



Human Foods Program

Risk Assessment of Foodborne Illness Associated with Pathogens from Produce Grown in Fields Amended with Untreated Biological Soil Amendments of Animal Origin – Part 1: Preharvest Exposure Assessment

Human Foods Program

Food and Drug Administration

U.S. Department of Health and Human Services

February 2026

Contributors

BSAAO development team

Hao Pang

David Oryang

David T. Ingram

Amir Mokhtari¹

Steven Duret²

Régis Pouillot³

Francisco Garces-Vega³

Yuhuan Chen

BSAAO project advisors

Jane Van Doren

Sherri Dennis

Acknowledgements

We thank Samir Assar and Mike Mahovic for sharing their expertise and providing invaluable feedback during the development of this work. We also thank Versar, Inc. for coordinating the external peer reviews, and the experts who participated in the external peer reviews of the risk assessment model.

¹Former FDA employee

²Former FDA ORISE fellow

³Contractor with FDA

Table of Contents

Contributors	2
Table of Contents	3
1. INTRODUCTION	5
2. METHODS	8
2.1 Field specifications and agricultural timeline	10
2.2 Prevalence and concentration of pathogens in untreated BSAAOs	11
2.3 Manure application and initial contamination condition in amended soil	14
2.4 Pathogen survival in amended soil	15
2.5 Transfer of pathogens from amended soils to crops.	18
2.6 Pathogen survival on produce crops grown in the field	20
2.7 Scenario analysis	22
2.7.1 <i>Baseline model with composted manure</i>	22
2.7.2 <i>Impact of time intervals between application of untreated BSAAO and harvest</i>	23
2.7.3 <i>Potential impact of runoff on pathogen contamination on crops</i>	24
2.7.4 <i>STEC non-O157 contamination on lettuce</i>	24
2.7.5 <i>Pathogen presence on produce that grows in the ground and on the ground</i>	25
2.8 Uncertainty analysis	28
2.8.1 <i>Initial contamination conditions</i>	30
2.8.2 <i>Survival model for pathogens in amended soils</i>	30
2.8.3 <i>Pathogen transfer models and splash radius</i>	32
2.8.4 <i>Survival rate for pathogens on crops</i>	32
3. RESULTS and DISCUSSION	34
3.1 Pathogen survival in amended soils	34
3.2 Pathogen survival on crops	38
3.3 Pathogen contamination on crops at the time of harvest	42
3.3.1 <i>Leafy greens</i>	42
3.3.2 <i>Produce that grows in the ground and on the ground</i>	56
3.4 Uncertainty analysis	60
3.5 Summary and conclusions	66
REFERENCES	68
Appendix A: Analysis of pathogen prevalence and concentration data from the manure surveys	77

Appendix B: Field trial for survival of <i>Escherichia coli</i> in chicken and rabbit feces.....	106
Appendix C: Development and performance evaluation of the transfer models quantifying the pathogen transfer from amended soils to produce crops via splash during rainfall or irrigation	110
Appendix D: Adjustment to the Bardsley et al. (2021), Murphy et al. (2024), and Franz et al. (2011) survival data for Weibull model derivation in uncertainty analysis for pathogen survival in amended soils.	112
Appendix E: Summary of dataset included and excluded in the analysis for survival of pathogens on lettuce grown in the field	119
Appendix F: Distribution of predicted pathogen concentration on lettuce heads from field amended with untreated BSAAO at the time of harvest by region and growing season.....	124
Appendix G: Additional results for predicted concentrations of <i>Salmonella</i> on lettuce.	127
Appendix H: Results for uncertainty analysis.	128

1. INTRODUCTION

Biological soil amendments of animal origin (BSAAOs) are biological soil amendments which consist, in whole or in part, of materials of animal origin (21 CFR 112.3). Untreated BSAAOs, such as raw bovine manure and raw poultry manure, are potential sources of human pathogens (e.g., *Escherichia coli* O157:H7 and *Salmonella*), which can be introduced into the production environment. Once introduced into the produce growing environment through soil amended with untreated BSAAO, human pathogens have been shown to survive for extended periods (Jiang et al., 2002; Islam et al., 2004a; Islam et al., 2004b). The rate of pathogen population decline over time in amended soils can be influenced by environmental factors such as soil moisture, temperature, and precipitation (Moynihan et al., 2013; Pang et al., 2020; Litt et al., 2021). Pathogen survival in amended soils is also dependent upon agricultural factors such as manure type, soil type, and irrigation regimen (Limoges et al., 2021; Bardsley et al., 2021). A recent study also found variability among the survival and die-off of different pathogen strains in BSAAO-amended soils (Bardsley et al., 2021).

Pathogens in amended soil can contaminate produce growing in the field through various mechanisms, including splash from irrigation or rainfall (Cevallos-Cevallos et al., 2012a; Cevallos-Cevallos et al., 2012b). Recent field trials have investigated the transfer of pathogens from animal fecal material onto lettuce plants (Atwill et al., 2015; Weller et al., 2017a; Jeamsripong et al., 2019). These studies indicated that pathogen transfer onto crops can occur under specific conditions, and the likelihood and rate of transfer can be affected by factors such as distance to fecal deposit and amount of irrigation water applied (Weller et al., 2017a; Jeamsripong et al., 2019). Recent field trials have also investigated the survival kinetics of

pathogens on the surface of produce under field conditions (Atwill et al., 2015; Chase et al., 2017; Weller et al., 2017b; Chase et al., 2019; Jearnsripong et al., 2019).

Produce growers generally amend their soils with untreated BSAAO for the purposes of enhancing and maintaining soil health as well as meeting crop nutrient demand. The feasibility for a farm to use untreated BSAAOs as a primary nutrient source depends on numerous factors, including availability, cost, and restrictions such as those from third party market agreements and whether there is required time interval(s) between application and harvest. The U.S. Food and Drug Administration (FDA), via the Produce Safety Rule, deferred action on an appropriate minimum application interval (or intervals) for untreated BSAAOs used in a manner that does not contact covered produce during application and minimizes the potential for contact with covered produce after application while the Agency pursued certain steps, including a risk assessment and research to supplement the science on an appropriate interval(s).

FDA is conducting a risk assessment to quantify the potential for human illness associated with the consumption of produce grown in soils amended with untreated BSAAO. FDA recently published models were incorporated into the risk assessment as integral components to describe survival of pathogens in soils amended with untreated BSAAO (Pang et al., 2020), and cross-contamination, pathogen die-off, and potential growth during processing, storage, and transportation (Mokhtari et al., 2018; Mokhtari et al., 2022). This risk assessment also considers available data and information on other relevant steps in the produce food safety continuum including: the initial prevalence and levels of pathogens in untreated BSAAO; pathogen transfer to and subsequent survival on produce grown in amended soils; consumption; and dose response. The BSAAO risk assessment will evaluate the impact of time intervals between application of untreated BSAAO and crop harvest, on the predicted risk of human

illness. This current document serves as part 1 of the two-part risk assessment report and focuses on preharvest exposure assessment. Other components of the risk assessment including postharvest exposure assessment, dose response, and risk characterization are provided in part 2 of the report.

2. METHODS

The BSAAO risk assessment model used lettuce as the primary commodity and evaluated the impact of different time intervals between application of untreated BSAAO and harvest (hereafter referred to as application intervals) at 45 days, 60 days, 90 days, and 120 days. Shiga-toxin producing *E. coli* (STEC, including STEC O157 and STEC non-O157 strains) in untreated bovine manure and *Salmonella* in untreated poultry manure were considered as the sources of contamination. Pathogen transfer via splash during irrigation and/or rainfall was modeled as a primary route of pathogen contamination onto produce growing in soils amended with BSAAO. A conceptual framework of the model to predict preharvest pathogen contamination on lettuce associated with application of untreated BSAAO is provided in Fig. 1. Modeling components included: the initial prevalence and levels of pathogens in untreated BSAAO; pathogen population dynamics in soils amended with untreated BSAAO; pathogen transfer to produce grown in amended soils; and pathogen population dynamics on produce surfaces. Published scientific literature was the primary source of data and information used to populate the model. FDA previously developed a Federal Register Notice (81 FR 11572, March 4, 2016) requesting scientific data, information, and comments that would assist the development of a risk assessment for produce grown in fields or other growing areas amended with untreated BSAAO (Docket number: FDA-2016-N-0321). FDA hosted a technical forum (February 2017) for scientists whose produce safety research was funded by the FDA as well as interested FDA staff research scientists, risk analysts, and technical and policy subject matter experts. We reviewed the received comments and relevant scientific data and information were considered during the development of the risk assessment model. Additional data from FDA commissioned studies that have not yet been published were also included, and relevant data and information about the

studies are provided in the Appendix. Detailed descriptions of data, equations, and assumptions used in the model are provided in the following sections.

The BSAAO risk assessment model was written in the open-source language R Version 3.5.2. The model code was written to be launched on parallelized processors using a high-performance computing cluster (Human Foods Program, FDA, College Park, MD).

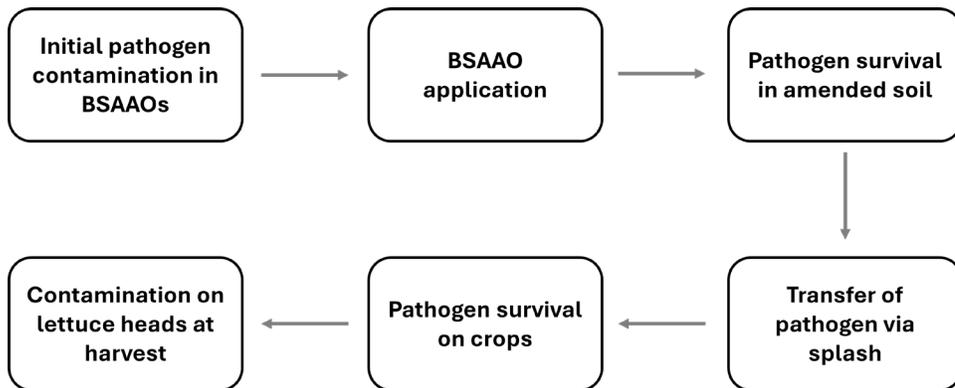


Fig. 1. Conceptual model to predict preharvest contamination of pathogens on lettuce heads associated with application of untreated BSAAO.

2.1 Field specifications and agricultural timeline

Lettuce production was modeled with virtual lettuce field specifications and growing practices created based on field studies (Atwill et al., 2015; Chase et al., 2019; Weller et al., 2017b; Jiamsripong et al., 2019) designed to represent certain commercial operations in the U.S. Fig. 2 illustrates the layout of the virtual lettuce fields. Specifically, the fields are 40 m wide \times 100 m long and are approximately 1 acre in size. Each field consisted of 40 beds, 0.6 m wide, separated by furrows 0.4 m wide. Within each bed, two rows of lettuce were spaced in parallel rows 0.3 m apart and lettuce plants were 0.4 m apart within each row. Given these specifications, each virtual field contains 80 rows and 250 plants per row, totaling 20,000 lettuce plants. Overhead sprinklers are spaced around the field with 10 m between sprinklers. Time for growth of lettuce plants (between planting and harvest) was assumed to be 45 days and simulated overhead irrigation occurred at intervals of 5 to 7 days with an intensity of 11 to 25 mm (Atwill et al., 2015; Chase et al., 2019; Weller et al., 2017b; Jiamsripong et al., 2019).

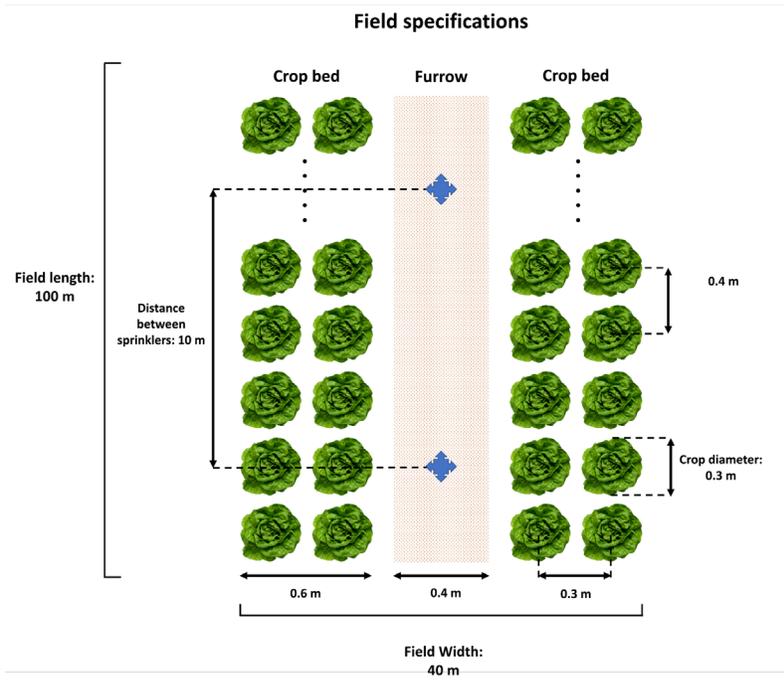


Fig. 2. Layout of the virtual lettuce fields.

2.2 Prevalence and concentration of pathogens in untreated BSAAOs

Data from recent FDA commissioned manure survey studies (Jay-Russel et al., 2018; Jay-Russel et al, 2023; Gartley et al., 2018; Baker et al., 2019; Dunn et al., 2022; Litt et al., 2025) were compiled to describe STEC O157 prevalence and concentration in untreated bovine manure and those of *Salmonella* in untreated poultry manure. Reported prevalence of positive samples varied greatly by region. For example, Dunn et al. (2022) reported an overall *Salmonella* prevalence of 6.7% (39 out of 490 samples) from poultry litter samples collected from southern U.S. states, whereas another study following the same sampling design and testing protocol reported that 28.6% of poultry litter samples collected from western U.S. states California and Arizona were positive for *Salmonella* (Jay-Russell et al., 2018). Therefore, separate analyses were performed using data from the three U.S. regions where manure survey studies were conducted: west, south, and Mid-Atlantic. For Mid-Atlantic region, prevalence of STEC O157 was set to be 0.6% (i.e., 1/161) as none of the 161 samples were found positive for STEC O157, representing a conservative assumption. Further analysis of the data from the abovementioned survey studies indicated significant clustering effect of positive samples and we performed additional data analysis following the approach reported in Jay-Russell et al. (2023) to take into account the clustering effect (see Appendix A). We also considered the potential seasonality of pathogen prevalence in untreated BSAAOs. Pathogen prevalence data were categorized by sampling season (winter-spring from December to May vs. summer-fall from June to November) based on the date when samples were collected. Then considering the clustering effect, the impact of sampling season on pathogen prevalence in manure was evaluated using the Donner and Rao-Scott test. If the impact of sampling season on prevalence was significant (Table 1), separate distributions were derived to describe prevalence for each season. Table 2 summarizes

the generated distributions that best represent the prevalence of pathogens in manure piles from each region considering seasonality.

Table 1. Impact of sampling season on prevalence of pathogens in untreated BSAAO.

Region	Pathogen	<i>p</i> – values ¹
West	<i>E. coli</i> O157:H7	0.036, 0.001
West	<i>Salmonella</i>	0.350, 0.356
South	<i>E. coli</i> O157:H7	0.183, 0.142
South	<i>Salmonella</i>	0.0001, 0.0002
Mid-Atlantic	<i>E. coli</i> O157:H7	NA ²
Mid-Atlantic	<i>Salmonella</i>	0.005, 0.006

¹*p*-value for the Donner test and the Rao-Scott test, where $p \leq 0.05$ indicates a significant difference in pathogen prevalence between the summer-fall and the winter-spring growing seasons.

²None of the samples were positive for *E. coli* O157:H7.

Table 2. Derived distributions for prevalence of pathogens in piles of untreated BSAAO.

Region	Pathogen	BSAAO type	Prevalence	Distribution	Reference
West	<i>E. coli</i> O157:H7	Bovine manure	Summer-fall: 23.3% Winter-spring: 9.7%	Summer-fall: Beta(0.070, 0.486) Winter-spring: Beta(0.19, 10.201)	Jay-Russell et al., 2018; Jay-Russell et al., 2023
West	<i>Salmonella</i>	Poultry manure	52.9%	Beta(0.231, 0.571)	Jay-Russell et al., 2018; Jay-Russell et al., 2023
South	<i>E. coli</i> O157:H7	Bovine manure	56.7%	Beta(0.534, 2.227)	Baker et al., 2019
South	<i>Salmonella</i>	Poultry manure	Summer-fall: 55.6% Winter-spring: 14.8%	Summer-fall: Beta(0.546, 1.760) Winter-spring: Beta(0.110, 2.611)	Dunn et al., 2022
Mid-Atlantic	<i>E. coli</i> O157:H7	Bovine manure	<0.6%*	NA	Gartley et al., 2018; Litt et al., 2025
Mid-Atlantic	<i>Salmonella</i>	Poultry manure	Summer-fall: 88.2% Winter-spring: 60.0%	Summer-fall: BetaBinomial(0.572, 0.346) Winter-spring: BetaBinomial(0.284, 0.666)	Gartley et al., 2018; Litt et al., 2025

*Estimation based on 0 positive out of 161 samples.

Concentrations of pathogens in positive manure samples also varied by region, ranging from -1.42 to 3.83 log₁₀ MPN/g and from -1.05 to 5.45 log₁₀ MPN/g for STEC O157 and *Salmonella* respectively. The relationships between the prevalence of positive samples and the levels of pathogens in the positive samples were investigated following the approach described in Jay-Russell et al. (2023). Seasonality in concentrations of pathogens in positive manure samples was evaluated and no significant difference was found between concentrations in samples collected from summer-fall season vs. winter-spring season; thus, we used the same concentration distribution for the same region regardless of differences in prevalence. For each dataset from the specific regions, if significant correlations between the prevalence of positive samples and the concentration in positive samples existed, normal distributions were derived to describe the concentration of pathogens where the mean of the normal distribution was determined with consideration of prevalence (Table 3). Otherwise, an empirical distribution was derived based on observations from the specific region. For Mid-Atlantic region, since none of the 161 samples were found positive for STEC O157, we assumed that STEC O157 levels in this region were the same as the levels in the other two regions, and the distribution of STEC O157 levels for this region was estimated using an empirical distribution based on concentration data collected from west and south regions (Table 3).

Table 3. Derived distributions for concentration of pathogens in untreated BSAAO.

Region	Pathogen	BSAAO type	Distribution or value (log ₁₀ MPN/g)	Reference
West	<i>E. coli</i> O157:H7	Bovine manure	lognormal(-0.78+1.74×prevalence, 1.16)	Jay-Russel et al., 2018; Jay-Russel et al., 2023
West	<i>Salmonella</i>	Poultry manure	Empirical distribution: Mean value: 0.64	Jay-Russel et al., 2018; Jay-Russel et al., 2023
South	<i>E. coli</i> O157:H7	Bovine manure	Empirical distribution: Mean value: -1.42	Baker et al., 2019
South	<i>Salmonella</i>	Poultry manure	Empirical distribution: Mean value: 2.32	Dunn et al., 2022
Mid-Atlantic	<i>E. coli</i> O157:H7	Bovine manure	Empirical distribution: Mean value: -0.87*	Gartley et al., 2018; Litt et al., 2025; Jay-Russel et al., 2018; Jay-Russel et al., 2023; Baker et al., 2019
Mid-Atlantic	<i>Salmonella</i>	Poultry manure	lognormal(-1.01+4.47×prevalence, 2.31)	Gartley et al., 2018; Litt et al., 2025

*Estimation based on reported concentration from west and south regions.

2.3 Manure application and initial contamination condition in amended soil

In the BSAAO risk assessment model, lettuce fields were divided into 0.1 m × 0.1 m field grids (totaling 400,000 grids per field) to characterize the spatial distribution of microbial pathogens within the growing field environment. In our model simulation, 10,000 kg of raw cattle or poultry manure were applied onto the 40 m × 100 m fields (i.e., an application rate of approximately 10,000 kg/acre) (Gravuer, 2016). Based on the prevalence of pathogens in the raw manure (Table 2), each field grid is designated as either contaminated or not contaminated:

$$n_{cgrid} = binomial(n_{grid}, P_{BSAAO}) \quad \text{Equation (1)}$$

where n_{cgrid} is the number of contaminated field grids; n_{grid} is the total number of grids within a field; and P_{BSAAO} is the prevalence of pathogens in raw manure. The amount (g) of raw manure in each field grid (g_{grid}) after application is calculated as:

$$g_{grid} = Poisson(n_{grid}, g_{mgrid}) \quad \text{Equation (2)}$$

where g_{mgrid} is the average amount of raw manure in a field grid (10,000 kg/400,000 = 25 g). The number of pathogen cells in a contaminated field grid is then calculated as:

$$C_{grid} = g_{grid} \times 10^{C_{BSAAO}} \quad \text{Equation (3)}$$

where C_{grid} is the number of pathogen cells in a contaminated field grid (CFU); C_{BSAAO} is the concentration of pathogens in raw manure (\log_{10} CFU/g).

2.4 Pathogen survival in amended soil

The survival model used in this risk assessment to estimate the survival of STEC O157 in soils amended with untreated BSAAO adapted the machine learning predictive model by Pang et al. (2020) that was developed based on data from longitudinal field survival trials conducted by Sharma et al. (2019). The predictive model considered the impact of various agricultural and environmental variables, such as soil amendment application methods (surface or tillage), amendment type (e.g., dairy or poultry manure), season, ambient temperature, precipitation, and soil moisture content, to predict the concentration of *E. coli* O157:H7 in amended soil over time under dynamic field conditions. Environmental data were retrieved from the Soil Moisture Visualizer at the Oak Ridge National Laboratory Distributed Active Archive Center for Biogeochemical Dynamics (ORNL DAAC) (https://daac.ornl.gov/cgi-bin/dsvviewer.pl?ds_id=1366) to calculate the environmental variables needed for the predictive model. Specifically, ambient temperature, precipitation, and soil moisture data for each region were retrieved and used to calculate the environmental variables during the production dates (i.e., from manure application to harvest). Environmental variables used in the survival model are listed in Table 4.

Table 4. Environmental explanatory variables used in the model for survival of *E. coli* O157:H7 in amended soil.

Environmental variables	Unit
Season	
Soil moisture content	%
Ambient temperature	°C
Average ambient temperature over the previous week	°C
Average ambient temperature since previous observation	°C
Precipitation	mm
Average daily precipitation over the previous week	mm
Average daily precipitation since previous observation	mm
Number of rainy days over the previous week	Days
Number of rainy days since previous observation	Days

Bardsley et al. (2021) investigated the survival of 12 different strains of *Salmonella* in soils amended with poultry litter and characterized the impact of strain, soil-type, and irrigation regimen on *Salmonella* survival. In this BSAAO risk assessment, data from the survival trials conducted by Bardsley et al. (2021) were fitted to log-linear and Weibull survival models. Goodness of fit of the two survival models was compared based on the Akaike Information Criterion (AIC) values. Curve fitting using the Weibull models generally resulted in lower AIC values and was therefore chosen to estimate the survival of *Salmonella* in soils amended with untreated BSAAO:

$$N_t = N_0 - \left(\frac{t}{D}\right)^p \quad \text{Equation (4)}$$

where N_0 is the cell count (expressed in \log_{10} CFU/g) at $t = 0$, and D and p are Weibull model parameters. Given the irrigation frequency used in the risk assessment (5-7 days), survival data for three *Salmonella* strains *S. Braenderup*, *S. Meleagridis*, and *S. Newport* that

implemented a weekly irrigation regimen were used for model development. *S. Braenderup*, *S. Meleagridis*, and *S. Newport* strains also showed significantly slower die-off rates compared to other strains evaluated in the survival trials (Bardsley et al., 2021). Therefore, to take into account the strain variability in *Salmonella* survival in amended soils, a total of 6 survival datasets (3 *Salmonella* strains \times 2 soil type) were used for model development, representing worst-case contamination scenarios. These survival datasets were fitted to the Weibull survival model and parameter values were generated for each *Salmonella* strain soil type combination (Table 5). Of note, we fitted all the *Salmonella* survival curves following inoculation in amended soils under daily irrigation (12 strains) or weekly irrigation (3 of the 12 strains) in further analysis to account for strain variability in *Salmonella* survival in amended soils (see additional details in section 2.7.2 and Appendix D).

Table 5. Parameter values for fitted Weibull models of *Salmonella* survival in amended soils.

Soil type	<i>Salmonella</i> Strain	D ¹	p ²
Sandy-loam	Braenderup	1.93±0.51	0.40±0.02
Sandy-loam	Newport	1.05±0.31	0.36±0.02
Sandy-loam	Meleagridis	0.38±0.21	0.25±0.02
Clay-loam	Braenderup	4.00±1.13	0.48±0.04
Clay-loam	Newport	56.36±5.03	0.97±0.05
Clay-loam	Meleagridis	16.69±3.11	0.62±0.05

^{1,2}Values followed by \pm indicate standard error.

The number of pathogens in field grids after manure application were used as the initial levels to calculate pathogen survival in amended soils. A field grid is considered positive if at least 1 CFU of pathogen is present. If the predicted number of pathogen cells in a field grid falls below 1 CFU (corresponds to approx. $-1.4 \log_{10}$ CFU/g considering an average amount of 25g of manure in a field grid), a Bernoulli process is used to determine if the grid becomes negative due

to pathogen die-off based on the predicted number of pathogen cells in the field grid, i.e., Bernoulli(C_{gridj}), where C_{gridj} is the number of pathogen cells (<1 CFU) in the field grid j .

2.5 Transfer of pathogens from amended soils to crops.

Pathogens in amended soils can potentially transfer onto crops grown in the field via splash during irrigation and rain events. Data specific to pathogen transfer from amended soils to crops are scarce. However, several published studies have examined the transfer of pathogens from animal feces in the field to lettuce during irrigation events and reported that factors such as distance from lettuce to fecal pellets and volume of irrigation applied may influence the likelihood of such transfer (Atwill et al., 2015; Weller et al., 2017a; Jeamsripong, 2015; Jeamsripong et al., 2019). In the BSAAO risk assessment model, we assumed that pathogen transfer mechanisms for amended soils are the same as those for animal feces in the field and data from the abovementioned feces transfer studies were used to quantify the number of pathogens transferred from amended soils to crops during irrigation and rainfall events. Specifically, a total of 655 data points were pooled from the studies and transfer coefficients were calculated as the number of pathogens recovered from lettuce immediately after irrigation divided by the number of pathogens in feces before irrigation. For trials where feces were left in the field for a time interval before irrigation occurred, levels were adjusted based on the field trial by Aminabadi et al. (2023) to account for possible changes in pathogen concentration in feces during these time intervals (see data in Appendix B). The overall presence of positive lettuce heads after irrigation was 58% and the average calculated transfer coefficient was 0.035%. Using the `xgboost` package in R, two gradient boosted tree models were developed to predict: (1) the likelihood of a pathogen transfer event (the transfer probability model) and (2) the pathogen transfer coefficients (proportion of pathogens transferred onto lettuce head) during

irrigation and rain events (the transfer coefficient model). The explanatory variables for both models were (1) distance (m) between contamination source and lettuce and (2) amount of irrigation applied/precipitation (mm). Data were split into training and testing datasets to develop the models and model parameters such as number of trees, tree depth, and learning rate were tuned via cross validation. Additional details on model development, training, parameter tuning, and performance evaluations are provided in Appendix C.

In the model, irrigation occurs every 5 to 7 days after planting as described in section 2.1 and raining events are identified using historical precipitation data of the simulated growing season for each region obtained from ORNL DAAC database (https://daac.ornl.gov/cgi-bin/dsviewer.pl?ds_id=1366). Splash radius (i.e., the maximum distance pathogens in a contaminated field grid can travel due to splash) is set to be 3 meters in the risk assessment based on the report by Jeamsripong (2015). During model simulation, pathogen transfer from soil to each lettuce head in the field was estimated when irrigation or rainfall occurred. For each lettuce head, contaminated grids within the 3-meter splash radius were identified and the distance to each contaminated grid was calculated. Then based on the distances to each contaminated grid and irrigation/ precipitation intensity, transfer probability and transfer coefficient were calculated for each contaminated grid within 3 meters of each lettuce head using the aforementioned transfer probability and transfer coefficient models. The number of pathogens transferred from all surrounding contaminated grids within the 3-meter splash radius onto each lettuce head was calculated as:

$$C_{ti} = \sum_{j=1}^{n_{si}} \text{binomial}(C_{0ji}, T_{cji} \times \text{Bernoulli}(T_{pji})) \quad \text{Equation (5)}$$

where C_{ii} is the number of pathogens transferred onto lettuce head i ; n_{si} is the number field grids within the splash radius (3 m) of lettuce i ; C_{0ji} is the number pathogens in field grid j before splash to lettuce i ; T_{cji} is the estimated transfer coefficient for field grid j to lettuce head i ; and T_{pji} is the estimated transfer probability for field grid j to lettuce head i .

