A QUANTITATIVE APPROACH TO UNDERSTANDING THE RISK OF FOODBORNE PATHOGEN CONTAMINATION ALONG THE ALTERNATIVE AND CONVENTIONAL BROILER SUPPLY CHAINS

by

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(Under the Direction of Abhinav Mishra)

ABSTRACT

The broiler supply chain is a complex system that contains several points where foodborne pathogen contamination can occur. Traditionally, broiler research has focused on the risk of *Salmonella* spp. and *Campylobacter* spp. contamination. Additionally, research has shown that *Listeria* spp. could be a pathogen of concern, especially for alternative broiler production systems. Alternative broiler production systems, such as organic or pastured systems, are characterized by alternative rearing practices, such as allowing birds outdoor access. Due to this, food safety risks may be different from those encountered during conventional production. Currently, the food safety risks for alternative production systems are not well characterized. The current study aimed to address knowledge gaps in food safety risks encountered in alternative broiler production and compare those with conventional production. Preharvest and processed broiler samples were collected from pastured poultry farms and processing centers in the southeastern United States. From these data, machine learning models were trained to predict *Listeria* and *Campylobacter* prevalence in feces and soil samples based on recent weather patterns. Models with area under the receiver operating characteristic curve (AUC) values of

>0.85 were obtained for each pathogen and sample type combination. Additionally, random forest models were constructed to predict *Listeria* spp. prevalence in pre- and postharvest samples based on farm practice variables. Both models had AUC >0.87. It was found that time of year and broiler age were important predictors of *Listeria* presence in preharvest samples and that brood feed and processing rinse water chlorination were important predictors in postharvest samples. In the next study, a systematic review and meta-analysis was performed to quantify the differences between conventional and alternative broiler productions systems in *Salmonella* and *Campylobacter* prevalence throughout the supply chain. *Campylobacter* prevalence was significantly higher in alternative environmental samples, but all other comparisons were not significantly different. Finally, a retail-to-consumption risk assessment model was used to compare risks from contaminated broiler meat produced conventionally and alternatively and prepared in-home in the United States. The model estimated that risk was slightly higher for alternatively-processed broiler meat. These results will help in risk-based decision making in the broiler industry.

INDEX WORDS: poultry, alternative farming, *Salmonella*, *Campylobacter*, *Listeria*, machine learning, risk assessment

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DEDICATION

I would like to dedicate this work to my mother and father, my sister, Allyson, and my girlfriend, Grace. None of this work would be possible without your continued love, patience, and support. Thank you.

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TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS v

LIST OF TABLES viii

LIST OF FIGURES xi

CHAPTER

1 INTRODUCTION 1

References 4

2 LITERATURE REVIEW 6

References 31

3 COMPARISON BETWEEN RANDOM FOREST AND GRADIENT BOOSTING MACHINE METHODS FOR PREDICTING *LISTERIA* SPP. PREVALENCE IN THE ENVIRONMENT OF PASTURED POULTRY FARMS 56

References 74

4 USING FARM PRACTICE VARIABLES AS PREDICTORS OF *LISTERIA* SPP. PREVALENCE IN PASTURED POULTRY FARMS 91

References 108

5 USING WEATHER PATTERNS TO FORECAST *CAMPYLOBACTER* PRESENCE IN THE ENVIRONMENT OF PASTURED POULTRY FARMS 127

References 142

6 PREVALENCE OF *SALMONELLA* SPP. AND *CAMPYLOBACTER* SPP. IN ALTERNATIVE AND CONVENTIONALLY PRODUCED CHICKEN IN THE UNITED STATES: A SYSTEMATIC REVIEW AND META-ANALYSIS..........155

References 173

7 ASSESSING THE RISK OF SALMONELLOSIS FROM CONSUMPTION OF CONVENTIONALLY AND ALTERNATIVELY PRODUCED BROILER MEAT PREPARED IN-HOME IN THE UNITED STATES..........206

References 225

8 SUMMARY AND CONCLUSIONS..........248

APPENDICES

A LIST OF THE METEOROLOGICAL PREDICTOR VARIABLES USED IN THE CONSTRUCTION OF RANDOM FOREST AND GRADIENT BOOSTING MACHINE MODELS GENERATED IN CHAPTER 3..........251

LIST OF TABLES

Page

Table 2.1: United States Department of Agriculture standards for organic poultry production according to 7 CFR §205 54

Table 2.2: Chicken-associated salmonellosis outbreaks in the United States and Puerto Rico during 2011-2018 55

Table 3.1: Comparison of the 11 all-natural, antibiotic-free, pastured broiler farms included in this study 80

Table 3.2: Number of samples and prevalence of positive *Listeria* spp. and *Listeria monocytogenes* environmental samples for each farm 81

Table 3.3: Effect of sample type on the prevalence of *Listeria* spp. and *Listeria monocytogenes* in environmental samples of pastured poultry farms 82

Table 3.4: Effect of season on the prevalence of *Listeria* spp. and *Listeria monocytogenes* in environmental samples of pastured poultry farms 83

Table 3.5: Predictive performance of all four models 84

Table 4.1: Comparison of the 11 all-natural, antibiotic-free, pastured broiler farms included in this study 114

Table 4.2: Predictors used in the preharvest (feces, soil) and final product whole carcass rinse (WCR) random forest models 115

Table 4.3: Broiler cold storage procedures before making the product available for consumers for each farm 118

Table 4.4: Effect of sample type on prevalence of *Listeria* spp. and *Listeria monocytogenes* in pastured poultry farm samples....119
Table 4.5: Prevalence of *Listeria* spp. by pastured poultry farm and sample type....120
Table 4.6: Predictive performance of the preharvest and whole carcass rinse (WCR) random forest models....121
Table 5.1: *Campylobacter* prevalence in fecal and soil samples collected from United States pastured poultry farms that were used for initial random forest model training and testing and forecast validation....147
Table 5.2: *Campylobacter* prevalence by season for samples in fecal and soil samples collected from United States pastured poultry farms that were used for initial random forest model training and testing....148
Table 5.3: Predictive performance of the generated random forest models....149
Table 5.4: Forecasting capabilities of the generated random forest models....150
Table 6.1: Summary of broiler farm environmental sampling studies used in meta-analysis....187
Table 6.2: Summary of broiler processing sampling studies used in meta-analysis....189
Table 6.3: Summary of retail broiler sampling studies used in meta-analysis....192
Table 6.4: *Salmonella* serotype prevalence in various sample types collected along the chicken supply chain....196
Table 6.5: Meta-analysis results for pathogen prevalence at different stages throughout the chicken supply chain subgrouped by type of production system....197
Table 6.6: The heterogeneity explained by various moderator variables in the combined meta-analysis models for each sample type and pathogen combination....199
Table 7.1: Description of QMRA inputs....233

Table 7.2: Studies containing primary *Salmonella* growth data on chicken used in secondary growth model fitting 239

Table 7.3: Cumulative distribution of *Salmonella* concentration in *Salmonella* positive chicken samples taken from the end of the production line 240

Table 7.4: Cross-contamination studies containing bacterial transfer rate data used in this study 241

Table 7.5: Median, 25th percentile, and 75th percentile estimated total risk per serving and total annual number of illnesses due to consumption of conventionally and alternatively produced broiler meat, as determined by the baseline QMRA model 242

Table 7.6: Median, 25th percentile, and 75th percentile estimates of total annual number of illnesses caused by "what-if" scenarios compared to the baseline QMRA model 243

LIST OF FIGURES

Page

Figure 1.1: General overview of the broiler supply chain with dissertation objectives overlaid 5

Figure 3.1: Receiver operating characteristic (ROC) curves for the feces random forest (solid line) and gradient boosting machine (dashed line) models. The diagonal line represents the line of no-discrimination, which represents a completely random guess 85

Figure 3.2: Receiver operating characteristic (ROC) curves for the soil random forest (solid line) and gradient boosting machine (dashed line) models. The diagonal line represents the line of no-discrimination, which represents a completely random guess 86

Figure 3.3: Variable importance plots for the feces (a) random forest model and (b) gradient boosting machine models. Variables were assigned a relative importance value on a scale of 0 to 100, where a higher importance score means higher relative importance. Variable names with a decimal are the average of that variable from the sampling day to X days prior, where X is the number of days before the sampling day 87

Figure 3.4: Partial dependency plots for the two most important variables for the feces random forest (a & b) and gradient boosting machine models (c & d) 88

Figure 3.5: Variable importance plots for the soil (a) random forest model and (b) gradient boosting machine models. Variables were assigned a relative importance value on a scale of 0 to 100, where a higher importance score means higher relative importance. Variable names with a decimal are the average of that variable from the sampling day to X days prior, where X is the number of days before the sampling day 89

Figure 3.6: Partial dependency plots for the two most important variables for the soil random forest (a & b) and gradient boosting machine models (c & d) 90

Figure 4.1: Variable importance plot for the preharvest sample random forest model. Relative importance is determined by average mean decrease in Gini and scaled from 0 to 100, where 100 is most important. SwineOnFarmY refers to farms that had swine present at the farm. EggSourceD refers to farms that received broiler eggs from Company D 122

Figure 4.2: Partial dependency plots (PDPs) for the two most important predictors in the preharvest sample random forest model. (a) DayOfYear represents the day of the year that samples were collected, numerically from 1 to 365. (b) FlockAgeWeeks represents the age of the flock in weeks at the time of sampling 123

Figure 4.3: Variable importance plot for the whole carcass rinse (WCR) final product random forest model. Relative importance is determined by average mean decrease in Gini and scaled from 0 to 100, where 100 is most important. BroodFeedCSW refers to brood feed with top 3 sources of protein as corn, soy, and wheat. RinseWaterChlorY refers to farms that chlorinated rinse water used during broiler processing. RinseWaterSourceWell refers to farms that used well water as the source for rinse water used during processing. EggSourceA refers to farms that received broiler eggs from Company A...................... 124

Figure 4.4: Partial dependency plots (PDPs) for the two most important predictors in the whole carcass rinse (WCR) final product random forest model. (a) Brood feed refers to up to the top 3 sources of protein in the brood feed. Ingredients include: barley (B), corn (C), oats (O), wheat (W), soy (S), cotton seed mill (M), peas (P). (b) RinseWaterChlor refers to whether the rinse water used during broiler processing was chlorinated or not. Levels for

this variable include: yes (Y), no (N), organic acids used instead of chlorine (OA), yes with vinegar (YV), certified organic rinse used as wash (COR)....125

Figure 4.5: Receiver operating characteristic (ROC) curves for the environmental (solid line) and whole carcass rinse (dashed line) sample random forest models. The diagonal line represents the line of no discrimination, or the expectation of a random guess. Curves above this line are considered better than random guess. The area under each curve is referred to as AUC....126

Figure 5.1: A schematic detailing predictions made to determine model accuracy based on forecasted weather. Underlined numbers represent the number of days before the sampling day the predictions were made. Boxed in days are days in which forecasted weather data were used, while days not boxed in represent days in which historical data were used for a given forecast day....151

Figure 5.2: Receiver operating characteristic (ROC) curves for the (a) feces and (b) soil random forest models. The dotted line represents the line of no discrimination, or the expectation of a random guess....152

Figure 5.3: Variable importance plot for the feces random forest model. Variables were assigned a relative importance value on a scale of 0 to 100, where a higher importance score means higher relative importance. Variable names with a decimal are the average of that variable from 1 day before sampling to X days before sampling, where X is the number of days before the sampling day. Variable names containing 'Before' represent variable values collected X days before sampling....153

Figure 5.4: Variable importance plot for the soil random forest model. Variables were assigned a relative importance value on a scale of 0 to 100, where a higher importance score means

higher relative importance. Variable names with a decimal are the average of that variable from 1 day before sampling to X days before sampling, where X is the number of days before the sampling day 154

Figure 6.1: Flow diagram of the systematic review process 200

Figure 6.2: Forest plots for the (a) *Campylobacter* spp. and (b) *Salmonella* spp. environmental sample subgroups 201

Figure 6.3: Forest plots for the (a) *Campylobacter* spp. and (b) *Salmonella* spp. rehang sample subgroups 202

Figure 6.4: Forest plots for the (a) *Campylobacter* spp. and (b) *Salmonella* spp. prechill sample subgroups 203

Figure 6.5: Forest plots for the (a) *Campylobacter* spp. and (b) *Salmonella* spp. postchill sample subgroups 204

Figure 6.6: Forest plots for the (a) *Campylobacter* spp. and (b) *Salmonella* spp. retail sample subgroups 205

Figure 7.1: Growth rates derived from primary growth data from Combase data sources (a). Secondary modeling of primary growth rates in temperature profiles from 5.9-37°C (b). The dotted line represents the 95% confidence interval and the dashed line represents the prediction interval 244

Figure 7.2: The distribution of final *Salmonella* concentration numbers in log CFU/g across all Monte Carlo iterations 245

Figure 7.3: Cumulative distribution functions for log *Salmonella* risk per serving for conventionally produced broiler meat (solid line) and alternatively produced broiler meat (dashed line) as predicted by the baseline QMRA model 246

Figure 7.4: Spearman's correlation coefficients showing the 8 most important model parameters in predicting the total *Salmonella* risk per serving of broiler meat 247

CHAPTER 1

INTRODUCTION

1.1 Broiler-related microbial concerns

Foodborne pathogens such as *Salmonella* spp. and *Campylobacter* spp. present a major concern for the broiler industry on a yearly basis, due to their association with broiler-related foodborne illnesses. Transport crates, environmental conditions, contaminated feed, meteorological factors, worker hygiene, and bird-to-bird pathogen transfer have all been identified as major preharvest contamination risk factors *(1, 3, 5)*. During processing, broiler carcasses can be contaminated with pathogenic bacteria due to leakage of fecal matter during processing steps *(2)*. Cross-contamination has also been identified as a major risk factor during processing and preparation *(6)*. Intervention strategies are implemented at the preharvest and postharvest levels to mitigate the risk of contamination of chicken products by these pathogenic bacteria.

1.2 Alternative broiler production

In recent years, increased demand for antibiotic-free or "natural" products has pushed consumers toward organic food products *(4, 7)*. This has impacted the poultry industry, where there has been increased demand for broiler meat harvested from alternative poultry farming production facilities, such as organic and pastured poultry systems *(8, 9)*. These types of operations are characterized by the lack of antibiotic use and the allowance of birds to access the outside environment. As such, birds are exposed to a less controlled environment, suggesting an increased risk of microbial contamination of the birds. Due to these conditions, the risks of

foodborne pathogens that are abundant in the natural environment, such as *Listeria* spp. and *Campylobacter* spp., and the risk factors associated with the presence of these pathogens need to be characterized for the broiler industry.

1.3 Project objectives

The goal of this dissertation was to utilize predictive microbiology techniques as a means to understand the risk of foodborne pathogen contamination throughout conventional and alternative (e.g. organic, pastured, free-range) broiler production chains. An emphasis was included on identifying risk factors associated with pathogen prevalence in pastured poultry farms and differences in risk characterization between different production systems. Specifically, objectives were:

1. **To use historic meteorological data to predict the prevalence of *Listeria* and *Campylobacter* in the environment of pastured poultry farms using machine learning models (Chapters 3 and 5).** Information on how weather patterns affect pathogen prevalence in outdoor poultry farms is limited, and these chapters presented models that showed what weather factors are most important in predicting prevalence.
2. **To use random forest models to predict the prevalence of *Listeria* in preharvest and postharvest samples based on pastured poultry farm practice variables (Chapter 4).** Relatively little is known about what risk factors are associated with *Listeria* presence at the poultry farm level when compared to other foodborne pathogens like *Salmonella* and *Campylobacter*. This chapter aimed to describe farm practice variables that are most important in predicting *Listeria* presence in pre- and postharvest samples.
3. **To provide estimates of *Salmonella* and *Campylobacter* prevalence at various points along the conventional and alternative broiler production chain through the use of**

systematic review and meta-analysis (Chapter 6). There was a need for a study that systematically quantified the differences in *Salmonella* and *Campylobacter* prevalence in conventional and alternative broiler production chains in the United States. As such, the purpose of this chapter was to address that need.

4. **To present a quantitative microbial risk assessment (QMRA) model to estimate the risk of salmonellosis acquired from the consumption of broiler meat in the United States annually (Chapter 7).** We identified that there were no QMRA models focused on predicting the risks of *Salmonella* from alternatively produced poultry meat. This chapter presents a QMRA model that estimates the number of salmonellosis cases annually from chicken prepared in-home in the United States and compares the risks of chicken from conventional and alternative production systems.

These objectives are displayed graphically in Fig. 1.1. The results of this dissertation will help benefit informed and risk-based decision making in the chicken industry.

1.4 References

1. Baggesen, D. L., J. E. Olsen, and M. Bisgaard. 1992. Plasmid profiles and phage types of *Salmonella typhimurium* isolated from successive flocks of chickens on three parent stock farms. *Avian Pathol.* 21:569-579.

2. Berrang, M. E., R. J. Buhr, J. A. Cason, and J. A. Dickens. 2001. Broiler carcass contamination with *Campylobacter* from feces during defeathering. *J. Food Prot.* 64:2063-2066.

3. Bull, S. A., V. M. Allen, G. Domingue, F. Jørgensen, J. A. Frost, R. Ure, R. Whyte, D. Tinker, J. E. L. Corry, and J. T. J. H. Gillard-King. 2006. Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. *Appl. Environ. Microbiol.* 72:645-652.

4. Dimitri, C., and L. Oberholtzer. 2009. Marketing US organic foods: Recent trends from farms to consumers. Economic Information Bulletin No. 58. United States Department of Agriculture, Economic Research Service.

5. Heyndrickx, M., D. Vandekerchove, L. Herman, I. Rollier, K. Grijspeerdt, and L. De Zutter. 2002. Routes for *Salmonella* contamination of poultry meat: Epidemiological study from hatchery to slaughterhouse. *Epidemiol. Infect.* 129:253-265.

6. Rasschaert, G., K. Houf, C. Godard, C. Wildemauwe, M. Pastuszczak-Frak, and L. De Zutter. 2008. Contamination of carcasses with *Salmonella* during poultry slaughter. *J. Food Prot.* 71:146-152.

7. Reisch, L., U. Eberle, and S. Lorek. 2013. Sustainable food consumption: An overview of contemporary issues and policies. *Sci. Pract. Pol.* 9:7-25.

8. Rothrock Jr., M. J., K. L. Hiett, J. Y. Guard, and C. R. Jackson. 2016. Antibiotic resistance patterns of major zoonotic pathogens from all-natural, antibiotic-free, pasture-raised broiler flocks in the southeastern United States. *J. Environ. Qual.* 45:593-603.

9. van Loo, E. J., V. Caputo, R. M. Nayga Jr, J.-F. Meullenet, and S. C. Ricke. 2011. Consumers' willingness to pay for organic chicken breast: Evidence from choice experiment. *Food Qual. Prefer.* 22:603-613.

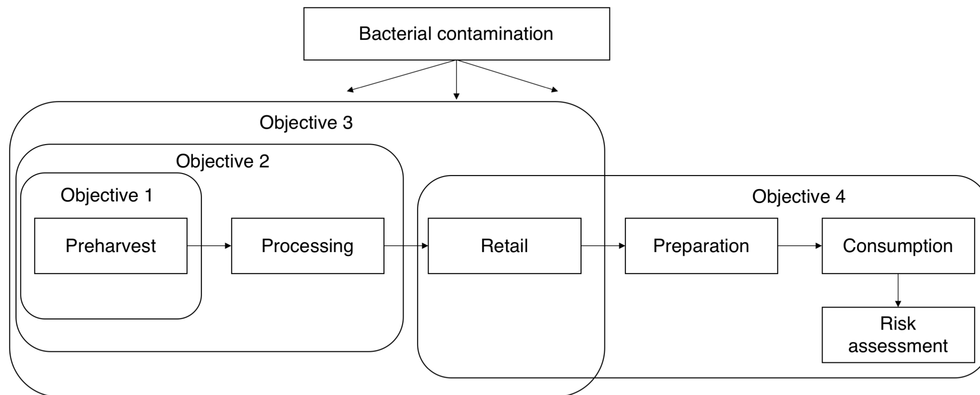

Figure 1.1 General overview of the broiler supply chain with dissertation objectives overlaid.

CHAPTER 2

LITERATURE REVIEW

2.1 Poultry production chain

2.1.1 Conventional poultry production

Conventional poultry farms are the main source of poultry meat and eggs worldwide. In 2013, experts estimated that conventional poultry farms accounted for 90-95% of the broiler production in the European Union *(204)*. Similarly, conventional livestock and poultry production accounted for approximately 88% of United States sales in 2015 *(86)*.

Conventional poultry farms are characterized by large, enclosed houses that contain a high density of birds. Castellini et al. *(42)* reported that an Italian conventional poultry farming system contained more birds per unit (15600) than an organic farming system (1000), and that, on average, the final broiler weight was higher in conventional systems, while mortality rate was lower. Part of this phenomenon is due to the use of antibiotics in conventional poultry production systems. Conventional poultry farms have used antibiotics for both therapeutic and prophylactic measures *(186, 216)*. Traditionally, conventional farms incorporated antibiotics into broiler feed to help stimulate growth and improve feed efficiency *(134, 200, 216)*. With recent advances on the study of antibiotic-resistant microorganisms, the United States has moved away from the prophylactic use of antibiotics, while the EU has banned their use as growth promoters in poultry feed *(41)*.

The conventional broiler production chain contains many opportunities for bacterial contamination from farm-to-fork *(93)*. Typically, one-day old broiler chicks are obtained from

hatching facilities and transported to grow out facilities, where they are reared for five to eight weeks before being slaughtered. Broilers are then further processed and transported to retail facilities. From here, broilers can be sold as whole carcasses, cut-up parts, or further processed chicken products. In 2015, it was forecasted that 11% of United States broilers were sold whole, 40% as cut-up parts, and 49% as further processed products *(147)*.

2.1.2 Alternative poultry production

Alternative types of poultry production operations include organic, pastured, and free-range systems. While the production chain is similar to conventional operations, alternative production systems are characterized by different rearing practices. For example, organic poultry farms are characterized by farms that rear birds without the use of antibiotics and allow the birds access to the outside (free-range), while pastured poultry operations require movable pens/housing that are moved to a fresh pasture on a daily basis *(7)*. Additionally, alternative poultry productions systems commonly use slow-growing bird breeds *(42, 68)*. Because of these practices, alternative poultry operations are faced with higher bird mortality rates, with necrotic enteritis being a particular problem *(68)*.

Organic farming has been traced back as far as the 1940s to writings of Sir Albert Howard and Lady Eve Balfour describing the practice *(120)*. Organic products became widely popular in the United States during the 2000s, when retail sales of organic foods increased from $3.6 billion in 1997 to $21.1 billion in 2008 *(61)*. In 2016, organic broiler chickens accounted for approximately $750 million in sales in the United States *(203)*. The rise in popularity is characterized by consumers' desire for sustainable food consumption and products that are considered "natural" *(178)*. Consumers have also shown the belief that organic foods are safer

and healthier than conventionally produced foods, but there has been no scientific evidence to prove this hypothesis *(104, 195, 206)*.

In 2002, the United States Department of Agriculture's (USDA) Agricultural Marketing Service (AMS) implemented the National Organic Program (NOP) to oversee the production of organic foods and implement uniform national regulations *(174)*. Currently, the USDA still oversees organic farming and provides mandates on labeling and production in the United States *(202)*. The USDA regulations for organic certification of poultry are contained in 7 CFR §205. The key points of these regulations are summarized in Table 2.1.

2.2 Microbiological concerns facing the poultry industry

2.2.1 Foodborne pathogen overview

2.2.1.1 *Salmonella* spp.

Salmonella spp. are gram-negative, rod-shaped, motile, facultative anaerobic bacteria that are part of the *Enterobacteriaceae* family. The *Salmonella* genus contains two species, *Salmonella enterica* and *Salmonella bongori*, with *S. enterica* consisting of 6 subspecies *(32)*. Within each subspecies, serotypes are determined based on surface and flagellar antigens *(32)*. There are currently greater than 2,500 serotypes of *Salmonella* with the majority belonging to *S. enterica* subsp. *enterica (32, 87)*. *Salmonella enterica* remains one of the most common causes of foodborne illness worldwide. In 2010, it was estimated that there are 93.8 million cases of gastrointestinal salmonellosis worldwide annually, 80.3 million of which are foodborne *(132)*.

The burden of *Salmonella* on the United States broiler industry is high. From 1998 to 2017, there were 298 salmonellosis outbreaks due to consumption of chicken, resulting in 7,881 illnesses, 905 hospitalizations, and 4 deaths *(52)*. Realistically, the number of illnesses caused by *Salmonella*-contaminated broiler meat and products is likely much higher, due to sporadic illness

events and unreported outbreak cases. A select number of recent multistate salmonellosis outbreaks due to consumption of chicken are described in Table 2.2. The high number of annual illnesses caused by *Salmonella* contamination of broiler meat underscores the importance of controlling for the organism.

Multiple serotypes of *Salmonella* were implicated in salmonellosis outbreaks in the United States from 2010 to 2019 (Table 2.2). A serotype of particular note is *Salmonella* I 4,[5],12:i:-, a monophasic variant of *Salmonella* Typhimurium *(73)*. This *Salmonella* serotype has been identified as an emerging disease-causing serotype of *Salmonella (144)*. Although sporadically isolated in the mid-1900s, the serotype did not gain much attention in the peer-reviewed literature until the late 1980s, when it was isolated from chicken carcasses in Portugal *(130)*. Reported illness data support the emergence of this serotype as a disease causing agent in the United States, as it was the 5th most common salmonellosis-causing serotype in the nation in 2016, compared to the 18th in 2002 *(48, 144)*. Some have suggested that the serotype is of primary concern for the pork industry *(27)*, but its isolation from chicken carcasses, ground chicken, and live chickens and foodborne illness outbreaks attributed to chicken contaminated with *Salmonella* I 4,[5],12:i:- show its major implication for the poultry industry *(50, 130, 220)*.

2.2.1.2 *Campylobacter* spp.

Campylobacter spp. are gram-negative, spiral-shaped, microaerophilic bacteria that are part of the *Campylobacteraceae* family. The *Campylobacter* genus consists of 25 species and 8 subspecies *(133)*. Of particular interest to the food industry are *C. jejuni* and *C. coli*, which can be isolated from all types of domestic livestock and some wild animals *(106)*. *Campylobacter* spp. have optimal growth ranges between 37-42°C and rarely grow at <30°C. Some thermotolerant strains of *C. jejuni* and *C. coli* have optimal growth ranges between 42-45°C.

These strains are thought to have adapted to the avian gastrointestinal tract, which is at a temperature of around 42°C *(163)*. Furthermore, the microaerophilic nature of *Campylobacter* spp. is potentially due to the lack of oxygen that exists in the avian gut *(163)*. *Campylobacter* spp. can remain viable in food products at temperatures as low as 4°C. While freezing reduces the viability of the cells, low levels of *Campylobacter* have been recovered in food products stored at temperatures as low as -20°C after several weeks *(6, 123)*

Campylobacter spp. are one of the leading causes of gastroenteritis worldwide. Sporadic cases and underreporting of cases make the annual burden of *Campylobacter* spp. hard to quantify, but according to Centers for Disease Control and Prevention (CDC) expert elicitation, there were 4,936 total outbreak cases as part of 120 foodborne-campylobacteriosis outbreaks in the United States from 1999 to 2008 *(17, 213)*. Evidence suggests that there has been a rise in the incidence of *Campylobacter* worldwide over the past decade, including rising rates in North America, Europe, and Australia *(114)*. In 2012, it was estimated that the annual cost of all *Campylobacter*-associated illnesses was approximately $1.7 billion, illustrating the high economic burden of the microorganism *(99)*.

Due to its presence in the gut of animals that are commonly used for food, *Campylobacter* is most often associated with poultry meat and products, unpasteurized milk, beef, and other meat products. Untreated water has also been frequently implicated as the cause of sporadic campylobacteriosis cases *(62, 102)*. Environmental samples, such as groundwater, can also harbor *Campylobacter (188)*.

2.2.1.3 Other pathogens

While most poultry safety-related research is focused on *Salmonella* spp. and *Campylobacter* spp., researchers have identified other organisms as potential concerns for the

poultry industry. In 2000 and 2002, there were multistate listeriosis outbreaks in the United States linked to the contamination of turkey deli meat *(85, 157)*. Currently, there have been no chicken-associated listeriosis outbreaks in the United States, but *Listeria* has been isolated from broiler farming environments, broiler meat, and broiler processing environments. In a survey of United States and United Kingdom foods, Gilbert et al. *(76)* found 12% of ready-to-eat (RTE) poultry product samples and 60% of raw chicken samples contaminated with *Listeria monocytogenes*. Conversely, Berrang et al. *(21)* did not consistently identify *L. monocytogenes* in raw, chilled broiler carcasses. Multiple studies have identified the organism in poultry processing and further processing plants *(23, 24)*. Loura et al. *(127)* showed that raw broiler meat, worker hands, and processing equipment were sources of contamination of *L. monocytogenes* in poultry processing plants. Understanding the routes of contamination in these types of poultry processing environments is of high importance to lower the risk of cross-contamination to fully cooked product. Limiting birds' exposure to the organism before processing could help reduce the risk of the entry of the organism into processing environments. More data need to be collected on the presence of *Listeria* spp., and specifically *L. monocytogenes*, in the environment of poultry farms. Golden et al. (2019) found *Listeria* spp. and *L. monocytogenes* in 15.9% and 1.8%, respectively, of preharvest (soil and feces) samples from pastured poultry farms. Due to the ubiquitous nature of *L. monocytogenes* in the environment and the rise in alternative poultry production methods, *L. monocytogenes* should be considered an emerging pathogen of concern for the poultry industry.

Arcobacter butzleri is another emerging foodborne pathogen in the food industry, characterized by its ability to cause gastroenteritis, bacteremia, and septicemia in humans and frequent isolation from animal-sourced foods *(8, 143)*. *Arcobacter* spp. are phylogenetically and

phenotypically very similar to *Campylobacter* spp. *(207)*. Poultry is considered a main source of *A. butzleri*, with pork and beef being other major sources *(98)*. *Arcobacter butzleri* has been commonly isolated from food processing environments, and particularly in slaughterhouses *(54, 69, 75)*. The organism has also shown the ability to form biofilms in food processing environments, which could act as a cross-contamination source to food products *(69)*. Due to its association with poultry, *A. butzleri* is a potential pathogen of concern for the poultry industry. In 2008, *A. butzleri* was implicated as the likely cause of a foodborne illness outbreak related to chicken consumption at a wedding in Wisconsin, resulting in 51 illnesses *(122)*. Studies have found it highly prevalent on broiler carcasses and processing equipment at various points during processing *(103, 197)* and in retail meat *(8, 115)*,

2.2.2 Preharvest contamination routes

2.2.2.1 Poultry as reservoirs for pathogenic bacteria

The gastrointestinal tract of poultry is a significant reservoir for *Salmonella* and *Campylobacter*, indicating why the two organisms have presented such a large public health risk for poultry-based food products. This is of major importance because organisms present in the gut of birds have the potential to spread to the outside of the bird during processing, posing a potential route of contamination for poultry meat. An understanding of the colonization properties of poultry-related foodborne pathogens is needed to help mitigate the risk of the organisms.

