857 to -3.857) results in a right-shifting of the effectiveness curve – relative to the baseline predictions – so that the proportional reduction in illnesses is larger for higher concentration thresholds and smaller for lower concentration thresholds. The opposite effect is noted when we decrease the mean initial contamination from -4.857 log10 to -5.957 log10; the effectiveness curve is shifted to the left relative to the baseline predictions. Nevertheless, the amplitude of the effectiveness curve (i.e., maximum effectiveness) for either increasing or decrease the mean of the initial contamination is similar to the baseline. It should be noted, however, that increasing or decreasing the mean of the initial contamination distribution substantially generates indefensible probability of illness per serving estimates that are too high or low.

Increasing the standard deviation (sigma) of the initial contamination distribution from 2.333 (baseline) to 3.333 creates higher and lower contamination levels in the distribution's tails. Consequently, the effectiveness of higher concentration thresholds is greater than the baseline model and smaller than the baseline for lower concentration thresholds. Decreasing the standard deviation of the initial contamination distribution to 1.333 reduces the frequencies of higher (and lower) contamination levels. Consequently, the public health effectiveness is reduced across the full range of concentration thresholds, although at very low concentration thresholds there is very little difference from the baseline.

Dose-response

Finally, to examine the effects of alternative dose-response functions, we use the lower- and upper-bounds for the C1 and C2 dose-response relationships (95 percent confidence limits in the uncertainty dimension – see **Appendix A**). For example, the lower bound percent reduction for a concentration threshold is estimated in the full model with default settings for all inputs except that the lower bound dose-response functions for C1 and C2 are used.

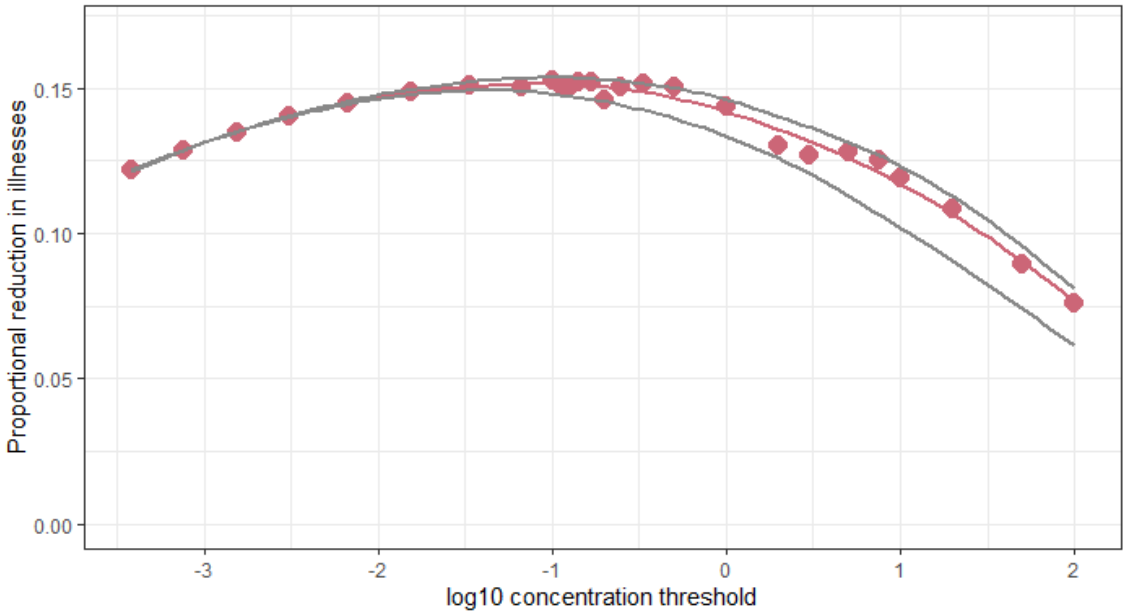

Figure 28: Analyzing the effect of alternate dose-response functions on the reduction in illnesses.

Using the lower bound dose-response relationship for both seroclusters C1 and C2 results in minimal shift in the proportional reduction in illnesses across a range of concentration thresholds. The upper bound dose-response relationship demonstrates a departure from linearity at lower doses compared to the baseline as well as the lower bound relationship. To that end, the public health effect estimated for the upper bound dose-response scenario is lower, particularly at higher concentration thresholds.

5.7 Uncertainty Analysis

The effects of uncertainty on the estimated number of illnesses prevented under different concentration thresholds is required. To motivate this analysis, we use the following equation and propagate uncertainty about each component to estimate an uncertainty distribution about illnesses prevented:

$$I_{avoided} \sim \left(1 - \frac{P_{new}(ill)}{P_{baseline}(ill)}\right) \lambda_{ill} \sim r \times \lambda_{ill}$$

Before the effects of a policy, the number of illnesses is modeled as

$$\lambda_{ill} \sim \frac{F \times B \times D \times u \times a}{C} \times T$$

where F=7600 is the typical annual number of *Salmonella* cases reported to FoodNet (CDC, 2022a), B=0.66 is the fraction of foodborne cases among all *Salmonella* cases (Beshearse, 2021), D=0.89 is the proportion of *Salmonella* cases acquired domestically (Scallan, 2011) and C=0.15 is the FoodNet catchment area fraction. The variable u is the under-diagnosis multiplier for *Salmonella* and is modeled as a $gamma\left(32.83,1/0.74\right)$ distribution (Ebel, 2012b). The variable a is the attribution fraction of *Salmonella* cases associated with consumption of turkey and is modeled as a $Pert\left(1.0,5.9,10.8\right)$ distribution(IFSAC, 2022) [6]. Finally, for comminuted turkey standards, the parameter T = 0.42 adjusts the total number of *Salmonella* illnesses associated with turkey (Lambertini, 2021).

Uncertainty about the proportional reduction in illnesses (r) is modeled as a $Pert\left(min,mode,max\right)$ distribution. For a given concentration threshold, the mode is the effectiveness estimated using our final product standards simulation model. The minimum value is estimated from the simulation model, but with the mean of the attenuation distribution set equal to +2 log10 (instead of -5 log10 as in the model scenario). The maximum value is the approximation we get when we assume the dose-response relationships are linear.

The summarized components of our uncertainty analysis for concentration thresholds of interest to FSIS risk managers demonstrate that the credible range for the proportional reduction in illnesses spans a wide range (**Table 27**).

Table 27: Description of the uncertainty distribution for the parameters used to estimate annual illnesses prevented.

Concentration threshold	**Variable**	**Comminuted turkey**
	λ_{ill} , mean (95% credible interval)	18,000 (7,000-32,000)
0.03 cfu/g	r , percent reduction	Pert(0.079, 0.151, 0.155)
1 cfu/g	r , percent reduction	Pert(0.035, 0.144, 0.164)
10 cfu/g	r , percent reduction	Pert(0.016, 0.119, 0.166)
100 cfu/g	r , percent reduction	Pert(0.006, 0.076, 0.165)

[6] The reference provides a mode and 90% confidence intervals directly. Minimum and maximum values are 99.9 percentiles estimated as $x_{0.999}=\tilde{x}\pm Z_{0.999}\left(\frac{x_{0.95}-\tilde{x}}{Z_{0.95}}\right)$, where Z_k is the k^{th} quantile of a $\mathrm{Normal}\left(0,1\right)$ distribution and $\tilde{x}$ is the reported mode.

Following Monte Carlo simulation (1 million iterations) of the product of the proportional reduction and the starting annual number of illnesses, we estimate distributions for annual illnesses prevented at the concentration thresholds of interest (**Table 28**). Results suggest substantial overlap in the 95 percent credibility intervals across progressively higher concentration thresholds. Overlapping credible intervals suggest that differences in the most likely effectiveness between different concentration thresholds may not be meaningful.

Table 28: Estimated annual illnesses prevented under final product standards for concentration thresholds of interest. Values are rounded to the nearest 100 illnesses.

Concentration threshold	**Annual illnesses prevented,** most likely (95% credible interval)
0.03 cfu/g	2500 (900 - 4500)
1 cfu/g	2300 (800 - 4400)
10 cfu/g	1900 (600 - 4000)
100 cfu/g	1400 (300 - 3200)

5.8 Discussion

Focusing on the direct effects of FSIS product testing and diversion of product lots, the developed risk assessment model predicts the average public health impact of concentration-based final product standards. Therefore, the average across repeated events should approach these model predictions; however, substantial fluctuations could be observed due to not accounting for variability between establishments or events (e.g., diversion of product units) and across time.

There is not enough *Salmonella* test-positive or enumeration data available for turkey carcasses to inform a reliable model. Generally speaking, there are two overarching explanations: (a) turkey industry on the whole is already achieving significantly low levels of *Salmonella* in carcass final products, or (b) sampling methods and diagnostic tests are not sensitive enough to detect concentrations of *Salmonella* on turkey carcasses unless they are at very high concentrations. Since turkeys are physically much larger than chickens, an alternative sampling method using sponges is employed where only 50 cm^2 sections of the carcass (back and thigh) are swabbed rather than rinsed. Additionally, the diagnostic assay has a LOD of 0.3 MPN/mL or 0.075 MPN/cm^2. Ideally, improving these testing measures could ultimately elucidate the nature of *Salmonella* on turkey carcasses.

On the other hand, approximately 17% of comminuted turkey lots are non-compliant, with a current LOD-based standard. Yet less than 3% of all lots would be diverted, on average. This results in about 2,500 illnesses prevented per year, if diverted lots are replaced by average lots. The analysis also

suggests that increasing the concentration-based threshold above the current LOD results in both a lower fraction of lots being diverted and more illnesses prevented (compared to the current LOD-based threshold). This effect occurs for small concentration threshold increases above the LOD standard, where lower risk lots are allowed to pass as opposed to diverting and replacing with potentially higher average risk lots.

In the event that any diverted lot could be replaced by a passing lot, the maximum number of illnesses prevented can be assessed under this microbial-based concentration standard. The maximum illness reduction, in this scenario, occurs using a threshold of 1cfu/2600g and amounts to approximately 3,000 illnesses prevented. This represents a nearly 17% reduction in comminuted turkey associated illnesses.

Chapter 6 Receiving Guidelines

The first risk management question states:

> What is the public health impact (change in illnesses, hospitalizations, and deaths) achieved by eliminating at receiving a proportion of turkey contaminated with specific levels of *Salmonella* and/or specific *Salmonella* subtypes?

As stated previously, FSIS does not have regulatory discretion in the pre-harvest environment, nor does it routinely collect data on the nature of flocks that are presented for slaughter—where FSIS jurisdiction begins. Specifically, FSIS does not have data on the *Salmonella* serotypes present on live birds, nor the *Salmonella* contamination levels. Further, FSIS does not have robust, generalizable data on the types of pre-harvest interventions, such as vaccination, employed by the live bird industry. FSIS has worked collaboratively with the University of Maryland through a Cooperative Agreement to obtain industry-collected data. At the time of writing, these efforts, while effective in laying the groundwork for future data sharing endeavors and have not been fruitful in producing new pre-harvest data for use in this risk assessment.

As a result, modeling of receiving guidelines was attempted using the rehang sample data. Given that there is no reliable information to parameterize a concentration distribution, risk management options that address *Salmonella* levels will not be assessed.

6.1 *Salmonella* at Rehang

The state of *Salmonella* at receiving is posited using rehang sampling data from the 2008-2009 FSIS Young Turkey Carcass microbiological baseline study due to the absence of any recent representative data for the turkey industry prior to rehang. Each pair (rehang and post-chill) of samples was obtained from a single flock or production lot and provides the earliest *Salmonella* data point in the slaughter process. That is, the data collected at rehang is used as a proxy for representing the situation or conditions at receiving. Of the 1,442 rehang carcass samples in the year-long study, 144 were positive for *Salmonella*, yielding a nearly 10% rate of detection, and less than half of these positive samples were quantifiable.

6.2 Serotype Distribution Description

Serovar Hadar was widely prevalent at rehang, amassing 40% of the *Salmonella* positive samples (**Figure 29**). Several other serovars (Saintpaul, Schwarzengrund, Agona, Albany, and Heidelberg) were identified in more than 5% of positive samples each and cover roughly another 37% cumulatively. The remaining 23% include 22 serotypes occurring sporadically. A single rehang sample was also confirmed as containing multiple serotypes. In terms of seroclusters, positive carcass samples at rehang depict an even distribution with the fraction in cluster 1 at roughly 0.53 and cluster 2 at 0.47. Without additional temporal data since the 1995 FSIS raw ground turkey microbiological baseline study, it is difficult to validate whether this distribution of serotypes (or clusters) on 144 samples is a reliable estimate of current rehang conditions in turkey carcasses across the industry.

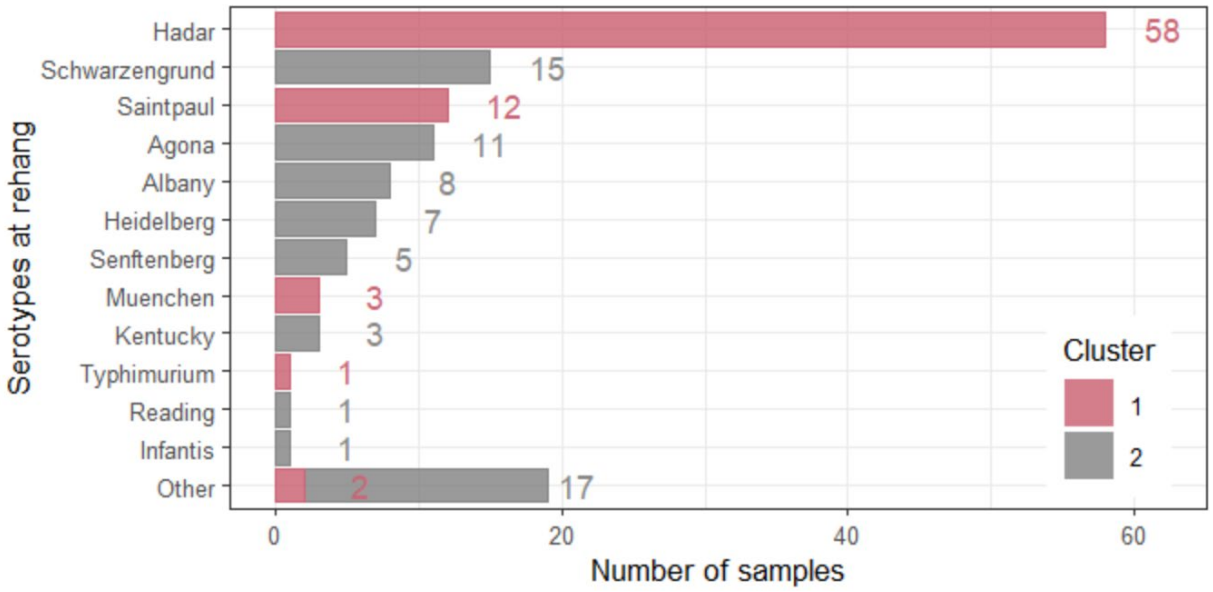

Figure 29: Frequency distribution of serotypes observed at rehang. Serotypes included in 'Other' were identified in only 1 or 2 samples.

6.3 Discussion

Data collected prior to slaughter is necessary to identify a representative population description of incoming turkey flocks. As stated previously, FSIS neither has regulatory oversight in the pre-harvest environment, nor routinely collects data on flocks prior to slaughter. However, in 2008-2009, FSIS conducted a microbiological baseline study on turkey carcasses that included a sampling point at rehang. Data collected during this period identified incoming turkey flocks containing some amount of contamination, although less than 10% of rehang samples were test-positive for *Salmonella*. Furthermore, a single rehang sample confirmed the presence of multiple serotypes in a sample, and ultimately, a flock. A recent poultry study observed that contaminated chicken carcasses typically contain multiple *Salmonella* serotypes (Thompson, 2018). This individual sample hypothesizes a potentially similar situation, although more data is certainly required to validate.

In the small fraction of rehang samples screened positive for *Salmonella*, serotype information describes an even split between serocluster occurrence, but less than half of these samples are quantifiable, indicating low levels of *Salmonella* (assuming a consistent and highly sensitive sampling method/diagnostic test). **Table 29** describes the distribution of quantifiable *Salmonella* at rehang where lower concentrations are much more frequent than higher concentrations.

Table 29: Distribution of quantifiable *Salmonella* at rehang.

MPN/cm^2	Number of samples at rehang	Number of samples at post-chill
<0.075	73	19
0.075 – 0.75	54	4
0.751-7.50	11	1
7.51-75.0	6	-
Undetermined (>28)	1	NA

Given the lack of preharvest data, analysis of receiving guidelines is limited to the earliest sampling point in the slaughter process (i.e., rehang) with sufficient data to be representative of the status of *Salmonella* contamination across the industry. This results in a constrained dataset consisting of 72 quantifiable samples at rehang (and correspondingly 5 quantifiable samples at post-chill) from 2008-2009 to convey the current conditions of incoming turkey flocks and the direct impact on public health downstream. Furthermore, the relationship between rehang and post-chill is inherently lost as described in Concordance section **5.2** Without data-based evidence of the relationship to final product contamination, no inferences can be made regarding the direct public health benefits of interventions or proposed guidelines at receiving are not fully reliable. A hypothetical scenario of serocluster distribution, using a crude approximation of reducing the higher virulence cluster 1 distribution, is provided for illustrative purposes in **Appendix B**, assuming one can substantiate the link to comminuted final product.

Chapter 7 Process Control

The third risk management question asks:

> *What is the public health impact of monitoring/enforcing process control from re-hang to post-chill? Monitoring could include analytes such as Enterobacteriaceae, Aerobic Count, or other indicator organisms, analysis could include presence/absence or levels and the monitoring could also include variability of actual result versus expected result, log reduction, absolute sample result, or other individual establishment specific criteria.*

The utility of measuring levels of indicator organisms at a single location in the slaughter process has been studied previously and found to be of limited effectiveness for controlling pathogenic bacteria (Altekruse, 2009), so multipoint sampling (e.g., rehang and post-chill) is required in the evaluation of process control, and thus, not assessed for parts and comminuted product. We address this question by analyzing the log reduction and presence/absence of two indicator organisms that can be effectively measured on turkey carcasses at different stages of the slaughter process, namely, AC and EB. FSIS has previously assessed the utility of using either AC and EB data from FSIS microbiological baseline studies (FSIS, 2009; FSIS, 2012) and concluded AC is a more practical indicator organism because concentrations are more likely to be above the LOD (Williams, 2015; Williams, 2017).

We analyze the scenario of underperforming establishments that adjust practices toward meeting a level of process control based on indicator organism metrics such as AC log reduction and EB elimination from rehang to post-chill. That is, by setting a log reduction or presence fraction target/guideline, we consider the overall prevalence that results.

7.1 Indicator organisms

Salmonella process control is analyzed using rehang and post-chill data from the 2008-2009 Young Turkey Carcass microbiological baseline study. Two indicator organisms are considered: AC and EB. The majority of establishments already achieve very positive rates of *Salmonella* on turkey carcasses which could in part be due to sampling issues. These indicator organisms, on the other hand, can be readily measured and quantified. In the following analysis, a comparison is made between *Salmonella* occurrence and indicator organism concentrations. A summary of the methods is provided in **Appendix C**.

Aerobic Counts

The AC log reduction by establishment is provided in **Figure 30** to illustrate a potential risk management option based on the change in average concentration of AC between re-hang and post-chill. Although, *Salmonella* occurrence is already quite low in most establishments, evidence suggests that establishments on the congregate have achieved high levels of average AC reduction. The overlayed lines represent a logistic regression model predicting the occurrence of *Salmonella* on post-chill samples as a function of average log10 AC reductions between re-hang and post-chill ($p < 1.4 \times 10^{-8}$). Establishment production volumes are also highlighted, using a threshold of 6 million birds to categorize high versus low volume. The number of samples per establishment during the 2008-2009 young turkey carcass microbiological baseline study was defined by volume category, with the most frequent sampling (five times per month) conducted at Category 1 establishments slaughtering more than 6

million young turkeys per year (i.e., high volume establishments). Low volume establishments cover the full spectrum of potential log reductions, but the extremes often arise based on limited samples.

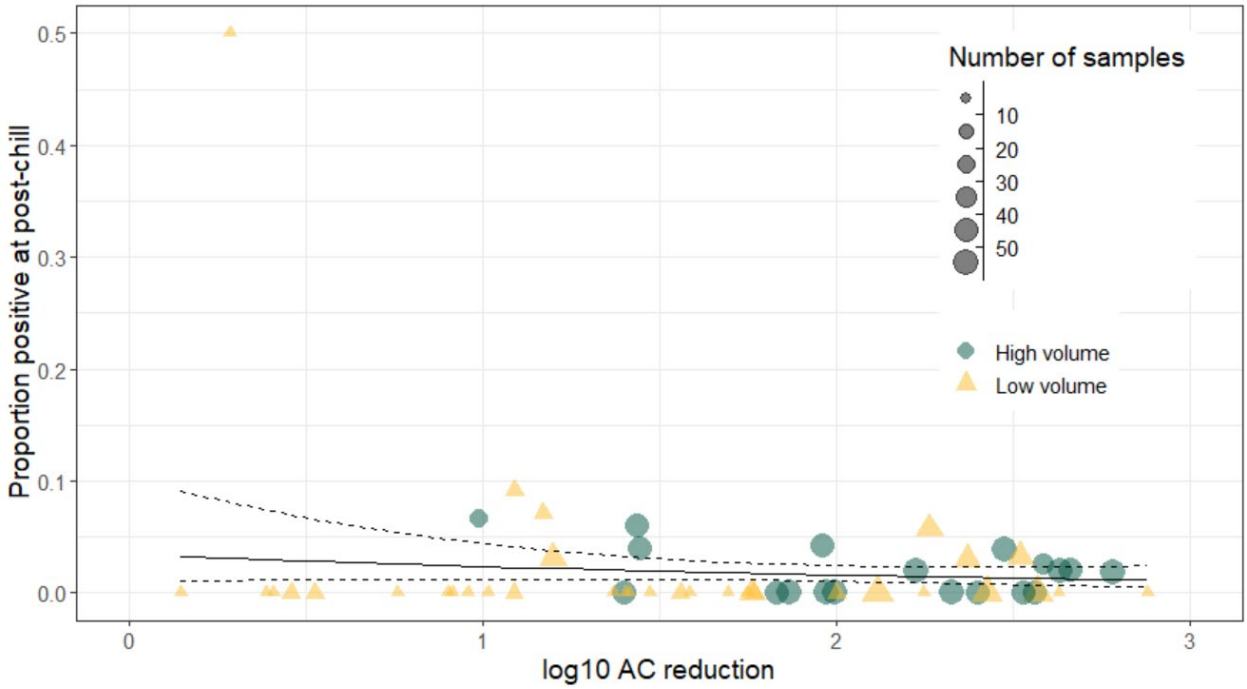

Figure 30: Average AC log reduction (from rehang to post-chill) by establishment versus post-chill *Salmonella* prevalence.

As an alternative, the LOD of the diagnostic assay for AC can be utilized. In the FSIS microbiological baseline study, the proportion of samples that had detectable AC reduced from 99.7% at rehang to 88% at post-chill.

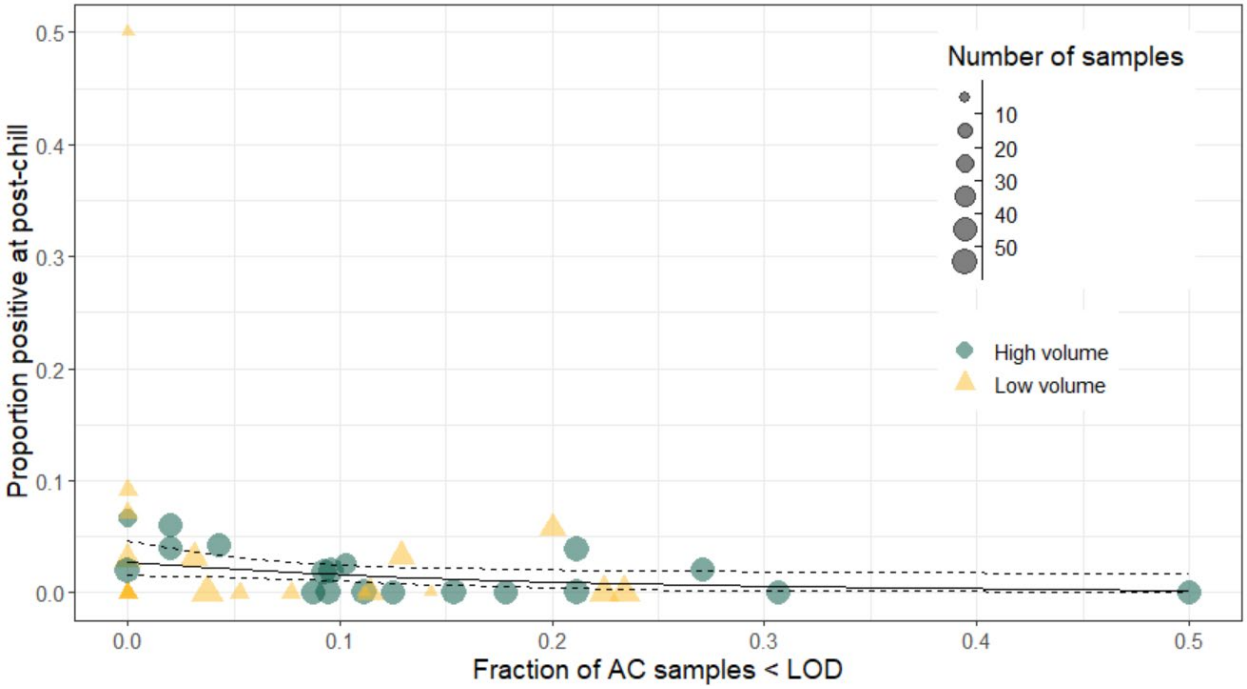

Figure 31 considers the relationship between the fraction of samples with no detectable AC at post-chill and the proportion positive for *Salmonella*. The discrepancy in detectable AC samples is highlighted in higher production volume establishments, which cover a range of 0 to 50%. A logistic regression model was fit to the data ($p < 2 \times 10^{-16}$).

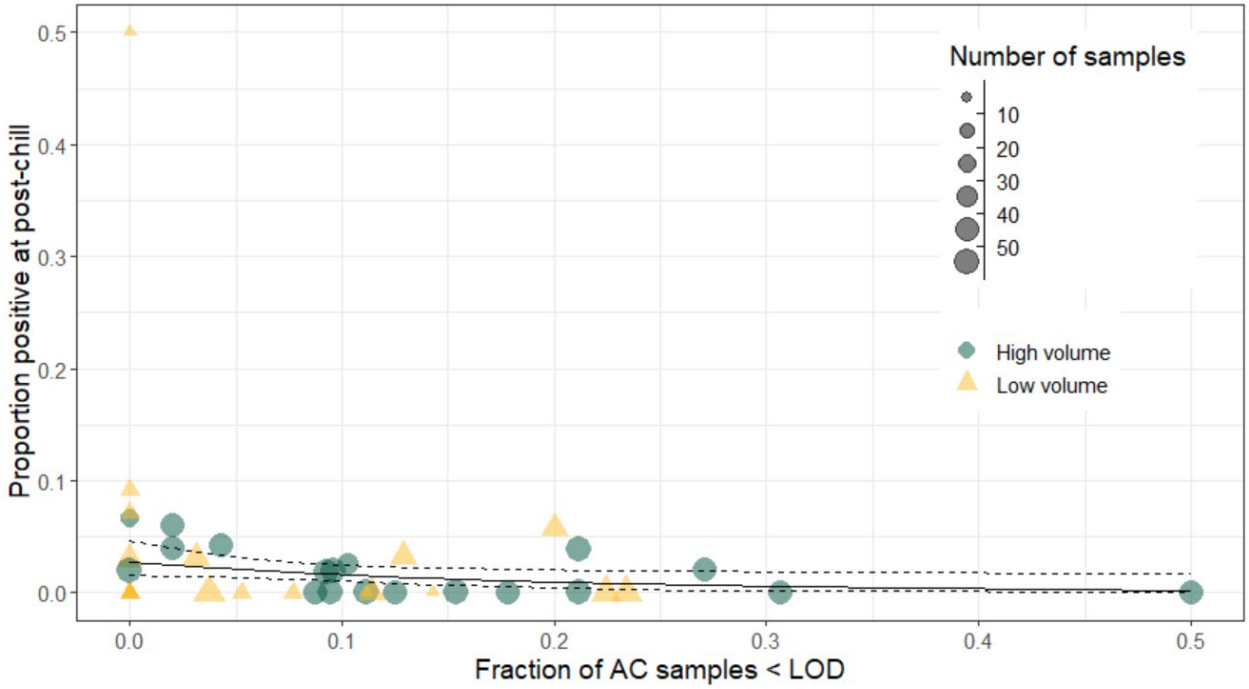

Figure 31: Comparing the fraction of AC samples below the LOD versus *Salmonella* prevalence at post-chill.

Enterobacteriaceae

The EB log reduction by establishment is provided in **Figure 32** to characterize a potential risk management option based on the change in average concentration of EB between re-hang and post-chill. There is less of a clearly defined relationship between EB reduction and *Salmonella* occurrence. However, a large fraction of post-chill samples has EB levels below the LOD. **Figure 33** investigates the relationship between the fraction of samples with EB below the LOD and the post-chill *Salmonella* prevalence on an establishment level basis with a logistic regression model fit to the data ($p < 3.3 \times 10^{-9}$).

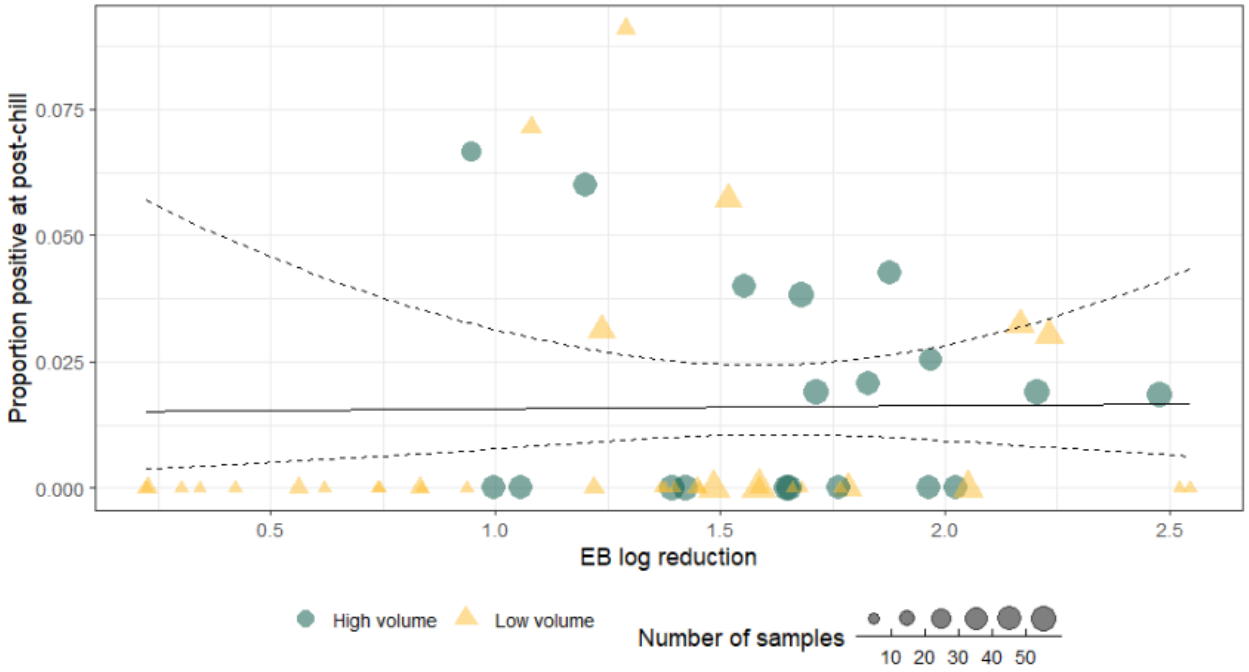

Figure 32: Average EB log reduction (from rehang to post-chill) by establishment versus post-chill *Salmonella* prevalence.