2.6 Pathogen survival on produce crops grown in the field

A literature search was conducted to gather information and data to describe the survival of STEC O157 and *Salmonella* on lettuce grown in the field. Datasets were derived from the survival experiments conducted under field conditions while data from controlled environment chamber or greenhouse trials were excluded due to differences in microbial behavior under such experimental conditions. In addition, given the need to model long-term survival of pathogens on lettuce grown in the field (i.e., 45 days between planting and harvest), survival trials with a duration less than 10 days between initial contamination event and last sampling of lettuce were further excluded (see Appendix E for details). Survival data retrieved from the literature search showed a variety of survival patterns, including monophasic die-off (log-linear), biphasic die-off, or curvature. Diversity of STEC O157 survival patterns on contaminated field lettuce was also reported by McKellar et al. (2014). Considering the complexity of survival patterns observed from survival studies, instead of fitting the data to a specific form of survival model, we described pathogen survival on crops using a daily die-off rate (\log_{10} CFU day⁻¹) calculated as:

$$D_r = \frac{C_{p0} - C_{pl}}{DPI} \quad \text{Equation (6)}$$

where D_r is the daily die-off rate (\log_{10} CFU day⁻¹); C_{p0} is the initial number of pathogens on crops (\log_{10} CFU); C_{pl} is the number of pathogens on crops on the last sampling day (\log_{10} CFU); DPI is the number of days post initial inoculation. Given its simplicity, using daily die-off rates

also allows for integration of the crop survival module to other modeling components, especially for the transfer models that calculate pathogen transfer during irrigation or rainfall events that occur during pathogen survival on crops.

Literature data were taken from tables or digitized from graphs using the “digitize” package in R to calculate the daily die-off rates. For survival of STEC O157 on lettuce grown in the field, data sets were retrieved from various trials conducted under a wide range of environmental conditions (e.g., different geographic regions, different lettuce cultivar, and different experimental procedures). Considerable differences in estimation of die-off rates were observed among the studies, the reported daily die-off rates range from 0.01 to 0.5 log₁₀ CFU day⁻¹ with a mean of 0.24 log₁₀ CFU day⁻¹. Empirical distributions based on the reported daily die-off rates retrieved from the literature search was used to describe the die-off of STEC O157 on lettuce grown in the field. On the contrary, only one *Salmonella* survival study met the criteria (i.e., field data with at least 10 days observation) where Islam et al. (2004b) reported an average daily die-off rate of 0.03 log₁₀ CFU day⁻¹ for *Salmonella* on lettuce, a significant slower die-off rate compared to those reported STEC O157. Improved survival (i.e., slower die-off) of *Salmonella* compared to STEC O157 was observed under field or controlled environments in several studies (Stine et al., 2005; Hutchison et al., 2008; Lopez-Velasco et al., 2015; Erickson et al., 2019; Belias et al., 2020). In the model, daily die-off rates for *Salmonella* were calculated based on data from Islam et al. (2004b). Additional scenarios were conducted to evaluate the uncertainty of *Salmonella* survival on crops (see section 2.8.4 and Appendix E for details). A lettuce head is considered positive, if at least 1 CFU of pathogen was present. If the estimated number of pathogens on a lettuce head falls below 1 CFU, a Bernoulli process is used to determine if the lettuce becomes negative due to pathogen die-off based on the predicted number

of pathogens transferred to the lettuce head in the field grid, i.e., Bernoulli(C_L), where C_L is the number of pathogen cells (<1 CFU) on the lettuce head.

2.7 Scenario analysis

2.7.1 Baseline model with composted manure

We estimated the concentration of pathogens on produce crops grown in fields amended with composted manure (i.e., treated BSAAO per 21 CFR §112.54(b)) at the time of harvest. Although human pathogens are known to survive the composting process (Chen et al., 2017; Kim et al., 2010), detection and enumeration of pathogens in finished composts are rarely reported. Ingram et al. (2009) found 6% of finished compost samples were positive for *Salmonella* at levels of at least 0.04 MPN/g (1 cell in 25g). Brinton et al. (2009) reported 6% of the compost samples had detectable STEC O157 and 3% contained *Salmonella* at a level of 0.45 MPN/g. In another study, Shepherd et al. (2010) reported that *Salmonella* was detected in 16.7% (4 out of 24 samples) of the finished compost samples while no STEC O157 was detected. Additionally, STEC O157 and *Salmonella* were not detected from samples in survey studies by Mao et al. (2021), Edrington et al. (2009), and Ramos et al. (2021). Based on these data, an empirical distribution weighted by the number of samples was used to describe the prevalence STEC O157 and *Salmonella* in treated BSAAO and a uniform distribution bounded by the range of reported levels was used to describe the concentrations of STEC O157 and *Salmonella* in treated BSAAO.

We developed a baseline model that implements a 0-day application interval (i.e., BSAAO application occurs on the same day of harvest) (hereafter referred as the zero-day baseline model). A zero-day application interval reflects the current Produce Safety Rule minimum required interval of 0 days for treated BSAAO (21 CFR 112.56) that meets treatment

requirements (21 CFR §112.54(b)). In addition to the zero-day baseline model, we also developed a baseline model that implements a 45-day interval between BSAAO application and harvest, reflecting recommended time interval by the California Leafy Green Products Handler Marketing Agreement (LGMA) regarding use of composted manure for leafy greens (LGMA, 2023).

2.7.2 Impact of time intervals between application of untreated BSAAO and harvest

We evaluated the impact of different application intervals at 0 day, 45 days, 60 days, 90 days, and 120 days using contamination data in untreated BSAAO from each of the three regions where commissioned manure survey studies were conducted (as described in section 2.2). In addition, to account for the potential seasonality in pathogen presence in BSAAOs and pathogen survival in amended soils, two produce growing seasons were simulated for each region: (1) summer fall season where BSAAO application occurs on March 1st; and (2) winter-spring season where BSAAO application occurs on August 1st. Specifically, pathogen prevalence/concentration data (as described in Section 2.2) and environmental data for pathogen survival (as described in section 2.4) for each growing season were used to generate outputs specific to each growing season. Predicted pathogen concentration per lettuce head (calculated as the log₁₀ values of the mean number of pathogens (CFU) across all lettuce heads in a 20,000 lettuce field) at the time of harvest was calculated as the model output for these application interval scenarios and for the baseline models. Predicted pathogen concentration per lettuce head from the untreated BSAAO scenarios with different application intervals were compared with results from the baseline models (i.e., treated BSAAO) to provide an estimate of the impact that application intervals could have on the presence of pathogens on produce from soils amended with untreated BSAAO.

2.7.3 Potential impact of runoff on pathogen contamination on crops

Runoff due to excessive rainfall or extreme weather conditions may carry and spread pathogens from contaminated sites such as manure piles or manured land to surrounding areas. In our model, we evaluated the potential impact of runoff on the estimated concentration of STEC O157 and *Salmonella* on lettuce heads at the time of harvest. For runoff scenarios, runoff occurs immediately after BSAAO application and runoff water evenly distributes pathogens in the amended soils to the entire field (i.e., all soil grids become contaminated at the same level after runoff event). Concentration of pathogens in soil grids after runoff was calculated as:

$$C_{grid} = (A_R \times P_{BSAAO} \times 10^{C_{BSAAO}}) / n_{grid}, \quad \text{Equation (17)}$$

where A_R is manure application rate (10,000 kg); P_{BSAAO} is the prevalence of pathogens in raw manure; C_{BSAAO} is the concentration of pathogens in raw manure (\log_{10} CFU/g); and n_{grid} is the total number of grids within a field.

2.7.4 STEC non-O157 contamination on lettuce

We also estimated concentration of STEC non-O157 on lettuce heads from fields amended with untreated bovine manure using data from the commissioned studies (Jay-Russel et al., 2018; Gartley et al., 2018; Baker et al., 2019). Following a similar approach described in section 2.2, prevalence of STEC non-O157 in untreated BSAAO was described using beta-distributions derived from prevalence data from each of the three regions and concentration of STEC non-O157 in untreated BSAAO was described by either normal distributions or empirical distributions derived from concentration data from each specific region with consideration of potential seasonality. Following a similar approach described in section 2.4, we developed Weibull models for survival of STEC non-O157 in amended soils using data from the study by

Murphy et al. (2024). Specifically, data specific to four STEC non-O157 strains from the survival trials conducted by Murphy et al. (2024) were retrieved and used to derive Weibull model parameters to describe STEC non-O157 survival in amended soils while accounting for variability (see additional details in Appendix D).

To establish baseline models for STEC non-O157 scenarios, prevalence of STEC non-O157 in untreated BSAAO was assigned a value of 4.9% based on the longitudinal study by Ramos et al. (2021) where 2 out of 41 commercial compost samples were found positive for STEC non-O157. Due to the lack of enumeration data for STEC non-O157, the level of STEC non-O157 in untreated BSAAOs was described using the same distribution as mentioned in section 2.7.1, assuming that the level of STEC non-O157 is similar to those of STEC O157 and *Salmonella* in untreated manure.

2.7.5 Pathogen presence on produce that grows in the ground and on the ground

In addition to lettuce as an example produce commodity that grows above the ground, we created two additional sets of scenarios following similar modeling approaches described in sections 2.1 to 2.6 to estimate pathogen presence in onion (as an example of produce that grows in the ground) and fresh-cut cantaloupe (as an example of produce commodity that grows on the ground) from soils amended with untreated BSAAO. For onion scenarios, the model considered STEC O157 in untreated bovine manure as the contamination source and pathogen transfer via direct contact with soil as the route of contamination. A conceptual framework for the onion model is provided in Fig.3. A virtual 1-acre onion field consisting of 40 beds with one row of onions per bed spaced 0.05 m apart was created reflecting typical onion production specifications that yield 80,000 onions per acre (<https://extension.psu.edu/onion-production>). Using the survival model described in section 2.4, concentration/prevalence of pathogens in amended soils

were estimated at the time of harvest. The number of pathogens that can transfer onto onion surfaces through contact with amended soils is not well documented. Islam et al. (2005) sampled onions grown in fields containing contaminated manure compost and sampled 100 g of soil surrounding the onions over time. Based on the reported level (\log_{10} CFU/g) of STEC O157 from onions and surrounding soil samples collected on the same day, we estimated the transfer rate as the ratio of onion sample to soil sample pathogen populations. The average calculated transfer rate was 0.2 (range from 0.08 to 0.33) and a triangular distribution derived based on these values was used to describe the proportion of pathogens transferred from the contaminated soil grids onto the onion surface that comes in direct contact in the model. Pathogen concentration and prevalence in soil grids were described following the same approach described in sections 2.3-2.4.

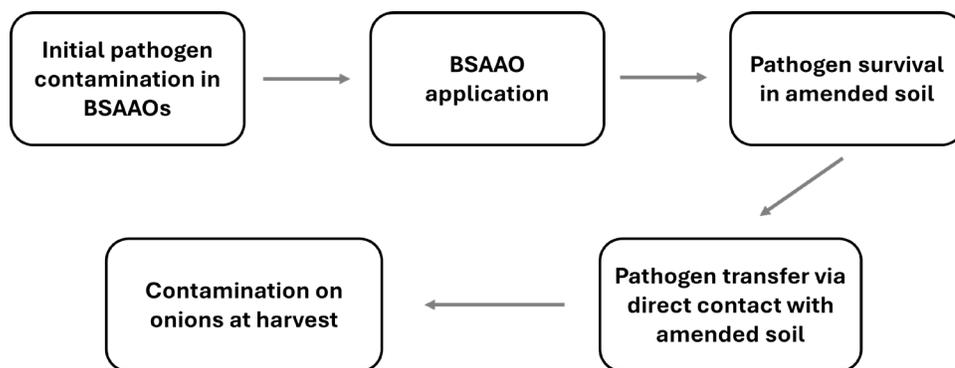


Fig. 3. Conceptual model to predict preharvest contamination of pathogens on onions associated with application of untreated BSAAO.

Similarly, for cantaloupe scenarios, the model considered *Salmonella* in untreated poultry manure as the source of contamination and considered both pathogen transfer from splash during rainfall (splash during irrigation was not modeled as cantaloupes are rarely irrigated using

overhead sprinklers) and pathogen transfer from direct contact with soil during harvest as the routes of contamination. A conceptual framework for the cantaloupe model is provided in Fig. 4. A virtual 1-acre cantaloupe field consisting of 40 beds with one row of cantaloupe spaced 1 m apart was created reflecting a typical cantaloupe production operation that yields 4,000 cantaloupes per acre (<https://www.producebluebook.com/know-your-produce-commodity/cantaloupe/>). Transfer of pathogens from amended soil to cantaloupes were modeled using the same transfer probability and transfer coefficient models described in section 2.5. Subsequent survival of *Salmonella* on cantaloupe surfaces following transfer was estimated based on the average daily die-off rate reported in Stine et al. (2005). Pathogen transfer rate from amended soil to cantaloupes grown on the field is not known. Considering that cantaloupes (grow on the ground) generally have a lower percentage of surface area that comes into direct contact with surrounding soils compared to onions (which grown in the ground), we assumed that the transfer rates for cantaloupes are 1/3 of those for onions. Therefore, a triangular distribution with a most likely value of 0.07, a minimum value of 0.03, and a maximum value of 0.11 was used to describe the proportion of pathogens transferred to cantaloupes from surrounding contaminated soil grids.

For both onion and cantaloupe scenarios, baseline models were developed for treated BSAAO with a 0-day or 45-day interval before harvest. Then various untreated BSAAO scenarios with different application intervals (0-day, 45-day, 60-day, 90-day, 120-day) across the three regions and two growing seasons were simulated and results were compared to the predicted concentration on crops from the baseline models.

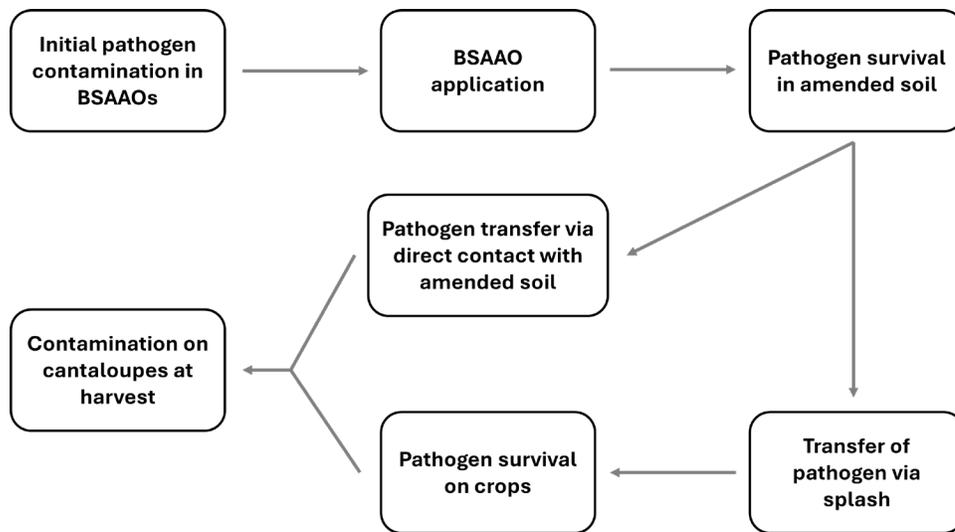


Fig. 4. Conceptual model to predict preharvest contamination of pathogens on cantaloupes associated with application of untreated BSAAO.

2.8 Uncertainty analysis

Table 6 summarizes sources of uncertainties and assumptions in the model. Following the approach described by Pérez-Rodríguez et al. (2017), we conducted uncertainty analyses by assigning an upper-bound value or a lower-bound value (or both) to selected input variables and then rerunning the model to evaluate the impact of such changes on pathogen concentration on produce crops at the time of harvest and the comparison with results from the baseline models. Field specification variables have minimal impact when comparing the estimated concentration on crops associated with untreated BSAAO versus treated BSAAO (as each scenario uses the exact same field specification settings) and were therefore excluded from uncertainty analysis. Variables evaluated in the uncertainty analysis included initial contamination conditions (prevalence and concentration) in untreated BSAAO, survival models for pathogens in amended

soils, pathogen transfer models, splash radius, and pathogen survival rates on crops grown in the field. We estimated the overall model uncertainty range by rerunning the model using the upper-bound or the lower-bound values for all abovementioned variables (based on their expected impact on model output) simultaneously. Analysis of uncertainty for each individual variable was also conducted by rerunning model using the upper or lower-bound value for the tested variable while keeping the original values for other variables. Detailed descriptions for uncertainty analysis are provided in the following sections.

Table 6. Description of sources of uncertainties in the model.

Variables	Description	Model assumptions	Expected impact on model output
Field specifications	Properties of the virtual produce field (e.g., length, width, spacing of crops, BSAAO application rate)	Field specifications are representative of typical produce growing operations in the U.S.	Minimal
Initial concentration	Initial concentration of STEC and <i>Salmonella</i> in untreated BSAAOs	Data from commissioned studies are representative for each region	+
Initial prevalence	Initial prevalence of STEC and <i>Salmonella</i> in untreated BSAAOs	Data from commissioned studies are representative for each region	+
Survival model - amended soils	Survival of STEC O157 and <i>Salmonella</i> in soils amended with untreated BSAAO	Models (Pang et al., 2020; Bardsley et al., 2021; Murphy et al. 2024) are valid to evaluate pathogen survival in soils amended with untreated BSAAO under field conditions	+
Transfer model	Pathogen transfer from soil to crops grown in the field during irrigation or rain events	Pathogen transfer is described using data for animal feces assuming the same mechanisms for amended soils. Pathogen transfer is influenced by irrigation/rain intensity and distance to contamination source. Predictive models are valid to evaluate pathogen transfer from amended soils to crops due to splash.	+
Splash radius	Maximum distance pathogen can transfer from soil to crops due to splash	Maximum splash radius was assumed to be 3 meters based on Jeamsripong et al. (2019)	+
Survival rates on crops	Survival of pathogens on crops grown in the field	Inclusion and exclusion criteria for literature search are appropriate and collected data are representative to estimate decline rates of pathogens on crops under field conditions	+

*”+” indicates a positive correlation with model results, e.g., upper-bound value leads to higher estimates. “-“ indicates a negative relationship between variable and model output, i.e., lower-bound value leads to higher estimates.

2.8.1 *Initial contamination conditions*

For uncertainty analysis of initial prevalence of STEC O157 in untreated bovine manure and *Salmonella* in untreated poultry manure, beta-binomial distribution parameter α for each region was assigned an upper-bound value equals to 200% of its original value (as shown in Table 2) and a lower-bound value equal to 50% of its original value. For Mid-Atlantic region where none of the 161 samples were found positive for STEC O157 and the prevalence was assumed to be 0.6% (i.e., 1/161), an upper-bound value of 1.2% (200% of the original value) and a lower-bound value of 0.3% (50% of the original value) were used.

Initial STEC O157 concentration in untreated bovine manure from west region and initial *Salmonella* concentration in untreated poultry manure from Mid-Atlantic region were described using lognormal distributions with the mean of the lognormal distribution as a function of prevalence in the original model simulations (Table 3). For uncertainty analysis, the slope of the prevalence/concentration function was assigned an upper-bound value equals to the mean estimated slope (as shown in Table 3) plus standard error and a lower-bound value equals to the mean minus standard error. For initial concentration of other pathogen/region combinations described by empirical distributions in the original model simulations (Table 3), upper-bound values were described by empirical distributions derived using values equal to 200% of the original concentration (CFU/g) and lower-bound values were described by empirical distributions derived using values equal to 50% of the original concentration (CFU/g).

2.8.2 *Survival model for pathogens in amended soils*

Survival of STEC O157 in amended soils was estimated using the main survival model from Pang et al. (2020) in the original model simulations. For uncertainty analysis, upper and lower-bound estimates of STEC O157 survival in amended soils were obtained using the upper-

bound and lower-bound models described in Pang et al. (2020). In addition, we also tested the impact of using an alternative modeling approach for STEC O157 survival in amended soils while considering the impact of strain variability. Specifically, we gathered data for survival of five STEC O157 strains in amended soils from the greenhouse study by Murphy et al. (2024) and data for survival of 18 STEC O157 strains from Franz et al. (2011). Gathered survival data were adjusted to account for the potential differences in survival impacted by irrigation regimen as reported in Murphy et al. (2024) (see additional details on survival data adjustments in Appendix D). Then Weibull survival models were developed with parameter values derived from the adjusted survival data from Murphy et al. (2024) and Franz et al. (2011) to predict pathogen contamination on produce crops.

For *Salmonella*, Weibull survival model parameter D (time required for a 90% reduction) was assigned upper-bound values equal to original parameter values (as shown in Table 5) plus the standard error and lower-bound values equal to original parameter values minus the standard error. In the study by Bardsley et al., (2021), survival of a total of 12 *Salmonella* strains were investigated. In the original model simulations, Weibull survival model parameters were based on the Bardsley et al. (2021) survival trials of three particular *Salmonella* strains (i.e., *S. Braenderup*, *S. Meleagridis*, and *S. Newport*) that implemented a weekly irrigation regimen. Bardsley et al. (2021) also investigated survival of a total of 12 *Salmonella* strains under daily irrigation regimen. Among the 12 *Salmonella* strains, *S. Braenderup*, *S. Meleagridis*, and *S. Newport* survival were monitored in both weekly and daily irrigation regimen trials, whereas the survival of nine other strains were observed only in daily irrigation regimen trials. To take into consideration of variability in survival of the other nine *Salmonella* strains observed from daily irrigation regimen trials by Bardsley, we compared the observed survival of *Salmonella* between

trials exposed to daily irrigation and trials exposed to weekly irrigation. Then survival data from daily irrigation trials were adjusted to account for potential differences in survival under a weekly irrigation regimen based on observations from strains that exposed to both daily and weekly irrigation in Bardsley et al. (2021) study (see additional details in Appendix D). Then Weibull model parameters were derived based on the adjusted survival data and were added to the pool of survival model parameters already reported in Table 5 to describe survival of *Salmonella* in amended soils (results based on considering the variability in survival of all 12 *Salmonella* strains are presented as an alternative scenario in the uncertainty analysis, see Table 23 below).

2.8.3 *Pathogen transfer models and splash radius*

We generated prediction intervals for the transfer coefficient model using conformal inference (Lei et al., 2018) implemented in the “probably” R package (Kuhn et al., 2024) and obtained the upper and lower-bound estimates for the number of pathogens transferred from surrounding soil grids onto crops to test the impact of uncertainties in pathogen transfer model on the model outputs. Splash radius (the maximum distance pathogens can travel due to splash) was set to be 3 m in the original model simulations based on observations from Jiamsripong (2015). In uncertainty analysis, we increased the splash radius to 4 m and tested its impact on model outputs.

2.8.4 *Survival rate for pathogens on crops*

Given the lack of studies that met the criteria for inclusion for *Salmonella* survival on crops, we loosened the inclusion criteria described in section 2.6 and included survival data from growth chamber/laboratory studies (Stine et al., 2005; Harapas et al., 2015; Lopez-Velasco et al., 2015; López-Gálvez et al., 2018; Erickson et al., 2019) and field studies with less than 10-day

observations (Erickson et al., 2019; Belias et al., 2020) for uncertainty analysis. An empirical distribution was derived based on the included *Salmonella* survival data with the loosened criteria and was used in the uncertainty analysis to test the impact of *Salmonella* crop survival rate on the predicted pathogen contamination on produce crops.

3. RESULTS and DISCUSSION

Predicted results from the preharvest exposure assessment across different scenarios are provided in the following sections. Key results presented in the report are also available in the interactive FDA BSAAO-Produce Risk Assessment Output Explorer (<https://pub-connect.foodsafetyrisk.org/content/6851ecb5-0122-45b7-af14-bd326ee07e6f>).

3.1 Pathogen survival in amended soils

Mean predicted concentrations of STEC O157 and *Salmonella* in amended soils (with bovine manure and poultry manure, respectively) over time after application of untreated BSAAO are shown in Fig. 5-6. Concentrations in Fig. 5-6 were the log₁₀ values of the average number of pathogens (CFU) across positive field soil grids. Despite varying patterns across three regions and two growing seasons, predicted concentrations of STEC O157 in amended soils generally declined over time between BSAAO application and harvest. Predicted STEC O157 levels in amended soil at the time of harvest decreased with increasing application interval and the longer the interval the larger the decrease (Fig. 5 and Table 7). Larger application intervals (e.g., increasing time between BSAAO application and harvest) allowed pathogens additional time to die-off in amended soils and led to decreased predicted levels and prevalence of pathogens available for transfer during rain or irrigation events. For example, compared to predicted level from a 45-day application interval, an application interval of 60 days allowed STEC O157 to decline from -0.33 log₁₀ CFU/g to -0.92 log₁₀ CFU/g based on results for south region in the winter-spring growing season (Table 9). And application intervals of 90 and 120 days further reduced the predicted level to -1.16 log₁₀ CFU /g and -1.30 log₁₀ CFU/g respectively, a 0.83-0.97 log₁₀ reduction compared to those from a 45-day application interval (Table 7).

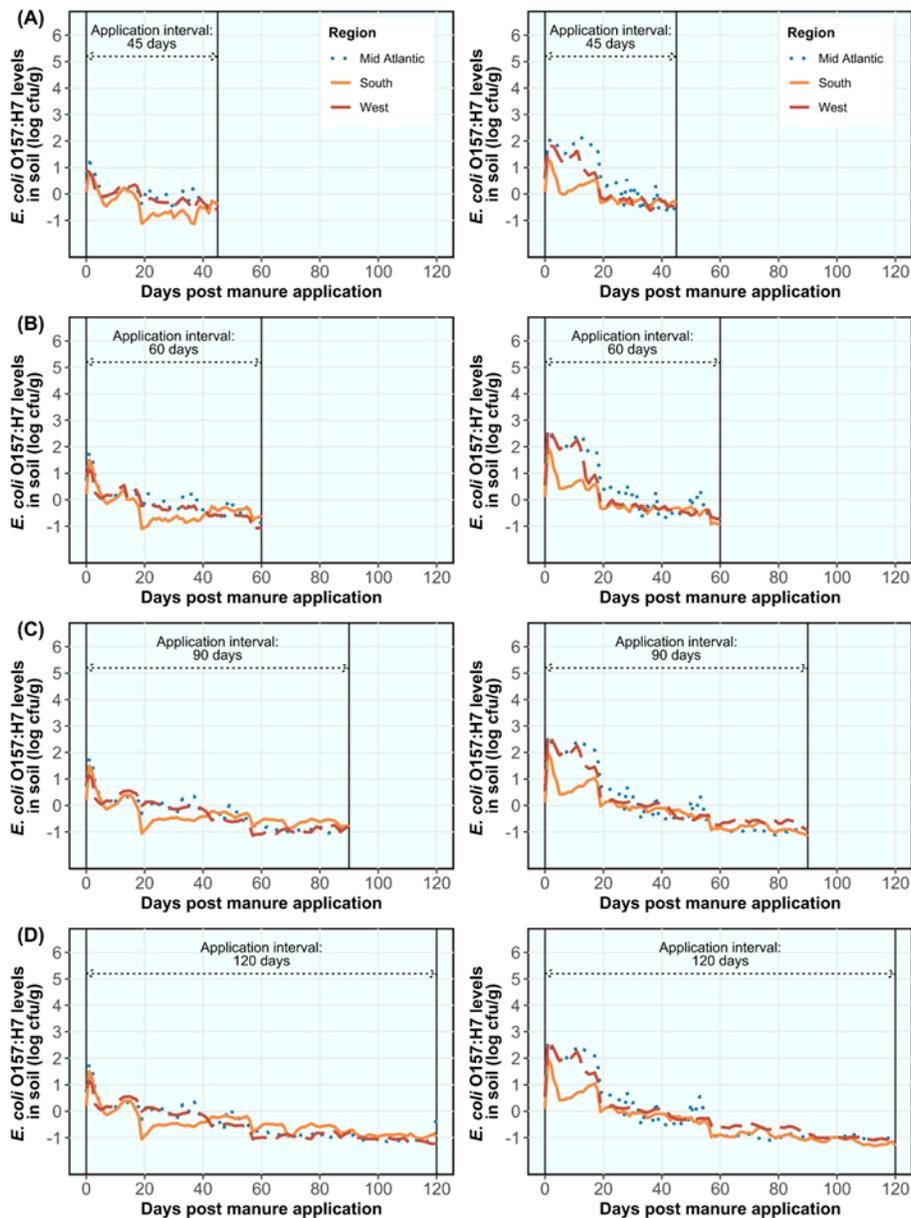


Fig. 5. Predicted mean *E. coli* O157:H7 concentration in amended soils over time during the summer-fall (left) and the winter-spring (right) growing season with an application interval of: (A) 45-day; (B) 60-day; (C) 90-day; and (D) 120-day.

Salmonella concentration in amended soils was also influenced by application intervals, where application intervals of 60, 90, and 120 days reduced the predicted level at the time of harvest by up to 1.35 log₁₀ CFU /g, compared to the predicted levels at an application interval of

45 days. The prevalence of STEC O157 and *Salmonella* contamination in the amended soils (expressed as % of field grids with at least 1 CFU) also decreased with increasing application intervals (from 60 to 120 days), compared to those with an application interval of 45 days (Table 9).