Campylobacter is a commensal microorganism in the gut of poultry and is mainly present in the cecum and colon *(19, 67)*. Colonization of the gut normally occurs approximately three weeks after bird hatching, with the presence of maternal antibodies identified as a potential cause of the delay *(94, 108, 182)*. Many studies have investigated the potential source of

Campylobacter that colonize the guts of birds. The external environment, previous poultry flocks, other domestic animals, contaminated water, and vertical transmission from parent birds have been suggested as potential major sources of contamination *(94, 165, 166, 168)*. Bull et al. *(35)* identified bird transport as a major contamination risk. Horizontal transfer of *Campylobacter* through bird feces has been identified as one of the major sources of flock contamination, and once a bird is contaminated, bird-to-bird transmission occurs rapidly *(105, 151, 189)*.

Unlike with *Campylobacter*, younger birds are more susceptible to *Salmonella* colonization than older birds *(11, 140)*. Early studies of Milner and Shaffer *(140)* showed that day-old chicks could be infected by as little as 5 *Salmonella* cells, while older birds were infected less frequently and required higher doses of *Salmonella* to be infected. Subsequently, *Salmonella* incidence in poultry decreases as the rearing time progresses *(121)*. Nurmi and Rantala *(155)* proposed that as birds grow in age, their intestinal microflora develops and becomes more resistant to colonization by pathogens like *Salmonella*, a phenomenon that has become known as competitive exclusion. Horizontal transmission of the organism has been identified as the major source for flock contamination *(93)*. Poor hygiene, feed contamination, contamination by small animals like rodents and insects, size of farm, and carryover from the previous flock have all been identified as other significant risk factors *(10, 93, 121, 192)*. Interestingly, Heyndrickx et al. *(93)* found no correlation between bird contamination during rearing and final product contamination, but instead identified fecal matter in transport crates as the major correlator of end product safety. Rasschaert et al. *(177)* also identified that gastrointestinal colonization of birds with *Salmonella* was not correlated with final product food safety and identified cross-contamination from slaughter equipment as the main source of

contamination. Despite this, poultry producers use *Salmonella* vaccines in young chicks to induce cell-mediated immunity and reduce the risk of further colonization of the gut by virulent *Salmonella (9)*. The use of probiotics/prebiotics and various feed additives have also been shown to lower the probability of *Salmonella* gut colonization *(38, 162, 210)*.

Studies have shown that poultry can serve as a potential reservoir for *Listeria* spp. Njagi et al. *(152)* reported that the intestinal tracts of chickens and other types of poultry can act as reservoirs for *Listeria* in live operations. Dhama et al. *(59)* further touched on this point and suggested that poultry can spread the organism into the litter and environment through fecal matter, representing an entryway into the poultry processing chain.

Due to the ubiquity of the organism, *Listeria* could pose a potentially increased risk to the alternative poultry industry, where birds are allowed access to the natural environment *(12, 142)*. While the risk is noted, Milillo et al. *(138)* found that only 7 of 399 (1.75%) of cecal samples from pasture-reared poultry were *Listeria*-positive and showed that samples were positive for *L. monocytogenes* and hemolytic *L. innocua*. These researchers indicated that *Listeria* was more frequently isolated from younger birds, indicating that as the birds' intestinal microflora matures, *Listeria* numbers decrease, but no follow up study was performed. Additionally, Locatelli et al. *(126)* isolated a higher number of *L. innocua* isolates from feces and soil samples collected from pastured poultry farms when compared with *L. monocytogenes* isolates, similar to conventional poultry farms.

2.2.2.2 Environmental contamination

As mentioned previously, pathogen prevalence in cages during transport of birds has been linked to a higher prevalence of pathogen contamination in the final product *(35, 93)*. Additionally, contaminated poultry litter, feed, and drinking water have been identified as

potential risk factors for increased pathogen risk in the final poultry product *(131, 205, 211)*. Contamination of these items can result from environmental factors, such as contaminated feces and soil, small animals, and poor worker hygiene. Because of this, subsequent measures have been taken by poultry farmers to improve biosecurity measures and to implement proper worker hygiene *(205)*. Due to the nature of alternative poultry operations, these factors can be harder to account for.

While the prevalence of foodborne pathogens in the environment of conventional poultry farms is well established, recent studies have statistically compared the two types of farms *(2, 116, 167, 190)*. Petkar et al. *(169)* reported that *Salmonella* survival in conventional and organic broiler feeds was not significantly different. Alali et al. *(2)* found that *Salmonella* contamination of fecal matter and bird feed was significantly lower in samples collected from organic farms when compared to conventional farms. This notion is supported by the work done by Siemon et al. *(190)*. Peng et al. *(167)* found various environmental samples from organic mixed-crop livestock farms were more contaminated with *Salmonella* than conventional poultry farms. Hoogenboom et al. *(101)* found no significant difference in *Salmonella* prevalence in the feces of organically-raised swine and conventionally-raised swine. Similarly, fecal samples collected from conventional and organic dairy farms were not significantly different in *Salmonella* prevalence *(70)*.

2.2.3 Harvest/processing contamination routes

2.2.3.1 Scalding

Scalding is used prior to defeathering of broiler carcasses primarily to help loosen the feathers of the bird. This step has been identified as a potential source of microbial contamination of birds via cross-contamination. Mulder et al. *(145)* found that cross-

contamination occurred during scalding when external contamination was introduced via dust and feathers. Controlling for bacterial load in the scalding water is imperative in preventing cross-contamination of bird carcasses. The reduction of organic matter in scalding water has been identified as a measure to reduce *E. coli* and coliform numbers *(107)*.

Traditionally, one-tank scalding systems containing 50-60°C water were used for this step, but time has given rise to other types of scalding systems including steam-scalding and three-tank, countercurrent scalders. Many studies have been conducted on the effect of the type of scalding operation used on the microbiological quality of carcasses. Steam-scalded carcasses were found to present significantly less coliforms than conventionally scalded carcasses *(164)*. The three-tank, countercurrent system is characterized by the use of three successive scalding tanks where the flow of water and carcasses move in the opposite direction, so that carcasses move into progressively cleaner water *(40)*. This system has been shown to improve the microbial quality of birds after scalding when compared to traditional systems *(109)*. This is likely due to the countercurrent flow of water and use of multiple tanks, where studies have found that coliform, *E. coli*, *Campylobacter*, and *Salmonella* numbers were reduced in successive tanks *(39, 209)*. Furthermore, when compared with *Enterobacteriaceae* numbers in a conventional single-tank scalding system, Veerkamp and Heemskerk *(209)* found lower numbers in the third tank of a countercurrent system.

2.2.3.2 Defeathering

After scalding, poultry carcasses are defeathered using automated machines with finger-like plucking appendages. Defeathering has been identified as a major potential source of microbial contamination. Nde et al. *(150)* found that *Salmonella* prevalence increased from 7% on freshly slaughtered turkeys to 16% on defeathered turkeys. Berrang et al. *(19)* found that one

of 120 broiler carcasses were *Campylobacter*-positive pre-defeathering compared with 95 of 120 carcasses post-defeathering.

Multiple studies have been conducted on the effect of cross-contamination during this step *(4, 5, 149, 173)*. Using an *E. coli* K12 marker, Allen et al. *(4)* found that cross-contamination during broiler defeathering was mainly attributed to aerosols, large droplets, and feathers. Furthermore, it was found that forward and backward contamination occurred when an inoculated broiler carcass was introduced to the process, but cross-contamination was highest when carcasses came from inoculated scalding water. Subsequent studies have identified feathers as a potential source of contamination, specifically of *Salmonella (5, 149, 176)*. Allen et al. *(5)* identified that defeathering reduced overall bacterial numbers on broiler carcasses but caused dispersion of a marker organism which caused forward and backward carcass contamination. Results from Nde et al. *(149)* support this, demonstrating through molecular subtyping that identical *Salmonella* isolates present on turkey feathers were found on defeathered turkey carcasses. Additionally, antibiotic-resistant strains of *Klebsiella pneumoniae* and *E. coli* isolated from defeathering machines before processing were isolated from broiler carcasses after processing *(173)*.

Berrang et al. *(19)* identified that contaminated fecal leakage during the defeathering process was a significant source of carcass contamination. Studies have analyzed the effects of various methods to overcome this problem. When a tampon device was applied to the inside of 120 broiler carcasses with subsequent cloacae suturing pre-defeathering, only 13 of the carcasses were *Campylobacter*-positive post-defeathering, compared to 95 of 120 *Campylobacter*-positive carcasses when defeathered conventionally *(19)*. A 50-cc dry sterile sponge plug was also identified as an effective way to prevent fecal leakage and subsequent *Campylobacter*

contamination *(22)*. Other types of control measures have been tested as well. Overall bacterial numbers were significantly lower in carcasses that were treated with 1% acetic acid after defeathering than a water control, with counts of 3.93 and 4.53 log CFU/carcass, respectively, but the effect of hydrogen peroxide (H_2O_2) was negligible *(60)*. An additional scalding step after defeathering had no significant reduction effect on *Campylobacter*, *E. coli*, and other coliforms *(20)*.

Research on the post-defeathering bacterial load on carcasses for alternative poultry operations is still limited. A recent study found that organically processed carcasses contained significantly less average *Campylobacter* CFU/unit than conventionally processed carcasses, with 1.6 log CFU/unit and 2.5 log CFU/unit, respectively *(13)*.

2.2.3.3 Evisceration

During evisceration, birds' viscera are removed by manual or automated methods. This involves the removal of the cloaca and rectum and the scooping out of the birds' entrails *(72)*. Cross-contamination during this processing step has been widely observed to occur by items such as contaminated evisceration equipment and poor worker hygiene. Contamination of equipment can occur when a bird's gastrointestinal tract is ruptured during evisceration, thus leading to leakage of fecal material. Leakage of fecal matter can also contaminate the skin of poultry during this step *(1)*. Lillard et al. *(125)* found that *Salmonella* incidence was significantly higher in eviscerated carcasses than in unprocessed control carcasses. Contrary to this finding, Nde et al. *(150)* found that there was no significant difference in *Salmonella* prevalence in pre- and post-eviscerated carcasses. Feed withdrawal is a measure that is taken where birds are not fed up to 12 h before slaughtering to try and reduce the amount of fecal matter present in the bird that could pose a potential contamination risk if leaked during evisceration *(36)*. It is important

that birds are not withheld feed for an extended period of time, as feed withdrawal lasting longer than 12 h can result in thinning of the intestinal wall, which presents a higher chance of rupturing during evisceration, increasing the likelihood of fecal leakage *(215)*. Control of the evisceration process through proper evisceration techniques, good worker hygiene, and feed withdrawal should result in carcasses with less fecal contamination and a subsequent reduction in bacterial pathogen risk.

2.2.3.4 Washing

After evisceration, poultry carcasses are often subjected to wash cycles to remove fecal and other organic matter from the surface and gut cavity of the carcasses. Numerous studies have shown that this step often leads to an overall reduction in bacterial numbers on poultry carcasses *(183, 198)*, but another study showed that subsequent washes with untreated water were ineffective at reducing *Campylobacter* numbers on carcasses *(16)*. Furthermore, introduction of contaminated carcasses to wash water poses a potential threat of cross-contamination. Numerous washing intervention strategies have been investigated to mitigate the risk during this processing step, including the use of antimicrobial chemicals and high temperature water.

The use of chlorine (sodium hypochlorite) during washing of poultry is widespread across the conventional poultry industry, but recent reports have suggested that sodium hypochlorite can interact with organic molecules on the surface of food products to produce harmful byproducts including haloquinones, halo-cyclopentene and cyclohexene derivatives *(34, 97)*. Although the use of chlorine is permitted in alternative poultry processing (Table 2.1), many processors have trended towards the use of other antimicrobials such as peracetic acid (PAA) and organic acid washes *(137)*. When compared to a 25-35 ppm chlorine wash, trisodium phosphate and acidified sodium chlorite washes reduced *Campylobacter* levels on carcasses by an

additional 1.03 and 1.26 log CFU/mL on average, respectively *(16)*. Chlorine dioxide (100 ppm) treatments provided up to 1.21 log CFU/g reductions of *Campylobacter* on poultry *(100)*. Various concentrations of oleic acid (2-10% wt/vol) applied to wash water had a significant effect in reducing aerobic bacteria, *Enterobacteriaceae*, and *Campylobacter (95)*. Other fatty acids have also been studied, and Hinton Jr and Ingram *(96)* found that a mixture of tripotassium phosphate (TPP) and lauric and myristic acids were highly effective towards gram negatives, gram positives, and yeasts, proving its potential use to improve the safety of poultry and cause reduction of potential spoilage organisms as well. Carcasses washed in potassium hydroxide and lauric acid solutions contained up to 1.55 log CFU/g less aerobic bacteria (based on total plate counts) than carcasses washed in distilled water *(97)*.

Electrolyzed water has been investigated as another potential alternative to traditional chlorine washes *(161, 214)*. Electrolyzed water (containing 25 mg/l of residual chlorine) reduced *Campylobacter* levels up to 3 log CFU/g on broilers compared to 1 log CFU/g after an untreated water was used *(161)*. Additionally, no viable *Campylobacter* cells were isolated from the wash water, as opposed to 4 log CFU/mL found in the untreated water after washing, showing its potential use in reducing cross-contamination risk during washing. More research needs to be conducted in this area to determine the large-scale applicability of this type of intervention.

2.2.3.5 Chilling

Before further processing or packaging, carcasses are subjected to a chilling process to lower the internal temperature of the bird. Primarily, two types of chilling processes are used in the industry: water-immersion chilling and air-chilling. Water immersion systems utilize a continuous flow of water to chill poultry carcasses, while air cooling systems utilize chill rooms or air blast tunnels for cooling *(3)*. Some processors make use of a water spray during the

beginning stages of air cooling. Water-immersion chilling is the primary chilling system used in the United States, while air-immersion systems are mainly used in the European Union *(25, 184)*. Several studies have found that both types of systems substantially reduce microbial load *(14, 26, 199)*, and a recently conducted meta-analysis found no significant difference in the microbial reduction efficacy of the two methods *(18)*.

Rapid-surface cooling has been investigated as a potential alternative system. A recent study showed that immersing carcasses in liquid nitrogen for 20 seconds reduced *Campylobacter* numbers by up to 1 log CFU/g *(37)*. However, no control was included to compare to traditional systems, nor was there any mention on how the cooling process affected the meat quality.

2.2.3.6 Differences in foodborne pathogen prevalence during alternative and conventional poultry processing

Key differences in the prevalence of foodborne pathogen contamination of poultry at various points in the conventional and alternative poultry processing chain need to be noted for accurate assessments of risk of the various pertinent pathogens. Early results from Luangtongkum et al. *(128)* showed that *Campylobacter* prevalence was high in the gastrointestinal tract of both organic and conventionally-raised, slaughter-age turkeys. The results were similar for broiler flocks, as Heuer et al. *(92)* found organic broiler flocks to have significantly higher *Campylobacter* prevalence compared to conventional flocks. *Salmonella* prevalence was also found to have a higher prevalence on organic, processed broiler carcasses when compared to conventional carcasses *(12)*.

With the rise in popularity of alternative poultry production systems and the rise of antibiotic-resistant bacteria, updated data are necessary, but are rather sparse in the scientific literature. Bailey et al. *(13)* found *Campylobacter* prevalence to decrease during broiler

processing for both organic and conventional production systems. Fecal matter and post-water chill carcasses of conventionally processed broilers had significantly higher *Campylobacter* prevalence than organically processed birds, but otherwise, prevalence levels were similar throughout the processing chain *(13)*. While the risk of foodborne pathogen isolation from poultry seems similar for both management systems, the complex and evolving nature of alternative poultry processing makes the need for more comprehensive studies very high.

2.2.4 Post-processing foodborne pathogen contamination of poultry and poultry products

After processing, poultry are portioned, packaged, and/or further processed into other products before they are delivered to retail establishments. Cross-contamination can occur during these steps, but proper hygiene control and cleaning and sanitizing of equipment are often effective at reducing the risk of cross-contamination of pathogenic bacteria *(135)*. After processing, it is also very important to control spoilage microorganisms to prevent off-flavors, odors, and spoilage of poultry meat due to growth of bacteria such as *Pseudomonas* spp. *(77, 156)*. Technologies such as modified atmosphere and active packaging can be useful to control the growth of microorganisms already present on the surface of poultry meat *(191)*. Quantifying the growth of various microbes on the surface of poultry meat after packaging has been well characterized due to the generation of accurate predictive models presented in the literature. Researchers have used *Pseudomonas* spp. as an indicator organism to determine remaining shelf-life based on storage conditions and cold-supply chain management *(63, 74, 175)*. Various models have been generated to predict pathogen growth in raw poultry *(64, 112, 158, 159)* and cooked poultry *(43, 111, 217)*.

Recent studies have provided the public with data on foodborne pathogen prevalence in retail poultry meat. A meta-analysis performed by Golden and Mishra *(80)* aggregated United

States surveys and reported retail *Campylobacter* prevalence estimates of 59 and 55% for conventional and alternative broiler meat, respectively, and *Salmonella* prevalence estimates of 19 and 23%, respectively. Estimated prevalence was not significantly different between production system for either pathogen observed.

2.2.5 Antibiotic resistance

For years, antibiotics have been used in the poultry industry in delivery systems such as feed additives for therapeutic, prophylactic, and growth stimulating properties *(134, 186, 200)*. Recent work has shown that, while antibiotic-resistant bacteria have always been present in nature, wide use of antibiotics has presented an opportunity for an increase in antibiotic-resistant pathogenic bacteria *(57)*. Antimicrobial resistance occurs when bacteria obtain resistance to select antimicrobials by processes including: gene mutation, acquiring transposons, and plasmid-mediated gene transfer *(58)*. Feed type has been shown to affect antimicrobial resistance, as Hegde et al. *(90)* found that resistance genes in the gut microbiome were more highly expressed in chickens reared on conventional diet when compared to organic. With the rise of consumer concern over antimicrobial-resistant bacteria, many consumers have opted for the purchase and consumption of organic food products *(55)*. However, research has shown that even though organic poultry are raised without antibiotics, this does not eliminate the presence of antibiotic-resistant bacteria in organic poultry meat and farms *(56, 141, 180)*. In a recent study by Rothrock Jr. et al. *(180)*, high prevalence of antibiotic-resistant isolates of *Listeria* (63.9%) and *Salmonella* (36.0%) were found in various sample types from pastured poultry farms in the southeastern United States.

Numerous studies have compared the prevalence of antibiotic-resistant bacteria on alternative and conventional retail broiler meat. Cui et al. *(56)* found that all *Salmonella*

Typhimurium isolates from conventional retail broiler meat were resistant to at least 5 of the tested antimicrobials, while 79% of *Salmonella* isolated from organic broiler meat were susceptible to all 17 tested antimicrobials. Lestari et al. *(124)* also found that *Salmonella* isolates from organic retail broiler meat were susceptible to a larger number of antibiotics than isolates from conventional chicken, but all isolates were resistant to amikacin, ceftriaxone, and ciprofloxacin. Other reports have found that up to 68% of *Salmonella* from pasture-raised broiler meat contained class I integrons, nonmobile genetic elements that have been linked to antimicrobial resistance, and all isolates were resistant to sulfisoxazole and novobiocin *(15, 136)*. In a recent study, it was reported that there was a statistically significant lower amount of multidrug-resistant strains of *Salmonella* in the environment of large-scale poultry farms that voluntarily withdrew antibiotics when compared to conventional large-scale poultry farms *(185)*. In various reports, *Salmonella* Kentucky has been the most isolated antibiotic-resistant serotype from broiler meat and the environment of poultry farms, with Hadar, Orion, and Enteritidis as other commonly isolated serotypes *(124, 136, 185)*.

The prevalence of other types of antibiotic-resistant bacteria has also been observed. Early reports by Luangtongkum et al. *(128)* found that less than 2% of *Campylobacter* strains isolated from organic broiler gastrointestinal tracts were resistant to fluoroquinolones compared to 46% of strains from conventional broilers, but a large number of the isolates from both conventional and organic broilers were resistant to tetracycline. Bailey et al. *(13)* presented similar results, finding 81.6% of *Campylobacter* isolates from various organic broiler processing steps to be resistant to tetracycline, compared with 65.3% of isolates from conventional farms. Noormohamed and Fakhr *(153)* isolated multidrug-resistant *Campylobacter* strains from both organic and conventional retail broiler meat. Both organic and conventional retail broiler meat

have been found to contain antibiotic-resistant enterococci *(118)*. Similarly, 41.7% of *Enterobacteriaceae* isolated from organic broiler meat were multidrug-resistant *(141)*. Additionally, organic broiler meat was found to be statistically indistinguishable in the number of antibiotic-resistant *E. coli* isolates when compared with conventional broiler meat *(139)*. These results show that although antibiotics are withheld from organically-raised birds, this does not necessarily guarantee the absence of antibiotic-resistant pathogenic bacteria from processed organic broiler meat.

2.3 Predictive microbiology and its use in the poultry industry

2.3.1 Machine learning

Machine learning (ML) is characterized by the use of machine algorithms to extract information and features out of big, complex datasets that could not be readily handled by traditional statistical approaches *(119)*. Typically, after data are collected and prepared, an ML model is chosen and trained on the training data. Commonly used ML models include ensemble decision tree algorithms like the random forest and gradient boosting machine models, support vector machine models, principal component analysis, neural network models, and Naïve Bayes models. During training, hyperparameter tuning and model selection take place. A hyperparameter is a value that is used in the fitting of a model but cannot be estimated directly from the provided data *(79)*. Cross-validation is often used in this step to improve hyperparameter choice efficacy. Finally, models are tested on a held-out test set from the original dataset or an independent dataset.

Machine learning has begun to make a large impact in the realm of food safety, with used such as predicting factors that are associated with increased pathogen prevalence in farming environments *(81, 160)*, increasing the effectiveness of risk assessment studies *(181)*, and

assisting in the prevention of food fraud worldwide *(29)*. Machine learning has been employed specifically in the poultry industry in many different applications. Soltani and Omid *(196)* presented decision tree and artificial neural network models that were capable of predicting the freshness class of chicken eggs. Golden et al. *(82)* used random forest models to identify major farm practice variables that were linked to a higher predicted prevalence of *Listeria* spp. in pre- and postharvest pastured poultry samples. Jianping et al. *(110)* developed an integrated Grey neural network model that could accurately predict the trend of antimicrobial resistance of *E. coli* in livestock and poultry.

2.3.1.1 Random forest

The random forest (RF) model is an ensemble decision tree method that was designed by Breiman *(31)*. This model is characterized by the growth of a large number of classification and regression trees (CART) *(30)*. During fitting of each tree, training data are first sampled using the non-parametric bootstrap method, and the chosen data are considered the in-bag sample, with the remaining data being the out-of-bag sample *(65)*. Next, a non-pruned CART is trained on the in-bag sample, using a randomly selected number of the predictors as potential split candidates at each node of the tree. The best splits are determined by the Gini index *(30)*. Trees are then evaluated on the out-of-bag samples to provide an estimate on performance and as a means to rank predictors in terms of importance to the model *(170)*.

Random forest models have seen use within the food and water safety fields. Barbut et al. *(14)* showed the versatility of RF models when comparing the epidemiology of *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- strains by determining the most important phenotypic and epidemiologic variables distinguishing the two strains. Smith et al. *(194)* used the model to find that migratory birds were the most likely cause of *E. coli* contamination of a Texas body of

water based on antibiotic resistance patterns. Lv and Liu *(129)* developed an RF model to rapidly and accurately predict arsenic contamination in rice crops. Vangay et al. *(208)* showed that the model was able to match the results of expert classification when determining *L. monocytogenes* persistence in retail deli meat environments and its potential use in environmental surveillance.

2.3.1.2 Gradient boosting machine

The gradient boosting machine (GBM) model is another type of ensemble decision tree method that was designed by Friedman *(71)*. While the decision trees that are part of RF models are all grown at once, trees grown as part of a GBM model are grown in a stepwise, additive way. Each model attempts to build on the weaknesses of the previous models, or "weak-learners", by generating new-learners that are maximally correlated with the negative gradient of the loss function *(146)*. Gradient boosting machine models have been widely used in other types of industries, but to our knowledge, the work provided in this dissertation is the first published study utilizing the model in a food safety context.

2.3.2 Meta-analysis

Meta-analysis is a tool that is used to aggregate the results of many individual studies and estimate the effect size of a treatment or intervention *(78)*. While meta-analysis has been widely used in the field of medicine and other industries since the 1970's, it is gaining in popularity in the food safety literature since recommendations for their use by Sargeant et al. *(187)* and Gonzales-Barron and Butler *(84)*. Various systematic review and meta-analysis studies have been conducted to estimate the prevalence of foodborne pathogens in poultry and poultry samples or effect of a treatment on pathogen load *(33, 83, 117, 201, 212, 219)*.

There are many aspects to a comprehensive meta-analysis. The first step is to conduct a systematic review of the literature *(84)*. In doing so, a clear research question needs to be stated,

considering the population and treatment/outcome of interest. Keywords relating to the formulated question must then be determined and searched for through applicable databases. Commonly used databases for scientific systematic reviews are PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), Web of Science (https://www.webofknowledge.com), and Google Scholar (https://scholar.google.com/). Additionally, inclusion criteria need to be set for potential inclusion of a research study in the final meta-analysis. Here, articles can be screened to make sure they fit the stated research question and provide the necessary quantitative and qualitative data. During this step, some researchers apply quality scores to articles to make sure they are of high enough quality for inclusion in the final meta-analysis, but studies have shown that quality scores can be highly problematic due to misleading rankings of studies *(91, 113)*. Following the screening of articles, data are extracted from eligible articles. This step is necessary to accumulate the results of each study, so an overall effect of a treatment can be estimated. It is also necessary to extract qualitative data during this step to potentially help identify sources of heterogeneity within the studies *(84)*. Finally, an overall effect size is estimated through the use of a fixed effect or random effect model. The effect size estimates the degree to which a specific type of phenomenon is present in the population of interest *(84)*. A fixed effect model is used when low amounts of between-study variability or heterogeneity is expected, or when it is assumed that all included studies share a common effect size and differences are only present due to sampling error *(28, 84)*. Random effect models are used when there is a high expected amount of heterogeneity or there is no reason to assume that the true effect size will be identical for all studies *(28)*.

2.3.3 Quantitative microbial risk assessment

Quantitative microbial risk assessments (QMRA) are widely used throughout the food industry as a tool to estimate the risk of foodborne biological hazards to human consumers. They allow for the mapping of foodborne pathogens throughout the complex supply chain of a food product. The first QMRA regarding food safety was conducted by Whiting and Buchanan *(218)* in predicting the risk of *Salmonella* Enteriditis infection due to human-consumption of pasteurized liquid eggs. There are four main steps to a QMRA: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization *(53)*. Hazard identification is used to identify the microbial hazard of interest as well as likely populations that would be impacted by the hazard *(172)*. Hazard characterization is then used to describe the hazard and its interaction with humans if consumed. This is where the dose-response relationship is determined *(172)*. Next, exposure assessment is used to determine the frequency in which the hazard is consumed by humans based on the consumption patterns of the food item of interest. Finally, risk characterization uses the results from the hazard characterization and exposure assessment to provide a final estimate of the number of illnesses caused by the food item of interest due to contamination of the pathogen of interest.

Numerous QMRAs estimating the risk of human campylobacteriosis and salmonellosis due to consumption of poultry meat are available in the scientific literature. The majority of these QMRAs focus on the risk of conventional poultry meat to the consumer, with the exception of the work presented by Rosenquist et al. *(179)*, who found that the risk of *Campylobacter* infection due to contaminated poultry meat was 1.7 times higher in Danish organically-produced meat when compared with conventionally-produced meat. To our knowledge, no QMRA has

been performed on the risk of *Salmonella* infection to humans due to the consumption of alternatively-produced poultry meat.

There are many approaches to constructing a poultry related QMRA model. Some models attempt to estimate the presence of pathogens throughout the entire farm-to-fork poultry continuum *(89, 148)*, while others focus on the retail-to-consumption part of the supply chain *(171, 193)*. Farm-to-fork type models require a comprehensive understanding of foodborne pathogen prevalence and behavior throughout the entire food chain. While this has been accomplished in QMRAs focused on conventionally-produced poultry, there are still data gaps in our knowledge of prevalence in alternative systems. In a meta-analysis performed by Golden and Mishra *(80)*, data were available to provide estimates of *Salmonella* and *Campylobacter* prevalence in alternative poultry farming environment and retail meat samples in the United States, but data were lacking to provide these estimates for pathogen prevalence in broiler carcass at various points during processing (i.e. rehang, prechill, postchill). An understanding of how bacterial numbers change during processing of alternatively-grown poultry is pertinent to the production of an accurate QMRA model. Similar studies to the work presented by Bailey et al. *(13)* should be adapted to track *Salmonella* throughout the alternative poultry processing supply chain. Additionally, a multi-state survey of the types of processing practices (e.g. type of washing system) that are utilized by the various types of alternative poultry production systems would be useful in QMRA construction. Similar surveys have been conducted for poultry processing facilities in the United States *(154)*, but distinctions should be made between the type of production facility. This would give risk assessors a better idea of the practices that are prominently in use in the United States and incorporate those factors into the QMRA.

2.4 References

1. Abu-Ruwaida, A. S., W. N. Sawaya, B. H. Dashti, M. Murad, and H. A. Al-Othman. 1994. Microbiological quality of broilers during processing in a modern commercial slaughterhouse in Kuwait. *J. Food Prot.* 57:887-892.

2. Alali, W. Q., S. Thakur, R. D. Berghaus, M. P. Martin, and W. A. Gebreyes. 2010. Prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. *Foodborne Pathog. Dis.* 7:1363-1371.

3. Allen, V. M., C. H. Burton, J. E. L. Corry, C. G. Mead, and D. B. Tinker. 2000. Investigation of hygiene aspects during air chilling of poultry carcases using a model rig. *Br. Poult. Sci.* 41:575-583.

4. Allen, V. M., M. H. Hinton, D. B. Tinker, C. Gibson, G. C. Mead, and C. M. Wathes. 2003. Microbial cross-contamination by airborne dispersion and contagion during defeathering of poultry. *Br. Poult. Sci.* 44:567-576.

5. Allen, V. M., D. B. Tinker, C. M. Wathes, and M. H. Hinton. 2003. Dispersal of micro-organisms in commercial defeathering systems. *Br. Poult. Sci.* 44:53-59.

6. Alter, T., and K. Scherer. 2006. Stress response of *Campylobacter* spp. and its role in food processing. *J. Vet. Med. B*. 53:351-357.

7. American Pastured Poultry Producers Association. 2017. Suggested Management Practices for Pasture-Raised Chicken, Eggs, and Turkeys. Available at: https://apppa.org/Pastured-Poultry-Management-Practices. Accessed 6 March 2020.

8. Atabay, H. I., F. Aydin, K. Houf, M. Sahin, and P. Vandamme. 2003. The prevalence of *Arcobacter* spp. on chicken carcasses sold in retail markets in Turkey, and identification of the isolates using SDS-PAGE. *Int. J. Food Microbiol.* 81:21-28.

9. Babu, U., M. Scott, M. J. Myers, M. Okamura, D. Gaines, H. F. Yancy, H. Lillehoj, R. A. Heckert, and R. B. Raybourne. 2003. Effects of live attenuated and killed *Salmonella* vaccine on T-lymphocyte mediated immunity in laying hens. *Vet. Immunol. Immunopathol.* 91:39-44.

10. Baggesen, D. L., J. E. Olsen, and M. Bisgaard. 1992. Plasmid profiles and phage types of *Salmonella typhimurium* isolated from successive flocks of chickens on three parent stock farms. *Avian Pathol.* 21:569-579.

11. Bailey, J. S. 1988. Integrated colonization control of *Salmonella* in poultry. *Poult. Sci.* 67:928-932.

12. Bailey, J. S., and D. E. Cosby. 2005. *Salmonella* prevalence in free-range and certified organic chickens. *J. Food Prot.* 68:2451-2453.