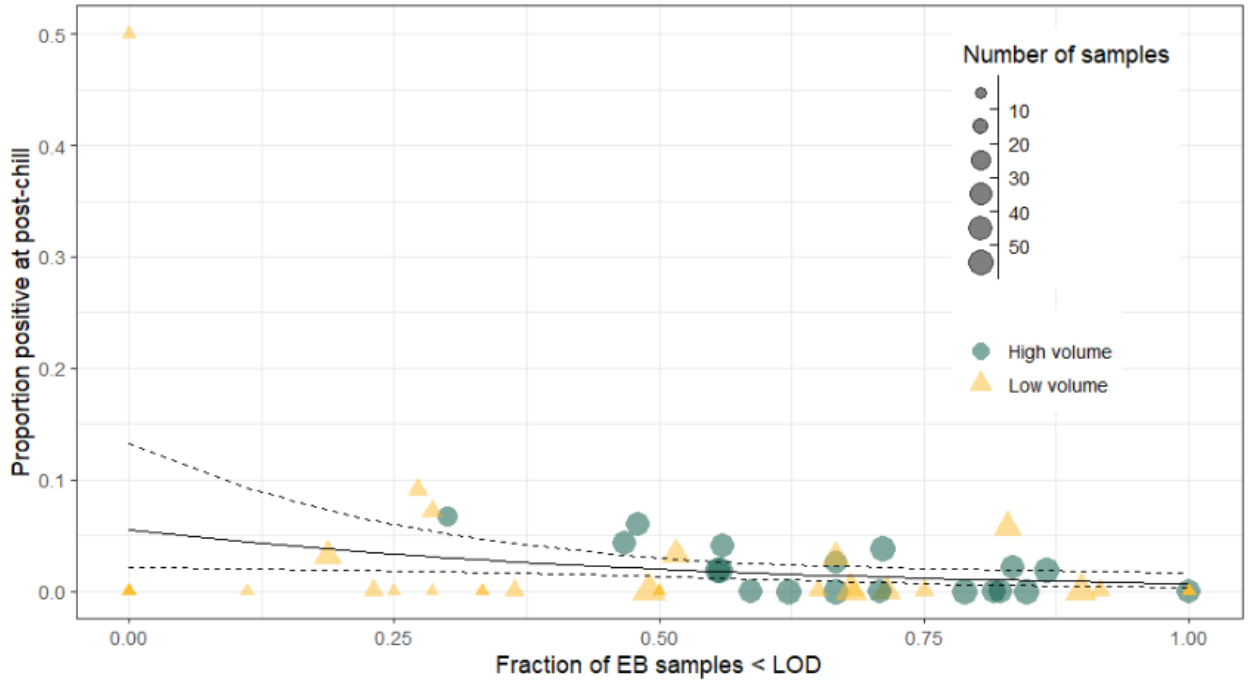

Figure 33: Comparing the fraction of EB samples below the LOD versus *Salmonella* prevalence at post-chill.

7.2 Modeling Approach

Given that the goal of the analysis is to assess the viability of replacing the existing *Salmonella* performance standards with an alternative framework, the same basic modeling structure is used. The

risk assessment model predicts the effect of imposing the new performance standard on all slaughter establishments. Once the performance standard is implemented, establishments will be subjected to the collection and testing of samples, and their results will be used to classify the establishment as either compliant or non-compliant with the standard. Assuming that prevalence is a stable characteristic of establishments, this classification creates two strata. On average, compliant establishments will have a lower prevalence of *Salmonella* compared to non-compliant establishments. Given that some fraction of establishments would initially fail the performance standard, and some fraction of these establishments would now be either voluntarily compelled or required to lower their carcass contamination frequency, some or all establishments would change their processing to become compliant. This change, from before and after implementation of the performance standard, is how the human health effect of the proposed performance standard is measured. Because it is assumed that there are two basic types of slaughter establishments (i.e., compliant and non-compliant), this approach is referred to as the "two-strata model" (Ebel, 2012a).

For performance standards based on a set of samples collected from the establishment, this analytic approach requires a fraction of production volume associated with establishments that initially pass (ω). Before the performance standard is implemented,

$$P_{baseline}\left(exp\right) = \omega P_{compliant}\left(exp\right) + \left(1-\omega\right) P_{noncompliant}\left(exp\right)$$

where $P_{compliant}\left(exp\right)$ and $P_{baseline}\left(exp\right)$ are the prevalence of contaminated carcasses among all slaughter establishments that would pass or fail the performance standard, respectively. $P_{baseline}\left(exp\right)$ is the current prevalence of *Salmonella*-positive samples. These values are estimated using a two-stage cluster sampling approach (Cochran, 1977). The weighting constant ω is the production volume produced by compliant establishments (i.e., establishments whose estimated average log10 AC reduction exceeds the value chosen for the AC-reduction performance standard).

Once the performance standard is implemented, and noncompliant establishments are identified, some fraction, α , of those establishments would change their production practices in order to pass the performance standard. We present values for two different compliance fractions, with the first one assuming that compliance with the performance standard is mandatory and all failing establishments will improve interventions sufficiently to meet the standard $\left(\alpha = 1\right)$. The second choice of compliance fraction assumes that the performance standards are not mandatory, but that half of failing establishments will add additional interventions to meet the standard $\left(\alpha = 0.5\right)$.

It is assumed that the additional reductions in all bacteria in failing establishments is such that the failing establishments would ultimately attain a prevalence of *Salmonella*-contaminated carcasses equal to those that pass the performance standard (i.e., $P_{compliant}\left(exp\right)$). Given this expected change, the estimated overall prevalence following implementation of the performance standard is given by;
$P_{new}\left(exp\right) = \left(\omega + \alpha - \omega\alpha\right) P_{compliant}\left(exp\right) + \left(1-\omega\right)\left(1-\alpha\right) P_{noncompliant}\left(exp\right)$

The choice of the appropriate cut-off values for both performance standards is determined by setting a specific reduction in the occurrence of pathogens. For this example, we will assume that the reduction target is informed by the Healthy People 2030 goal of a 25% reduction in human illnesses (HHS, 2020) and further assume that reductions in the occurrence of pathogen contaminated samples are

proportional to reductions in cases of salmonellosis.

The new weighted average of the prevalence among compliant and noncompliant establishments is calculated after some fraction of those establishments initially non-compliant have changed their practices so that they are compliant with the performance standard. For a mandatory standard, $P_{new}(exp) = P_{compliant}(exp)$ because all noncompliant establishments are expected to change their production processes to match those of the compliant establishments.

7.3 Results

Three indicator organism-based standards are investigated.

AC-reduction standard

The proposed AC-reduction standard would set a minimum value for the difference in average log10 AC concentrations between rehang and post-chill.

Figure 34 considers applying AC log reduction requirements from minimal 0.1 log10 to 3.0 log10. In the simulation, compliant, $P_{compliant}(exp)$, and non-compliant, $P_{noncompliant}(exp)$, classes are identified in gray and red, respectively, and a mixed compliance fraction in the case of non-mandatory standards $(\alpha = 0.5)$. The vertical lines denote the log reductions that achieve the intended goal. The first vertical line (orange) at a 1 log10 reduction in AC concentrations corresponds to an enforceable standard that requires all establishments to achieve that level of reduction. If adoption of the standard is voluntary, the overall log reduction must be set higher at a 2.3 log10 reduction in AC concentrations (green vertical line) to offset the additional *Salmonella* that enters the food supply from establishments that do not adopt the standard.

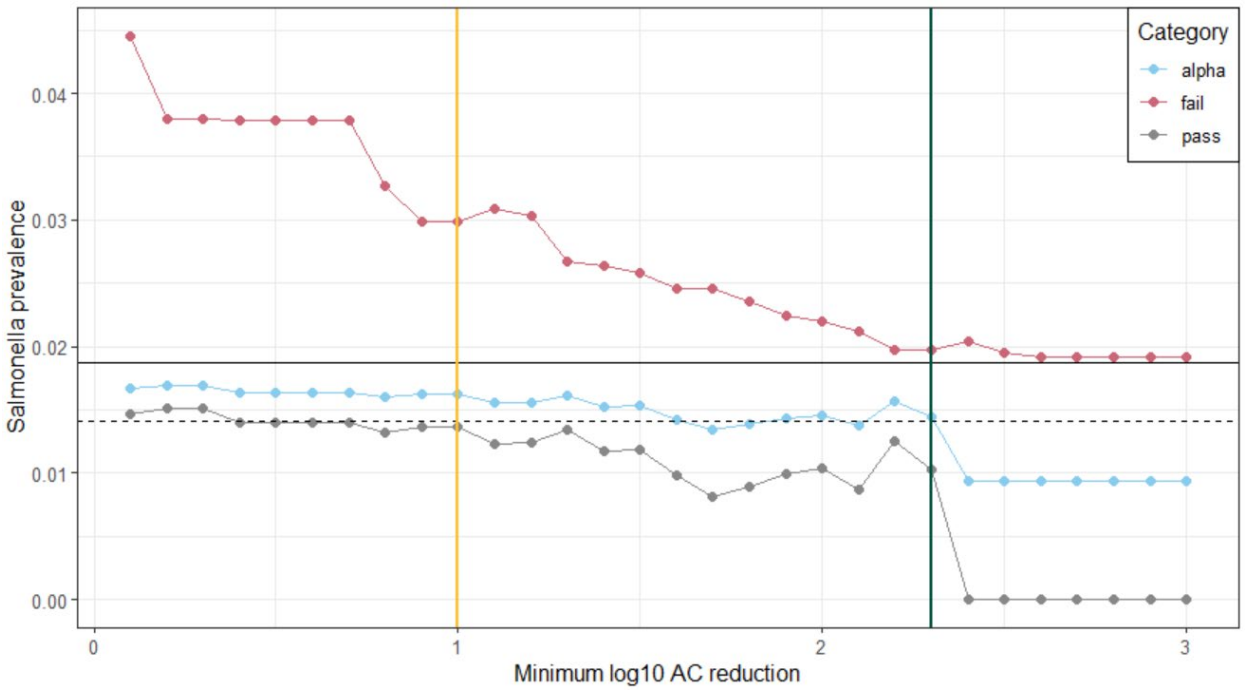

Figure 34: *Salmonella* prevalence in establishments classified (passing or failing) as a function of a range of log10 AC reduction process control scenarios. The horizontal lines represent the estimated prevalence (solid) and a 25 percent reduction (dashed).

Next, the relationship of the production volume achieving the AC reduction criterion is assessed.

Figure 35 demonstrates the proportion of the industry that would pass given the simulated average AC reduction requirements. Across the entire range, the proportion of production volume that is initially passing is generally greater than the proportion of establishments. This phenomenon occurs because *Salmonella* contamination tends to be inversely related to production volume, so smaller establishments have a higher occurrence of pathogen contamination and lower contribution to the overall industry volume.

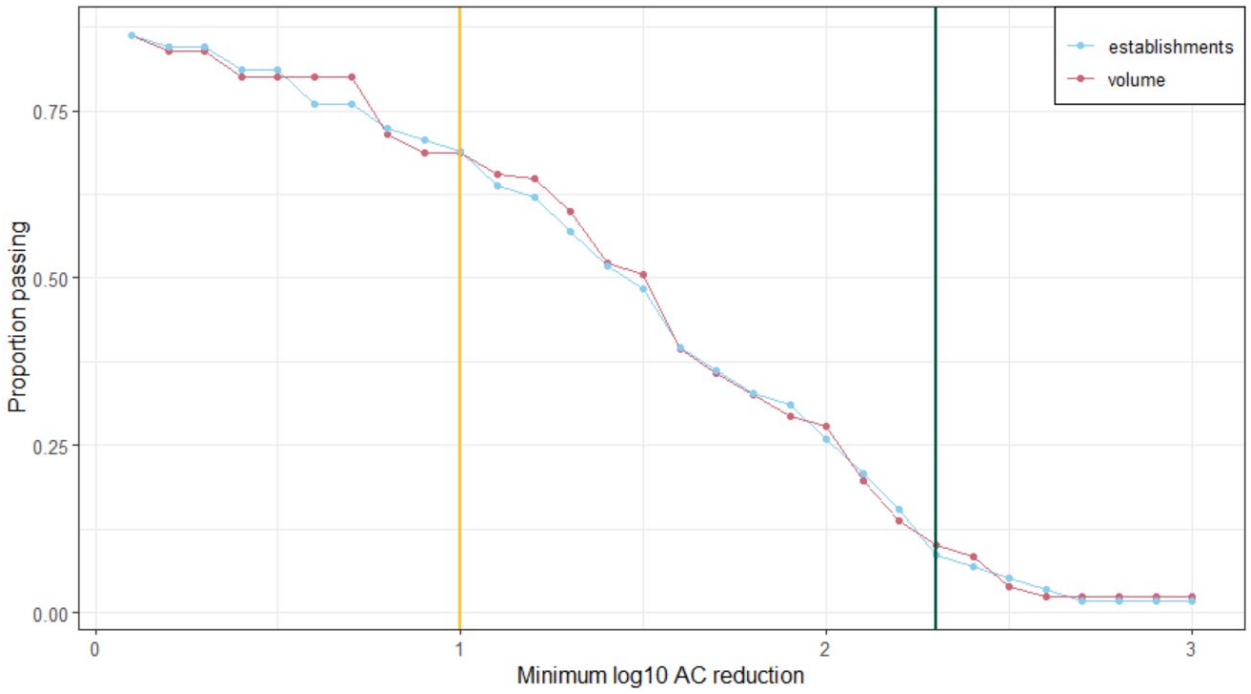

Figure 35: Proportion of production volume and number of establishments passing minimum log10 AC reduction process control scenarios.

Since the inception of performance standards in the mid-1990s, FSIS has always considered a) whether the performance standards were technically feasible and b) the chance that a passing establishment was misclassified as failing. These considerations ensured that a performance standard did not place an unreasonable burden on the industry (FSIS, 1997). Technical feasibility has been ensured by never setting performance standards lower than what some reasonable fraction of the industry was already achieving.

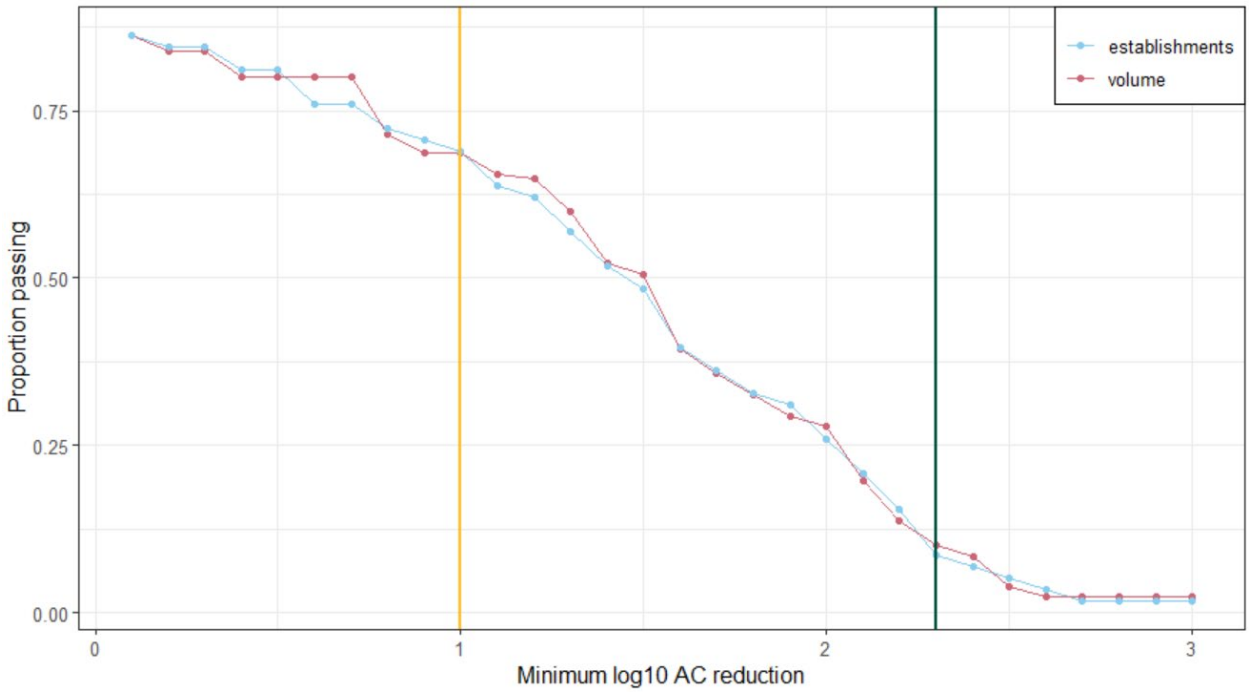

Figure 35Figure 35 demonstrates that the possible AC reduction standards are already technically feasible.

The issue of misclassification of establishments under an AC reduction standard is less straight forward because there can be multiple measures of this concept. Given that interest lies in replacing the existing prevalence-based standards with an AC-based standard, a natural measure of misclassification is determining if establishments that are currently passing the prevalence-based performance standard would also pass the AC reduction standard (i.e., the sensitivity of the new standard) and similarly an establishment failing the AC reduction standard would also be currently failing the prevalence-based standard (i.e., specificity).

Figure 36 provides the estimated sensitivities and specificities of an AC reduction standard across the simulated range of possible log10 AC reductions. In general, a good performance standard has both a high sensitivity (Se) and specificity (Sp) value. In this analysis, 94.8% of establishments are already passing prevalence-based performance standards; that is, there are 3 establishments that did not meet the allowable positive proportion limit based on the sampling rate in the microbiological baseline study. The specificity, subsequently, results in a stepwise function when these establishments as each achieves the AC-reduction standard. The sum of the two performance characteristics (Se+Sp) is maximized early at low AC log reductions (0.0-0.5 log10) and peaks again as the establishments pass the prevalence-based performance standards (1.5 and 2.0). For the AC log reductions that are predicted to achieve a desired 25% reduction, the sensitivity is approximately 70% at the enforceable standard and drops to 9% for the voluntary standard.

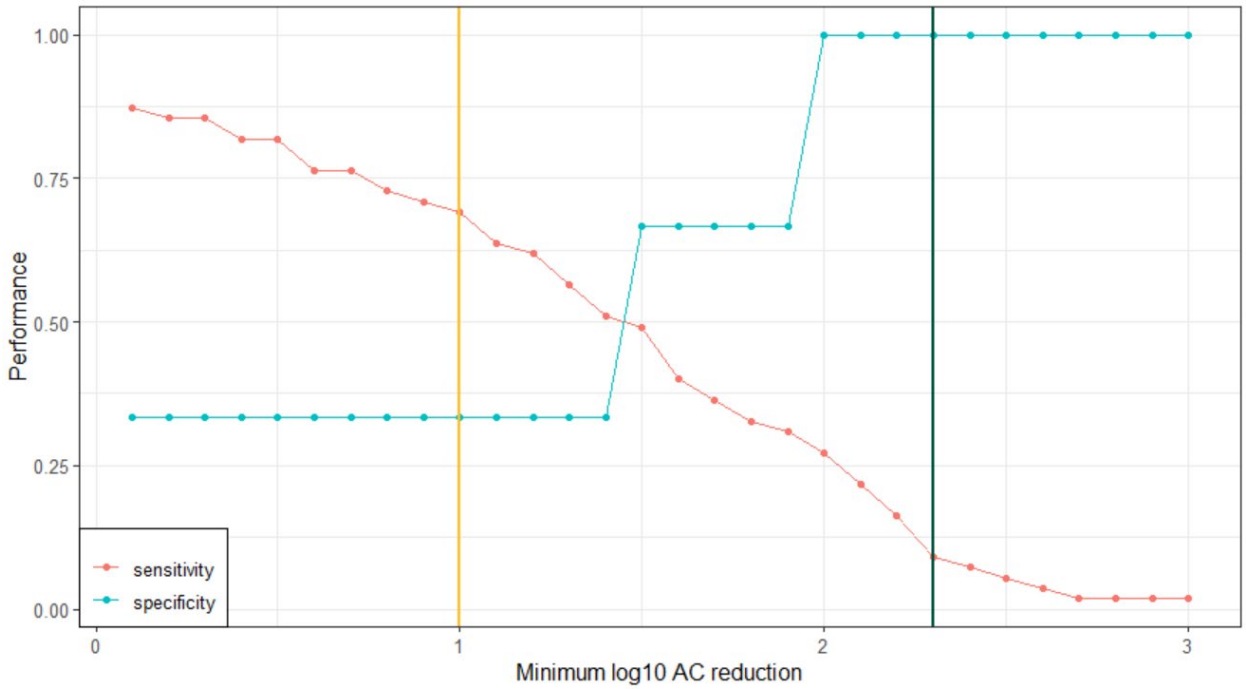

Figure 36: Approximation of performance metrics – sensitivity (passing both/passing prevalence-based performance standard) and specificity (failing both/failing prevalence-based performance standard) – for process control scenarios setting a minimum log10 AC reduction criteria.

AC-elimination standard

The proposed AC-elimination standard would set a minimum fraction of post-chill samples where no AC is observed with consideration of the current diagnostic assay.

Figure 37 considers applying detectable AC requirements. As in the previous AC standard simulation, compliant, $P_{compliant}(exp)$, and non-compliant, $P_{noncompliant}(exp)$, classes are identified in gray and red, respectively, and a mixed compliance fraction in the case of non-mandatory standards ($\alpha = 0.5$). The vertical lines denote the AC samples that achieve the intended goal. The first vertical line (orange) at 20% corresponds to an enforceable standard for all establishments. If adoption of the standard is voluntary, the overall target must be set at 30% (green vertical line) to offset the additional *Salmonella* that enters the food supply from establishments that do not adopt the standard.

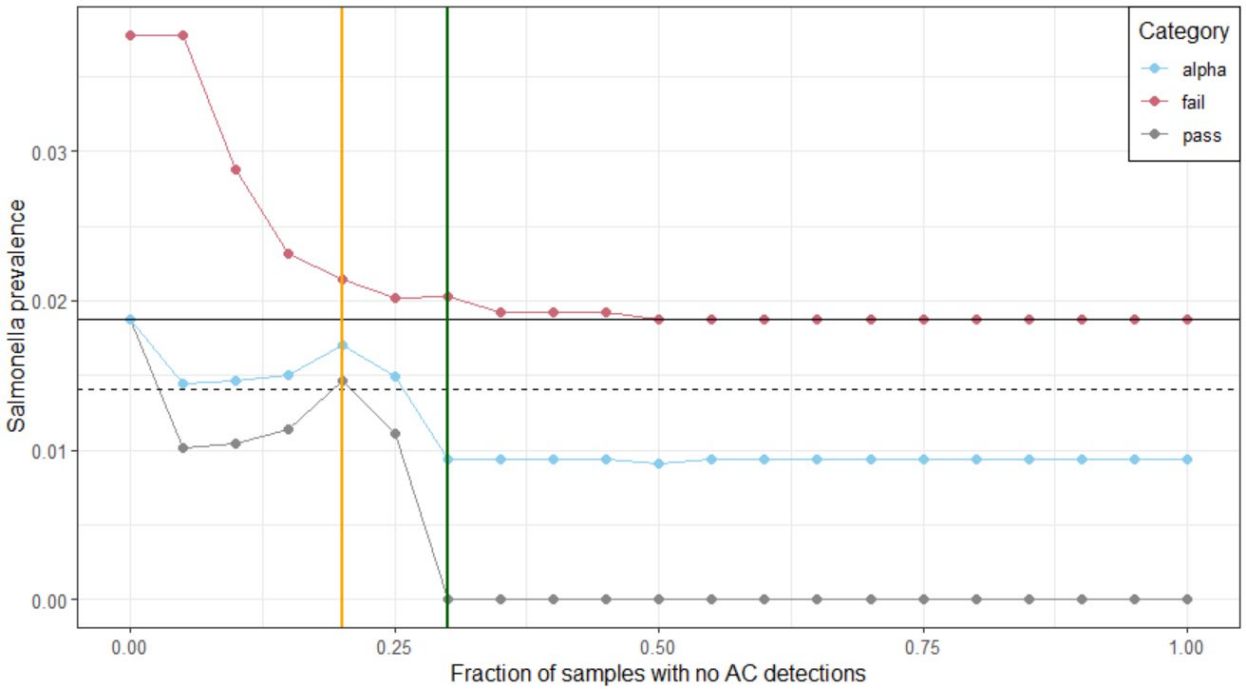

Figure 37: *Salmonella* prevalence in establishments classified (passing or failing) based on process control as a function of the proportion of AC detections (i.e., AC elimination target). The horizontal lines represent the estimated prevalence (solid) and a 25 percent reduction (dashed).

Figure 38 provides the proportion of the industry that would be passing the AC-elimination standard across the range of values. For the enforceable standard, 30% of production volume and about 14% of establishments are already passing the standard that meets the 25% reduction goal. Similar to the AC-reduction standard, the proportion of production volume that is initially passing is generally greater than the proportion of establishments.

Figure 39 provides the estimated sensitivities and specificities of an AC-elimination standard across the range of possible samples where no AC was detected. Noting again that 94.8% of establishments are already passing prevalence-based performance standards, the specificity, subsequently, results in a stepwise function when these establishments as each achieves the AC-elimination standard.

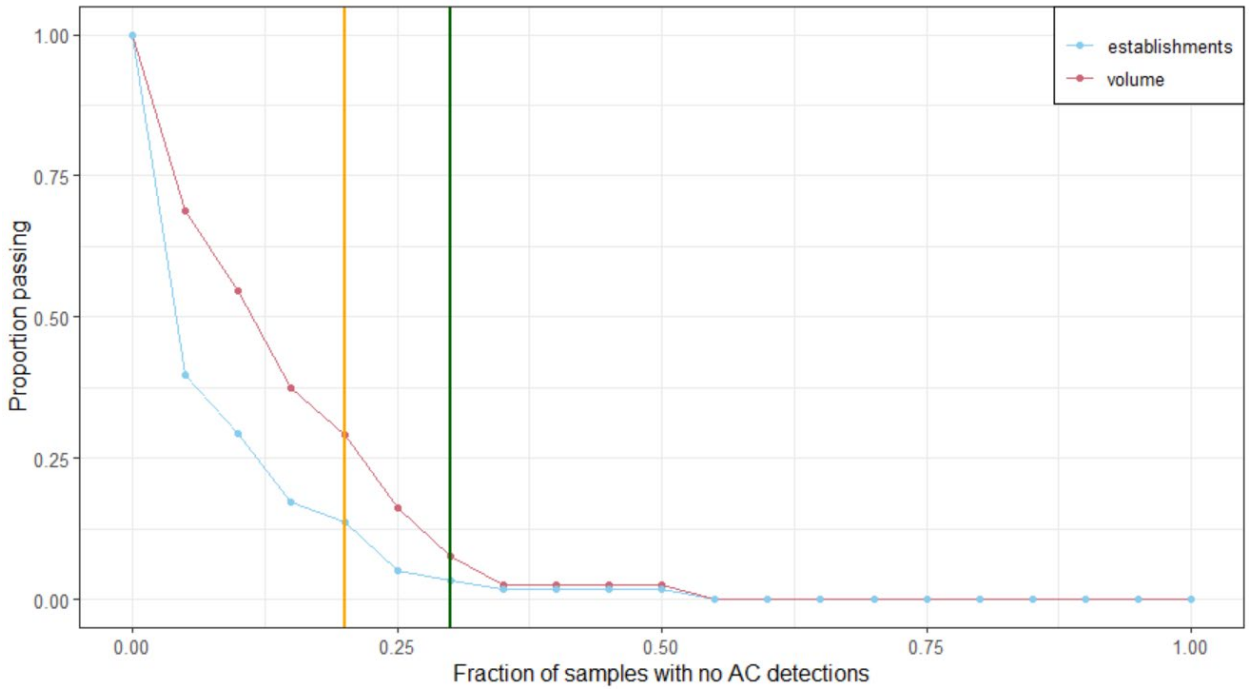

Figure 38: Proportion of production volume and number of establishments passing a process control criteria based on non-detections of AC at varying percentages.

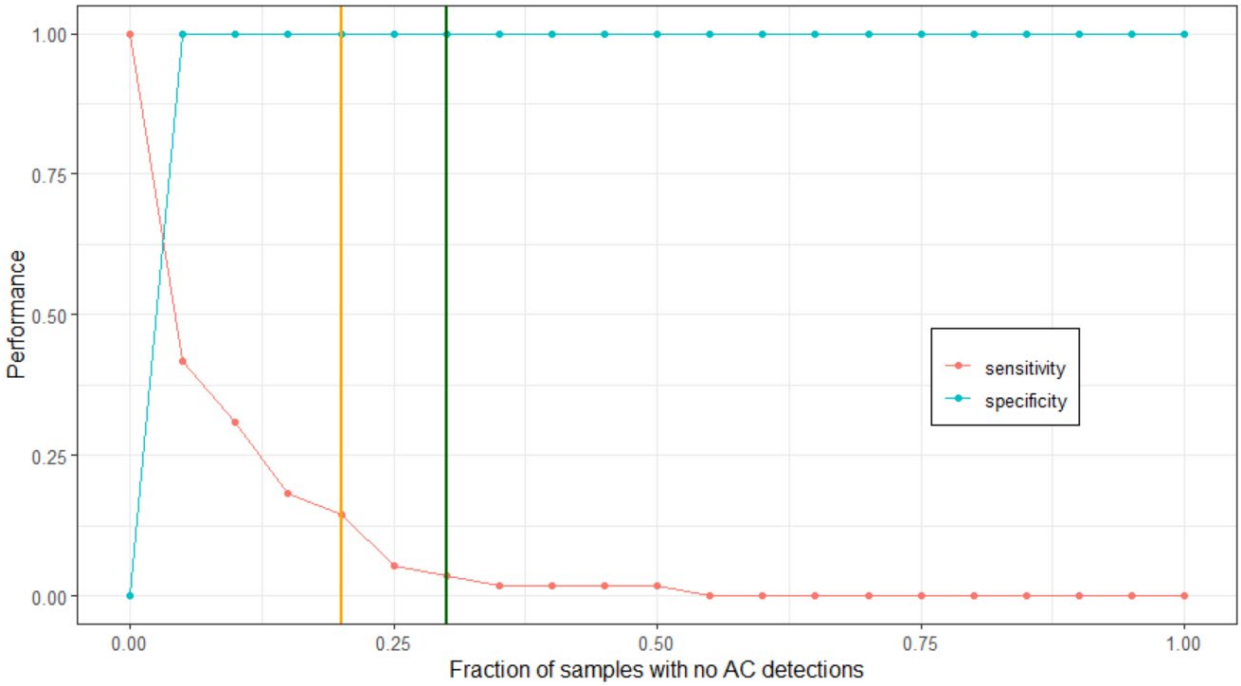

Figure 39: Approximation of performance metrics – sensitivity (passing both/passing prevalence-based performance standard) and specificity (failing both/failing prevalence-based performance standard) – for process control scenarios aimed at eliminating AC (i.e. limit detections of AC).