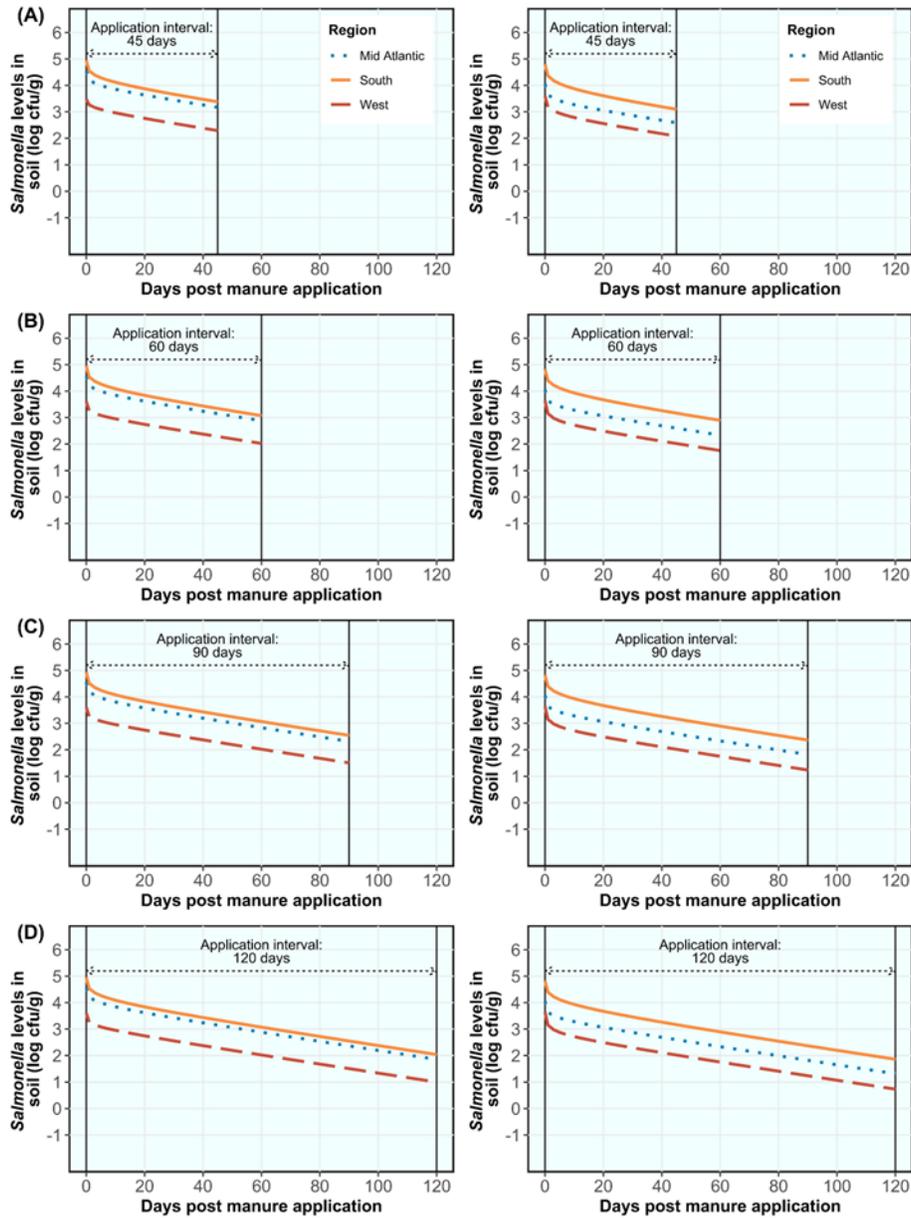


Fig. 6. Predicted mean *Salmonella* concentration in amended soils over time during the summer-fall (left) and the winter-spring (right) growing season with an application interval of: (A) 45-day; (B) 60-day; (C) 90-day; and (D) 120-day.

Table 7. Predicted mean prevalence and level of STEC O157 and *Salmonella* in amended soils at the time of harvest.

Growing season	Region	Application interval (days)	STEC O157 Prevalence (%)	STEC O157 Concentration* (log ₁₀ CFU/g)	<i>Salmonella</i> Prevalence (%)	<i>Salmonella</i> Concentration* (log ₁₀ CFU/g)
Summer- fall	Mid-Atlantic	45	0.41	-0.37	42.9	3.17
Summer- fall	Mid-Atlantic	60	0.40	-0.81	40.2	2.88
Summer- fall	Mid-Atlantic	90	0.39	-0.91	34.7	2.32
Summer- fall	Mid-Atlantic	120	0.36	-1.04	31.6	1.86
Summer- fall	South	45	14.9	-0.40	14.2	3.38
Summer- fall	South	60	14.4	-0.59	13.3	3.07
Summer- fall	South	90	14.4	-0.75	11.0	2.55
Summer- fall	South	120	14.3	-0.96	10.6	2.04
Summer- fall	West	45	11.5	-0.57	11.0	2.29
Summer- fall	West	60	11.3	-0.75	9.0	2.02
Summer- fall	West	90	11.3	-1.06	6.3	1.51
Summer- fall	West	120	11.3	-1.20	4.7	1.00
Winter-spring	Mid-Atlantic	45	0.41	-0.69	16.7	2.59
Winter-spring	Mid-Atlantic	60	0.40	-0.90	14.5	2.34
Winter-spring	Mid-Atlantic	90	0.39	-0.99	12.0	1.82
Winter-spring	Mid-Atlantic	120	0.37	-1.07	9.8	1.31
Winter-spring	South	45	15.4	-0.33	2.1	3.10
Winter-spring	South	60	15.0	-0.92	2.1	2.90
Winter-spring	South	90	14.9	-1.16	1.8	2.37
Winter-spring	South	120	12.3	-1.30	1.7	1.86
Winter-spring	West	45	1.6	-0.49	10.4	2.08
Winter-spring	West	60	1.4	-0.69	8.4	1.75
Winter-spring	West	90	1.4	-0.92	6.5	1.24
Winter-spring	West	120	1.4	-1.08	4.9	0.73

*Calculated as the log₁₀ values of the average number of pathogens (CFU) across all positive field soil grids.

3.2 Pathogen survival on crops

For lettuce, with a 45-day growth period, these BSAAO application intervals translate to planting on day 0 (same day the BSAAO is applied), 15, 45, or 75 days after the BSAAO is applied, respectively. Fig. 7-8 display the predicted mean concentrations of STEC O157 and *Salmonella* on lettuce grown in the field throughout the 45-day growing period. Concentrations in Fig. 7-8 were the \log_{10} values of the average number of pathogens (CFU) across positive lettuce heads in a field. For both STEC O157 and *Salmonella*, concentration on crops generally increased initially after planting due to pathogen transfer from splash events (indicated by spikes observed from the curves shown in Fig. 7 and Fig. 8) and then started declining over time indicating subsequent die-off of pathogens on produce and less pathogen transfer onto crops as pathogen concentrations decreased in amended soils over time (as shown in Fig. 5-6).

Larger application intervals resulted in lower prevalence and concentrations of STEC O157 or *Salmonella* on lettuce at the time of harvest (Table 8). For example, given an application interval of 45 days, STEC O157 was predicted to be present on 0.155% of lettuce heads at an average level of $-0.55 \log_{10}$ CFU/head at the time of harvest based on data from the west region under the winter-spring season, whereas the predicted prevalence and level of STEC O157 on lettuce heads decreased to 0.004% and $-0.83 \log_{10}$ CFU/head, respectively, when application interval increased to 120 days (Table 8). Similarly, predicted prevalence of *Salmonella* contaminated lettuce heads decreased by up to 6.5-fold and predicted level of *Salmonella* on contaminated lettuce heads was reduced by up to $1.4 \log_{10}$ CFU/head at larger application intervals of 60, 90, and 120 days compared to results from scenarios with an application interval of 45 days (Table 8).

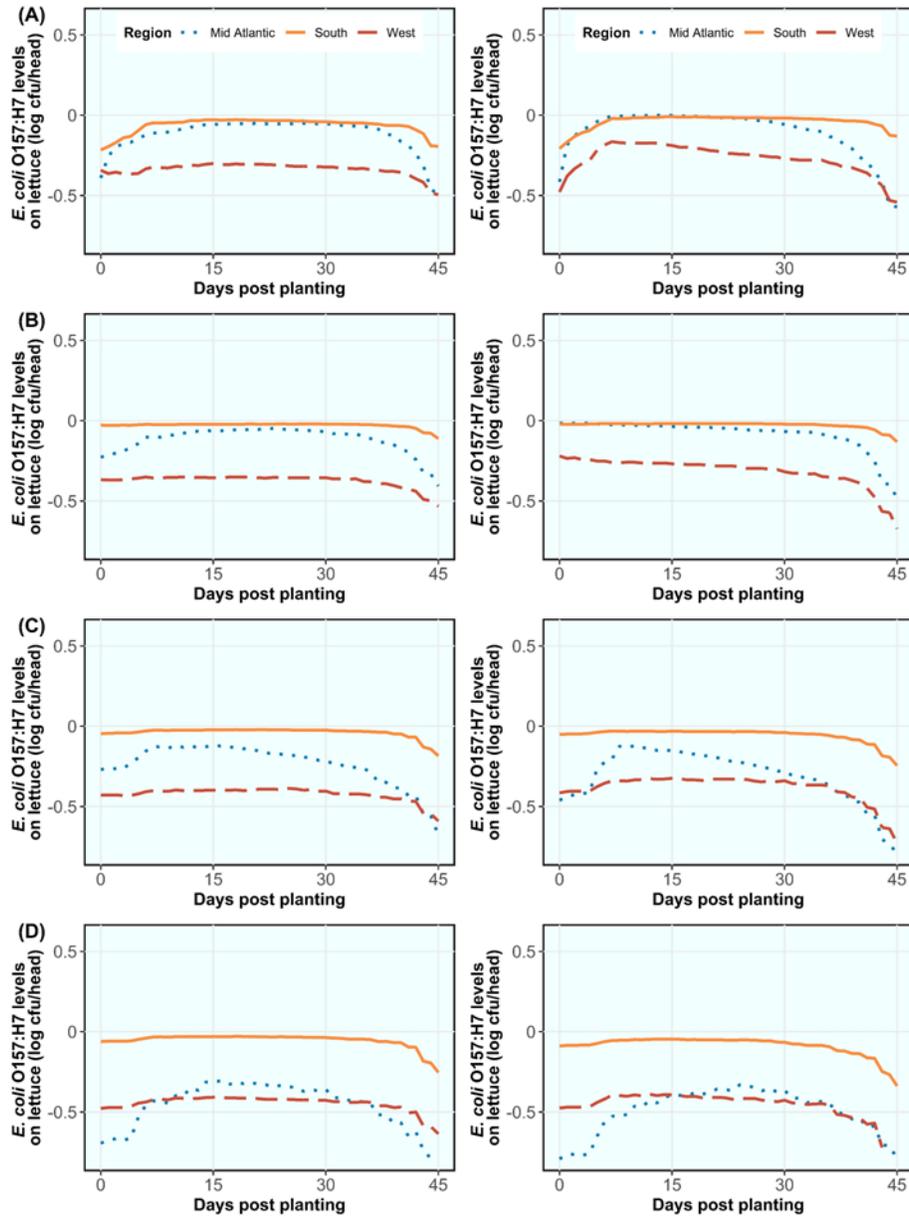


Fig. 7. Predicted mean concentration of *E. coli* O157:H7 on lettuce grown in the field from planting to harvest during the summer-fall (left) and the winter-spring (right) growing season. Time intervals between application of untreated BSAAO and harvest: (A) 45 days; (B) 60 days; (C) 90 days; and (D) 120 days. The x-axis represents the number of days between crop planting and harvest (45 days after crops were planted into the amended field regardless of application interval).

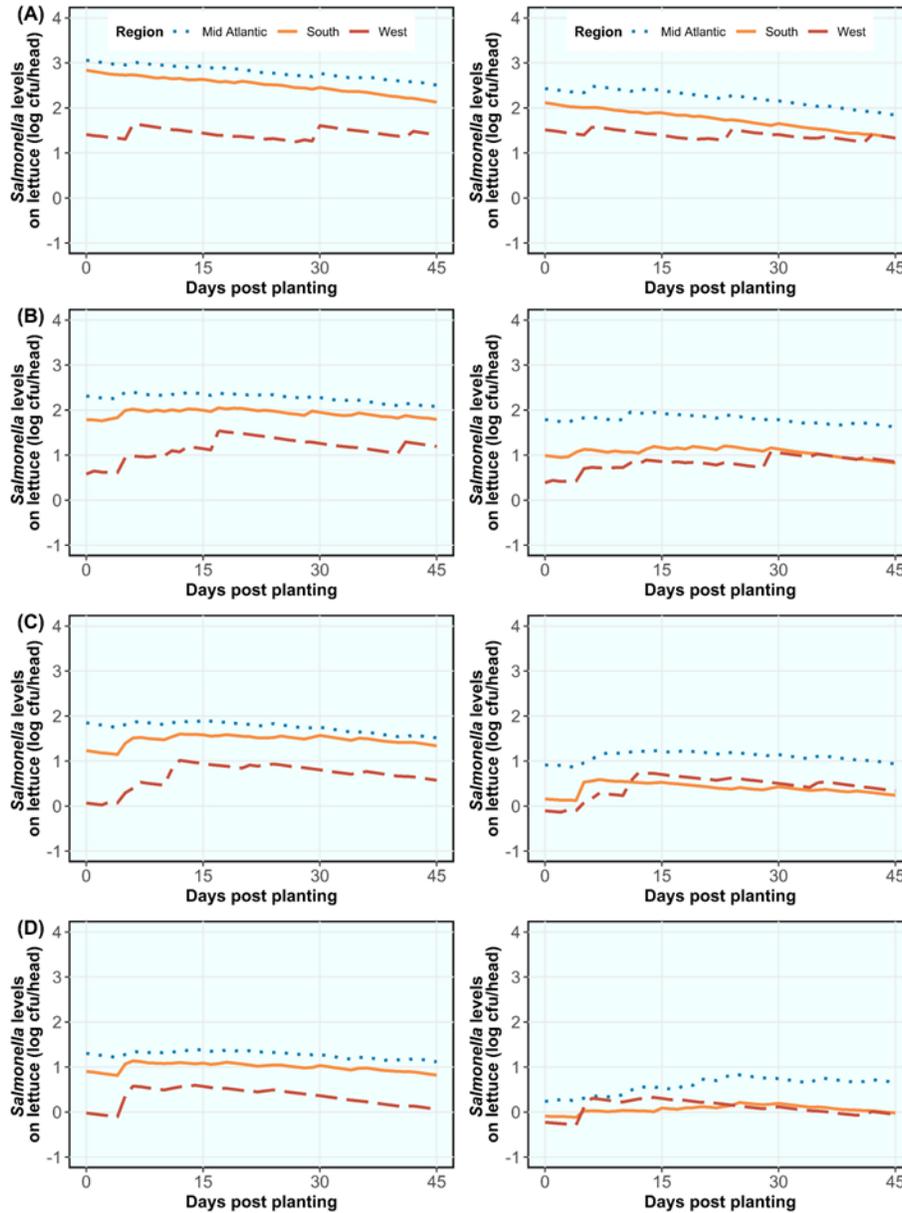


Fig. 8. Predicted mean concentration of *Salmonella* on lettuce grown in the field from planting to harvest during the summer-fall (left) and the winter-spring (right) growing season. Time intervals between application of untreated BSAAO and harvest: (A) 45 days; (B) 60 days; (C) 90 days; and (D) 120 days. The x-axis represents the number of days between crop planting and harvest (45 days after crops were planted into the amended field regardless of application interval).

Table 8. Mean predicted prevalence and level of *E. coli* O157:H7 and *Salmonella* on lettuce at the time of harvest.

Growing season	Region	Application interval (days)	STEC O157 Prevalence (%)	STEC O157 Concentration* (log ₁₀ CFU/+head)	<i>Salmonella</i> Prevalence (%)	<i>Salmonella</i> Concentration* (log ₁₀ CFU/+head)
Summer- fall	Mid-Atlantic	45	0.008	-0.50	48.6	2.51
Summer- fall	Mid-Atlantic	60	0.007	-0.41	35.0	2.08
Summer- fall	Mid-Atlantic	90	0.002	-0.69	24.5	1.52
Summer- fall	Mid-Atlantic	120	0.001	-0.87	17.3	1.12
Summer- fall	South	45	0.069	-0.20	47.4	2.13
Summer- fall	South	60	0.133	-0.11	37.5	1.79
Summer- fall	South	90	0.083	-0.19	24.0	1.34
Summer- fall	South	120	0.057	-0.26	17.0	0.82
Summer- fall	West	45	0.604	-0.51	13.4	1.40
Summer- fall	West	60	0.195	-0.54	5.77	1.19
Summer- fall	West	90	0.065	-0.60	3.64	0.57
Summer- fall	West	120	0.048	-0.65	2.65	0.07
Winter-spring	Mid-Atlantic	45	0.108	-0.58	21.4	1.84
Winter-spring	Mid-Atlantic	60	0.024	-0.49	13.3	1.62
Winter-spring	Mid-Atlantic	90	0.002	-0.82	9.15	0.94
Winter-spring	Mid-Atlantic	120	0.001	-0.81	6.68	0.66
Winter-spring	South	45	0.265	-0.13	22.9	1.33
Winter-spring	South	60	0.181	-0.13	14.6	0.82
Winter-spring	South	90	0.063	-0.25	8.66	0.24
Winter-spring	South	120	0.027	-0.34	5.50	-0.02
Winter-spring	West	45	0.155	-0.55	14.4	1.33
Winter-spring	West	60	0.025	-0.69	6.76	0.85
Winter-spring	West	90	0.008	-0.75	3.94	0.33
Winter-spring	West	120	0.004	-0.83	2.36	-0.05

*Calculated as the log₁₀ values of the average number of pathogens (CFU) across positive lettuce heads in a field.

3.3 Pathogen contamination on crops at the time of harvest

3.3.1 Leafy greens

3.3.1.1 Predicted concentrations of STEC O157:H7 and *Salmonella* on crops at the time of harvest

The mean predicted concentrations of pathogens on lettuce heads from fields amended with untreated BSAAOs at the time of harvest for different application intervals are presented in Table 9. As shown in Table 9, the mean predicted concentrations of STEC O157 on lettuce from fields amended with untreated bovine manure varied by region. For example, with a 0-day application interval and the summer-fall growing season, the mean predicted concentration was $-1.015 \log_{10}$ CFU/head using data from the west region, whereas the mean predicted concentration for south region was -1.931 (Table 9). STEC O157 concentrations on lettuce also varied by growing seasons, especially for west region where pathogen prevalence differed significantly between the summer-fall and the winter-spring (Table 9). Despite the differences across regions and growing seasons, the highest predicted STEC O157 concentrations on lettuce were observed from scenarios with 0-day application intervals (i.e., no time interval between manure application and harvest) and application intervals of 45, 60, 90, and 120 days drastically reduced the predicted STEC O157 concentrations on lettuce at the time of harvest (Table 9). Compared to the mean predicted STEC O157 concentrations with a 0-day application interval, a 120-day application interval provided 1.90 to 1.94 log reduction for the Mid-Atlantic region, 1.31 to 1.64 log reduction for the south region, and 2.29 to 3.42 log reduction for the west region (Table 9).

Regional differences and seasonality in mean predicted pathogen concentrations on lettuce at the time of harvest were also evident for *Salmonella* and untreated poultry manure.

Scenarios using data from the Mid-Atlantic region had the highest mean predicted concentrations and predicted concentrations were generally higher for the summer-fall season especially for Mid-Atlantic and south region where difference in *Salmonella* prevalence between growing seasons in untreated BSAAO was significant (Table 9). Similar to the trend observed from results for STEC O157 and bovine manure, larger application intervals reduced the mean predicted concentrations of *Salmonella* across different regions and growing seasons. Compared to the predictions from 0-day application interval scenarios, a 120-day application interval reduced *Salmonella* concentrations on lettuce by 1.72 to 2.18 log for the Mid-Atlantic region, 1.50 to 2.48 log for the south region, and 1.53 to 1.70 log for the west region (Table 9). Mean predicted *Salmonella* concentrations on lettuce were also generally higher compared to concentrations of STEC O157 at the time of harvest (Table 9).

We also investigated the distribution of predicted pathogen contamination on lettuce heads. Results (see Table F1-F2 in Appendix F) show that distributions of pathogen contamination are highly right-skewed as evidenced by mean prediction being greater than the median prediction and located much closer to the 97.5th percentile than to the 2.5th percentile. This indicates that predicted pathogen concentrations from the majority of simulated lettuce fields are low and simulated lettuce fields that resulted in high predicted concentrations on lettuce are rare.

Table 9. Mean predicted concentrations of STEC O157, *Salmonella*, and STEC non-O157 on lettuce heads from field amended with untreated BSAAO at the time of harvest by region and growing season.

Growing season	Region	Application interval (days)	STEC O157 Concentration* (log ₁₀ CFU/head)	<i>Salmonella</i> Concentration* (log ₁₀ CFU/head)
Summer- fall	Mid-Atlantic	0	-3.047	3.153
Summer- fall	Mid-Atlantic	45	-4.112	2.506
Summer- fall	Mid-Atlantic	60	-4.177	2.082
Summer- fall	Mid-Atlantic	90	-4.672	1.510
Summer- fall	Mid-Atlantic	120	-4.983	1.101
Summer- fall	South	0	-1.931	2.893
Summer- fall	South	45	-2.866	2.128
Summer- fall	South	60	-3.069	1.789
Summer- fall	South	90	-3.148	1.329
Summer- fall	South	120	-3.239	0.788
Summer- fall	West	0	0.043	1.537
Summer- fall	West	45	-1.690	1.390
Summer- fall	West	60	-2.629	1.181
Summer- fall	West	90	-3.154	0.546
Summer- fall	West	120	-3.300	0.006
Winter-spring	Mid-Atlantic	0	-3.047	2.575
Winter-spring	Mid-Atlantic	45	-3.151	1.837
Winter-spring	Mid-Atlantic	60	-3.597	1.620
Winter-spring	Mid-Atlantic	90	-4.770	0.922
Winter-spring	Mid-Atlantic	120	-4.949	0.639
Winter-spring	South	0	-1.931	2.123
Winter-spring	South	45	-2.529	1.323
Winter-spring	South	60	-2.708	0.795
Winter-spring	South	90	-3.192	0.146
Winter-spring	South	120	-3.573	-0.195
Winter-spring	West	0	-2.227	1.537
Winter-spring	West	45	-2.522	1.316
Winter-spring	West	60	-3.576	0.823
Winter-spring	West	90	-4.070	0.278
Winter-spring	West	120	-4.437	-0.163

*Calculated as the log₁₀ values of the average number of pathogens (CFU) across all lettuce heads in a field.

Predicted pathogen concentrations on lettuce at the time of harvest from the baseline models are summarized in Table 10. The zero-day baseline model predicted a mean STEC O157 concentration of $-3.660 \log_{10}$ CFU per lettuce head from fields amended with treated BSAAO. As a comparison, the predicted mean concentration from the 45-day baseline model for STEC O157 increased to $-3.114 \log_{10}$ CFU per head. The increase is likely due to the predicted initial increase in STEC O157 concentration in amended soils following BSAAO application (as shown in Fig 5). Compared to a zero-day interval where BSAAO was applied on the same day of harvest, a 45-day interval also provides more opportunities for splash events (i.e., rain and irrigation) that can bring pathogens in the amended soils onto crops. For *Salmonella*, the 45-day baseline models predicted lower mean values of pathogen concentrations on lettuce from fields amended with treated BSAAOs when compared to the predictions from zero-day baseline models. The underlying greenhouse survival data for *Salmonella* (Bardsley et al., 2021) did not indicate initial increases in concentration following BSAAO application. Additional field trials might be needed to further investigate *Salmonella* survival under field conditions.

Mean predicted pathogen concentrations on lettuce from untreated BSAAO scenarios were compared to the results from the baseline models across three regions (i.e., Mid-Atlantic, south, and west) and two growing seasons (i.e., summer-fall and winter-spring) (Fig. 9). As seen in Fig. 9A, predicted STEC O157 concentrations on lettuce varied by region and growing season. For the summer-fall growing season, predicted STEC O157 concentrations on lettuce for west and south region were greater than zero-day baseline model predictions across all interval scenarios (zero-day or 45- to 120-days) while risk estimates from Mid-Atlantic region remained lower than the baseline model results with non-zero application intervals (Fig. 9A). For the winter-spring season, predicted STEC O157 concentrations from all three regions were higher

than zero-day baseline model predictions with either zero-day, 45-day, or 60-day application intervals. However, a 90- or 120-day interval reduced the predicted concentration of STEC O157 to lower than the zero-day baseline model predictions for the Mid-Atlantic and west regions. When compared to the 45-day baseline models, predicted STEC O157 concentrations on lettuce from all three regions decreased to below baseline model predictions only at 120-day interval for the summer-fall growing season and fell below baseline model predictions at both 90- and 120-day interval for the winter-spring growing season (Fig 9A). For *Salmonella*, predicted concentrations on lettuce were reduced drastically as application interval increases from 0 to 45 and up to 120 days. However, predicted *Salmonella* concentration remained greater than the predicted concentration from the zero-day or the 45-day baseline models at all intervals (Fig. 9B).

The mean predicted pathogen concentrations on lettuce with different application intervals across all regions and growing seasons are provided in Table 11. For STEC O157, an application interval of 120 days reduced the mean concentration from untreated BSAAO scenarios to lower than the zero-day baseline model predictions (Table 13). When compared to the 45-day baseline model, both 90-day and 120- day intervals reduced the mean predicted STEC O157 concentration on lettuce from untreated BSAAO scenarios to below baseline model predictions (Table 11). For *Salmonella*, mean predicted concentrations on lettuce from fields amended with untreated BSAAO remained greater than the results from both baseline models at all application intervals ranging from 45 to 120 days (Table 11). We performed additional simulations to predict the mean concentration of *Salmonella* on lettuce at the time of harvest with application intervals greater than 120 days. Results (Appendix G) show that predicted

Salmonella concentrations on lettuce from fields amended with untreated poultry manure are lower than the zero-day baseline model predictions with an application interval of 600 days.

Table 10. Mean predicted concentrations of STEC O157 and *Salmonella* on lettuce at the time of harvest for the baseline models.

Baseline model interval (days)	Pathogen	Mean pathogen concentration on lettuce heads (\log_{10} CFU/head)*
0	STEC O157	-3.660
45	STEC O157	-3.114
0	<i>Salmonella</i>	-3.660
45	<i>Salmonella</i>	-4.606

*Calculated as the \log_{10} values of the average number of pathogens (CFU) across all lettuce heads in a field.

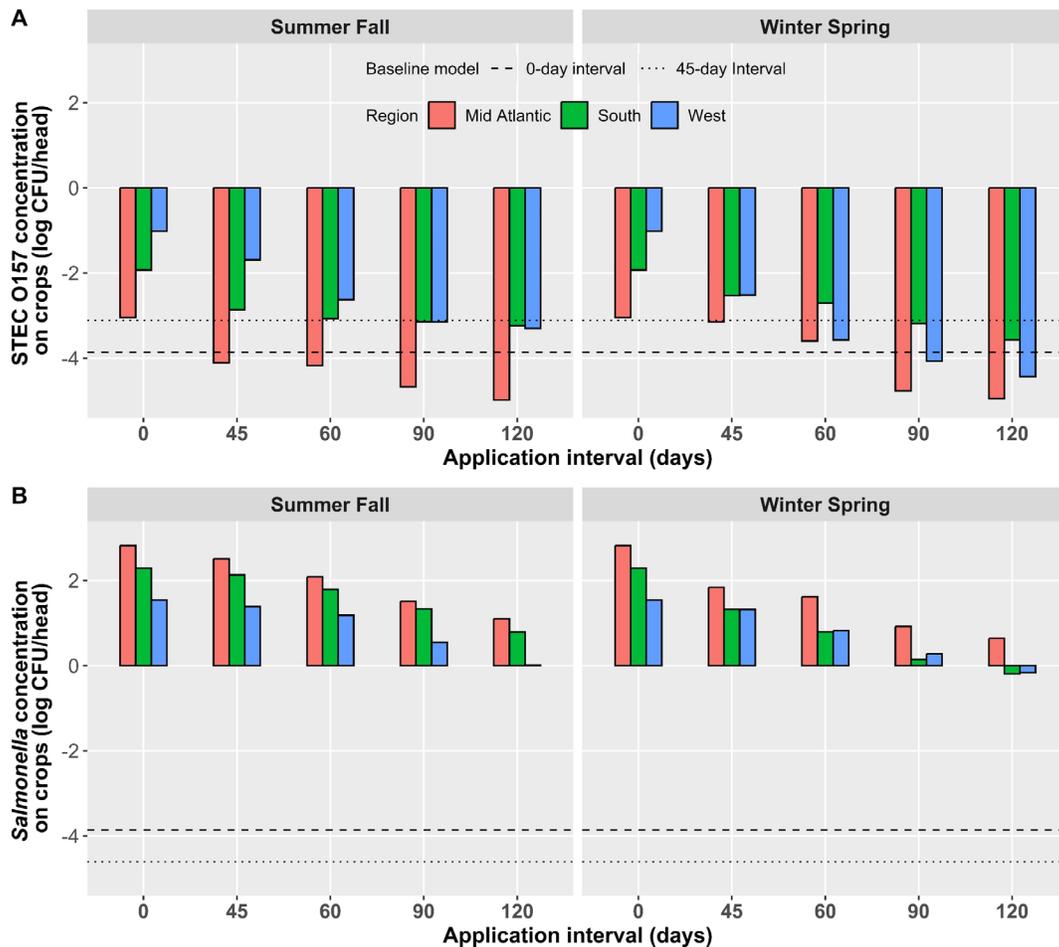


Fig. 9. Mean predicted concentrations of (A) STEC O157 and (B) *Salmonella* lettuce from field amended with untreated BSAAO at the time of harvest by region and growing season; Dashed lines represent results from the zero-day baseline models; Dotted lines represent results from the 45-day baseline models.

Table 11. Mean predicted concentrations of STEC O157 and *Salmonella* on lettuce heads grown in fields (20,000 lettuce heads) amended with untreated BSAAO at the time of harvest.