13. Bailey, M. A., R. M. Taylor, J. S. Brar, S. C. Corkran, C. Velásquez, E. Novoa Rama, H. F. Oliver, and M. Singh. 2018. Prevalence and antimicrobial resistance of *Campylobacter* from antibiotic-free broilers during organic and conventional processing. *Poult. Sci.* 98:1447-1454.

14. Barbut, S., L. F. Moza, F. Nattress, B. Dilts, and C. O. Gill. 2009. The microbiological conditions of air-or water-chilled carcasses produced at the same poultry packing plant. *J. Appl. Poult. Res.* 18:501-507.

15. Barlow, R. S., J. M. Pemberton, P. M. Desmarchelier, and K. S. Gobius. 2004. Isolation and characterization of integron-containing bacteria without antibiotic selection. *Antimicrob. Agents Chemother.* 48:838-842.

16. Bashor, M. P., P. A. Curtis, K. M. Keener, B. W. Sheldon, S. Kathariou, and J. A. Osborne. 2004. Effects of carcass washers on *Campylobacter* contamination in large broiler processing plants. *Poult. Sci.* 83:1232-1239.

17. Batz, M. B., S. Hoffmann, and J. G. Morris. 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. *J. Food Prot.* 75:1278-1291.

18. Belluco, S., L. Barco, A. Roccato, and A. Ricci. 2016. *Escherichia coli* and *Enterobacteriaceae* counts on poultry carcasses along the slaughterline: A systematic review and meta-analysis. *Food Control*. 60:269-280.

19. Berrang, M. E., R. J. Buhr, J. A. Cason, and J. A. Dickens. 2001. Broiler carcass contamination with *Campylobacter* from feces during defeathering. *J. Food Prot.* 64:2063-2066.

20. Berrang, M. E., J. A. Dickens, and M. T. Musgrove. 2000. Effects of hot water application after defeathering on the levels of *Campylobacter*, coliform bacteria, and *Escherichia coli* on broiler carcasses. *Poult. Sci.* 79:1689-1693.

21. Berrang, M. E., C. E. Lyon, D. P. Smith, and J. K. Northcutt. 2000. Incidence of *Listeria monocytogenes* on pre-scald and post-chill chicken. *J. Appl. Poult. Res.* 9:546-550.

22. Berrang, M. E., R. J. Meinersmann, and E. S. Adams. 2018. Shredded sponge or paper as a cloacal plug to limit broiler carcass *Campylobacter* contamination during automated defeathering. *J. Appl. Poult. Res.* 27:483-487.

23. Berrang, M. E., R. J. Meinersmann, J. F. Frank, and S. R. Ladely. 2010. Colonization of a newly constructed commercial chicken further processing plant with *Listeria monocytogenes. J. Food Prot.* 73:286-291.

24. Berrang, M. E., R. J. Meinersmann, J. F. Frank, D. P. Smith, and L. L. Genzlinger. 2005. Distribution of *Listeria monocytogenes* subtypes within a poultry further processing plant. *J. Food Prot.* 68:980-985.

25. Berrang, M. E., R. J. Meinersmann, D. P. Smith, and H. Zhuang. 2008. The effect of chilling in cold air or ice water on the microbiological quality of broiler carcasses and the population of *Campylobacter. Poult. Sci.* 87:992-998.

26. Blank, G., and C. Powell. 1995. Microbiological and hydraulic evaluation of immersion chilling for poultry. *J. Food Prot.* 58:1386-1388.

27. Bone, A., H. Noel, S. Le Hello, N. Pihier, C. Danan, M. E. Raguenaud, S. Salah, H. Bellali, V. Vaillant, and F. X. Weill. 2010. Nationwide outbreak of *Salmonella enterica* serotype 4, 12: i:-infections in France, linked to dried pork sausage, March-May 2010. *Eurosurveillance*. 15:1-3.

28. Borenstein, M., L. Hedges, and H. Rothstein. 2007. Meta-analysis: Fixed effect vs. random effects. Available at: https://www.meta-analysis.com/downloads/M-a_f_e_v_r_e_sv.pdf. Accessed 6 November 2019.

29. Bouzembrak, Y., and H. J. Marvin. 2016. Prediction of food fraud type using data from Rapid Alert System for Food and Feed (RASFF) and Bayesian network modelling. *Food Control*. 61:180-187.

30. Breiman, L. 1984. Classification and regression trees. Routledge, New York.

31. Breiman, L. 2001. Random forests. *Mach. Learn.* 45:5-32.

32. Brenner, F. W., R. G. Villar, F. J. Angulo, R. Tauxe, and B. Swaminathan. 2000. *Salmonella* nomenclature. *J. Clin. Microbiol.* 38:2465-2467.

33. Bucher, O., A. M. Farrar, S. C. Totton, W. Wilkins, L. A. Waddell, B. J. Wilhelm, S. A. McEwen, A. Fazil, and A. Rajić. 2012. A systematic review-meta-analysis of chilling interventions and a meta-regression of various processing interventions for *Salmonella* contamination of chicken. *Prev. Vet. Med.* 103:1-15.

34. Bull, R. J., D. A. Reckhow, X. Li, A. R. Humpage, C. Joll, and S. E. Hrudey. 2011. Potential carcinogenic hazards of non-regulated disinfection by-products: haloquinones, halo-cyclopentene and cyclohexene derivatives, N-halamines, halonitriles, and heterocyclic amines. *Toxicology*. 286:1-19.

35. Bull, S. A., V. M. Allen, G. Domingue, F. Jørgensen, J. A. Frost, R. Ure, R. Whyte, D. Tinker, J. E. L. Corry, and J. T. J. H. Gillard-King. 2006. Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. *Appl. Environ. Microbiol.* 72:645-652.

36. Buncic, S., and J. Sofos. 2012. Interventions to control *Salmonella* contamination during poultry, cattle and pig slaughter. *Food Res. Int.* 45:641-655.

37. Burfoot, D., J. Hall, K. Nicholson, K. Holmes, C. Hanson, S. Handley, and E. Mulvey. 2016. Effect of rapid surface cooling on *Campylobacter* numbers on poultry carcasses. *Food Control*. 70:293-301.

38. Carter, A., M. Adams, R. M. La Ragione, and M. J. Woodward. 2017. Colonisation of poultry by *Salmonella* Enteritidis S1400 is reduced by combined administration of *Lactobacillus salivarius* 59 and *Enterococcus faecium* PXN-33. *Vet. Microbiol.* 199:100-107.

39. Cason, J. A., and A. Hinton Jr. 2006. Coliforms, *Escherichia coli*, *Campylobacter*, and *Salmonella* in a counterflow poultry scalder with a dip tank. *Int. J. Poult. Sci.* 5:846-849.

40. Cason, J. A., A. D. Whittemore, and A. D. Shackelford. 1999. Aerobic bacteria and solids in a three-tank, two-pass, counterflow scalder. *Poult. Sci.* 78:144-147.

41. Castanon, J. I. R. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. *Poult. Sci.* 86:2466-2471.

42. Castellini, C., S. Bastianoni, C. Granai, A. Dal Bosco, and M. Brunetti. 2006. Sustainability of poultry production using the emergy approach: Comparison of conventional and organic rearing systems. *Agric. Ecosyst. Environ.* 114:343-350.

43. Castillejo-Rodriguez, A. M., R. M. García Gimeno, G. Z. Cosano, E. B. Alcalá, and M. R. Rodríguez Pérez. 2002. Assessment of mathematical models for predicting *Staphylococcus aureus* growth in cooked meat products. *J. Food Prot.* 65:659-665.

44. Centers for Disease Control and Prevention. 2012. Multistate outbreak of human *Salmonella* Heidelberg infections linked to "Kosher Broiled Chicken Livers" from Schreiber Processing Corporation (Final update). Available at: https://www.cdc.gov/salmonella/2011/chicken-liver-1-11-2012.html. Accessed 14 February 2019.

45. Centers for Disease Control and Prevention. 2014. Multistate outbreak of multidrug-resistant *Salmonella* Heidelberg infections linked to Foster Farms Brand Chicken (Final update). Available at: https://www.cdc.gov/salmonella/heidelberg-10-13/index.html. Accessed 14 February 2019.

46. Centers for Disease Control and Prevention. 2015. Multistate outbreak of drug-resistant *Salmonella* Enteritidis infections linked to raw, frozen, stuffed chicken entrees produced by Barber Foods (Final update). Available at: https://www.cdc.gov/salmonella/frozen-chicken-entrees-07-15/index.html. Accessed 14 February 2019.

47. Centers for Disease Control and Prevention. 2015. Outbreak of *Salmonella* Enteritidis infections linked to raw, frozen, stuffed chicken entrees produced by Aspen Foods (Final update). Available at: https://www.cdc.gov/salmonella/frozen-chicken-entrees-part2-07-15/index.html. Accessed 14 February 2019.

48. Centers for Disease Control and Prevention. 2016. National enteric disease surveillance: *Salmonella* annual report, 2016. Available at: https://www.cdc.gov/nationalsurveillance/pdfs/2016-Salmonella-report-508.pdf. Accessed 14 February 2019.

49. Centers for Disease Control and Prevention. 2018. Multistate outbreak of *Salmonella* Typhimurium linked to chicken salad (Final update). Available at: https://www.cdc.gov/salmonella/typhimurium-02-18/index.html. Accessed 14 February 2019.

50. Centers for Disease Control and Prevention. 2018. Outbreak of *Salmonella* infections linked to chicken (Final update). Available at: https://www.cdc.gov/salmonella/chicken-08-18/index.html. Accessed 14 February 2019.

51. Centers for Disease Control and Prevention. 2019. Outbreak of multidrug-resistant *Salmonella* infections linked to raw chicken products. Available at: https://www.cdc.gov/salmonella/infantis-10-18/index.html. Accessed 2 December 2019.

52. Centers for Disease Control and Prevention. 2020. National Outbreak Reporting System (NORS), Outbreaks per state, *Salmonella*, chicken. Available at: https://wwwn.cdc.gov/norsdashboard/. Accessed 17 January 2020.

53. Codex Alementarius Comission. 2015. Procedural manual, Twenty-fourth edition. Available at: http://www.fao.org/3/a-i5079e.pdf. Accessed 1 January 2020.

54. Collado, L., and M. J. Figueras. 2011. Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter. Clin. Microbiol. Rev.* 24:174-192.

55. Crandall, P. G., S. Seideman, S. C. Ricke, C. A. O'Bryan, A. F. Fanatico, and R. Rainey. 2009. Organic poultry: Consumer perceptions, opportunities, and regulatory issues. *J. Appl. Poult. Res.* 18:795-802.

56. Cui, S., B. Ge, J. Zheng, and J. Meng. 2005. Prevalence and antimicrobial resistance of *Campylobacter* spp. and *Salmonella* serovars in organic chickens from Maryland retail stores. *Appl. Environ. Microbiol.* 71:4108-4111.

57. D'Costa, V. M., C. E. King, L. Kalan, M. Morar, W. W. Sung, C. Schwarz, D. Froese, G. Zazula, F. Calmels, and R. Debruyne. 2011. Antibiotic resistance is ancient. *Nature*. 477:457.

58. Davies, J., and D. Davies. 2010. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* 74:417-433.

59. Dhama, K., A. K. Verma, S. Rajagunalan, A. Kumar, R. Tiwari, S. Chakraborty, and R. Kumar. 2013. *Listeria monocytogenes* infection in poultry and its public health importance with special reference to food borne zoonoses. *Pak. J. Biol. Sci.* 16:301-308.

60. Dickens, J. A., and A. D. Whittemore. 1997. Effects of acetic acid and hydrogen peroxide application during defeathering on the microbiological quality of broiler carcasses prior to evisceration. *Poult. Sci.* 76:657-660.

61. Dimitri, C., and L. Oberholtzer. 2009. Marketing US organic foods: Recent trends from farms to consumers. Economic Information Bulletin No. 58. United States Department of Agriculture, Economic Research Service.

62. Domingues, A. R., S. M. Pires, T. Halasa, and T. Hald. 2012. Source attribution of human campylobacteriosis using a meta-analysis of case-control studies of sporadic infections. *Epidemiol. Infect.* 140:970-981.

63. Dominguez, S. A., and D. W. Schaffner. 2007. Development and validation of a mathematical model to describe the growth of *Pseudomonas* spp. in raw poultry stored under aerobic conditions. *Int. J. Food Microbiol.* 120:287-295.

64. Dominguez, S. A., and D. W. Schaffner. 2008. Modeling the growth of *Salmonella* in raw poultry stored under aerobic conditions. *J. Food Prot.* 71:2429-2435.

65. Efron, B., and R. J. Tibshirani. 1994. An introduction to the bootstrap. Chapman and Hall/CRC Press, Boca Raton, FL.

66. Electronic Code of Federal Regulations. 2018. Title 7, Subtitle B, Chapter I, Subchapter M, Part 205. Available at: https://www.ecfr.gov/cgi-bin/text-idx?tpl=/ecfrbrowse/Title07/7cfr205_main_02.tpl. Accessed 21 February 2019.

67. Epps, S., R. Harvey, M. Hume, T. Phillips, R. Anderson, and D. Nisbet. 2013. Foodborne *Campylobacter*: Infections, metabolism, pathogenesis and reservoirs. *Int. J. Environ. Res. Public Health*. 10:6292-6304.

68. Fanatico, A. C., C. M. Owens, and J. L. Emmert. 2009. Organic poultry production in the United States: Broilers. *J. Appl. Poult. Res.* 18:355-366.

69. Ferreira, S., M. J. Fraqueza, J. A. Queiroz, F. C. Domingues, and M. Oleastro. 2013. Genetic diversity, antibiotic resistance and biofilm-forming ability of *Arcobacter butzleri* isolated from poultry and environment from a Portuguese slaughterhouse. *Int. J. Food Microbiol.* 162:82-88.

70. Fossler, C. P., S. J. Wells, J. B. Kaneene, P. L. Ruegg, L. D. Warnick, J. B. Bender, S. M. Godden, L. W. Halbert, A. M. Campbell, and A. M. G. Zwald. 2004. Prevalence of *Salmonella* spp on conventional and organic dairy farms. *J. Am. Vet. Med. Assoc.* 225:567-573.

71. Friedman, J. H. 2002. Stochastic gradient boosting. *Comput. Stat. Data Anal.* 38:367-378.

72. Fries, R. 2002. Reducing *Salmonella* transfer during industrial poultry meat production. *Worlds Poult. Sci. J.* 58:527-540.

73. Garaizar, J., S. Porwollik, A. Echeita, A. Rementeria, S. Herrera, R. M.-Y. Wong, J. Frye, M. A. Usera, and M. McClelland. 2002. DNA microarray-based typing of an atypical monophasic *Salmonella enterica* serovar. *J. Clin. Microbiol.* 40:2074-2078.

74. Ghollasi-Mood, F., M. Mohsenzadeh, M. R. Hoseindokht, and M. Varidi. 2017. Quality changes of air-packaged chicken meat stored under different temperature conditions and mathematical modelling for predicting the microbial growth and shelf life. *J. Food Saf.* 37:e12331.

75. Giacometti, F., A. Lucchi, A. Di Francesco, M. Delogu, E. Grilli, I. Guarniero, L. Stancampiano, G. Manfreda, G. Merialdi, and A. Serraino. 2015. *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* circulation in a dairy farm and sources of milk contamination. *Appl. Environ. Microbiol.* 81:5055-5063.

76. Gilbert, R. J., K. L. Miller, and D. Roberts. 1989. *Listeria monocytogenes* and chilled foods. *Lancet*. 333:383-384.

77. Gill, C. O., and K. G. Newton. 1978. The ecology of bacterial spoilage of fresh meat at chill temperatures. *Meat Sci.* 2:207-217.

78. Glass, G. V. 1976. Primary, secondary, and meta-analysis of research. *Educ. Res.* 5:3-8.

79. Goel, P. K., and M. H. Degroot. 1981. Information about hyperparameters in hierarchical models. *J. Am. Stat. Assoc.* 76:140-147.

80. Golden, C. E., and A. Mishra. 2020. Prevalence of *Salmonella* spp. and *Campylobacter* spp. in alternative and conventionally produced chicken in the United States: A systematic review and meta-analysis. *J. Food Prot.*:(In Press).

81. Golden, C. E., M. J. Rothrock Jr, and A. Mishra. 2019. Comparison between random forest and gradient boosting machine methods for predicting *Listeria* spp. prevalence in the environment of pastured poultry farms. *Food Res. Int.* 122:47-55.

82. Golden, C. E., M. J. Rothrock Jr, and A. Mishra. 2019. Using farm practice variables as predictors of *Listeria* spp. prevalence in pastured poultry farms. *Front. Sustain. Food Syst. 3: 15. doi: 10.3389/fsufs*.

83. Goncalves-Tenorio, A., B. Silva, V. Rodrigues, V. Cadavez, and U. Gonzales-Barron. 2018. Prevalence of pathogens in poultry meat: A meta-analysis of European published surveys. *Foods*. 7:69.

84. Gonzales-Barron, U., and F. Butler. 2011. The use of meta-analytical tools in risk assessment for food safety. *Food Microbiol.* 28:823-827.

85. Gottlieb, S. L., E. C. Newbern, P. M. Griffin, L. M. Graves, R. M. Hoekstra, N. L. Baker, S. B. Hunter, K. G. Holt, F. Ramsey, and M. Head. 2006. Multistate outbreak of listeriosis linked to turkey deli meat and subsequent changes in US regulatory policy. *Clin. Infect. Dis.* 42:29-36.

86. Greene, C., G. Ferreira, A. Carlson, B. Cooke, and C. Hitaj. 2017. Growing organic demand provides high-value opportunities for many types of producers. Available at: https://www.ers.usda.gov/amber-waves/2017/januaryfebruary/growing-organic-demand-provides-high-value-opportunities-for-many-types-of-producers/. Accessed 16 December 2019.

87. Grimont, P. A. D., and F.-X. Weill. 2007. Antigenic formulae of the *Salmonella* serovars. Available at: https://www.pasteur.fr/sites/default/files/veng_0.pdf. Accessed 26 February 19.

88. Grinnell, M., G. Provo, N. Marsden-Haug, K. A. Stigi, E. DeBess, B. Kissler, E. Crarey, H. Tate, J. Pringle, and J. Grass. 2013. Outbreak of *Salmonella* Heidelberg infections linked to a single poultry producer—13 states, 2012–2013. *Morb. Mortal. Wkly. Rep.* 62:553.

89. Hartnett, E., L. Kelly, D. Newell, M. Wooldridge, and G. Gettinby. 2001. A quantitative risk assessment for the occurrence of *Campylobacter* in chickens at the point of slaughter. *Epidemiol. Infect.* 127:195-206.

90. Hegde, N. V., S. Kariyawasam, and C. DebRoy. 2016. Comparison of antimicrobial resistant genes in chicken gut microbiome grown on organic and conventional diet. *Vet. Anim. Sci.* 1:9-14.

91. Herbison, P., J. Hay-Smith, and W. J. Gillespie. 2006. Adjustment of meta-analyses on the basis of quality scores should be abandoned. *Journal of Clinical Epidemiology*. 59:1249-e1.

92. Heuer, O. E., K. Pedersen, J. Andersen, and M. Madsen. 2001. Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. *Lett. Appl. Microbiol.* 33:269-274.

93. Heyndrickx, M., D. Vandekerchove, L. Herman, I. Rollier, K. Grijspeerdt, and L. De Zutter. 2002. Routes for *Salmonella* contamination of poultry meat: Epidemiological study from hatchery to slaughterhouse. *Epidemiol. Infect.* 129:253-265.

94. Hiett, K. L., N. J. Stern, P. Fedorka-Cray, N. A. Cox, M. T. Musgrove, and S. Ladely. 2002. Molecular subtype analyses of *Campylobacter* spp. from Arkansas and California poultry operations. *Appl. Environ. Microbiol.* 68:6220-6236.

95. Hinton Jr, A., and K. D. Ingram. 2000. Use of oleic acid to reduce the population of the bacterial flora of poultry skin. *J. Food Prot.* 63:1282-1286.

96. Hinton Jr, A., and K. D. Ingram. 2005. Microbicidal activity of tripotassium phosphate and fatty acids toward spoilage and pathogenic bacteria associated with poultry. *J. Food Prot.* 68:1462-1466.

97. Hinton Jr, A., J. Northcutt, J. Cason, D. Smith, and K. Ingram. 2007. Bacterial populations of broiler carcasses washed in mixtures of potassium hydroxide and lauric acid. *J. Appl. Poult. Res.* 16:387-391.

98. Ho, H. T. K., L. J. A. Lipman, and W. Gaastra. 2006. *Arcobacter*, what is known and unknown about a potential foodborne zoonotic agent. *Vet. Microbiol.* 115:1-13.

99. Hoffmann, S., M. B. Batz, and J. G. Morris Jr. 2012. Annual cost of illness and quality-adjusted life year losses in the United States due to 14 foodborne pathogens. *J. Food Prot.* 75:1292-1302.

100. Hong, Y., and K. B. Song. 2009. Inhibition of pathogenic bacteria inoculated on raw chicken by aqueous chlorine dioxide treatment. *Ital. J. Food Sci.* 21:106-109.

101. Hoogenboom, L. A. P., J. G. Bokhorst, M. D. Northolt, L. P. L. Van de Vijver, N. J. G. Broex, D. J. Mevius, J. A. C. Meijs, and J. Van Der Roest. 2008. Contaminants and microorganisms in Dutch organic food products: a comparison with conventional products. *Food Addit. Contam.* 25:1195-1207.

102. Hopkins, R. S., R. Olmsted, and G. R. Istre. 1984. Endemic *Campylobacter jejuni* infection in Colorado: Identified risk factors. *Am. J. Public Health*. 74:249-250.

103. Houf, K., L. De Zutter, J. Van Hoof, and P. Vandamme. 2002. Occurrence and distribution of *Arcobacter* species in poultry processing. *J. Food Prot.* 65:1233-1239.

104. Hughner, R. S., P. McDonagh, A. Prothero, C. J. Shultz, and J. Stanton. 2007. Who are organic food consumers? A compilation and review of why people purchase organic food. *J. Consum. Behav.* 6:94-110.

105. Humphery, T. J., A. Henley, and D. G. Lanning. 1993. The colonization of broiler chickens with *Campylobacter jejuni*: Some epidemiological investigations. *Epidemiol. Infect.* 110:601-607.

106. Humphrey, T., S. O'Brien, and M. Madsen. 2007. Campylobacters as zoonotic pathogens: A food production perspective. *Int. J. Food Microbiol.* 117:237-257.

107. Incili, G. K., and M. Çalicioğlu. 2018. Change in scalding fluids by time in poultry slaughterhouse and its effect on microbiological quality of carcasses. *J. Food Saf.* 38:e12485.

108. Jacobs-Reitsma, W., A. Van de Giessen, N. Bolder, and R. Mulder. 1995. Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. *Epidemiol. Infect.* 114:413-421.

109. James, W. O., J. C. Prucha, R. L. Brewer, J. W. O. Williams, W. A. Christensen, A. M. Thaler, and A. T. Hogue. 1992. Effects of countercurrent scalding and postscald spray on the bacteriologic profile of raw chicken carcasses. *J. Am. Vet. Med. Assoc.* 201:705-708.

110. Jianping, L. I., M. A. Hengtao, X. U. Ding, F. Pengxing, Y. Shenghui, and Y. Zheng. 2019. Prediction of the trend of antimicrobial resistance of *Escherichia coli* in livestock and poultry. p. 1. *In*, 2019 ASABE Annual International Meeting American Society of Agricultural and Biological Engineers.

111. Juneja, V. K., H. Marks, L. Huang, and H. Thippareddi. 2011. Predictive model for growth of *Clostridium perfringens* during cooling of cooked uncured meat and poultry. *Food Microbiol.* 28:791-795.

112. Juneja, V. K., M. V. Melendres, L. Huang, V. Gumudavelli, J. Subbiah, and H. Thippareddi. 2007. Modeling the effect of temperature on growth of *Salmonella* in chicken. *Food Microbiol.* 24:328-335.

113. Jüni, P., A. Witschi, R. Bloch, and M. Egger. 1999. The hazards of scoring the quality of clinical trials for meta-analysis. *Jama*. 282:1054-1060.

114. Kaakoush, N. O., N. Castaño-Rodríguez, H. M. Mitchell, and S. M. Man. 2015. Global epidemiology of *Campylobacter* infection. *Clin. Microbiol. Rev.* 28:687-720.

115. Kabeya, H., S. Maruyama, Y. Morita, T. Ohsuga, S. Ozawa, Y. Kobayashi, M. Abe, Y. Katsube, and T. Mikami. 2004. Prevalence of *Arcobacter* species in retail meats and antimicrobial susceptibility of the isolates in Japan. *Int. J. Food Microbiol.* 90:303-308.

116. Kassem, I. I., O. Kehinde, A. Kumar, and G. Rajashekara. 2017. Antimicrobial-resistant *Campylobacter* in organically and conventionally raised layer chickens. *Foodborne Pathog. Dis.* 14:29-34.

117. Kerr, A. K., A. M. Farrar, L. A. Waddell, W. Wilkins, B. J. Wilhelm, O. Bucher, R. W. Wills, R. H. Bailey, C. Varga, and S. A. McEwen. 2013. A systematic review-meta-analysis and meta-regression on the effect of selected competitive exclusion products on *Salmonella* spp. prevalence and concentration in broiler chickens. *Prev. Vet. Med.* 111:112-125.

118. Kilonzo-Nthenge, A., A. Brown, S. N. Nahashon, and D. Long. 2015. Occurrence and antimicrobial resistance of enterococci isolated from organic and conventional retail chicken. *J. Food Prot.* 78:760-766.

119. Kim, S., Z. Yu, R. M. Kil, and M. Lee. 2015. Deep learning of support vector machines with class probability output networks. *Neural Netw.* 64:19-28.

120. Klonsky, K., and L. Tourte. 1998. Organic agricultural production in the United States: Debates and directions. *Am. J. Agric. Econ.* 80:1119-1124.

121. Lahellec, C., and P. Colin. 1985. Relationship between serotypes of Salmonellae from hatcheries and rearing farms and those from processed poultry carcases. *Br. Poult. Sci.* 26:179-186.

122. Lappi, V., J. R. Archer, E. Cebelinski, F. Leano, J. M. Besser, R. F. Klos, C. Medus, K. E. Smith, C. Fitzgerald, and J. P. Davis. 2013. An outbreak of foodborne illness among attendees of a wedding reception in Wisconsin likely caused by *Arcobacter butzleri*. *Foodborne Pathog. Dis.* 10:250-255.

123. Lee, A., S. C. Smith, and P. J. Coloe. 1998. Survival and growth of *Campylobacter jejuni* after artificial inoculation onto chicken skin as a function of temperature and packaging conditions. *J. Food Prot.* 61:1609-1614.

124. Lestari, S. I., F. Han, F. Wang, and B. Ge. 2009. Prevalence and antimicrobial resistance of *Salmonella* serovars in conventional and organic chickens from Louisiana retail stores. *J. Food Prot.* 72:1165-1172.

125. Lillard, H. S., D. Hamm, and J. E. Thomson. 1984. Effect of reduced processing on recovery of foodborne pathogens from hot-boned broiler meat and skin. *J. Food Prot.* 47:209-212.

126. Locatelli, A., M. A. Lewis, and M. J. Rothrock Jr. 2017. The distribution of *Listeria* in pasture-raised broiler farm soils Is potentially related to University of Vermont medium enrichment bias toward *Listeria innocua* over *Listeria monocytogenes*. *Front. Vet. Sci.* 4:227.

127. Loura, C. A. C., R. C. C. Almeida, and P. F. Almeida. 2005. The incidence and level of *Listeria* spp. and *Listeria monocytogenes* contamination in processed poultry at a poultry processing plant. *J. Food Saf.* 25:19-29.

128. Luangtongkum, T., T. Y. Morishita, A. J. Ison, S. Huang, P. F. McDermott, and Q. Zhang. 2006. Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. *Appl. Environ. Microbiol.* 72:3600-3607.

129. Lv, J., and X. Liu. 2011. Predicting arsenic concentration in rice plants from hyperspectral data using random forests. p. 601-606. *In*, Advances in Multimedia, Software Engineering and Computing Vol. 1 Springer.

130. Machado, J., and F. Bernardo. 1990. Prevalence of *Salmonella* in chicken carcasses in Portugal. *J. Appl. Bacteriol.* 69:477-480.

131. Maciorowski, K. G., F. T. Jones, S. D. Pillai, and S. C. Ricke. 2004. Incidence, sources, and control of food-borne *Salmonella* spp. in poultry feeds. *Worlds Poult. Sci. J.* 60:446-457.

132. Majowicz, S. E., J. Musto, E. Scallan, F. J. Angulo, M. Kirk, S. J. O'brien, T. F. Jones, A. Fazil, and R. M. Hoekstra. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin. Infect. Dis.* 50:882-889.

133. Man, S. M. 2011. The clinical importance of emerging *Campylobacter* species. *Nat. Rev. Gastroenterol.* 8:669-685.

134. McEwen, S. A., and P. J. Fedorka-Cray. 2002. Antimicrobial use and resistance in animals. *Clin. Infect. Dis.* 34:S93-S106.

135. Mead, G. C., W. R. Hudson, and M. H. Hinton. 1995. Effect of changes in processing to improve hygiene control on contamination of poultry carcasses with *Campylobacter*. *Epidemiol. Infect.* 115:495-500.

136. Melendez, S. N., I. Hanning, J. Han, R. Nayak, A. Clement, A. Wooming, P. Hererra, F. Jones, S. Foley, and S. Ricke. 2010. *Salmonella enterica* isolates from pasture-raised poultry exhibit antimicrobial resistance and class I integrons. *J. Appl. Microbiol.* 109:1957-1966.

137. Micciche, A. C., P. M. Rubinelli, J. A. Wages, and S. C. Ricke. 2018. Source of water and potential sanitizers and biological antimicrobials for alternative poultry processing food safety applications. *Front. Sustain. Food Syst.* 2:82.

138. Milillo, S. R., J. C. Stout, I. B. Hanning, A. Clement, E. D. Fortes, H. C. Den Bakker, M. Wiedmann, and S. C. Ricke. 2012. *Listeria monocytogenes* and hemolytic *Listeria innocua* in poultry. *Poult. Sci.* 91:2158-2163.

139. Millman, J. M., K. Waits, H. Grande, A. R. Marks, J. C. Marks, L. B. Price, and B. A. Hungate. 2013. Prevalence of antibiotic-resistant *E. coli* in retail chicken: Comparing conventional, organic, kosher, and raised without antibiotics. *F1000Res.* 2:155.

140. Milner, K. C., and M. F. Shaffer. 1952. Bacteriologic studies of experimental *Salmonella* infections in chicks. *J. Infect. Dis.* 90:81-96.

141. Miranda, J. M., M. Guarddon, B. I. Vázquez, C. A. Fente, J. Barros-Velazquez, A. Cepeda, and C. M. Franco. 2008. Antimicrobial resistance in *Enterobacteriaceae* strains isolated from organic chicken, conventional chicken and conventional turkey meat: A comparative survey. *Food Control*. 19:412-416.

142. Miranda, J. M., B. I. Vazquez, C. A. Fente, P. Calo-Mata, A. Cepeda, and C. M. Franco. 2008. Comparison of antimicrobial resistance in *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* strains isolated from organic and conventional poultry meat. *J. Food Prot.* 71:2537-2542.

143. Mor-Mur, M., and J. Yuste. 2010. Emerging bacterial pathogens in meat and poultry: An overview. *Food Bioproc. Tech.* 3:24.