EB-elimination standard

The risk management option for an EB-elimination criterion would set a minimum fraction of post-chill samples where no EB is detected with consideration of the LOD of the current diagnostic assay in a similar manner as the AC-elimination process control criterion. **Figure 40** considers applying detectable EB requirements. The vertical lines denote the EB samples that achieve the intended goal. The first vertical line (orange) at 45% corresponds to an enforceable standard for all establishments. If adoption of the standard is voluntary, the overall target must be set higher at 80% (green vertical line) to offset the additional *Salmonella* that enters the food supply from establishments that do not adopt the standard.

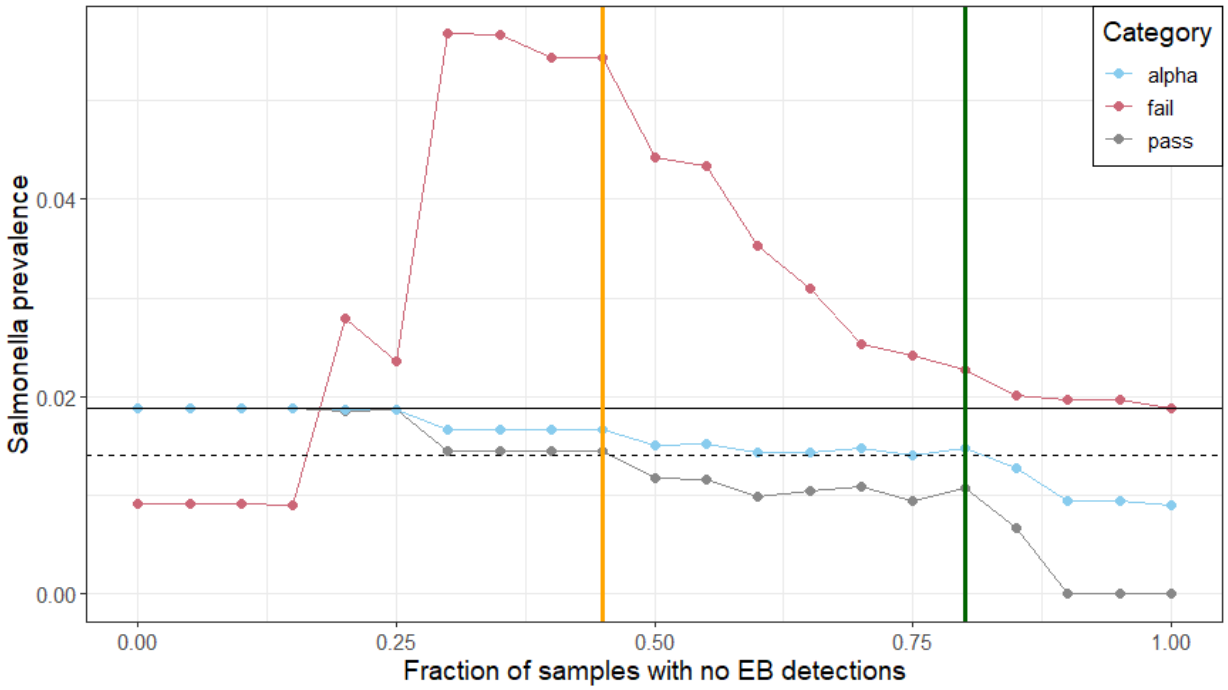

Figure 40: *Salmonella* prevalence in establishments classified (passing or failing) based on process control as a function of the proportion of EB detections (i.e., EB elimination target). The horizontal lines represent the estimated prevalence (solid) and a25 percent reduction (dashed).

Figure 41 provides the proportion of the industry that would be passing the EB-elimination standard across the range of values. For the enforceable standard, 89% of production volume and about 66% of establishments are already passing the standard that meets the 25% reduction goal. **Figure 42** provides the estimated sensitivities and specificities of an EB-elimination standard across the range of possible samples where no EB was detected. The sum of the two performance characteristics (Se+Sp) is maximized at 30%, dropping slightly at the enforceable standard of 45%.

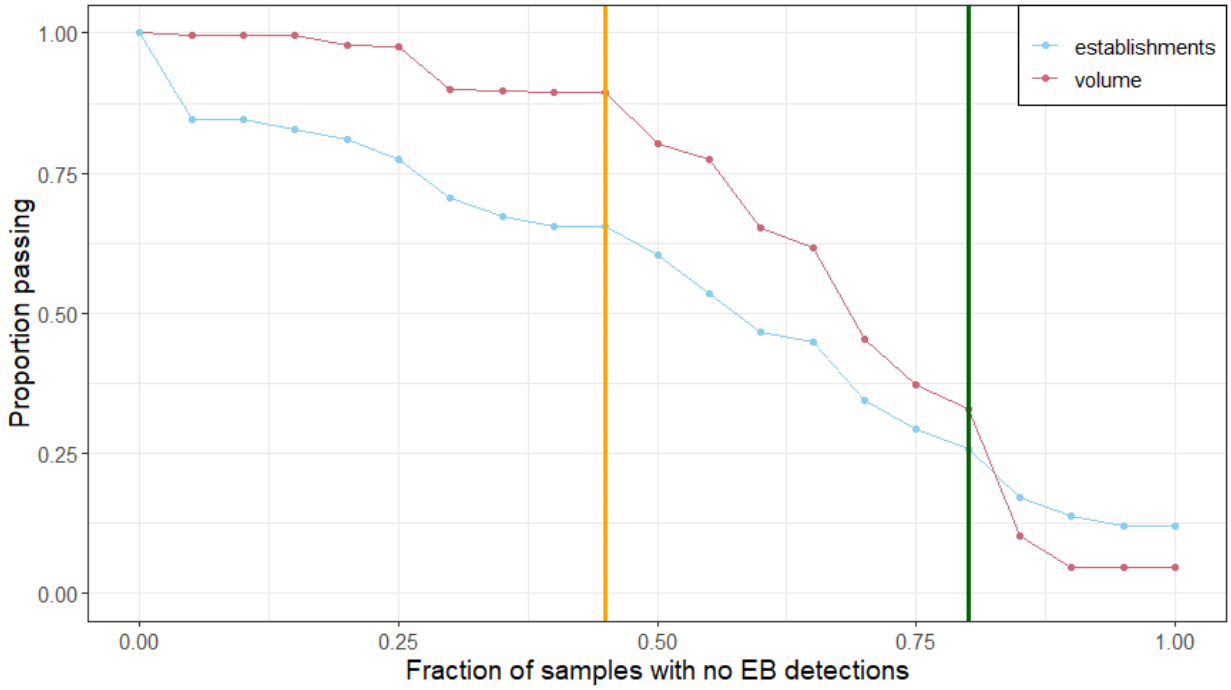

Figure 41: Proportion of production volume and number of establishments passing a process control criteria based on non-detections of EB at varying percentages.

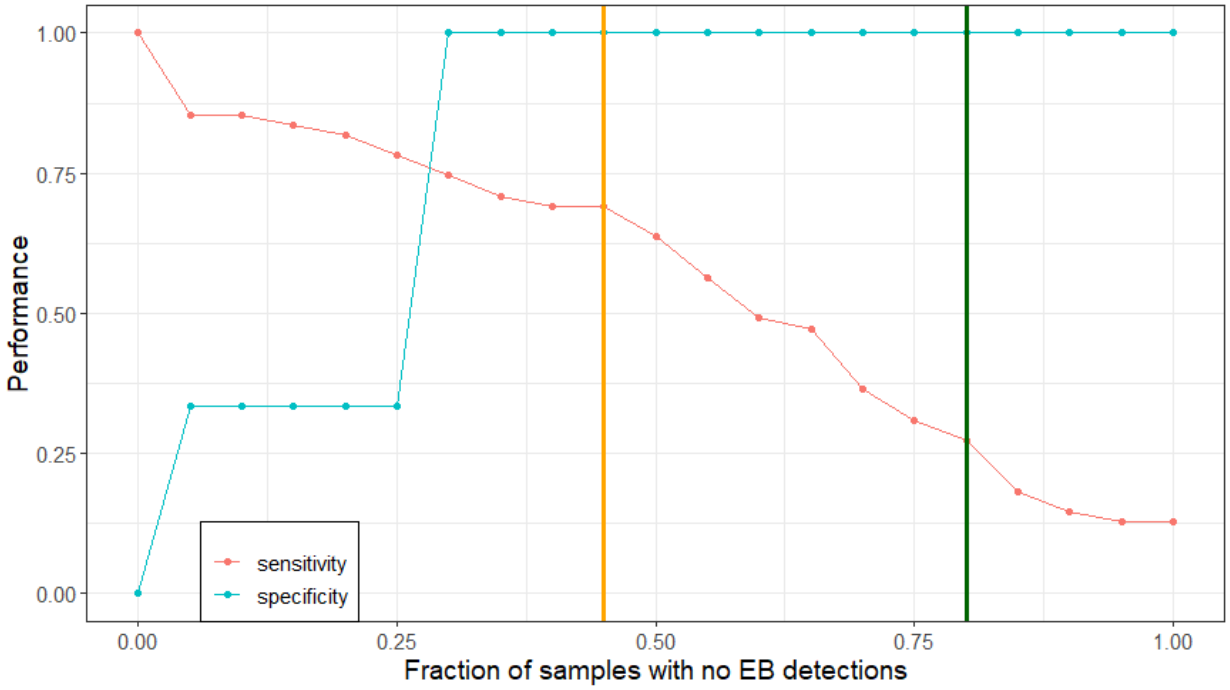

Figure 42: Approximation of performance metrics – sensitivity (passing both/passing prevalence-based performance standard) and specificity (failing both/failing prevalence-based performance standard) – for process control scenarios aimed at eliminating EB (i.e., limit detections of EB).

7.4 Discussion

For the indicator organisms AC and EB, average concentration reductions of approximately 2 logs were

observed between rehang and post-chill throughout the 2008 young turkey carcass microbiological baseline study. However, no significant relationships were observed between *Salmonella* prevalence and log reductions in EB. Hence, three process control standards were investigated on the available data from the FSIS 2008-2009 turkey carcass microbiological baseline: (a) an AC-reduction standard that sets a minimum threshold for the average change in log10 AC between rehang and post-chill, (b) AC-elimination standard or similarly (c) an EB-elimination standard that sets a minimum fraction of post-chill samples where AC or EB (respectively) is not detected at post-chill (i.e., presence/absence threshold).

The proposed enforceable standards (orange vertical lines in all figures) will reduce an already low prevalence (1.9% in 2008-09, solid horizontal line) of *Salmonella* in turkey carcasses even further. AC-reduction and EB-elimination approaches are roughly equivalent in performance (i.e., similar sensitivities). Yet, the EB elimination approach attains better performance metrics (Se+Sp) as the enforceable standard due to a higher specificity. To that end, establishments that are already passing the prevalence-based performance standard typically also pass the proposed indicator organism standard for AC-reduction and EB-elimination (sensitivity) except in AC-elimination standard. Further, the rare establishment that is failing the prevalence-based performance standard is also not compliant with the AC reduction, or more noticeably, the EB elimination requirement; however, these establishments are meeting the AC-elimination standard (specificity).

One drawback on using these metrics to distinguish between the proposed indicator organism standards is that most establishments in the 2008-2009 FSIS microbiological baseline study are substantially passing the prevalence standards (94.8%), whereas only a few establishments are not (disregarding the minimum sample count requirement); and these select establishments have a greater influence on specificity measures. Hence, the differences in specificity are somewhat arbitrary, yielding a comparably equal impact on performance in reducing *Salmonella* prevalence, when considering sensitivity alone. Additionally, the elimination standards also have a key advantage of employing a single sample to assess process control, rather than the two-point sampling requirements for AC-reduction control.

A voluntary standard would necessitate more stringent criteria (i.e., larger AC log reduction or smaller fraction of EB presence at post-chill) to achieve the 25% reduction in *Salmonella* prevalence on post-chill turkey carcasses(which amounts to a prevalence less than 1.5%). However, this is not a huge benefit compared to the mandatory standard, which reduces *Salmonella* to approximately 1.75% prevalence.

It is important to note that the turkey carcass prevalence-based standards are continually significantly met across the industry with very few (< 10) *Salmonella*-positive samples arising annually. Moreover, since the 2009-2009 FSIS microbiological baseline study, *Salmonella* prevalence on turkey carcasses has decreased (see **Table 18**); i.e., from 0.02 in 2008 to <0.005 in 2021. Current data on indicator organisms is required to validate the relevancy of these proposed standards (and updated/realistic public health benefit estimates) as process control may have significantly improved to attain such few test-positives on turkey carcasses over the last 10 years. Given this dramatic reduction in *Salmonella* prevalence on turkey carcasses over the last 10 years, the process control results may not be applicable to current conditions and analysis of data collected using more sensitive assays may be necessary. Nevertheless**,** indicator organisms were readily measured and quantified compared to *Salmonella* levels at both sampling locations.

Chapter 8 Discussion

The risk assessment provides answers to two of the following risk management questions: final product standards in comminuted turkey and process control in turkey carcasses* (assuming conditions remain similar to 2008-2009 baselines). Due to current data limitations, it was not feasible to develop reliable estimates on the public health impact for other scenarios.

> ***Risk Management Question #1:*** *What is the public health impact (change in illnesses, hospitalizations, and deaths) achieved by eliminating at receiving a proportion of chicken contaminated with specific levels of Salmonella and/or specific Salmonella subtypes?*

As the receiving step only occurs when animals enter slaughter establishments, scenarios for this risk management question could only be preliminarily assessed for turkey carcasses based on a limited historical microbiological baseline study.

FSIS does not have regulatory discretion in the pre-harvest environment, nor does it routinely collect data on the nature of flocks that are presented for slaughter—where FSIS jurisdiction begins. Specifically, FSIS does not have data on the *Salmonella* serotypes present on live birds, nor the *Salmonella* contamination levels. Further, FSIS does not have robust, generalizable data on the types of pre-harvest interventions, such as vaccination, employed by the live bird industry.

Given the lack of robust data, it was not possible to estimate the public health impact of performance standards at receiving that address either *Salmonella* levels or serotype. Of the 1,442 rehang carcass samples from 58 establishments in the year-long study, 144 were positive for *Salmonella*, yielding a nearly 10% rate of detection, and less than half of these positive samples were quantifiable. That said, attempts were made to develop a hypothetical model (**Appendix B**) of cluster distributions based on limited data regarding turkey carcasses at rehang, that would allow for the development of performance standards at receiving. This hypothetical model does demonstrate that focusing on reducing the higher virulence cluster distribution at receiving could have an improved effect on reducing illnesses. However, it is not advisable at this time to make risk management decisions based on this cursory model, though further exploration of this approach warrants future consideration.

> ***Risk Management Question #2:*** *What is the public health impact (change in illnesses, hospitalizations, and deaths) achieved by eliminating final product contaminated with specific levels of Salmonella and/or specific Salmonella subtypes?*

This question was the major point of interest for FSIS risk managers and stakeholders. As such, the bulk of model development was geared toward answering it. The model assesses only changes in overall illnesses, including those that result in hospitalization and deaths, but does not explore direct changes to hospitalizations and deaths.

Concentration-Based Final Product Standards

Turkey Carcasses

FSIS does not currently enumerate Agency PR/HACCP turkey carcass samples. This fact, combined with the very low prevalence rates of *Salmonella*, made a reliable estimate of the current concentration distribution of *Salmonella* on post-chill carcasses infeasible. Additionally, in the FSIS 2008-2009 young

turkey carcass microbiological baseline study, less than 5% of rehang samples and less than 1% of post-chill samples were quantifiable. The lack of ample data limits FSIS' ability to assess a concentration threshold performance standard for turkey carcasses.

That said, examination of foodborne illness data from the CDC—and related IFSAC estimates of *Salmonella* attribution to turkey—indicates that a not insignificant number of *Salmonella* illnesses in the population are attributed to turkey. Therefore, it follows logically that some *Salmonella* must exist on turkey carcasses, but it perhaps is not being identified adequately through FSIS' current sampling techniques. Consequently, FSIS is presently assessing the viability of its current *Salmonella* sampling program for turkey carcasses.

Turkey Parts

FSIS does not currently, nor has historically, collected enumeration data on turkey parts, which makes the development of a concentration-based performance standard infeasible at present.

Comminuted Turkey

Unlike turkey carcasses and parts, it is possible to address the final product standard risk management question for comminuted turkey. A comminuted turkey performance standard that diverts test-positive lots based on a concentration threshold of 1CFU/15g is the most effective risk management option, with 2,700 illnesses prevented annually, which equates to slightly over 15% of the approximately 18,000 comminuted turkey illnesses estimated to occur annually. A comminuted turkey performance standard that diverts test-positive lots based on a concentration threshold (at the current LOD, or screening level) of 0.033 cfu/g is a similarly effective risk management option, with 2,500 illnesses prevented annually, which equates to 14% of the approximately 18,000 comminuted turkey illness that occur annually. These estimates do not fully take into account fluctuations in *Salmonella* levels across establishments and time, but repeated simulations converge toward the average. **Table 30** estimates the number of annual illnesses prevented based on concentration thresholds (i.e., microbial criteria) in final product comminuted turkey.

Table 30: Estimated annual illnesses prevented under final product standards for concentration thresholds of interest in comminuted turkey.

Concentration threshold	**Annual illnesses prevented,** most likely (95% credible interval)
0.03 cfu/g	2500 (700 – 4900)
1 cfu/g	2300 (600 – 4800)
10 cfu/g	2000 (500 – 4300)
100 cfu/g	1400 (200 – 3500)

A major assumption of this modeling approach is that consumer demand for raw turkey products will continually be met by the industry, and so every lot removed (as a result of a new standard) will

ultimately be replaced by another average lot. While this approach differs from other modeling approaches described in the scientific literature, FSIS believes this approach represents a more realistic assessment of the current turkey industry and, therefore, the identified public health benefits.

Serotype-Based Final Product Standards

Lack of robust sampling at rehang (or any earlier sampling point in process prior to post-chill), made it infeasible to estimate the public health impact of performance standards that focus on serotype for all turkey products, as the underlying mixture of serotypes in a lot (i.e., within a flock or day of production) could not be validated. Theoretical mixtures could be evaluated, however, it is not advisable for risk management decisions. Furthermore, a serotype-based approach would target a subset of the failing lots from the concentration-based final standard defined at the screening level.

> ***Risk Management Question #3:*** *What is the public health impact of monitoring/enforcing process control from rehang to post-chill? Monitoring could include analytes such as Enterobacteriaceae, Aerobic Count, or other indicator organisms, analysis could include presence/absence or levels and the monitoring could also include variability of actual result versus expected result, log reduction, absolute sample result, or other individual establishment specific criteria.*

Process control scenarios were assessed for the turkey slaughter industry. Monitoring was interpreted as the effect of a log reduction or elimination of indicator organisms from rehang to post-chill. Analyses of the 2008 FSIS microbiological baseline data indicates that the turkey industry was consistently observing AC and EB in the majority of rehang samples (>95%), and simultaneously, achieving a large reduction in AC and EB. Given the further decrease in *Salmonella* across the turkey industry since this study and the lack of current data, it is assumed that establishments are still achieving 1-2 log reductions in these indicator organisms. This finding, however, demonstrates that any new performance standards that rely on changes in process control would be limited in its ability to reduce the overall burden of *Salmonella* illnesses from turkey.

Further complicating efforts to achieve significant decreases in *Salmonella* illnesses from turkey is the fact that, as has been the case historically, indicator organisms are not strongly correlated with the presence of *Salmonella* at post-chill or a log reduction in EB. As a result of these weak relationships between indicator organisms (i.e., AC and EB) and *Salmonella* prevalence, it follows that the correlation between AC or EB and *Salmonella* serotypes or levels is also weak. Therefore, it was not possible to assess the risk management question regarding the public health impact (illnesses, hospitalizations, and deaths) of monitoring/enforcing process control from rehang to post-chill in the same manner as it was estimated for final product standards which incorporate *Salmonella* contamination distributions.

Consequently, this analysis instead focused on assessing the potential of two process control performance standards to achieve the 25% Healthy People 2030 (HP2030) illness reduction targets for *Salmonella* (HHS2020).

Three process control standards were investigated on the available data from 2008-2009:

1. an AC-reduction standard that sets a minimum threshold for the average change in log10 AC between rehang and post-chill,

2. an AC-elimination standard that sets a minimum fraction of post-chill samples where AC is not observed with the current assay (i.e., samples below the LOD identifying presence/absence),

and

3. an EB-elimination standard that sets a minimum fraction of post-chill samples where EB is not observed with the current assay (i.e., samples below the LOD identifying presence/absence)

Scenarios were run assuming underperforming establishments adjust their practices toward meeting a level of control according to the indicator organism metrics listed above from rehang to post-chill. That is, by setting a log reduction or presence fraction target/guideline, the overall prevalence that results from that change can be assessed.

Utilizing the 2008-2009 microbiological baseline data, mandatory standards of AC-reduction (requiring 1log10 reduction), AC-elimination (requiring less than 0.20 samples with AC detections), and a EB-elimination standard (requiring less than 0.45 samples with EB detections) would achieve the targeted Healthy People 2030 25% reduction in S*almonella* prevalence; from approximately 2% to 1.5%.

If these standards were voluntary, higher standards (i.e., larger AC log reduction or smaller fraction of EB presence at post-chill) would be required to achieve the 25% reduction in prevalence (which amounts to a prevalence less than 1.5%). However, this is not a huge benefit compared to the mandatory standard, which reduces to approximately 1.75% prevalence.

That said, the elimination standards for either AC or EB require only 1 sample at post-chill to implement, rather than 2 samples to test a reduction in AC, so implementation of an elimination standard is more practical from a logistical and cost perspective. It is important to note that an underlying assumption for these elimination standards necessitates that indicator organisms remain consistently present (and at high levels) on incoming carcasses.

Further complicating the analysis, the prevalence of *Salmonella* on turkey carcasses has dropped substantially, with a current rate of less than 1%. Turkey carcass establishments, therefore, are likely still achieving (or even exceeding) these indicator targets, making for little room for improvement in the industry. Further, regardless of how many samples are required (1 for elimination v. 2 for reduction), the expected cost of these efforts should be considered carefully in light of the minimal impact on public health.

Nevertheless**,** indicator organisms were readily measured and quantified compared to *Salmonella* levels at both sampling locations. Future analyses would require more current information to validate appropriate targets for AC and EB.

> ***Risk Management Question #4:*** *What is the public health impact of implementing combinations of the risk management options listed above?*

The fourth risk management question was not answered by the analyses summarized in this document. Analytical challenges and data gaps prevented a full treatment of combination scenarios. Cursory explanations for how scenarios could be combined are outlined in the chicken risk assessment (available here) where more data were available, along with some of the current data.

In short, from a risk management perspective, it is possible to combine receiving, processing and final product standards. As none of these possibilities could be modeled in turkey products, it is unwarranted to explore further until the baseline research gaps are addressed, as discussed in section **8.1** .

The process control analysis is focused on predicting changes in *Salmonella* prevalence following the imposition of indicator organism log reduction standards. As such, it is not amenable directly to combining with final product standards. Nevertheless, it is feasible that, given sufficient data to fit an initial turkey carcass contamination concentration distribution for use in the final product standards, that distribution could be adjusted to account for predicted changes in *Salmonella* prevalence from a process control standard. As explained in Ebel and Williams (2015), such an adjustment could be scalar (only influencing the lognormal parameter) or non-scalar (influencing both and lognormal parameters). Such adjustments are only warranted if the process control standards are mandatory and based on sufficient testing that limits establishment misclassification.

8.1 Research Needs

The following research needs were identified during the development of the risk assessment in raw turkey products. The following list is not prioritized, however, a central challenge to the risk assessment is:

1. Quantification of *Salmonella* in turkey products
   IFSAC estimates the percentage of foodborne *Salmonella* illnesses associated with turkey products was roughly 5.9% based on a model incorporating 2016-2020 outbreak data. However, *Salmonella* is rarely detected on raw turkey carcasses, FSIS does not currently sample turkey parts so their *Salmonella* prevalence is unknown, and the comminuted turkey prevalence hovers at around 16% across the industry every year (which implies a genuine presence on carcasses). Without reliable and consistent sampling programs, *Salmonella* cannot be detected, much less quantified, on a majority of turkey products leaving slaughter facilities.

   Turkey carcass sampling: Currently, inspectors take a sponge sample of small areas (i.e., 5cm x 10cm) of the back and of the thigh of a turkey carcass. These sponge samples create a composite that will determine the pathogen status of the carcass. Under this current sampling approach, less than 20 carcass samples test positive for *Salmonella* every year, although more than 1,800 samples are collected on average. **Appendix B** describes the prevalence in turkey products across time in more detail.

   Sponge sampling likely underestimates the prevalence (and ultimately the levels) of *Salmonella* on turkey carcasses. One of FSIS' research priorities and studies is to improve methods for sampling turkey carcasses, and at the time this risk assessment was authored, a study was underway in collaboration with USDA-ARS (FSIS, 2023a). Other researchers have examined different approaches to recover/quantify *Salmonella* in turkey carcasses including boot swabs or fecal sampling, alternative rinse methods, and pooled samples(Arnold, 2009; McEvoy, 2005). A reliable sampling method regarding for turkey carcasses and parts is critical to evaluating the current state of *Salmonella* across turkey products.

   Turkey parts sampling: FSIS does not currently, nor has historically, regularly collected enumeration data on turkey parts regarding *Salmonella* contamination. A consistent sampling

source would allow for a greater understanding of *Salmonella* contamination on this turkey product.

2. Within Flock *Salmonella* Variability

   The lack of data to determine within lot and between lot variability of bacterial occurrence and levels in turkey products severely limits the ability to assess the effects of diversion options, in particular for the final product standards in this risk assessment.

   Serotype Mixtures: Analysis of the FSIS 2008-2009 Turkey Microbiological Baseline (FSIS, 2010) sampling data with two data points (rehang and post-chill) per turkey flock indicates that multiple *Salmonella* serotypes can occur in flocks. However, data is still limited due to turkey carcasses rarely testing positive for *Salmonella*. Furthermore, while it is possible that some flocks do not contain a single, dominant serotype, no data exist that describes what other per flock mixtures of serotypes may be present or how prevalent they are in the U.S. poultry population.

   Per Unit Salmonella Population: It is plausible that multiple serotypes are present on a given carcass, part, or comminuted unit, both within flock/lot (Rasamsetti 2021, Siceloff 2022) or a single sample (Cox 2019, Rasamsetti 2023, Thompson 2018) Such a microbial profile at each product level could shed light on population *Salmonella* variability.

3. Industry Response to an Adulteration Status for *Salmonella*
   While it can be postulated that any regulation that declares a pathogen an adulterant will have an indirect effect on the turkey industry's pathogen control measures, no data is available at this time describing the magnitude of that effect. Therefore, only the direct of effect of such a risk management approach was assessed in this document.

   More clarity and insight could be gained from data describing interventions that target levels of *Salmonella* and their efficacy. While some serotype-specific interventions are known (e.g., vaccination), their current usage and effectiveness is not well understood and no data capturing industry-wide usage are available at this time. This data is necessary to model changes to industry-wide usage of such interventions.

   The declaration of *Salmonella* as an adulterant may lead to an industry-wide shift of control measures on the same scale as the STEC O157 policy (FSIS, 2002), however no after-action analyses of the STEC shift are available at this time. One feature that is well understood, is the change in lotting practices on the basis of STEC microbial independence in response to the introduction of the STEC O157 adulterant policy change. This risk assessment used the average industry lot sizes for turkey products (flocks and days production), but future research on this topic may refine lot size on the basis of *Salmonella* survival capability throughout slaughter and processing.

4. Efficacy of Preharvest Interventions

NACMF 2023 response (NACMCF, 2023) outlined the microbiological criteria that could be established to encourage control of *Salmonella* at preharvest, but there remains little concrete data as to the effectiveness of existing preharvest interventions and the nature/breadth of their usage across the industry. Given the lack of data in this arena, future risk assessments would benefit from both data collection, a systematic literature review, and data extraction in the style of (Wang, 2023).

5. <u>*Salmonella* virulence capacity</u>

   The genetic basis of *Salmonella* virulence has not been fully elucidated and is likely to be complex. Virulence genes in *Salmonella* are heavily influenced by gene acquisition facilitated by horizontal gene transfer and gene loss through pseudo-gene formation. The clustering approach undertaken in this risk assessment relied on the presence/absence of Enterobacteriaceae virulence gene markers without directly accounting for their biological function. As research into *Salmonella* virulence factors and their gene functions continues to develop, clustering should be revisited to ensure the reliability/consistency, and potentially, the resolution. Additionally, outbreak data was used to validate the constructed seroclusters to estimate relative risk to public health. Further exploration into the individual strains within broad seroclusters would continue to improve future risk assessment analyses.

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Appendix A EpiX Analytics' Report "Using genomics to identify nontyphoidal *Salmonella* serovars of concern, and estimating dose-response models amenable to risk assessments in poultry"

Using genomics to identify nontyphoidal *Salmonella* serovars of concern, and estimating dose-response models amenable to risk assessments in poultry

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Executive summary

Objectives

This report describes the work performed by EpiX Analytics as part of a cooperative agreement with the United States Department of Agriculture's Food Safety and Inspection Service (FSIS) and the University of Maryland. The overarching goal of the agreement was to provide expertise and analysis to be used by FSIS as part of two risk assessments aimed at evaluating public health impact of different microbial criteria applied post-harvest in chicken and turkey (poultry) meat.

The work performed by EpiX Analytics had the following objectives:

1. Use genomics to classify serovars into groups (clusters) based on *virulence*[7] similarities
2. Use dose-response (DR) models for the serovar clusters identified under objective 1.

Methodology

The work was based on patent pending methodology originally developed by our team for application to *Salmonella* risk assessments in beef [1–3], and is summarized in **Figure 1**.

We collated and processed genetic sequences from *S. enterica* isolates from humans, animals, and animal products in the US, and performed genomic analyses to create a catalog of virulence genes for each isolate. We then employed machine learning methods to estimate the closeness of the isolates based on their virulence genes and used statistical classification methods to allocate isolates to two groups (clusters) by their virulence. We then used epidemiological data from the CDC (FoodNet) to externally validate the differences in epidemiological outcomes of virulence between the groups. We also evaluated the robustness of cluster assignments for all strains.