Pathogen	Application interval (days)	Concentration on lettuce heads (log₁₀ CFU/head)¹	Comparison with the zero-day baseline model²	Comparison with the 45-day baseline model
STEC O157	0	-0.936	+	+
STEC O157	45	-2.389	+	+
STEC O157	60	-2.975	+	+
STEC O157	90	-3.429	+	-
STEC O157	120	-3.677	-	-
<i>Salmonella</i>	0	2.552	+	+
<i>Salmonella</i>	45	1.750	+	+
<i>Salmonella</i>	60	1.382	+	+
<i>Salmonella</i>	90	0.789	+	+
<i>Salmonella</i>	120	0.363	+	+

¹Calculated as the log₁₀ values of the mean number of pathogens (CFU) across all lettuce heads in a field; ²“+”Indicates risk estimates that are greater than baseline model predictions; “-”Indicates risk estimates that are less than baseline model predictions.

3.3.1.2 Impact of runoff on pathogen contamination on crops

Predicted concentrations of STEC O157 and *Salmonella* on lettuce heads at the time of harvest from runoff scenarios for each region and each season at different application intervals are summarized in Table 12. Compared to scenarios without runoff (Table 9), impact of runoff on pathogen contamination varied by region and growing season. For example, runoff increased STEC O157 concentrations on lettuce for the Mid-Atlantic and west regions for both growing seasons whereas runoff resulted in reduced estimated concentration for south region for both growing seasons, assuming the runoff spread the pathogen across the field, but no additional contamination was introduced into the field. These observed differences were likely due to the difference in initial contamination prevalence and concentration in manure as runoff could spread pathogens across field but lower the concentration on initially contaminated soil grids. The mean concentration estimates across regions and seasons are provided in Table 13 and the trends when compared to the baseline models remain consistent with the original model scenarios without runoff for both pathogens.

Table 12. Mean predicted concentrations of STEC O157 and *Salmonella* on lettuce at the time of harvest from runoff scenarios by region and season at different application intervals by region and growing season.

Growing season	Region	Application interval (days)	STEC O157 concentration (log ₁₀ CFU/head)*	<i>Salmonella</i> concentration (log ₁₀ CFU/head)*
Summer- fall	Mid-Atlantic	0	-3.047	3.153
Summer- fall	Mid-Atlantic	45	-2.713	2.458
Summer- fall	Mid-Atlantic	60	-2.752	2.215
Summer- fall	Mid-Atlantic	90	-3.244	1.591
Summer- fall	Mid-Atlantic	120	-3.693	1.033
Summer- fall	South	0	-1.953	2.893
Summer- fall	South	45	-3.431	2.124
Summer- fall	South	60	-3.272	1.783
Summer- fall	South	90	-3.463	1.237
Summer- fall	South	120	-3.962	0.799
Summer- fall	West	0	-0.869	1.443
Summer- fall	West	45	-1.620	1.111
Summer- fall	West	60	-2.339	0.917
Summer- fall	West	90	-2.871	0.370
Summer- fall	West	120	-3.354	0.120
Winter-spring	Mid-Atlantic	0	-3.047	2.575
Winter-spring	Mid-Atlantic	45	-1.269	2.173
Winter-spring	Mid-Atlantic	60	-1.670	1.987
Winter-spring	Mid-Atlantic	90	-3.247	1.392
Winter-spring	Mid-Atlantic	120	-3.538	0.368
Winter-spring	South	0	-1.953	2.123
Winter-spring	South	45	-2.551	1.308
Winter-spring	South	60	-3.132	0.749
Winter-spring	South	90	-3.615	0.139
Winter-spring	South	120	-4.219	-0.310
Winter-spring	West	0	-1.474	1.443
Winter-spring	West	45	-1.626	1.152
Winter-spring	West	60	-2.455	0.892
Winter-spring	West	90	-3.081	0.622
Winter-spring	West	120	-3.639	-0.067

*Calculated as the log₁₀ values of the average number of pathogens (CFU) across all lettuce heads in a field.

Table 13. Mean predicted pathogen concentration on lettuce from runoff scenarios across region and seasons at different time intervals.

Pathogen	Application interval (days)	Mean predicted pathogen concentration on lettuce¹	Comparison with the zero-day baseline model²	Comparison with the 45-day baseline model
STEC O157	0	-1.565	+	+
STEC O157	45	-1.743	+	+
STEC O157	60	-2.274	+	+
STEC O157	90	-3.181	+	-
STEC O157	120	-3.872	-	-
<i>Salmonella</i>	0	2.664	+	+
<i>Salmonella</i>	45	2.012	+	+
<i>Salmonella</i>	60	1.758	+	+
<i>Salmonella</i>	90	1.170	+	+
<i>Salmonella</i>	120	0.566	+	+

¹Calculated as the log₁₀ values of the average number of pathogens (CFU) across all lettuce heads in a field; ²“+”Indicates risk estimates that are greater than baseline model predictions; “-”Indicates risk estimates that are less than baseline model predictions.

3.3.1.3 Predicted concentration of STEC non-O157 on lettuce at the time of harvest

None of the sampling data from the three regions suggested significant impact of season (summer-fall vs. winter-spring) on the presence of STEC non-O157 in bovine manure.

Therefore, predicted concentrations of STEC non-O157 on lettuce at the time of harvest were similar across two growing seasons (Table 14). Contamination of STEC non-O157 on lettuce varied by region and application intervals of 45 to 120 days reduced the predicted concentration on lettuce compared to the results from 0-day application intervals (Table 14). Overall, the predicted STEC non-O157 concentrations on lettuce were also higher compared to the predicted concentrations of STEC O157 on lettuce at the time of harvest. The difference is likely contributed by the difference in prevalence and concentration in untreated manure between STEC non-O157 and STEC O157. For example, none of the 161 samples from the Mid-Atlantic region were found positive for STEC O157 whereas STEC non-O157 was found in over 19% of the 161 samples (Litt et al., 2025). Concentration of STEC non-O157 ranged from -1.05 to 4.87 \log_{10} MPN/g from west region whereas STEC O157 concentration ranged from -1.05 to 3.04 \log_{10} MPN/g (Jay-Russel et al., 2018; Jay-Russel et al., 2023).

The baseline models predicted a mean STEC non-O157 concentration of -3.417 \log_{10} CFU and -4.914 \log_{10} CFU per lettuce head from fields amended with treated BSAAO for zero-day and 45-day application intervals respectively. Compared to the baseline models across three regions and two growing seasons, STEC non-O157 concentrations on lettuce from untreated BSAAO scenarios were higher than the predicted concentrations obtained from all baseline model results except for south region with a 120-day interval in both growing seasons when compared to the zero-day baseline model (Fig. 10).

Table 14. Mean predicted concentrations of STEC non-O157 on lettuce heads from field amended with untreated BSAAO at the time of harvest by region and growing season.

Growing season	Region	Application interval (days)	STEC non-O157 Concentration (log ₁₀ CFU/head)
Summer- fall	Mid-Atlantic	0	2.916
Summer- fall	Mid-Atlantic	45	1.332
Summer- fall	Mid-Atlantic	60	0.659
Summer- fall	Mid-Atlantic	90	-0.284
Summer- fall	Mid-Atlantic	120	-1.014
Summer- fall	South	0	0.105
Summer- fall	South	45	-2.129
Summer- fall	South	60	-2.231
Summer- fall	South	90	-2.980
Summer- fall	South	120	-3.793
Summer- fall	West	0	1.567
Summer- fall	West	45	0.308
Summer- fall	West	60	-0.373
Summer- fall	West	90	-1.367
Summer- fall	West	120	-1.877
Winter-spring	Mid-Atlantic	0	2.916
Winter-spring	Mid-Atlantic	45	1.329
Winter-spring	Mid-Atlantic	60	0.528
Winter-spring	Mid-Atlantic	90	-0.383
Winter-spring	Mid-Atlantic	120	-1.020
Winter-spring	South	0	0.105
Winter-spring	South	45	-2.116
Winter-spring	South	60	-2.108
Winter-spring	South	90	-3.057
Winter-spring	South	120	-4.072
Winter-spring	West	0	1.567
Winter-spring	West	45	0.300
Winter-spring	West	60	-0.458
Winter-spring	West	90	-1.208
Winter-spring	West	120	-1.818

*Calculated as the log₁₀ values of the average number of pathogens (CFU) across all lettuce heads in a field.

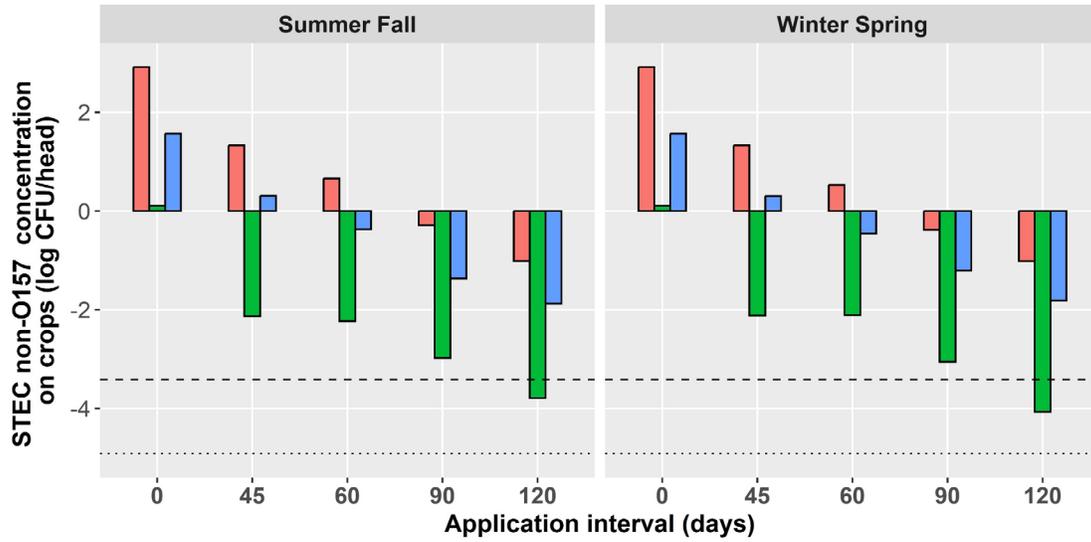


Fig. 10. Mean predicted concentrations of STEC non-O157 on lettuce from field amended with untreated BSAAO at the time of harvest by region and growing season; Dashed lines represent results from the zero-day baseline models; Dotted lines represent results from the 45-day baseline models.

3.3.2 *Produce that grows in the ground and on the ground*

Mean predicted STEC O157 concentrations on onions from fields amended with bovine manure and *Salmonella* concentrations on cantaloupes from fields amended with poultry manure at different application intervals are summarized by region and growing season in Table 15. Predicted STEC O157 concentrations on onions and predicted *Salmonella* concentrations both varied by region and season and decreased with increasing application intervals (Fig. 11). When compared to the baseline models (Table 16), an interval of 45 to 120 days was predicted to reduce the mean STEC O157 concentrations on onions across all regions and growing seasons (summarized in Table 17) to lower than the zero-day baseline model predictions. As a comparison, mean predicted STEC O157 concentrations on onions remained higher than the results from the 45-day baseline models regardless of interval length (Table 17). Predicted *Salmonella* concentrations on cantaloupe from all untreated BSAAO scenarios were higher than both baseline models' predictions (Table 17).

Table 15. Mean predicted concentration of STEC O157 on onions and *Salmonella* on cantaloupes from field amended with untreated BSAAO at the time of harvest by region and growing season.

Growing season	Region	Application interval (days)	STEC O157 concentration on onions (log ₁₀ CFU/onion)*	<i>Salmonella</i> concentration on cantaloupes (log ₁₀ CFU/cantaloupe)*
Summer- fall	Mid-Atlantic	0	-0.890	4.238
Summer- fall	Mid-Atlantic	45	-6.328	3.392
Summer- fall	Mid-Atlantic	60	-6.529	3.135
Summer- fall	Mid-Atlantic	90	-7.600	2.597
Summer- fall	Mid-Atlantic	120	-8.469	2.042
Summer- fall	South	0	-0.027	4.598
Summer- fall	South	45	-3.457	2.965
Summer- fall	South	60	-3.951	2.759
Summer- fall	South	90	-5.011	2.228
Summer- fall	South	120	-5.947	1.689
Summer- fall	West	0	1.448	3.467
Summer- fall	West	45	-1.787	2.301
Summer- fall	West	60	-2.124	1.944
Summer- fall	West	90	-3.138	1.441
Summer- fall	West	120	-4.101	1.080
Winter-spring	Mid-Atlantic	0	-0.890	3.705
Winter-spring	Mid-Atlantic	45	-6.332	2.871
Winter-spring	Mid-Atlantic	60	-6.530	2.603
Winter-spring	Mid-Atlantic	90	-7.631	2.114
Winter-spring	Mid-Atlantic	120	-8.468	1.591
Winter-spring	South	0	-0.027	3.717
Winter-spring	South	45	-3.457	2.359
Winter-spring	South	60	-3.951	2.089
Winter-spring	South	90	-5.011	1.571
Winter-spring	South	120	-5.947	1.063
Winter-spring	West	0	-0.526	3.467
Winter-spring	West	45	-3.997	1.995
Winter-spring	West	60	-5.325	1.733
Winter-spring	West	90	-6.483	1.233
Winter-spring	West	120	-7.719	0.693

*Calculated as the log₁₀ values of the average number of pathogens (CFU) across all onions (80,000 per field) or cantaloupes (4,000 per field) in a field.

Table 16. Mean predicted concentration of STEC O157 on onions and *Salmonella* on cantaloupes at the time of harvest for the baseline models.

Baseline model interval (days)	Pathogen	Produce type	Mean predicted pathogen concentration on crops (\log_{10} CFU/crop)*
0	STEC O157	Onion	-2.539
45	STEC O157	Onion	-8.120
0	<i>Salmonella</i>	Cantaloupe	-2.231
45	<i>Salmonella</i>	Cantaloupe	-3.899

*Calculated as the \log_{10} values of the average number of pathogens (CFU) across all onions (80,000 per field) or cantaloupes (4,000 per field) in a field.

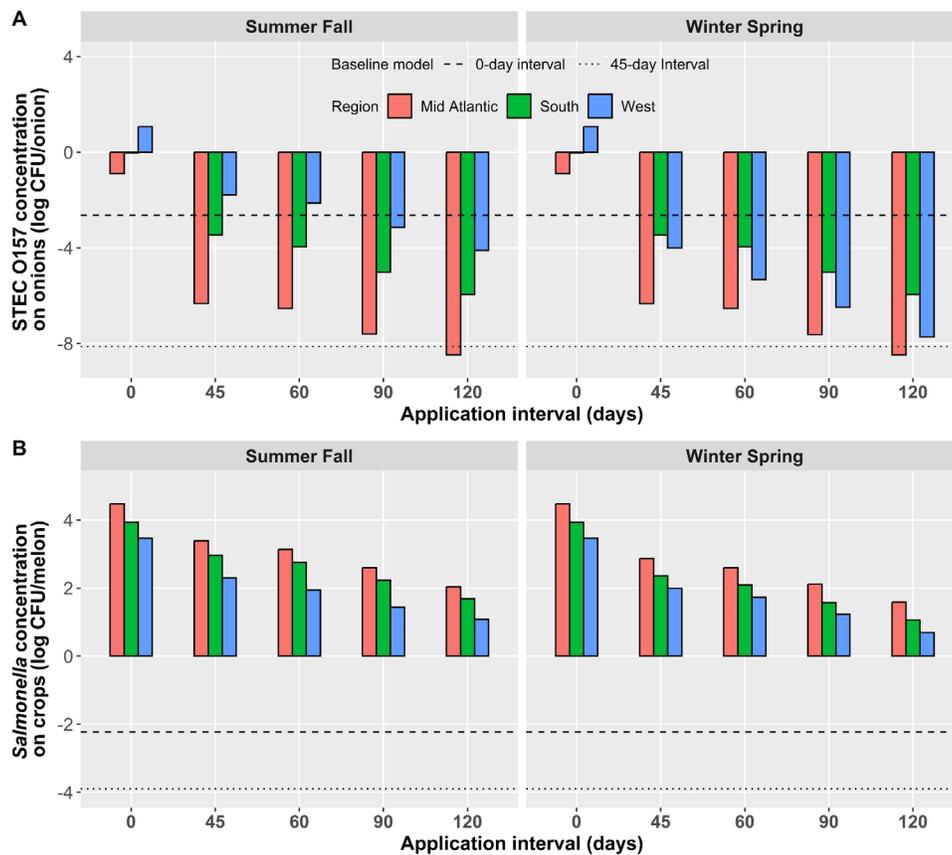


Fig. 11. Concentration of (A) STEC O157 on onions and (B) *Salmonella* on cantaloupes from field amended with untreated BSAAO at the time of harvest; Dashed lines represent results from the zero-day baseline models; Dotted lines represent results from the 45-day baseline models.

Table 17. Mean predicted concentrations of STEC O157 on onions and *Salmonella* on cantaloupes at the time of harvest across region and seasons.

Scenario	Application interval (days)	Mean predicted pathogen concentration on crops (log ₁₀ CFU/crop) ¹	Comparison with the zero-day baseline model ²	Comparison with the 45-day baseline model
Onion/STEC O157	0	0.453	+	+
Onion/STEC O157	45	-2.544	+	+
Onion/STEC O157	60	-2.889	-	+
Onion/STEC O157	90	-3.905	-	+
Onion/STEC O157	120	-4.867	-	+
Cantaloupe/ <i>Salmonella</i>	0	3.968	+	+
Cantaloupe/ <i>Salmonella</i>	45	2.803	+	+
Cantaloupe/ <i>Salmonella</i>	60	2.547	+	+
Cantaloupe/ <i>Salmonella</i>	90	2.027	+	+
Cantaloupe/ <i>Salmonella</i>	120	1.495	+	+

¹Calculated as the log₁₀ values of the average number of pathogens (CFU) across all onions (80,000 per field) or cantaloupes (4,000 per field) in a field; ²“+”Indicates risk estimates that are greater than baseline model predictions; “-”Indicates risk estimates that are less than baseline model predictions.

3.4 Uncertainty analysis

The estimated overall model uncertainty range considering model uncertainty sources is provided in Table 18 and uncertainty analysis results for individual variables are summarized in Table 19 for STEC O157 and Table 20 for *Salmonella*, where mean predicted pathogen concentrations were calculated as the average of the unweighted mean predicted pathogen concentrations on crops from the three regions and two growing seasons. Note that the baseline predictions for some of the uncertainty scenarios might be different from those for the original model scenarios (as reported in Table 10) as the uncertainty scenarios also affected baseline model predictions. As an example, the 45-day baseline model results for soil survival model uncertainty scenarios were different compared to the original baseline results in Table 10 as upper-bound and lower-bound models were used to estimate survival of STEC O157 in soils amended with treated manure. Detailed results for each region are provided in Appendix H. Consistent with the expected impact on model output (Table 6), scenarios with upper-bound values led to higher predicted pathogen concentrations and those with lower-bound values resulted in lower predicted pathogen concentrations when compared to the original model simulations for each tested uncertainty variable. Despite the differences in predicted concentrations, the impact of application intervals was consistent with the original model simulations where larger application intervals reduced the predicted pathogen concentrations. When compared to results from the baseline models, most of the tested variables had a low impact and the overall comparison trend is consistent with the observations from the original model simulations. For STEC O157 in untreated bovine manure, predicted concentrations on lettuce generally remained higher than the results from baseline models until application interval

increased to 120 days. For *Salmonella* in untreated poultry manure, predicted concentrations were higher than BSAAO baseline model predictions across all evaluated application intervals.

The predicted STEC O157 concentrations on lettuce are more sensitive to changes in initial contamination prevalence and initial contamination level in untreated bovine manure. When upper-bound initial contamination prevalence and initial contamination level values were used, predicted upper-bound STEC O157 concentrations remained higher than the 0-day interval baseline model prediction even with an interval of 120 days; however, given the prevalence uncertainty range, the upper-bound predicted concentrations for an application interval of 90- or 120-days are lower than the 45-day baseline model predictions (Table 19). Additionally, when lower-bound initial contamination prevalence values were used, a 90-day interval was able to reduce the predicted STEC O157 concentration to below the zero-day baseline model predictions, as opposed to an interval of 120 days in the original model simulations (Table 19). These results suggest that initial contamination conditions are influential factors when assessing pathogen contamination on produce from fields amended with untreated BSAAOs. In the model, we took into consideration the regional differences in pathogen contamination conditions in untreated BSAAO by modeling three regions separately. Additional manure survey studies and quantitative data on pathogen prevalence and levels, when become available, can be incorporated to reduce the uncertainty in the initial contamination conditions in untreated BSAAO. The longitudinal study by Ramos et al. (2021) assessed the prevalence and persistence of pathogens including STEC and *Salmonella* in soil amended with manure from animal origins across different regions in the U.S. Although positive manure samples were not enumerated for pathogens (and thus no enumeration data for use in our risk assessment), the longitudinal study

by Ramos et al. (2021) provided multi-regional data for pathogen prevalence in manure that we incorporated to estimate the baseline prevalence for STEC non-O157 in our model.

When an alternative Weibull model was used for survival of STEC O157 in amended soils, the impact of application interval on predicted STEC O157 concentration and the comparison trend with the baseline models remained consistent with the original model simulations (Table 19). The random forest survival models used in the original model simulations, albeit limited by the use of an attenuated strain of STEC O157 (which was found to have die-off rate comparable to a cocktail of three non-pathogenic *E. coli* strains, referred to as the *E. coli* TVS cocktail) instead of pathogenic strains, had the advantage of being based on survival data from field trials that reflect the diversity of produce preharvest environmental conditions (Sharma et al., 2019). As a comparison, the Weibull survival models used in uncertainty analysis were limited by simulated conditions in a greenhouse setting but the limitation was balanced by the benefit of using pathogenic strains (typically not used in field studies) in addition to the *E. coli* TVS cocktail, and investigating variability in survival of five individual STEC O157 strains in two soil types (Murphy et al., 2024). Furthermore, under the same greenhouse conditions, Murphy et al. found that pathogenic *E. coli* strains were nondetectable earlier (i.e., died off earlier) than the non-pathogenic *E. coli* TVS cocktail, where comparable \log_{10} reduction was observed either earlier (for most of the STEC O157-soil type combinations) or at the same time period (for some of the STEC O157-soil type combinations) as that for the *E. coli* TVS cocktail. The fact that predicted STEC O157 concentrations on lettuce for scenarios with application intervals of 60, 90, and 120-days are lower based on the alternative Weibull survival model than the random forest survival model (based on data for non-pathogenic

E. coli strains) is not unexpected because of the faster die-off of pathogenic STEC strains than the non-pathogenic *E. coli* strains observed under greenhouse conditions.

Results also indicate that our model is sensitive to *Salmonella* survival rate on crops grown in the field as the predicted *Salmonella* concentrations on lettuce were reduced drastically when alternative criteria were used (Table 20). In the original model simulations, only one study met the criteria for inclusion and the average decline rate is 0.03 log₁₀ CFU day⁻¹, significantly slower compared to those of STEC O157. For uncertainty analysis, with the additional inclusion of data from laboratory/greenhouse studies and field trials with less than 10-day observations, the average daily die-off rate for *Salmonella* on lettuce increased to 0.28 log₁₀ CFU day⁻¹, approximately 10 times faster than the value used in original model simulations, and that contributed to the drastic reduction in predicted *Salmonella* concentration. Given the discrepancies between field and laboratory/greenhouse survival data, additional field studies may improve the understanding of *Salmonella* survival on crops under field conditions over long term. Nonetheless, when comparing to the results from the baseline models, the trend remains consistent with the original model simulations as predicted *Salmonella* concentrations remained higher than baseline model predictions across all application intervals, suggesting that the results are robust to the uncertainties in *Salmonella* survival rate on crops.

Table 18. Estimated overall model uncertainty range.

Interval (Day)	Range of predicted level of STEC O157 on lettuce (min, max) ¹	Range of predicted level of <i>Salmonella</i> on lettuce (min, max) ¹
45	(-4.014, -1.156)	(-0.831, 3.726)
60	(-4.472, -1.820)	(-1.290, 3.435)
90	(-5.042, -2.733)	(-2.090, 2.928)
120	(-5.080, -3.151)	(-2.635, 2.433)

¹Calculated as the log₁₀ values of the average number of pathogens (CFU) across all crops in a field.

Table 19. Summary of uncertainty analysis results for STEC O157.

Tested parameters	Test scenario ¹	Interval (Day)	Predicted concentration ²	Zero-day baseline results	45-day baseline results	Comparison to zero-day baseline ³	Comparison to 45-day baseline ³
Original model scenarios	Model default	45	-2.389	-3.660	-3.114	+	+
Original model scenarios	Model default	60	-2.975	-3.660	-3.114	+	+
Original model scenarios	Model default	90	-3.429	-3.660	-3.114	+	-
Original model scenarios	Model default	120	-3.877	-3.660	-3.114	-	-
Initial prevalence	UB	45	-2.081	-3.660	-3.114	+	+
Initial prevalence	UB	60	-2.685	-3.660	-3.114	+	+
Initial prevalence	UB	90	-3.568	-3.660	-3.114	+	-
Initial prevalence	UB	120	-3.784	-3.660	-3.114	-	-
Initial prevalence	LB	45	-2.930	-3.660	-3.114	+	+
Initial prevalence	LB	60	-3.366	-3.660	-3.114	+	-
Initial prevalence	LB	90	-4.202	-3.660	-3.114	-	-
Initial prevalence	LB	120	-4.405	-3.660	-3.114	-	-
Initial level	UB	45	-2.514	-3.660	-3.114	+	+
Initial level	UB	60	-2.837	-3.660	-3.114	+	+
Initial level	UB	90	-3.786	-3.660	-3.114	-	-
Initial level	UB	120	-3.854	-3.660	-3.114	-	-
Initial level	LB	45	-2.837	-3.660	-3.114	+	+
Initial level	LB	60	-3.368	-3.660	-3.114	+	-
Initial level	LB	90	-4.267	-3.660	-3.114	-	-
Initial level	LB	120	-4.445	-3.660	-3.114	-	-
Soil survival model	UB	45	-1.721	-3.660	-2.905	+	+
Soil survival model	UB	60	-2.148	-3.660	-2.905	+	+
Soil survival model	UB	90	-2.960	-3.660	-2.905	+	-
Soil survival model	UB	120	-3.346	-3.660	-2.905	+	-
Soil survival model	LB	45	-3.109	-3.660	-3.947	+	+
Soil survival model	LB	60	-3.463	-3.660	-3.947	+	+
Soil survival model	LB	90	-4.148	-3.660	-3.947	-	-
Soil survival model	LB	120	-4.307	-3.660	-3.947	-	-
Soil survival model	Alternative ⁴	45	-1.456	-3.660	-4.011	+	+
Soil survival model	Alternative	60	-1.990	-3.660	-4.011	+	+
Soil survival model	Alternative	90	-2.973	-3.660	-4.011	+	+
Soil survival model	Alternative	120	-4.040	-3.660	-4.011	-	-
Maximum transfer radius	UB	45	-2.636	-3.660	-3.758	+	+
Maximum transfer radius	UB	60	-3.064	-3.660	-3.758	+	+
Maximum transfer radius	UB	90	-3.599	-3.660	-3.758	+	+
Maximum transfer radius	UB	120	-3.950	-3.660	-3.758	-	-
Transfer model	UB	45	-2.482	-3.431	-3.167	+	+
Transfer model	UB	60	-2.885	-3.431	-3.167	+	+
Transfer model	UB	90	-3.389	-3.431	-3.167	+	-
Transfer model	UB	120	-3.719	-3.431	-3.167	-	-
Transfer model	LB	45	-3.100	-3.946	-3.730	+	+
Transfer model	LB	60	-3.603	-3.946	-3.730	+	+
Transfer model	LB	90	-4.233	-3.946	-3.730	-	-
Transfer model	LB	120	-4.645	-3.946	-3.730	-	-

¹UB: upper-bound parameter values; LB: lower-bound parameter values. ²Calculated as the log₁₀ values of the average number of pathogens (CFU) across all crops in a field. ³"+"Indicates risk estimates that are greater than baseline model predictions; "-"Indicates risk estimates that are less than baseline model predictions. ⁴Alternative Weibull survival model for survival of STEC O157 in amended soils.

Table 20. Summary of uncertainty analysis results for *Salmonella*.