144. Moreno Switt, A. I., Y. Soyer, L. D. Warnick, and M. Wiedmann. 2009. Emergence, distribution, and molecular and phenotypic characteristics of *Salmonella enterica* serotype 4, 5, 12: i:–. *Foodborne Pathog. Dis.* 6:407-415.

145. Mulder, R. W. A. W., L. W. J. Dorresteijn, and J. Van Der Broek. 1978. Cross-contamination during the scalding and plucking of broilers. *Br. Poult. Sci.* 19:61-70.

146. Natekin, A., and A. Knoll. 2013. Gradient boosting machines, a tutorial. *Front. Neurorobot.* 7:21.

147. National Chicken Council. 2015. How broilers are marketed. Available at: https://www.nationalchickencouncil.org/about-the-industry/statistics/how-broilers-are-marketed/. Accessed 19 February 2019.

148. Nauta, M., I. Van Der Fels-Klerx, and A. Havelaar. 2005. A poultry-processing model for quantitative microbiological risk assessment. *Risk Anal.* 25:85-98.

149. Nde, C. W., J. M. McEvoy, J. S. Sherwood, and C. M. Logue. 2007. Cross contamination of turkey carcasses by *Salmonella* species during defeathering. *Poult. Sci.* 86:162-167.

150. Nde, C. W., J. S. Sherwood, C. Doetkott, and C. M. Logue. 2006. Prevalence and molecular profiles of *Salmonella* collected at a commercial turkey processing plant. *J. Food Prot.* 69:1794-1801.

151. Newell, D. G., and C. Fearnley. 2003. Sources of *Campylobacter* colonization in broiler chickens. *Appl. Environ. Microbiol.* 69:4343-4351.

152. Njagi, L. W., P. G. Mbuthia, L. C. Bebora, P. N. Nyaga, U. Minga, and J. E. Olsen. 2004. Carrier status for *Listeria monocytogenes* and other *Listeria* species in free range farm and market healthy indigenous chickens and ducks. *East Afr. Med. J.* 81:529-533.

153. Noormohamed, A., and M. K. Fakhr. 2014. Prevalence and antimicrobial susceptibility of *Campylobacter* spp. in Oklahoma conventional and organic retail poultry. *Open Microbiol. J.* 8:130.

154. Northcutt, J. K., and D. R. Jones. 2004. A survey of water use and common industry practices in commercial broiler processing facilities. *J. Appl. Poult. Res.* 13:48-54.

155. Nurmi, E., and M. Rantala. 1973. New aspects of *Salmonella* infection in broiler production. *Nature*. 241:210-211.

156. Nychas, G.-J. E., P. N. Skandamis, C. C. Tassou, and K. P. Koutsoumanis. 2008. Meat spoilage during distribution. *Meat Sci.* 78:77-89.

157. Olsen, S. J., M. Patrick, S. B. Hunter, V. Reddy, L. Kornstein, W. R. MacKenzie, K. Lane, S. Bidol, G. A. Stoltman, and D. M. Frye. 2005. Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. *Clin. Infect. Dis.* 40:962-967.

158. Oscar, T. P. 2006. Validation of a tertiary model for predicting variation of *Salmonella* Typhimurium DT104 (ATCC 700408) growth from a low initial density on ground chicken breast meat with a competitive microflora. *J. Food Prot.* 69:2048-2057.

159. Oscar, T. P. 2017. Neural network models for growth of *Salmonella* serotypes in ground chicken subjected to temperature abuse during cold storage for application in HACCP and risk assessment. *Int. J. Food Sci. Technol.* 52:214-221.

160. Pang, H., R. McEgan, A. Mishra, S. A. Micallef, and A. K. Pradhan. 2017. Identifying and modeling meteorological risk factors associated with pre-harvest contamination of *Listeria* species in a mixed produce and dairy farm. *Food Res. Int.* 102:355-363.

161. Park, H., Y.-C. Hung, and R. E. Brackett. 2002. Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing. *Int. J. Food Microbiol.* 72:77-83.

162. Park, J. H., and I. H. Kim. 2014. Supplemental effect of probiotic *Bacillus subtilis* B2A on productivity, organ weight, intestinal *Salmonella* microflora, and breast meat quality of growing broiler chicks. *Poult. Sci.* 93:2054-2059.

163. Park, S. F. 2002. The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. *Int. J. Food Microbiol.* 74:177-188.

164. Patrick, T. E., T. Goodwin, J. Collins, R. Wyche, and B. Love. 1972. Steam versus hot-water scalding in reducing bacterial loads on the skin of commercially processed poultry. *Appl. Environ. Microbiol.* 23:796-798.

165. Pearson, A. D., M. Greenwood, T. D. Healing, D. Rollins, M. Shahamat, J. Donaldson, and R. R. Colwell. 1993. Colonization of broiler chickens by waterborne *Campylobacter jejuni. Appl. Environ. Microbiol.* 59:987-996.

166. Pearson, A. D., M. H. Greenwood, R. Feltham, T. D. Healing, J. Donaldson, D. M. Jones, and R. R. Colwell. 1996. Microbial ecology of *Campylobacter jejuni* in a United Kingdom chicken supply chain: Intermittent common source, vertical transmission, and amplification by flock propagation. *Appl. Environ. Microbiol.* 62:4614-4620.

167. Peng, M., S. Salaheen, J. A. Almario, B. Tesfaye, R. Buchanan, and D. Biswas. 2016. Prevalence and antibiotic resistance pattern of *Salmonella* serovars in integrated crop-livestock farms and their products sold in local markets. *Environ. Microbiol.* 18:1654-1665.

168. Petersen, L., and A. Wedderkopp. 2001. Evidence that certain clones of *Campylobacter jejuni* persist during successive broiler flock rotations. *Appl. Environ. Microbiol.* 67:2739-2745.

169. Petkar, A., W. Q. Alali, M. A. Harrison, and L. R. Beuchat. 2011. Survival of *Salmonella* in organic and conventional broiler feed as affected by temperature and water activity. *Agric. Food Anal. Bacteriol.* 1:175-185.

170. Philibert, A., M.-L. Desprez-Loustau, B. Fabre, P. Frey, F. Halkett, C. Husson, B. Lung-Escarmant, B. Marçais, C. Robin, C. Vacher, and D. Makowski. 2011. Predicting invasion success of forest pathogenic fungi from species traits. *J. Appl. Ecol.* 48:1381-1390.

171. Pouillot, R., B. Garin, N. Ravaonindrina, K. Diop, M. Ratsitorahina, D. Ramanantsoa, and J. Rocourt. 2012. A risk assessment of campylobacteriosis and salmonellosis linked to chicken meals prepared in households in Dakar, Senegal. *Risk Anal.* 32:1798-1819.

172. Pradhan, A. K., A. Mishra, and H. Pang. 2019. Predictive microbiology and microbial risk assessment. *In* M.P. Doyle, F. Diez-Gonzalez, and C. Hill (ed.), Food Microbiology: Fundamentals and Frontiers, 5th. ASM Press, Washington, D.C.

173. Projahn, M., P. von Tippelskirch, T. Semmler, S. Guenther, T. Alter, and U. Roesler. 2019. Contamination of chicken meat with extended-spectrum beta-lactamase producing-*Klebsiella pneumoniae* and *Escherichia coli* during scalding and defeathering of broiler carcasses. *Food Microbiol.* 77:185-191.

174. Raab, C., and D. Grobe. Date 2005. Consumer knowledge and perceptions about organic food *J. Ext.* 43. Available from: https://www.joe.org/joe/2005august/rb3.php. Accessed 26 February 2019.

175. Raab, V., S. Bruckner, E. Beierle, Y. Kampmann, B. Petersen, and J. Kreyenschmidt. 2008. Generic model for the prediction of remaining shelf life in support of cold chain management in pork and poultry supply chains. *J. Chain Network Sci.* 8:59-73.

176. Rasschaert, G., K. Houf, and L. De Zutter. 2007. Impact of the slaughter line contamination on the presence of *Salmonella* on broiler carcasses. *J. Appl. Microbiol.* 103:333-341.

177. Rasschaert, G., K. Houf, C. Godard, C. Wildemauwe, M. Pastuszczak-Frak, and L. De Zutter. 2008. Contamination of carcasses with *Salmonella* during poultry slaughter. *J. Food Prot.* 71:146-152.

178. Reisch, L., U. Eberle, and S. Lorek. 2013. Sustainable food consumption: An overview of contemporary issues and policies. *Sci. Pract. Pol.* 9:7-25.

179. Rosenquist, H., L. Boysen, A. L. Krogh, A. N. Jensen, and M. Nauta. 2013. *Campylobacter* contamination and the relative risk of illness from organic broiler meat in comparison with conventional broiler meat. *Int. J. Food Microbiol.* 162:226-230.

180. Rothrock Jr., M. J., K. L. Hiett, J. Y. Guard, and C. R. Jackson. 2016. Antibiotic resistance patterns of major zoonotic pathogens from all-natural, antibiotic-free, pasture-raised broiler flocks in the southeastern United States. *J. Environ. Qual.* 45:593-603.

181. Ru, G., M. I. Crescio, F. Ingravalle, C. Maurella, D. Gregori, C. Lanera, D. Azzolina, G. Lorenzoni, N. Soriani, S. Zec, P. Berchialla, S. Mercadante, F. Zobec, M. Ghidina, S. Baldas, B. Bonifacio, A. Kinkopf, D. Kozina, L. Nicolandi, and L. Rosat. 2017. Machine learning techniques applied in risk assessment related to food safety. *EFSA Supporting Publications*. 14:1254E.

182. Sahin, O., Q. Zhang, J. C. Meitzler, B. S. Harr, T. Y. Morishita, and R. Mohan. 2001. Prevalence, antigenic specificity, and bactericidal activity of poultry anti-*Campylobacter* maternal antibodies. *Appl. Environ. Microbiol.* 67:3951-3957.

183. Sakhare, P. Z., N. M. Sachindra, K. P. Yashoda, and D. N. Rao. 1999. Efficacy of intermittent decontamination treatments during processing in reducing the microbial load on broiler chicken carcass. *Food Control*. 10:189-194.

184. Sanchez, M. X., W. M. Fluckey, M. M. Brashears, and S. R. McKee. 2002. Microbial profile and antibiotic susceptibility of *Campylobacter* spp. and *Salmonella* spp. in broilers processed in air-chilled and immersion-chilled environments. *J. Food Prot.* 65:948-956.

185. Sapkota, A. R., E. L. Kinney, A. George, R. M. Hulet, R. Cruz-Cano, K. J. Schwab, G. Zhang, and S. W. Joseph. 2014. Lower prevalence of antibiotic-resistant *Salmonella* on large-scale US conventional poultry farms that transitioned to organic practices. *Sci. Total Environ.* 476:387-392.

186. Sapkota, A. R., L. Y. Lefferts, S. McKenzie, and P. Walker. 2007. What do we feed to food-production animals? A review of animal feed ingredients and their potential impacts on human health. *Environ. Health Perspect.* 115:663-670.

187. Sargeant, J. M., A. Rajic, S. Read, and A. Ohlsson. 2006. The process of systematic review and its application in agri-food public-health. *Prev. Vet. Med.* 75:141-151.

188. Schaffter, N., J. Zumstein, and A. Parriaux. 2004. Factors influencing the bacteriological water quality in mountainous surface and groundwaters. *Acta Hydrochim. Hydrobiol.* 32:225-234.

189. Shreeve, J. E., M. Toszeghy, M. Pattison, and D. G. Newell. 2000. Sequential spread of *Campylobacter* infection in a multipen broiler house. *Avian Dis.* 44:983-988.

190. Siemon, C. E., P. B. Bahnson, and W. A. Gebreyes. 2007. Comparative investigation of prevalence and antimicrobial resistance of *Salmonella* between pasture and conventionally reared poultry. *Avian Dis.* 51:112-117.

191. Skandamis, P. N., and G.-J. E. Nychas. 2002. Preservation of fresh meat with active and modified atmosphere packaging conditions. *Int. J. Food Microbiol.* 79:35-45.

192. Skov, M. N., O. Angen, M. Chriel, J. E. Olsen, and M. Bisgaard. 1999. Risk factors associated with *Salmonella enterica* serovar *typhimurium* infection in Danish broiler flocks. *Poult. Sci.* 78:848-854.

193. Smadi, H., and J. M. Sargeant. 2013. Quantitative risk assessment of human salmonellosis in Canadian broiler chicken breast from retail to consumption. *Risk Anal.* 33:232-248.

194. Smith, A., B. Sterba-Boatwright, and J. Mott. 2010. Novel application of a statistical technique, Random Forests, in a bacterial source tracking study. *Water Res.* 44:4067-4076.

195. Sofos, J. N. 2008. Challenges to meat safety in the 21st century. *Meat Sci.* 78:3-13.

196. Soltani, M., and M. Omid. 2015. Detection of poultry egg freshness by dielectric spectroscopy and machine learning techniques. *LWT.* 62:1034-1042.

197. Son, I., M. D. Englen, M. E. Berrang, P. J. Fedorka-Cray, and M. A. Harrison. 2007. Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. *Int. J. Food Microbiol.* 113:16-22.

198. Stopforth, J. D., R. O'connor, M. Lopes, B. Kottapalli, W. E. Hill, and M. Samadpour. 2007. Validation of individual and multiple-sequential interventions for reduction of microbial populations during processing of poultry carcasses and parts. *J. Food Prot.* 70:1393-1401.

199. Svobodová, I., G. Bořilová, R. Hulánková, and I. Steinhauserová. 2012. Microbiological quality of broiler carcasses during slaughter processing. *Acta Vet. Brno*. 81:37-42.

200. Threlfall, E. J., L. R. Ward, J. A. Frost, and G. A. Willshaw. 2000. The emergence and spread of antibiotic resistance in food-borne bacteria. *Int. J. Food Microbiol.* 62:1-5.

201. Totton, S. C., A. M. Farrar, W. Wilkins, O. Bucher, L. A. Waddell, B. J. Wilhelm, S. A. McEwen, and A. Rajić. 2012. A systematic review and meta-analysis of the effectiveness of biosecurity and vaccination in reducing *Salmonella* spp. in broiler chickens. *Food Res. Int.* 45:617-627.

202. United States Department of Agriculture. 2016. About the national organic program. Available at: https://www.ams.usda.gov/publications/content/about-national-organic-program. Accessed 16 February 2019.

203. United States Department of Agriculture. 2017. Certified organic survey, 2016 summary. Available at: https://www.nass.usda.gov/Publications/Todays_Reports/reports/census17.pdf. Accessed 16 December 2019.

204. van Horne, P. L. M., and N. Bondt. 2013. Competitiveness of the EU poultry meat sector. Available at: https://library.wur.nl/WebQuery/wurpubs/fulltext/292607. Accessed 19 February 2019.

205. van Immerseel, F., L. De Zutter, K. Houf, F. Pasmans, F. Haesebrouck, and R. Ducatelle. 2009. Strategies to control *Salmonella* in the broiler production chain. *Worlds Poult. Sci. J.* 65:367-392.

206. van Loo, E. J., V. Caputo, R. M. Nayga Jr, J.-F. Meullenet, and S. C. Ricke. 2011. Consumers' willingness to pay for organic chicken breast: Evidence from choice experiment. *Food Qual. Prefer.* 22:603-613.

207. Vandamme, P., and J. De Ley. 1991. Proposal for a new family, *Campylobacteraceae. Int. J. Syst. Evol. Microbiol.* 41:451-455.

208. Vangay, P., J. Steingrimsson, M. Wiedmann, and M. J. Stasiewicz. 2014. Classification of *Listeria monocytogenes* persistence in retail delicatessen environments using expert elicitation and machine learning. *Risk Anal.* 34:1830-1845.

209. Veerkamp, C. H., and W. Heemskerk. 1992. Counter-current multi-stage scalding. p. 30-32. *In*, Broiler Industry, 55. Watt Publishing Co., Mt. Morris, IL.

210. Vermeulen, K., J. Verspreet, C. Courtin, F. Haesebrouck, R. Ducatelle, and F. Van Immerseel. 2017. Reduced particle size wheat bran is butyrogenic and lowers *Salmonella* colonization, when added to poultry feed. *Vet. Microbiol.* 198:64-71.

211. Volkova, V. V., R. H. Bailey, and R. W. Wills. 2009. *Salmonella* in broiler litter and properties of soil at farm location. *PLOS ONE*. 4:e6403.

212. Voss-Rech, D., L. Potter, C. S. L. Vaz, D. I. B. Pereira, L. A. Sangioni, A. C. Vargas, and S. de Avila Botton. 2017. Antimicrobial resistance in nontyphoidal *Salmonella* isolated from human and poultry-related samples in Brazil: 20-year meta-analysis. *Foodborne Pathog. Dis.* 14:116-124.

213. Wagenaar, J. A., N. P. French, and A. H. Havelaar. 2013. Preventing *Campylobacter* at the source: Why is it so difficult? *Clin. Infect. Dis.* 57:1600-1606.

214. Wang, H., J. Qi, D. Duan, Y. Dong, X. Xu, and G. Zhou. 2018. Combination of a novel designed spray cabinet and electrolyzed water to reduce microorganisms on chicken carcasses. *Food Control*. 86:200-206.

215. Warriss, P. D., L. J. Wilkins, S. N. Brown, A. J. Phillips, and V. Allen. 2004. Defaecation and weight of the gastrointestinal tract contents after feed and water withdrawal in broilers. *Br. Poult. Sci.* 45:61-66.

216. Wegener, H. C. 2003. Antibiotics in animal feed and their role in resistance development. *Curr. Opin. Microbiol.* 6:439-445.

217. Wei, Q. K., T. J. Fang, and W. C. Chen. 2001. Development and validation of growth model for *Yersinia enterocolitica* in cooked chicken meats packaged under various atmosphere packaging and stored at different temperatures. *J. Food Prot.* 64:987-993.

218. Whiting, R. C., and R. L. Buchanan. 1997. Development of a quantitative risk assessment model for *Salmonella enteritidis* in pasteurized liquid eggs. *Int. J. Food Microbiol.* 36:111-125.

219. Young, I., A. Rajić, B. J. Wilhelm, L. Waddell, S. Parker, and S. A. McEwen. 2009. Comparison of the prevalence of bacterial enteropathogens, potentially zoonotic bacteria and bacterial resistance to antimicrobials in organic and conventional poultry, swine and beef production: a systematic review and meta-analysis. *Epidemiol. Infect.* 137:1217-1232.

220. Zamperini, K., V. Soni, D. Waltman, S. Sanchez, E. C. Theriault, J. Bray, and J. J. Maurer. 2007. Molecular characterization reveals *Salmonella enterica* serovar 4,[5], 12: i:− from poultry is a variant Typhimurium serovar. *Avian Dis.* 51:958-964.

Table 2.1 United States Department of Agriculture standards for organic poultry production according to 7 CFR §205[a]

Factor	Key points	CFR section
Origin of livestock	All birds intended for slaughter/egg production must be under organic management by the second day of life	§205.236
Livestock feed	All feed, feed additives, and feed supplements must be 100% certified organic; water additives must be consistent with the regulations in §205.603	§205.237
Livestock health care	Animals must be kept in low stress environments; no hormones can be used to induce growth; no antibiotics can be used to treat birds that will be marketed as organic	§205.238
Livestock living conditions	Animals must have access to the outside, unless due to inclement weather; housing must provide room for exercise, direct sunlight, fresh air, shade, shelter, and adequate ventilation, supply of clean water, and sanitation	§205.239
Carcass washes	Carcass wash water can contain chlorine levels permitted by FDA[b] and EPA[c], but must be followed by a rinse with potable water that does not exceed 4 ppm chlorine	§205.102
Marketing and labeling	Products represented as "100% organic" or "organic" must include handler information and "Certified by ___" statements naming the appropriate certifying agency	§205.303
Record keeping	Accurate records must be kept on an ongoing basis; common records to be kept: feed receipts/certificates, sales records, production records, mortality/cull records	§205.103

[a] Source: Electronic Code of Federal Regulations *(66)*
[b] Food and Drug Administration (FDA)
[c] Environmental Protection Agency (EPA)

Table 2.2 Chicken-associated salmonellosis outbreaks in the United States and Puerto Rico during 2011-2018

Year	**Food source**	**Serovar**	**Cases**	**Deaths**	**Reference**
2011	Kosher broiled chicken livers	Heidelberg	190	0	*(44)*
2012-2013	Chicken	Heidelberg	134	0	*(88)*
2013-2014	Chicken	Heidelberg	634	0	*(45)*
2015	Raw, frozen, stuffed chicken	Enteritidis	15	0	*(46)*
2015	Raw, frozen, stuffed chicken	Enteritidis	5	0	*(47)*
2018	Chicken salad	Typhimurium	265	1	*(49)*
2018	Chicken	I 4,[5],12:i:-	25	1	*(50)*
2018	Chicken	Infantis	129	10	*(51)*

CHAPTER 3

COMPARISON BETWEEN RANDOM FOREST AND GRADIENT BOOSTING MACHINE METHODS FOR PREDICTING *LISTERIA* SPP. PREVALENCE IN THE ENVIRONMENT OF PASTURED POULTRY FARMS[1]

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[1] Golden, C.E., M.J. Rothrock, Jr., and A. Mishra. 2019. *Food Research International*. 122:47-55. Reprinted here with permission of the publisher.

Abstract

Foodborne pathogens such as *Listeria* spp. have the ability to survive and multiply in poultry farming environments, which provides a route of contamination for poultry processing environments and final poultry products. An understanding of the effect of meteorological variables on the prevalence of *Listeria* spp. in the farming environment is lacking. Soil and fecal samples were collected from 11 pastured poultry farms from 2014 to 2017. Random forest (RF) and gradient boosting machine (GBM) predictive models were generated to describe and predict *Listeria* spp. prevalence in fecal and soil samples based on meteorological factors at the farming location. In this study, we attempted to demonstrate the use of GBM models in a food safety context and compare their use to RF models. Both feces models performed very well, with area under the curve (AUC) values of 0.905 and 0.855 for the RF and GBM models, respectively. The soil GBM model outperformed the RF model with AUCs of 0.873 and 0.700, respectively. The developed models can be used to predict the prevalence of *Listeria* spp. in pastured poultry farm environments and should be of great use to poultry farmers, producers, and risk managers.

3.1 Introduction

Foodborne pathogens such as *Listeria* spp., *Salmonella* spp., and *Campylobacter* spp. are of great concern to the poultry industry due to their association with poultry meat products *(36, 48, 58)*. An understanding of factors that lead to the contamination of poultry meat and products with these pathogens needs to be obtained to ensure the safety of these products. Much is known about *Salmonella* and *Campylobacter* contamination of poultry, but there is little information on the occurrence of *Listeria* spp. in poultry farming environments and raw and ready-to-eat (RTE) poultry products at the consumer level in the United States *(53)*.

Listeria monocytogenes is a gram-positive foodborne pathogen that causes listeriosis in humans. It is abundant in the natural environment and relatively hardy under adverse conditions *(38)*. Additionally, when it is introduced to processing environments, it can establish a niche and remain persistent *(11)*. *Listeria monocytogenes* has proven to be a significant risk in the ready-to-eat (RTE) meat industry, with multiple listeriosis outbreaks occurring in the last 20 years *(14, 22, 23, 42, 61)*. In the United States, from 2010 to 2016, there were 18 multistate foodborne illness outbreaks attributed to *Listeria* resulting in 324 illnesses, 294 hospitalizations, and 65 deaths *(12)*. While *L. monocytogenes* is not as common as *Salmonella* and *Campylobacter*, its high mortality rate (30%) proves its significance to the food industry *(3)*. It is important to limit *Listeria* spp. on raw product to avoid the risk of bringing the organism into raw and RTE processing facilities.

Organic and pastured poultry farms have been growing in popularity over the past decade *(26)*. Pastured poultry farms are characterized by poultry that are reared on an open pasture in open-air, movable pens *(55)*. This differs from organic poultry living conditions, as organic production only requires that birds have access to the outdoors *(26)*. Due to the fact that pasture-raised birds are exposed to the outdoor environment, an increase in foodborne pathogen transmission routes exist when compared to conventional houses, such as the presence of other farm animals *(56)*, pests, and meteorological events *(52)*. Furthermore, foodborne pathogens such as *Listeria* spp. have shown the ability to survive for extended periods of time in the soil of farming environments *(17)*. Thus, it is important for pastured poultry producers to understand what factors are most important in controlling pathogen prevalence within the farming environment.

It has been shown that meteorological variables are significantly associated with foodborne pathogens at the farm level in produce farm environments *(28, 44, 57)*. Studies have shown that temperature *(28, 57)*, precipitation *(44, 57, 60)*, and wind speed *(44)* have been linked to the isolation of various foodborne pathogens on produce farms. Limited data are available in terms of the effect of meteorological variables on foodborne pathogen prevalence in poultry farm environments. Considering the unique qualities of pastured poultry farms, it is vital to characterize the importance of meteorological events on the prevalence of foodborne pathogens in these environments.

The purpose of the current study was to characterize the importance of meteorological variables in terms of the prevalence of *Listeria* spp. in soil and fecal samples in pastured poultry farms in the southeast US. Specifically, random forest (RF) and gradient boosting machine (GBM) models were used to predict prevalence of *Listeria* spp. in soil and fecal samples based on meteorological data. Due to limited use of these types of models in the food microbiology literature, the two methods were also compared for effectiveness. Additionally, effect of season and sample type were explored. The information from the current study should provide poultry farmers, producers, and risk managers information to help minimize foodborne pathogen contamination of their product.

3.2 Materials and methods

3.2.1 Description of data and study area

A longitudinal study was conducted on 43 flocks of broilers on 11 farms in the southeastern United States from March 2014 to November 2017. The major broiler management practices for each farm are shown in Table 3.1. All broiler flocks at each farm were pasture-

raised using temporary fences and moveable pens, considered all-natural or certified all-natural, and were never administered antibiotics.

3.2.2 Sample collection

On each sampling day, fecal and soil samples were collected from the pasture where the flock was currently residing at the time of sampling. Fecal samples were collected from fresh fecal droppings in the sampling site. If the farm was a multi-use farm, soil and fecal samples were also collected from sites where the other types of animals were residing. Soil samples were collected from the surface (0-7 cm). Sterile scoops were used to collect both types of samples, with gloves and scoops being changed after each sample. The pasture area was divided into 5 separate sections and 5 subsamples in each section were pooled into a single sample for each section (5 total fecal and 5 total soil samples were collected on each sampling day). Samples were pooled due to high variability and low expected pathogen population size among environmental samples *(4, 54)*. The total amount of sample collected for each field sample was at least 25 g. All samples were collected in the field and returned to the lab in a cooler packed in ice for immediate processing. For each flock, sampling occurred 3 times during grow-out: (i) within a few days of being placed on the pasture, (ii) halfway through their time on pasture, and (iii) on the day which the flock was processed. A total of 1,537 samples (770 fecal and 767 soil samples) were used in the current study.

3.2.3 *Listeria* spp. enrichment and isolation

To prepare both feces and soil samples for homogenization, 3 g from each subsample were combined in filter stomacher bags (Seward Laboratory Systems, Inc., Davie, FL) and diluted 1:3 in 10 mM phosphate buffered saline (PBS). Samples were then stomached for 60 s. Enrichment and isolation of *Listeria* from soil and fecal samples were performed using a

modified version of the USDA-FSIS MLG 8.10 method *(59)*. Three g of fresh soil or feces was added to 9 mL of buffered peptone water (BPW; Acumedia, Lansing, MI) in a filtered stomacher bag and shaken vigorously for 30 s. These bags were incubated overnight at 35°C to serve as a pre-enrichment. Following pre-enrichment, a primary enrichment was made in University of Vermont Modified *Listeria* Enrichment Broth (UVM; Remel, Lenexa, KS) and a secondary enrichment was made in Fraser Broth (Oxoid CM0895, Basingstoke, UK). Both the primary and secondary enrichments required overnight incubation at 30°C. One loopful (~10 µL) of the FB enrichment was streaked onto *Listeria* selective agar (LSA; Oxoid CM0856, Basingstoke, UK) for the isolation of *Listeria* spp. colonies. These plates were incubated overnight at 30°C. If *Listeria* colonies were isolated, three *Listeria*-like colonies were picked and used for further testing. From this, stock colonies were prepared by growing *Listeria* isolates in trypticase soy broth (TSB; Acumedia, Lansing, MI) at 37°C. Cell pellets were then centrifuged, washed in sterile water, and suspended in a brain heart infusion (BHI; Acumedia, Lansing, MI) broth containing 25% glycerol. Aliquots of 300 µL were distributed to microtubes and stored at -80°C until needed.

3.2.4 Speciation of *Listeria* spp.

Species of presumptive *Listeria* spp. colonies was determined by using multiplex PCR as described previously *(35)*. Briefly, two multiplex PCR reactions were conducted, and the sample species was determined. Samples were speciated as *L. ivanovii*, *L. grayi*, *L. innocua, L. welshimeri,* or *L. monocytogenes.* Samples that were classified as *L. monocytogenes* underwent further serotyping analysis, following the procedure from Doumith et al. *(16)*. Briefly, one *L. monocytogenes* isolate was thoroughly mixed with 25 µL pCR media containing: 1X EconoTaq PLUS 2X Master Mix (Lucigen Corporation, Middleton), 1 µM of each *lmo0737*, *ORF2819* and

ORF2110 reverse and forward primers, 1.5 µM of *lmo1118* reverse and forward primers, 0.2 µM of *prs* reverse and forward primers and qs water. PCR reactions were then run and following completion, the PCR products were mixed with 3 µL of BlueJuice™ loading buffer (Invitrogen, Carlsbad, CA) and separated on a 2% E-gel® with SYBR-safe™ (Invitrogen, Carlsbad, CA) along with 12 µL of E-Gel™ 1 kb Plus DNA Ladder (Invitrogen, Carlsbad, CA).

3.2.5 Meteorological data collection

For each sample collection date at each farm, relevant meteorological variables were obtained. Meteorological data were collected from Weather Underground (wunderground.com; Ann Arbor, MI) from the nearest weather station to each farm location. Meteorological variables obtained included: temperature (°C; minimum, maximum, and daily average), wind speed (m/s; maximum and daily average), gust speed (m/s; maximum), humidity (%; minimum, maximum, and daily average), and precipitation (mm). Weather data were collected for the sampling day, as well as 1 and 2 days before the sampling day. These data represented the short-term effect of the weather variables. Additionally, averages of all variables between the sampling day and from 1 to 7 days before the sampling day were calculated. These data represented the over-time effect of weather *(28)*. In total, there were 100 meteorological variables (Appendix A). For some dates and locations where precipitation data were missing from Weather Underground, data were obtained from the National Oceanic Atmospheric Administration (NOAA) weather database (https://www.ncdc.noaa.gov/cdo-web/).

3.2.6 Statistical analysis

For all statistical analyses, fecal and soil samples were analyzed separately unless otherwise stated. All statistical analyses were performed in R (version 3.4.0; R Foundation for Statistical Computing, Vienna, Austria).

Fisher's exact test was performed to test for significant differences in *Listeria* spp. and *L. monocytogenes* prevalence by sample type. Fisher's exact test was also used to compare *Listeria* spp. prevalence across season, with the Holm-Bonferroni correction used for each pairwise comparison *(27)*. Both types of samples were combined for this analysis. An alpha level of 0.05 was used, and any adjusted *p* value less than 0.05 was considered statistically significant.