We estimated a DR model (*i.e.,* function), linking the number of ingested bacteria to the probability of illness, for the higher virulence cluster using literature data. Subsequently, we scaled a DR model for the lower virulence cluster so that the observed overrepresentation of strains from the higher virulence cluster in outbreaks in the US was preserved when using the DR models. We achieved this scaling by estimating risk multipliers that adjusted for the relative risks of illness from exposures to serovars belonging to different clusters, resulting from consumption of poultry.

[7] Note that in *Salmonella* microbiology, the term *virulence* is used to describe loci that affect both infectivity and virulence. For consistency with the literature, we use the term *virulence* here but both infectivity and virulence are incorporated in the clustering methods, and then further quantified in the step used to adjust the DR functions.

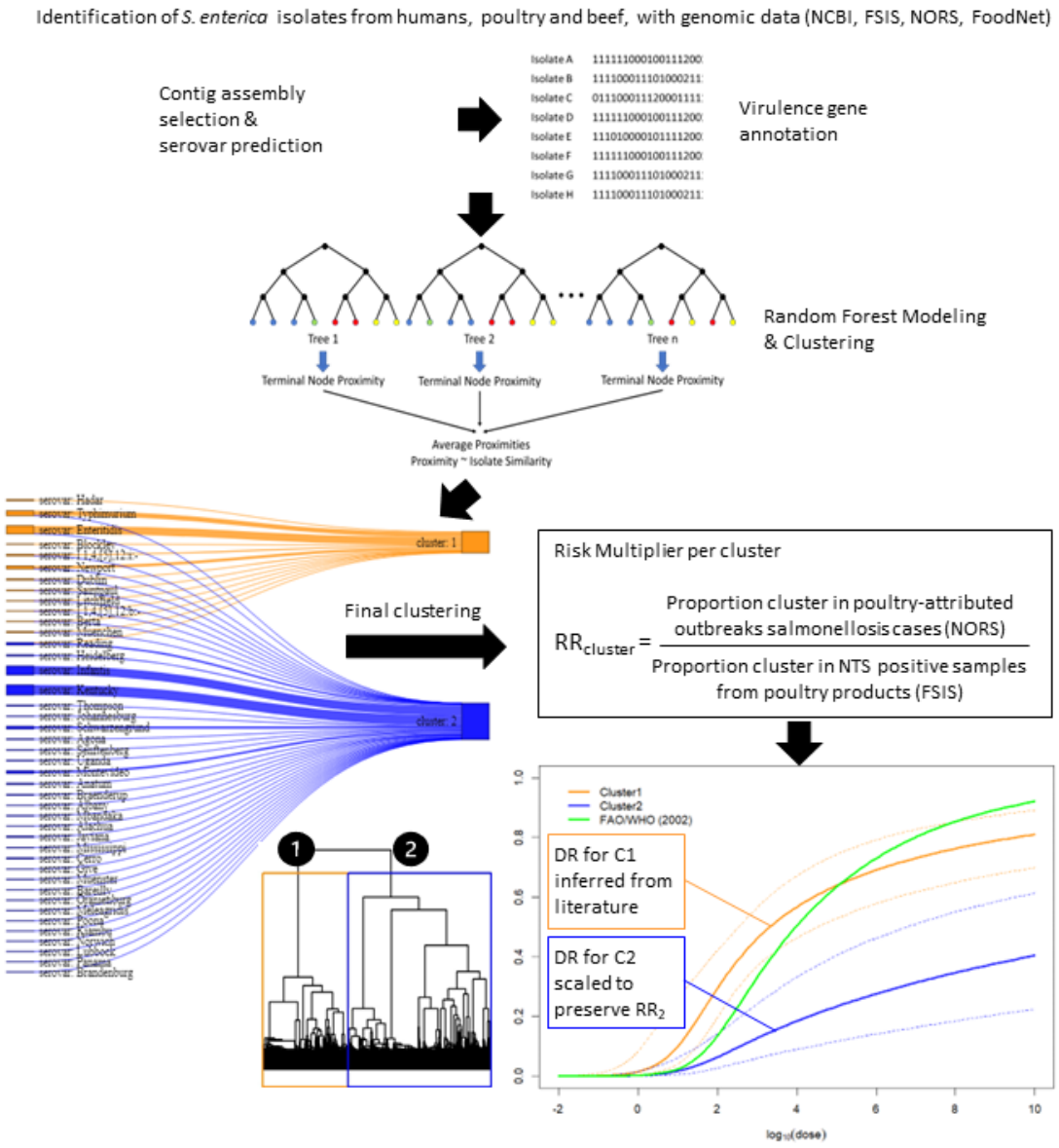

Figure 1: Overview of the analysis performed by EpiX Analytics. First, *S. enterica* isolates were retrieved from NCBI. After serovar prediction and virulence factor gene annotation, isolate assemblies were subjected to unsupervised random forest and hierarchical clustering to determine two virulence groups. Next, poultry and human salmonellosis surveillance data were used to construct risk multipliers used to scale dose-response models describing the two virulence clusters.

Results

We allocated 40,038 *S. enterica* isolates to clusters from the 61,670 isolates initially compiled from human clinical, beef, and poultry isolation sources. The allocation of serovars was stable and robust for two, three, and four clusters. Serovars composing Cluster 1 (the "higher virulence" cluster) remained consistent when allocating isolates to 2-4 clusters and was primarily composed of Enteritidis, Typhimurium, Newport, *S.* I 1,4,[5],12:i:-, and Dublin. Most remaining serovars were assigned to a single "lower virulence" cluster (Cluster 2). When we increased the number of clusters from two to three, the

majority (98%) of Kentucky isolates separated into their own cluster (Cluster 3). Kentucky remained on its own when we increased the number of clusters to four, and most Infantis isolates (88%) formed their own cluster.

Using multiple clustering methods, we allocated 13,106 (99%) of isolates from FSIS poultry *Salmonella* sampling programs to a cluster, allowing us to estimate the risk multipliers with high precision. For example, 33% [95% Confidence Interval: 31-35%] of serovars in poultry belonged to Cluster 1 ("higher virulence" cluster), while we estimated that 71% [58-83%] of human cases attributed to poultry were caused by Cluster 1 serovars. This resulted in a risk multiplier that is 2.1 times higher for Cluster 1 than that without knowing the strain belonged to Cluster 1. The reverse occurred for Cluster 2 ("lower virulence" cluster), where the infection risk was 2.6 times lower than that without knowing the strain belonged to Cluster 2.

The risk multipliers were robust to different modeling choices and type of data used, as established via a sensitivity analysis.

The risk assessment team from FSIS reviewed the clustering results together with the accompanying risk multipliers and decided to proceed with the results for two clusters (*k*=2, Cluster 1 and Cluster 2). Therefore, we estimated DR models for two clusters, which had remarkably different infection risks. For example, for serovars in Cluster 1, an average of 10,000 *Salmonella* cells had roughly 57% chance of resulting in an infection. In contrast, for serovars in Cluster 2, the maximum evaluated dose of 1.00E+10 cells resulted in approximately a 40% risk of infection. Table 1 summarizes the risk multipliers and top five isolates for both clusters.

Table 1: Summary of the five most frequent serovars by cluster and cluster-specific multipliers

"Higher virulence" Cluster 1 (*n*=15,788) Risk multiplier: 2.1 [1.7, 2.5]	"Lower virulence" Cluster 2 (*n*=24,250) Risk multiplier: 0.38 [0.21, 0.58]
Enteritidis, n=5,502	Kentucky, n=6,412
Typhimurium, n=3,403	Infantis, n=5,603
Newport, n=2,724	Montevideo, n=1,531
I 4,5,[5],12:i:-, n=970	Schwarzengrund, n=1,528
Dublin, n=696	Reading, n=1,273

The resulting DR models for the two clusters were provided in the form of functions in the R statistical language amenable for direct integration into FSIS' risk assessment models.

Conclusions

The methodology used in this project provides an objective, science-based framework to estimate heterogeneity in the virulence of serovars and incorporate these differences into quantitative risk assessments. The genomic grouping was validated against epidemiological data, and the model estimates were robust to different analytical and data assumptions.

Introduction

Much of the previous investigations into *Salmonella enterica* subspecies *enterica* virulence mechanisms focus upon Typhimurium as a model organism for *Salmonella* pathogenesis [4–7]. However, given the genomic and phenotypic diversity observed within *Salmonella*, we contend that some virulence models, especially site-specific gene mutations, may not be broadly applicable across the genus. To remediate this, we sought to identify genomic markers which correspond to virulence potential from a curated database of virulence genes identified from Enterobacteriaceae (family containing *Salmonella*, *Escherichia coli*, *Yersinia pestis*, *etc*.). Virulence genes in *Salmonella* are heavily influenced by gene acquisition facilitated by horizontal gene transfer [8,9] and gene loss through pseudo-gene formation [10,11]. Therefore, our goal was to cluster, or group, serovars based upon current virulence gene carriage of isolates commonly implicated in human disease in the U.S.

Accounting for different virulence in serovar groups into a quantitative risk assessment requires having group-specific DR models. The Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) derived a *Salmonella* DR in 2002 using outbreak data [12]. This DR model didn't consider strain variability, *i.e.,* all *Salmonella* serovar were considered equally virulent. Teunis et al. (2010) used a more sophisticated DR framework using outbreak data to fit a DR model but found no differences between serotypes and susceptibility categories [13]. Re-analyzing those outbreak data with a more flexible approach, Teunis (2022) focused on the major serotypes Enteritidis and Typhimurium, showing that Typhimurium was less infectious and has a lower probability of causing acute illness in infected subjects, but the authors didn't provide a DR model that could be used for the majority of other serotypes of public health concern [14]. Thus, our second goal was to determine DR functions for each cluster that could be used in *Salmonella* risk assessments in poultry.

Materials and Methods

Virulence clusters

Our first objective was to use genomics to group *enterica* isolates based on genetic markers of virulence (virulence factors). First, we collected *enterica* isolate genomes originating from humans, beef, and poultry (*i.e.*, chicken and turkey). Next, we assigned a serovar to each isolate and annotated the isolate assemblies with a custom database of virulence factor. To generate clusters, we fitted an unsupervised random forest model to measure isolate similarity based on the presence of virulence factors and then grouped isolates into clusters. These methods follow our previous (patent pending) work grouping *enterica* isolates from human and beef sources by virulence factors [3]. Below, we briefly outline each step within the isolate clustering protocol and provide additional references, which describe our methods in detail.

Contig assembly selection and quality criteria

S. enterica assemblies from bovine-, chicken-, and turkey-associated isolates came from three primary sources: 1) BioProject PRJNA242847 (FSIS HACCP samples), 2) BioProject PRJNA292666 (FSIS NARMS isolates), and 3) BioProject PRJNA292661 (FDA NARMS isolates). We searched the metadata for the above BioProjects for isolation sources specified as bovine-, chicken-, and turkey-associated or beef, chicken, and turkey origin.

We identified *enterica* isolates associated with human clinical cases from BioProject PRJNA230403 (CDC PulseNet). We included sporadic, domestically acquired *enterica* isolates from the FoodNet active surveillance network. However, we did not consider outbreak cases from FoodNet in the initial

unsupervised random forest. Rather, beef-, chicken-, and turkey-attributed outbreak isolates instead came from the National Outbreak Reporting System (NORS) dataset.

We performed quality control measures on the resultant isolate assembly dataset and applied the following exclusion criteria to generate the final assembly dataset: 1) there was no pre-computed assembly on NCBI, 2) SKESA v. 2.2 assembler did not construct the assembly, 3) > 300 contigs represented the assembly, and 4) the contig n50 < 25,000 base pairs. Finally, any serovar that represented less than 50 isolates was removed from the final assembly dataset.

Serovar prediction

The *Salmonella in silico* Typing Resource (SISTR) assigned a putative serovar to each isolate assembly [15]. 1,077 assemblies failed the subsequent quality control step within the SISTR software, but all 330 genes for the core genome multilocus sequence typing (cgMLST) scheme used to assign a serovar within the software were present within these assemblies. We retained assemblies with all 330 cgMLST loci, even if they failed SISTR software quality control, because they contained all loci necessary for the assignment of a putative serovar to an assembly. The final assembly dataset used as input for the unsupervised random forest model included 36,647 *enterica* assemblies and represented 42 serovars.

Virulence gene annotation

To determine the virulence gene catalogue carried by each *Salmonella* isolate assembly, a custom database of putative virulence factors from *Salmonella*, *Escherichia*, *Shigella*, and *Yersinia* was collated from the virulence factor database (VFDB)[16] and putative virulence factors from *Salmonella*, *Escherichia*, and *Shigella* from Bacterial and Viral Bioinformatics Resource Center (BV-BRC)[17]. We combined amino acid sequences of the open reading frames (ORF) with a reference proteome of *Salmonella* Typhimurium LT2 (https://www.uniprot.org/proteomes/UP000001014) and made the database non-redundant by clustering the open reading frames at 0.90 global identity using cd-hit [18]. We then passed the resultant database to Prokka using the "--proteins" option to specify the database as the primary annotation database in the software pipeline [19]. Additionally, to ensure consistent ORF predictions between assemblies, we trained a model using Prodigal [20] on the chromosome of the reference *Salmonella* Typhimurium LT2 assembly ASM694v2 (https://www.ncbi.nlm.nih.gov/assembly/GCF_000006945.2/) and passed to Prokka using the command "--prodigaltf". We then parsed gene annotations from the resultant Prokka annotation tables to determine the presence/absence of virulence factor genes from the VFDB and BV-BRC non-redundant database in each isolate assembly.

Random Forest model construction

After annotation of the isolate assemblies with the custom virulence factor database, we used the resultant Prokka outputs and constructed a count matrix of virulence genes for each assembly. We excluded putative virulence loci present in more than 95% of assemblies or which were found in fewer than 10 assemblies, which resulted in a final database of 193 loci. Next, we generated row similarity (isolate relatedness) by fitting an unsupervised random forest (10,000 trees, using 60 features loci at each split) to the count matrix of virulence loci (36,647 assemblies x 193 virulence factors) using the randomForest package in R [21].

Grouping isolates and assessing cluster stability

We converted the row-wise proximity matrix (isolate relatedness) output from the random forest model to a distance matrix (1 – similarity) and subjected it to agglomerative clustering using Ward's method [22]. We used the "hclust" functionality from the stats package in R to perform clustering and

bootstrapping via the "Ward.D2" method [23]. Although numerous packages are available in R which can carry out this analysis, due to the computational requirements of clustering a distance matrix for 36,647 isolates, hclust was chosen due to ease of constructing parallel functions. The number of clusters was applied to the resulting trees using the "cutree" function.

The unsupervised random forest algorithm is agnostic to the biological meaning of the virulence factor genes and will cluster observations solely based on similarity. To ensure that the clusters we found were the result of repeatable virulence factor patterns, we conducted scenario analyses to investigate the stability of the clustered results based on varying the number of *k* clusters (*i.e*., k = 2, 3, and 4). Each scenario analysis used 1,000 bootstrap iterations of the distance matrix generated from 36,647 resampled isolates which were then each clustered using the same methods. Multiple measures of stability were applied to assess the consistency of the cluster formation per bootstrap: 1) Jaccard similarity of per-isolate grouping into the same categories over a bootstrap, 2) tendency within a serotype to switch to a different cluster based on majority of isolates swapping, and 3) fraction of serotype isolates assigned to the same cluster. Cluster stability was defined as a Jaccard similarity ≥ 0.75[24].

Following the initial assignment based on unsupervised clustering, isolates which were initially excluded due to low numbers (*i.e.,* < 50) of the total serotype were assigned to clusters using a *supervised* random forest method, where isolates' clusters and their virulence factors were used as a training dataset. The supervised method is also agnostic to serotype, based only on the virulence factors of the clustered training isolates and the non-clustered isolates. This ultimately brought the number of isolates allocated to clusters to 40,038.

DR adjustment by cluster

Use of Risk Multipliers

Following oral exposure to a *Salmonella* strain *s,* the probability of becoming ill[8] given that the strain *s* belongs to cluster C_i may be written, according to Bayes theorem Equation 1) :

Equation 1

$$Pr(ill\ |s \in C_i) = Pr(ill) \times \frac{Pr(s \in C_i|ill)}{Pr(s \in C_i)}$$

We can use epidemiological data to estimate the marginal ratio $\frac{\Pr(s \in C_i|\text{ill})}{\Pr(s \in C_i)}$, over a population by computing the ratio of the proportion of individuals that are sick from a strain from Cluster C_i and the proportion of individuals that ingested a strain of Cluster C_i. We will focus specifically on individuals that acquired salmonellosis from consumption of poultry.

Using NORS and FSIS data, we estimated RR_i, the ratio of the proportion of estimated outbreak cases attributed to poultry linked to Cluster C_i with the proportion of estimated strains of Cluster C_i in poultry, as a proxy of $\frac{\Pr(s \in C_i|\text{ill})}{\Pr(s \in C_i)}$ in Equation 1.

Salmonella in poultry

We used data from the FSIS ground chicken (HC_CH_COM01), chicken parts,

[8] Notice that for simplicity here we assume that the probability of illness given infection is unity. Thus, "ill" and "infected" is used interchangeably but can be addressed separately.

(HC_CPT_LBW01/LO_CPT_LBW01), chicken carcasses (HC_CH_CARC01/LO_CH_CARC01), turkey carcasses (HC_TU_CARC01/LO_TU_CARC01), and ground turkey (HC_TU_COM01/LO_TU_COM01) sampling programs from 2016 to 2021. The isolates were assigned to one of the clusters using the following process:

- If the isolate was used in the previously described clustering process, we assigned the isolate to the cluster as it was allocated at that step;
- For isolates not included in the initial clustering process (i.e., serovars with less than 50 isolates), if it was possible to perform a complete virulence gene annotation for the isolate, we used the cluster predicted by a supervised random forest estimated from the previous classification;
- If it was not possible to perform a complete virulence gene annotation (*e.g.*, the isolate was not sent to NCBI, no assembly, *etc.*), we used a classification based on the isolate's serovar. This classification of cluster per serovar was obtained from the previous supervised random forest. In this case, we tested two modes of assignment.
    - Best cluster: we assigned the isolate to the cluster where the majority of the isolates of its serovar was assigned. For example, assuming two clusters, if the supervised random forest predicted that 20% of the strains of serovar *x* fell in Cluster 1 and 80% in Cluster 2, we set all strains of serovar *x* not yet allocated to a cluster in Cluster 2, or;
    - Proportion cluster: we allocated a value for each isolate, equal to the proportion of strains of its serovar predicted by the supervised random forest. In the previous example, strains of serovar *x* not yet allocated to a cluster would be assigned a value of 0.2 for Cluster 1 and 0.8 for Cluster 2.
- If the isolate's serovar was not one for which any other isolate with a sequence also existed, and therefore, was not sorted by any of the three steps above using genetic information, it was assigned to the lower virulence (e.g., Cluster 2 if using two clusters), assuming that the rarity of this serotype suggests it does not have high infectivity or pose a high probability of exposure.

We estimated the proportion of *Salmonella* in each cluster considering within-program weights (FSIS sampling) based on establishment production volumes and between-program weights based on total consumption rates per product. These total consumption rates per product led to a weight of 11% for chicken carcasses, 6% for ground chicken, and the remaining (83%) for chicken parts. For turkey, the weights were 75% for carcasses and 25% for ground for product (weight between programs). We applied a final weight of 5/1 for chicken *vs.* turkey. All weights were provided by FSIS.

To give a lower weight to older data that might be less representative of the current situation, we used a recency weighting as described by Batz et al. (2021)[25]. The weight was 1 for data collected between 01/01/2017 and 12/31/2021 (5 years of collection). The weight for previous data decayed daily using a decay parameter of 5/7 per year (S3).

We used a non-parametric bootstrap to incorporate data uncertainty into our estimates.

Clinical cases attributed to poultry

To determine the proportion of cases attributed to chicken and turkey, we used the 1,616 recorded outbreaks in NORS from 2/4/2009 to 4/9/2021 (local report dates). Of these outbreaks, 792 have an identified food source. Chicken or turkey-attributed outbreaks were categorized as "definitive, "probable" or "possible" depending on the following NORS dataset fields: "CAFC", "FoodName", "CommoditizedFoodOrIngredient", and "IngredientName" (Supplemental figure 1).

Outbreaks classified as “definite” chicken or turkey met at least one of the following criteria:

1. CAFC = Chicken or Turkey;
2. CAFC = NA or Multiple, “FoodName” or “CommoditizedFoodOrIngredient” only contains one food and that one food is chicken or turkey;
3. CAFC = NA or Multiple, “IngredientName” only contains one ingredient, and that one ingredient is chicken/turkey.

Forty-seven of the 1,616 outbreaks included in this analysis are attributed to multiple serotypes based on samples from patients, food, and the environment. Therefore, unique outbreak-serotype combinations, or “sub-outbreaks” were used to group the outbreak-associated illnesses as is shown in the Results section regarding multipliers. The breakdown of these sub-outbreaks and their attribution to poultry is shown in detail in **Figure 3**. The total number of these sub-outbreaks extracted from the NORS databases was 1,690. We assigned a weight to each sub-outbreak equal to the proportion of strains of this serovar isolated within this outbreak.

Attribution to cluster

We assigned the strains associated with the sub-outbreaks to a cluster using a method similar to the one used for the FSIS isolates, that is 1) if isolates were used in the clustering process, using the resulting cluster assignment ; 2) if not, using the supervised random forest if a complete virulence gene annotation was obtained; 3) if not, using a classification based on its serovar (with “Best cluster” or “Proportion cluster” assignments).

Weight per outbreak (considering the number of cases per outbreak)

We tested three methods to weight each outbreak:

- Outbreak counts transformation: Applying a weight equal to 1 (potentially weighted for sub-outbreaks) to each outbreak, we used the number of outbreaks as the outcome. (*i.e.,* we didn’t consider the number of cases per outbreak);
- Estimated primary cases transformation: using the number of cases per outbreak (potentially weighted for sub-outbreaks) as estimated in the NORS database through the “estimated primary cases” field;
- IFSAC transformation: we considered the number of cases using the predicted value of a mixed-effects model, adapted from the method used by the US Interagency Food Safety Analytics Collaboration (IFSAC) for foodborne pathogen attribution based on outbreak data [25] (S3).

We also implemented a recency weighting on the outbreak data, like the one used for FSIS data. Lastly, we considered an adjustment for differential underdiagnosed cases according to severity of illness based on the proportion of bloody diarrhea reported per cluster. The adjustment was similar to the one used by Scallan et al. (2011)[26] (S4).

We used a non-parametric bootstrap to incorporate part of the epistemic uncertainty stemming from data. We did the bootstrap sampling at the level of the sub-outbreak level. The proportion of outbreaks attributed to each cluster was sampled from a Dirichlet distribution using a Bayesian framework, with Jeffrey’s (Dirichlet(α_1 = 0.5, …, α_k = 0.5)) priors. When using the IFSAC transformation, we applied the mixed model to a bootstrap sample of the complete NORS database. The procedure also considered the uncertainty linked to the sub-outbreak allocation. The uncertainty around the underdiagnosing was similar to the one used in Scallan et al. (2011)[26] (S4).

Comparison with FoodNet data

To corroborate the cluster proportion (*i.e.*, proportion of strains from each cluster) using outbreak data linked to poultry, we compared this proportion with the one that is observed for sporadic cases in the U.S. For that purpose, we identified sporadic, domestically acquired enterica isolates from the FoodNet active surveillance network (Specimen Collection Date from 01/01/2000 to 12/31/2019) as described in the previous section. We assigned the cases to the various clusters and applied recency weights using the procedure described previously for FSIS data and NORS data [25]. Recency weights were particularly meaningful for this dataset since it dates back to the year 2000. We also considered the underdiagnosed factor to estimate the proportion of sporadic cases associated to the various clusters in a similar procedure as described for NORS data. Note that sporadic cases in the FoodNet database are not assigned to a given food or food commodities.

Dose-response Models

DR model for Cluster 1 (" higher virulence")

The DR model for Cluster 1 (including Enteritidis and Typhimurium) utilized outbreak data associated to these serovars. We reproduced the Teunis et al.(2010)[13] *Salmonella* DR derivation using Teunis et al.(2008)[27] and Teunis et al.(2010)[13] framework. This framework considers an exact beta-Poisson model of infection for a given dose in a hierarchical model, *i.e*. where α and β parameters follow a variability distribution from outbreak to outbreak.

Contrary to Teunis et al. (2008) framework [27], we used a beta-Poisson model to directly calculate the probability of illness resulting from *Salmonella* exposure and thus, did not consider Teunis et al. (2008)'s model of illness given infection.[9]

We used the data provided in table 1 from Teunis et al.(2010)[13], limited to data from strains belonging to Cluster 1 strains (Enteritidis and Typhimurium), as updated in Teunis (2022)[14] . Using the R nimble package [28], and following Teunis et al.(2008)'s framework [27], we used a Bayesian hierarchical model where the transformed parameters ω and ζ follow a normal distribution from outbreak to outbreak, where $\omega_o = \text{logit}(u_o)$ and $\zeta_o = \log(v_o)$, with $u_o = \alpha_o/(\alpha_o+ \beta_o)$ and $v_o = \alpha_o + \beta_o$, α_o and β_o being the parameters of an exact beta-Poisson DR for outbreak o. As in Teunis et al. (2010)[13], we considered heterogeneity in the distribution of the bacteria per meal (negative binomial distribution with parameters dose, the mean dose, and a dispersion parameter r, see Teunis et al. 2008 [27]), and hence used a ${}_2F_1$ hypergeometric confluent function of the second kind.[10] The resulting marginal probability of infection is $1-{}_2F_1(\alpha_o, r_o, \alpha_o+ \beta_o, -d_o/r_o)$. See S5 for the derivation.

We obtained the posterior distributions for the hyperparameters of the beta-Poisson models that we can use to derive:

- the variability of αs and βs
- the uncertainty of the DR models

From Cluster 1 to other clusters

If we have a distribution of the exposure in the population, we can derive a DR for the less virulent

[9] Our tests suggested an overparametrized model when the infection and the illness model were considered, since no data were available for the number of infected individuals for the S. Enteritidis and S. Typhimurium outbreaks.

[10] The ${}_2F_1$ hypergeometric confluent function was rewritten using the `nimble` framework from the GNU Scientific Library (gsl) C++ library (https://www.gnu.org/software/gsl/).

strains (*e.g.*, Cluster 2) under the condition:

Equation 2

$$\int DR_{cl2}(d|\alpha',\beta')f(d) = \frac{RR_2}{RR_1}\int DR_{cl1}(d|\alpha,\beta)g(d),$$

Where $DR_{cl2}(d|\alpha',\beta')$ is the beta-Poisson DR for strains of Cluster 2, $DR_{cl1}(d|\alpha,\beta)$ is the DR for strains of Cluster 1, $f(d)$ is the density of the ingested doses of *Salmonella* of cluster 2 from poultry in the US, $g(d)$ is the density of the ingested doses of *Salmonella* of Cluster 1 from poultry in the US, RR_2 is the risk multiplier for strains of Cluster 2 and RR_1 is the risk multiplier for strains of Cluster 1. We'll assume that $f(d) = g(d)$ for all *d*, that is that the density of the ingested dose is the same whatever the cluster (see Assumptions and Discussion).

FSIS provided our team with a lognormal (LN) distribution (base 10) [11] *LN*(-3.037117, 1.279985) representing the distribution of *Salmonella* in raw poultry, and an attenuation distribution *LN*(-5.00, 1.91)[29]. This distribution adjusts the initial dose distribution by the combined effect of cooking, mixing, partitioning, cross-contamination, growth and consumption while considering variability in cooking practices. Under the reasonable assumption of independence between the original distribution and the attenuation, we can assume that $f(d)$ is a lognormal (base 10) distribution $LN\left(-3.037117 - 5.00, \sqrt{1.279985^2 + 1.91^2}\right)$.

As we have one equation (Equation 2) and two parameters (α',β'), an infinite number of solutions are possible. Following Teunis et al. (2010)[13], and Thébault et al.[12] (2013)[30], we assume that the strain variability impacts the mean $u = \alpha/(\alpha+\beta)$ of the underlying beta distribution in the beta-Poisson DR model, but that the parameter $v = \alpha + \beta$ is shared by all strains. Equation 2 can then be solved numerically.

We consider that for each set of (variable) beta-Poisson parameters for cluster 1 correspond a set of beta-Poisson parameters for Cluster 2 that fulfills the relationship stated in Equation 2, preserving $\alpha + \beta = \alpha' + \beta'$. So, for each pair of (variable) α and β parameters, we have corresponding α' and β' parameters for Cluster 2.

We start from the output of the Bayesian process (empirical posterior distributions for ωm, ζm, ωsd, ζsd and correlation between ω_o *and* ζ_o), and derived 5,001 sets (uncertainty) of 5,000 sets (variability) of αs and βs parameters for Cluster 1. Within each iteration of uncertainty, we find the corresponding set of α' and β' that would fulfill Equation 2 for each of the 5,000 sets, using one iteration of the bootstrap sample for the RR. We repeat the process over the 5,001 iterations of uncertainty. We obtain 5,001 sets (uncertainty) of 5,000 sets (variability) of α's and β's for Cluster 2.

As, ultimately, we are interested in the mean risk (over strains, within a cluster) for a given dose and its confidence interval, we integrated this mean DR numerically using a Monte Carlo simulation. Given the computational complexity of the full DR model, we fitted a polynomial model on the obtained DR so that the model is almost instant to integrate and fully portable.

[11] $x \sim LN(\mu, \sigma)$ if $\log_{10}(x) \sim N(\mu, \sigma)$

[12] For Norovirus. Note however that P. Teunis was a co-author, so we can't consider those assumptions as having been taken independently.

Results

Serovar assignment

In total, there are over 400,000 *Salmonella* isolates housed in the Pathogen Detection Network hosted by NCBI. We extracted 61,670 isolates from the four previously described BioProjects. Based on the exclusion criteria described in the methods section, we further reduced the number of extracted isolates to a final analysis set of 36,647 *enterica* assemblies representing human clinical cases in the US and poultry and beef associated isolates. Within this dataset, which was used as the unsupervised random forest input, 18.4% (6,751) assemblies came from US human clinical infections, 15.2% (5,586) represented isolates from bovine sources, and the remaining 66.3% (24,310) isolates originated from poultry. We assigned a cluster to an additional 3,391 isolates that were initially excluded via supervised random forest, bringing the total number of isolates allocated to a cluster to 40,038.

Serovar assignment for *k*=2, 3, and 4 clusters are provided in Table 2. The serovars composing Cluster 1 remained consistent at the three levels of *k* (**Figure 2**). When *k* was increased from 2 to 3, the majority (98%) of Kentucky isolates separated into their own cluster (Cluster 3, when k=3), while Infantis belonged to Cluster 2. Kentucky remained on its own when *k* was increased to 4 (Cluster 4, when k=4) and most Infantis isolates (88%) formed their own cluster (Cluster 3, when k=4). The remaining serovars comprising Cluster 2 in the *k*=2 designation continued to cluster together as *k* increased to 3 and 4. Isolates (*i.e*., non-serotyped) which were not assigned a serovar due to missing "O" or "H" antigens (*n*=26) may comprise a group of diverse serovars, which split between Cluster 1 and 2 for all levels of *k* based on supervised random forest.