Tested parameters	Test scenario ¹	Interval (Day)	Predicted concentration ²	Zero-day baseline results	45-day baseline results	Comparison to zero-day baseline ³	Comparison to 45-day baseline ³
Original model scenarios	Model default	45	1.750	-3.660	-4.606	+	+
Original model scenarios	Model default	60	1.382	-3.660	-4.606	+	+
Original model scenarios	Model default	90	0.789	-3.660	-4.606	+	+
Original model scenarios	Model default	120	0.363	-3.660	-4.606	+	+
Initial prevalence	UB	45	1.907	-3.660	-4.606	+	+
Initial prevalence	UB	60	1.684	-3.660	-4.606	+	+
Initial prevalence	UB	90	1.153	-3.660	-4.606	+	+
Initial prevalence	UB	120	0.629	-3.660	-4.606	+	+
Initial prevalence	LB	45	1.564	-3.660	-4.606	+	+
Initial prevalence	LB	60	0.823	-3.660	-4.606	+	+
Initial prevalence	LB	90	0.464	-3.660	-4.606	+	+
Initial prevalence	LB	120	-0.267	-3.660	-4.606	+	+
Initial level	UB	45	2.696	-3.660	-4.606	+	+
Initial level	UB	60	2.146	-3.660	-4.606	+	+
Initial level	UB	90	1.877	-3.660	-4.606	+	+
Initial level	UB	120	1.245	-3.660	-4.606	+	+
Initial level	LB	45	1.606	-3.660	-4.606	+	+
Initial level	LB	60	1.115	-3.660	-4.606	+	+
Initial level	LB	90	0.682	-3.660	-4.606	+	+
Initial level	LB	120	0.346	-3.660	-4.606	+	+
Soil survival model	UB	45	1.695	-3.660	-4.290	+	+
Soil survival model	UB	60	0.994	-3.660	-4.290	+	+
Soil survival model	UB	90	0.505	-3.660	-4.290	+	+
Soil survival model	UB	120	0.408	-3.660	-4.290	+	+
Soil survival model	LB	45	1.631	-3.660	-5.141	+	+
Soil survival model	LB	60	1.035	-3.660	-5.141	+	+
Soil survival model	LB	90	0.505	-3.660	-5.141	+	+
Soil survival model	LB	120	0.063	-3.660	-5.141	+	+
Soil survival model	Alternative ⁴	45	1.85	-3.660	-5.893	+	+
Soil survival model	Alternative	60	1.535	-3.660	-5.893	+	+
Soil survival model	Alternative	90	0.862	-3.660	-5.893	+	+
Soil survival model	Alternative	120	0.345	-3.660	-5.893	+	+
Maximum transfer radius	UB	45	1.87	-3.660	-3.758	+	+
Maximum transfer radius	UB	60	1.47	-3.660	-3.758	+	+
Maximum transfer radius	UB	90	0.837	-3.660	-3.758	+	+
Maximum transfer radius	UB	120	0.381	-3.660	-3.758	+	+
Transfer model	UB	45	1.986	-3.431	-3.167	+	+
Transfer model	UB	60	1.561	-3.431	-3.167	+	+
Transfer model	UB	90	0.889	-3.431	-3.167	+	+
Transfer model	UB	120	0.398	-3.431	-3.167	+	+
Transfer model	LB	45	1.205	-3.946	-3.730	+	+
Transfer model	LB	60	0.946	-3.946	-3.730	+	+
Transfer model	LB	90	0.539	-3.946	-3.730	+	+
Transfer model	LB	120	0.241	-3.946	-3.730	+	+
Survival model - crops	Alternative ⁵	45	-0.907	-3.660	-5.999	+	+
Survival model - crops	Alternative	60	-0.852	-3.660	-5.999	+	+
Survival model - crops	Alternative	90	-1.858	-3.660	-5.999	+	+
Survival model - crops	Alternative	120	-2.103	-3.660	-5.999	+	+

¹UB: upper-bound parameter values; LB: lower-bound parameter values. ²Calculated as the log₁₀ values of the average number of pathogens (CFU) across all crops in a field. ³“+”Indicates risk estimates that are greater than baseline model predictions; “-”Indicates risk estimates that are less than baseline model predictions. ⁴Alternative Weibull survival model for *Salmonella* survival rate on crops as described in 2.11.2. ⁵Alternative inclusion criteria for *Salmonella* survival rate on crops.

3.5 Summary and conclusions

In summary, we developed a risk assessment model framework to predict pathogen contamination on produce crops from fields amended with treated and untreated BSAAO. Using data specific to three different regions (Mid-Atlantic, south, and west) and two growing seasons (summer-fall and winter-spring), we assessed the impact of various application intervals on the predicted contamination of different pathogens on different produce commodities from fields amended with different types of BSAAO. In general, application intervals (from 45 days to 120 days) greatly reduced the mean predicted pathogen concentrations on produce from fields amended with untreated BSAAO; however, the predictions varied greatly by region and by growing season. When compared to the baseline model with treated BSAAO, a 120-day application interval was predicted to reduce the mean predicted STEC O157 concentrations on lettuce from fields amended with untreated bovine manure to below the baseline models' predictions. However, predicted *Salmonella* concentrations on lettuce from fields amended with untreated poultry manure remained above the predictions from treated BSAAO even at intervals of up to 120 days. Mean concentrations of STEC O157 on onions harvested from fields after an application interval of 45 to 120 days post application of untreated bovine manure were predicted to be lower than the predicted STEC O157 concentration from treated BSAAO with a zero-day interval but higher than those from treated BSAAO with a 45-day interval. Mean predicted *Salmonella* concentrations on cantaloupes from fields amended with untreated poultry manure were higher than the predicted *Salmonella* concentrations from treated BSAAO even when an interval of up to 120 days between application and harvest was used.

The model and results are limited to three example produce commodities (lettuce, onion, and cantaloupe), three pathogen/untreated BSAAO combinations (STEC O157 in untreated

bovine manure, STEC non-O157 in untreated bovine manure, and *Salmonella* in untreated poultry manure), and geographically to the U.S. The virtual produce production systems in the model were created with specifications and agricultural practices reflecting some commercial produce farm operations in the U.S. While result trends are expected to be robust, quantitative results (e.g., predicted pathogen concentrations on produce crops) are expected to differ for produce farms with different specifications and following different agricultural practices (e.g., types of irrigation). The model and results represent our best estimates using best available data and information. Uncertainty analysis results suggest that the results and conclusions are robust to the uncertainty. Additional data on pathogen contamination in untreated BSAAO from other geographic regions, pathogen survival data in amended soils, and pathogen transfer and subsequent survival on produce crops, when these become available, could be incorporated into the model to extend and improve model predictions.

REFERENCES

- Aminabadi, P., Pang, H., Chen, Y., Ingram, D., & Jay-Russell, M. (2023). Survival of generic *E. coli* and *E. coli* O157 in chicken, pig, and rabbit scats: growth chamber and field trial experiments and data analysis. Manuscript to be submitted.
- Atwill, E. R., Chase, J. A., Oryang, D., Bond, R. F., Koike, S. T., Cahn, M. D., Anderson, M., Mokhtari, A., & Dennis, S. (2015). Transfer of *Escherichia coli* O157:H7 from simulated wildlife scat onto romaine lettuce during foliar irrigation. *Journal of Food Protection*, 78(2), 240-247.
- Baker, C. A., De, J., Bertoldi, B., Dunn, L., Chapin, T., Jay-Russell, M., Danyluk, M. D., & Schneider, K. R. (2019). Prevalence and concentration of stx+ *E. coli* and *E. coli* O157 in bovine manure from Florida farms. *PloS one*, 14(5), e0217445.
- Bardsley, C. A., Weller, D. L., Ingram, D. T., Chen, Y., Oryang, D., Rideout, S. L., Strawn, L. K. (2021). Strain, soil-type, irrigation regimen, and poultry litter influence *Salmonella* survival and die-off in agricultural soils. *Frontiers in Microbiology*, 12, 590303.
- Belias, A. M., Sbodio, A., Truchado, P., Weller, D., Pinzon, J., Skots, M., Allende, A., Munther, D., Suslow, T., Wiedmann, M., & Ivanek, R. (2020). Effect of weather on the die-off of *Escherichia coli* and Attenuated *Salmonella enterica* Serovar Typhimurium on preharvest leafy greens following irrigation with contaminated water. *Applied and Environmental Microbiology*, 86(17), e00899-20.
- Brinton, W. F., Jr, Storms, P., & Blewett, T. C. (2009). Occurrence and levels of fecal indicators and pathogenic bacteria in market-ready recycled organic matter composts. *Journal of Food Protection*, 72(2), 332–339.

California Leafy Green Products Handler Marketing Agreement (LGMA). (2023). Commodity specific food safety guidelines for the production and harvest of lettuce and leafy greens.

Available at: https://lgma-assets.sfo2.digitaloceanspaces.com/downloads/CURRENT-PUBLISHED-VERSION_CA-LGMA-Metrics_2023.09.20_FINAL.pdf.

Cevallos-Cevallos, J. M., Gu, G., Danyluk, M. D., & van Bruggen, A. H. (2012a). Adhesion and splash dispersal of *Salmonella enteric* Typhimurium on tomato leaflets: Effects of rdar morphotype and trichome density. *International Journal of Food Microbiology*, 160(1), 58-64.

Cevallos-Cevallos, J. M., Danyluk, M. D., Gu, G., Vallad, G. E., & van Bruggen, A. H. (2012b). Dispersal of *Salmonella* Typhimurium by rain splash onto tomato plants. *Journal of Food Protection*, 75(3), 472-479.

Chase, J. A., Atwill, E. R., Partyka, M. L., Bond, R. F., & Oryang, D. (2017). Inactivation of *Escherichia coli* O157:H7 on romaine lettuce when inoculated in a fecal slurry matrix. *Journal of Food Protection*, 80(5), 792–798.

Chase, J. A., Partyka, M. L., Bond, R. F., & Atwill, E. R. (2019). Environmental inactivation and irrigation-mediated regrowth of *Escherichia coli* O157:H7 on romaine lettuce when inoculated in a fecal slurry matrix. *PeerJ*, 7, e6591.

Chen, Z., & Jiang, X. (2017). Microbiological safety of animal wastes processed by physical heat treatment: an alternative to eliminate human pathogens in biological soil amendments as recommended by the Food Safety Modernization Act. *Journal of Food Protection*, 80(3), 392–405.

- Chen, Z., Biswas, S., Aminabadi, P., Stackhouse, J. W., Jay-Russell, M. T., & Pandey, P. K. (2019). Prevalence of *Escherichia coli* O157 and *Salmonella* spp. in solid bovine manure in California using real-time quantitative PCR. *Letters in Applied Microbiology*, 69(1), 23–29.
- Dunn, L. L., Sharma, V. K., Chapin, T. K., Friedrich, L. M., Larson, C. C., Rodrigues, C., Jay-Russell, M. T., Schneider, K. R., & Danyluk, M. D. (2022). The prevalence and concentration of *Salmonella enterica* in poultry litter in the southern United States. *PLoS ONE*, 17(5), e0268231.
- Edrington, T. S., Fox, W. E., Callaway, T. R., Anderson, R. C., Hoffman, D. W., & Nisbet, D. J. (2009). Pathogen prevalence and influence of composted dairy manure application on antimicrobial resistance profiles of commensal soil bacteria. *Foodborne Pathogens and Disease*, 6(2), 217–224.
- Erickson, M. C., Liao, J. Y., Payton, A. S., Cook, P. W., Den Bakker, H. C., Bautista, J., & Pérez, J. C. D. (2019). Pre-harvest internalization and surface survival of *Salmonella* and *Escherichia coli* O157:H7 sprayed onto different lettuce cultivars under field and growth chamber conditions. *International Journal of Food Microbiology*, 291, 197–204.
- Franz, E., van Hoek, A. H., Bouw, E., & Aarts, H. J. (2011). Variability of *Escherichia coli* O157 strain survival in manure-amended soil in relation to strain origin, virulence profile, and carbon nutrition profile. *Applied and environmental microbiology*, 77(22), 8088–8096.
- Gartley, S., Ramos, T., Nyarko, E., de Souza, T. R., Jay-Russell, M., Chen, Y., et al. (2018). Manure pathogen survey of *Salmonella* and Shiga toxin-producing *Escherichia coli* in untreated poultry and cattle manure of the Mid-Atlantic region. IAFP Annual Meeting 2018. Available at: <https://iafp.confex.com/iafp/2018/onlineprogram.cgi/Paper/18447>.

Gravuer, K., & Gunasekara, A. (2016). Compost application rates for California croplands and rangelands for a CDFA Healthy Soils Incentives Program. *Sacramento, California*.

Available at:

https://www.cdfa.ca.gov/oefi/efasap/docs/CompostApplicationRate_WhitePaper.pdf.

Harapas, D., Premier, R., Tomkins, B., Hepworth, G., & Ajlouni, S. (2015). Shoot Injury Increases the Level of Persistence of *Salmonella enterica* Serovar Sofia and *Listeria innocua* on Cos Lettuce and of *Salmonella enterica* Serovar Sofia on Chive. *Journal of Food Protection*, 78(12), 2150–2155.

Hutchison, M. L., Avery, S. M., & Monaghan, J. M. (2008). The air-borne distribution of zoonotic agents from livestock waste spreading and microbiological risk to fresh produce from contaminated irrigation sources. *Journal of Applied Microbiology*, 105(3), 848–857.

Ingram, D. T. (2009). *Assessment of foodborne pathogen survival during production and pre-harvest application of compost and compost tea* (dissertation). University of Maryland, College Park.

Islam, M., Doyle, M. P., Phatak, S. C., Millner, P., & Jiang, X. (2004). Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. *Journal of Food Protection*, 67(7), 1365–1370.

Islam M., Morgan J., Doyle M. P., Phatak S. C., Millner P., Jiang X. (2004b). Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathogens and Disease*, 1, 27–35.

- Islam, M., Morgan, J., Doyle, M. P., Phatak, S. C., Millner P., Jiang X. (2005). Survival of *Escherichia coli* O157:H7 in soil and on carrots and onions grown in fields treated with contaminated manure composts or irrigation water. *Food Microbiology*, 22(1), 63-70.
- Jay-Russell, M., Chen, Y., Rivadeneira, P., Pouillot, R., Aminabadi, P., Pandey, P., Bell, R. L., Oryang, D., Ingram, D., Kniel, K., & Van Doren, J. (2018). Prevalence and levels of Shiga toxin-producing *Escherichia coli* and *Salmonella* in untreated cattle and poultry manure in the west coast of United States. IAFP Annual Meeting 2018. Available at: <https://iafp.confex.com/iafp/2018/onlineprogram.cgi/Paper/18452>.
- Jay-Russell, M., Aminabadi, P., Chen, Y., Pouillot, R., Pandey, P., Ingram, D., Oryang, D., Kniel, K., & Van Doren, J. (2023). Quantifying the variability in the prevalence and levels of Shiga toxin-producing *Escherichia coli* in untreated cattle and manure in the west coast of United States. Manuscript to be submitted.
- Jeamsripong, S. (2015). *In-field transfer and survival of indicator Escherichia coli from wildlife feces to romaine lettuce, in the Salinas valley, California* (dissertation). University of California, Davis.
- Jeamsripong, S., Chase, J. A., Jay-Russell, M. T., Buchanan, R. L., & Atwill, E. R. (2019). Experimental in-field transfer and survival of *Escherichia coli* from animal feces to romaine lettuce in salinas valley, California. *Microorganisms*, 7(10), 408.
- Jiang, X., Morgan, J., & Doyle, M.P. (2002). Fate of *Escherichia coli* O157:H7 in manure-amended soil. *Applied and Environmental Microbiology*, 68, 2605-2609.
- Kim, J., Luo, F., & Jiang, X. (2009). Factors impacting the regrowth of *Escherichia coli* O157:H7 in dairy manure compost. *Journal of Food Protection*, 72(7), 1576–1584.

- Kuhn, M., Vaughan, F., & Ruiz, E. (2024). probably: Tools for Post-Processing Predicted Values. R package version 1.0.3. Available at <https://CRAN.R-project.org/package=probably>
- Lei, J., G'Sell, M., Rinaldo, A., Tibshirani, R. J., & Wasserman, L. (2018). Distribution-Free Predictive Inference for Regression. *Journal of the American Statistical Association*, *113*(523), 1094–1111.
- Limoges, M., Neher, D. A., Weicht, T. R., Millner, P., Sharma, M., & Donnelly, C. W. (2021). Differential survival of generic *E. coli* and *Listeria* spp. in northeastern U.S. soils amended with dairy manure compost, poultry litter compost, and heat-treated poultry pellets and fate in raw edible radish crops. *Journal of Food Protection*, *85*(12), 1708-1715.
- Litt, P. K., Kelly, A., Omar, A. N., Johnson, G., Vinyard, B. T., Kniel, K. E., & Sharma, M. (2021). Temporal and agricultural factors influence *Escherichia coli* survival in soil and transfer to cucumbers. *Applied and Environmental Microbiology*, *87*, e02418-2420.
- Litt, P. K., Gartley, S., Kelly, A., Ramos, T., Nyarko, E., de Souza, T. R., Jay-Russell, M., Chen, Y., Aminabadi, P., Ingram, D., Severson, D., & Kniel, K. E. (2025). Prevalence of Shiga-toxigenic *Escherichia coli* in bovine manure in the Mid-Atlantic region of the United States. *Microorganisms*, *13*(2), 419
- López-Gálvez, F., Gil, M. I., & Allende, A. (2018). Impact of relative humidity, inoculum carrier and size, and native microbiota on *Salmonella* ser. Typhimurium survival in baby lettuce. *Food Microbiology*, *70*, 155–161.
- Lopez-Velasco, G., Tomás-Callejas, A., Sbodio, A. O., Pham, X. K., Wei, P., Diribsa, D., & Suslow, T. V. (2015). Factors affecting cell population density during enrichment and

- subsequent molecular detection of *Salmonella enterica* and *Escherichia coli* O157:H7 on lettuce contaminated during field production. *Food Control*, 54, 165-175.
- Mao, Y., Akdeniz, N., & Nguyen, T. H. (2021). Quantification of pathogens and antibiotic resistance genes in backyard and commercial composts. *Science of the Total Environment*, 797, 149197.
- McKellar, R. C., Pérez-Rodríguez, F., Harris, L. J., Moyne, A. L., Blais, B., Topp, E., Bezanson, G., Bach, S., & Delaquis, P. (2014). Evaluation of different approaches for modeling *Escherichia coli* O157:H7 survival on field lettuce. *International journal of food microbiology*, 184, 74–85.
- Mokhtari, A., Oryang, D., Chen, Y., Pouillot, R., & Van Doren, J. (2018). A mathematical model for pathogen cross-contamination dynamics during the postharvest processing of leafy greens. *Risk Analysis*, 38(8), 1718-1737.
- Mokhtari, A., Pang, H., Santillana Farakos, S., Davidson, G. R., Williams, E. N., & Van Doren, J. M. (2022). Evaluation of potential impacts of free chlorine during washing of fresh-cut leafy greens on *Escherichia coli* O157:H7 cross-contamination and risk of illness. *Risk Analysis*, 42(5), 966–988.
- Mokhtari, A., Pang, H., Santillana Farakos, S., McKenna, C., Crowley, C., Cranford, V., Bowen, A., Phillips, S., Madad, A., Obenhuber, D., & Van Doren, J. M. (2023). Leveraging risk assessment for foodborne outbreak investigations: the quantitative risk assessment-epidemic curve prediction model. *Risk Analysis*, 43(2), 324–338.
- Moynihan, E., Richards, K.G., Ritz, K., Tyrrel, S., & Brennan, F.P. (2013). Impact of soil type, biology and temperature on the survival of non-toxigenic *Escherichia coli* O157. *Biology and Environment: Proceedings of the Royal Irish Academy*, 113B, 41-46.

- Murphy, C. M., Weller, D. L., Bardsley, C. A., Ingram, D. T., Chen, Y., Oryang, D., Rideout, S. L., & L. K. Strawn. (2024). Survival of twelve pathogenic and generic *Escherichia coli* strains in agricultural soils as influenced by strain, soil type, irrigation regimen, and soil amendment. *Journal of Food Protection*, 87(10), 100343.
- Pang, H., Mokhtari, A., Chen, Y., Oryang, D., Ingram, D.T., Sharma, M., Millner, P.D. and Van Doren, J.M. (2020), A predictive model for survival of *Escherichia coli* O157:H7 and generic *E. coli* in soil amended with untreated animal manure. *Risk Analysis*, 40, 1367-1382.
- Pang, H., Pouillot, R., & Van Doren, J. M. (2023). Quantitative risk assessment-epidemic curve prediction model for leafy green outbreak investigation. *Risk Analysis*, 43(9), 1713-1732.
- Pérez-Rodríguez, F., Carrasco, E., Bover-Cid, S., Jofré, A., & Valero, A. (2017). Closing gaps for performing a risk assessment on *Listeria monocytogenes* in ready-to-eat (RTE) foods: activity 2, a quantitative risk characterization on *L. monocytogenes* in RTE foods; starting from the retail stage. *EFSA Supporting Publication*, 14(7):EN-1252.
- Sharma, M., Millner, P. D., Hashem, F., Vinyard, B. T., East, C. L., Handy, E. T., White, K., Stonebraker, R., & Cotton, C. P. (2019). Survival of *Escherichia coli* in manure-amended soils is affected by spatiotemporal, agricultural, and weather factors in the Mid-Atlantic United States. *Applied and environmental microbiology*, 85(5), e02392-18.
- Shepherd, M. W., Jr, Liang, P., Jiang, X., Doyle, M. P., & Erickson, M. C. (2010). Microbiological analysis of composts produced on South Carolina poultry farms. *Journal of Applied Microbiology*, 108(6), 2067–2076.

- Stine, S. W., Song, I., Choi, C. Y., & Gerba, C. P. (2005). Effect of relative humidity on preharvest survival of bacterial and viral pathogens on the surface of cantaloupe, lettuce, and bell peppers. *Journal of Food Protection*, 68(7), 1352-1358.
- Weller, D. L., Kovac, J., Kent, D. J., Roof, S., Tokman, J. I., Mudrak, E., Kowalcyk, B., Oryang, D., Aceituno, A., & Wiedmann, M. (2017a). *Escherichia coli* transfer from simulated wildlife feces to lettuce during foliar irrigation: a field study in the northeastern United States. *Food Microbiology*, 68, 24–33.
- Weller, D. L., Kovac, J., Roof, S. E., Kent, D. J., Tokman, J. I., Kowalcyk, B. B., Oryang, D., Ivanek, R., Aceituno, A. F., Sroka, C. J., & Wiedmann, M. (2017b). Survival of *Escherichia coli* on lettuce under field conditions encountered in the northeastern United States. *Journal of Food Protection*, 80(7), 1214-1221.

Appendix A: Analysis of pathogen prevalence and concentration data from the manure surveys

Prevalence and concentration data from the FDA commissioned manure survey studies conducted in west (Jay-Russel et al., 2018; Jay-Russel et al., 2023), south (Baker et al., 2019; Dunn et al., 2022), and Mid-Atlantic (Gartley et al., 2018; Litt et al., 2025) region were collected. Raw data from two unpublished commissioned studies were provided below (Table A1-A4).

Table A1. Presence and concentration of *Salmonella* in manure samples from Mid-Atlantic region(Gartley et al., 2018; Litt et al., 2025).

ID	Sampling date	Presence	Concentration (MPN/g)
Farm 1	012617	Positive	0.089
Farm 1	012617	Negative	NA
Farm 1	012617	Negative	NA
Farm 1	012617	Negative	NA
Farm 1	012617	Positive	0.089
Farm 1	012617	Positive	72
Farm 1	012617	Negative	NA
Farm 2	012617	Negative	NA
Farm 2	012617	Negative	NA
Farm 2	012617	Negative	NA
Farm 2	012617	Negative	NA
Farm 2	012617	Negative	NA
Farm 2	012617	Negative	NA
Farm 2	012617	Negative	NA
Farm 3	012617	Negative	NA
Farm 3	012617	Negative	NA
Farm 3	012617	Negative	NA
Farm 3	012617	Negative	NA
Farm 3	012617	Negative	NA
Farm 3	012617	Negative	NA
Farm 4	012617	Positive	33
Farm 4	012617	Negative	NA
Farm 4	012617	Negative	NA
Farm 4	012617	Negative	NA
Farm 4	012617	Negative	NA
Farm 4	012617	Negative	NA
Farm 5	012617	Negative	NA
Farm 5	012617	Positive	2.20E+02
Farm 5	012617	Negative	NA
Farm5	012617	Positive	0.089
Farm 5	012617	Negative	NA
Farm 5	012617	Negative	NA
Farm 5	012617	Positive	0.089
Farm 6	012617	Negative	NA
Farm 6	012617	Negative	NA

Farm 14	060117	Negative	NA
Farm 14	060117	Positive	0.089
Farm 15	060117	Negative	NA
Farm 15	060117	Negative	NA
Farm 15	060117	Negative	NA
Farm 15	060117	Negative	NA
Farm 15	060117	Negative	NA
Farm 15	060117	Negative	NA
Farm 15	060117	Negative	NA
Farm 15	060117	Negative	NA
Farm 16	060117	Positive	5.5
Farm 16	060117	Positive	0.51
Farm 16	060117	Positive	0.089
Farm 16	060117	Positive	0.089
Farm 16	060117	Positive	2.80E+05
Farm 16	060117	Positive	5.70E+04
Farm 16	060117	Positive	7.20E+03
Farm 17	060117	Positive	0.089
Farm 17	060117	Negative	NA
Farm 17	060117	Negative	NA
Farm 17	060117	Negative	NA
Farm 17	060117	Negative	NA
Farm 17	060117	Positive	>2.80E+05
Farm 17	060117	Negative	NA
Farm 18	060117	Positive	>2.80E+05
Farm 18	060117	Positive	>2.80E+05
Farm 18	060117	Positive	>2.80E+05
Farm 18	060117	Positive	2.80E+05
Farm 18	060117	Positive	2.80E+05
Farm 18	060117	Positive	2.30E+04
Farm 18	060117	Positive	1.20E+03
Farm 19	060117	Positive	4.80E+04
Farm 19	060117	Positive	1.40E+05
Farm 19	060117	Negative	NA
Farm 19	060117	Positive	>2.80E+05
Farm 19	060117	Positive	>2.80E+05
Farm 19	060117	Positive	>2.80E+05
Farm 19	060117	Positive	5.70E+04
Farm 20	060117	Positive	32
Farm 20	060117	Positive	1.2
Farm 20	060117	Negative	NA
Farm 20	060117	Positive	7.70E+04
Farm 20	060117	Positive	>2.80E+05
Farm 20	060117	Positive	>2.80E+05
Farm 20	060117	Positive	>2.80E+05
Farm 1	052417	Positive	3.3
Farm 1	052417	Negative	NA
Farm 1	052417	Negative	NA
Farm 1	052417	Negative	NA
Farm 1	052417	Negative	NA
Farm 1	052417	Negative	NA
Farm 1	052417	Negative	NA
Farm 2	052417	Negative	NA
Farm 2	052417	Negative	NA
Farm 2	052417	Positive	1.9
Farm 2	052417	Negative	NA
Farm 2	052417	Negative	NA
Farm 2	052417	Positive	3.9
Farm 2	052417	Negative	NA
Farm 3	052417	Negative	NA

Farm 3	052417	Negative	NA
Farm 3	052417	Negative	NA
Farm 3	052417	Negative	NA
Farm 3	052417	Negative	NA
Farm 3	052417	Negative	NA
Farm 3	052417	Negative	NA
Farm 4	052417	Negative	NA
Farm 4	052417	Negative	NA
Farm 4	052417	Negative	NA
Farm 4	052417	Negative	NA
Farm 4	052417	Negative	NA
Farm 4	052417	Negative	NA
Farm 4	052417	Negative	NA
Farm 4	052417	Negative	NA
Farm 4	052417	Negative	NA
Farm 5	052417	Negative	NA
Farm 5	052417	Negative	NA
Farm 5	052417	Negative	NA
Farm5	052417	Negative	NA
Farm 5	052417	Negative	NA
Farm 5	052417	Negative	NA
Farm 5	052417	Negative	NA
Farm 5	052417	Negative	NA
Farm 6	052417	Negative	NA
Farm 6	052417	Negative	NA
Farm 6	052417	Negative	NA
Farm 6	052417	Negative	NA
Farm 6	052417	Negative	NA
Farm 6	052417	Negative	NA
Farm 6	052417	Negative	NA
Farm 6	052417	Negative	NA
Farm 7	053017	Negative	NA
Farm 7	053017	Positive	0.089
Farm 7	053017	Positive	4.6
Farm 7	053017	Negative	NA
Farm 7	053017	Positive	1.20E+04
Farm 7	053017	Positive	0.089
Farm 7	053017	Positive	2.80E+05
Farm 8	053017	Positive	2.80E+05
Farm 8	053017	Positive	2.80E+05
Farm 8	053017	Positive	2.80E+05
Farm 8	053017	Positive	2.80E+05
Farm 8	053017	Positive	0.089
Farm 8	053017	Positive	0.089
Farm 8	053017	Positive	2.80E+05
Farm 9	053017	Positive	11
Farm 9	053017	Positive	3.2
Farm 9	053017	Positive	13
Farm 9	053017	Negative	NA
Farm 9	053017	Positive	3.6
Farm 9	053017	Positive	6.7
Farm 9	053017	Positive	1.10E+03
Farm 10	052417	Negative	NA
Farm 10	052417	Negative	NA
Farm 10	052417	Negative	NA
Farm 10	052417	Negative	NA
Farm 10	052417	Negative	NA
Farm 10	052417	Negative	NA
Farm 10	052417	Negative	NA
Farm 11	053017	Positive	2.80E+05
Farm 11	053017	Positive	2.80E+05
Farm 11	053017	Positive	2.80E+05
Farm 11	053017	Positive	2.80E+05