Two different types of tree-based modeling were used to determine rules that classified the presence or absence of *Listeria* spp. in environmental samples. The first method was the random forest (RF) method. This method fits a large number of classification trees to a data set, then combines the predictions from all trees to present a final predictive model. The RF method utilizes two sources of randomization during construction of each tree that make it desirable over single-tree methods such as classification and regression tree *(7, 45, 46)*. First, data are randomly selected using the non-parametric bootstrap method *(19)*. The data collected this way are considered the in-bag samples, and the data that remain are the out-of-bag samples. The CART method is then used to build a non-pruned tree with the in-bag samples where a small number of explanatory variables are randomly chosen to determine the best split at each node. Each node split was based on the Gini index *(7)*. The out-of-bag samples are used as an estimate of performance of each tree and provide a way to rank the importance of the explanatory variables through the calculation of mean decrease accuracy *(45)*.

The second method of tree-based modeling used was the gradient boosting machine (GBM) method *(20, 21)*. The GBM is step-wise, additive type model that sequentially fits new tree-based models. Each fitted model at every step attempts to compensate for the shortcomings of the previous fitted models. The existing models are referred to as "weak learners" *(39)*. The GBM algorithm is unique in that it attempts to generate new base-learners that are maximally

correlated with the negative gradient of the loss function, based on the previously assembled trees *(39)*.

All model fitting and associated analyses were performed with the *caret* package in R *(32)*. Within the "train()" function of the *caret* package, method = "rf" was used to train RF models and method = "gbm" was used to train GBM models. Each data set was partitioned into a training and testing set using random sampling that preserved the overall class distribution of the data. Data sets contained one categorical independent variable, the presence or absence of *Listeria* spp., and 100 meteorological predictor variables (Appendix A). The training set contained 80% of the observations that were used for model selection and tuning, and the testing set contained the other 20% of the observations that were used for model performance evaluation. Within the training set, both model types were trained using 10-fold cross validation. Due to the imbalance in class distribution (positive and negative prevalence observations), the synthetic minority over-sampling (SMOTE) technique was used *(13)*. The SMOTE technique is a method that over-samples the minority class to attempt to achieve better classifier performance. This method was used within the cross-validation step. From the cross-validation results, the best combination of hyperparameters were chosen for each model, and the model was trained to the entire training set with the tuned hyperparameters. For RF models, one hyperparameter, mtry, was tuned, which represents the number of variables as candidates to randomly sample at each split. For the RF feces model, mtry = 51, and for the RF soil model, mtry = 100. For GBM models, four hyperparameters were tuned: n.trees (the total number of trees to fit), interaction.depth (maximum depth of each tree), shrinkage (learning rate), and n.minobsinnode (minimum number of observations in terminal nodes of tree) *(33)*. For the GBM feces model, n.trees = 135, interaction.depth = 10, shrinkage = 0.1, and n.binosmode = 10. For the GBM soil

model, n.trees = 125, interaction.depth = 10, shrinkage = 0.1, and n.binosmode = 10. The receiver operating characteristic (ROC) metric was used to tune the hyperparameters. Variable importance was calculated for each final model. For RF models, the variable importance measure was the mean decrease Gini as described by *(8, 9)*. For GBM models, variable importance is determined by a variable's average relative influence across all trees generated by the GBM algorithm *(20, 51)*. Variable importance was scaled on a scale of 0 to 100, where a higher number represents higher variable importance. For each model, partial dependency plots were generated using the *pdp* package for the two most important variables as determined by the above criteria *(24)*. Model code can be accessed on GitHub (https://github.com/cgolden1993/Dissertation).

The testing set was used to evaluate the performance of each final trained model. Receiver operating characteristic (ROC) curves, area under the curve (AUC), sensitivity, and specificity were used as measurements of predictive performance for each model *(6)*. The R package *pROC* was used to generate ROC curves, *Metrics* was used to analyze AUC, and *caret* was used to calculate sensitivity and specificity.

3.3 Results

A total of 1,537 environmental samples were collected from pastured poultry farms from March 2014 to November 2017. Across all of the sampled farms, there were 245 (15.9%) *Listeria* spp. positive environmental samples (Table 3.2). Of these 245 positive samples, there were 28 *L. monocytogenes* positive samples. Farm D accounted for the majority of the *L. monocytogenes* samples with 17 positive environmental samples. Each farm had at least 1 *Listeria* spp. positive sample, with percent positive samples ranging from 3.3-60.0%.

Table 3.3 presents the prevalence of *Listeria* spp. and *L. monocytogenes* for each sample type, feces and soil, across all farms. Overall, 15.7% of fecal samples and 16.2% of soil samples were positive for *Listeria* spp, and 1.7% of fecal samples and 2.0% of soil samples were positive for *L. monocytogenes*. There was no significant difference in prevalence among sample type for either *Listeria* spp. ($p = 0.834$) nor *L. monocytogenes* ($p = 0.708$). Species characterization of all *Listeria* spp. positive samples is shown in Appendix B.

The effect of season on *Listeria* spp. and *L. monocytogenes* prevalence is shown in Table 3.4. For both sample types, the prevalence of *Listeria* spp. positive samples was significantly higher (adjusted-$p < 0.001$) in the spring than in the summer and fall. Spring samples contained 40.7% and 38.7% positive *Listeria* spp. samples for fecal and soil samples, respectively. No other season had prevalence greater than 10.1%. Likely due to the low sample size of winter samples, there was no significant difference (adjusted-$p > 0.05$) between winter and spring samples for both sample types.

Predictive performance of all four models were tested on the held-out test set for each type of model. Table 3.5 displays the confusion matrices and AUC statistics for all four models. The RF model performed slightly better than the GBM model in terms of AUC, with AUCs of 0.905 and 0.855, respectively. In a food safety context, it is important for predictive models to limit the number of false negatives so that positive contamination events (or positive samples) are not missed. This makes model sensitivity very important. To this case, the feces RF model had a higher sensitivity (0.792) than the GBM model (0.708). These results are mirrored in the receiver operating characteristic (ROC) curves for each feces model (Fig. 3.1). For the soil models, the GBM model vastly outperformed the RF model in all statistics. The GBM model had AUC, sensitivity, and specificity statistics of 0.873, 0.615, and 0.873, respectively, compared to 0.700,

0.538, and 0.825 for the RF model, respectively. Again, the ROC curves (Fig. 3.2) reflect these results. Both models seem viable to handle new independent fecal data, but the GBM model is the only appropriate generated model to handle new soil data.

Random forest and GBM models were constructed for both fecal and soil samples. Both types of models incorporated all 100 predictor variables. Figure 3 depicts the variable importance plots for the RF and GBM feces models. Temperature and humidity were the most important variables in the feces RF model, accounting for the top 5 predictors in terms of relative importance (Fig. 3.3a). Average minimum temperature between the sampling day and four days prior was the most important variable with a relative importance of 100, followed by the average minimum temperature between the sampling day and three days prior with a relative importance score of 50.6. Partial dependency plots (PDP) were generated for these two variables to display the dependence between the outcome and the variable of interest, marginalizing the values over the rest of the predictors (Fig. 3.4a and 3.4b). For both variables, probability of *Listeria* spp. isolation was highest at temperatures from approximately 0-18°C, before dropping at temperatures greater than 18°C. The top five feces GBM model predictors in terms of relative importance contained temperature, humidity, and wind speed variables. Like the RF model, the GBM model ranked the average minimum temperature between the sampling day and four days prior as the most important variable, followed by the average humidity between the sampling day and three days prior (Fig. 3.3b). Those two variables had relative importance scores of 100 and 95.5, respectively. Figure 4c and Figure 4d depict the PDPs for the top two important feces GBM predictors. According to the feces GBM model, probability of *Listeria* spp. isolation was highest at average minimum temperatures 0-18°C (Fig. 3.4c). For the average humidity between the

sampling day and three days prior predictor, isolation probability was highest at lower humidity levels (15-50%) before decreasing and slightly increasing at 80% (Fig. 3.4d).

Figure 5 reports the variable importance plots for the soil RF and GBM models. Both models contained humidity, temperature, and wind speed predictors in the top five most important predictors. The RF model (Fig. 3.5a) ranked the average humidity between the sampling day and three days prior and the minimum temperature two days prior to the sampling day the top two most important models with relative important scores of 100 and 70.9, respectively. The RF model stated that the probability of *Listeria* spp. isolation was at its highest between average humidity levels of 15-55% (Fig. 3.6a). Also, according to the RF model, *Listeria* spp. isolation probability was highest at minimum temperatures between 10-18°C (Fig. 3.6b). For the GBM model, the average humidity between the sampling day and three days prior and the average minimum temperature between the sampling day and two days prior were the two most important predictors, with relative importance scores of 100 and 82.6, respectively (Fig. 3.5b). According to the GBM model, probability of *Listeria* spp. isolation was highest at average humidity levels of about 15-55% between the sampling day and three days prior (Fig. 3.6c). The GBM model also predicted that when the average minimum temperature between the sampling day and two days prior was between about 12-17°C, probability of *Listeria* spp. isolation was at its highest (Fig. 3.6d). Both models ranked the average humidity between the sampling day and three days prior as the most important variable and agreed that minimum temperature was also an important predictor.

3.4 Discussion

The current study proposed random forest (RF) and gradient boosting machine (GBM) models that can be used to predict the probability of *Listeria* spp. isolation in fecal and soil

samples in pastured poultry farming environments based off of meteorological data. The goals of the study were to identify meteorological variables that are associated with an increased probability of *Listeria* spp. isolation in the environment of pastured poultry farms. The use of RF and CART models in a food safety context has been widely characterized in the literature *(2, 28, 44, 57)*. Gradient boosting machine models have been shown to have use in food analysis, but no studies have been conducted on their use in food safety *(10, 25)*. To this end, the current study also looked to evaluate their use in the classification of foodborne pathogen prevalence and compare their performance to RF models.

Another thing to consider when utilizing training and test sets is class imbalance. Most machine learning algorithms are not inherently built to handle such class imbalance. In our case, fecal and soil samples only contained 15.7% and 16.2% of positive samples, respectively. Models that are trained on imbalanced data tend to favor prediction of the majority class, unless the model has substantial evidence to predict otherwise. This leads to very accurate predictions on the majority class, but inaccurate predictions on the minority class *(31)*. Traditional accuracy measures, like AUC of ROC curves, tend to underestimate this effect *(47)*. There are two approaches to address this issue. One is to develop a model that is insensitive to the class distribution of the training set *(50)*. Another method, like what was used in the current study, is to resample the training data, so that either the majority class is under represented, the minority class is over represented, or a combination of the two using a method like the SMOTE method *(13, 31)*. The SMOTE method has been shown to lead to more accurate classification than traditional over and under sampling techniques *(13)*. It is important to note that resampling only applies to the training set. To accurately predict model performance, test data should reflect the

class imbalance that is seen in the “real world”. In order to allow for direct model comparison, the same resampled data were used for each feces and soil model.

In terms of AUC, all generated models performed acceptably (AUC > 0.70), with the exception of the soil RF model (Table 3.5). Both feces models had AUCs greater than 0.85, with the RF model performing slightly better. For the soil models, the GBM model vastly outperformed the RF model, with AUC scores of 0.873 and 0.700, respectively. These results show that both RF and GBM models can both have success in predicting the prevalence of foodborne pathogens in poultry farming environments based off of meteorological data. The results also suggest that these types of models can have use in other food safety applications.

Although both RF and GBM are both tree-based models, the methods differ on the way they are constructed and internally evaluated *(8, 20, 34)*. Because of this, variable importance rankings can differ among different types of models trained on the same data. Interestingly, for both the soil and feces models, the RF and GBM models agreed on the most important variable for each type of environmental sample. For feces models, the average minimum temperature between the sampling day and four days prior was determined to be the most important variable (Fig. 3.3). This was by far the most important variable in the RF model, with no other variable above 55 relative importance. For the feces GBM model, the average humidity between the sampling day and three days prior was almost as important. Both soil models ranked the average humidity between the sampling day and three days prior as the most important variable (Fig. 3.5). Both the RF and GBM soil models had a minimum temperature variable as the second most important predictor. In all, average humidity, minimum temperature, and max wind speed were the most important variables in terms of prediction *Listeria* spp. prevalence in fecal and soil samples in pastured poultry farming environments. *Listeria* spp. are able to survive temperatures

as low as 1-2°C, whereas similar pathogens like *Salmonella* and *E. coli* are not as likely to thrive, so it is not surprising to see this variable as a key predictor of *Listeria* spp. in the environment *(18, 30)*. This contrasts the findings by Pang et al. *(44)*, where temperature was not found to be a significant predictor of *Listeria* spp. in the environment of mixed produce and dairy farms according to a RF model.

It is important to note that many of the predictors used during model development were highly correlated. As such, variable importance rankings and partial dependency plots could potentially have been impacted by such multicollinearity. Previous studies have shown that multicollinearity is handled well by both RF and GBM models *(15, 37)*, but the potential impact of multicollinearity on final model interpretation in the current study could be seen as a limitation.

The effect of wind speed on foodborne pathogen transmission has been well documented in the literature. Pang et al. *(44)* reported that increased average wind speed at two days before sampling was related to higher predicted probability of *Listeria* spp. in the mixed produce and dairy farms. Due to the open nature of pastured poultry farms, the transmission routes for foodborne pathogens in these environments are very similar to produce farms. Foodborne pathogens can be transmitted by contaminated dust, aerosols, and wind-driven fecal matter *(1, 43, 49)*.

Although data were only collected from pastured poultry type farms, the types of farms sampled from varied greatly (Table 3.1). Farms differed in scale of the farm, flock size, and whether or not the farm is used for other purposes (i.e. if the farm houses other types of animals). This large data set from different farms should be representative of the many different types of pastured poultry farms in the United States. Because of this, the models generated in the current

study should be applicable to diverse pastured poultry farms. It is not clear whether meteorological factors would be predictive of foodborne pathogen prevalence in the flocks and environment of traditional poultry farms, as research on this topic is limited. Studies have shown that weather events did not have a significant effect on *Campylobacter* prevalence in broiler flocks in traditional poultry farming environments *(5)*. This may be due to the fact that most conventional poultry farms are temperature and humidity controlled.

The current study presented RF and GBM models that are capable of predicting the prevalence of *Listeria* spp. in the environment of pastured poultry farms. Both models performed similarly for the feces models, but the soil GBM model outperformed the soil RF model. These results show that both types of machine learning algorithms can have use in a food safety context, if used correctly. Gradient boosting machine models take longer than RF models to construct, due to the increased number of hyperparameters to tune and the sequential nature in which the models are built. The two types of models have been compared in other fields, with varying results. Gradient boosting machine models have been shown that they have better predictive capability than RF models in genomic selection *(41)*. Random forest models outperformed GBM models in soil total nitrogen and carbon predictions *(40)*.

Studies have shown that the presence of *Listeria* spp. is often indicative of conditions that support and increase the likelihood of *L. monocytogenes* presence *(28)*. Furthermore, it is important to control the prevalence of foodborne pathogens, like *Listeria*, in the environment of farming environments because a contaminated farm environment could be a route of contamination for the poultry processing environment, poultry feed, and further processing or retail environments *(29)*. The generated models present meteorological factors that are predicted to increase the likelihood of *Listeria* spp. contamination in the environment of pastured poultry

farms. The findings can assist farmers and risk managers in important variables to account for to reduce the risk of *Listeria* contamination in the environment of their farms.

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3.6 References

1. Baertsch, C., T. Paez-Rubio, E. Viau, and J. Peccia. 2007. Source tracking aerosols released from land-applied class B biosolids during high-wind events. *Appl. Environ. Microbiol.* 73:4522-4531.

2. Barco, L., M. Mancin, M. Ruffa, C. Saccardin, C. Minorello, P. Zavagnin, A. A. Lettini, J. E. Olsen, and A. Ricci. 2012. Application of the random forest method to analyse epidemiological and phenotypic characteristics of *Salmonella* and *Salmonella* Typhimurium strains. *Zoonoses Public Health*. 59:505-512.

3. Berche, P. 2005. Pathophysiology and epidemiology of listeriosis. *B. Acad. Nat. Med. Paris*. 189:507-16.

4. Bergholz, P. W., J. D. Noar, and D. H. Buckley. 2011. Environmental patterns are imposed on the population structure of *Escherichia coli* after fecal deposition. *Appl. Environ. Microbiol.* 77:211-219.

5. Berrang, M. E., R. J. Meinersmann, S. R. Ladely, and N. A. Cox. 2017. *Campylobacter* detection in broiler ceca at processing: A three-year, 211-flock survey. *J. Appl. Poult. Res.* 26:154-158.

6. Bradley, A. P. 1997. The use of the area under the ROC curve in the evaluation of machine learning algorithms. *Pattern Recogn.* 30:1145-1159.

7. Breiman, L. 1984. Classification and regression trees. Routledge, New York.

8. Breiman, L. 2001. Random forests. *Mach. Learn.* 45:5-32.

9. Breiman, L. 2002. Manual on setting up, using, and understanding random forests v3.1. Available at: https://www.stat.berkeley.edu/~breiman/Using_random_forests_V3.1.pdf. Accessed 16 October 2018.

10. Brillante, L., F. Gaiotti, L. Lovat, S. Vincenzi, S. Giacosa, F. Torchio, S. R. Segade, L. Rolle, and D. Tomasi. 2015. Investigating the use of gradient boosting machine, random forest and their ensemble to predict skin flavonoid content from berry physical–mechanical characteristics in wine grapes. *Comput. Electron. Agric.* 117:186-193.

11. Carpentier, B., and O. Cerf. 2011. Persistence of *Listeria monocytogenes* in food industry equipment and premises. *Int. J. Food Microbiol.* 145:1-8.

12. Centers for Disease Control and Prevention. 2018. Outbreak of *Listeria* infections linked to deli ham. Available at: https://www.cdc.gov/listeria/outbreaks/countryham-10-18/index.html. Accessed 11 November 2018.

13. Chawla, N. V., K. W. Bowyer, L. O. Hall, and W. P. Kegelmeyer. 2002. SMOTE: Synthetic minority over-sampling technique. *J. Artif. Intell. Res.* 16:321-357.

14. Dev Kumar, G., R. C. Williams, S. S. Sumner, and J. D. Eifert. 2016. Effect of ozone and ultraviolet light on *Listeria monocytogenes* populations in fresh and spent chill brines. *Food Control*. 59:172-177.

15. Ding, C., D. Wang, X. Ma, and H. Li. 2016. Predicting short-term subway ridership and prioritizing its influential factors using gradient boosting decision trees. *Sci. Pract. Pol.* 8:1100.

16. Doumith, M., C. Buchrieser, P. Glaser, C. Jacquet, and P. Martin. 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.* 42:3819-3822.

17. Dowe, M. J., E. D. Jackson, J. G. Mori, and C. R. Bell. 1997. *Listeria monocytogenes* survival in soil and incidence in agricultural soils. *J. Food Prot.* 60:1201-1207.

18. Doyle, M. P. 1989. Foodborne bacterial pathogens. Marcel Dekker, Inc., New York.

19. Efron, B., and R. J. Tibshirani. 1994. An introduction to the bootstrap. Chapman and Hall/CRC Press, Boca Raton, FL.

20. Friedman, J. H. 2001. Greedy function approximation: A gradient boosting machine. *Ann. Stat.* 29:1189-1232.

21. Friedman, J. H. 2002. Stochastic gradient boosting. *Comput. Stat. Data Anal.* 38:367-378.

22. Frye, D. M., R. Zweig, J. Sturgeon, M. Tormey, M. LeCavalier, I. Lee, L. Lawani, and L. Mascola. 2002. An outbreak of febrile gastroenteritis associated with delicatessen meat contaminated with *Listeria monocytogenes. Clin. Infect. Dis.* 35:943-949.

23. Gottlieb, S. L., E. C. Newbern, P. M. Griffin, L. M. Graves, R. M. Hoekstra, N. L. Baker, S. B. Hunter, K. G. Holt, F. Ramsey, and M. Head. 2006. Multistate outbreak of listeriosis linked to turkey deli meat and subsequent changes in US regulatory policy. *Clin. Infect. Dis.* 42:29-36.

24. Greenwell, B. M. 2017. pdp: An R package for constructing partial dependence plots. *R J.* 9:421-436.

25. Hazir, M. H. M., A. R. M. Shariff, and M. D. Amiruddin. 2012. Determination of oil palm fresh fruit bunch ripeness—Based on flavonoids and anthocyanin content. *Ind. Crops Prod.* 36:466-475.

26. Hilimire, K. 2012. The grass is greener: Farmers' experiences with pastured poultry. *Renew. Agric. Food Syst.* 27:173-179.

27. Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* 6:65-70.

28. Ivanek, R., Y. T. Gröhn, M. T. Wells, A. J. Lembo, B. D. Sauders, and M. Wiedmann. 2009. Modeling of spatially referenced environmental and meteorological factors influencing the probability of *Listeria* species isolation from natural environments. *Appl. Environ. Microbiol.* 75:5893-5909.

29. Ivanek, R., Y. T. Gröhn, and M. Wiedmann. 2006. *Listeria monocytogenes* in multiple habitats and host populations: Review of available data for mathematical modeling. *Foodborne Pathog. Dis.* 3:319-336.

30. Junttila, J. R., S. I. Niemelä, and J. Hirn. 1988. Minimum growth temperatures of *Listeria monocytogenes* and non-haemolytic *Listeria. J. Appl. Bacteriol.* 65:321-327.

31. Kubat, M., R. C. Holte, and S. Matwin. 1998. Machine learning for the detection of oil spills in satellite radar images. *Mach. Learn.* 30:195-215.

32. Kuhn, M. 2008. Building predictive models in R using the caret package. *J. Stat. Softw.* 28:1-26.

33. Kuhn, M. 2018. The caret package. Available at: http://topepo.github.io/caret/index.html. Accessed 16 October 2018.

34. Liaw, A., and M. Wiener. 2002. Classification and regression by randomForest. *R News*. 2:18-22.

35. Locatelli, A., M. A. Lewis, and M. J. Rothrock Jr. 2017. The distribution of *Listeria* in pasture-raised broiler farm soils is potentially related to University of Vermont medium enrichment bias toward *Listeria innocua* over *Listeria monocytogenes*. *Front. Vet. Sci.* 4:227.

36. Loura, C. A. C., R. C. C. Almeida, and P. F. Almeida. 2005. The incidence and level of *Listeria* spp. and *Listeria monocytogenes* contamination in processed poultry at a poultry processing plant. *J. Food Saf.* 25:19-29.

37. Matsuki, K., V. Kuperman, and J. A. Van Dyke. 2016. The Random Forests statistical technique: An examination of its value for the study of reading. *Sci. Stud. Read.* 20:20-33.

38. Montville, T. J., K. R. Matthews, and K. E. Kniel. 2012. Food microbiology: An introduction. ASM Press, Washington D.C.

39. Natekin, A., and A. Knoll. 2013. Gradient boosting machines, a tutorial. *Front. Neurorobot.* 7:21.

40. Nawar, S., and A. Mouazen. 2017. Comparison between random forests, artificial neural networks and gradient boosted machines methods of on-line vis-NIR spectroscopy measurements of soil total nitrogen and total carbon. *Sensors*. 17:2428.

41. Ogutu, J. O., H. Piepho, and T. Schulz-Streeck. 2011. A comparison of random forests, boosting and support vector machines for genomic selection. *BMC Proc.* 5:1-5.

42. Olsen, S. J., M. Patrick, S. B. Hunter, V. Reddy, L. Kornstein, W. R. MacKenzie, K. Lane, S. Bidol, G. A. Stoltman, and D. M. Frye. 2005. Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. *Clin. Infect. Dis.* 40:962-967.

43. Oni, R. A., M. Sharma, and R. L. Buchanan. 2015. Survival of *Salmonella enterica* in dried turkey manure and persistence on spinach leaves. *J. Food Prot.* 78:1791-1799.

44. Pang, H., R. McEgan, A. Mishra, S. A. Micallef, and A. K. Pradhan. 2017. Identifying and modeling meteorological risk factors associated with pre-harvest contamination of *Listeria* species in a mixed produce and dairy farm. *Food Res. Int.* 102:355-363.

45. Philibert, A., M.-L. Desprez-Loustau, B. Fabre, P. Frey, F. Halkett, C. Husson, B. Lung-Escarmant, B. Marçais, C. Robin, C. Vacher, and D. Makowski. 2011. Predicting invasion success of forest pathogenic fungi from species traits. *J. Appl. Ecol.* 48:1381-1390.

46. Prasad, A. M., L. R. Iverson, and A. Liaw. 2006. Newer classification and regression tree techniques: Bagging and random forests for ecological prediction. *Ecosystems*. 9:181-199.

47. Provost, F., and T. Fawcett. 2001. Robust classification for imprecise environments. *Mach. Learn.* 42:203-231.

48. Rajan, K., Z. Shi, and S. C. Ricke. 2017. Current aspects of *Salmonella* contamination in the US poultry production chain and the potential application of risk strategies in understanding emerging hazards. *Crit. Rev. Microbiol.* 43:370-392.

49. Ravva, S. V., C. Z. Sarreal, and R. E. Mandrell. 2011. Bacterial communities in aerosols and manure samples from two different dairies in central and Sonoma valleys of California. *PLOS ONE*. 6:e17281.

50. Riddle, P., R. Segal, and O. Etzioni. 1994. Representation design and brute-force induction in a Boeing manufacturing domain. *Appl. Artif. Intell.* 8:125-147.

51. Ridgeway, G. 2007. Generalized boosted models: A guide to the gbm package. Available at: http://www.saedsayad.com/docs/gbm2.pdf. Accessed 16 October 2018.

52. Rodenburg, T. B., M. C. Van Der Hulst-Van Arkel, and R. P. Kwakkel. 2004. *Campylobacter* and *Salmonella* infections on organic broiler farms. *NJAS Wagening. J. Life Sci.* 52:101-108.

53. Rothrock Jr, M. J., M. L. Davis, A. Locatelli, A. Bodie, T. G. McIntosh, J. R. Donaldson, and S. C. Ricke. 2017. *Listeria* occurrence in poultry flocks: Detection and potential implications. *Front. Vet. Sci.* 4:125.

54. Semenov, A. V., E. Franz, L. van Overbeek, A. J. Termorshuizen, and A. H. C. van Bruggen. 2008. Estimating the stability of *Escherichia coli* O157:H7 survival in manure-amended soils with different management histories. *Environ. Microbiol.* 10:1450-1459.

55. Siemon, C. E., P. B. Bahnson, and W. A. Gebreyes. 2007. Comparative investigation of prevalence and antimicrobial resistance of *Salmonella* between pasture and conventionally reared poultry. *Avian Dis.* 51:112-117.

56. Skovgaard, N., and C. Morgen. 1988. Detection of *Listeria* spp. in faeces from animals, in feeds, and in raw foods of animal origin. *Int. J. Food Microbiol.* 6:229-242.

57. Strawn, L. K., E. D. Fortes, E. A. Bihn, K. K. Nightingale, Y. T. Gröhn, R. W. Worobo, M. Wiedmann, and P. W. Bergholz. 2013. Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. *Appl. Environ. Microbiol.* 79:588-600.

58. Umaraw, P., A. Prajapati, A. K. Verma, V. Pathak, and V. Singh. 2017. Control of *Campylobacter* in poultry industry from farm to poultry processing unit: A review. *Crit. Rev. Food Sci. Nutr.* 57:659-665.

59. United States Department of Agriculture. 2017. Isolation and identification of *Listeria monocytogenes* from red meat, poultry, ready-to-eat siluriformes (fish) and egg products, and environmental samples. Available at: https://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES. Accessed 19 November 2018.

60. Weller, D., M. Wiedmann, and L. K. Strawn. 2015. Spatial and temporal factors associated with an increased prevalence of *Listeria monocytogenes* in spinach fields in New York State. *Appl. Environ. Microbiol.* 81:6059-6069.

61. Zhu, M., M. Du, J. Cordray, and D. U. Ahn. 2005. Control of *Listeria monocytogenes* contamination in ready-to-eat meat products. *Compr. Rev. Food Sci. Food Saf.* 4:34-42.

Table 3.1 Comparison of the 11 all-natural, antibiotic-free, pastured broiler farms included in this study

Farm	Breed	No. of flocks	Flock size	Multi-use farm?[a]	Animal types[b]
A	Freedom Ranger	10	>500	Yes	Layers, Swine, Beef cattle, Sheep
B	Freedom Ranger, Cornish Cross	5	50-75	Yes	Layers, Swine, Horses, Goats
C	Freedom Ranger	1	50-75	No	n/a
D	Freedom Ranger	1	50-75	No	n/a
E	Freedom Ranger, Cornish Cross	5	50-75	Yes	Layers, Swine, Beef cattle, Sheep
F	Freedom Ranger	2	>500	Yes	Layers
G	Freedom Ranger, Cornish Cross	9	100-500	Yes	Layers, Swine, Goats
H	Freedom Ranger, Cornish Cross	2	50-75	Yes	Layers
I	Freedom Ranger	4	100-500	Yes	Layers, Beef cattle, Goats
J	Freedom Ranger	2	>500	Yes	Layers, Swine, Beef cattle, Sheep
K	Cornish Cross	2	50-75	Yes	Layers, Swine

[a] This column represents whether the poultry farm is used to raise other animals than broilers.
[b] If the farm is used to raise other animals, this column represents the other types of animals present on the farm. If a farm was not a multi-use farm, it was given the term not applicable (n/a).

Table 3.2 Number of samples and prevalence of positive *Listeria* spp. and *Listeria monocytogenes* environmental samples for each farm

Farm	No. of samples	No. (%) of positive samples	
		Listeria spp.	*L. monocytogenes*
A	331	55 (16.6)	2 (0.6)
B	180	50 (27.8)	0 (0)
C	30	2 (6.7)	0 (0)
D	30	18 (60.0)	17 (56.7)
E	213	19 (8.9)	0 (0)
F	80	10 (12.5)	0 (0)
G	263	20 (7.6)	5 (1.9)
H	80	16 (20.0)	2 (2.5)
I	180	20 (11.1)	1 (0.6)
J	120	34 (28.3)	1 (0.8)
K	30	1 (3.3)	0 (0)
Total	1537	245 (15.9)	28 (1.8)

Table 3.3 Effect of sample type on the prevalence of *Listeria* spp. and *Listeria monocytogenes* in environmental samples of pastured poultry farms

Sample type	No. of samples	No. (%) of positive samples[a]	
		Listeria spp.	*L. monocytogenes*
Feces	770	121 (15.7) A	13 (1.7) A
Soil	767	124 (16.2) A	15 (2.0) A
Total	1537	245 (15.9)	28 (1.8)

[a] Different letters within a column represent statistically significant different values (p values < 0.05 according to Fisher's exact test)

Table 3.4 Effect of season on the prevalence of *Listeria* spp. and *Listeria monocytogenes* in environmental samples of pastured poultry farms

Sample type	Season	No. of samples	No. (%) of positive samples	
			***Listeria* spp.**[a]	***L. monocytogenes***
Feces	Spring	182	74 (40.7) A	13 (7.1)
	Summer	428	35 (8.2) B	0 (0)
	Fall	150	11 (7.3) B	0 (0)
	Winter	10	1 (10.0) AB	0 (0)
Soil	Spring	181	70 (38.7) A	9 (5.0)
	Summer	426	43 (10.1) B	6 (1.4)
	Fall	150	11 (7.3) B	0 (0)
	Winter	10	0 (0) AB	0 (0)
Total		1537	245 (15.9)	28 (1.8)

[a] Different letters within a column and sample type represent statistically significant different values (Holm-Bonferroni corrected p values < 0.05 according to Fisher's exact test)

Table 3.5 Predictive performance of all four models

Predictions		Actual: Positive	Actual: Negative	Sensitivity	Specificity	AUC[a]
Feces RF	Positive	19	17	0.792	0.868	0.905
	Negative	5	112			
Feces GBM	Positive	17	10	0.708	0.923	0.855
	Negative	7	119			
Soil RF	Positive	14	22	0.538	0.825	0.700
	Negative	12	104			
Soil GBM	Positive	16	16	0.615	0.873	0.873
	Negative	10	110			

[a] AUC, area under the receiver operating characteristic (ROC) curve

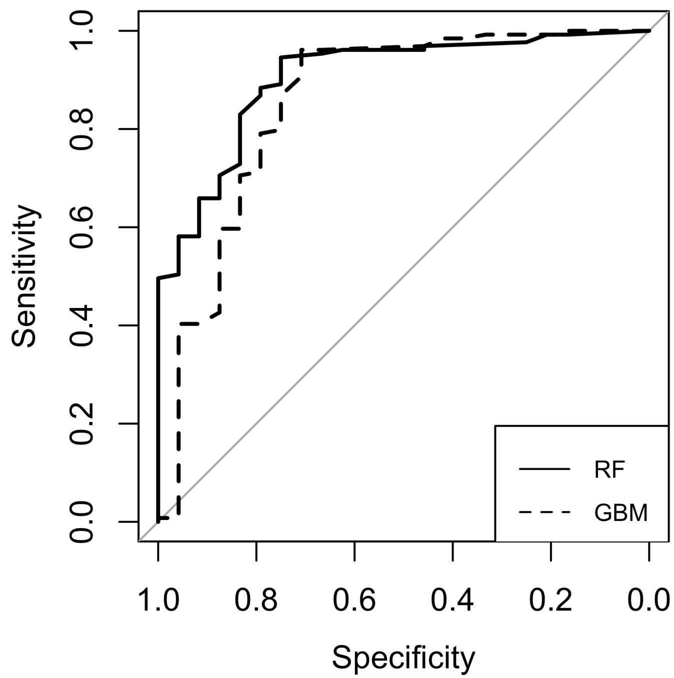

Figure 3.1 Receiver operating characteristic (ROC) curves for the feces random forest (solid line) and gradient boosting machine (dashed line) models. The diagonal line represents the line of no-discrimination, which represents a completely random guess.