Table 2 includes the serotype names as reassigned using the SISTR methodology, and therefore does not capture all unique partial serotypes that might be found in the FSIS and NORS datasets. If new data is added to this analysis, the serotypes should be characterized using genetic information to assign a cluster to the new isolates, or the cluster should be assigned to the isolate via supervised random forest.

Table 2: Serovar cluster assignments for k= 2, 3 and 4.

		2 Clusters (*k*=2)		3 Clusters (*k*=3)			4 Clusters (*k*=4)			
Serovar	*n*	Cluster 1	Cluster 2	Cluster 1	Cluster 2	Cluster 3	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Adelaide	40		100%		100%			100%		
Agbeni	8		100%		100%			100%		
Agona	406		100%		100%			100%		
Alachua	67		100%		100%			100%		
Albany	88		100%		100%			100%		
Anatum	673		100%		100%			100%		
Baildon	6	100%		100%			100%			
Bareilly	143	5%	95%	5%	95%		5%	95%		
Berta	193	98%	2%	98%	2%		98%	2%		
Blockley	163	100%		100%			100%			
Bovismorbificans	56	100%		100%			100%			
Braenderup	525	1%	99%	1%	99%		1%	99%		
Brandenburg	111		100%		100%			100%		
Calabar	1		100%		100%			100%		
Carrau	93		100%		100%			100%		
Cerro	591		100%		100%			100%		
Chailey	7	100%		100%			100%			
Chester	2		100%		100%			100%		
Concord	6		100%		100%			100%		
Cubana	2		100%		100%			100%		
Dublin	697	100%		100%			100%			
Duisburg	1		100%		100%			100%		
Eastbourne	10		100%		100%			100%		
Enteritidis	5510	100%		100%			100%			
Gaminara	1		100%		100%			100%		
Gateshead	1		100%		100%			100%		
Give	164		100%		100%			100%		
Goldcoast	1		100%		100%			100%		
Hadar	558	100%		100%			100%			
Hartford	3		100%		100%			100%		
Heidelberg	728		100%		100%			100%		
Hillingdon	1	100%		100%			100%			
Hvittingfoss	1		100%		100%			100%		
I 1,4,[5],12:b:-	108	95%	5%	95%	5%		95%	5%		
I 1,4,[5],12:i:-	987	98%	2%	98%	2%		98%	2%		
Idikan	1		100%		100%			100%		
Infantis	5604		100%		100%			12%	88%	
Javiana	971		100%		100%			100%		
Johannesburg	158		100%		100%			100%		

Serovar	n	Cluster 1	Cluster 2	Cluster 1	Cluster 2	Cluster 3	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Kentucky	6413		100%		2%	98%		2%		98%
Kiambu	65		100%		100%			100%		
Koessen	3		100%		100%			100%		
Leiden	4		100%		100%			100%		
Litchfield	120	98%	2%	98%	2%		98%	2%		
Livingstone	1		100%		100%			100%		
Lomalinda	4		100%		100%			100%		
Lubbock	103		100%		100%			100%		
Manhattan	4	100%		100%			100%			
Mbandaka	370		100%		100%			100%		
Meleagridis	138		100%		100%			100%		
Miami	47		100%		100%			100%		
Mississippi	263		100%		100%			100%		
Montevideo	1533		100%		100%			100%		
Muenchen	607	94%	6%	94%	6%		94%	6%		
Muenster	258		100%		100%			100%		
Newport	2740	99%	1%	99%	1%		99%	1%		
Norwich	78		100%		100%			100%		
Ohio	11		100%		100%			100%		
Okatie	1		100%		100%			100%		
Oranienburg	203	1%	99%	1%	99%		1%	99%		
Panama	66		100%		100%			100%		
Poona	57		100%		100%			100%		
Potsdam	1		100%		100%			100%		
Reading	1299	2%	98%	2%	98%		2%	98%		
Rissen	6		100%		100%			100%		
Rubislaw	24		100%		100%			100%		
Saintpaul	612	100%		100%			100%			
Sandiego	2		100%		100%			100%		
Schwarzengrund	1528		100%		100%			100%		
Senftenberg	327		100%		100%			100%		
Stanley	47	100%		100%			100%			
Telelkebir	12		100%		100%			100%		
Thompson	549		100%		100%			100%		
Typhimurium	3421	99%	1%	99%	1%		99%	1%		
Uganda	356		100%		100%			100%		
Urbana	18		100%		100%			100%		
Vinohrady	14		100%		100%			100%		
Virchow	7		100%		100%			100%		
Weltevreden	14		100%		100%			100%		
Non-serotyped	26	31%	69%	31%	69%		31%	69%		

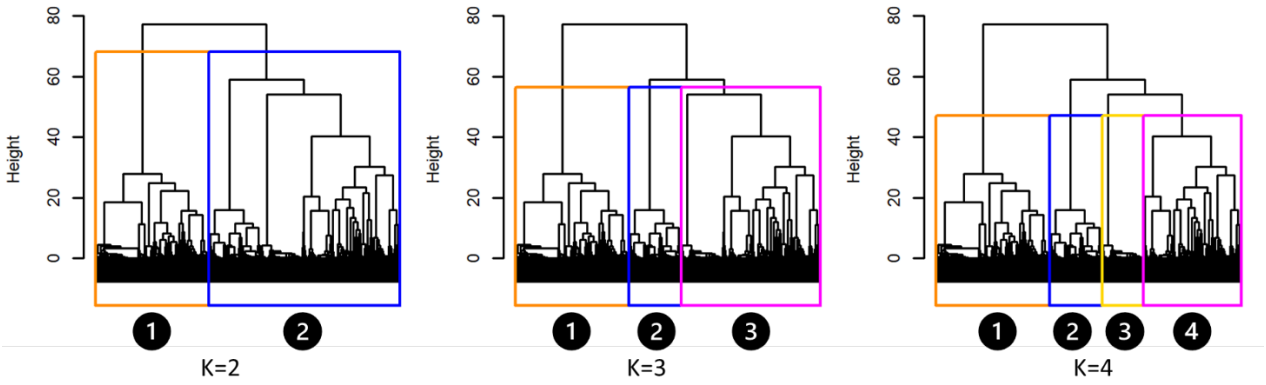

Figure 2: Dendrograms showing isolate groupings for k=2,3, and 4. Numbers correspond to cluster number listed in **Table 2** headings above. Note that Cluster 1 remains consistent as k increases.

Robustness of serovar assignments

Jaccard Similarity

The mean bootstrap Jaccard similarity for all clusters within the *k*=2, 3, and 4 designations was above the 0.75 threshold, indicating cluster stability.

Serotype Switching

Berta (*n*=193) and Saintpaul (*n*=612) isolates switched clusters within the bootstrap samples most often. For *k*=2,3 and 4, the percent of Berta and Saintpaul isolates that switched from Cluster 1 to Cluster 2 in more than 5% of the bootstraps was 100% and 79.4%, respectively. Among the remaining serovars, the level of isolate switching in the bootstrap samples was low, indicating stability of serovar cluster assignments and relatively low variation in virulence factors within serotypes.

Division of serovars between multiple clusters

For *k*=4, a large majority (≥95%) of isolates fall into a single cluster with the remarkable exception of Infantis for which 12% of the isolates (672/5,604) are classified as Cluster 2 while 88% (4,932/5,604) are classified as Cluster 3. However, for *k*=2 and *k*=3, Infantis does not split between clusters and all isolates reside within Cluster 2. The genes most responsible for the split of Infantis isolates into two clusters when *k*=4 are located on the pESI megaplasmid [31]. Most notably, these genes are necessary to produce yersiniabactin, which is a siderophore dependent iron uptake system [32].

Multiplier Estimation

Results

A total of 13,537 isolates were extracted from the previously described FSIS sampling program databases (chicken carcasses, parts and comminuted and turkey carcasses and comminuted) using our selection criteria, resulting in 13,241 recency-weighted strains. We were able to assign a cluster *via* random forest or supervised random forest to 9,578 of these weighted isolates. Using serovar assignments on the remaining strains, we allocated 13,106 (99%) weighted isolates to a cluster.

The weighted (recency, establishment and between products) proportion of strains in poultry is

provided for *k*=2 (Table 3) and *k* = 3 (Table 4). The proportions of strains in outbreaks were derived using our baseline scenario based on the previously described IFSAC transformation, outbreak recency weighting (5 years), and outbreaks definitively attributed to chicken and turkey. Serovars without assemblies were assigned to a cluster based on the previously described "proportion cluster" method.

Table 3: Multipliers for k = 2 (Estimate [bootstrap 95%CI])

	Cluster 1	Cluster 2	Not Assigned
Proportion in Poultry	0.33 [0.31, 0.35]	0.66 [0.64; 0.68]	0.010 [0.006; 0.017]
Proportion in outbreaks	0.71 [0.58; 0.83]	0.25 [0.14; 0.38]	0.039 [0.012; 0.081]
Multiplier	2.1 [1.7; 2.5]	0.38 [0.21; 0.58]	3.9 [1.1; 9.1]

Table 4: Multipliers for k = 3 (Estimate [bootstrap 95%CI])

	Cluster 1	Cluster 2	Cluster 3	Not Assigned
Proportion in Poultry	0.33 [0.31, 0.35]	0.42 [0.40; 0.44]	0.24 [0.23; 0.25]	0.010 [0.006; 0.017]
Proportion in outbreaks	0.71 [0.58; 0.82]	0.25 [0.14; 0.38]	0.002 [0.000; 0.021]	0.038 [0.012; 0.080]
Multiplier	2.1 [1.7; 2.5]	0.60 [0.34; 0.90]	0.01 [0.000; 0.088]	3.9 [1.1; 9.2]

Forty-seven of the 1,616 outbreaks included in this analysis are attributed to multiple serotypes based on samples from patients, food, and the environment, and some serotypes differ in cluster assignment. Of the 47 outbreaks linked to more than one serotype, 22 had at least two serotypes which sorted into different clusters (when *k*=2). Three of these 22 multi-cluster outbreaks were linked to chicken and none to turkey. Therefore, unique outbreak-serotype combinations, or "sub-outbreaks" were used to group the outbreak-associated illnesses (**Figure 3**). The total number of these sub-outbreaks extracted from the NORS databases was 1,690 - 216 of which were attributed to poultry (191 using our "definitively" definition). To each sub-outbreak, we assigned a cluster: first via random forest (*n*=51), then by supervised random forest (*n*=9), and then by assignment according to serotype (*n*=134), so that a total of 194 sub-outbreaks attributed to poultry were included (**Figure 3**). Applying the recency weight system reduced the influence of sub-outbreaks which occurred before 2017, so that the apparent weighted number of sub-outbreaks used were 118 (108 of these were definitively linked to poultry).

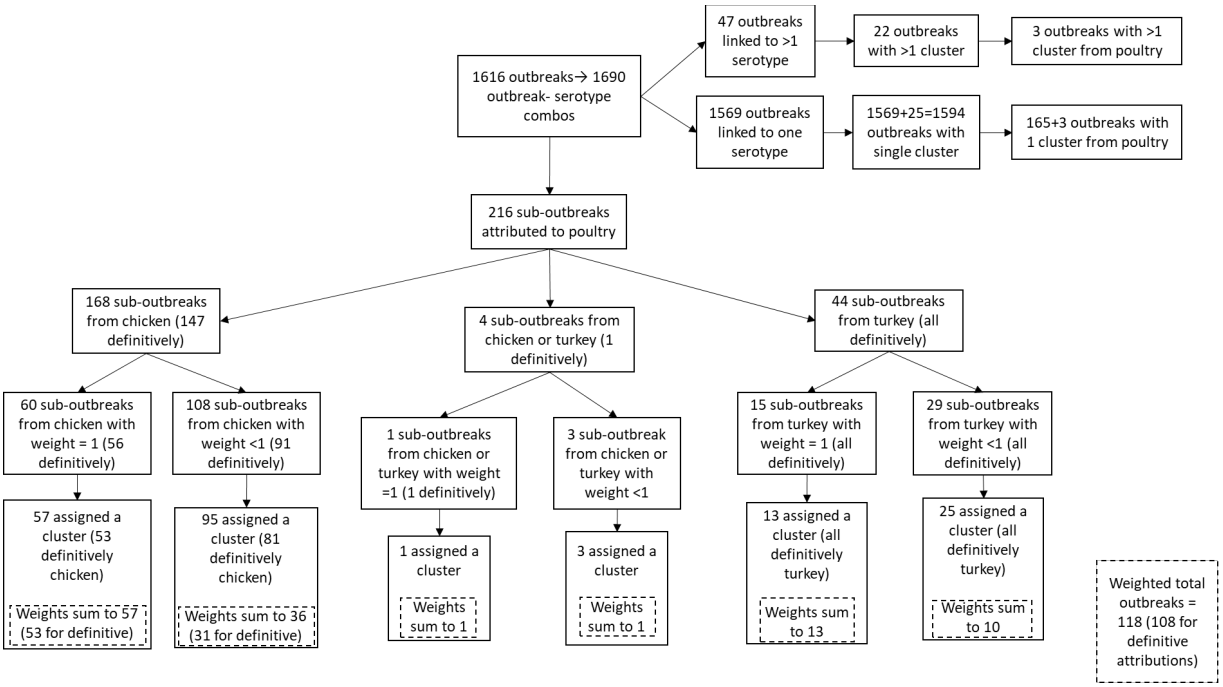

Figure 3: Distribution of outbreaks, sub-outbreaks, and weighted total outbreaks used from NORS data.

We collated 132,326 sporadic, domestically acquired cases the FoodNet database. Applying the recency weight led to 43,882 weighted cases as a majority of cases were recorded before 2017. We assigned a cluster *via* unsupervised random forest or supervised random forest for 6,133 of these weighted cases. Ultimately, using serovar, we assigned a cluster to 37,679 weighted cases (86%). For k=3, the proportion of cases attributed to each cluster was 63% [CI95%: 62; 63%] 37% [37; 38%] and 0.13% [0.09; 0.17%] for Cluster 1, 2, and 3, respectively, which is comparable to what was observed from the NORS outbreak data. For this estimation, we were unable to assign a large number of isolates (15%) to a given cluster.

Assessing sensitivity of risk multipliers to modeling options

We tested how the risk multipliers changed under the various options described for this analysis. The results were robust to modeling options, with the most impactful options being not using recency weighting in the data, or only using turkey data (Table 5).

Table 5: Sensitivity of risk multipliers to different modeling and data transformation options

	Cluster 1	**Cluster 2**
Baseline*	2.1 [1.7; 2.5]	0.38 [0.21; 0.58]
Outbreak counts transformation	2.0 [1.6; 2.4]	0.39 [0.24; 0.55]
Estimated Primary cases transformation	2.0 [1.3; 2.5]	0.51 [0.22; 0.84]
No recency weighting	1.8 [1.4; 2.1]	0.53 [0.36; 0.70]
Recency weight starting to decrease after 1 year	2.4 [1.8; 2.9]	0.32 [0.14; 0.58]
Turkey only	1.7 [0.77; 3.0]	0.65 [0.22; 1.10]
Chicken only	2.2 [1.8; 2.6]	0.32 [0.16; 0.52]
Do not weight different products	2.4 [1.9; 2.9]	0.36 [0.21; 0.56]
Outbreaks Definitively or Probably attributed to poultry	2.1 [1.8; 2.4]	0.39 [0.24; 0.55]
Use best Cluster	2.1 [1.7; 2.5]	0.38 [0.21; 0.59]

* Baseline: IFSAC transformation, recency weighting (5 years), use chicken data, use turkey data, use FSIS weights for different products, use outbreaks definitively attributed to chicken (resp. turkey), use proportion of cluster. Results from unattributed isolates not presented. Bootstrap used 1001 iterations.

Interpretation of risk multipliers

Recall Equation 1: $\Pr(\text{ill} \mid s \in C_i) = \Pr(\text{ill}) \times \frac{\Pr(s \in C_i \mid \text{ill})}{\Pr(s \in C_i)}$, that allows us to calculate the probabilities of illness following exposure to *Salmonella* from a given cluster.

The multipliers allow us to state that if we have an exposure from a strain belonging to Cluster 1 (e.g., Enteriditis), the risk of illness is 2.1 times [CI95%: 1.7; 2.5] higher than the probability of illness prior to knowing that the strain belonged to Cluster 1. Similarly, knowing that the strain is from Cluster 2 informs that the risk of illness is 1/0.38 = 2.63 times [CI95%: 1/0.58 = 1.74; 1/0.21 = 4.76] lower than the probability of illness without knowing the strain belonged to Cluster 2.

A note on FSIS' decision on the number of clusters to use for further analysis

Our team participated in weekly calls and discussed with FSIS the different results of the analysis. Particular attention was paid to the robustness of the allocation of serovars based on different number of clusters used, and how this translated in different risk multipliers. As described earlier, most of the serovars were stable and the allocations changed mostly for Infantis and Kentucky when increasing the number of clusters.

Using the information that we provided for 2-4 clusters combined with the risk multipliers, FSIS decided to proceed with the DR model adjustments for two clusters. Below we provide a summary of the serovars and multipliers for Cluster 1 ("higher virulence") and Cluster 2 ("lower virulence") (Table 6).

Table 6: Summary of the five most frequent serovars by cluster and cluster-specific multipliers

"Higher virulence" Cluster 1 (*n*=15,788) Risk multiplier: 2.1 [1.7, 2.5]	**"Lower virulence" Cluster 2** (*n*=24,250) Risk multiplier: 0.38 [0.21, 0.58]
Enteritidis, n=5,502	Kentucky, n=6,412
Typhimurium, n=3,403	Infantis, n=5,603
Newport, n=2,724	Montevideo, n=1,531
I 4,5,[5],12:i:-, n=970	Schwarzengrund, n=1,528
Dublin, n=696	Reading, n=1,273

Dose-response models

Figure 4 illustrates the fit of the DR model to outbreak data from Cluster 1, using data from Teunis et al. (2022)[14], which resulted in large uncertainty and very large variability in the DR models.

Here, "dose" is the parameter of a Poisson distribution considering serving-to-serving variability. Hence the dose is not an integer value as it represents an average or intensity parameter.

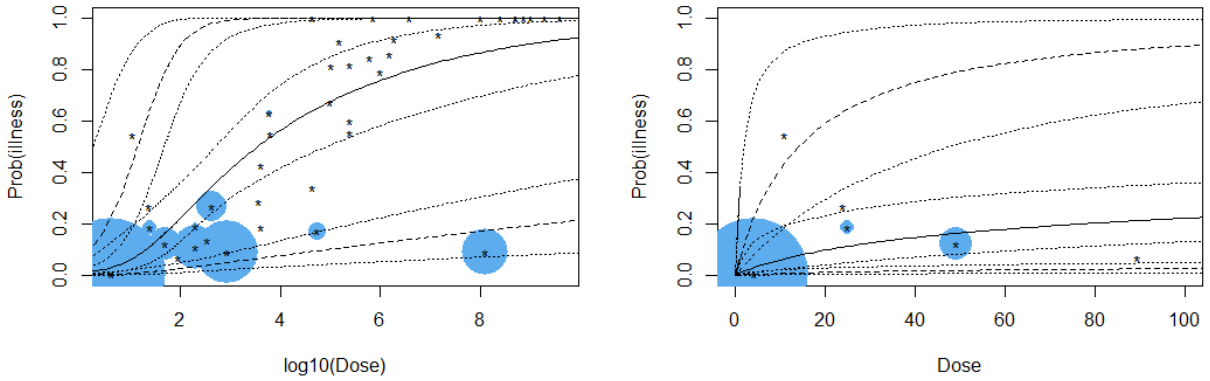

Figure 4: DR model fitted to Teunis (2022) data. Asterisks represent the proportion of individuals exposed to *S*. Enteritidis or *S*. Typhimurium that became ill from individual outbreaks. with blue radius proportional to the number of individuals in the outbreak. Curves (from bottom to top) represents: 1) the 2.5th uncertainty of the 2.5th variability, 2) the median of the 2.5th variability, 3) the 97.5th uncertainty of the 2.5th variability, 4) the 2.5th uncertainty of the median variability, 5 plain black) the median (uncertainty) of the median (variability), 6) the 97.5th uncertainty of the median variability, 7) the 2.5th uncertainty of the 97.5th variability, 8) the median uncertainty of the 97.5th variability, 9) the 97.5th uncertainty of the 97.5th variability. left: x is the $\log_{10}$(dose), right: x is the dose, up to 100 bacteria.

Figure 5 illustrates the DR model for *Salmonella* from Cluster 1 and Cluster 2. The value is the marginal

probability of infection after integration over strains within a cluster[13], over doses (Poisson distribution with intensity "dose") and over individuals[14]. We also include the FAO/WHO (2002) DR model for comparison.

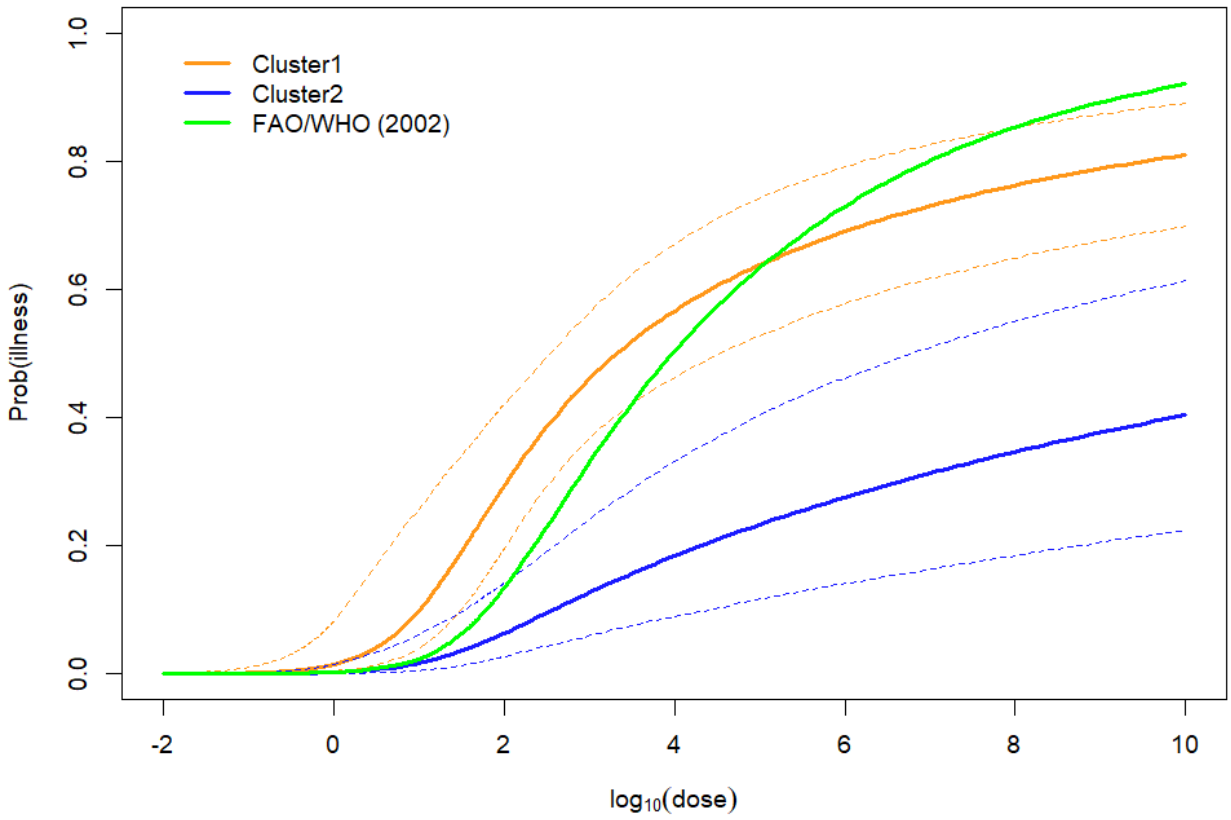

Figure 5: Average (over strains) DR model for Cluster 1 (orange), Cluster 2 (blue), compared to FAO/WHO (2002) dose response (green). Plain lines: best estimates (median of the values in the uncertainty dimension). Dotted line: 95% confidence intervals (2.5th and 97.5th quantiles in the uncertainty dimension).

Figure 6 provides a "zoomed-in" view of the DR model for lower doses and using a $\log_{10}$ y-axis. Note the (log-log) linearity of the DR model at these low doses, and the similarity between the Cluster 1 and FAO/WHO DR curves.

[13] As the model is integrated over strains, these values should not be used to estimate the expected number of cases for a given outbreak, where a single strain is involved.

[14] The integration over the dose (assuming a Poisson distribution) and over the individuals (assuming a beta distribution) are considered in the use of the underlying beta-Poisson DR function. The integration over strains within a cluster was done by averaging 5000 beta-Poisson DR models considering α and β strain-to-strain variability.

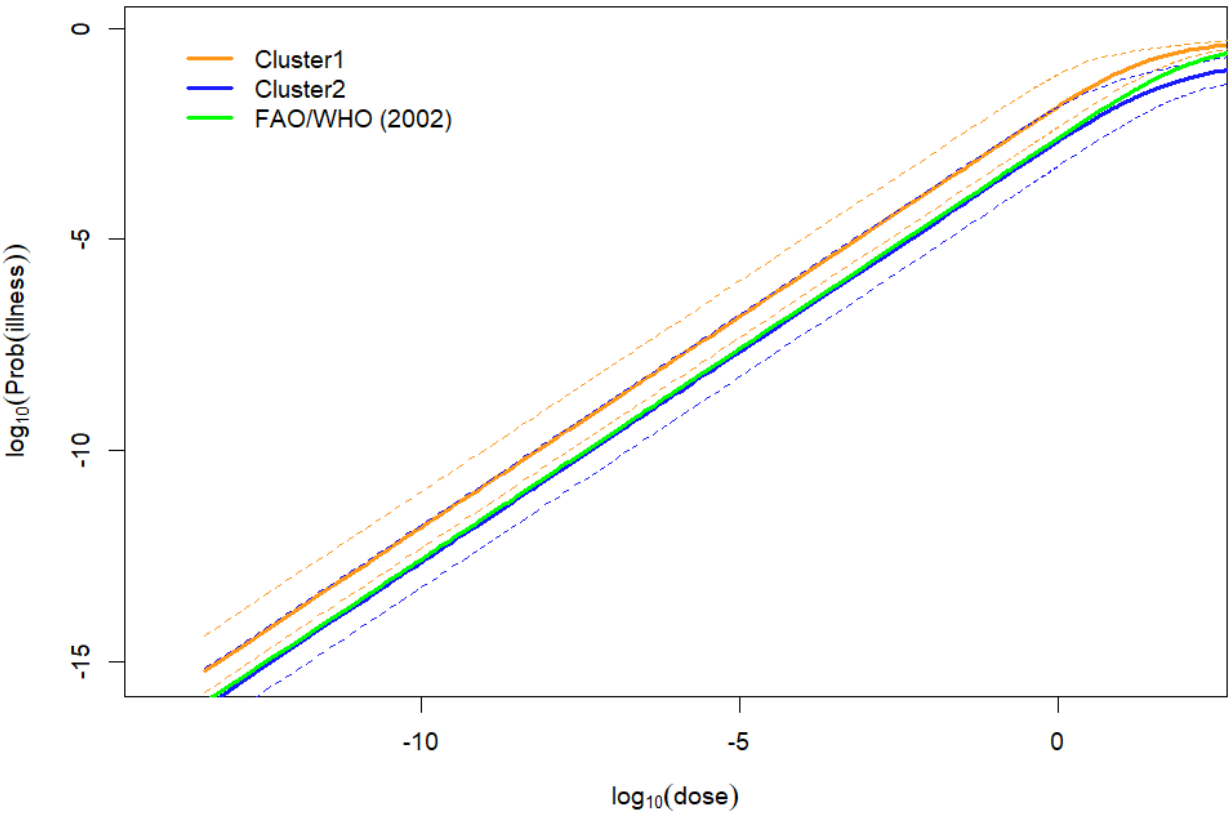

Figure 6: Average (over strains) DR model for Cluster 1 (orange), Cluster 2 (blue), compared to FAO/WHO (2002) DR (green). Note: the DR model for Cluster 2 is mostly hidden by the FAO/WHO DR. Plain lines: best estimates (median of the values in the uncertainty dimension). Dotted line: 95% confidence intervals (2.5th and 97.5th quantiles in the uncertainty dimension).

Table 7 provides the coefficients for a polynomial approximation that can be used to derive the DR model for Cluster 1 and Cluster 2 without having to repeat the inference. We checked the fit of the polynomial function for a dose ranging from 10^{-14} to 10^{10} cfu. Using these figures, the probability of illness for a given dose of *Salmonella* from a given cluster can be obtained using[15]:

Equation 3 $Prob(illness) = coef1 \times ln(Dose+1) + coef2 \times (ln(Dose+1))^2 + \ldots + coef9 \times (ln(Dose+1))^9.$

Where "Dose" is the intensity parameter of the Poisson distribution describing the number of bacteria from serving to serving in the subpopulation of interest. To achieve reliable precision in this calculation, we recommend using the R functions provided as an output of this project.

[15] ln is $\log_e$ (logarithm, base e)

Table 7: Polynomial regression of the probability of infection as a function of the dose, for strains from Cluster 1 and 2 (2 Cluster). Validated from dose = 0 bacteria to dose = 1E10 bacteria.

	Cluster 1			Cluster 2/2		
	Estimate	Lower CI95%	Upper CI95%	Estimate	Lower CI95%	Upper CI95%
ln(Dose*+1)	1.677793E-02	8.028926E-03	1.044496E-01	2.547083E-03	8.850745E-04	1.635746E-02
$(\ln(Dose+1))^2$	-8.965997E-04	-8.483557E-03	2.859753E-02	-1.917973E-04	-7.198445E-04	6.578363E-03
$(\ln(Dose+1))^3$	9.486076E-03	8.090451E-03	-2.025913E-02	1.247473E-03	6.752766E-04	-2.176502E-03
$(\ln(Dose+1))^4$	-2.710090E-03	-1.421906E-03	5.193496E-03	-1.920443E-04	-5.763324E-05	5.905104E-04
$(\ln(Dose+1))^5$	3.473447E-04	9.426093E-05	-7.006729E-04	8.414753E-06	-5.246505E-06	-8.899356E-05
$(\ln(Dose+1))^6$	-2.460849E-05	-9.720838E-07	5.396930E-05	3.616453E-07	1.131561E-06	7.362400E-06
$(\ln(Dose+1))^7$	9.981556E-07	-1.788357E-07	-2.386504E-06	-4.844387E-08	-7.339216E-08	-3.394596E-07
$(\ln(Dose+1))^8$	-2.176171E-08	8.410247E-09	5.642269E-08	1.711068E-09	2.146805E-09	8.220825E-09
$(\ln(Dose+1))^9$	1.981074E-10	-1.155037E-10	-5.529915E-10	-2.095305E-11	-2.413967E-11	-8.166917E-11

* dose is the parameter of the Poisson parameter describing the distribution of dose from serving to serving in number of bacteria, ln is logarithm in base *e*. The formula has no intercept.