Farm 11	053017	Positive	2.80E+05
Farm 11	053017	Positive	4.6
Farm 11	053017	Positive	2.80E+05
Farm 12	053017	Positive	2.80E+05
Farm 12	053017	Positive	2.80E+05
Farm 12	053017	Positive	2.80E+05
Farm 12	053017	Positive	2.80E+05
Farm 12	053017	Positive	2.80E+05
Farm 12	053017	Positive	2.80E+05
Farm 12	053017	Positive	2.80E+05
Farm 13	080117	Negative	NA
Farm 13	080117	Positive	0.089
Farm 13	080117	Negative	NA
Farm 13	080117	Negative	NA
Farm 13	080117	Negative	NA
Farm 13	080117	Negative	NA
Farm 13	080117	Negative	NA
Farm 14	070517	Negative	NA
Farm 14	070517	Negative	NA
Farm 14	070517	Positive	1.9
Farm 14	070517	Positive	8.2
Farm 14	070517	Positive	0.089
Farm 14	070517	Negative	NA
Farm 14	070517	Positive	1.20E+03
Farm 15	070517	Negative	NA
Farm 15	070517	Negative	NA
Farm 15	070517	Negative	NA
Farm 15	070517	Positive	4.6
Farm 15	070517	Positive	0.089
Farm 15	070517	Positive	0.089
Farm 15	070517	Positive	1.20E+03
Farm 16	070517	Positive	18
Farm 16	070517	Positive	0.089
Farm 16	070517	Positive	32
Farm 16	070517	Negative	NA
Farm 16	070517	Negative	NA
Farm 16	070517	Negative	NA
Farm 16	070517	Positive	4.60E+02
Farm 17	070517	Positive	0.089
Farm 17	070517	Positive	0.089
Farm 17	070517	Positive	0.089
Farm 17	070517	Positive	0.089
Farm 17	070517	Positive	0.089
Farm 17	070517	Positive	0.46
Farm 17	070517	Positive	0.51
Farm 18	073117	Positive	2.80E+05
Farm 18	073117	Positive	4.50E+03
Farm 18	073117	Positive	4.80E+04
Farm 18	073117	Positive	2.80E+05
Farm 18	073117	Positive	32
Farm 18	073117	Positive	0.089
Farm 18	073117	Positive	2.80E+05
Farm 19	073117	Positive	1.40E+05
Farm 19	073117	Positive	1.20E+04
Farm 19	073117	Positive	4.80E+04
Farm 19	073117	Positive	7.20E+02
Farm 19	073117	Positive	2.50E+03
Farm 19	073117	Positive	8.20E+03
Farm 19	073117	Positive	7.20E+02

Farm 20	073117	Positive	2.30E+04
Farm 20	073117	Positive	7.20E+03
Farm 20	073117	Positive	2.30E+04
Farm 20	073117	Positive	3.20E+02
Farm 20	073117	Positive	4.60E+03
Farm 20	073117	Positive	4.60E+03
Farm 20	073117	Positive	4.80E+04
Farm 21	091018	Negative	NA
Farm 21	091018	Negative	NA
Farm 21	091018	Negative	NA
Farm 21	091018	Negative	NA
Farm 21	091018	Negative	NA
Farm 21	091018	Negative	NA
Farm 21	091018	Negative	NA
Farm 21	091018	Negative	NA
Farm 21	091018	Negative	NA
Farm 22	091018	Negative	NA
Farm 22	091018	Negative	NA
Farm 22	091018	Negative	NA
Farm 22	091018	Negative	NA
Farm 22	091018	Negative	NA
Farm 22	091018	Negative	NA
Farm 22	091018	Positive	11

313	120916	Negative	NA	Negative	NA
313	120916	Positive	0.089	Negative	NA
313	120916	Negative	NA	Negative	NA
313	120916	Negative	NA	Negative	NA
313	120916	Negative	NA	Negative	NA
313	120916	Positive	2.7	Negative	NA
314	071216	Negative	NA	Negative	NA
314	071216	Positive	>4.20E+05	Negative	NA
314	071216	Negative	NA	Negative	NA
314	071216	Positive	2.40E+03	Negative	NA
314	071216	Negative	NA	Negative	NA
314	071216	Positive	>4.20E+05	Negative	NA
314	071216	Negative	NA	Negative	NA
314	120916	Negative	NA	Negative	NA
314	120916	Negative	NA	Negative	NA
314	120916	Negative	NA	Negative	NA
314	120916	Positive	6.5	Negative	NA
314	120916	Positive	1.30E+03	Negative	NA
314	120916	Negative	NA	Negative	NA
314	120916	Negative	NA	Negative	NA
411	092817	Negative	NA	Negative	NA
411	092817	Negative	NA	Negative	NA
411	092817	Negative	NA	Negative	NA
411	092817	Positive	1.4	Negative	NA
411	092817	Negative	NA	Negative	NA
411	092817	Negative	NA	Negative	NA
411	092817	Negative	NA	Negative	NA
411	053118	Negative	NA	Negative	NA
411	053118	Negative	NA	Negative	NA
411	053118	Negative	NA	Negative	NA
411	053118	Negative	NA	Negative	NA
411	053118	Negative	NA	Negative	NA
411	053118	Positive	1.8	Negative	NA
411	053118	Negative	NA	Negative	NA
412	092817	Negative	NA	Negative	NA
412	092817	Negative	NA	Negative	NA
412	092817	Negative	NA	Negative	NA
412	092817	Negative	NA	Negative	NA
412	092817	Negative	NA	Negative	NA
412	092817	Positive	0.43	Negative	NA
412	053118	Negative	NA	Negative	NA
412	053118	Positive	120	Negative	NA
412	053118	Positive	4.60E+03	Negative	NA
412	053118	Negative	NA	Negative	NA
412	053118	Positive	4.5	Negative	NA
412	053118	Negative	NA	Negative	NA
412	053118	Positive	0.56	Negative	NA

Table A3. Presence and concentration of *Salmonella* in manure samples from west region (Jay-Russel et al., 2018; Jay-Russel et al., 2023).

ID	Sampling date	Presence	Concentration (MPN/g)
AZ-0E64O9GL	2/12/2018	Negative	0
AZ-0E64O9GL	2/12/2018	Negative	0
AZ-0E64O9GL	2/12/2018	Negative	0
AZ-0E64O9GL	2/12/2018	Negative	0
AZ-0E64O9GL	2/12/2018	Negative	0
AZ-0E64O9GL	2/12/2018	Negative	0
AZ-0E64O9GL	2/12/2018	Negative	0
AZ-0E64O9GL	8/24/2017	Positive	0.089
AZ-0E64O9GL	8/24/2017	Positive	0.51
AZ-0E64O9GL	8/24/2017	Positive	2.2
AZ-0E64O9GL	8/24/2017	Positive	12
AZ-0E64O9GL	8/24/2017	Positive	28
AZ-0E64O9GL	8/24/2017	Positive	550
AZ-0E64O9GL	8/24/2017	Positive	3200
AZ-4566S1B9	2/12/2018	Negative	0
AZ-4566S1B9	2/12/2018	Negative	0
AZ-4566S1B9	2/12/2018	Negative	0
AZ-4566S1B9	2/12/2018	Negative	0
AZ-4566S1B9	2/12/2018	Negative	0
AZ-4566S1B9	2/12/2018	Negative	0
AZ-4566S1B9	2/12/2018	Negative	0
AZ-4566S1B9	8/24/2017	Negative	0
AZ-4566S1B9	8/24/2017	Positive	0.089
AZ-4566S1B9	8/24/2017	Positive	0.089
AZ-4566S1B9	8/24/2017	Positive	3.2
AZ-4566S1B9	8/24/2017	Positive	4
AZ-4566S1B9	8/24/2017	Positive	2300
AZ-4566S1B9	8/24/2017	Positive	280000
AZ-587S16SC	3/14/2018	Negative	0
AZ-587S16SC	3/14/2018	Negative	0
AZ-587S16SC	3/14/2018	Positive	0.089
AZ-587S16SC	3/14/2018	Positive	0.089
AZ-587S16SC	3/14/2018	Positive	0.45
AZ-587S16SC	3/14/2018	Positive	1
AZ-587S16SC	3/14/2018	Positive	2.5
AZ-587S16SC	9/18/2017	Positive	4.6
AZ-587S16SC	9/18/2017	Positive	42
AZ-587S16SC	9/18/2017	Positive	72
AZ-587S16SC	9/18/2017	Positive	720
AZ-587S16SC	9/18/2017	Positive	1900
AZ-587S16SC	9/18/2017	Positive	4300
AZ-587S16SC	9/18/2017	Positive	4300
AZ-78KJ7AQ4	2/12/2018	Positive	0.089
AZ-78KJ7AQ4	2/12/2018	Positive	0.089
AZ-78KJ7AQ4	2/12/2018	Positive	0.089
AZ-78KJ7AQ4	2/12/2018	Positive	0.089
AZ-78KJ7AQ4	2/12/2018	Positive	0.51
AZ-78KJ7AQ4	2/12/2018	Positive	48
AZ-78KJ7AQ4	2/12/2018	Positive	54
AZ-78KJ7AQ4	9/18/2017	Positive	220
AZ-78KJ7AQ4	9/18/2017	Positive	310
AZ-78KJ7AQ4	9/18/2017	Positive	430
AZ-78KJ7AQ4	9/18/2017	Positive	1900
AZ-78KJ7AQ4	9/18/2017	Positive	2300
AZ-78KJ7AQ4	9/18/2017	Positive	8500

AZ-R49749DL	3/12/2018	Negative	0
AZ-R49749DL	9/20/2017	Negative	0
AZ-R49749DL	9/20/2017	Negative	0
AZ-R49749DL	9/20/2017	Negative	0
AZ-R49749DL	9/20/2017	Positive	0.089
AZ-R49749DL	9/20/2017	Positive	4.3
AZ-R49749DL	9/20/2017	Positive	220
AZ-R49749DL	9/20/2017	Positive	7200
AZ-Y8X8XBQ9	3/13/2018	Negative	0
AZ-Y8X8XBQ9	3/13/2018	Negative	0
AZ-Y8X8XBQ9	3/13/2018	Negative	0
AZ-Y8X8XBQ9	3/13/2018	Negative	0
AZ-Y8X8XBQ9	3/13/2018	Negative	0
AZ-Y8X8XBQ9	3/13/2018	Negative	0
AZ-Y8X8XBQ9	3/13/2018	Negative	0
AZ-Y8X8XBQ9	9/21/2017	Negative	0
AZ-Y8X8XBQ9	9/21/2017	Positive	0.089
AZ-Y8X8XBQ9	9/21/2017	Positive	0.51
AZ-Y8X8XBQ9	9/21/2017	Positive	0.51
AZ-Y8X8XBQ9	9/21/2017	Positive	2.2
AZ-Y8X8XBQ9	9/21/2017	Positive	12
AZ-Y8X8XBQ9	9/21/2017	Positive	72
AZ-YO7H6W4O	3/27/2018	Negative	0
AZ-YO7H6W4O	3/27/2018	Negative	0
AZ-YO7H6W4O	3/27/2018	Negative	0
AZ-YO7H6W4O	3/27/2018	Negative	0
AZ-YO7H6W4O	3/27/2018	Negative	0
AZ-YO7H6W4O	7/11/2017	Positive	0.089
AZ-YO7H6W4O	7/11/2017	Positive	0.089
AZ-YO7H6W4O	7/11/2017	Positive	0.089
AZ-YO7H6W4O	7/11/2017	Positive	0.089
AZ-YO7H6W4O	7/11/2017	Positive	0.089
AZ-YO7H6W4O	7/11/2017	Positive	0.51
AZ-YO7H6W4O	7/11/2017	Positive	0.51
CA-cE34KRek	3/28/2018	Negative	0
CA-cE34KRek	3/28/2018	Negative	0
CA-cE34KRek	3/28/2018	Negative	0
CA-cE34KRek	3/28/2018	Negative	0
CA-cE34KRek	3/28/2018	Negative	0
CA-cE34KRek	3/28/2018	Negative	0
CA-cE34KRek	3/28/2018	Negative	0
CA-cE34KRek	5/3/2017	Negative	0
CA-cE34KRek	5/3/2017	Negative	0
CA-cE34KRek	5/3/2017	Negative	0
CA-cE34KRek	5/3/2017	Negative	0
CA-cE34KRek	5/3/2017	Negative	0
CA-cE34KRek	5/3/2017	Positive	0.089
CA-cE34KRek	5/3/2017	Positive	0.51
CA-mO20qsPD	4/4/2017	Negative	0
CA-mO20qsPD	4/4/2017	Negative	0
CA-mO20qsPD	4/4/2017	Negative	0
CA-mO20qsPD	4/4/2017	Negative	0
CA-mO20qsPD	4/4/2017	Negative	0
CA-mO20qsPD	4/4/2017	Negative	0
CA-mO20qsPD	4/4/2017	Positive	2.7
CA-nk89ciDS	1/31/2018	Negative	0
CA-nk89ciDS	1/31/2018	Negative	0

CA-nk89ciDS	1/31/2018	Negative	0
CA-nk89ciDS	1/31/2018	Negative	0
CA-nk89ciDS	1/31/2018	Negative	0
CA-nk89ciDS	1/31/2018	Negative	0
CA-nk89ciDS	1/31/2018	Negative	0
CA-nk89ciDS	7/25/2018	Negative	0
CA-nk89ciDS	7/25/2018	Negative	0
CA-nk89ciDS	7/25/2018	Negative	0
CA-nk89ciDS	7/25/2018	Negative	0
CA-nk89ciDS	7/25/2018	Negative	0
CA-nk89ciDS	7/25/2018	Positive	0.089
CA-nk89ciDS	7/25/2018	Positive	0.089
CA-on82WYXy	6/6/2016	Negative	0
CA-on82WYXy	6/6/2016	Negative	0
CA-on82WYXy	6/6/2016	Negative	0
CA-on82WYXy	6/6/2016	Negative	0
CA-on82WYXy	6/6/2016	Negative	0
CA-on82WYXy	6/6/2016	Negative	0
CA-on82WYXy	6/6/2016	Negative	0
CA-pB23IELB	3/30/2017	Negative	0
CA-pB23IELB	3/30/2017	Negative	0
CA-pB23IELB	3/30/2017	Negative	0
CA-pB23IELB	3/30/2017	Negative	0
CA-pB23IELB	3/30/2017	Negative	0
CA-pB23IELB	3/30/2017	Negative	0
CA-pB23IELB	3/30/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	1/25/2017	Negative	0
CA-pB23IELB	7/31/2018	Negative	0
CA-pB23IELB	7/31/2018	Negative	0
CA-pB23IELB	7/31/2018	Positive	0.062
CA-pB23IELB	7/31/2018	Positive	0.089
CA-pB23IELB	7/31/2018	Positive	0.089
CA-pB23IELB	7/31/2018	Positive	0.089
CA-pB23IELB	7/31/2018	Positive	0.46
CA-pB23IELB	7/31/2018	Positive	1.2
CA-pB23IELB	7/31/2018	Positive	3.1
CA-pB23IELB	7/31/2018	Positive	4.5
CA-pB23IELB	7/31/2018	Positive	22
CA-pB23IELB	7/31/2018	Positive	43
CA-pB23IELB	7/31/2018	Positive	390
CA-pB23IELB	7/31/2018	Positive	720
CA-pf53ALCS	3/12/2018	Negative	0
CA-pf53ALCS	3/12/2018	Negative	0
CA-pf53ALCS	3/12/2018	Negative	0
CA-pf53ALCS	3/12/2018	Negative	0
CA-pf53ALCS	3/12/2018	Negative	0

CA-qT89PELK	4/18/2017	Negative	0
CA-qT89PELK	4/18/2017	Negative	0
CA-qT89PELK	4/18/2017	Negative	0
CA-qT89PELK	4/18/2017	Negative	0
CA-qT89PELK	4/18/2017	Negative	0
CA-qT89PELK	4/18/2017	Negative	0
CA-qT89PELK	4/18/2017	Negative	0
CA-qT89PELK	4/18/2017	Negative	0
CA-qT89PELK	4/18/2017	Negative	0
CA-qT89PELK	4/18/2017	Negative	0
CA-qT89PELK	4/18/2017	Negative	0
CA-qT89PELK	4/18/2017	Negative	0
CA-qT89PELK	4/18/2017	Negative	0
CA-S8LQ5OU3	4/10/2018	Negative	0
CA-S8LQ5OU3	4/10/2018	Negative	0
CA-S8LQ5OU3	4/10/2018	Negative	0
CA-S8LQ5OU3	4/10/2018	Negative	0
CA-S8LQ5OU3	4/10/2018	Negative	0
CA-S8LQ5OU3	4/10/2018	Negative	0
CA-S8LQ5OU3	4/10/2018	Negative	0
CA-S8LQ5OU3	8/22/2017	Negative	0
CA-S8LQ5OU3	8/22/2017	Negative	0
CA-S8LQ5OU3	8/22/2017	Negative	0
CA-S8LQ5OU3	8/22/2017	Negative	0
CA-S8LQ5OU3	8/22/2017	Negative	0
CA-S8LQ5OU3	8/22/2017	Negative	0
CA-S8LQ5OU3	8/22/2017	Negative	0
CA-S8LQ5OU3	8/22/2017	Negative	0
CA-td22zMWj	3/15/2018	Negative	0
CA-td22zMWj	3/15/2018	Negative	0
CA-td22zMWj	3/15/2018	Negative	0
CA-td22zMWj	3/15/2018	Negative	0
CA-td22zMWj	3/15/2018	Negative	0
CA-td22zMWj	3/15/2018	Negative	0
CA-td22zMWj	3/15/2018	Negative	0
CA-td22zMWj	3/15/2018	Negative	0
CA-td22zMWj	3/15/2018	Negative	0
CA-td22zMWj	3/28/2017	Negative	0
CA-td22zMWj	3/28/2017	Negative	0
CA-td22zMWj	3/28/2017	Negative	0
CA-td22zMWj	3/28/2017	Negative	0
CA-td22zMWj	3/28/2017	Negative	0
CA-td22zMWj	3/28/2017	Negative	0
CA-td22zMWj	3/28/2017	Negative	0
CA-td22zMWj	3/28/2017	Negative	0
CA-td22zMWj	3/28/2017	Negative	0
CA-td22zMWj	3/28/2017	Negative	0
CA-td22zMWj	3/28/2017	Positive	0.089
CA-td22zMWj	3/28/2017	Positive	0.089
CA-td22zMWj	3/28/2017	Positive	0.91
CA-td22zMWj	3/28/2017	Positive	3.2
CA-td22zMWj	3/28/2017	Positive	46
CA-Xo17YqnT	2/7/2017	Positive	0.51
CA-Xo17YqnT	2/7/2017	Positive	4.3
CA-Xo17YqnT	2/7/2017	Positive	12
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0

CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	9/25/2017	Negative	0
CA-Xv53IWgp	5/2/2017	Positive	0.089
CA-Xv53IWgp	5/2/2017	Positive	0.089
CA-Xv53IWgp	5/2/2017	Positive	0.089
CA-Xv53IWgp	5/2/2017	Positive	0.089
CA-Xv53IWgp	5/2/2017	Positive	0.089
CA-Xv53IWgp	5/2/2017	Positive	0.089
CA-Xv53IWgp	5/2/2017	Positive	0.089
CA-Xv53IWgp	5/2/2017	Positive	0.51
CA-Xv53IWgp	5/2/2017	Positive	1
CA-Xv53IWgp	5/2/2017	Positive	1.2
CA-Xv53IWgp	5/2/2017	Positive	2.1
CA-Xv53IWgp	5/2/2017	Positive	4.6
CA-Xv53IWgp	5/2/2017	Positive	4.6
CA-Xv53IWgp	5/2/2017	Positive	98

Table A4. Presence and concentration of STEC O157 and STEC non-O157 in manure samples from west region (Jay-Russel et al., 2018; Jay-Russel et al., 2023).

ID	Sampling date	Presence non-O157	Concentration non-O157 (MPN/g)	Presence O157 (MPN/g)	Concentration O157 (MPN/g)
AZ-2QF0EMOW	7/10/2017	Negative	0	Negative	0
AZ-2QF0EMOW	7/10/2017	Negative	0	Negative	0
AZ-2QF0EMOW	7/10/2017	Negative	0	Positive	0.089
AZ-2QF0EMOW	7/10/2017	Negative	0	Negative	0
AZ-2QF0EMOW	7/10/2017	Negative	0	Negative	0
AZ-2QF0EMOW	7/10/2017	Negative	0	Negative	0
AZ-2QF0EMOW	7/10/2017	Negative	0	Negative	0
AZ-2QF0EMOW	3/27/2018	Negative	0	Negative	0
AZ-2QF0EMOW	3/27/2018	Positive	0.089	Negative	0
AZ-2QF0EMOW	3/27/2018	Negative	0	Negative	0
AZ-2QF0EMOW	3/27/2018	Negative	0	Negative	0
AZ-2QF0EMOW	3/27/2018	Negative	0	Negative	0
AZ-2QF0EMOW	3/27/2018	Negative	0	Negative	0
AZ-2QF0EMOW	3/27/2018	Negative	0	Negative	0
AZ-2QF0EMOW	3/27/2018	Negative	0	Negative	0
AZ-31GOK76E	8/7/2017	Negative	0	Negative	0
AZ-31GOK76E	8/7/2017	Negative	0	Negative	0
AZ-31GOK76E	8/7/2017	Negative	0	Negative	0
AZ-31GOK76E	8/7/2017	Negative	0	Negative	0
AZ-31GOK76E	8/7/2017	Negative	0	Negative	0
AZ-31GOK76E	8/7/2017	Negative	0	Negative	0
AZ-31GOK76E	8/7/2017	Negative	0	Negative	0
AZ-31GOK76E	8/7/2017	Negative	0	Negative	0
AZ-31GOK76E	3/19/2018	Negative	0	Negative	0
AZ-31GOK76E	3/19/2018	Negative	0	Negative	0
AZ-31GOK76E	3/19/2018	Positive	0.089	Negative	0
AZ-31GOK76E	3/19/2018	Positive	1.9	Negative	0
AZ-31GOK76E	3/19/2018	Positive	22	Negative	0
AZ-31GOK76E	3/19/2018	Negative	0	Negative	0
AZ-31GOK76E	3/19/2018	Negative	0	Negative	0
AZ-5EZ2IR9X	9/19/2017	Negative	0	Negative	0
AZ-5EZ2IR9X	9/19/2017	Positive	1.8	Negative	0
AZ-5EZ2IR9X	9/19/2017	Negative	0	Negative	0
AZ-5EZ2IR9X	9/19/2017	Negative	0	Negative	0
AZ-5EZ2IR9X	9/19/2017	Negative	0	Negative	0
AZ-5EZ2IR9X	9/19/2017	Positive	12000	Negative	0
AZ-5EZ2IR9X	9/19/2017	Positive	77000	Negative	0
AZ-5EZ2IR9X	3/12/2018	Positive	9.4	Negative	0
AZ-5EZ2IR9X	3/12/2018	Positive	240	Negative	0
AZ-5EZ2IR9X	3/12/2018	Negative	0	Negative	0
AZ-5EZ2IR9X	3/12/2018	Negative	0	Negative	0
AZ-5EZ2IR9X	3/12/2018	Positive	4.2	Negative	0
AZ-5EZ2IR9X	3/12/2018	Positive	11	Negative	0
AZ-5EZ2IR9X	3/12/2018	Positive	120	Negative	0
AZ-97K60HVT	7/13/2017	Negative	0	Negative	0
AZ-97K60HVT	7/13/2017	Negative	0	Negative	0
AZ-97K60HVT	7/13/2017	Negative	0	Negative	0
AZ-97K60HVT	7/13/2017	Negative	0	Negative	0
AZ-97K60HVT	7/13/2017	Negative	0	Negative	0
AZ-97K60HVT	7/13/2017	Negative	0	Negative	0
AZ-97K60HVT	3/5/2018	Negative	0	Negative	0
AZ-97K60HVT	3/5/2018	Negative	0	Negative	0
AZ-97K60HVT	3/5/2018	Negative	0	Negative	0
AZ-97K60HVT	3/5/2018	Negative	0	Negative	0
AZ-97K60HVT	3/5/2018	Negative	0	Negative	0

AZ-L6448HV0	3/26/2018	Positive	1.8	Negative	0
AZ-L6448HV0	3/26/2018	Negative	0	Negative	0
AZ-L6448HV0	3/26/2018	Positive	0.089	Negative	0
AZ-L6448HV0	3/26/2018	Negative	0	Negative	0
AZ-L6448HV0	3/26/2018	Negative	0	Negative	0
AZ-L6448HV0	3/26/2018	Negative	0	Negative	0
AZ-L6448HV0	7/10/2017	Negative	0	Negative	0
AZ-L6448HV0	7/10/2017	Negative	0	Negative	0
AZ-L6448HV0	7/10/2017	Positive	0.089	Positive	3.2
AZ-L6448HV0	7/10/2017	Negative	0	Negative	0
AZ-L6448HV0	7/10/2017	Negative	0	Negative	0
AZ-L6448HV0	7/10/2017	Positive	0.51	Positive	0.51
AZ-RyJLIWPQ	3/13/2018	Negative	0	Negative	0
AZ-RyJLIWPQ	3/13/2018	Negative	0	Negative	0
AZ-RyJLIWPQ	3/13/2018	Negative	0	Negative	0
AZ-RyJLIWPQ	3/13/2018	Negative	0	Negative	0
AZ-RyJLIWPQ	3/13/2018	Negative	0	Negative	0
AZ-RyJLIWPQ	3/13/2018	Negative	0	Negative	0
AZ-RyJLIWPQ	3/13/2018	Negative	0	Negative	0
AZ-RyJLIWPQ	9/21/2017	Positive	0.089	Negative	0
AZ-RyJLIWPQ	9/21/2017	Negative	0	Negative	0
AZ-RyJLIWPQ	9/21/2017	Positive	0.089	Negative	0
AZ-RyJLIWPQ	9/21/2017	Positive	0.089	Negative	0
AZ-RyJLIWPQ	9/21/2017	Negative	0	Negative	0
AZ-RyJLIWPQ	9/21/2017	Positive	0.089	Negative	0
AZ-RyJLIWPQ	9/21/2017	Negative	0	Negative	0
AZ-V0OX570C	3/19/2018	Negative	0	Negative	0
AZ-V0OX570C	3/19/2018	Negative	0	Negative	0
AZ-V0OX570C	3/19/2018	Negative	0	Negative	0
AZ-V0OX570C	3/19/2018	Negative	0	Negative	0
AZ-V0OX570C	3/19/2018	Negative	0	Negative	0
AZ-V0OX570C	3/19/2018	Negative	0	Negative	0
AZ-V0OX570C	8/7/2017	Negative	0	Negative	0
AZ-V0OX570C	8/7/2017	Negative	0	Negative	0
AZ-V0OX570C	8/7/2017	Negative	0	Negative	0
AZ-V0OX570C	8/7/2017	Positive	0.51	Positive	4.6
AZ-V0OX570C	8/7/2017	Negative	0	Negative	0
AZ-V0OX570C	8/7/2017	Negative	0	Negative	0
AZ-V8GTZZ7L	7/13/2017	Negative	0	Negative	0
AZ-V8GTZZ7L	7/13/2017	Negative	0	Negative	0
AZ-V8GTZZ7L	7/13/2017	Negative	0	Negative	0
AZ-V8GTZZ7L	7/13/2017	Negative	0	Negative	0
AZ-V8GTZZ7L	7/13/2017	Negative	0	Negative	0
AZ-V8GTZZ7L	7/13/2017	Negative	0	Negative	0
AZ-VN55V187	5/23/2017	Negative	0	Negative	0
AZ-VN55V187	5/23/2017	Negative	0	Negative	0
AZ-VN55V187	5/23/2017	Positive	0.089	Negative	0
AZ-VN55V187	5/23/2017	Negative	0	Negative	0
AZ-VN55V187	5/23/2017	Positive	0.089	Negative	0
AZ-VN55V187	5/23/2017	Negative	0	Negative	0
AZ-VN55V187	5/23/2017	Negative	0	Negative	0
AZ-VN55V187	5/23/2017	Positive	0.089	Negative	0
AZ-VN55V187	5/23/2017	Negative	0	Negative	0
AZ-VN55V187	5/23/2017	Positive	0.089	Negative	0