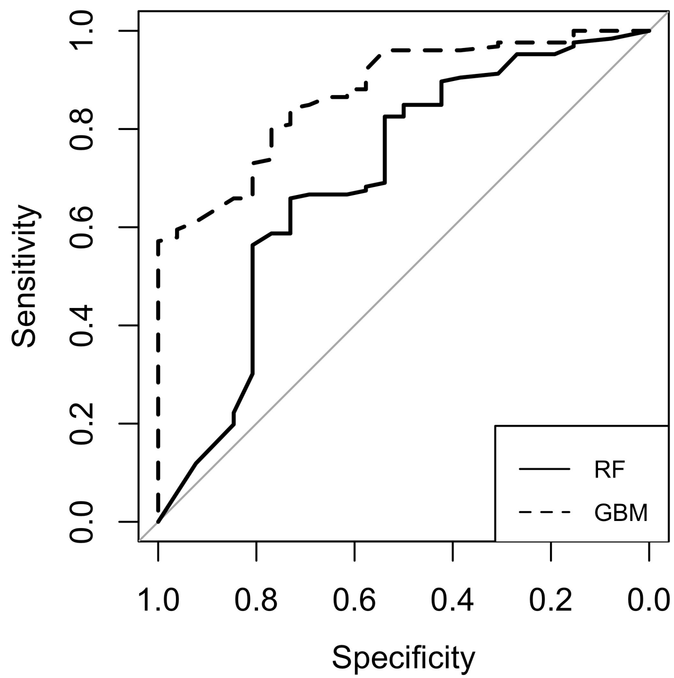

Figure 3.2 Receiver operating characteristic (ROC) curves for the soil random forest (solid line) and gradient boosting machine (dashed line) models. The diagonal line represents the line of no-discrimination, which represents a completely random guess.

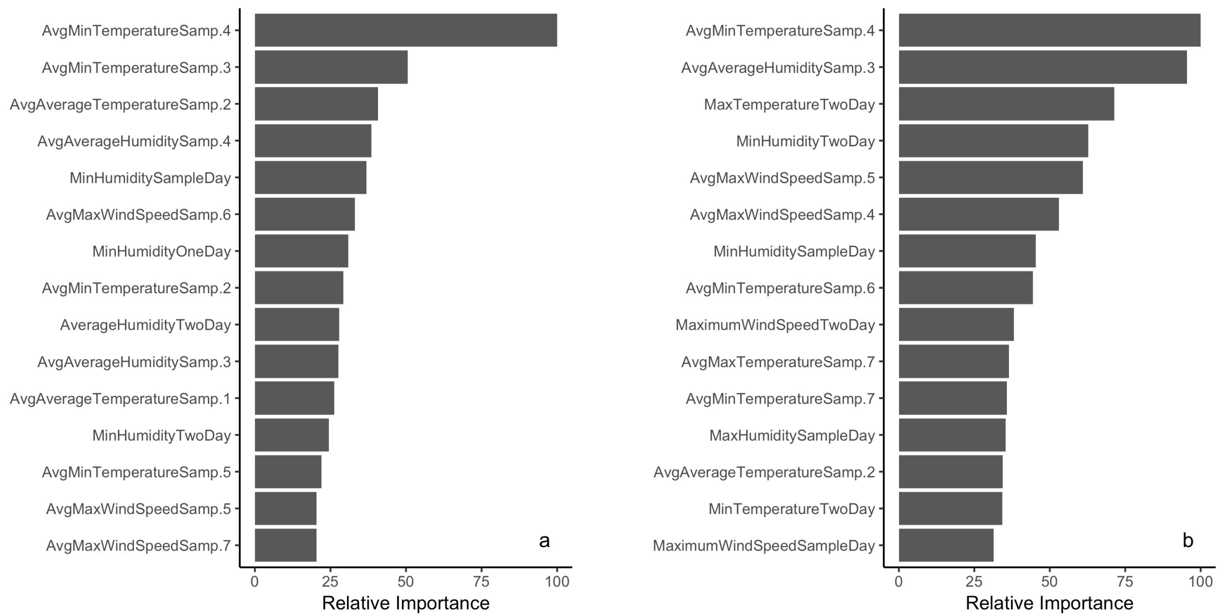

Figure 3.3 Variable importance plots for the feces (a) random forest model and (b) gradient boosting machine models. Variables were assigned a relative importance value on a scale of 0 to 100, where a higher importance score means higher relative importance. Variable names with a decimal are the average of that variable from the sampling day to X days prior, where X is the number of days before the sampling day.

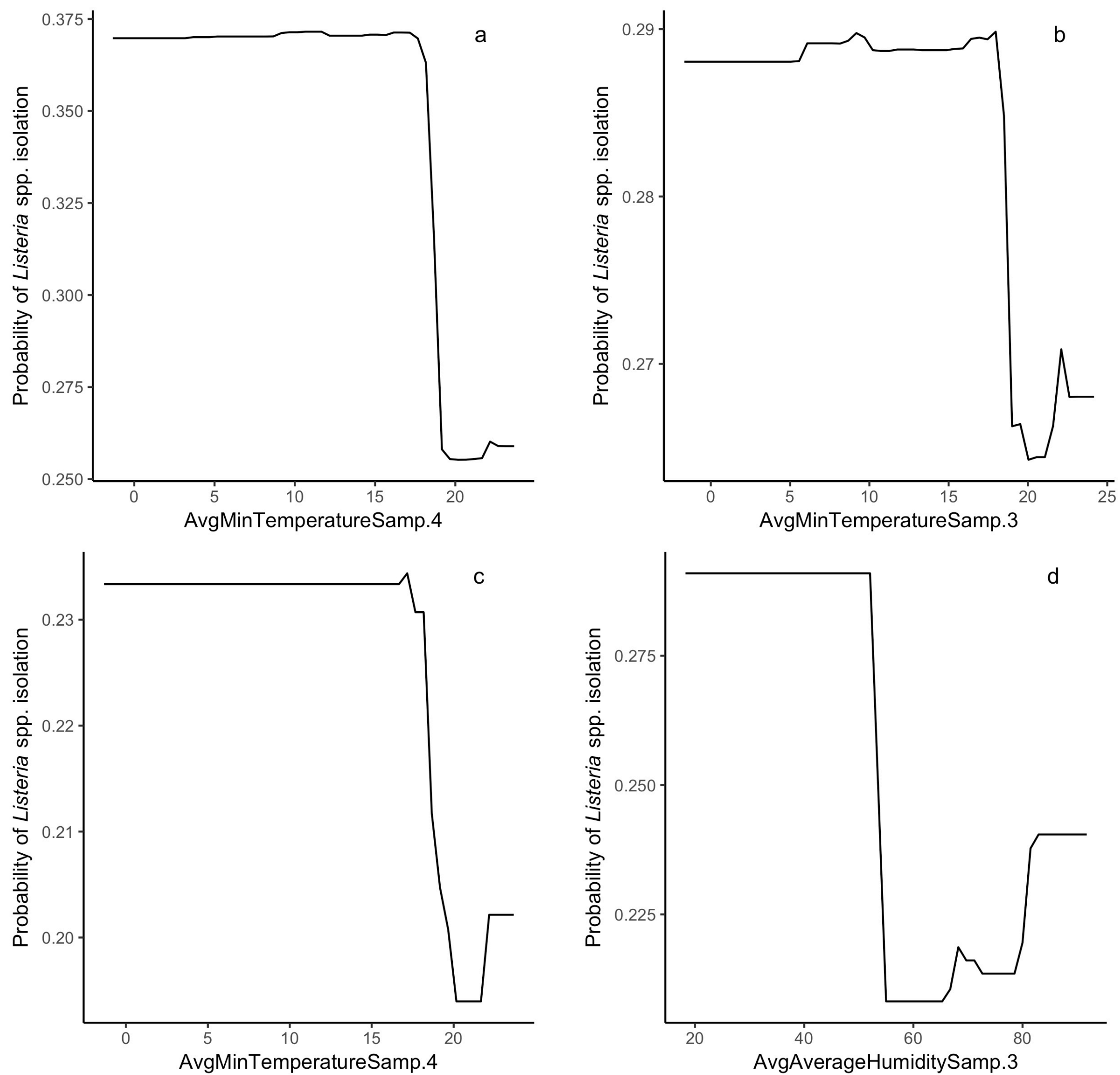

Figure 3.4 Partial dependency plots for the two most important variables for the feces random forest (a & b) and gradient boosting machine models (c & d).

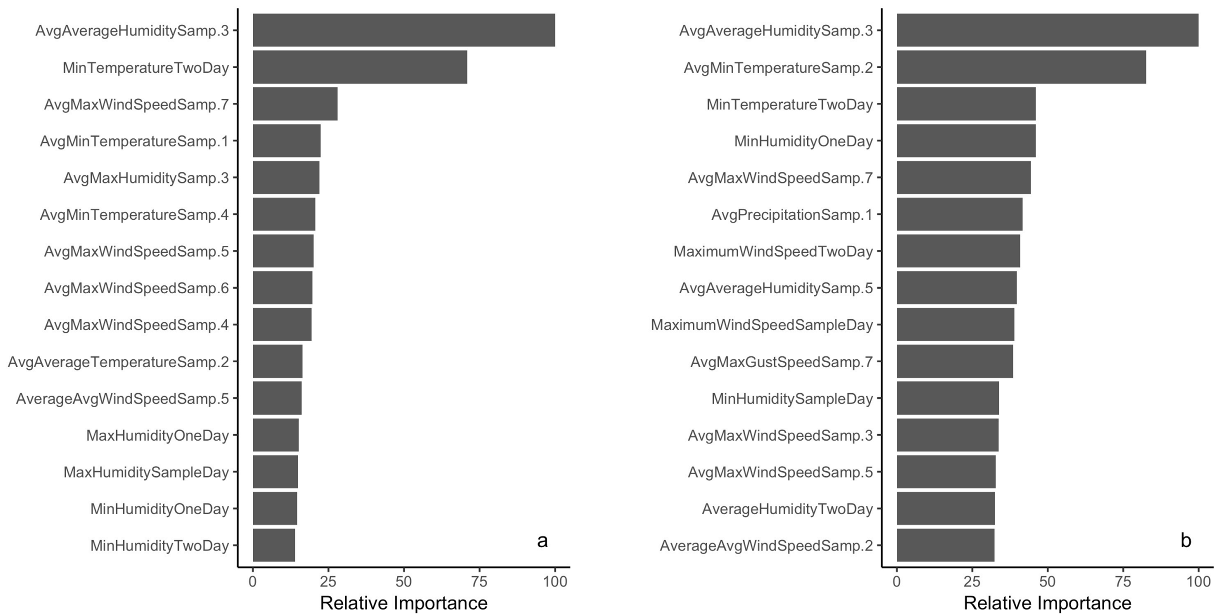

Figure 3.5 Variable importance plots for the soil (a) random forest model and (b) gradient boosting machine models. Variables were assigned a relative importance value on a scale of 0 to 100, where a higher importance score means higher relative importance. Variable names with a decimal are the average of that variable from the sampling day to X days prior, where X is the number of days before the sampling day.

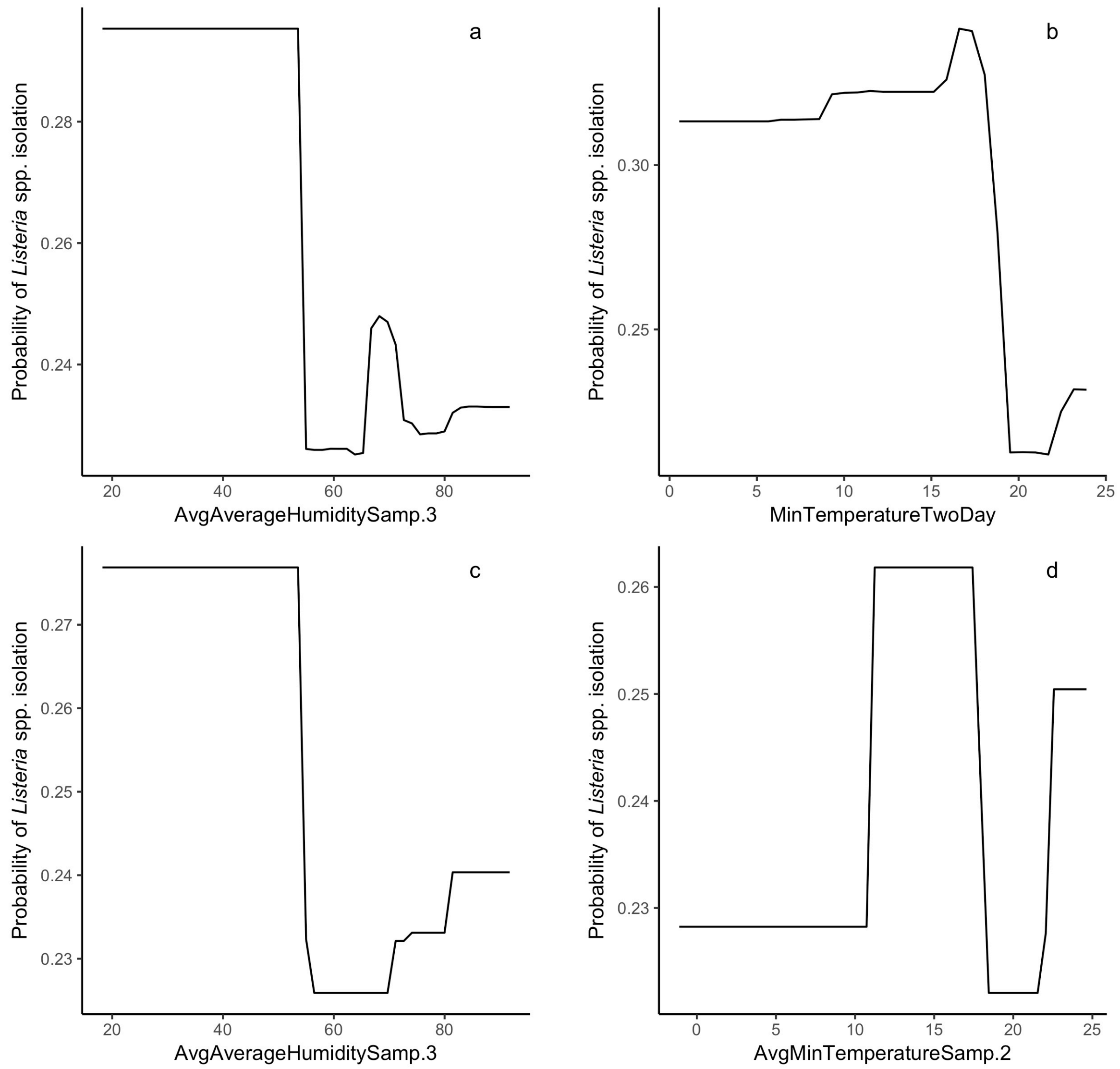

Figure 3.6 Partial dependency plots for the two most important variables for the soil random forest (a & b) and gradient boosting machine models (c & d).

CHAPTER 4

USING FARM PRACTICE VARIABLES AS PREDICTORS OF *LISTERIA* SPP. PREVALENCE IN PASTURED POULTRY FARMS [1]

[1] Golden, C.E., M.J. Rothrock, Jr., and A. Mishra. 2019. *Frontiers in Sustainable Food Systems*. 122:47-55. Reprinted here with permission of the publisher.

Abstract

Predictive models offer food scientists, farmers, and processors tools to help identify variables that lead to an increase in the food safety risk of a product. Foodborne pathogens, such as *Listeria* spp., pose a major problem for the pastured poultry industry. Currently, there is a lack of understanding of what farm practices lead to higher prevalence of *Listeria* spp. This study constructed random forest (RF) models to predict the prevalence of *Listeria* spp. in pastured poultry farming environments and the final broiler product based on major farm practices and variables. Fecal, soil, and whole carcass rinse samples were collected from 11 farms in the southeastern United States and evaluated for *Listeria* spp. presence. The preharvest sample RF model identified the time of year and age of the broiler flock at time of sampling as factors of increased probability of *Listeria* spp. presence in fecal and soil samples. The final product RF model identified brood feed and the presence of chlorine in processing rinse water as the two most important variables associated with an increased likelihood of *Listeria* spp. presence. Both the preharvest and postharvest RF models performed well on a held-out test set, with area under the receiver operating characteristic curve values of 0.876 and 0.887, respectively. The presented models showed the usefulness of RF models in a food safety context. Both RF models will help pastured poultry farmers and processors guide control strategies to manage *Listeria* contamination in pastured poultry farms and products.

4.1 Introduction

Listeria spp. are gram-positive, ubiquitous organisms that have been found in a variety of environments, including agricultural and farming, food processing, and retail environments *(1, 34, 46)*. *Listeria* spp. are hardy organisms that have shown the ability to establish a niche once introduced to an environment, allowing for persistence within that environment *(9)*. *Listeria*

monocytogenes is a foodborne pathogen that belongs to the *Listeria* genus that causes listeriosis in humans. The pathogen has been shown to be a significant foodborne risk in the meat and poultry industry, especially with ready-to-eat (RTE) products *(13, 21, 22, 39, 56)*. From 2010-2016, there were 18 multistate foodborne illness outbreaks attributed to *L. monocytogenes*, resulting in 324 illnesses and 65 deaths *(10)*.

Although much is known about the impact and prevalence of *Salmonella* spp. and *Campylobacter* spp. throughout the poultry supply chain, relatively little is known about the prevalence of *Listeria* spp. *(44)*. Studies have shown that the presence of *Listeria* spp. in the environment often indicates an environment that supports and increases the likelihood of *L. monocytogenes* presence and survival *(28)*. Additionally, studies have shown that the occurrence of *L. monocytogenes* in food products is mainly due to cross contamination from processing and retail environments, emphasizing the importance of eliminating transfer of the organism into a processing facility from the outside environment *(20, 32)*. *Listeria* can colonize the intestines of poultry and spread into bedding and environment through poultry feces *(14, 37)*. This is a potential entry pathway into a poultry processing plant, if not controlled for. Therefore, it is important for poultry producers and processors to understand what factors are most important in controlling *Listeria* spp. prevalence in the environment and postharvest product of pastured poultry farms.

In recent years, pastured poultry and other alternative poultry products have increased in popularity in the United States *(26)*. Pastured poultry farms are characterized by poultry flocks that are reared in open-air, movable pens *(48)*. Pens are often rotated to fresh pasture to encourage forage intake *(45)*. Flock sizes vary, but often contain less than 3,000 birds *(19)*. Although broilers reared on these types of farms can cost up to 200% more than traditionally

raised broilers, US consumers have shown a willingness to pay more for organic chicken meat *(38, 54)*. Compared to conventional broiler farms, research on the food safety of pastured poultry farms is limited. An understanding of the impact of different types of pastured poultry farm practices on *Listeria* spp. prevalence in the environment and final broiler product is important to poultry farmers and producers.

In the current study, we evaluated preharvest and processed broiler samples collected from pastured poultry farms from 2014-2017 and constructed random forest (RF) models to predict *Listeria* spp. prevalence based on various farm practice variables, including: feed type, egg source, and broiler breed. The presented models and information will be useful for poultry farmers, processors, and risk managers to minimize the risk of *Listeria* spp. contamination.

4.2 Materials and Methods

4.2.1 Farm sampling design

A longitudinal study was conducted on 43 flocks of broilers across 11 pastured poultry farms in the southeastern United States from March 2014 to November 2017. All 11 farms reared their broiler flocks in movable pens with temporary fences. A brief description of the size and scale of each farm is contained in Table 4.1. Data were collected for 40 major farm practice variables (Table 4.2) over a flock's lifecycle and all samples were evaluated for the presence of *Listeria* spp.

4.2.2 Sample collection

The following samples were collected along the farm-to-fork continuum for each flock to analyze for the presence of *Listeria* spp.: (i) feces, (ii) pasture soil, and (iii) whole carcass rinse (WCR) directly after processing from each farm. If a farm was multi-use and contained other

types of animals, environmental samples were collected from the area of residence of the other animals as well (Table 4.1).

Preharvest samples (feces and soil) were taken 3 times throughout a flock's lifecycle: (i) within a few days of being placed on the pasture, (ii) halfway through their time on the pasture, and (iii) on the day in which the flock was processed. Processing samples were only taken when the flock had reached the processing point. In all, there were 1,867 samples from 43 flocks of birds.

On each sampling day, fecal and soil samples were collected from the area in which the flock was residing. Fecal samples were collected from fresh fecal droppings at the sampling site. Soil samples were collected from the surface of the pasture (0-7 cm). Each sample consisted of at least 25 g. Sterile scoops and gloved hands were used to collect each type of environmental sample, with all equipment being changed after each collected sample. During sampling, the pasture area was divided into 5 areas. In each area 5 subsamples were taken and pooled into one sample to account for low expected pathogen population size *(3, 47)*.

To assess the prevalence of *Listeria* spp. on the final broiler product, 25 carcasses were sampled after processing, packaging, and cold storage of the carcasses according to the practices followed by each farm (Table 4.3). Each carcass was placed in an individual sterile sample bag. Carcasses were rinsed with 100 mL of 10 mM phosphate-buffered saline (PBS) and the bags were vigorously shaken. Whole carcass rinses from 5 carcasses were combined into 1 pooled sample, creating 5 pooled samples (n = 25) in total. Carcasses were then returned to the processor to be packed, stored, and distributed in the appropriate fashion for that farm.

All fecal, soil, and WCR samples were transferred to a microbiological lab on ice and processed within 2 h of collection. Once in the lab, no further preparation was performed with the WCR samples.

All work related to flocks on farm and broiler processing were performed by the farmers or the processing facility workers, not the researchers or the technicians. Preharvest samples were not collected directly from live birds and all postharvest samples were taken post-mortem, therefore, no animal care and use review process was required for the current study.

4.2.3 *Listeria* spp. enrichment and isolation

Listeria spp. enrichment and isolation followed a modified version of the USDA-FSIS MLG 8.10 method *(53)*. To prepare the fecal and soil samples for pre-enrichment, 3 g (feces or soil) was added to a filtered stomacher bag (Seward Laboratory Systems, Inc., Davie, FL) and diluted 1:3 buffered peptone water (BPW; Acumedia, Lansing, MI). Samples were then stomached for 60 s. All sample bags were then incubated at 35°C overnight. This step acted as a pre-enrichment. Following pre-enrichment, two subsequent primary enrichments were carried out first in University of Vermont Modified Listeria Enrichment Broth (UVM; Remel, Lenexa, KS) incubated at 30°C for 24 h and then into Fraser Broth (FB; Oxoid, Basingstoke, UK) incubated at 30°C for 24. For both, tubes were incubated at 30°C for 24 h. Following primary enrichment, one loopful (~10 µL) of the FB was streaked onto *Listeria* selective agar (LSA; Oxoid, Basingstoke, UK), and plates were incubated at 30°C overnight. If present, 3 presumptive *Listeria* colonies were picked from each plate and kept for further analysis.

4.2.4 *Listeria* characterization by PCR

Speciation of *Listeria* was carried out using the procedures described by Huang et al. *(27)* and Locatelli et al. *(33)*. Briefly, two multiplex PCR reactions were conducted, and samples were

types as *L. innocua, L. welshimeri, L. monocytogenes, L. grayi,* or *L. ivanovii.* If a sample was typed as *L. monocytogenes*, it underwent further testing to determine the serovar, using the methods described by Doumith et al. *(15)*. In short, one *L. monocytogenes* isolate was thoroughly mixed with 25 µL PCR media containing: 1X EconoTaq PLUS 2X Master Mix (Lucigen Corporation, Middleton), 1 µM of each *lmo0737*, *ORF2819* and *ORF2110* reverse and forward primers, 1.5 µM of *lmo1118* reverse and forward primers, 0.2 µM of *prs* reverse and forward primers and qs water. Following completion of PCR, products were mixed with 3 µL of BlueJuice™ loading buffer (Invitrogen, Carlsbad, CA) and separated on a 2% E-gel® with SYBR-safe™ (Invitrogen, Carlsbad, CA) along with 12 µL of E-Gel™ 1 kb Plus DNA Ladder (Invitrogen, Carlsbad, CA).

4.2.5 Statistical analyses

All statistical analyses were performed in R (Version 3.4.0; R Foundation for Statistical Computing, Vienna, Austria). Fisher's exact test was used to compare *Listeria* spp. prevalence among different sample types. p values less than 0.05 were considered statistically significant. The *caret* package was used for model training and analysis *(30)*. Model code can be accessed on GitHub (https://github.com/cgolden1993/Dissertation).

The random forest (RF) model is an ensemble, tree-based, machine learning (ML) model that was introduced by Breiman *(7)*. This method utilizes a large number of unpruned classification and regression trees *(6)* and two sources of randomization during construction *(41, 42)*. During construction of each tree, data are randomly chosen using the non-parametric bootstrap sampling method *(18)*, and trees are constructed on those data. These observations represent the in-bag sample. Trees are then built using the CART method, with a number of randomly selected variables (m_{try}) chosen at each split to determine the best split according to the

Gini index *(6)*. Each tree's performance is then evaluated on the leftover out-of-bag samples. This step helps to rank the importance of each predictor through the calculation of mean decrease accuracy *(41)*. Finally, all trees are aggregated into a final RF model. For classification problems, the model passes new data through each tree in the forest and counts the number of "votes" for each classification result *(7)*. The result with the most "votes" represents the final prediction.

Random forest models were built for both the preharvest (feces and soil) and WCR sample data to predict the presence or absence of *Listeria* spp. based on the predictors presented in Table 4.2. For the WCR model, all 40 predictors were used, but for the preharvest samples, only non-processing specific predictors were used, since these samples were collected before any processing occurred. In all, there were 1,637 preharvest samples and 230 WCR samples. Each data set was first split into training and testing sets using stratified random sampling to preserve the overall class distribution. The training set contained 80% of observations and the test set contained the remaining 20%. Models were trained on the training data, while the test set was held out to resemble an independent, "real-world" data set to evaluate the performance of the final model.

To choose an appropriate value for m_{try}, RF models were trained by the training set using various values for m_{try} and 10-fold cross-validation. From these results, the m_{try} value with the highest receiver operating characteristic (ROC) statistic was chosen and the model was retrained by the training set with the appropriate m_{try} value to construct the final RF model. For the preharvest model, an m_{try} of 53 was used, and for the WCR model, an m_{try} of 35 was used. Variable importance was determined in each model using the mean decrease in Gini as described by Breiman *(7, 8)*. Variables were ranked by relative importance on a scale of 0 to 100, where a

score of 100 represents the most important variable. Partial dependency plots (PDP) were constructed for the 2 most important variables in each model using the *pdp* package *(23)*.

For preharvest and WCR data sets, there were far more negative observations than positive observations. To correct for this class imbalance, the synthetic minority over-sampling technique (SMOTE) was used *(12)*. The SMOTE method generates synthetic minority class samples to attempt to achieve higher classifier performance. This method was used within the cross-validation step and was only applied to the training set.

After the final models were constructed, each model's performance was evaluated on the held-out test set. Each model was used to predict *Listeria* spp. prevalence given the predictor data and model predictions were compared to observed values. Models were evaluated using area under the ROC curves (AUC) *(5)*, sensitivity, and specificity.

4.3 Results

The prevalence of *Listeria* spp. in all samples collected in the current study was 17.4% (Table 4.4). Out of the 1,867 total samples, 37 (2.0%) were positive for *L. monocytogenes*. Among sample types, there was no significant difference in the presence of *Listeria* spp. (p = 0.11) or in the prevalence of *L. monocytogenes* (p = 0.58). The WCR samples had the highest prevalence of both *Listeria* spp. and *L. monocytogenes* (22.2% and 2.6%, respectively). The total prevalence of *Listeria* spp. in preharvest samples was 16.7%.

Farm-by-farm *Listeria* spp. prevalence results are presented in Table 4.5. For preharvest samples, each farm had at least one *Listeria* spp. positive sample with prevalence values ranging from 6.7% to 60%. Farm D had the highest percentage of positive samples, with 18 of the 30 samples positive. Of these 18 samples, 17 were positive for *L. monocytogenes*. For WCR samples, *Listeria* spp. prevalence values ranged from 0% to 80%, with Farm D having the

highest percentage of *Listeria* spp. positive WCR samples. There were 3 farms that did not have any positive WCR samples.

Random forest models were constructed for preharvest and WCR samples. The variable importance plot containing the top 8 most important predictors as defined by the preharvest RF model is illustrated in Fig. 4.1. The model predicted that day of year was by far the most important variable in predicting *Listeria* spp. preharvest prevalence of a pastured poultry farm with a relative importance score of 100, compared to 31.0 and 13.8 for flock age at time of sampling and the number of years that a farm has been operating, respectively. Partial dependency plots (PDPs) were constructed for the day of year and flock age variables (Fig. 4.2). Generally, predicted probability of *Listeria* spp. isolation was highest during generally colder temperature days of the year (days 100-175 and 275-325) and lowest during generally warmer parts of the year (Fig. 4.2a). There is some variability within the plot, but the variable's importance relative to other variables suggest its importance in the model. The RF model also predicted that probability of *Listeria* spp. preharvest isolation of pastured poultry farms is highest when the flock is between 5 and 10 weeks old and decreases substantially after 10 weeks (Fig 2b).

The variable importance plot for the WCR RF model is shown in Fig. 4.3. The model ranked brood feed whose top three ingredients were corn, soy, and wheat, as the most important predictor of *Listeria* spp. prevalence on the final broiler product from pastured poultry farms with a relative importance score of 100. The second most important indicator was chlorinated rinse water used during the processing of broilers with a relative importance score of 83.8. No other variable had a relative importance score of over 25. The model predicted that the marginal effect of corn/soy/wheat brood feed on the predicted *Listeria* spp. outcome was much higher than

the other brood feeds (Fig. 4.4a). Similarly, the marginal effect of chlorinated processing rinse water on the predicted final *Listeria* spp. prevalence outcome was much higher than the other values for that predictor (Fig. 4.4b).