In addition to the figures illustrating the DR curves, we provide a summary of the probability of illness by cfu (dose) for the two clusters (Table 8). Only a finite set of dose values are provided in the table, but Equation 3 can be used to derive this probability for any dose.

Table 8: Probability of illness as a function of the dose for the two cluster.

Dose	Cluster 1			Cluster 2		
	Estimate	Lower CI95%	Upper CI95%	Estimate	Lower CI95%	Upper CI95%
1.00E-14	1.68E-16	8.02E-17	1.04E-15	2.55E-17	8.84E-18	1.63E-16
1.00E-13	1.68E-15	8.02E-16	1.04E-14	2.55E-16	8.84E-17	1.63E-15
1.00E-12	1.68E-14	8.03E-15	1.04E-13	2.55E-15	8.85E-16	1.64E-14
1.00E-11	1.68E-13	8.03E-14	1.04E-12	2.55E-14	8.85E-15	1.64E-13
1.00E-10	1.68E-12	8.03E-13	1.04E-11	2.55E-13	8.85E-14	1.64E-12
1.00E-09	1.68E-11	8.03E-12	1.04E-10	2.55E-12	8.85E-13	1.64E-11
1.00E-08	1.68E-10	8.03E-11	1.04E-09	2.55E-11	8.85E-12	1.64E-10
1.00E-07	1.68E-09	8.03E-10	1.04E-08	2.55E-10	8.85E-11	1.64E-09
1.00E-06	1.68E-08	8.03E-09	1.04E-07	2.55E-09	8.85E-10	1.64E-08
1.00E-05	1.68E-07	8.03E-08	1.04E-06	2.55E-08	8.85E-09	1.64E-07
1.00E-04	1.68E-06	8.03E-07	1.04E-05	2.55E-07	8.85E-08	1.64E-06
1.00E-03	1.68E-05	8.02E-06	1.04E-04	2.55E-06	8.84E-07	1.64E-05
1.00E-02	1.67E-04	7.91E-05	1.04E-03	2.53E-05	8.74E-06	1.63E-04
1.00E-01	1.60E-03	6.95E-04	1.02E-02	2.42E-04	7.84E-05	1.62E-03
1.00	1.38E-02	3.87E-03	8.05E-02	2.05E-03	4.78E-04	1.39E-02
10.00	9.96E-02	4.22E-02	0.26	1.66E-02	5.16E-03	6.08E-02
100.00	0.29	0.19	0.41	6.25E-02	2.61E-02	0.14
1000.00	0.46	0.36	0.56	0.13	5.87E-02	0.24
10000.00	0.57	0.47	0.68	0.18	9.00E-02	0.33
100000.00	0.64	0.53	0.74	0.23	0.12	0.40
1.00E+06	0.69	0.57	0.78	0.27	0.14	0.46
1.00E+07	0.73	0.62	0.83	0.31	0.16	0.51
1.00E+08	0.76	0.65	0.85	0.35	0.18	0.55
1.00E+09	0.79	0.67	0.87	0.38	0.20	0.58
1.00E+10	0.81	0.69	0.88	0.40	0.22	0.61

Assumptions, their implications, and justification

We provide a list of the main assumptions made to derive our estimates (Table 9). Next to each assumption we list their implications for the work and possible use in a quantitative risk assessment, and our justification(s) for making the assumption with references, where applicable.

Table 9: Table of assumptions made during phases of project with implications and justifications for making assumptions

Index	Assumption	Meaning / implications	Justification / Comment
Serovar clustering			
1	Serovar virulence cluster does not depend on host species (e.g., chicken vs. turkey)	Isolates from multiple species can be included in the same clustering analysis, to increase power of analysis without significantly affecting results.	Prior analysis indicates that when isolates from multiple species [3] (i.e., humans and bovine animals) are included in the clustering algorithm, isolates categorize into the same clusters regardless of the species of origin.
2	Only pre-assembled isolate contigs from NCBI were annotated. We assume isolates that were not pre-assembled were missing at random and would not change the results of the clustering.	By using only pre-assembled contigs, our analysis is faster, while still being complete.	Including NCBI isolates that were not pre-assembled would have taken weeks to months to download and assemble. Thus, this approach was unfeasible given the time frame of this project.
Derivation of Multipliers – Use of Multipliers as posterior probability			
3	Serotype proportion inferred from FSIS data are representative of the serotype proportion in the US poultry product supply	The risk multipliers derived from surveillance data apply to the entire US poultry product supply	See FSIS works.
4	Outbreak-based attribution of Salmonellosis to different food sources based on CDC data from NORS is representative from cases of *Salmonellosis* in the US population	The risk multipliers derived from surveillance data apply to the US population	See IFSAC works. There is empirical support for the comparability of sporadic and outbreak-associated foodborne illnesses [33]. Also, the CDC reports similar salmonella attribution to poultry using outbreak data, vs a genetic method using sporadic data [34].

Use of Multipliers as Dose-Response adjustment			
5	Concentration of *Salmonella* on product does not differ between serovar clusters	No need to adjust D-R calculations for different distributions of level	Sub-analysis of FSIS MPN data from ground beef and poultry (results not shown) indicates level of *Salmonella* did not differ significantly by cluster.
6	*Salmonella* inactivation and growth don't differ according to the cluster	The same attenuation factor was applied to NTS nontyphoidal *Salmonella* (NTS) in all clusters	Literature suggests that *Salmonella* resistance to heat treatment and growth parameter varies more within serotypes than between serotypes. We couldn't find in the literature any relevant research suggesting a significant differences in growth and/or inactivation according to serovar. Furthermore, as the type of NTS in product would be unknown, it is reasonable to assume that the process, cooking, and handling of animal meats would be the same regardless of the serovar present.
7	*Salmonella* inactivation and growth don't differ according to the product (chicken, turkey, parts, ground, carcasses)	There is no need to explicitly include this in the analysis, since it would be the same for all products	Note: because of a different harborage of clusters per product, this assumption has to be extended to these products (inactivation and growth shouldn't differ according to the product, because it would lead to a difference according to the cluster).
8	Data collected in Teunis(2022) are representative of Cluster 1 isolates in the U.S.	The risk multipliers can be applied to Cluster 1 serovars from Tenuis 2022, to adjust the DR models	In absence of similar studies focused solely in the US, Tenuis 2022 provides the best estimate available for a DR model relevant to Cluster 1 serovars [14]. The possibility of bias in the resulting DR models exists. However, no gold standard dose-response is available to test for such bias.
9	For a given strain, the DR model (probability of illness) follows a beta-Poisson DR	The beta-Poisson DR is applicable to *Salmonella* strains used in this study	FAO/WHO 2002 [35].

10	Strain variability impacts the mean $u = \alpha/(\alpha + \beta)$ of the underlying beta distribution in the beta-Poisson dose response DR model, but the parameter $v = \alpha + \beta$ is shared by all strains	These assumptions provide bounds in the estimation of beta-Poisson parameters	Assumption used in Teunis et al., 2010, and Thébault et al., 2013 [13,30].

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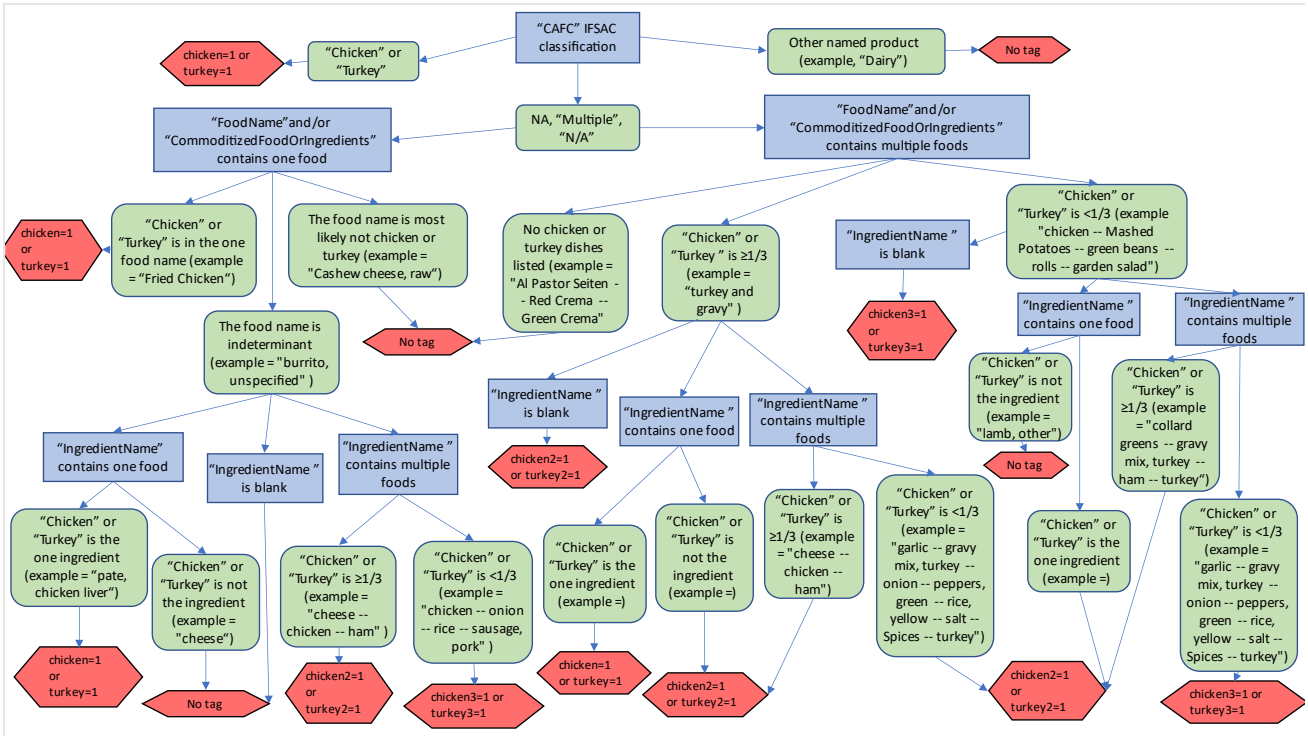
Supplementary materials

This section contains more details on the methodology and computations used in our analysis. This section is not self-explanatory and should be reviewed in combination with the full report.

S1: Outbreak attribution

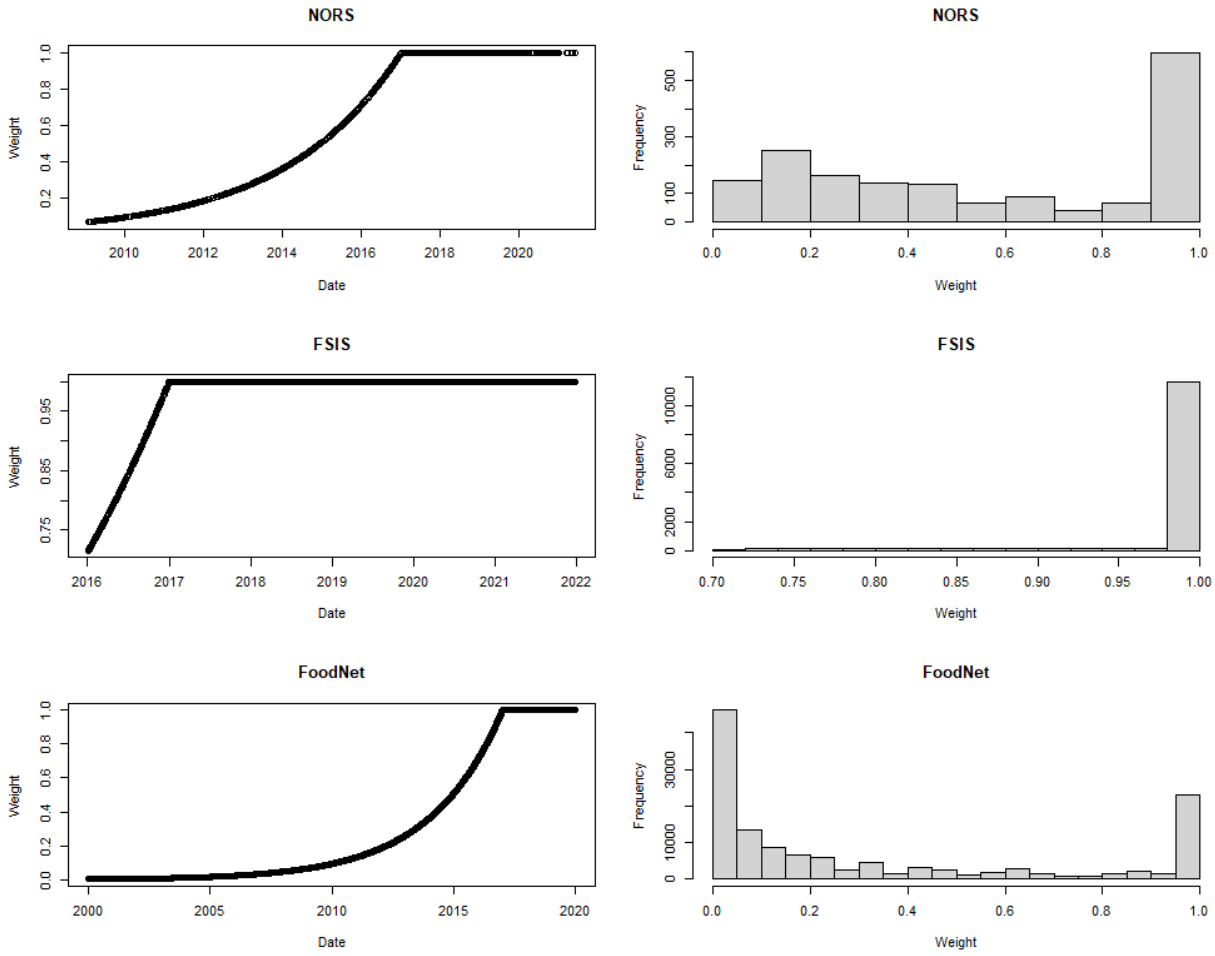
Chicken or turkey-attributed outbreaks were categorized as "definitive, "probable" or "possible" depending on the following NORS dataset fields: "CAFC", "FoodName", "CommoditizedFoodOrIngredient", and "IngredientName. Supplemental figure 1 shows the flow diagram used to attribute an outbreak to poultry and assign a level of certainty.



Supplemental figure 1: Criteria for NORS attribution to poultry. Chicken/turkey= definite attribution, chicken2/turkey2= probable attribution and chicken3/turkey3=possible attribution

S2: Recency Weight

The equation [25] is written $w(x) = \begin{cases} (5/7)^{\left(\frac{maxDate-x}{365}\right)-5} & \text{if } \left(\frac{maxDate-x}{365}\right)-5 > 0 \\ 1 & \text{if } \left(\frac{maxDate-x}{365}\right)-5 \leq 0 \end{cases}$, where *maxDate* is the number of days between a reference day and 12/31/2021, and *x* is the number of days between this reference day and the date considered. Supplemental figure 2 illustrates the evolution of the weight with the date in the NORS, the FSIS and the FoodNet data.



Supplemental figure 2: left: weight as a function of the date, right: histogram of weights for the NORS (top), FSIS (middle) and FoodNet (bottom) data.

S3: Modeling outbreak cases

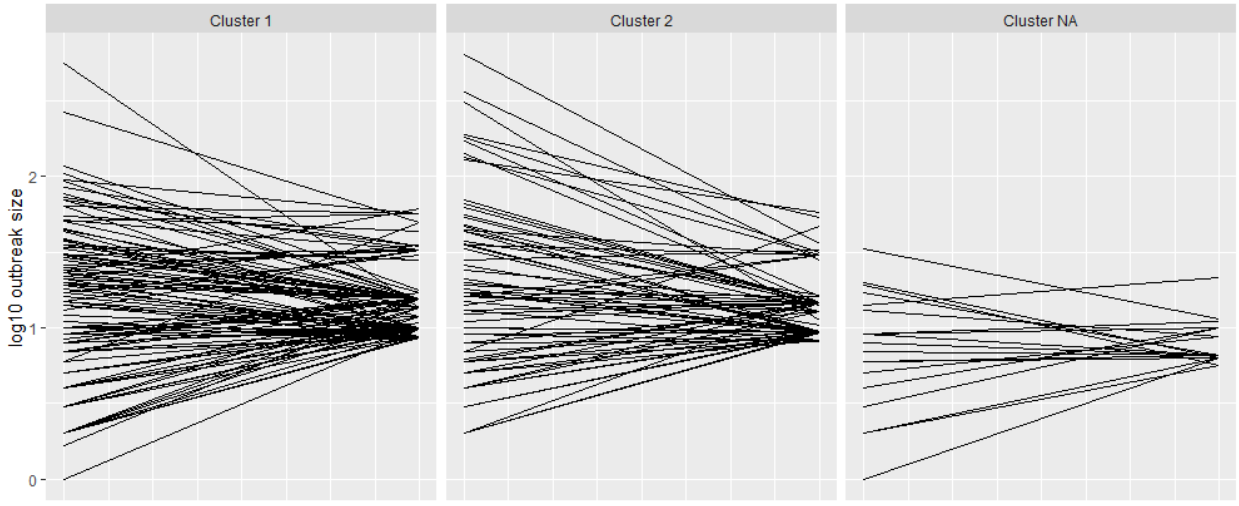
As cases per outbreak can vary widely, we adapted the method used by Batz et al., 2021 [25] to provide a more robust prediction of outbreak cases (i.e., smoothing extreme values). We used the following linear mixed model:

$$\log(Y_{i,j}) = \mu + FC + TP + MS + \zeta_i + \varepsilon_{ij}$$

with :

- Y the estimated number of Primary Cases (eventually weighted for sub-outbreaks) associated to cluster i; Note that we are interested in primary cases (rather than total cases, as in Batz et al. (2021)[25] ;
- FC: the food category (fixed effect). We used 17 IFSAC categories, including “multiple” and “NA”s;
- TP: the type of preparation (fixed effect) using 5 categories as described in Batz et al, 2011;
- MS: multistate (Binary);
- $\zeta_i \sim N(0, \sigma_1^2)$ is a random effect associated to the cluster i;
- $\varepsilon_{ij} \sim N(0, \sigma^2)$ is the error, independently.

Supplemental figure 3 illustrates how the model shrinks extreme values to more central ones.



Supplemental figure 3: number of cases (log_{10}) before (left) and after (right) use of the model, according to the Cluster (example: 3 clusters. Cluster 3 is not represented as no NORS outbreak was assigned to this cluster).

S4: Underdiagnosed cases according to severity

In order to consider the differential underdiagnosis according to case severity, we used the method developed by Scallan et al. (2011)[26]. Following this method, we assume that the sensitivity of laboratory test followed a Pert (min=0.6, mode=0.7, max=0.9)[36]; that the proportion of clinical laboratories routinely testing stool samples for *Salmonella* followed a Pert(0.94, 0.97, 1); that the proportion of respondents who submitted a stool specimen among persons with bloody diarrhea followed a Pert(0.11, 0.36, 0.62); that this proportion among persons without bloody diarrhea followed a Pert(0.12, 0.19, 0.25); that the proportion of individual who sought medical care among persons with bloody diarrhea followed a Pert(0.19, 0.35, 0.51); and those without bloody diarrhea followed a Pert(0.15, 0.18, 0.20). We also apply this differentiated underreporting factor (on average: 1 case out of 13 for bloody diarrhea vs. 1 case out of 44 for non-bloody diarrhea) to the various clusters. For the proportion of bloody diarrhea, we use, as an uncertainty distribution, a Beta distribution under the assumption of a prior proportion of bloody diarrhea equal to a Beta(0.5, 0.5) (i.e., Jeffrey's prior).

S5: Bayesian Inference model for Cluster 1

The model is written as following. For each outbreak o:

$$p_o = 1 - {}_2F_1(\alpha_o, r_o, \alpha_o + \beta_o, -d_o / r_o)$$

$$x_o \sim \text{binomial}(\text{size}=f n_o, \text{prob}=p_o)$$

with r_o, d_o, n_o and x_o, the data provided in appendix, and

$$\alpha_o = u_o \times v_o$$

$$\beta_o = (1-u_o) \times v_o$$

$$u_o = \exp(\omega_o) / (1+\exp(\omega_o))$$

$v_o = \exp(\zeta_o)$

ω_o ~ normal(mean= ωm, sd= ωsd)

ζ_o ~ normal(mean= ζm, sd= ζsd)

The prior distributions were flat, centered around the values estimated from Teunis et al (2010):

ωm ~ normal(mean= -5.9, sd=8)

ζm ~ normal(mean= 1.15, sd=8)

ωsd ~ uniform(min=0, max=4)

ζsd ~ uniform(min=0, max=4)

In Teunis (2022), one outbreak appears to have a right-censored dose (“>2.4E5” cfu, and one has a left-censored dose (‘"<3.60E3" cfu). We adapted our model to deal with these censored values (considering a flat lognormal(11, 10) prior distribution for the doses).

Appendix B Data and Supplemental Analysis

The current state of *Salmonella* in turkey is estimated via data collected in FSIS' Pathogen Reduction, Hazard Analysis and Critical Control Points (PR/HACCP) Verification Sampling Program and supplemented by historical baselines, where applicable, to address the risk management questions.

PR/HACCP Verification Sampling

During FSIS' routine PR/HACCP Verification Sampling program from 2016 to 2021, the concentration of *Salmonella* was estimated using presence/absence for both carcass and comminuted samples, as well as enumeration for various *Salmonella*-positive comminuted turkey products. All samples were selected randomly at a post-chill location. As a part of FSIS' Verification Program, the Agency assesses whether establishments meet *Salmonella* performance standards within a 52-week window and assigns a category status (1, 2, or 3) to each establishment. Follow-up sampling occurs in establishments approximately 30 days after receiving Category 3 status and corrective actions have been implemented.

A total of 18,650 samples (10,909 carcass and 7,741 comminuted) were collected and analyzed from 110 different turkey establishments. Turkey carcasses were routinely sampled, averaging approximately 1800 samples per year across 45 establishments (**Figure 43**).

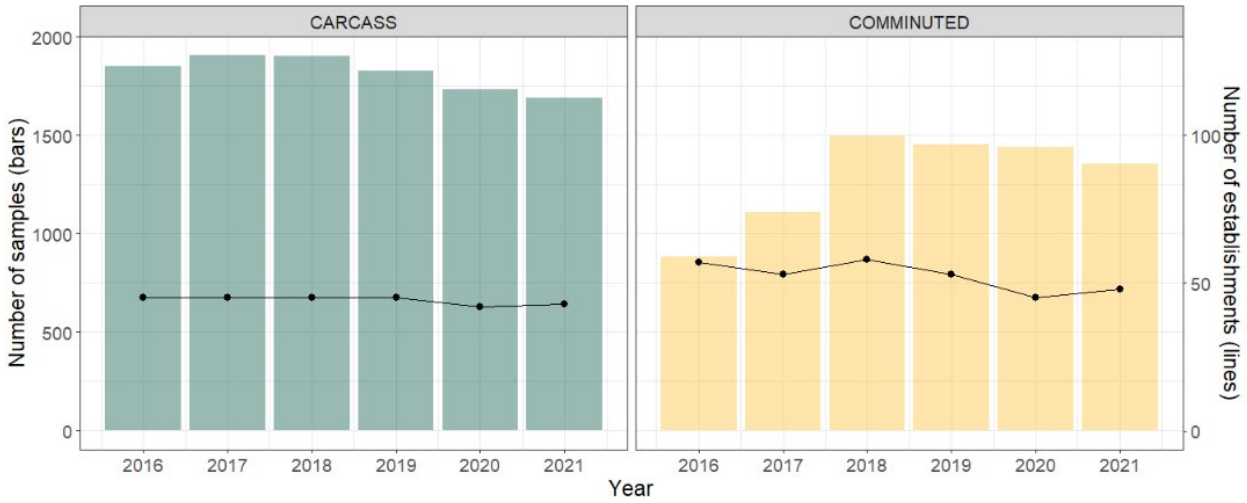


Figure 43: Routine PR/HACCP Verification Program sampling distribution by year.

FSIS enumerated a subset of comminuted turkey samples during this program period. The subsample of enumerated samples consisted of samples analyzed at one of the three FSIS laboratories. These samples are assumed to be a random subsample of comminuted turkey samples because the location of sample collection does not determine the laboratory at which the samples are analyzed (i.e., the overnight courier service ships all samples to a central location in Memphis, Tennessee and then ships the samples to one of the three laboratories. Thus, there is no advantage to having to a laboratory serve a specific geographic region of the country). Samples collection in 2020 were not used in the analysis because not all establishments were consistently tested prior to the implementation of performance standards in 2016 and the "most probable number" (MPN) analysis was not consistently performed.

No data was collected prior to rehang.

Salmonella Initiative Program Data

The FSIS *Salmonella* Initiative Program (SIP) data was not included in the turkey analysis as data from only a single establishment was consistently collected.

FSIS Microbiological Baselines

Current PR/HACCP data lacks information on *Salmonella* at (and prior to) rehang. Therefore, turkey microbiological baseline studies that sampled at rehang and post-chill were considered to investigate process control as well as receiving guidelines.

Young Turkey Carcass Microbiological baseline

An overarching objective in the 2008-2009 Young Turkey Carcass Baseline Study was to determine a national prevalence estimate for *Salmonella* on young turkey carcasses, as well as to investigate the level of reduction between rehang and post-chill. A total of 2,884 young turkey carcass swab samples were collected from 58 different establishments at two points in the production process – rehang and post-chill – and collected from two separate shifts. The concentration of *Salmonella* was estimated using presence/absence. Samples confirmed positive were subject to follow up quantitative testing using the MPN estimation method and processed using WGS to identify the *Salmonella* serotype. Swab samples were also analyzed for microbial indicator organisms (Aerobic count, Enterobacteriaceae, generic E. *coli*, total coliforms) to assess sanitation and process control.

This dataset is included as FSIS does not currently collect data in its PR/HACCP sampling programs regarding *Salmonella* on turkey carcass at rehang or another early point in the slaughter process, prior to post-chill.

Raw Ground Turkey Microbiological baseline

In 1995, a microbiological baseline study was implemented to assess the national prevalence and microbial contamination in ground turkey. A total of 310 (1-pound final product) samples were collected from 40 different establishments over two 7-week intervals (January to March and September to November) in 1995. MPN enumeration used a 25-gram portion of the original sample.

This dataset is included to serve as a retrospective comparison to current conditions; particularly, in terms of levels and serotypes.

Salmonella prevalence across time

Since 2016, PR/HACCP post-chill sampling typically detects *Salmonella* in less than 1% of turkey carcasses and roughly 16% of comminuted turkey every year. However, appropriately estimating the prevalence of *Salmonella* requires taking into account the sampling rate and production volume at each establishment. The prevalence of *Salmonella* $\left(\hat{P}\right)$ was estimated in **Table 31** using the design-based paradigm described previously. During this six-year period, the overall prevalence was rather stable, particularly in carcasses, whereas the prevalence in comminuted turkey has decreased since a peak in 2018.

Table 31: *Salmonella* prevalence statistics.

Commodity	Year	s Number of positive samples	n Total number of samples	$\hat{P}$	$var\left[\hat{P}\right]$	95% confidence interval
Carcass	2016	16	1842	0.0086	0.000007	(0.0043, 0.0143)
	2017	16	1904	0.0056	0.000003	(0.0028, 0.0091)
	2018	14	1903	0.0048	0.000002	(0.0024, 0.0082)
	2019	10	1825	0.0035	0.000002	(0.0014, 0.0065)
	2020	18	1731	0.0093	0.000006	(0.0051, 0.0149)
	2021	8	1690	0.0028	0.000001	(0.0011, 0.0053)
Comminuted	2016	117	878	0.1630	0.00031	(0.1301, 0.1988)
	2017	134	1110	0.1437	0.00018	(0.1182, 0.1711)
	2018	250	1493	0.2539	0.00022	(0.2254, 0.2835)
	2019	287	1456	0.2228	0.00048	(0.1813, 0.2672)
	2020	236	1440	0.1833	0.00015	(0.1597, 0.2083)
	2021	193	1355	0.1763	0.00051	(0.1343, 0.2227)

Every year, nearly 2,000 samples are collected on carcasses, yet less than 20 screened positive for *Salmonella*. One hypothesis for the low prevalence of *Salmonella* found on turkey carcasses could be a result of the sampling procedure.

Serotype-based final product scenario

Taking a retrospective look at recent FSIS; PR/HACCP post-chill data (2016-2021), a risk management option can be preliminarily investigated regarding lot diversions of higher virulence *Salmonella* serovars such as Hadar. It is important to note that a serotype-based final product option requires additional data to accurately model the underlying distribution of *Salmonella* serotypes or seroclusters within a lot (i.e., limited paired sampling data for turkey products). Nevertheless, the scenario explored in this supplemental section considers the general impact of diverting product based on higher virulence serotypes.

Of particular interest, in addition to Hadar, are Muenchen and Typhimurium, which are also assigned to the higher virulence cluster 1 serotypes, and have been increasing detected in comminuted turkey (**Figure 13**). These serovars rank high in the 2020 FoodNet annual summaries of *Salmonella* serotypes linked to human illness (**Table 19**).

The occurrence rate of Hadar, Muenchen and Typhimurium by production volume is depicted in **Figure 44** per establishment to illustrate the number of hypothetical diversions due to higher virulence serotypes detected in post-chill samples across the industry. In 2021, there were 47 comminuted turkey final product samples that screened positive for *Salmonella* and detected a target higher virulence serotype (i.e., Hadar, Muenchen, or Typhimurium). As production volume increases, the rate of theoretical diversions from previous years rises based on historical serotype data and as a result of more frequent sampling of higher volume establishments.

Figure 44: Detections of Hadar, Muenchen, and Typhimurium in comminuted turkey final product samples by establishment production volume between 2016 and 2021. The triangles highlight establishments with no diversions based on a screening test (0.003 cfu/g) and serotyping result of Hadar, Muenchen or Typhimurium.

Cluster distribution reduction scenario

Consider the scenario where preharvest measures or interventions prior to receiving could influence the *Salmonella* serocluster distribution of flocks entering a slaughter establishment to be processed for comminuted product. While no specific information is available to explain the occurrence of serotypes in flocks, some major simplifying assumptions allow for a crude approximation of the effect of removing flocks where a larger fraction of higher virulence *Salmonella* serotypes are identified. To illustrate the broad effects of this hypothetical scenario, we also assume that the average proportion of higher virulence cluster 1 versus lower virulence cluster 2 serotypes in any lot/flock directed to processing for comminuted turkey final product is approximately 0.3 and 0.7, respectively, as observed in FSIS' post-chill *Salmonella* testing data.

Our model for final product standards provides a naïve description of the effect of incrementally reducing the share of all *Salmonella* that are higher virulence cluster 1 (**Figure 45**). If the model parameter describing the fraction of *Salmonella* that are higher virulence cluster 1 is progressively reduced, we calculate a linear reduction in illnesses that extends from 0 to nearly 10,000 salmonellosis cases prevented per year.