AZ-VN55V187	5/23/2017	Positive	0.089	Negative	0
AZ-VN55V187	5/23/2017	Positive	0.089	Negative	0
AZ-VN55V187	5/23/2017	Negative	0	Negative	0
CA-aR08srTs	8/1/2018	Negative	0	Positive	85
CA-aR08srTs	8/1/2018	Negative	0	Positive	68
CA-aR08srTs	8/1/2018	Negative	0	Positive	2.1
CA-aR08srTs	8/1/2018	Negative	0	Positive	21
CA-aR08srTs	8/1/2018	Negative	0	Positive	32
CA-aR08srTs	8/1/2018	Negative	0	Positive	6.4
CA-aR08srTs	8/1/2018	Negative	0	Positive	1100
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Positive	0.089	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-aR08srTs	11/15/2016	Negative	0	Negative	0
CA-DT9J4OFZ	4/11/2018	Negative	0	Negative	0
CA-DT9J4OFZ	4/11/2018	Negative	0	Negative	0
CA-DT9J4OFZ	4/11/2018	Negative	0	Negative	0
CA-DT9J4OFZ	4/11/2018	Negative	0	Negative	0
CA-DT9J4OFZ	4/11/2018	Negative	0	Negative	0
CA-DT9J4OFZ	4/11/2018	Negative	0	Negative	0
CA-DT9J4OFZ	4/11/2018	Negative	0	Negative	0
CA-DT9J4OFZ	8/8/2017	Negative	0	Negative	0
CA-DT9J4OFZ	8/8/2017	Negative	0	Negative	0
CA-DT9J4OFZ	8/8/2017	Positive	1.2	Negative	0
CA-DT9J4OFZ	8/8/2017	Negative	0	Negative	0
CA-DT9J4OFZ	8/8/2017	Negative	0	Negative	0
CA-DT9J4OFZ	8/8/2017	Negative	0	Negative	0
CA-DT9J4OFZ	8/8/2017	Negative	0	Negative	0
CA-E04RHY9M	8/15/2017	Negative	0	Negative	0
CA-E04RHY9M	8/15/2017	Negative	0	Negative	0
CA-E04RHY9M	8/15/2017	Negative	0	Negative	0
CA-E04RHY9M	8/15/2017	Negative	0	Negative	0
CA-E04RHY9M	8/15/2017	Negative	0	Negative	0
CA-E04RHY9M	8/15/2017	Negative	0	Negative	0
CA-E04RHY9M	8/15/2017	Negative	0	Negative	0
CA-E04RHY9M	8/15/2017	Negative	0	Negative	0
CA-EU10BNPR	7/31/2018	Negative	0	Negative	0
CA-EU10BNPR	7/31/2018	Negative	0	Positive	5
CA-EU10BNPR	7/31/2018	Negative	0	Positive	100
CA-EU10BNPR	7/31/2018	Negative	0	Positive	41
CA-EU10BNPR	7/31/2018	Negative	0	Positive	120
CA-EU10BNPR	7/31/2018	Negative	0	Negative	0
CA-EU10BNPR	7/31/2018	Negative	0	Negative	0
CA-EU10BNPR	11/15/2017	Negative	0	Negative	0
CA-EU10BNPR	11/15/2017	Negative	0	Positive	0.089
CA-EU10BNPR	11/15/2017	Negative	0	Positive	0.089
CA-EU10BNPR	11/15/2017	Negative	0	Positive	0.089
CA-EU10BNPR	11/15/2017	Negative	0	Positive	0.089
CA-EU10BNPR	11/15/2017	Negative	0	Positive	0.089
CA-EU10BNPR	11/15/2017	Negative	0	Positive	0.089

CA-wU30WEPK	2/7/2017	Negative	0	Negative	0
CA-wU30WEPK	2/7/2017	Negative	0	Negative	0
CA-wU30WEPK	10/31/2017	Negative	0	Negative	0
CA-wU30WEPK	10/31/2017	Negative	0	Negative	0
CA-wU30WEPK	10/31/2017	Negative	0	Negative	0
CA-wU30WEPK	10/31/2017	Negative	0	Negative	0
CA-wU30WEPK	10/31/2017	Negative	0	Negative	0
CA-wU30WEPK	10/31/2017	Negative	0	Negative	0
CA-wU30WEPK	10/31/2017	Negative	0	Negative	0
CA-wU30WEPK	10/31/2017	Negative	0	Negative	0
CA-XQ0F9AJK	3/28/2018	Positive	6.6	Negative	0
CA-XQ0F9AJK	3/28/2018	Positive	0.51	Negative	0
CA-XQ0F9AJK	3/28/2018	Negative	0	Negative	0
CA-XQ0F9AJK	3/28/2018	Positive	4300	Negative	0
CA-XQ0F9AJK	3/28/2018	Negative	0	Negative	0
CA-XQ0F9AJK	3/28/2018	Positive	46	Negative	0
CA-XQ0F9AJK	3/28/2018	Negative	0	Negative	0
CA-XQ0F9AJK	8/9/2017	Negative	0	Negative	0
CA-XQ0F9AJK	8/9/2017	Negative	0	Negative	0
CA-XQ0F9AJK	8/9/2017	Negative	0	Negative	0
CA-XQ0F9AJK	8/9/2017	Negative	0	Negative	0
CA-XQ0F9AJK	8/9/2017	Negative	0	Negative	0
CA-XQ0F9AJK	8/9/2017	Negative	0	Negative	0
CA-XQ0F9AJK	8/9/2017	Negative	0	Negative	0
CA-XQ0F9AJK	8/9/2017	Negative	0	Negative	0
CA-XQ0F9AJK	8/9/2017	Negative	0	Negative	0
CA-Y262WW181	4/10/2018	Negative	0	Negative	0
CA-Y262WW181	4/10/2018	Negative	0	Negative	0
CA-Y262WW181	4/10/2018	Negative	0	Negative	0
CA-Y262WW181	4/10/2018	Negative	0	Negative	0
CA-Y262WW181	4/10/2018	Negative	0	Negative	0
CA-Y262WW181	4/10/2018	Negative	0	Negative	0
CA-Y262WW181	4/10/2018	Negative	0	Negative	0
CA-Y262WW181	8/21/2017	Positive	460	Negative	0
CA-Y262WW181	8/21/2017	Positive	830	Negative	0
CA-Y262WW181	8/21/2017	Positive	460	Negative	0
CA-Y262WW181	8/21/2017	Positive	410	Negative	0
CA-Y262WW181	8/21/2017	Positive	390	Negative	0
CA-Y262WW181	8/21/2017	Positive	19000	Negative	0
CA-Y262WW181	8/21/2017	Positive	25	Negative	0
CA-zf57hNXB	6/23/2016	Negative	0	Negative	0
CA-zf57hNXB	6/23/2016	Negative	0	Negative	0
CA-zf57hNXB	6/23/2016	Negative	0	Negative	0
CA-zf57hNXB	6/23/2016	Negative	0	Negative	0
CA-zf57hNXB	6/23/2016	Negative	0	Negative	0
CA-zf57hNXB	6/23/2016	Negative	0	Negative	0
CA-zf57hNXB	6/23/2016	Negative	0	Negative	0
CA-zf57hNXB	6/23/2016	Negative	0	Negative	0
CA-zf57hNXB	10/24/2016	Negative	0	Negative	0
CA-zf57hNXB	10/24/2016	Positive	0.089	Negative	0
CA-zf57hNXB	10/24/2016	Negative	0	Negative	0
CA-zf57hNXB	10/24/2016	Negative	0	Negative	0
CA-zf57hNXB	10/24/2016	Negative	0	Negative	0
CA-zf57hNXB	10/24/2016	Negative	0	Negative	0
CA-zf57hNXB	10/24/2016	Negative	0	Negative	0

Prevalence and concentration data were further analyzed to describe the prevalence and concentration of pathogens in untreated BSAAO in the risk assessment model. We followed the data analysis approach by Jay-Russell et al. (2023) and a brief description of the approach is provided below. With the sampling design of the FDA commissioned manure survey studies, most farms or facilities were visited more than once on different dates and a set of seven samples was taken during each of the visits (typically from a manure pile). Prevalence was first estimated by assuming independence of samples (regardless of farm, pile, and date) and was then compared it to the testing results with regard to the occurrence of multiple positive samples in a pile. If significant clustering was found, prevalence was estimated by analyzing data at the pile/farm/date level rather than at the individual samples level. Then distributions for the likelihood of detecting one or more positive samples in a set of seven samples were derived to describe the prevalence of positive manure piles. Correlation between the prevalence of positive manure piles and the concentration in positive samples were evaluated and if significant correlation exist, distributions were derived with consideration of prevalence to describe the concentration of pathogens.

In the risk assessment model, we applied the above approach and analyzed manure survey data from each of the aforementioned FDA commissioned studies for Shiga-toxin producing *E. coli* (STEC) O157:H7, STEC non-O157, and *Salmonella*. Below is an illustration of the analysis using the manure STEC O157 prevalence and concentration data reported in Jay-Russell et al. (2018) and Jay-Russell et al. (2023) as an example.

The overall prevalence for STEC O157 is 8.1% (42 out of 518 samples). Assuming independence of samples, prevalence of positive manure piles is expected to be $1-(1-0.081)^7 = 44.7\%$ given the overall prevalence of 8.1% and the probability of 0 to 7 positives obtained from

a set of 7 samples from a pile would be 55.3%, 34.2%, 9.05%, 1.3%, 0.12%, 0.01%, 0.00%, and 0.00%, respectively. However, observed prevalence for positive manure piles is 17.6% and three piles have 6 or 7 positive samples, which are not consistent with the calculated probability (i.e., 44.7% for positive manure pile and close to 0 probability to have 6 or 7 positives from a set of 7 samples) assuming independence of samples. Therefore, data suggested clustering and data analysis were conducted at the pile/farm/date level rather than at the individual sample level. Pile level prevalence data were fitted to a beta-binomial distribution and fitness was tested using the goodness of fit test. Expected number of positives from a set of 7 samples from a pile obtained from the derived beta-distribution was compared versus the observations from sampling to examine fitness of the distribution. The derived beta-binomial distribution that best represent the prevalence of STEC O157 in a manure pile is provided below:

$$\text{BetaBinomial}(0.0588, 0.643)$$

Using the above distribution, predicted overall prevalence of positive manure pile is 18.0%, and the probability of finding 0 to 7 positives from a set of 7 samples in a pile is 82.0%, 5.0%, 3.0%, 2.0%, 2.0%, 2.0%, 2.0%, and 2.0%, respectively. These values are consistent with the observed data.

Among the positive samples, STEC O157 concentration varies from -1.05 to 3.04 \log_{10} MPN/g. A linear regression analysis was conducted and results indicate a significant ($p < 0.05$) correlation between the concentration and the number of positive in a set of 7 samples: the greater the number of positives, the higher the mean concentration. Based on results from the linear regression, a normal distribution was derived where the mean was expressed as a function of prevalence of positive piles to describe the concentration in a positive pile (\log_{10} MPN/g):

Normal(-0.78+1.74 × prevalence, 1.16)

Predictions from the derived distribution is generally consistent with the enumeration data, where the median level for STEC O157 is significantly higher in a pile when 4 positive samples were detected than when 3 positive samples were detected. Similarly, the median level for STEC O157 is significantly higher in a pile when 7 positive samples were detected than when 4, 3 or 1 positive sample were detected.

Reference:

- Baker, C. A., De, J., Bertoldi, B., Dunn, L., Chapin, T., Jay-Russell, M., Danyluk, M. D., & Schneider, K. R. (2019). Prevalence and concentration of stx+ *E. coli* and *E. coli* O157 in bovine manure from Florida farms. *PloS one*, *14*(5), e0217445.
- Dunn, L. L., Sharma, V. K., Chapin, T. K., Friedrich, L. M., Larson, C. C., Rodrigues, C., Jay-Russell, M. T., Schneider, K. R., & Danyluk, M. D. (2022). The prevalence and concentration of *Salmonella enterica* in poultry litter in the southern United States. *PLoS ONE*, *17*(5), e0268231.
- Gartley, S., Ramos, T., Nyarko, E., de Souza, T. R., Jay-Russell, M., Chen, Y., et al. (2018). Manure pathogen survey of *Salmonella* and shiga toxin-producing *Escherichia coli* in untreated poultry and cattle manure of the Mid-Atlantic region. IAFP Annual Meeting 2018. Available at: <https://iafp.confex.com/iafp/2018/onlineprogram.cgi/Paper/18447>.
- Jay-Russell, M., Chen, Y., Rivadeneira, P., Pouillot, R., Aminabadi, P., Pandey, P., Bell, R. L., Oryang, D., Ingram, D., Kniel, K., & Van Doren, J. (2018). Prevalence and levels of Shiga toxin-producing *Escherichia coli* and *Salmonella* in untreated cattle and poultry manure in the west coast of United States. IAFP Annual Meeting 2018. Available at: <https://iafp.confex.com/iafp/2018/onlineprogram.cgi/Paper/18452>.
- Jay-Russell, M., Aminabadi, P., Chen, Y., Pouillot, R., Pandey, P., Ingram, D., Oryang, D., Kniel, K., & Van Doren, J. (2023). Quantifying the variability in the prevalence and levels

of Shiga toxin-producing *Escherichia coli* in untreated cattle and manure in the west coast of United States. Manuscript to be submitted.

Litt, P. K., Gartley, S., Kelly, A., Ramos, T., Nyarko, E., de Souza, T. R., Jay-Russell, M., Chen, Y., Aminabadi, P., Ingram, D., Severson, D., & Kniel, K. E. (2025). Prevalence of Shiga-toxigenic *Escherichia coli* in bovine manure in the Mid-Atlantic region of the United States. *Microorganisms*, *13*(2), 419.

Appendix B: Field trial for survival of *Escherichia coli* in chicken and rabbit feces.

Field trials were conducted to investigate the survival of *E. coli* in chicken and rabbit feces (Aminabadi et al., 2023). Data from the field trials were used to account for possible change in pathogen concentration in feces in certain trials from the transfer studies by Atwill et al. (2015), Weller et al. (2017a), Jeamsripong (2015), and Jeamsripong et al. (2019) where feces were left in the field for various time intervals before irrigation. Using this data allows for correct characterization of transfer probability and coefficients due to splash during irrigation.

Chicken and rabbit feces were collected two days prior to the trial. Rifampicin resistance *E. coli* (TVS355) was grown in Tryptic Soy Broth (TSB) supplemented with rifampicin (50 µg/mL). After determining the enrichment concentration, feces were inoculated to achieve final concentration of 10^4 CFU/g and 10^7 CFU/g in low and high inoculation treatments respectively. Field was flagged and scats were placed in the randomly assigned designated spot. Then each day at 7:00 AM one scat per spot was collected aseptically followed by transporting to the lab under refrigeration to be processed the same day. For microbial analysis, samples were weighted and suspended in 45 mL of PBS supplemented with rifampicin (50 µg/mL). To calculate the concentration (MPN/g), each sample was diluted in Tryptic Soy Broth (TSB) supplemented with rifampicin (50 µg/mL) into six dilutions (1:10, 1:10³, 1:10⁵, 1:10⁷, 1:10⁹ and 1:10¹¹) with two replications using a 12 well 84 mL reservoir followed by incubation in 42°C for 20 hours. Data from the field trials were provided in Table B1.

Table B1. *E. coli* concentration in chicken and rabbit feces over time under field condition.

Feces type	Rep	Treatment	Day	<i>E. coli</i> concentration (MPN/g)
Chicken	1	Low	0	3.57E+04
Chicken	2	Low	0	3.29E+06
Chicken	3	Low	0	4.22E+04
Chicken	4	Low	0	4.14E+04
Rabbit	1	Low	0	4.14E+04
Rabbit	2	Low	0	4.06E+06
Rabbit	3	Low	0	3.70E+04
Rabbit	4	Low	0	3.57E+04
Chicken	1	High	0	2.62E+08
Chicken	2	High	0	3.76E+06
Chicken	3	High	0	4.50E+10
Chicken	4	High	0	3.51E+08
Rabbit	1	High	0	5.37E+09
Rabbit	2	High	0	4.50E+08
Rabbit	3	High	0	5.56E+09
Rabbit	4	High	0	3.09E+08
Chicken	1	Low	1	1.39E+02
Chicken	2	Low	1	2.57E+04
Chicken	3	Low	1	3.00E+04
Chicken	4	Low	1	2.57E+04
Rabbit	1	Low	1	1.13E+06
Rabbit	2	Low	1	8.28E+04
Rabbit	3	Low	1	9.86E+04
Rabbit	4	Low	1	1.46E+06
Chicken	1	High	1	6.90E+04
Chicken	2	High	1	9.00E+06
Chicken	3	High	1	7.67E+06
Chicken	4	High	1	1.09E+08
Rabbit	1	High	1	8.28E+08
Rabbit	2	High	1	1.25E+10
Rabbit	3	High	1	6.90E+08
Rabbit	4	High	1	3.15E+10
Chicken	1	Low	2	3.38E+04
Chicken	2	Low	2	3.86E+04
Chicken	3	Low	2	3.86E+04
Chicken	4	Low	2	3.18E+04
Rabbit	1	Low	2	2.04E+06
Rabbit	2	Low	2	1.38E+05
Rabbit	3	Low	2	1.04E+07
Rabbit	4	Low	2	1.61E+06
Chicken	1	High	2	1.38E+03

Chicken	2	High	2	1.04E+03
Chicken	3	High	2	1.09E+11
Chicken	4	High	2	1.80E+04
Rabbit	1	High	2	1.15E+11
Rabbit	2	High	2	1.22E+09
Rabbit	3	High	2	5.31E+06
Rabbit	4	High	2	1.29E+09
Chicken	1	Low	3	3.86E+04
Chicken	2	Low	3	0.00E+00
Chicken	3	Low	3	2.19E+02
Chicken	4	Low	3	4.15E+04
Rabbit	1	Low	3	2.04E+06
Rabbit	2	Low	3	1.70E+04
Rabbit	3	Low	3	1.38E+03
Rabbit	4	Low	3	8.74E+03
Chicken	1	High	3	1.22E+03
Chicken	2	High	3	4.50E+06
Chicken	3	High	3	2.19E+06
Chicken	4	High	3	7.96E+08
Rabbit	1	High	3	1.33E+10
Rabbit	2	High	3	1.09E+09
Rabbit	3	High	3	0.00E+00
Rabbit	4	High	3	2.78E+10
Chicken	1	Low	4	4.15E+04
Chicken	2	Low	4	0.00E+00
Chicken	3	Low	4	2.57E+04
Chicken	4	Low	4	3.18E+04
Rabbit	1	Low	4	4.85E+08
Rabbit	2	Low	4	9.86E+04
Rabbit	3	Low	4	1.73E+05
Rabbit	4	Low	4	1.22E+07
Chicken	1	High	4	1.53E+04
Chicken	2	High	4	1.91E+04
Chicken	3	High	4	1.09E+07
Chicken	4	High	4	3.50E+06
Rabbit	1	High	4	1.13E+10
Rabbit	2	High	4	1.59E+09
Rabbit	3	High	4	5.59E+08
Rabbit	4	High	4	1.83E+12

The average changes in concentrations on sampling day 1 to day 4 post inoculation (day 0) were calculated for each feces type (i.e., chicken and rabbit) and the values were used to adjust the concentrations in feces just before irrigation from certain trials in the transfer studies where feces samples were left in the field for various time intervals before irrigation. Then, the adjusted concentration values in feces before irrigation were used to calculate the proportion of pathogens transferred on the crops in the transfer studies (Atwill et al., 2015; Weller et al., 2017a; Jeamsripong, 2015; Jeamsripong et al., 2019).

Reference:

- Atwill, E. R., Chase, J. A., Oryang, D., Bond, R. F., Koike, S. T., Cahn, M. D., Anderson, M., Mokhtari, A., & Dennis, S. (2015). Transfer of *Escherichia coli* O157:H7 from simulated wildlife scat onto romaine lettuce during foliar irrigation. *Journal of Food Protection*, 78(2), 240-247.
- Jeamsripong, S. (2015). *In-field transfer and survival of indicator Escherichia coli from wildlife feces to romaine lettuce, in the Salinas valley, California* (dissertation). University of California, Davis.
- Jeamsripong, S., Chase, J. A., Jay-Russell, M. T., Buchanan, R. L., & Atwill, E. R. (2019). Experimental in-field transfer and survival of *Escherichia coli* from animal feces to romaine lettuce in salinas valley, California. *Microorganisms*, 7(10), 408
- Weller, D. L., Kovac, J., Kent, D. J., Roof, S., Tokman, J. I., Mudrak, E., Kowalcyk, B., Oryang, D., Aceituno, A., & Wiedmann, M. (2017a). *Escherichia coli* transfer from simulated wildlife feces to lettuce during foliar irrigation: a field study in the northeastern United States. *Food microbiology*, 68, 24–33.

Appendix C: Development and performance evaluation of the transfer models quantifying the pathogen transfer from amended soils to produce crops via splash during rainfall or irrigation

The transfer probability model predicts the probability of pathogen transfer (i.e., if pathogen transfer occurred) from amended soils to crops during a rainfall or an irrigation event and the transfer coefficient model predicts what percentage of pathogens transferred onto the crops when such transfer occurred. Both models are gradient boosted tree models developed using the xgboost package in R as a function of two explanatory variables: (1) distance (m) between contamination source and lettuce and (2) amount of irrigation applied/precipitation (mm). Collected data were split and 75% of the data were used for model development, and the remaining 25% were reserved for measuring model performance. Model hyperparameters including number of trees, tree depth, learn rate, and loss reduction were tuned through a grid using cross validation.

Using the area under the receiver operating characteristic curve (AUC-ROC) as the measurement, the hyperparameter values that resulted in best model performance for the transfer probability model was identified (Table C1). The best transfer probability model has an AUC-ROC value of 0.93 and the ROC curve is shown in Fig. C1. The transfer coefficient model was evaluated using root mean squared error (rmse) as the measurement, and the hyperparameter set for the best transfer coefficient model was listed in Table C2.

Table C-1. Hyperparameter values for the best transfer probability model

Number of trees	Minimum data points in a node	Tree depth	Learning rate	Loss reduction
842	17	3	0.133	0.000112

Table C-2. Hyperparameter values for the best transfer coefficient model

Number of trees	Minimum data points in a node	Tree depth	Learning rate	Loss reduction
633	34	1	0.217	2.48×10^{-9}

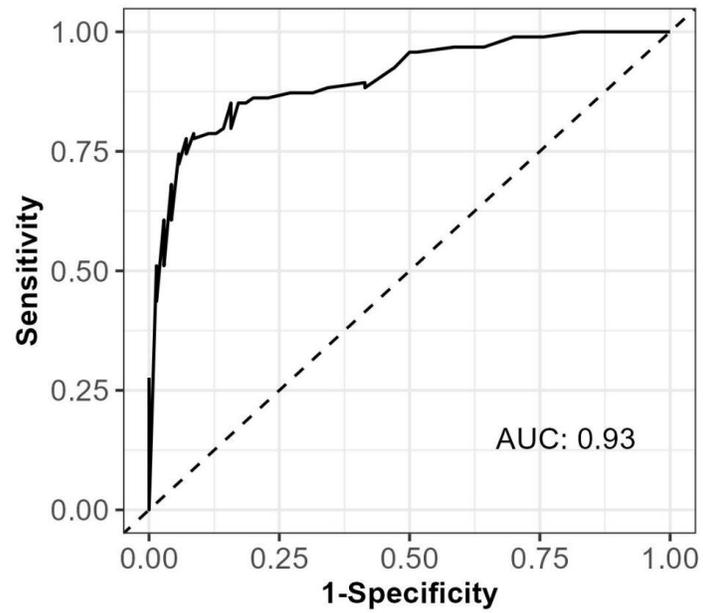


Fig. C-1. Receiver operating characteristic (ROC) curve for the transfer probability model

Appendix D: Adjustment to the Bardsley et al. (2021), Murphy et al. (2024), and Franz et al. (2011) survival data for Weibull model derivation in uncertainty analysis for pathogen survival in amended soils.

Bardsley et al. (2021) reported significantly longer survival of *Salmonella* in poultry litter amended soils that received weekly irrigation compared to daily irrigation based on trials using three strains (*S. Braenderup*, *S. Newport*, and *S. Meleagridis*) that were exposed to different irrigation regimens. In addition, Bardsley et al. (2021) examined survival of nine other *Salmonella* strains under daily irrigation regimen and observed significantly different survival and die-off rates among different strains. Considering the significant impact of irrigation regimen on *Salmonella* survival, survival data from trials by Bardsley et al. (2021) that received daily irrigation should not be used directly in our risk assessment considering our model's 5-7 day irrigation frequency. We further evaluated the difference between trials exposed to weekly irrigation and trials exposed to daily irrigation under both soil types (i.e., sandy loam and clay loam) considered in the study by Bardsley et al. (2021). Fig. D1 shows the comparison between the fitted *Salmonella* survival curves from weekly and daily irrigation trials. Variability among different *Salmonella* strains were similar between trials exposed to weekly and daily irrigation for clay-loam soil type (Fig. D1A,) whereas daily irrigation trials showed larger strain variability in survival compared to weekly irrigation trials for sandy-loam soil type (Fig. D1B).

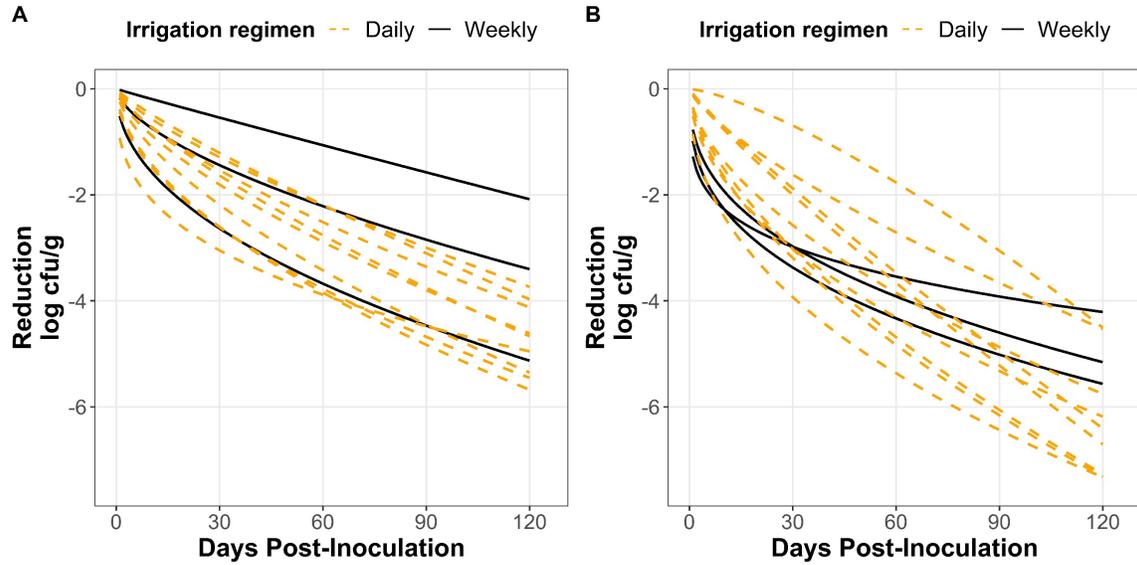


Fig. D1. Comparison between the fitted *Salmonella* survival curves from weekly and daily irrigation trials in (A) clay-loam soils and (B) sandy-loam soils from Bardsley et al. (2021).

Therefore, to account for strain variability observed from on survival trials that received daily irrigation, we adjusted *Salmonella* levels reported by Bardsley et al. (2021) for the nine other strains that only exposed to daily irrigation based on the observed differences in *Salmonella* concentration between weekly and daily irrigation regimen trials in sandy-loam soils using *S. Braenderup*, *S. Newport*, and *S. Meleagridis* strains, assuming the survival of nine other *Salmonella* strains that exposed only to daily irrigation will encounter similar impact when exposed to weekly irrigation. Specifically, for each of the three strains (i.e., *S. Braenderup*, *S. Newport*, and *S. Meleagridis* strains), the average difference between *Salmonella* concentration from weekly irrigation trials and daily irrigation trials in sandy-loam soils was calculated at each sampling point. Then, the calculated difference in *Salmonella* concentration values at each sampling point were used to adjust the concentrations at each sampling point for the other nine strains that only exposed to daily irrigation. Weibull model parameters were then derived based on the adjusted survival data and parameter values are summarized in Table D1.

Table D1. Parameter values for fitted Weibull models of *Salmonella* survival in amended soils based on adjusted survival data from Bardsley et al. (2021).

Soil type	<i>Salmonella</i> Strain	D	p
Sandy-loam	A (Muenchen)	1.53	0.37
Sandy-loam	B (Newport)	10.54	0.60
Sandy-loam	C (Poona)	0.24	0.23
Sandy-loam	D (Saintpaul)	1.08	0.28
Sandy-loam	E (Montevideo)	1.00	0.34
Sandy-loam	F (Enteritidis)	60.05	1.33
Sandy-loam	G (4, 12 i -)	0.08	0.23
Sandy-loam	H (Javiana)	9.94	0.41
Sandy-loam	J (Paratyphi B)	8.78	0.59

Murphy et al. (2024) reported significantly longer survival of STEC O157 and STEC non-O157 strains in bovine manure amended sandy loam soils that received weekly irrigation compared to daily irrigation based on trials using one STEC O157 strain and one STEC non-O157 that exposed to different irrigation regimens. Murphy et al. (2024) also observed significantly different survival among 4 other STEC O157 strains and 3 other STEC non-O157 exposed to daily irrigation. Following the same approach described above, we evaluated the difference between trials exposed to weekly irrigation and trials exposed to daily irrigation under both soil types (i.e., sandy-loam and clay-loam) considered in the study by Murphy et al. (2021) (Fig. D2). For both STEC O157 and STEC non-O157, survival curves were similar between trials exposed to weekly and daily irrigation for clay loam soil type (Fig. D2A&Fig. D2C,) whereas weekly irrigation trials showed slower die-off compared to daily irrigation trials for sandy loam soil type (Fig. D2B&Fig. D2D). Then, we adjusted STEC O157 and STEC non-O157 levels reported by Murphy et al. (2024) from trials that only exposed to daily irrigation and

Weibull model parameters were derived based on the adjusted survival data from daily irrigation trials. Table D2 summarizes the derived Weibull model parameters for the adjusted daily irrigation trials from Murphy et al. (2024).