For both models, model performance was evaluated on a held-out test set. This test set was not used in training of the model, and is meant to resemble an independent, "real-world" data set. Confusion matrices for both models were generated to illustrate the comparison of model predictions and observed outcomes (Table 4.6). It is important to consider that in this context, false negatives are much more costly than false positives, as having a model that incorrectly misses positive contamination results can be more harmful than one that incorrectly predicts contamination. Thus, model sensitivity is of vast importance. The preharvest RF model had a sensitivity of 0.778 and a specificity of 0.846. Of the 326 model predictions, there were only 12 false negatives, but the model incorrectly predicted a positive result 42 times, indicating that the model appears to be fail-safe. To further illustrate these results, ROC curves and area under the ROC curve (AUC) were used (Fig. 4.5). According to this statistic, the preharvest RF model performed exceptionally with an AUC of 0.876. The WCR model had a sensitivity of 0.800 and specificity of 0.886. Only 2 of the 45 test samples were false negatives. Additionally, the WCR RF model performed very well according to the ROC curve, with an AUC of 0.887 (Fig. 4.6).

4.4 Discussion

Currently, there is a lack of understanding on the impact and prevalence of *Listeria* spp. in pastured poultry farming environments and products *(44)*. The current study aimed to construct accurate predictive models that can be used to predict the presence or absence of *Listeria* spp. in the preharvest pastured poultry farm environment and in the final broiler product

and examine the prevalence of the pathogen across 11 southeastern United States farms. Doing so would help identify major farm management variables (Table 4.2) that are associated with a higher risk of *Listeria* spp. presence, as predicted by the models.

4.4.1 The risk of *Listeria* to pastured poultry farmers

There have been several foodborne illness outbreaks due to *Listeria monocytogenes* contamination of deli turkey and other types of RTE meats *(39)*. This signals its potential significance in the chicken and RTE chicken product industry. It is important to control for the pathogen within a farming environment, because favorable *Listeria* spp. environments are indicative of conditions that increase the risk of *Listeria monocytogenes (28)*. This means that an environment that is contaminated with *Listeria* could be a source of contamination for downstream processing areas *(29)*. Furthermore, poultry can act as reservoirs for the organism *(14, 37)*. Therefore, it is important for farmers and processors to be aware of farm management practices that can lead to an increased risk of *Listeria* spp. contamination in the environment of pastured poultry farms and in the final broiler product, as this is the point closest to the meat being available at the consumer level.

4.4.2 Weather and flock age are likely to impact the presence of *Listeria* spp. in the environment of pastured poultry farms

The RF model generated from preharvest sample data identified the day of the year that the sample was collected as the most important variable in classifying a sample as positive or negative for the presence of *Listeria* spp. While specific weather data were not reported for each sampling day in this study, the RF model predicted that days at the early and late parts of the year were associated with a higher probability of *Listeria* spp. isolation in the environment (Fig. 4.2a). We only sampled from day 90 to day 350 of the year that did not include the winter

season, and thus, our model does not extrapolate the results for the days corresponding to this season. Days 100-175 showed exceptionally high predicted risk. These days of the year are associated with the spring season. It was previously found that there was a higher prevalence of *L. monocytogenes* in processing environments during spring and winter months *(25)*. It follows that if there is a higher probability of *Listeria* isolation in the environment during cold weather, there is a higher probability of *Listeria* being brought into a processing facility during these times.

The increased probability of *Listeria* isolation during cold weather months could be due to the fact that the pathogen is more resistant to lower temperatures. For example, *Listeria* are able to grow at temperatures as low as 1°C, whereas enteric foodborne pathogens are not *(16)*. Strawn et al. *(50)* found that *L. monocytogenes* had a higher prevalence in above freezing, cooler temperatures in the environment of fruit and vegetable farms. Further data need to be collected to study the effect of temperature on *Listeria* prevalence in pastured poultry farms.

It is important to consider that the presented preharvest RF model may be region specific, as data were collected only from southeastern United States pastured poultry farms. It is likely that the day of year variable is important due to weather patterns that are occurring during those times. Because weather patterns over the course of a year are region specific, this model might not be appropriate for all regions. Despite this, the presented RF model suggests that farmers and processors have a higher awareness of *Listeria* during colder, above freezing weather.

Flock age was the second most important variable in the preharvest RF model (Fig. 4.1). *Listeria* spp. isolation from the environment was predicted to have the highest probability when a flock was between 5-10 weeks old. As a flock increases in age, changes occur in the flock's gut

microbiota, and it has been found that *Listeria* spp. levels decrease in the intestine of poultry as the bird's intestinal microbiota develops *(35)*.

4.4.3 Farm management practices can impact *Listeria* spp. prevalence in the final broiler product

The presented final WCR RF model identified brood feed formulation as the most important explanatory variable in predicting *Listeria* spp. presence in the final product (Fig. 4.3). Flocks whose brood feed's 3 main sources of protein were corn, soy, and wheat had a significantly higher probability than the other 5 types of feed (Table 4.2). After hatching, the intestinal microbiota of a chicken develops rapidly *(11, 31)*. As such, the type of feed that a chick receives early on its life can have a profound effect on its gut microbiota *(31)*. This is an especially important point to consider for pastured poultry and organic farms that utilize feeds with very few additives. If present in the external environment, *Listeria* could contaminate a flock's food source, and be present throughout a bird's lifespan. It is important to note of the possibility that type of feed could just be important to our model. Rather, our model identified it as an important predictor of *Listeria* spp. presence, but not necessarily the cause of contamination.

The use of chlorinated rinse water during broiler processing was identified as the second most important variable in the final broiler product RF model (Fig. 4.3). Studies have shown that gram-positive organisms, such as *Listeria monocytogenes*, are more resistant to chlorine than gram-negative bacteria *(55)*. Thus, it is possible that chlorine is more effective at reducing gram-negative bacteria during rinsing of broilers during processing, and this might create a less competitive environment for *Listeria* to survive and grow during cold storage. Organic acids, on the other hand, have been shown to be more effective against gram-positive bacteria *(49)*.

Further research needs to be conducted to see if the use of chlorinated rinse water during poultry processing leads to a more favorable environment for *Listeria*.

Our model did not identify major pastured poultry farm management practices such as type of processing unit and skin on/skin off processing as important predictors of *Listeria* spp. presence. While no studies have examined the effect of these practices on *Listeria* prevalence, there have been conflicting results on the effect of these variables on other foodborne pathogens. Trimble et al. *(52)* found that *Salmonella* prevalence was significantly higher on carcasses processed on-farm compared to carcasses processed at a USDA-inspected facility, while there were no significant differences in *Campylobacter* prevalence. For traditionally-reared broilers, Berrang et al. *(4)* found that skin-on and skin-off broiler parts had no significant difference in *Campylobacter* and *Escherichia coli* numbers. Currently, there are no data indicating the difference of foodborne pathogen prevalence in skin-on and skin-off broilers reared on pastured poultry farms.

4.4.4 Random forest model performance

Machine learning models have been shown to have use in the food safety industry, with several studies being published on the performance of random forest models *(2, 24, 40)* and classification and regression tree models *(28, 36, 50)* in a food safety context. Benefits to using RF models is that they are robust to outliers and skewed data, provide variable importance rankings, and compute an unbiased out-of-bag error estimate *(43)*.

Machine learning models are often tested on how they perform at classifying independent, new observations that were not used during training of the model. In many biological and research settings, it can be too costly to obtain a new testing set, so data sets are split into training and testing sets *(17)*. Models are then evaluated by prediction performance on

the testing set and evaluated by metrics such as ROC curves and AUC *(5)*. In the current study, both RF models performed well with respect to AUC, with both scoring > 0.85. (Table 4.6).

It is important to note that the sample size for the final product WCR model was small, which might have impacted the prediction performance estimation. It would be of great use to obtain a new, independent data set from other pastured poultry farms to confirm the usefulness of the generated RF model. The small sample size shouldn't have a large effect on the variable importance rankings, though (Fig. 4.3). Strobl and Zeileis *(51)* reported that importance measures from models trained on smaller data sets were not significantly different from those trained on larger data sets.

4.5 Conclusions

Random forest models were generated to classify pastured poultry preharvest and final product samples as positive or negative for *Listeria* spp. Our model identified time of year as a potential indicator of preharvest presence of *Listeria* spp. on pastured poultry farms. Additionally, corn/soy/wheat brood feed and rinse water chlorination were associated with a higher probability of *Listeria* spp. isolation on the final product WCR, as predicted by our model. Due to the variation in the types of pastured poultry farms sampled from Table 4.1, the information provided by our models could be representative of many different types of pastured poultry and similar organic type farms, although a greater geographic diversity of farms is needed to test this hypothesis. This study showed the use of RF models at predicting pathogen presence and should assist farmers and processors be aware of factors that are associated with a higher risk of *Listeria* contamination on pastured poultry farms.

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4.7 References

1. Ahmed, O. M., P. Pangloli, C. Hwang, S. Zivanovic, T. Wu, D. D'Souza, and F. A. Draughon. 2015. The occurrence of *Listeria monocytogenes* in retail ready-to-eat meat and poultry products related to the levels of acetate and lactate in the products. *Food Control*. 52:43-48.

2. Barco, L., M. Mancin, M. Ruffa, C. Saccardin, C. Minorello, P. Zavagnin, A. A. Lettini, J. E. Olsen, and A. Ricci. 2012. Application of the random forest method to analyse epidemiological and phenotypic characteristics of *Salmonella* and *Salmonella* Typhimurium strains. *Zoonoses Public Health*. 59:505-512.

3. Bergholz, P. W., J. D. Noar, and D. H. Buckley. 2011. Environmental patterns are imposed on the population structure of *Escherichia coli* after fecal deposition. *Appl. Environ. Microbiol.* 77:211-219.

4. Berrang, M. E., S. R. Ladely, and R. J. Buhr. 2001. Presence and level of *Campylobacter*, coliforms, *Escherichia coli*, and total aerobic bacteria recovered from broiler parts with and without skin. *J. Food Prot.* 64:184-188.

5. Bradley, A. P. 1997. The use of the area under the ROC curve in the evaluation of machine learning algorithms. *Pattern Recogn.* 30:1145-1159.

6. Breiman, L. 1984. Classification and regression trees. Routledge, New York.

7. Breiman, L. 2001. Random forests. *Mach. Learn.* 45:5-32.

8. Breiman, L. 2002. Manual on setting up, using, and understanding random forests v3.1. Available at: https://www.stat.berkeley.edu/~breiman/Using_random_forests_V3.1.pdf. Accessed 16 October 2018.

9. Carpentier, B., and O. Cerf. 2011. Persistence of *Listeria monocytogenes* in food industry equipment and premises. *Int. J. Food Microbiol.* 145:1-8.

10. Centers for Disease Control and Prevention. 2018. Outbreak of *Listeria* infections linked to deli ham. Available at: https://www.cdc.gov/listeria/outbreaks/countryham-10-18/index.html. Accessed 11 November 2018.

11. Chambers, J. R., and J. Gong. 2011. The intestinal microbiota and its modulation for *Salmonella* control in chickens. *Food Res. Int.* 44:3149-3159.

12. Chawla, N. V., K. W. Bowyer, L. O. Hall, and W. P. Kegelmeyer. 2002. SMOTE: Synthetic minority over-sampling technique. *J. Artif. Intell. Res.* 16:321-357.

13. Dev Kumar, G., R. C. Williams, S. S. Sumner, and J. D. Eifert. 2016. Effect of ozone and ultraviolet light on *Listeria monocytogenes* populations in fresh and spent chill brines. *Food Control*. 59:172-177.

14. Dhama, K., A. K. Verma, S. Rajagunalan, A. Kumar, R. Tiwari, S. Chakraborty, and R. Kumar. 2013. *Listeria monocytogenes* infection in poultry and its public health importance with special reference to food borne zoonoses. *Pak. J. Biol. Sci.* 16:301-308.

15. Doumith, M., C. Buchrieser, P. Glaser, C. Jacquet, and P. Martin. 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.* 42:3819-3822.

16. Doyle, M. P. 1989. Foodborne bacterial pathogens. Marcel Dekker, Inc., New York.

17. Dupuy, A., and R. M. Simon. 2007. Critical review of published microarray studies for cancer outcome and guidelines on statistical analysis and reporting. *J. Natl. Cancer Inst.* 99:147-157.

18. Efron, B., and R. J. Tibshirani. 1994. An introduction to the bootstrap. Chapman and Hall/CRC Press, Boca Raton, FL.

19. Elkhoraibi, C., M. Pitesky, N. Dailey, and D. Niemeier. 2017. Operational challenges and opportunities in pastured poultry operations in the United States. *Poult. Sci.* 96:1648-1650.

20. Ferreira, V., M. Wiedmann, P. Teixeira, and M. J. Stasiewicz. 2014. *Listeria monocytogenes* persistence in food-associated environments: Epidemiology, strain characteristics, and implications for public health. *J. Food Prot.* 77:150-170.

21. Frye, D. M., R. Zweig, J. Sturgeon, M. Tormey, M. LeCavalier, I. Lee, L. Lawani, and L. Mascola. 2002. An outbreak of febrile gastroenteritis associated with delicatessen meat contaminated with *Listeria monocytogenes*. *Clin. Infect. Dis.* 35:943-949.

22. Gottlieb, S. L., E. C. Newbern, P. M. Griffin, L. M. Graves, R. M. Hoekstra, N. L. Baker, S. B. Hunter, K. G. Holt, F. Ramsey, and M. Head. 2006. Multistate outbreak of listeriosis linked to turkey deli meat and subsequent changes in US regulatory policy. *Clin. Infect. Dis.* 42:29-36.

23. Greenwell, B. M. 2017. pdp: An R package for constructing partial dependence plots. *R J.* 9:421-436.

24. Gu, W., A. R. Vieira, R. M. Hoekstra, P. M. Griffin, and D. Cole. 2015. Use of random forest to estimate population attributable fractions from a case-control study of *Salmonella enterica* serotype Enteritidis infections. *Epidemiol. Infect.* 143:2786-2794.

25. Guerini, M. N., D. M. Brichta-Harhay, S. D. Shackelford, T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, T. L. Wheeler, and M. Koohmaraie. 2007. *Listeria* prevalence and *Listeria monocytogenes* serovar diversity at cull cow and bull processing plants in the United States. *J. Food Prot.* 70:2578-2582.

26. Hilimire, K. 2012. The grass is greener: Farmers' experiences with pastured poultry. *Renew. Agric. Food Syst.* 27:173-179.

27. Huang, B., S. Eglezos, B. A. Heron, H. Smith, T. Graham, J. Bates, and J. Savill. 2007. Comparison of multiplex PCR with conventional biochemical methods for the identification of *Listeria* spp. isolates from food and clinical samples in Queensland, Australia. *J. Food Prot.* 70:1874-1880.

28. Ivanek, R., Y. T. Gröhn, M. T. Wells, A. J. Lembo, B. D. Sauders, and M. Wiedmann. 2009. Modeling of spatially referenced environmental and meteorological factors influencing the probability of *Listeria* species isolation from natural environments. *Appl. Environ. Microbiol.* 75:5893-5909.

29. Ivanek, R., Y. T. Gröhn, and M. Wiedmann. 2006. *Listeria monocytogenes* in multiple habitats and host populations: Review of available data for mathematical modeling. *Foodborne Pathog. Dis.* 3:319-336.

30. Kuhn, M. 2008. Building predictive models in R using the caret package. *J. Stat. Softw.* 28:1-26.

31. Lan, Y., M. W. A. Verstegen, S. Tamminga, and B. A. Williams. 2005. The role of the commensal gut microbial community in broiler chickens. *Worlds Poult. Sci. J.* 61:95-104.

32. Lianou, A., and J. N. Sofos. 2007. A review of the incidence and transmission of *Listeria monocytogenes* in ready-to-eat products in retail and food service environments. *J. Food Prot.* 70:2172-2198.

33. Locatelli, A., M. A. Lewis, and M. J. Rothrock Jr. 2017. The distribution of *Listeria* in pasture-raised broiler farm soils is potentially related to University of Vermont medium enrichment bias toward *Listeria innocua* over *Listeria monocytogenes*. *Front. Vet. Sci.* 4:227.

34. Martín, B., A. Perich, D. Gómez, J. Yangüela, A. Rodríguez, M. Garriga, and T. Aymerich. 2014. Diversity and distribution of *Listeria monocytogenes* in meat processing plants. *Food Microbiol.* 44:119-127.

35. Milillo, S. R., J. C. Stout, I. B. Hanning, A. Clement, E. D. Fortes, H. C. Den Bakker, M. Wiedmann, and S. C. Ricke. 2012. *Listeria monocytogenes* and hemolytic *Listeria innocua* in poultry. *Poult. Sci.* 91:2158-2163.

36. Mokhtari, A., H. C. Frey, and L.-A. Jaykus. 2006. Application of classification and regression trees for sensitivity analysis of the *Escherichia coli* O157:H7 food safety process risk model. *J. Food Prot.* 69:609-618.

37. Njagi, L. W., P. G. Mbuthia, L. C. Bebora, P. N. Nyaga, U. Minga, and J. E. Olsen. 2004. Carrier status for *Listeria monocytogenes* and other *Listeria* species in free range farm and market healthy indigenous chickens and ducks. *East Afr. Med. J.* 81:529-533.

38. O'Bryan, C. A., P. Crandall, D. Jaroni, S. C. Ricke, and K. E. Gibson. 2017. Assessment of nitrogen and phosphorus loads present in environments impacted by alternative poultry processing operations utilized in pasture-raised poultry production. *Renew. Agric. Food Syst.* 32:33-42.

39. Olsen, S. J., M. Patrick, S. B. Hunter, V. Reddy, L. Kornstein, W. R. MacKenzie, K. Lane, S. Bidol, G. A. Stoltman, and D. M. Frye. 2005. Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. *Clin. Infect. Dis.* 40:962-967.

40. Pang, H., R. McEgan, A. Mishra, S. A. Micallef, and A. K. Pradhan. 2017. Identifying and modeling meteorological risk factors associated with pre-harvest contamination of *Listeria* species in a mixed produce and dairy farm. *Food Res. Int.* 102:355-363.

41. Philibert, A., M.-L. Desprez-Loustau, B. Fabre, P. Frey, F. Halkett, C. Husson, B. Lung-Escarmant, B. Marçais, C. Robin, C. Vacher, and D. Makowski. 2011. Predicting invasion success of forest pathogenic fungi from species traits. *J. Appl. Ecol.* 48:1381-1390.

42. Prasad, A. M., L. R. Iverson, and A. Liaw. 2006. Newer classification and regression tree techniques: Bagging and random forests for ecological prediction. *Ecosystems*. 9:181-199.

43. Rodriguez-Galiano, V. F., B. Ghimire, J. Rogan, M. Chica-Olmo, and J. P. Rigol-Sanchez. 2012. An assessment of the effectiveness of a random forest classifier for land-cover classification. *ISPRS J. Photogramm. Remote Sens.* 67:93-104.

44. Rothrock Jr, M. J., M. L. Davis, A. Locatelli, A. Bodie, T. G. McIntosh, J. R. Donaldson, and S. C. Ricke. 2017. *Listeria* occurrence in poultry flocks: Detection and potential implications. *Front. Vet. Sci.* 4:125.

45. Salatin, J. 1993. Pastured poultry profits. Polyface, Swoope, VA.

46. Sasaki, Y., M. Haruna, M. Murakami, M. Hayashida, N. Takahashi, T. Urushiyama, K. Ito, and Y. Yamada. 2014. Contamination of poultry products with *Listeria monocytogenes* at poultry processing plants. *J. Vet. Med. Sci.* 76:129-132.

47. Semenov, A. V., E. Franz, L. van Overbeek, A. J. Termorshuizen, and A. H. C. van Bruggen. 2008. Estimating the stability of *Escherichia coli* O157:H7 survival in manure-amended soils with different management histories. *Environ. Microbiol.* 10:1450-1459.

48. Siemon, C. E., P. B. Bahnson, and W. A. Gebreyes. 2007. Comparative investigation of prevalence and antimicrobial resistance of *Salmonella* between pasture and conventionally reared poultry. *Avian Dis.* 51:112-117.

49. Skřivanová, E., M. Marounek, V. Benda, and P. Březina. 2006. Susceptibility of *Escherichia coli*, *Salmonella* sp. and *Clostridium perfringens* to organic acids and monolaurin. *Vet. Med. (Praha)*. 51:81-88.

50. Strawn, L. K., E. D. Fortes, E. A. Bihn, K. K. Nightingale, Y. T. Gröhn, R. W. Worobo, M. Wiedmann, and P. W. Bergholz. 2013. Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. *Appl. Environ. Microbiol.* 79:588-600.

51. Strobl, C., and A. Zeileis. 2008. Danger: High power!—Exploring the statistical properties of a test for random forest variable importance. p. 59-66. *In* P. Brito (ed.), COMPSTAT 2008: Proceedings in Computational Statistics Physica Verlag, Heidelberg.

52. Trimble, L. M., W. Q. Alali, K. E. Gibson, S. C. Ricke, P. Crandall, D. Jaroni, and M. Berrang. 2013. *Salmonella* and *Campylobacter* prevalence and concentration on pasture-raised broilers processed on-farm, in a mobile processing unit, and at small USDA-inspected facilities. *Food Control*. 34:177-182.

53. United States Department of Agriculture. 2017. Isolation and identification of *Listeria monocytogenes* from red meat, poultry, ready-to-eat siluriformes (fish) and egg products, and environmental samples. Available at: https://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES. Accessed 19 November 2018.

54. van Loo, E. J., V. Caputo, R. M. Nayga Jr, J. Meullenet, and S. C. Ricke. 2011. Consumers' willingness to pay for organic chicken breast: Evidence from choice experiment. *Food Qual. Prefer.* 22:603-613.

55. Virto, R., P. Manas, I. Alvarez, S. Condon, and J. Raso. 2005. Membrane damage and microbial inactivation by chlorine in the absence and presence of a chlorine-demanding substrate. *Appl. Environ. Microbiol.* 71:5022-5028.

56. Zhu, M., M. Du, J. Cordray, and D. U. Ahn. 2005. Control of *Listeria monocytogenes* contamination in ready-to-eat meat products. *Compr. Rev. Food Sci. Food Saf.* 4:34-42.

Table 4.1 Comparison of the 11 all-natural, antibiotic-free, pastured broiler farms included in this study

Farm	Breed	No. of flocks	Flock size	Multi-use farm?	Animal types	Processing
A	Freedom Ranger	10	>500	Yes	Layers, Swine, Beef cattle, Sheep	USDA-inspected facility
B	Freedom Ranger, Cornish Cross	5	50-75	Yes	Layers, Swine, Horses, Goats	On-farm (skin-off)
C	Freedom Ranger	1	50-75	No	n/a	On-farm (skin-on)
D	Freedom Ranger	1	50-75	No	n/a	On-farm (skin-on)
E	Freedom Ranger, Cornish Cross	5	50-75	Yes	Layers, Swine, Beef cattle, Sheep	On-farm (skin-on)
F	Freedom Ranger	2	>500	Yes	Layers	USDA-inspected facility
G	Freedom Ranger, Cornish Cross	9	100-500	Yes	Layers, Swine, Goats	USDA-inspected facility
H	Freedom Ranger, Cornish Cross	2	50-75	Yes	Layers	On-farm (skin-on)
I	Freedom Ranger	4	100-500	Yes	Layers, Beef cattle, Goats	USDA-inspected facility and on-farm (skin-on)
J	Freedom Ranger	2	>500	Yes	Layers, Swine, Beef cattle, Sheep	USDA-inspected facility
K	Cornish Cross	2	50-75	Yes	Layers, Swine	On-farm (skin-on)

Table 4.2 Predictors used in the preharvest (feces, soil) and final product whole carcass rinse (WCR) random forest models

Variable	Description	Levels/unit
AvgNumBirds	Average number of birds that the farm handled in one year	Numeric
AvgNumFlocks	Average number of flocks that the farm handled in one year	Numeric
YearsFarming	Number of years the farm had been operating at the time of sampling	Numeric (years)
EggSource	Source of broiler eggs	6 levels: Company A, B, C, D, E, F
BroodBedding	Type of bedding broilers received during brooding	3 levels: pasture-based brooder (PB), wood shavings (WS), saw-dust/shredded paper (SDSP)
BroodFeed	Up to top 3 sources of protein in brooding feed	6 levels: barley, wheat, oats (BWO); corn, soy, wheat (CSW); wheat, corn (WC); wheat and grain byproducts (W); corn, soy, oats (CSO); peas, corn, oats (PCO)
BrGMOFree	Was the brood feed GMO free?	2 levels: yes (Y), no (N)
BrSoyFree	Was the brood feed soy free?	2 levels: yes (Y), no (N)
BrMedicated	Was the brood feed medicated?	2 levels: yes (Y), no (N)
BroodClean-Frequency	How often the brooding area was cleaned	7 levels: 3Days, all in/all out (AIAO), daily, deep litter method (DLM), mobile, weekly, yearly
AvgAgeTo-Pasture	Average age broilers were put on pasture	Numeric (weeks)
PastureHousing	Type of pasture housing environment	4 levels: chicken tractor (CT), chicken tractor with fencing (CTF),

		chicken tractor free range (CTFR), chicken tractor with fencing (2 tractors; CTF2)
FreqHousing-Move	How often the pasture area was moved	2 levels: daily, every 2 days
AlwaysNew-Pasture	Was the pasture always moved to a brand-new pasture area	2 levels: yes (Y), no (N)
PastureFeed	Up to top 3 sources of protein in pasture feed	7 levels: barley, wheat, oats (BWO); corn, soy, wheat (CSW); wheat, corn (WC); wheat and grain byproducts (W); corn, soy, oats (CSO); corn, cotton seed mill, wheat (CMW); peas, corn, oats (PCO)
PaGMOFree	Was the pasture feed GMO free?	2 levels: yes (Y), no (N)
PaSoyFree	Was the pasture feed soy free?	2 levels: yes (Y), no (N)
PaMedicated	Were broilers medicated while on pasture?	2 levels: yes (Y), no (N)
LayersOnFarm	Were layers present on the farm?	2 levels: yes (Y), no (N)
CattleOnFarm	Were cattle present on the farm?	2 levels: yes (Y), no (N)
SwineOnFarm	Were swine present on the farm?	2 levels: yes (Y), no (N)
GoatsOnFarm	Were goats present on the farm?	2 levels: yes (Y), no (N)
SheepOnFarm	Were sheep present on the farm?	2 levels: yes (Y), no (N)
WaterSource	Water source for broilers during grow-out	3 levels: public, rain, well
FreqBird-Handling	How often chickens were handled on pasture	2 levels: daily, only if needed (OIN)

AnyABXUse	Were antibiotics ever used on the broilers	2 levels: yes (Y), no (N)
LengthFeed-RestrixProcess	Length of feed restriction before processing	Numeric (hours)
DayOfYear	Day of the year samples were collected on	Numeric (days)
FlockAgeWeeks	Age of flock at time of sampling	Numeric (weeks)
Breed	Breed of broilers used	2 levels: Freedom Ranger (FR), Cornish Cross (CC)
FlockSize	Number of birds in the sampled flock	Numeric
ProcessingType[a]	Where the broilers were processed	2 levels: farm, plant
SkinOnOff[a]	Skin-on or off processing facility	2 levels: on, off
ScalderTempC[a]	Temperature of water (°C) used during scalding of birds during processing	7 levels: 55, 60, 63, 65, 71, 82, none
RinseWaterSource[a]	Source of water used for carcass rinsing during processing	2 levels: public, well
RinseWaterChlor[a]	Was the rinse water chlorinated?	2 levels: yes (Y), no (N)
ChillingMethod[a]	Type of chilling used for carcasses after processing	2 levels: water, air
TransportTime[a]	Length of time to transport broilers to processors (if necessary)	Numeric (hours)
StorageTempC[a]	Temperature that carcasses were stored at before reception by customer	Numeric (°C)
StorageTimeD[a]	Amount of time carcasses were stored for before reception by customer	Numeric (days)

[a] Variables were only used in the WCR model

Table 4.3 Broiler cold storage procedures before making the product available for consumers for each farm

Farm	Storage temperature (°C)	Average storage time (Days)
A	4	1
B[a]	4	2
B[a]	-20	2
C	-20	11
D	-20	13
E	4	0.2
F	4	1
G	-20	30.25
H	-20	30.5
I	-20	8.75
J	4	1
K	4	0

[a] Farm B used two different carcass storage methods over the course of the study

Table 4.4 Effect of sample type on prevalence of *Listeria* spp. and *Listeria monocytogenes* in pastured poultry farm samples

Sample type	**No. of samples**	**No. (%) of positive samples**	
		***Listeria* spp.**	***L. monocytogenes***
Feces	820	133 (16.2)	14 (1.7)
Soil	817	140 (17.1)	17 (2.1)
WCR[a]	230	51 (22.2)	6 (2.6)
Total	1867	324 (17.4)	37 (2.0)

[a] Whole carcass rinse (WCR)

Table 4.5 Prevalence of *Listeria* spp. by pastured poultry farm and sample type

Farm	Preharvest samples		WCR samples	
	No. of samples	**No. (%) of positive *Listeria spp.* samples**	**No. of samples**	**No. (%) of positive *Listeria spp.* samples**
A	331	55 (16.6)	50	0 (0)
B	180	50 (27.8)	25	13 (52)
C	30	2 (6.7)	5	0 (0)
D	30	18 (60)	5	4 (80)
E	213	19 (8.9)	25	1 (4.0)
F	80	10 (12.5)	10	0 (0)
G	273	24 (8.8)	40	2 (5.0)
H	80	16 (20)	10	5 (50)
I	200	31 (15.5)	40	24 (60)
J	120	34 (28.3)	10	1 (10)
K	100	14 (14)	10	1 (10)
Total	1637	273 (16.7)	230	51 (22.2)

Table 4.6 Predictive performance of the preharvest and whole carcass rinse (WCR) random forest models

Predictions		Actual		Sensitivity	Specificity	AUC[a]
		Positive	**Negative**			
Preharvest model	Positive	42	42	0.778	0.846	0.876
	Negative	12	230			
WCR model	Positive	8	4	0.800	0.886	0.887
	Negative	2	31			

[a] AUC, area under the receiver operating characteristic (ROC) curve

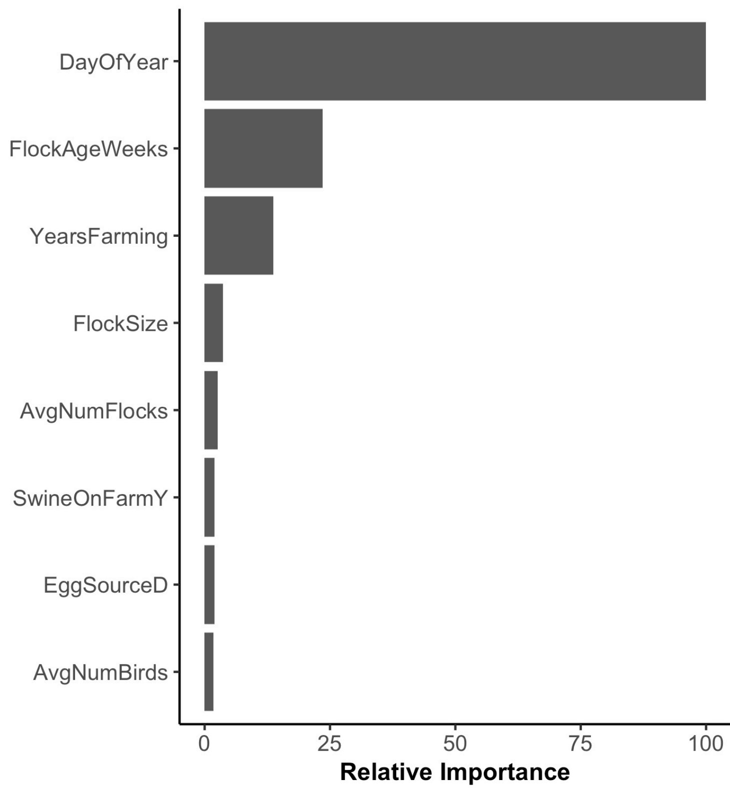

Figure 4.1 Variable importance plot for the preharvest sample random forest model. Relative importance is determined by average mean decrease in Gini and scaled from 0 to 100, where 100 is most important. SwineOnFarmY refers to farms that had swine present at the farm. EggSourceD refers to farms that received broiler eggs from Company D.