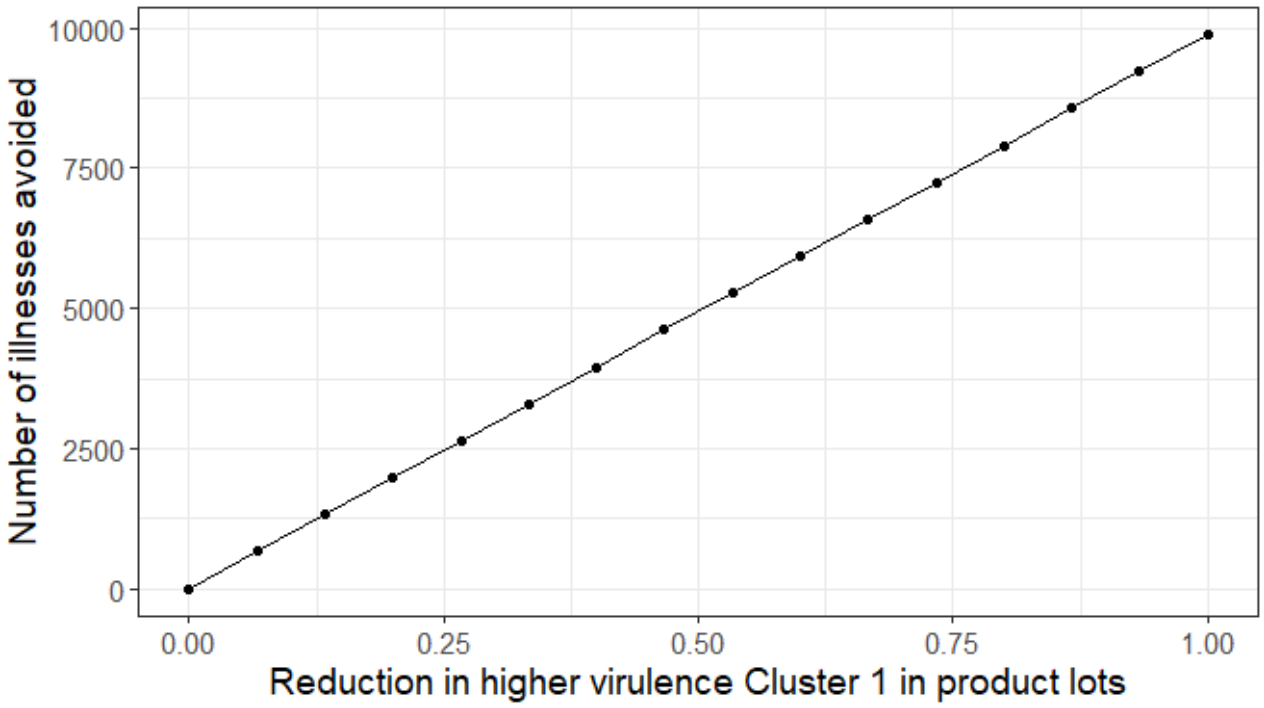


Figure 45: General impacts of reducing the fraction of higher virulence cluster 1 *Salmonella* serotypes in product lots.

Serotype Geography

Incoming turkey flocks are generally local to slaughter establishments. Partitioning the U.S. into Atlantic, Southeast, South Central, Midwest, and Mountain West regions, the distribution of production is heavily favored to the central U.S. (**Figure 46**). The average sampling rate for each establishment was 25 samples over the duration of the 2008-2009 young turkey microbiological baseline study. Higher volume establishments that slaughtered more than 6 million young turkeys annually were sampled more frequently (targeting five samples per month) and averaged around 49 samples per higher production volume establishment. On the other hand, lower volume establishments were sampled less frequently and ranged between 1 and 35 samples total in the year-long (August 2008-July 2009) study.

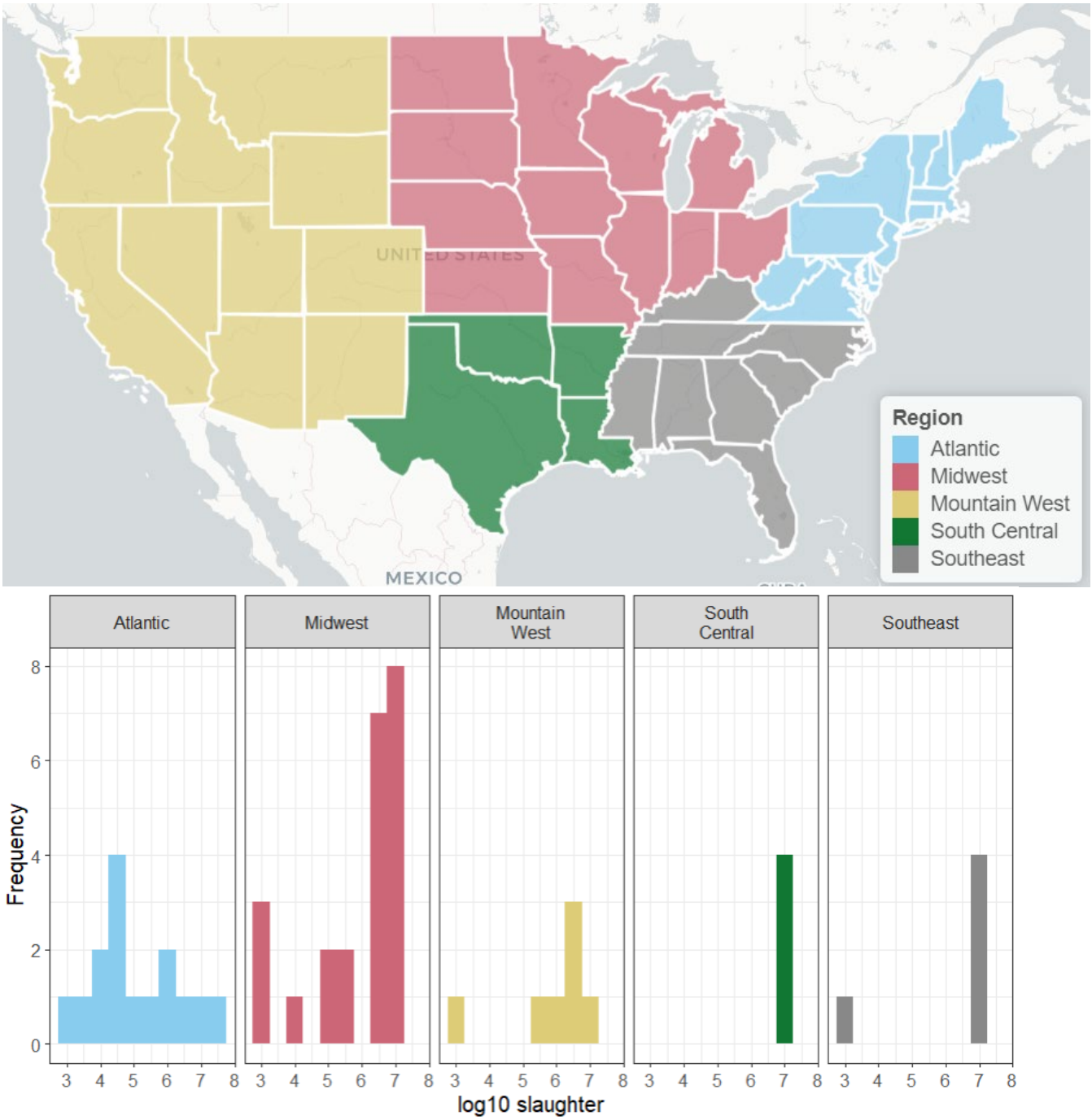


Figure 46: Distribution of annual production volume for turkey slaughter establishments by region on a log10 scale.

Larger volume establishments in this microbiological baseline study are primarily located in the Midwest region (**Figure 46**), and hence, direct the overall serotype proportions at rehang (**Figure 47**). Yet, serotype proportions vary widely in the other regions albeit with fewer positive samples. For example, the South Central and Mountain West regions observed 6 and 9 positive samples at rehang, respectively, that included only 4 and 5 different serotypes, yielding distributions that were dramatically different than the Midwest region.

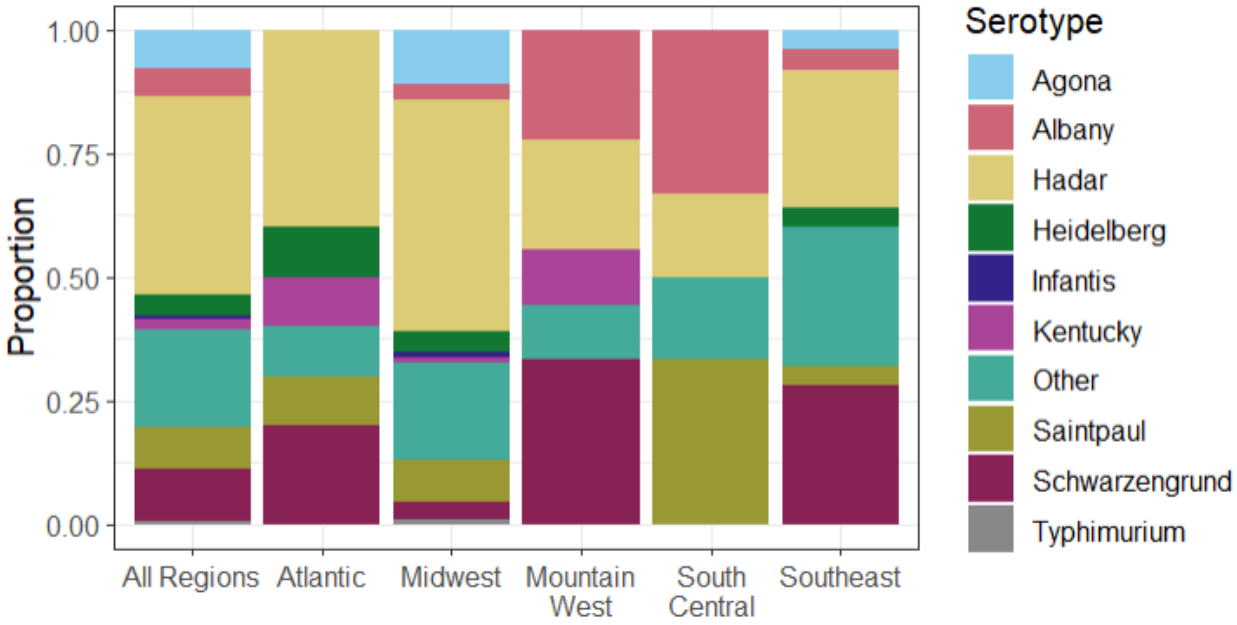


Figure 47: Serotypes by region identified in turkey carcasses at rehang.

NHANES Turkey Consumption Data Analysis

Data on the consumption of turkey in the U.S. were obtained from the National Health and Nutrition Examination Survey (NHANES). The NHANES program suspended field operations in March 2020 due to the 2019 coronavirus disease (COVID-19) pandemic. As a result, data collection for the NHANES 2019-2020 cycle was not completed and the collected data are not nationally representative. Therefore, data collected from 2019 to March 2020 were combined with data from the NHANES 2017-2018 cycle to form a nationally representative sample of NHANES 2017-March 2020 pre-pandemic data.

All NHANES participants are eligible for two 24-hour dietary recall interviews. The first dietary recall interview is collected in-person in the Mobile Examination Center and the second interview is collected by telephone 3 to 10 days later. In the 2017-March 2020 pre-pandemic sample, 12,634 participants provided complete dietary intakes for Day 1. Of those providing the Day 1 data, 10,830 provided complete dietary intakes for Day 2. The NHANES Day 1 weights, adjusted for non-response and daily variability and the SAS code examples given on the NHANES website were used in the analysis (CDC, 2022b).

The full results of this analysis are below. The risk assessment used the GRM50 estimates to derive empirical probability of illness estimates.

Estimates for Turkey

Table 32 has the percent of the US population consuming turkey commodities on an average day according to NHANES day 1 records. **Table 33** has the servings per day as percent of the US population consuming turkey commodities on an average day according to NHANES day 1 records. According to **Table 32**, about 17.8% of the population consumes turkey daily of which 3.4% consists of turkey parts with 13.3% comminuted turkey and with the remainder of ground turkey. Or alternatively and arguably more accurate is the percentage of 17.8% turkey broken down to 3.4% parts and 14.5% combined ground and comminuted turkey.

Table 32: The percent of the US population consuming turkey commodities per day.

Commodity	Consumers	SEM	Percent
TURKEY	57,496,400	4,770,062	17.8
PARTS	10,813,993	1,385,547	3.4
GROUND	4,065,955	760,569	1.3
COMM	42,616,452	3,625,029	13.2
GC=GROUND+COMM	46,682,407	4,019,348	14.5
PARTS+GROUND+COMM	57,496,400	4,770,062	17.8
PARTS+GC	57,496,400	4,770,062	17.8
US POPULATION	322,281,961		

According to **Table 33**, about 19.8% of the US population servings are consumed as 100% of turkey servings on a daily average of 63,678,677 servings per day of which about a 52.6% consists of turkey parts servings on a daily average of 33,515,155 servings per day with 38.7% comminuted turkey servings on a daily average of 24,627,698 servings per day and with the remainder of ground turkey. Or alternatively and arguably more accurate is the percentage of 100% turkey servings broken down to 63,678,677 servings per day with 52.6% parts with a daily average of 33,515,155 servings per day and 47.4% combined ground and comminuted turkey servings at 30,163,522 servings per day.

Table 33: The percent of the US population consuming turkey commodities per day.

Commodity	Servings per Day	Standard Deviation	Population%	Servings%
TURKEY	63,678,677	5,282,961	19.8	100.0
PARTS	33,515,155	2,848,240	10.4	52.6
GROUND	5,535,823	866,640	1.7	8.7
COMM	24,627,698	2,870,151	7.6	38.7
GC=GROUND+COMM	30,163,522	3,159,465	9.4	47.4
PARTS+GROUND+COMM	63,678,676	5,282,961	19.8	100.0
PARTS+GC	63,678,677	5,282,961	19.8	100.0
US POPULATION	322,281,961			

Table 34 shows average daily turkey consumption in grams of turkey commodity on a population basis. The average over all turkey containing food codes as a high and low estimate average taken as all the "chicken or turkey" food codes (44.9% of all 180 turkey food codes) are all turkey or alternatively contain 50% turkey. This means the total average grams equals 100.0 with the averages for parts, ground, and comminuted summing to that value. **Table 35** shows the average percent with an overall average of 12.7% for turkey parts, 81.8% for comminuted turkey, and 5.5% for ground turkey. Or alternatively and arguably more accurate is the percentage of 100% turkey broken down to 12.7% parts and 87.3% combined ground and comminuted.

Table 34: The average daily turkey consumption in grams of turkey commodity on a population basis.

	Average Daily Consumption from Day 1 NHANES for Population					
Commodity	**GRM[a]**	**SEM[a]**	**GRM50[b]**	**SEM[b]**	**AVEGRM[c]**	**SEM[c]**
TURKEY	120.3	3.7	79.7	2.3	100.0	2.2
PARTS	14.5	1.8	10.9	1.6	12.7	1.2
GROUND	6.9	1.3	4.1	0.7	5.5	0.7
COMM	98.9	3.6	64.7	2.2	81.8	2.1
GC=GROUND+COMM	105.8	3.9	68.8	2.2	87.3	2.3
PARTS+GROUND+COMM	120.3	2.5	79.7	1.6	100.0	1.5
PARTS+GC	120.3	4.1	79.7	2.5	100.0	2.4

a Grams consumed per day without subtracting chicken or turkey food codes
b Grams consumed per day subtracting 50% of grams per day for chicken or turkey food codes
c Average grams per day for a and b

Table 35: The average daily consumption percentage.

	Average Daily Consumption Percent from Day 1 NHANES		
Commodity	**GRM Percent**	**GRM50 Percent**	**AVE Percent**
TURKEY	100.0	100.0	100.0
PARTS	12.1	13.6	12.7
GROUND	5.7	5.2	5.5
COMM	82.2	81.2	81.8
GROUND + COMM	87.9	86.4	87.3

Table 36 shows average daily consumption in grams turkey commodity by consumer domain. This means that the denominator of the average is only from the part of the U.S. population that consumed the parts, ground, or comminuted turkey.

Table 36: Average daily consumption in grams turkey commodity by consumer domain.

	Average Daily Consumption from Day 1 NHANES for Commodity Domain					
Commodity Domain	**GRM[a]**	**SEM[a]**	**GRM50[b]**	**SEM[b]**	**AVEGRM[c]**	**SEM[c]**
TURKEY	120.3	3.7	79.7	2.3	100.0	3.1
PARTS	75.6	6.2	56.6	5.9	66.1	6.0
GROUND	95.1	10.5	57.3	6.1	76.2	8.6
COMM	130.7	4.5	85.4	2.4	108.1	3.6
GC=GROUND+COMM	128.4	4.1	83.5	2.3	105.9	3.3

a Grams consumed per day without subtracting chicken or turkey food codes
b Grams consumed per day subtracting 50% of grams per day for chicken or turkey food codes
c Average grams per day for a and b

Table 37 shows the percentiles of average daily turkey consumption as high, low, and average values.

Table 37: The percentiles of average daily turkey consumption as high, low, and average values.

	TURKEY GRM		TURKEYGRM50		AVE Turkey GRM	
PERCENTILE	**GRAMS**	**SEM**	**GRAMS**	**SEM**	**GRAMS**	**SEM**
1%	1.2	0.2	0.6	0.1	0.9	0.2
2.5%	2.5	0.5	1.3	0.2	1.9	0.4
5%	5.4	1.0	2.7	0.6	4.0	0.8
10%	17.4	2.3	10.4	0.9	13.9	1.7
20%	32.9	1.7	25.0	1.6	29.0	1.7
50%	84.0	3.4	60.7	2.8	72.4	3.1
Mean	120.3	3.7	79.7	2.3	100.0	3.1
80%	183.0	12.5	113.4	2.9	148.2	9.1
90%	276.9	17.4	171.3	5.3	224.1	12.9
95%	366.0	19.1	226.3	12.5	296.1	16.1
97.5%	504.0	25.9	272.9	14.1	388.4	20.9
99%	603.8	35.7	313.8	21.1	458.8	29.3

Figure 48 shows the distribution approximation to the average total daily grams turkey consumption. The best fit is for a gamma distribution. The percentiles are shown in **Table 38**. The percentiles do not exactly match those in **Table 36** because the distribution takes the average uncertainty of all or 50% turkey in the "chicken or turkey" food codes to be 75%. These percentiles and means are nearly identical to the average of the two separate turkey total grams and turkey grams50 distributions.

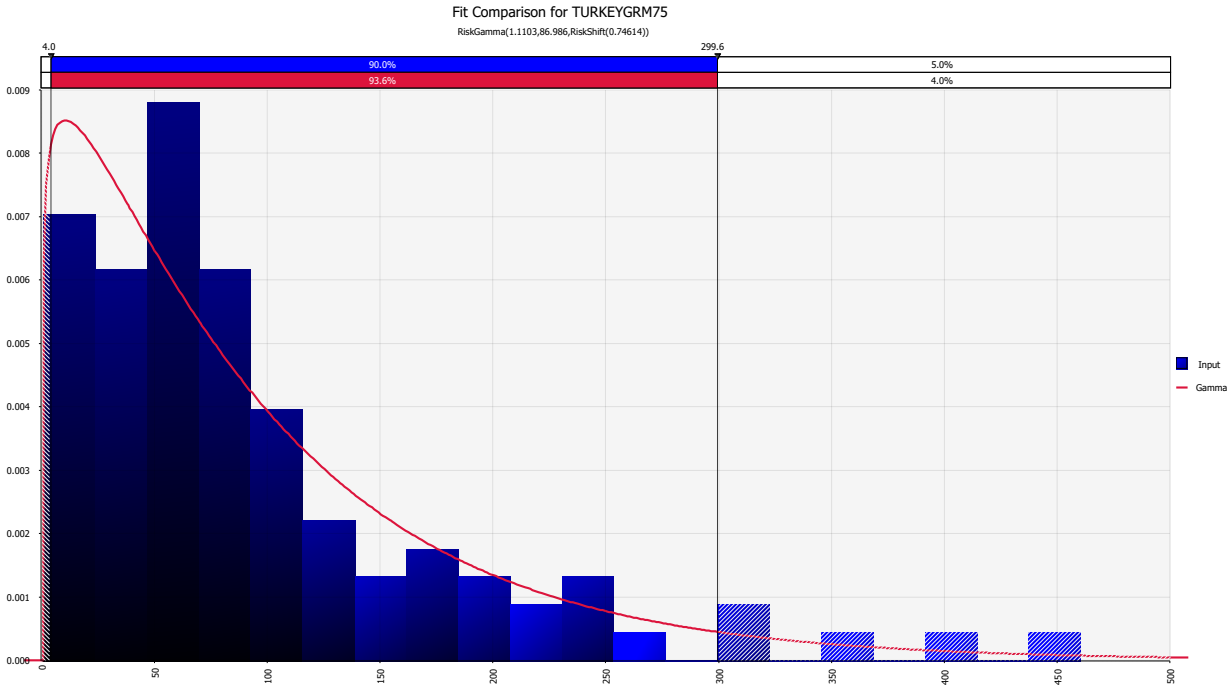


Figure 48: The distribution approximation to the average total daily grams turkey consumption.

Table 38: The percentiles of the gamma distribution.

	Turkey Grams75										
Stats	Input	Gamma	Pecentiles	Input	Gamma	Pecentiles	Input	Gamma	Pecentiles	Input	Gamma
Minimum	0.9	0.7	1%	0.9	2.2	40%	58.4	53.1	80%	150.8	154.8
Maximum	460.4	∞	5%	4.0	7.1	45%	64.0	61.4	85%	180.6	180.9
Mean	97.3	97.3	10%	14.2	13.0	50%	69.0	70.4	90%	226.0	217.5
Mode	≈112.03	10.3	15%	22.0	18.9	55%	82.7	80.2	95%	299.6	279.6
Median	69.0	70.4	20%	28.0	25.1	60%	84.6	91.2	99%	460.4	422.9
Std Dev	89.4	91.7	25%	37.8	31.5	65%	94.9	103.6			
Skewness	1.8	1.9	30%	46.6	38.3	70%	112.0	117.8			
Kurtosis	6.7	8.4	35%	55.7	45.4	75%	122.0	134.5			

Appendix C Theory

***Salmonella* prevalence estimation methods**

It is necessary to estimate the prevalence of sample units (i.e., carcasses and comminuted samples) where *Salmonella* is present at concentrations above the LOD of the assay. This requires weighting the sampling information from each establishment to account for the large range of establishment production volumes. Using carcasses as an example, the target population consists of the V carcasses produced during the period of interest. Associated with each carcass is one or more attributes of interest, denoted Y_k, and the production volume for the establishment where slaughter occurred. The objective of a survey is to estimate some function of the population total, which is defined as

$$T_y = \sum_{k=1}^{V} Y_k .$$

For most food-safety applications the target parameter is the population mean $\frac{T_y}{V}$. When estimating the prevalence of a pathogen, $Y_k = 1$ when the pathogen is present and 0 otherwise. In other applications, it could be the information regarding serotype.

There are two approaches to describing the estimation strategy using a design-based inferential paradigm. The sample design assumes that a sample of slaughter establishments (clusters) is selected for testing and samples of the commodity of interest are collected from each selected establishment. This is a typical application of two-stage cluster sampling, where establishments represent the clusters (Cochran, 1977; Särndal, 1992). The sample design for selecting a sample from M establishments will define a first-stage probability of selection,

$$P\left(\text{establishment } j \text{ is selected}\right) = \pi_{1j}, j = 1,...M .$$

The Horvitz-Thompson estimator (Cochran, 1977; Fuller, 2009) can be used to estimate a population total and is given by

$$\hat{Y} = \sum_{j=1}^{m} \frac{\hat{Y}_j}{\pi_{1j}},$$

where m is the number of establishments sampled, π_{1j} represents the probability of selecting establishment j, and $\hat{Y}_j$ is the estimator for the total of the target parameter in the establishment. Note, however, $\pi_{1j} = 1$ because samples will be collected from all M establishments.

For all sampling programs, FSIS collected samples within an establishment at regular intervals, so an assumption of systematic sampling is reasonable. Sampling within establishment j yields n_j samples and sample unit (e.g., a carcass) i has a second-stage probability of inclusion of $\pi_{2i} = \frac{n_j}{V_j}$, where V_j is the total number of units produced by that establishment. The key difference between simple random sampling and the more systematic nature of the FSIS sample design is that the joint inclusion probability for all samples within the sample period (e.g., weekly) is $\pi_{2,ii'} = 0$. The Horvitz-Thompson estimator (Cochran, 1977; Fuller, 2009) of the total for the test outcomes in establishment j is

$$\hat{Y}_j = \sum_{i=1}^{n_j} \frac{y_{ij}}{\pi_{2i}}.$$

For this example, $y_{ij} = 1$ when a sample tests positive for a *Salmonella* and 0 otherwise, so $\hat{Y}_j$ is the estimator of the total number of test-positive carcasses across the entire volume of production in establishment j.

Therefore, in the case where y_{ij} is binary, the estimator of the proportion of test-positive carcasses is

$$\hat{P}_j = \hat{Y}_j = \frac{\hat{Y}_j}{V_j}$$

Alternatively, if y_{ij} is the pathogen count per unit volume (e.g., *Salmonella* colony forming units per milliliter (cfu/mL)), then $\hat{Y}_j$ is the average microbial count per unit volume across all sample units produced by establishment j.

The total across all establishments is

$$\hat{Y} = \sum_{j=1}^{m} \sum_{i=1}^{n_j} \frac{y_{ij}}{\pi_{2i}\pi_{1j}}.$$

Given that $\pi_j = 1$ because samples are collected from all establishments, the population total for y is estimated by

$$\hat{Y} = \sum_{j=1}^{M} \sum_{i=1}^{n_j} \frac{y_{ij}}{\pi_{2i}\pi_{1j}} = \sum_{j=1}^{M} \sum_{i=1}^{n_j} \frac{V_j y_{ij}}{n_j}$$

and

$$\hat{\bar{Y}} = \frac{1}{V} \sum_{j=1}^{M} \sum_{i=1}^{n_j} \frac{V_j y_{ij}}{n_j}$$

is a design-unbiased estimator of the mean. When the target parameter is the prevalence of an indicator organism of pathogen, the estimator can be written as

$$\hat{P} = \sum_{j=1}^{M} \frac{V_j}{V} \hat{P}_j = \frac{1}{V} \sum_{j=1}^{M} \frac{V_j s_j}{n_j},$$

where s_j is the number of positive samples in establishment j.

When the estimation strategy is viewed as an application of two-stage cluster sampling, the variance estimator for the population total is given by

$$var\left[\hat{Y}\right] = M\left(M-m\right)\frac{\hat{\sigma}^2_{between}}{m} + \frac{M}{m}\sum_{j=1}^{m} V_j\left(V_j - n_j\right)\frac{\hat{\sigma}^2_{within}}{n_j}.$$

Noting that $M = m$ and $V_j \gg n_j$ for all FSIS performance standards applications yields

$$var\left[\hat{Y}\right] = \sum_{j=1}^{M}\left(\frac{V_j}{V}\right)^2 \frac{\hat{\sigma}^2_{within}}{n_j}$$

because the contribution of the between-cluster sampling variance is zero.
If the population parameter of interest is the proportion of *Salmonella*-positive carcasses in the population, the variance estimator is given by

$$var\left[\hat{Y}\right] = \sum_{j=1}^{M}\left(\frac{V_j}{V}\right)^2 \frac{p_j\left(1-p_j\right)}{n_j - 1},$$

where p_j is the proportion of positive samples in establishment j.

Concentration estimation for comminuted product

FSIS has enumerated a subset of comminuted chicken and turkey performance standards samples since 2015. The subsample of enumerated samples consisted of samples that were analyzed at one of the FSIS three laboratories. These samples are assumed to be a random subsample of comminuted chicken and turkey samples because the location of sample collection does not determine the laboratory at which the samples are analyzed (i.e., the overnight courier service ships all samples to a central location in Memphis, Tennessee and then ships the samples to one of the three laboratories. Thus, there is no advantage to having to a laboratory serve a specific geographic region of the country).

Samples collection in 2015 and 2020 were not used in the analysis because not all establishments were consistently tested prior to the implementation of performance standards in 2016 and the MPN analysis was not consistently performed. The comminuted chicken dataset consisted of 1815 samples of which 387 were positive on the screening test. The comminuted turkey dataset consisted of 1178 samples of which 156 were positive on the screening test.

Given the lower priority of MPN analysis, some samples were not analyzed due to limited staffing in the laboratory. The number of samples that were not analyzed was 21 and 1 for the comminuted chicken and turkey datasets respectively. These missing results were addressed by randomly imputing sample results from those samples with results.

A weighted maximum likelihood routine was used to fit a lognormal distribution to comminuted chicken and turkey datasets. The estimated parameters for were $\hat{\mu}_{comm,chick} = \text{-}3.700$, $\hat{\sigma}_{comm.chick} = 1.949$ and $\hat{\mu}_{comm,turk} = -4.857$, $\hat{\sigma}_{comm.turk} = 2.333$. The implied prevalence for the two commodities, derived from the cumulative distribution of the lognormal evaluated at the LOD=1/325, are 0.271 and 0.157 comminuted chicken and turkey, respectively. The estimates are similar to the prevalence estimates for calendar year 2021, which were 0.280 and 0.153 for comminuted chicken and turkey, respectively.

Indicator organism reduction estimation

In previous studies of indicator organisms, a correlation was observed between an establishment's reduction in the average concentrations of AC between re-hang and post-chill and the occurrence of generic *E.coli* and pathogenic bacteria, with establishments that had larger reductions tending to have both lower concentrations and occurrence of pathogens (Williams, 2015; Williams, 2017). When indicator organisms are present in nearly

all samples, the average reduction is simply calculated as

$$\Delta_{APC,j} = \frac{1}{n_j}\sum_{i=1}^{n_j} y_{rh,i} - \frac{1}{n_j}\sum_{i=1}^{n_j} y_{pc,i},$$

Where $y_{*,i}$ is the concentration of sample *i* from establishment *j*. When the fraction of samples that are below the LOD is small, simple ad hoc adjustments such as substituting ½ of the LOD for samples where AC was not found, are reasonable solutions. Similarly, some samples can have levels that exceed the assay's ability to enumerate the sample. When this occurs infrequently, the ad hoc solution of substituting twice the upper limit of quantification is reasonable. Simple ad hoc adjustments are inappropriate and create large biases in the concentration estimates, unless used sparingly (Helsel, 2009; Helsel, 2010).

To account for the large fraction of samples below the LOD, a maximum likelihood routine was used fit a lognormal distribution to the data for each establishment while accounting for the censored observations (Williams, 2014). The $\hat{\mu}_j$ derived from the fitted distribution is used to estimate the average AC concentration for every establishment. The average log reduction, accounting for the censored data, is estimated as

$$\Delta_{APC,j} = \frac{1}{n_j}\sum_{i=1}^{n_j} y_{rh,i} - \hat{\mu}_j.$$

Modeling the relationship between prevalence and concentration

Risk assessments that evaluate the difference between performance standards approaches based on prevalence or concentration can give the impression that the two approaches are inherently different (Lambertini, 2019). Nevertheless, both approaches are related since once a flock enters slaughter reductions in prevalence are achieved by applying interventions (e.g., the incorporation of an organic acid spray) that also result in log reductions compared to baseline scenarios. In the same manner, if incoming concentrations of *Salmonella* are similar at two establishments, but the establishments achieve different log reductions in pathogen contamination, then the establishments will attain different prevalence at final product given by $1 - F(\log(1/30), \mu, \sigma)$.