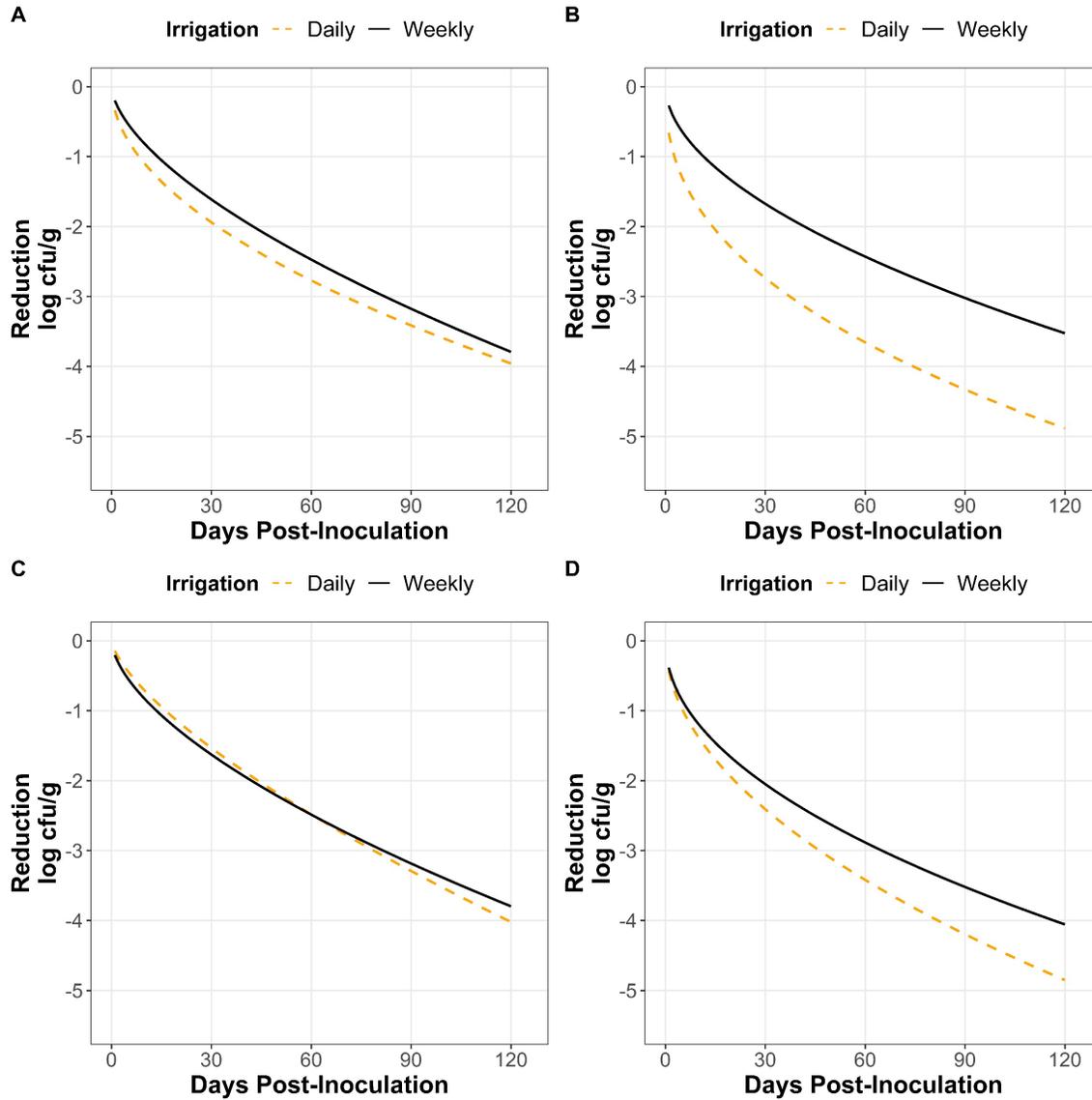


Fig. D2. Comparison between the fitted survival curves from weekly and daily irrigation trials for (A) STEC O157 strains in clay loam soils, (B) STEC O157 strains in sandy loam soils, (C) STEC non-O157 strains in clay loam soils, and (D) STEC non-O157 strains in sandy loam soils from Murphy et al. (2024).

Table D2. Parameter values for fitted Weibull models of STEC O157 and STEC non-O157 survival in amended soils based on adjusted survival data from Murphy et al. (2024).

Soil type	Strains	D	p
Sandy-loam	A (STEC O157)	3.13	0.46
Sandy-loam	C (STEC O157)	34.51	1.10
Sandy-loam	E (STEC O157)	8.62	0.46
Sandy-loam	K (STEC O157)	4.23	0.56
Sandy-loam	F (STEC non-O157)	21.91	0.84
Sandy-loam	G (STEC non-O157)	14.73	0.68
Sandy-loam	L (STEC non-O157)	9.44	0.61

Franz et al. (2011) also reported significant variability in the survival of 18 STEC O157 strains in manure-amended soils. Trials from Franz et al. (2011) showed larger strain variability in survival compared to trials from Murphy et al. (2024) under similar conditions (i.e., sandy soil and daily irrigation regimen) (Fig. D3). Then, following the same approach described above, we adjusted STEC O157 levels reported by Franz et al. (2011) and Weibull model parameters were derived based on the adjusted survival data to account for potential variability observed under weekly irrigation regimen. Table D3 summarizes the derived Weibull model parameters for the adjusted survival trials from Franz et al. (2011).

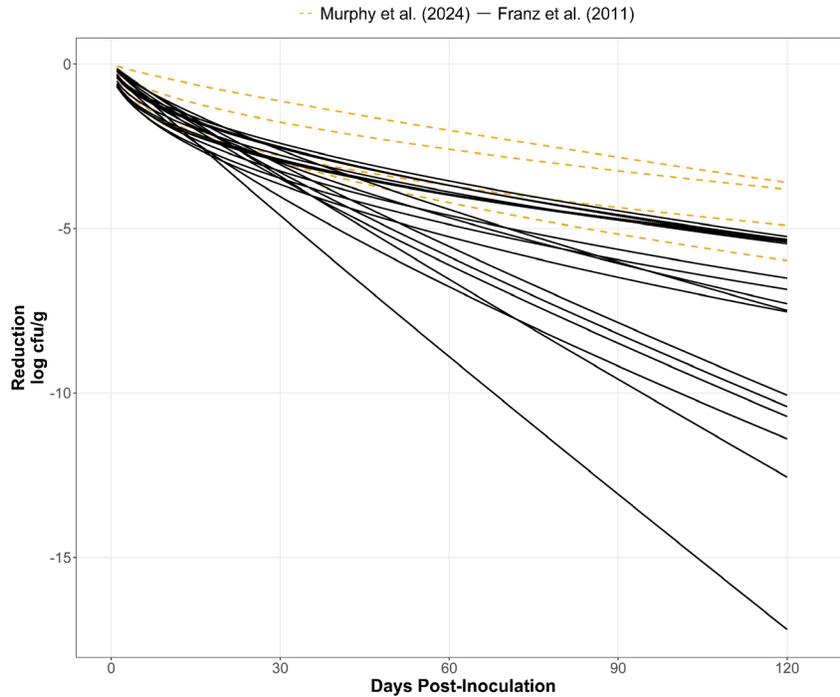


Fig. D2. Comparison between the fitted survival curves for STEC O157 strains from Murphy et al. (2024) trials (dashed lines) and Franz et al. (2011) trials (solid lines). Curves for Franz et al. (2011) trials were generated based on Weibull model parameter values reported in Fig. 1 in Franz et al. (2011).

Table D3. Parameter values for fitted Weibull models of STEC O157 survival in amended soils based on adjusted survival data from Franz et al. (2011).

D	p
9.79	0.73
14.53	0.66
21.33	0.83
26.96	1.21
33.30	1.07
17.87	1.15
11.92	1.00
17.15	1.23
16.14	1.11
11.12	1.16
29.73	1.01
14.15	0.66
28.30	0.97
20.23	1.00
10.35	0.70
16.48	0.72
13.13	0.75
20.26	1.21

Reference:

Bardsley, C. A., Weller, D. L., Ingram, D. T., Chen, Y., Oryang, D., Rideout, S. L., Strawn, L.

K. (2021). Strain, soil-type, irrigation regimen, and poultry litter influence *Salmonella* survival and die-off in agricultural soils. *Frontiers in Microbiology*, *12*, 590303.

Franz, E., van Hoek, A. H., Bouw, E., & Aarts, H. J. (2011). Variability of *Escherichia coli*

O157 strain survival in manure-amended soil in relation to strain origin, virulence profile, and carbon nutrition profile. *Applied and environmental microbiology*, *77*(22), 8088–8096.

Murphy, C. M., Weller, D. L., Bardsley, C. A., Ingram, D. T., Chen, Y., Oryang, D., Rideout, S.

L., & L. K. Strawn. (2024). Survival of twelve pathogenic and generic *Escherichia coli* strains in agricultural soils as influenced by strain, soil type, irrigation regimen, and soil amendment. *Journal of Food Protection*, *87*(10), 100343.

Appendix E: Summary of dataset included and excluded in the analysis for survival of pathogens on lettuce grown in the field

Table E1. Summary of dataset included and excluded for survival of *E. coli* O157:H7 on lettuce grown in the field.

Condition	No. of sampling days post inoculation	Reference	Decision
Field	27	Erickson et al., 2010	Include
Field	35	Moyne et al., 2011	Include
Field	21	Bezanson et al., 2011	Include
Field	10	Chase et al., 2019	Include
Field	10	Jeamsripong et al., 2019	Include
Field	56	Islam et al., 2004a	Include
Field	10	Weller et al., 2017	Include
Field	28	Oliverira et al., 2012	Include
Growth chamber	28	Erickson et al., 2014	Exclude
Growth chamber	8	Erickson et al., 2019	Exclude
Growth chamber	14	Stine et al., 2005	Exclude
Field	7	Moyne et al., 2020	Exclude
Field	4	Chase et al., 2017	Exclude
Field	9	Erickson et al., 2019	Exclude

Table E2. Summary of dataset included and excluded for survival of *Salmonella* on lettuce grown in the field.

Condition	No of sampling days post inoculation	Reference	Decision
Field	63	Islam et al., 2004b	Include
Growth chamber	14	Stine, et al., 2005	Exclude*
Growth chamber	7	López-Gálvez et al., 2018	Exclude*
Growth chamber	21	Harapas et al., 2015	Exclude*
Growth chamber	10	Lopez-Velasco et al., 2015	Exclude*
Growth chamber	8	Erickson et al., 2019	Exclude*
Field	4	Belias et al., 2020	Exclude*
Field	9	Erickson et al., 2019	Exclude*

*Included in the uncertainty analysis (see section 2.11.4)

Reference:

- Belias, A. M., Sbodio, A., Truchado, P., Weller, D., Pinzon, J., Skots, M., Allende, A., Munther, D., Suslow, T., Wiedmann, M., & Ivanek, R. (2020). Effect of weather on the die-off of *Escherichia coli* and Attenuated *Salmonella enterica* Serovar Typhimurium on preharvest leafy greens following irrigation with contaminated water. *Applied and environmental microbiology*, 86(17), e00899-20.
- Bezanson, G., Delaquis, P., Bach, S., McKellar, R., Topp, E., Gill, A., Blais, B., & Gilmour, M. (2012). Comparative examination of *Escherichia coli* O157:H7 survival on romaine lettuce and in soil at two independent experimental sites. *Journal of Food Protection*, 75(3), 480-487.

- Chase, J. A., Atwill, E. R., Partyka, M. L., Bond, R. F., & Oryang, D. (2017). Inactivation of *Escherichia coli* O157:H7 on romaine lettuce when inoculated in a fecal slurry matrix. *Journal of food protection*, 80(5), 792–798.
- Chase, J. A., Partyka, M. L., Bond, R. F., & Atwill, E. R. (2019). Environmental inactivation and irrigation-mediated regrowth of *Escherichia coli* O157:H7 on romaine lettuce when inoculated in a fecal slurry matrix. *PeerJ*, 7, e6591.
- Erickson, M. C., Liao, J. Y., Payton, A. S., Cook, P. W., Den Bakker, H. C., Bautista, J., & Pérez, J. C. D. (2019). Pre-harvest internalization and surface survival of *Salmonella* and *Escherichia coli* O157:H7 sprayed onto different lettuce cultivars under field and growth chamber conditions. *International journal of Food Microbiology*, 291, 197–204.
- Erickson, M. C., Webb, C. C., Davey, L. E., Payton, A. S., Flitcroft, I. D., Doyle, M. P. (2014). Internalization and fate of *Escherichia coli* O157:H7 in leafy green phyllosphere tissue using various spray conditions. *Journal of Food Protection*, 77(5):713-721.
- Erickson, M. C., Webb, C. C., Diaz-Perez, J. C., Payton, A. S., Silvoy, J.J., Davey, L., Payton, A. S., Liao, J., Ma, L., Doyle, M. P. (2010). Surface and internalized *Escherichia coli* O157:H7 on field-grown spinach and lettuce treated with spray-contaminated irrigation water. *Journal of Food Protection*, 73(6):1023-1029.
- Harapas, D., Premier, R., Tomkins, B., Hepworth, G., & Ajlouni, S. (2015). Shoot Injury Increases the Level of Persistence of *Salmonella enterica* Serovar Sofia and *Listeria innocua* on Cos Lettuce and of *Salmonella enterica* Serovar Sofia on Chive. *Journal of Food Protection*, 78(12), 2150–2155.
- Islam, M., Doyle, M. P., Phatak, S. C., Millner, P., & Jiang, X. (2004a). Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown

- in fields treated with contaminated manure composts or irrigation water. *Journal of Food Protection*, 67(7), 1365–1370.
- Islam, M., Morgan, J., Doyle, M. P., Phatak, S. C., Millner P., Jiang X. (2004b). Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathogens and Disease*, 1, 27–35.
- Jeamsripong, S., Chase, J. A., Jay-Russell, M. T., Buchanan, R. L., & Atwill, E. R. (2019). Experimental in-field transfer and survival of *Escherichia coli* from animal feces to romaine lettuce in salinas valley, California. *Microorganisms*, 7(10), 408.
- López-Gálvez, F., Gil, M. I., & Allende, A. (2018). Impact of relative humidity, inoculum carrier and size, and native microbiota on *Salmonella* ser. Typhimurium survival in baby lettuce. *Food microbiology*, 70, 155–161.
- Lopez-Velasco, G., Tomas-Callejas, Sbodio, A. O., Pham, X., Wei, Polly., Diribsa, Dawit., & Suslow, T. V. (2015). Factors affecting cell population density during enrichment and subsequent molecular detection of *Salmonella enterica* and *Escherichia coli* O157:H7 on lettuce contaminated during field production. *Food Control*, 54, 165-175.
- Moyne, A. L., Sudarshana, M. R., Blessington, T., Koike, S. T., Cahn, M. D., & Harris, L. J. (2011). Fate of *Escherichia coli* O157:H7 in field-inoculated lettuce. *Food Microbiology*, 28(8):1417-1425.
- Moyne, A. L., Blessington, T., Williams, T. R., Koike, S. T., Cahn, M. D., Marco, M. L., & Harris, L. J. (2020). Conditions at the time of inoculation influence survival of attenuated *Escherichia coli* O157:H7 on field-inoculated lettuce. *Food Microbiology*, 85, 103274.

Oliveira, M., Viñas, I., Usall, J., Anguera, M., & Abadias, M. (2012). Presence and survival of *Escherichia coli* O157:H7 on lettuce leaves and in soil treated with contaminated compost and irrigation water. *International Journal of Food Microbiology*, 156(2), 133–140.

Stine, S. W., Song, I., Choi, C. Y., & Gerba, C. P. (2005). Effect of relative humidity on preharvest survival of bacterial and viral pathogens on the surface of cantaloupe, lettuce, and bell peppers. *Journal of Food Protection*, 68(7), 1352-1358 .

Weller, D. L., Kovac, J., Roof, S. E., Kent, D. J., Tokman, J. I., Kowalcyk, B. B., Oryang, D., Ivanek, R., Aceituno, A. F., Sroka, C. J., & Wiedmann, M. (2017). Survival of *Escherichia coli* on lettuce under field conditions encountered in the northeastern United States. *Journal of Food Protection*, 80(7), 1214-1221.

Appendix F: Distribution of predicted pathogen concentration on lettuce heads from field amended with untreated BSAAO at the time of harvest by region and growing season

Table F1. Mean, median, 2.5th and 97.5th percentile of predicted STEC O157 concentration on lettuce heads from field amended with untreated BSAAO at the time of harvest*.

Growing season	Region	Application interval (days)	Mean predicted concentration (CFU/head)	2.5 th percentile of predicted concentration (CFU/head)	Median predicted concentration (CFU/head)	97.5 th percentile of predicted concentration (CFU/head)
Summer- fall	Mid-Atlantic	45	7.73×10^{-5}	0	0	8.26×10^{-4}
Summer- fall	Mid-Atlantic	60	6.65×10^{-5}	0	0	5.76×10^{-4}
Summer- fall	Mid-Atlantic	90	2.13×10^{-5}	0	0	2.00×10^{-4}
Summer- fall	Mid-Atlantic	120	1.04×10^{-5}	0	0	1.00×10^{-4}
Summer- fall	South	45	0.001	0	1.00×10^{-4}	6.24×10^{-3}
Summer- fall	South	60	8.53×10^{-4}	0	2.00×10^{-4}	1.04×10^{-2}
Summer- fall	South	90	7.11×10^{-4}	0	1.00×10^{-4}	8.40×10^{-3}
Summer- fall	South	120	5.77×10^{-4}	0	5.00×10^{-5}	5.43×10^{-3}
Summer- fall	West	45	0.020	0	0	3.11×10^{-2}
Summer- fall	West	60	0.002	0	0	1.44×10^{-2}
Summer- fall	West	90	7.01×10^{-4}	0	0	4.66×10^{-3}
Summer- fall	West	120	5.01×10^{-4}	0	0	4.48×10^{-3}
Winter-spring	Mid-Atlantic	45	7.06×10^{-4}	0	0	1.31×10^{-2}
Winter-spring	Mid-Atlantic	60	2.53×10^{-4}	0	0	2.40×10^{-3}
Winter-spring	Mid-Atlantic	90	1.70×10^{-5}	0	0	2.00×10^{-4}
Winter-spring	Mid-Atlantic	120	1.12×10^{-5}	0	0	1.00×10^{-4}
Winter-spring	South	45	0.003	0	2.25×10^{-4}	2.67×10^{-2}
Winter-spring	South	60	0.002	0	1.50×10^{-4}	1.97×10^{-2}
Winter-spring	South	90	6.43×10^{-4}	0	5.00×10^{-5}	6.85×10^{-3}
Winter-spring	South	120	2.67×10^{-4}	0	0	3.10×10^{-3}
Winter-spring	West	45	0.003	0	0	5.67×10^{-3}
Winter-spring	West	60	2.65×10^{-4}	0	0	1.25×10^{-3}
Winter-spring	West	90	8.51×10^{-5}	0	0	6.00×10^{-4}
Winter-spring	West	120	3.66×10^{-5}	0	0	2.50×10^{-4}

Table F2. Mean, median, 2.5th and 97.5th percentile of predicted *Salmonella* concentration on lettuce heads from field amended with untreated BSAAO at the time of harvest*.

Growing season	Region	Application interval (days)	Mean predicted concentration (CFU/head)	2.5 th percentile of predicted concentration (CFU/head)	Median predicted concentration (CFU/head)	97.5 th percentile of predicted concentration (CFU/head)
Summer- fall	Mid-Atlantic	45	320.627	0	0.470	4188
Summer- fall	Mid-Atlantic	60	120.781	0	0.133	2071
Summer- fall	Mid-Atlantic	90	32.359	0	2.50×10 ⁻⁴	283
Summer- fall	Mid-Atlantic	120	12.618	0	0	221
Summer- fall	South	45	134.276	0	0.322	950
Summer- fall	South	60	61.518	0	0.119	547
Summer- fall	South	90	21.330	0	4.25×10 ⁻⁴	182
Summer- fall	South	120	6.138	0	1.50×10 ⁻⁴	40.7
Summer- fall	West	45	24.547	0	2.50×10 ⁻⁴	14.1
Summer- fall	West	60	15.171	0	0	4.98
Summer- fall	West	90	3.516	0	0	2.84
Summer- fall	West	120	1.014	0	0	0.583
Winter-spring	Mid-Atlantic	45	68.707	0	1.50×10 ⁻⁴	200
Winter-spring	Mid-Atlantic	60	41.687	0	0	47.7
Winter-spring	Mid-Atlantic	90	8.356	0	0	14.9
Winter-spring	Mid-Atlantic	120	4.355	0	0	4.48
Winter-spring	South	45	21.038	0	5.00×10 ⁻⁵	138
Winter-spring	South	60	6.237	0	0	34.8
Winter-spring	South	90	1.400	0	0	5.40
Winter-spring	South	120	0.638	0	0	3.64
Winter-spring	West	45	20.701	0	3×10 ⁻⁴	13.6
Winter-spring	West	60	6.653	0	0	4.87
Winter-spring	West	90	1.897	0	0	2.55
Winter-spring	West	120	0.687	0	0	0.614

Table F3. Mean, median, 2.5th and 97.5th percentile of predicted STEC non-O157 concentration on lettuce heads from field amended with untreated BSAAO at the time of harvest*.

Growing season	Region	Application interval (days)	Mean predicted concentration (CFU/head)	2.5 th percentile of predicted concentration (CFU/head)	Median predicted concentration (CFU/head)	97.5 th percentile of predicted concentration (CFU/head)
Summer- fall	Mid-Atlantic	45	21.5	0	0.004	242
Summer- fall	Mid-Atlantic	60	4.560	0	0.003	30.4
Summer- fall	Mid-Atlantic	90	0.520	0	3.75×10 ⁻⁴	2.60
Summer- fall	Mid-Atlantic	120	0.097	0	0	0.627
Summer- fall	South	45	0.007	0	0	0.043
Summer- fall	South	60	0.006	0	0	0.012
Summer- fall	South	90	0.001	0	0	0.001
Summer- fall	South	120	1.61×10 ⁻⁴	0	0	1.50×10 ⁻⁴
Summer- fall	West	45	2.03	0	5.00×10 ⁻⁵	2.06
Summer- fall	West	60	0.424	0	0	1.22
Summer- fall	West	90	0.043	0	0	0.116
Summer- fall	West	120	0.013	0	0	0.025
Winter-spring	Mid-Atlantic	45	21.3	0	0.003	183
Winter-spring	Mid-Atlantic	60	3.37	0	0.002	22.4
Winter-spring	Mid-Atlantic	90	0.414	0	2.50×10 ⁻⁴	3.85
Winter-spring	Mid-Atlantic	120	0.095	0	0	0.606
Winter-spring	South	45	0.008	0	0	0.047
Winter-spring	South	60	0.008	0	0	0.010
Winter-spring	South	90	8.77×10 ⁻⁴	0	0	0.001
Winter-spring	South	120	8.47×10 ⁻⁵	0	0	1.76×10 ⁻⁴
Winter-spring	West	45	2.00	0	2.50×10 ⁻³	1.93
Winter-spring	West	60	0.348	0	0	1.16
Winter-spring	West	90	0.062	0	0	0.147
Winter-spring	West	120	0.015	0	0	0.021

Appendix G: Additional results for predicted concentrations of *Salmonella* on lettuce.

Table G1. Mean predicted concentrations of *Salmonella* on lettuce heads from field amended with untreated BSAAO at application intervals from 180 to 600 days.

Application interval (days)	<i>Salmonella</i> Concentration* (log ₁₀ CFU/head)
180	-0.377
240	-1.112
360	-2.920
480	-3.684
600	-4.062

Appendix H: Results for uncertainty analysis.

Detailed results from uncertainty analysis are shown in the tables below.

Table H1. Uncertainty analysis – impact of initial contamination prevalence in untreated BSAAOs on the predicted pathogen concentration on lettuce at the time of harvest¹.

Region/ scenario	Interval (day)	Results for STEC O157 using upper- bound values	Results for STEC O157 using lower- bound values	Results for <i>Salmonella</i> using upper-bound values	Results for <i>Salmonella</i> using lower-bound values
Mid Atlantic	45	-2.405	-3.057	2.303	1.295
Mid Atlantic	60	-3.070	-3.769	2.032	0.263
Mid Atlantic	90	-4.413	-4.972	1.496	-0.291
Mid Atlantic	120	-4.602	-5.176	0.906	-0.677
South	45	-2.450	-2.897	1.585	1.952
South	60	-2.564	-3.081	1.556	1.249
South	90	-3.071	-3.578	1.044	0.907
South	120	-3.434	-4.006	0.660	0.134
West	45	-1.388	-2.835	0.434	-0.164
West	60	-2.422	-3.249	0.076	-0.406
West	90	-3.219	-4.057	-0.600	-0.884
West	120	-3.316	-4.033	-0.892	-1.275

¹Calculated as the log₁₀ values of the average number of pathogens (CFU) across all crops in a field.

Table H2. Uncertainty analysis – impact of initial contamination level in untreated BSAAOs on the predicted pathogen concentration on lettuce at the time of harvest¹.

Region/ scenario	Interval (day)	Results for STEC O157 using upper- bound values	Results for STEC O157 using lower- bound values	Results for <i>Salmonella</i> using upper-bound values	Results for <i>Salmonella</i> using lower-bound values
Mid Atlantic	45	-2.660	-2.700	1.454	1.084
Mid Atlantic	60	-3.465	-3.374	0.830	0.595
Mid Atlantic	90	-4.706	-4.729	0.472	0.167
Mid Atlantic	120	-4.921	-4.844	-0.038	-0.161
South	45	-2.490	-3.876	3.172	1.581
South	60	-2.756	-4.227	2.621	1.310
South	90	-3.405	-4.909	2.353	1.088
South	120	-3.502	-4.972	1.722	0.857
West	45	-1.433	-0.967	2.446	1.578
West	60	-2.289	-2.503	2.273	1.276
West	90	-3.248	-3.163	1.492	0.871
West	120	-3.440	-3.518	0.841	0.779

¹Calculated as the log₁₀ values of the average number of pathogens (CFU) across all crops in a field.

Table H3. Uncertainty analysis – impact of soil survival model on the predicted pathogen concentration on lettuce at the time of harvest¹.

Region/ scenario	Interval (day)	Results for STEC O157 using upper- bound values	Results for STEC O157 using lower- bound values	Results for <i>Salmonella</i> using upper-bound values	Results for <i>Salmonella</i> using lower-bound values
Mid Atlantic	45	-2.339	-4.437	2.137	2.093
Mid Atlantic	60	-2.795	-4.593	1.769	1.619
Mid Atlantic	90	-3.804	-5.119	1.309	1.019
Mid Atlantic	120	-4.223	-5.260	0.866	0.459
South	45	-1.569	-2.697	1.553	1.454
South	60	-1.729	-3.009	1.094	0.726
South	90	-2.511	-3.553	0.514	0.202
South	120	-2.840	-3.645	0.123	-0.314
West	45	-1.256	-2.193	1.394	1.345
West	60	-1.919	-2.787	0.118	0.759
West	90	-2.566	-3.773	-0.309	0.295
West	120	-2.976	-4.015	0.235	0.043

¹Calculated as the log₁₀ values of the average number of pathogens (CFU) across all crops in a field.

Table H4. Uncertainty analysis – impact of splash radius on the predicted pathogen concentration on lettuce at the time of harvest¹.

Region/ scenario	Interval (day)	Results for STEC O157 using upper-bound values	Results for <i>Salmonella</i> using upper-bound values
Mid Atlantic	45	-3.281	2.321
Mid Atlantic	60	-3.655	1.969
Mid Atlantic	90	-4.446	1.291
Mid Atlantic	120	-4.807	0.899
South	45	-2.656	1.844
South	60	-2.620	1.374
South	90	-2.949	0.783
South	120	-3.297	0.307
West	45	-1.970	1.446
West	60	-2.917	1.066
West	90	-3.402	0.438
West	120	-3.745	-0.061

¹Calculated as the log₁₀ values of the average number of pathogens (CFU) across all crops in a field.

Table H5. Uncertainty analysis – impact of pathogen transfer models on the predicted pathogen concentration on lettuce at the time of harvest¹.

Region/ scenario	Interval (day)	Results for STEC O157 using upper- bound values	Results for STEC O157 using lower- bound values	Results for <i>Salmonella</i> using upper-bound values	Results for <i>Salmonella</i> using lower-bound values
Mid Atlantic	45	-3.089	-3.860	2.465	1.490
Mid Atlantic	60	-3.441	-4.300	2.091	1.270
Mid Atlantic	90	-4.187	-5.230	1.371	0.831
Mid Atlantic	120	-4.527	-5.650	0.955	0.579
South	45	-2.501	-3.120	1.958	1.190
South	60	-2.467	-3.080	1.460	0.885
South	90	-2.777	-3.470	0.831	0.504
South	120	-3.105	-3.880	0.326	0.197
West	45	-1.855	-2.320	1.536	0.931
West	60	-2.747	-3.430	1.132	0.686
West	90	-3.204	-4.000	0.465	0.282
West	120	-3.526	-4.400	-0.086	-0.052

¹Calculated as the log₁₀ values of the average number of pathogens (CFU) across all crops in a field.

Table H6. Uncertainty analysis – impact of *Salmonella* survival rate on crops on the predicted pathogen concentration on lettuce at the time of harvest¹.

Region/ scenario	Interval (day)	Results for STEC O157 using alternative survival rate values
Mid Atlantic	45	0.085
Mid Atlantic	60	-0.033
Mid Atlantic	90	-0.82
Mid Atlantic	120	-0.783
South	45	-1.000
South	60	-0.287
South	90	-2.289
South	120	-2.828
West	45	-1.807
West	60	-2.236
West	90	-2.465
West	120	-2.697

¹Calculated as the log₁₀ values of the average number of pathogens (CFU) across all crops in a field.

²Alternative: using alternative inclusion criteria for *Salmonella* survival rate on crops.