This phenomenon can also be expressed probabilistically as an application of Bayes Theorem. Consider a distribution that describes the log10-transformed contamination distribution with parameters μ and σ. Assume that σ remains roughly constant, and so, to simplify the notation, express this distribution as $P(\mu)$. Next consider that a sample has concentration x that is greater than a threshold value denoted by d. Bayes Theorem yields the relationship

$$P(\mu \mid x > d) = \frac{P(x > d \mid \mu)P(\mu)}{P(x > d)}.$$

The duality of the relationship between concentration and prevalence is demonstrated by noting that the probability of $x > d$ can be replaced by $x > LOD$, which is the event that the sample is positive on the screening test, with $P(x > LOD) = P(test+)$ and $P(test+) = 1 - F(LOD, \hat{\mu}, \hat{\sigma})$.

Methods for modeling illnesses

FSIS is interested in evaluating the effectiveness of different approaches to reducing illnesses associated with *Salmonella* contaminated poultry. The first step in the risk assessment process is to define new probabilistic models to address potential risk management scenarios. To simplify the development, most of the probabilistic

components will be replaced by fixed values.

The starting point for the risk assessment is the concept that the annual number of illnesses is the product of the probability of illness per serving times the number of servings, so

$$I = N_{\text{servings}} P(ill),$$

where $N_{servings}$ is the number of servings of turkey consumed per year and $P(ill)$ is the probability of illness per serving. The total number of illnesses from chicken are determined by the estimated total number of domestically acquired foodborne cases of salmonellosis (Scallan, 2011) multiplied by the attribution fraction (IFSAC, 2019). The number of servings can be estimated using the estimated per capita weight of turkey available for consumption (USDA-ERS, 2021) times the average serving size (**Appendix B**). Given that the motivation for revised performance standards is driven by a lack of observed changes in overall cases of salmonellosis reported by FoodNet, the probability of illness per servings should logically be directly tied to CDC illness estimates, which imposes the requirement that

$$P(ill) \approx I / N_{servings}.$$

This formulation for the probability of illness will be referred to as the attribution-based probability of illness per serving.

Interest lies in addressing specific serotypes or groups of serotypes, indexed by $g = 1,...G$, so the illnesses are decomposed by

$$I = I_1 + I_2 + ..., I_G = N_{servings,1} P_1(ill) + N_{servings,2} P_2(ill) +, ... N_{servings,G} P_G(ill)$$

A reasonable method for estimating the number of serving contaminated with serotype g is to use the fraction *Salmonella*-positive samples where serotype s_g is identified divided by the number of all *Salmonella*-positive samples S, so

$$N_{servings,g} = \frac{s_g}{S} N_{servings} = p_g N_{servings}.$$

Estimates of I_g can be derived by considering the attribution fraction for different serotypes/serogroups, as was the case in the original attribution study (Painter, 2013).

While the probability of illness per servings will be required to "match" the observed value $P(ill) = I / N_{servings}$, the risk assessment model will need an additional level of detail so that changes in the levels of contamination can be assessed.

There is no evidence to suggest that any flock is truly free of *Salmonella* contamination (De Villena, 2022), thus the preferred parameterization of $P(ill)$ assumes that all servings have the potential for some level of contamination, so that the random variable describing dose D describes the average number of pathogens in each serving. Note that because D describes an average concentration, it is possible for these concentration values to be much less than 1 organism per serving. The average concentration of *Salmonella* follows a

distribution with probability density $f(D)$. The probability that a random person will become ill, given a microbial dose of average concentration D, is $P(ill|D)$. Averaging across all possible doses yields the probability of a person becoming ill. When D describes an average dose, the probability of illness given exposure described by a continuous dose distribution is

$$\tilde{P}(ill) = \int_0^\infty P(ill \mid D) f(D)\, dD = \int_0^\infty R(D) f(D)\, dD,$$

where $R(D)$ is the dose-response function and the ~ sign indicates that this probability of illness is derived from a dose-response function.

Epi*X* Analytics supplied beta-Poisson dose-response functions that are appropriate for continuous dose distributions where the input variable is the average concentration per serving. What will be unique for this risk assessment is that specific serotypes will be grouped into a small number of clusters based on the estimated pathogenicity of the serotype. Let's assume there are three clusters representing high, medium, and low pathogenicity. Then the number of illnesses associated with a highly virulent serotype is

$$N_{servings,s}\tilde{P}_s(ill) = \int_0^\infty R_s(D) f_s(D)\, dD.$$

An assessment of the broiler microbiological baseline data (FSIS, 2009) found insufficient evidence to reject the hypothesis of significant differences in the levels of contamination between serotypes assumed to be in the high pathogenicity cluster compared to the low pathogenicity cluster, so it is reasonable to assume $f_g(D) = f_{g'}(D)$ for all serotype clusters.

Note that the dose-dependent probability of illness per serving has some inherent limitations, with the most obvious one being that the dose at the point of consumption is unknown. The second limitation is that it is difficult to model the changes between the last point at which the product is sampled.

Models for describing consumption dose distribution.

Data to directly estimate the parameters of the dose distribution at consumption (i.e., $f(D) = f(D_{consump})$) are typically only available for a small number of outbreaks. This risk assessment will use data collected at the end of production, which is represented as $f(D_{test})$. The lognormal distribution is appealing for describing microbial data from different locations in the food chain (Chen, 2001; Commeau, 2012; Gonzales-Barron, 2011; Pouillot, 2013; Williams, 2015). Furthermore, the lognormal distribution is mathematically convenient for scaling the concentration to account for sampling volumes and efficiencies (Williams, 2010; Williams, 2011), modeling the effects of cooking (Bassett, 2010), growth (Shorten, 2006) and cross contamination (Chen, 2001). A lognormal distribution is obtained asymptotically even if intermediate processes that modify a lognormal distribution are not themselves lognormal (Mitzenmacher, 2003). This result is important because even if some intermediate processes are not lognormally distributed, it is reasonable to assume that $f(D_{consump})$ follows a lognormal distribution.

If the focus of a risk assessment is to determine changes in risk due to measurements taken at the end of production, then the lognormal distribution allows modification of $f(D_{test})$ through a single component by

modeling $\log_{10}(D_{consump}) = \log_{10}(D_{test}) + \log_{10}(D_{atten})$, where $f\left(D_{atten}\right)$ is a lognormal distribution describing the cumulative change in average microbial level between production and consumption (i.e., it combines the effects of mixing, growth, partitioning, cooking and other processes). Assuming independence between concentration at the end of production and magnitude of attenuation, the mean and standard deviation of the consumption distribution can be computed directly from the means and variances of the distributions for D_{test} and D_{atten} (i.e., $\mu_{test}, \mu_{atten}, \sigma^2_{test}, \sigma^2_{atten}$), where $\mu_{consump} = \mu_{test} + \mu_{atten}$ and $\sigma_{consump} = \sqrt{\sigma^2_{test} + \sigma^2_{atten}}$. In this case, μ and σ refer to the mean and standard deviation of the $\log_{10}$ transform of the random variable (e.g., $\log_{10}\left(D_{test}\right) \sim Normal\left(\mu,\sigma\right)$ and $D_{test} \sim Lognormal\left(\mu,\sigma\right)$).

Appendix D Industry Data Sharing and Analysis

To provide support for this risk assessment, the Food Safety and Inspection Service (FSIS) entered into a cooperative agreement (FSIS-02152022) with the University of Maryland, Joint Institute for Food Safety and Applied Nutrition (UMD-JIFSAN).[16] While FSIS routinely collects microbiological data across the poultry industry and that data is sufficient to conduct quantitative risk assessments, the breadth of FSIS' *Salmonella* Framework (FSIS, 2022b) was an opportunity to gather additional data. UMD-JIFSAN served as a neutral intermediary for voluntary sharing of existing industry data in a confidential and secure manner.

UMD-JIFSAN Facilitated Data Sharing

The UMD-JIFSAN, in collaboration with Structured Partnerships and the Institute for the Advancement of Food and Nutrition Sciences (IFSANS), held over 70 meetings with poultry industry organizations, FSIS, and other interested stakeholders from July 2022 through September 2023. The UMD-JIFSAN team established working groups with industry and FSIS, including scientists and lawyers, to clarify data criteria, establish mechanisms to securely provide anonymized industry data, and a legal mechanism that secured industry anonymity, as outlined in the goals of the data sharing effort under the FSIS cooperative agreement. This dialogue resulted in signed agreements between industry and UMD-JIFSAN facilitating sharing of available industry data on *Salmonella* levels in ground turkey with FSIS.

Industry Data

UMD-JIFSAN provided monthly data summaries describing industry test results for *Salmonella* in raw ground turkey samples collected from 15 establishments between July 2019 and June 2022 (**Table 39**).

A total of 1,065 raw ground turkey samples were collected, following FSIS' sampling protocols (FSIS, 2013; FSIS, 2021), with 12 to 80 samples collected in most months. One sample was missing collection month and year. No samples were provided during the second quarter of 2020 during the COVID-19 pandemic. In March 2020, the onset of the COVID-19 pandemic, and June 2022, the last month of the study, there were two exceptions where only one sample was collected and analyzed. Sample submissions were scheduled based on establishment production volume. Based on information shared from UMD-JIFSAN, each aseptically collected 500-gram sample was taken following the final intervention outlined in the establishment's Hazard Analysis and Critical Control Point plan and were indicative of finished product. Samples were shipped overnight to the laboratory in Styrofoam coolers with ice packs. Upon receipt at the laboratory, samples were inspected for integrity and temperature, and stored at 4 °C.

[16] FSIS requested proposals for a cooperative agreement to facilitate industry data sharing and provide dose-response modeling support for FSIS risk assessments for *Salmonella* in poultry FSIS *Constituent Update* announcements: February 18, 2022. March 4, 2022, April 11, 2022, April 15, 2022, and May 6, 2022. FSIS held a webinar, *Salmonella in Poultry Webinar for the Cooperative Agreement Announcement* (March 18, 2022), to answer questions related to the cooperative agreement and included Final Frequently Asked Questions as part of Cooperative Agreement FSIS-02152022. On July 1, 2022, FSIS announced the agency had signed a cooperative agreement with UMD-JIFSAN to develop quantitative risk assessments for *Salmonella* in poultry designed to inform specific risk management questions.

Table 39: Industry-collected data summary variables provided to FSIS by UMD-JIFSAN.

Available Summary Values (monthly)
Month
Sample Count
MPN Mean (no adjustment for censored data)
MPN Standard Deviation (no adjustment for censored data)
Salmonella Positive Count
Salmonella Negative Count
Maximum MPN Observed

*FSIS was provided with monthly summaries which included: counts for samples and *Salmonella* presumptive positives, and summary statistics (mean and standard deviation) of MPN (most probable number) results. The MPN summary statistics consider all samples (i.e., all non-detects are zeros as well as those below LOQ via MPN), but do not account for any differences in sampling rates nor volume weighting. Instead, censored data techniques should be used to account for the lower limit of detection of the presence/absence test for *Salmonella* (Helsel, 2005; Helsel, 2011).

Of the raw ground turkey samples screened for *Salmonella,* 267 were presumptive positive and further enumerated following FSIS' laboratory testing protocol (FSIS, 2022a). Samples were screened for *Salmonella* using the FSIS polymerase chain reaction (PCR) protocol. Raw ground turkey samples (325 ± 5 grams) were incorporated with Buffered Peptone Water (1625 ± 5 milliliters) then aerobically incubated at 42 ± 1 °C for 24 hours. The enriched samples were analyzed using the Gene-up *Salmonella* 2, an AOAC approved test method for detection of *Salmonella* species. PCR presumptive positive samples were further enumerated using the FSIS MPN procedure. The standard 3-tube FSIS Microbiological Laboratory Guidebook method given in Appendix 2.05 was used.

Comparing FSIS and Industry Data

FSIS compared the industry data shared by UMD-JIFSAN with the *Salmonella* counts and levels observed in the agency's routine verification testing of *Salmonella* in comminuted[17] turkey. Although FSIS discontinued routine MPN enumeration of *Salmonella* in raw comminuted turkey in 2020, FSIS considered data from January 2016 through December 2022 for comparison (e.g., positivity rates). During this timeframe, FSIS collected 8,982 raw comminuted turkey samples from 58 establishments, averaging 5 samples per month collected from larger, higher-volume establishments and 2 samples per month from smaller, lower-volume establishments. Of these samples, 157 *Salmonella* positive samples were enumerated by MPN through 2019.

Table 40 summarizes the annual establishment, sample, and *Salmonella* positive sample counts for both industry and FSIS data from 2016 through 2022. It should be noted that while the industry data reflected fewer samples from fewer establishments compared to the FSIS data, the industry data has a consistently higher average *Salmonella* positive rate than the FSIS data. This dynamic has been visually represented in **Figure 49**. Standard box plot visualizations of the distribution of *Salmonella* MPN data are provided below to compare FSIS and industry findings on *Salmonella* levels in comminuted turkey. **Figure 50** is an annual representation of the available MPN data. **Figure 51** is a quarterly representation from quarter 1 of 2019 through quarter 4 of 2020. Only in quarters 3 and 4 of 2019 did industry and FSIS data collection coincide. In those two quarters, the industry-collected *Salmonella* MPN data is noticeably higher than FSIS values. From 2021 onward, industry-collected *Salmonella* MPN has a central tendency more comparable to FSIS findings from 2017 through 2019,

[17] FSIS samples ground poultry product as part of comminuted poultry sampling programs and as such this is the most appropriate dataset for use in this comparison. Note, however, that other poultry products fall into the category including those with added ingredients.

nonetheless the fourth quartile is higher than that of the FSIS findings.

Table 40: Description of industry and FSIS *Salmonella* occurrence in comminuted turkey data.

Data Source	Year	Establishment Count	Sample Count	*Salmonella* Positive Sample Count	Positive Rate
Industry Data					
	2019*	≤ 15	253	69	27%
	2020*	≤ 15	244	60	26%
	2021	≤ 15	369	96	26%
	2022*	≤ 15	198	38	19%
	All	15	1065	263	25%
FSIS PR/HACCP					
	2016	57	878	117	13%
	2017	53	1110	134	12%
	2018	58	1493	250	17%
	2019	53	1456	287	20%
	2020	45	1440	236	16%
	2021	48	1355	193	14%
	2022	42	1250	195	16%

*Data collection was not for the full year.

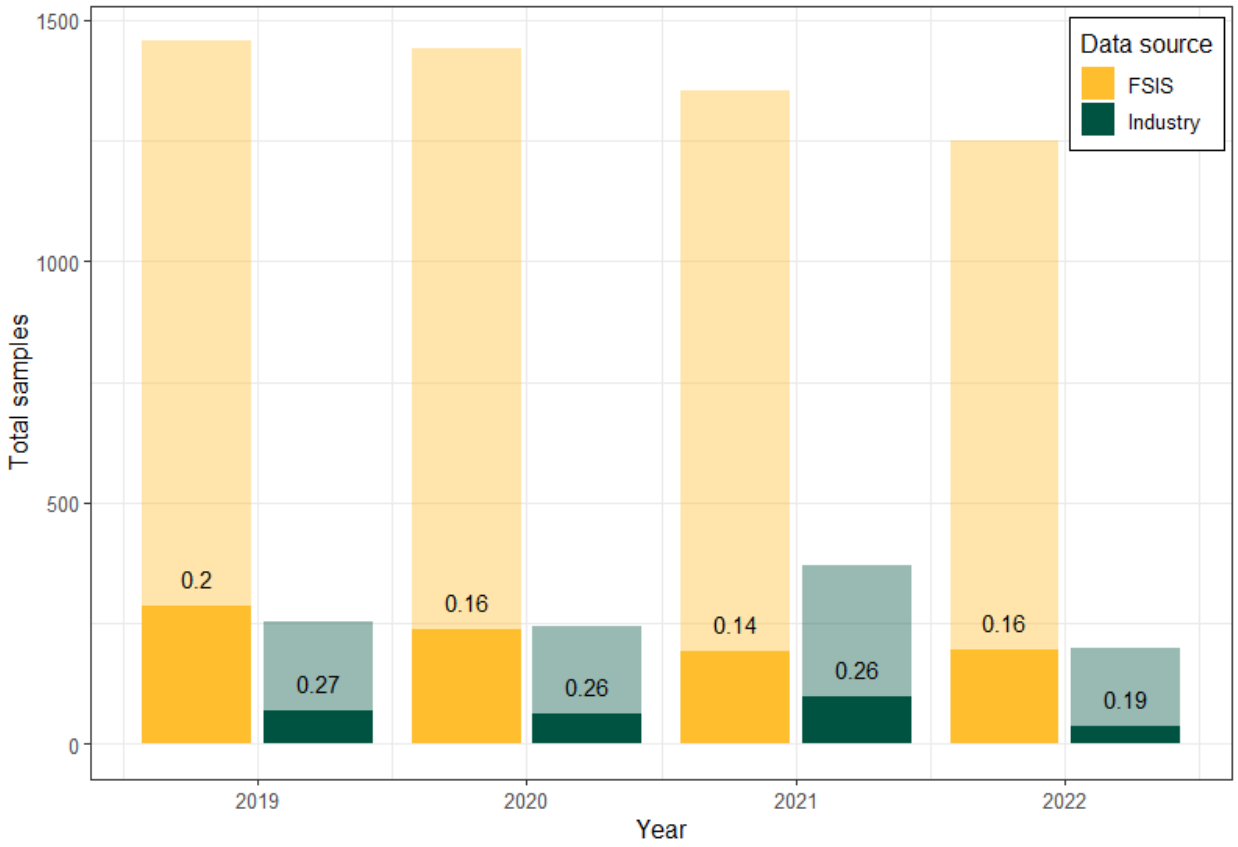


Figure 49: Bar chart of sampling count (lighter shade) and *Salmonella* positive count (darker shade) for FSIS and industry-collected data during the overlapping timeframe (2019-2022). The numbers indicate the proportion.

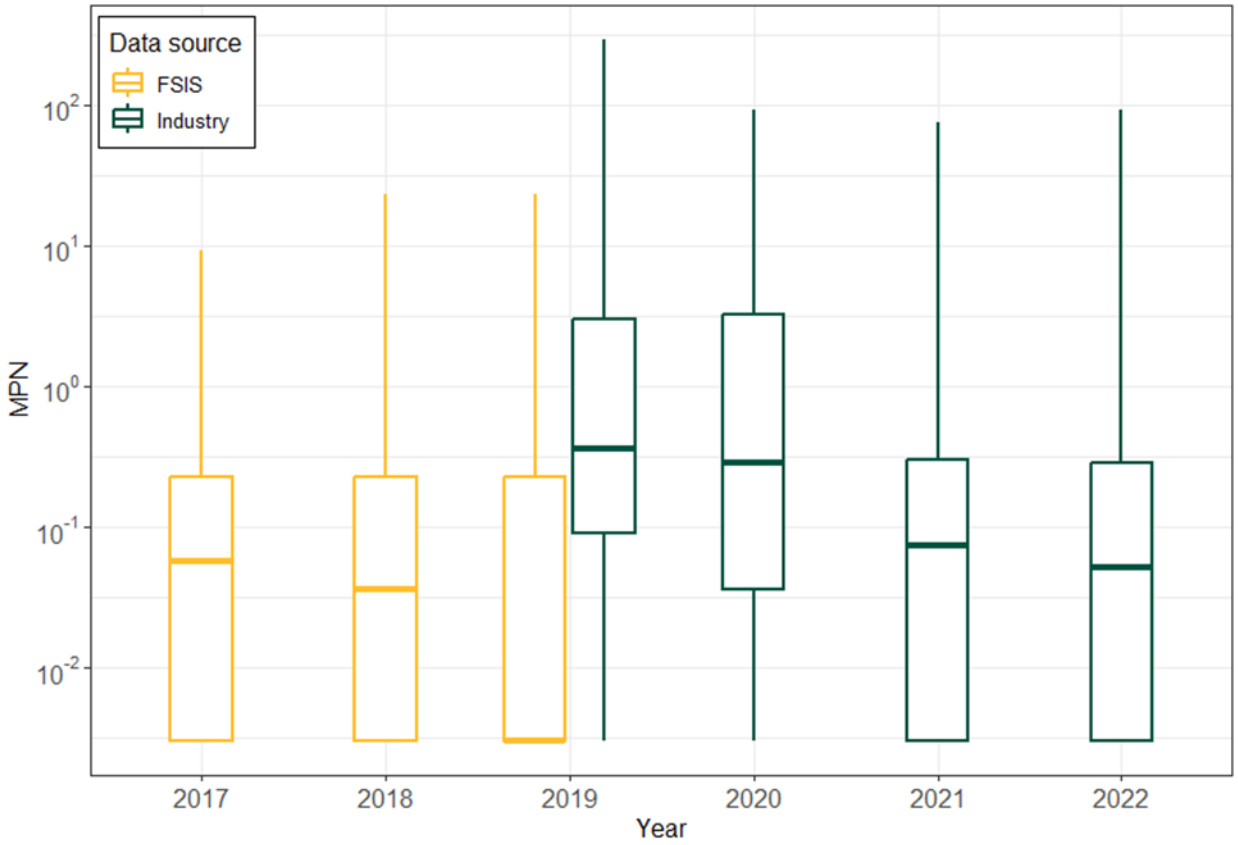


Figure 50: Box plots representing distribution of *Salmonella* MPN data annually from 2017-2022 for FSIS-collected (represented in yellow) and industry-collected ground turkey samples (represented in green). Any presumptive *Salmonella* positives with missing MPN values were assumed as < 1 cfu/325 g for visualization.

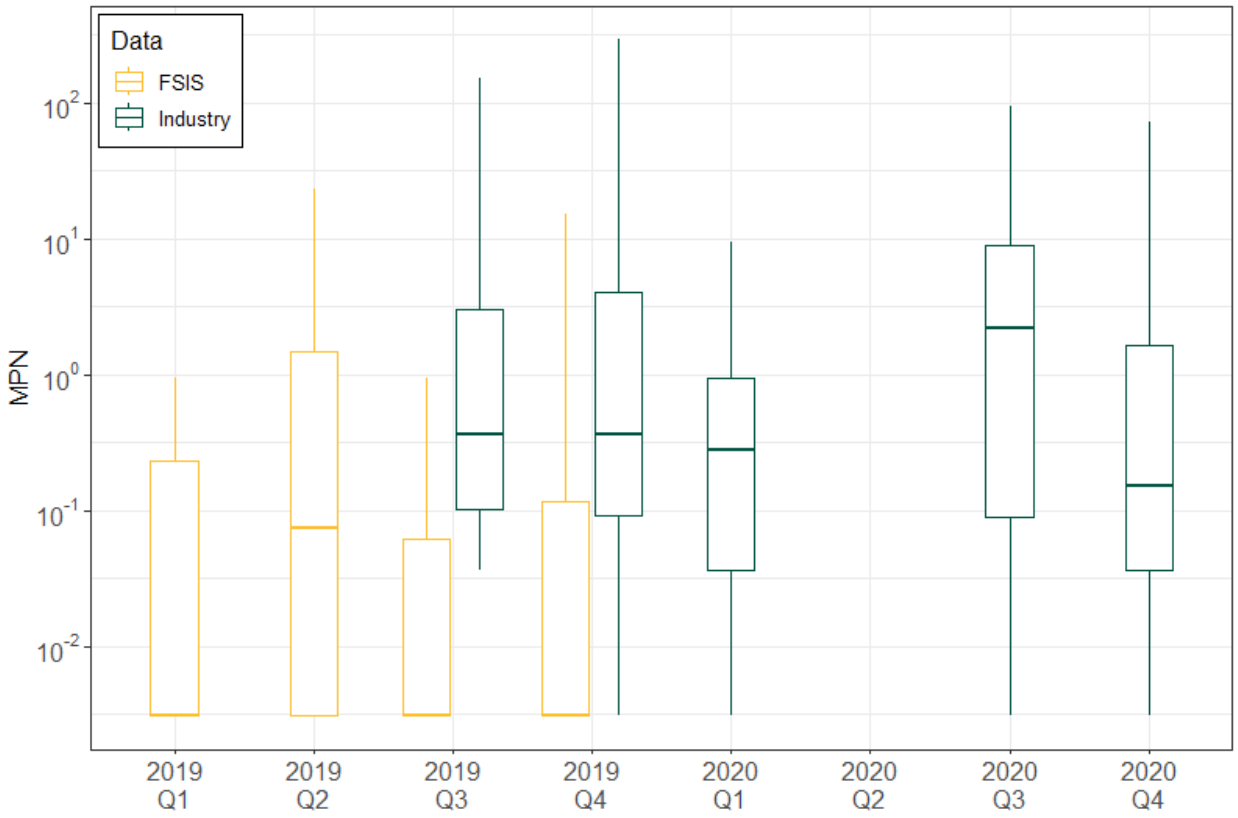


Figure 51: Box plots representing quarterly distribution of *Salmonella* MPN levels from quarter 1, 2019 through quarter 4, 2020 for FSIS (represented in yellow) and industry (represented in green). Any resumptive *Salmonella* positives with missing MPN values were assumed as < 1 cfu/325 g for visualization.

Risk Assessment Scenario Analysis: Public Health Impact Using Industry Data

FSIS conducted a preliminary scenario analysis of the predicted public health impact of establishing a 10 cfu/g threshold level for comminuted turkey using industry data shared by UMD-JIFSAN.

To describe the *Salmonella* contamination rate of the overall comminuted turkey industry, it is necessary to volume-weight the industry data, as shared by UMD-JIFSAN, and use this to generate a volume-weighted lognormal distribution. In the absence of this metadata, the scenario presented here is limited to the comparison of the industry-collected data with different contamination distributions, as in the final product standards model's sensitivity analysis described in **Section 5.6** .

This analysis relied on specific assumptions about the production volume of the industry-collected *Salmonella* data for comminuted turkey. Scenarios with initial contamination with a larger mean (mu) and larger standard deviation (sigma) than the baseline (i.e., estimated contamination distribution from FSIS data) were considered to best capture a scenario where the industry-collected data, with its higher *Salmonella* positive rate and right-shifted MPN—reflective of higher *Salmonella* levels—are representative of the entire comminuted turkey industry.

FSIS' preliminary analysis showed a slightly higher reduction in *Salmonella* illnesses using industry data compared to FSIS data. As shown in **Figure 52** there is a modest increase in the reduction of illness with this *Salmonella* contamination distribution change, with no more than a 3% greater reduction in illnesses in the

scenario of a 10 cfu/g *Salmonella* threshold.

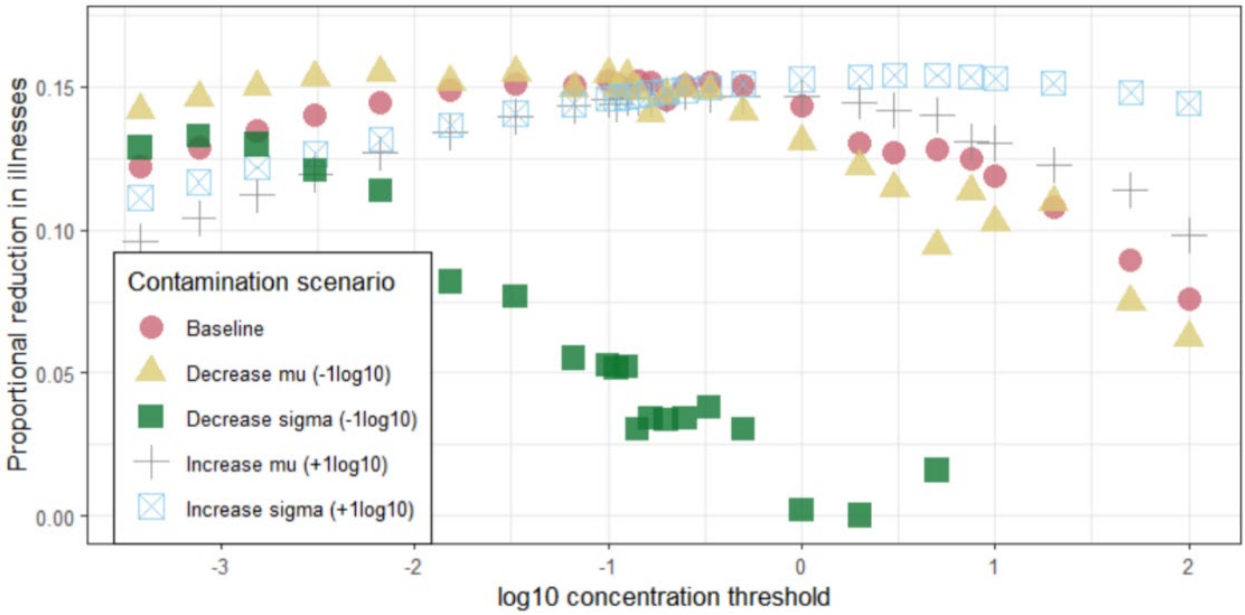


Figure 52: Analyzing the effect of initial *Salmonella* contamination distribution in comminuted turkey adjustments on the predicted reduction in foodborne illness.

This scenario, however, does not align with existing epidemiological data (i.e., they are outside the bounds of reasonable probability of illness per serving estimates and cannot be validated by existing epidemiological data).

Discussion

The cooperative agreement with UMD-JIFSAN provided a valuable proof of concept for industry data sharing (Stumpf 2023). This effort yielded 4-years of available industry data on *Salmonella* levels in ground turkey, in a secure and confidential manner, for use in this risk assessment. These data were compared to FSIS' data on *Salmonella* occurrence and levels in comminuted turkey. A slightly higher illness reduction is predicted using industry data compared to FSIS data, but the risk of illness per servings using the industry data do not align with the epidemiological evidence. Further information about the representativeness of the industry data is needed.

In general, the industry-collected data exhibited higher *Salmonella* positive rates and levels than FSIS data. Additional metadata further characterizing the industry data could identify the cause of this difference. It could be that the industry-collected data reflected a portion of the industry with higher levels of *Salmonella* in ground turkey or an increasing contamination trend FSIS had not yet identified through its own data. Factors including differences in sample collection timeframes, frequency of sampling and testing during the pandemic, and data summary conventions (e.g., censored data methods) further confound interpretation of the data differences between FSIS and industry-collected data. Direct comparison of data from the same establishment would provide insight and narrow the list of explanatory variables. This suggests the need to further explore the role of an intermediary organization like UMD-JIFSAN in data evaluation and curation to enhance the quality of the industry data that is shared for risk assessment.

While this analysis resulted in epidemiological model outcomes that do not align with the empirical data, additional information on the production volume represented by the industry-collected data could be used to produce more representative *Salmonella* contamination distributions and enhance the utility of industry-

collected data in risk assessment.

There is value to public-private data sharing partnerships and those partnerships are most effective when a protected mechanism for data sharing from a legal perspective exists, the process of deciding on the specific data needs is iterative, and data sharing is motivated by analytical requirements needed to support rulemaking. The findings and lessons learned from the UMD-JIFSAN cooperative agreement can serve as a tangible guide to support additional industry data sharing efforts.