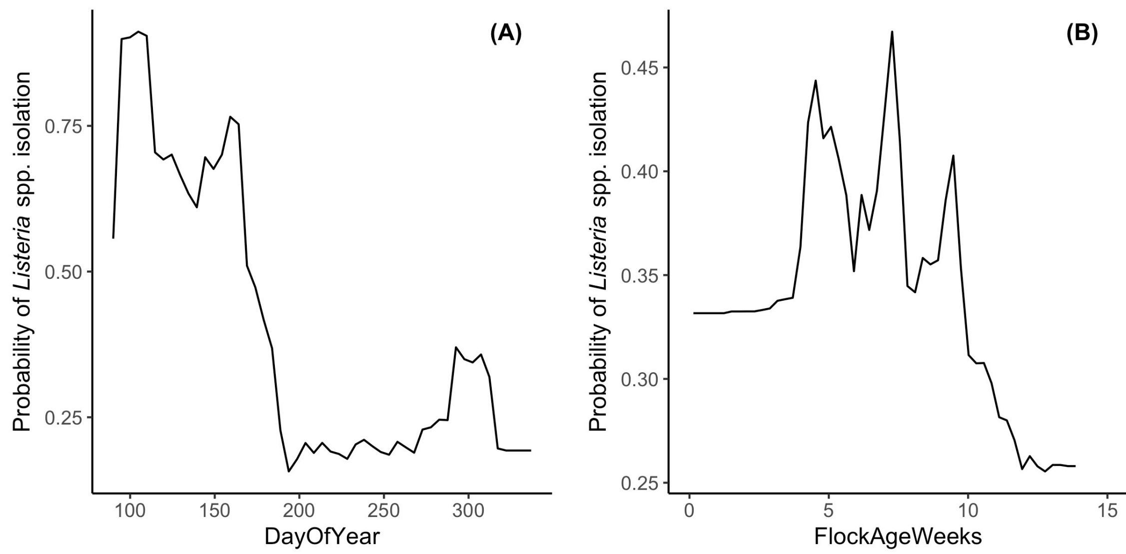

Figure 4.2 Partial dependency plots (PDPs) for the two most important predictors in the preharvest sample random forest model. (a) DayOfYear represents the day of the year that samples were collected, numerically from 1 to 365. (b) FlockAgeWeeks represents the age of the flock in weeks at the time of sampling.

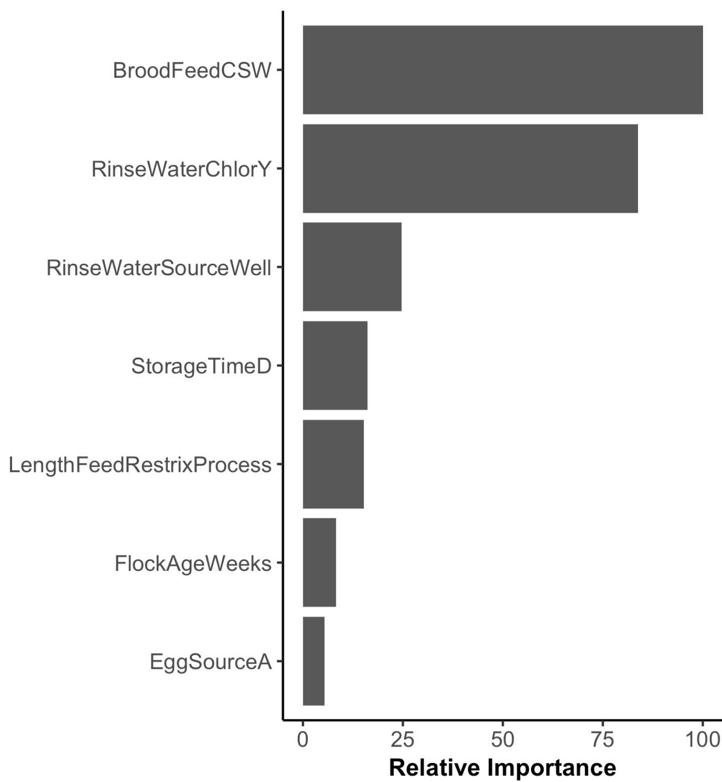

Figure 4.3 Variable importance plot for the whole carcass rinse (WCR) final product random forest model. Relative importance is determined by average mean decrease in Gini and scaled from 0 to 100, where 100 is most important. BroodFeedCSW refers to brood feed with top 3 sources of protein as corn, soy, and wheat. RinseWaterChlorY refers to farms that chlorinated rinse water used during broiler processing. RinseWaterSourceWell refers to farms that used well water as the source for rinse water used during processing. EggSourceA refers to farms that received broiler eggs from Company A.

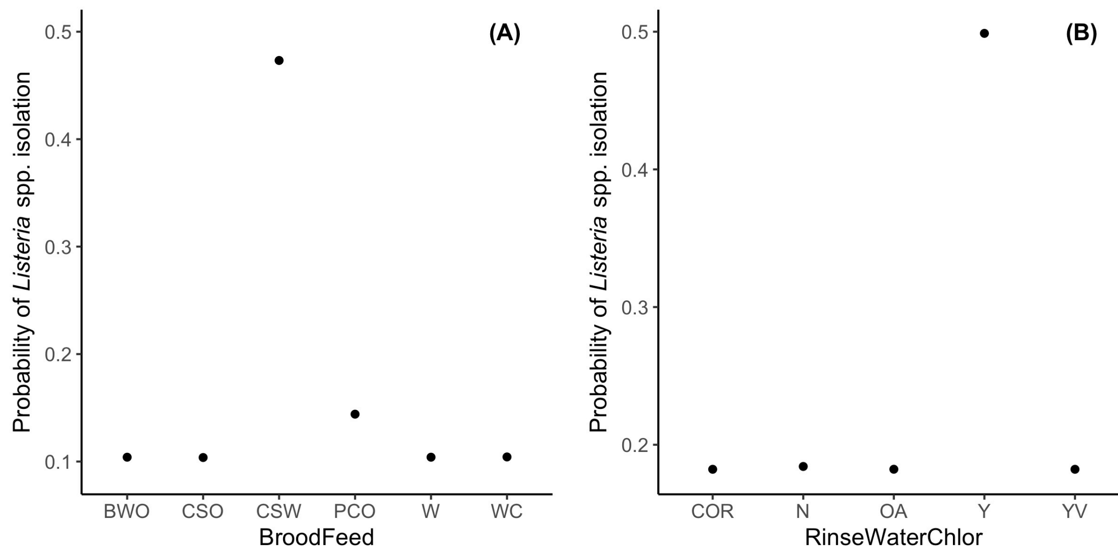

Figure 4.4 Partial dependency plots (PDPs) for the two most important predictors in the whole carcass rinse (WCR) final product random forest model. (a) Brood feed refers to up to the top 3 sources of protein in the brood feed. Ingredients include: barley (B), corn (C), oats (O), wheat (W), soy (S), cotton seed mill (M), peas (P). (b) RinseWaterChlor refers to whether the rinse water used during broiler processing was chlorinated or not. Levels for this variable include: yes (Y), no (N), organic acids used instead of chlorine (OA), yes with vinegar (YV), certified organic rinse used as wash (COR).

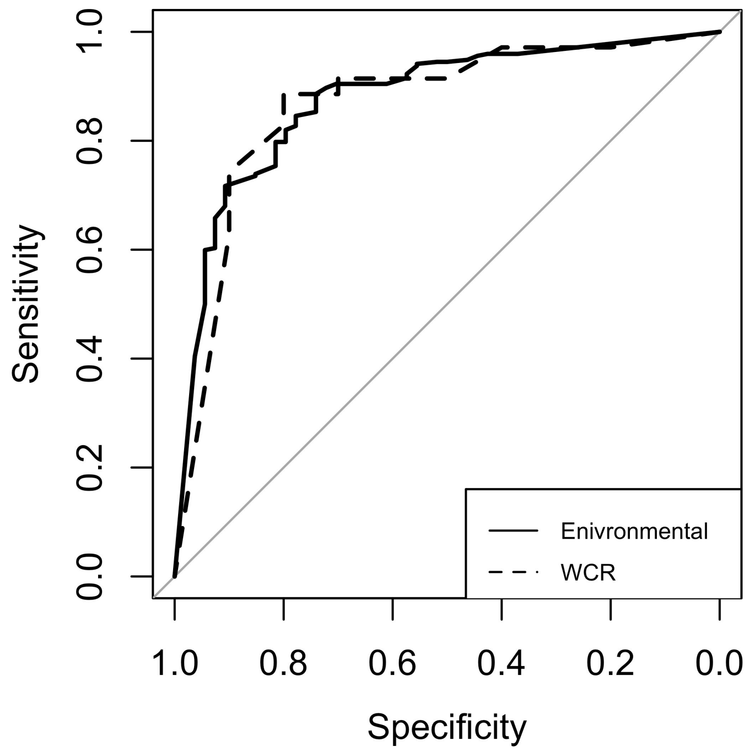

Figure 4.5 Receiver operating characteristic (ROC) curves for the environmental (solid line) and whole carcass rinse (dashed line) sample random forest models. The diagonal line represents the line of no discrimination, or the expectation of a random guess. Curves above this line are considered better than random guess. The area under each curve is referred to as AUC.

CHAPTER 5

USING WEATHER PATTERNS TO FORECAST *CAMPYLOBACTER* PRESENCE IN THE ENVIRONMENT OF PASTURED POULTRY FARMS

[1] Golden, C.E., M.J. Rothrock, Jr., A. Gupta, and A. Mishra. To be submitted to *International Journal of Food Microbiology*.

Abstract

Campylobacter is one of the leading causes of gastroenteritis worldwide. It presents an annual problem for the poultry industry, where, from 1998-2017 in the United States, 279 foodborne illness outbreaks linked to poultry consumption were reported to the Centers for Disease Control and Prevention. It is important to control for the organism in the environment of broiler farms, as environmental contamination provides a means for broiler flock contamination. For this purpose, it is necessary to have an understanding of the conditions that are linked to high environmental *Campylobacter* prevalence. Random forest models were constructed from fecal and soil samples collected during a 2014-2017 longitudinal study of 11 southeastern United States pastured poultry farms. Each model included 80 predictors, made up of different combinations of observed weather patterns. After training, models were evaluated for predictive performance on a held-out 20% test set. Both the feces and soil models performed well in terms of area under the receiver operating characteristic curve (AUC), with AUC values of 0.96 and 0.89, respectively. During the 2019 growing season, additional fecal and soil samples were collected to evaluate the models' ability to predict *Campylobacter* prevalence using weather forecasts made up to seven days ahead. For both samples, accuracy was not affected by the number of days before sampling that predictions were made. On average, the feces model did not tend to over or underpredict prevalence with bias % scores ranging from 87.3 to 106.4% across the seven days of predictions, whereas the soil model tended to underpredict prevalence with bias % scores ranging from 66.0 to 75.8%. The generated models provide poultry farmers and producers a tool to better perform *Campylobacter* surveillance and ability to make risk-based decisions.

5.1 Introduction

Campylobacter spp. are gram-negative, spiral-shaped, microaerophilic microorganisms that are commonly found in the intestine of animals used for human food. *Campylobacter* is one of the leading causes of foodborne gastroenteritis worldwide, and incidence of campylobacteriosis has been increasing over time in North America, Europe, and Asia *(21)*. The organism has presented a specific problem for the poultry industry, showing the ability to colonize the gastrointestinal tract of birds from an early age *(5, 12)*. This can lead to the potential for public health impact, if not properly controlled throughout the poultry supply chain. From 1998-2017 in the United States, there were 279 identified campylobacteriosis outbreaks linked to poultry consumption that resulted in 2,902 illnesses, 152 hospitalizations, and 1 death, according to the Centers for Disease Control and Prevention's (CDC) National Outbreak Reporting System *(9)*. The actual number is likely much larger, as Scallan et al. *(33)* estimated an under-diagnosis multiplier of 30.3 for illnesses caused by *Campylobacter* spp. in the United States.

It is important to control *Campylobacter* within the environment of poultry farms, as the external environment can be a source of flock contamination. Specifically, bird feces has been shown to be a major source of flock contamination, with bird-to-bird horizontal transfer occurring rapidly after initial contamination *(18, 27, 37)*. Other potential risk factors include contaminated bedding, feed, and drinking water, which can potentially result from contaminated soil, insects, rodents and other small animals, and poor worker hygiene *(24, 44, 45)*. Controlling for these risk factors becomes even more imperative when considering alternative poultry production systems, such as pastured and free-range poultry systems, in which birds have more access to the external environment than in a conventional poultry operation.

The correlation between weather events and *Campylobacter* prevalence in poultry samples or incidence of human campylobacteriosis incidence has begun to be explored, with varying results *(6, 15, 30, 39)*. To our knowledge, research has been limited to observing past contamination events rather than forecasting them. A predictive model that uses weather forecasting data to predict *Campylobacter* contamination in farm environments could be of great use to poultry farmers and processors by providing a tool that provides information to mitigate the risk of *Campylobacter* by identifying important meteorological risk factors and alerting farmers when the probability of finding the organism is high. Similar models have been presented for *Listeria* spp. in pastured poultry farms *(13)*.

Machine learning provides a means to develop predictive models with complex datasets that traditional statistical methods are not optimized to handle *(22)*. Random forest (RF) models are an example of a commonly used machine learning algorithm that has shown promise in predicting pathogen presence in many food safety-related scenarios *(1, 13, 14, 19, 28, 38)*. The RF method is a tree-based modeling method that fits a specified number of classification and regression trees to training data, and model predictions are made by averaging the decision between all of the trees *(8)*. The "voting" scheme used by RF models represents one of its greatest advantages when compared to other machine learning methods, due to the fact that it is relatively robust to outliers, can handle high-dimensional data, and incorporates randomization into tree construction *(8)*.

The current study was undertaken to develop RF models that could be used to predict *Campylobacter* presence in fecal and soil samples from pastured poultry farms in the United States. The study was completed in two phases. The first phase consisted of training and testing predictive models based on historical meteorological data. Models were then tested for their

forecasting capabilities by using weather forecasts to predict *Campylobacter* presence. The generated models will assist poultry farmers understand important meteorological risk factors linked to *Campylobacter* contamination and are tools that can be used to predict high-risk conditions based on forecasted and historical weather data.

5.2 Materials and methods

5.2.1 Sample collection

Sample collection was performed in two phases. Phase one consisted of a longitudinal study from March 2014 to November 2017, where 43 flocks of broilers from 11 pastured poultry farms in the southeastern United States were followed through each flock's lifecycle. Fecal and soil samples were collected at three points during a flock's lifecycle: (i) within a few days of the birds being placed on the pasture (range: 16-47 days after birth), (ii) mid-way through grow-out (range: 36-69 days), and (iii) on the day a flock was processed (range: 55-97 days). If the farm contained other types of animals, samples were also collected from these animals to get an understanding of the overall *Campylobacter* prevalence on the farm. This group of samples was used in model construction and initial testing. In total, there were 772 soil and 774 fecal samples collected that were used. Additionally, four farms were visited during August to November of the 2019 growing season, where we collected 90 fecal and 70 soil samples across 18 and 14 sampling days, respectively. These samples were intended to act as an independent dataset for testing the predictive accuracy of the developed models at predicting *Campylobacter* spp. presence in fecal and soil samples based on seven-day weather forecasts.

On each sampling day, fecal and soil samples were collected from the area where a flock resided on the previous day. The sampling site was divided into five sections; five subsamples were collected and pooled from each section. Samples were pooled due to the high variability

expected from each subsample and for the possibility that there would be low numbers of *Campylobacter (3, 36)*. Fecal samples were fresh droppings at the sampling site. Soil samples were collected by scooping surface level soil (approximately 0-7 cm from the surface) into sterile bags. Sterile scoops were used for each sample, and scoops and gloves were changed after each sample. All pooled samples were at least 25 g. Samples were immediately transferred on ice to a laboratory for processing within 3 hours.

5.2.2 Sample preparation

Upon arrival in the laboratory, samples were prepared as previously described by Rothrock and Locatelli *(32)*. Briefly, 3 g from each subsample were combined in a filtered stomacher bag (Seward Laboratory Systems, Inc., Davie, FL) and diluted 1:3 with 10 mM phosphate buffered saline (PBS) and stomached for 1 min. Next, 100 μL of stomached sample was plated onto Campy-Cefex agar and incubated at 42 ± 1°C in microaerophilic conditions (85% N_2, 10% CO_2, 5% O_2) for 36-48 h *(40)*. Putative *Campylobacter* colonies were enumerated for each plate, and up to 5 suspected colonies were transferred to *Brucella* agar supplemented with 10% lysed horse blood for confirmation and incubated as previously described. For model development purposes, samples were classified as positive if countable colonies were found during Campy-Cefex plating.

5.2.3 Historical weather data collection

On each sampling date during phase one sampling, weather data were collected for the zip code of the sampled farm. These data were used to represent the predictors of *Campylobacter* presence during model development. Temperature (°C; minimum, maximum, and daily average), wind speed (m/s; maximum and daily average), wind gust speed (m/s; maximum), humidity (%; minimum, maximum, and daily average), and precipitation (mm) values were collected for one

day before sampling up to seven days before sampling. To represent short-term weather effects, values for one and two days before the sampling day were specifically noted *(13)*. Additionally, averages between one day before sampling and each day up to seven days before sampling were calculated to represent the over-time effect of weather *(20)*. This resulted in a total of 80 predictors. During this phase, historical weather data were collected from Weather Underground (https://www.wunderground.com/) and the National Oceanic Atmospheric Administration weather database (https://www.ncdc.noaa.gov/cdo-web/).

5.2.4 Model development

Fisher's exact test was used to compare prevalence of *Campylobacter* across season and sample type. The Holm-Bonferroni correction was used for each seasonal pairwise comparison *(17)*. For all statistical analyses, p values less than 0.05 were considered statistically significant.

Before model fitting, data for each sample type were split into training and testing sets using stratified random sampling. The training set included 80% of the data, with the testing set containing the remaining 20%. To correct for class imbalance within the soil testing set, the synthetic minority oversampling technique (SMOTE) was used to generate synthetic minority class samples to improve model performance *(10)*. Random forest models were trained on the training set of each type of preharvest sample, and the testing set was held-out for model evaluation. Models were trained using the RandomForestClassifier function from Scikit-Learn 0.21.2 *(31)*. A randomized grid search with 10-fold cross-validation including the following hyperparameters was used for model tuning and selection: "max_depth" (maximum tree depth), "max_features" (number of predictors to consider when determining the best split), "min_samples_split" (minimum number of samples required for a split), "min_samples_leaf" (minimum number of samples present in a leaf node), "criterion" (metric to evaluate the quality

of a split), and “n_estimators” (number of trees grown). Randomized grid searches have been shown to be more effective than an exhaustive grid search for hyperparameter tuning *(4)*. The receiver operating characteristic (ROC) metric was used to evaluate the grid search. For the feces model, the best fit hyperparameters were “max_depth” = “None”, “max_features” = 80, “min_samples_split” = 8, “min_samples_leaf” = 1, “criterion” = “entropy”, and “n_estimators” = 500. For the soil model, “max_depth” = “None”, “max_features” = 41, “min_samples_split” = 4, “min_samples_leaf” = 1, “criterion” = “entropy”, and “n_estimators” = 800. Variable importance plots were then generated to determine the most important predictors in each model. Finally, the testing set was used to determine the accuracy of each RF model.

5.2.5 Forecast data collection and forecasting validation

Phase two samples were used to test model performance at predicting *Campylobacter* presence based on forecasted weather. Predictions were made for each of seven days leading up to the sampling day. During each day, various combinations of forecasted and actually observed weather were explored. For example, predictions made seven days before the sampling day used the seven-day forecast made on the seventh day before the sampling day to represent the 80 predictors used in the model. Conversely, predictions made one day before the sampling day used the forecasted weather for that day and 6 days of historical weather data to represent the 80 model predictors. This concept is further explained in Fig. 5.1. Based on the inputted weather data, prevalence probabilities were obtained from both models for each forecast day. During this phase, forecasted and historical weather data were collected from the Dark Sky API (https://darksky.net/dev).

5.2.6 Statistical analysis

All statistical analysis was performed in Python 3.7.3 (https://www.python.org/). Receiver operating characteristic (ROC) curves, area under the ROC curve (AUC), sensitivity, and specificity were used to quantify initial model accuracy on the held-out test set *(7)*. To determine the forecasting ability of the models, predicted prevalence probabilities for each forecast day were compared with observed sample day prevalence. Mean absolute deviation (MAD) and bias % were calculated for each forecast day and sample type combination:

$$\text{MAD} = \frac{1}{n}\sum_{i=1}^{n}|\text{predicted prevalence} - \text{observed prevalence}| \quad (1)$$

$$\text{Bias \%} = \frac{\sum_{i=1}^{n}\text{predicted prevalence}}{\sum_{i=1}^{n}\text{observed prevalence}} \quad (2)$$

where *n* represented the number of sample days for each sample type. A one-way analysis of variance (ANOVA) was performed to determine if forecast day had a significant effect on MAD. Model code can be accessed on GitHub (https://github.com/cgolden1993/Dissertation).

5.3 Results

Campylobacter prevalence values for fecal and soil samples collected during both phases of sample collection are shown in Table 5.1. In model development and testing, *Campylobacter* prevalence was significantly greater ($p < 0.0001$) for fecal samples than soil samples. Similar *Campylobacter* prevalence was observed for samples used in model construction/testing and in forecast validation. However, soil samples used during forecast validation had a significantly greater ($p < 0.001$) *Campylobacter* prevalence than samples used during model construction/testing.

A seasonal breakdown of *Campylobacter* prevalence for fecal and soil samples used during model construction/testing is shown in Table 5.2. For fecal samples, there was no

seasonal effect ($p = 0.67$). For soil samples, spring prevalence was significantly higher (adjusted $p < 0.001$) than summer and fall prevalence.

The predictive performance of both models was tested on the held-out test set (Table 5.3). These statistics are intended to detail model accuracy on an independent dataset with similar structure to what was used to construct the models. The feces model performed very well with an AUC of 0.959 (Fig. 5.2a). Additionally, it showed the ability to accurately predict true positives while minimizing the number of false negatives, with a sensitivity value of 0.957. The soil model also performed well on the held-out test set (AUC = 0.886; Fig 5.2b), but only had a sensitivity of 0.772.

All 80 predictors were ranked in terms of their importance for both models. The two most important predictors for the feces model were the average maximum wind gust speed between the day before sampling and six days prior and the average humidity between the day before sampling and seven days prior (Fig. 5.3). All 5 of the top predictors were related to maximum wind gust speed and average humidity. For the soil model, the top two predictors were average temperature one day prior to sampling and maximum humidity two days before sampling (Fig. 5.4).

Additionally, the ability for both models to predict *Campylobacter* based on forecasted weather was tested (Table 5.4). Mean absolute deviation represented the average difference between the predicted prevalence and the observed prevalence. For both feces and soil models, forecasting day did not have a significant effect on MAD, as determined by one-way ANOVA. For both models, across all forecast days, MAD was between 0.29 and 0.36. These numbers are likely impacted by the days in which 0% prevalence and 100% prevalence was observed, as model predictions ranged from 12 to 75%, and thus were not fit to model such extreme sampling

days. Bias % was also assessed for each model to determine whether the models tended to over or under-predict based on forecasted weather data. Values above 100% reflected fail-safe predictions and values below 100% reflected fail-dangerous predictions. For the feces model, bias % values ranged from 87.3 to 106.4%. This shows that the model did not tend to favor over or under-predictions. On the other hand, the soil model tended to under-predict prevalence across all seven forecast days with values ranging from 66 to 75.8%. This could be due to the fact that *Campylobacter* prevalence was significantly higher in forecast validation samples when compared to the samples used during model construction/testing.

5.4 Discussion

One of the most important factors in limiting *Campylobacter* contamination of broilers is to reduce the birds' exposure to the microorganism. The outside environment has been identified as a major source of contamination, and bird-to-bird transmission of the microorganism can occur rapidly once a flock becomes infected *(18, 27, 37)*. This fact is especially important to consider for alternative production systems, like pastured poultry production, as birds are allowed more access to the outside environment than conventionally produced birds. While surveys have been conducted to analyze *Campylobacter* prevalence in environmental samples *(2, 16, 23, 35, 42, 43)*, research on causes of environmental contamination are still lacking. The current study utilized a machine learning approach to determine key meteorological factors that are associated with prevalence of *Campylobacter* in fecal and soil samples collected from pastured poultry farms. The generated models can act as a tool to help enhance environmental surveillance of *Campylobacter* based on recently observed and predicted weather by providing poultry producers recommendations on when to employ strategies that could potentially decrease birds' exposure to the pathogen. Risk-mitigation strategies could include moving birds to higher

topographical areas in case of precipitation events, more intensive environmental sampling, and changing the area of pasture or outdoor area that the birds are residing.

Two types of environmental samples were collected from poultry farms in the current study. Both fecal and soil samples have been recognized as potential sources of contamination for broilers *(29)*. Separate random forest models were constructed for each sample type due to the significant difference in prevalence observed in the samples that were used for model construction and testing (Table 5.1). Similar *Campylobacter* fecal prevalence was found by Berghaus et al. *(2)*, who isolated the organism in 51.4% of fecal samples. In the current study, year of collection did not have an effect on *Campylobacter* prevalence in fecal samples. However, *Campylobacter* soil prevalence was significantly higher in 2019 samples than in 2014-2017 samples (Table 5.1). Other surveys have been conducted to observe *Campylobacter* prevalence in soil samples. Trimble et al. *(43)* isolated *Campylobacter* from 64.3% of soil samples collected from pastured poultry farms. Conversely, Schets et al. *(34)* only isolated *Campylobacter* from 7.4% of soil samples collected from broiler farms with positive broiler flocks. These results show the variability in *Campylobacter* prevalence numbers for soil samples collected from broiler farms. This means that the proposed model could have potentially been biased from the samples used during model construction. It is possible that a larger number of samples need to be collected in the future to better capture the distribution of soil prevalence numbers.

The effect of seasonality was determined for fecal and soil samples that were included in model construction/testing (Table 5.2). For fecal samples, no effect of season was observed, but significantly more positive soil samples were found in the springtime. Many other studies have attempted to quantify the seasonality of *Campylobacter* in broiler-related samples. Willis and

Murray *(48)* found *Campylobacter* prevalence in retail broiler meat to be highest from May to October. Another study found retail *Campylobacter* prevalence to peak in May *(26)*. Despite these findings, studies have found that seasonal patterns in *Campylobacter* prevalence on chicken do not match up with the seasonal patterns in campylobacteriosis cases in the United States and Europe *(26, 47)*.

Similar approaches to the current study have been followed in other food safety-focused studies *(13, 28, 41)*. The usefulness of the random forest modeling approach to predict pathogen prevalence was shown by Golden et al. *(13)* and Pang et al. *(28)*, who used the model to effectively predict *Listeria* spp. prevalence in broiler and produce farming environments, respectively, based on recently observed weather patterns. The predictive performance of models generated in the current study are similar to these studies, with AUC values > 0.88 (Table 5.3). Similarly, Strawn et al. *(41)* used temporal and meteorological data to construct classification trees to predict *Salmonella* and *Listeria monocytogenes* prevalence on produce farms. Due to the possibility of covariation among predictors used, the authors used principal component analysis (PCA) as part of their modeling approach. One of the advantages of random forest models is that the model is robust to this kind of multicollinearity *(25)*, therefore PCA was not used in the current study.

The results of the current study demonstrate the ability to predict *Campylobacter* prevalence in fecal and soil samples based on observed weather patterns. For the feces model, maximum wind gust speed and average humidity variables were identified as the most important meteorological predictors. Relative humidity was previously shown to be an important factor in determining *Campylobacter* prevalence in laying hens *(11)* and wind gust speed has been shown to be associated with *Salmonella* prevalence in fecal and soil samples collected from pastured

poultry farms *(19)*. Similarly, humidity was deemed important by our models in predicting soil *Campylobacter* prevalence. Minimum and maximum temperature were also identified as important meteorological factor. Wallace et al. *(46)* also identified minimum and maximum temperature to be significant factors in relation to *Campylobacter* prevalence in chickens.

To our knowledge, this is the first time that predictive models have been tested to predict foodborne pathogen prevalence in farming environments based on weather forecasts. Due to the unpredictability of weather forecasts and the lack of publicly available, reliable data beyond seven days in advance, predictions were made from one to seven days before the day of sampling, using combinations of forecasted and observed weather data, to determine if predictions made closer to the sampling day were more accurate than those made earlier. Bias % for the feces model ranged from 87-106%, suggesting good prediction ability of the model. This reflects the high model sensitivity observed during initial model testing. The soil model, on the other hand, had lower bias %, as compared to the feces model. As mentioned previously, this could be due to the significant difference in *Campylobacter* prevalence observed in samples used during model construction and those used in forecasting validation. A benefit of machine learning models is that the models can be updated when introduced to more data. Thus, generated models can continue to learn from prevalence data that will be collected in the future, to better handle data with high variability. One limitation to the current study was that, due to limited resources, only 5 samples were collected on each sampling day, giving prevalence possibilities of 0, 20, 40, 60, 80, and 100%. This makes it possible that each model was penalized more heavily in regard to MAD than it would have been if more samples had been collected during each sampling day. With more data collected in the future, the prediction ability of the feces and soil models may be enhanced. The feces model was robust in terms of bias %

with predictions made up to seven days in advance, but the soil model performed better if predictions were made closer to the sampling day.

In conclusion, two random forest models were generated to predict *Campylobacter* prevalence in fecal and soil samples collected from southeastern United States pastured poultry farms based on weather data. Models performed well on a held-out test set when testing their ability to predict prevalence based on historically observed weather data. The generated models will help provide poultry farmers and producers with a means to make risk-based decisions when trying to control for environmental *Campylobacter* contamination.

5.5 Acknowledgements

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5.6 References

1. Barbut, S., L. F. Moza, F. Nattress, B. Dilts, and C. O. Gill. 2009. The microbiological conditions of air-or water-chilled carcasses produced at the same poultry packing plant. *J. Appl. Poult. Res.* 18:501-507.

2. Berghaus, R. D., S. G. Thayer, B. F. Law, R. M. Mild, C. L. Hofacre, and R. S. Singer. 2013. Enumeration of *Salmonella* and *Campylobacter* spp. in environmental farm samples and processing plant carcass rinses from commercial broiler chicken flocks. *Appl. Environ. Microbiol.* 79:4106-4114.

3. Bergholz, P. W., J. D. Noar, and D. H. Buckley. 2011. Environmental patterns are imposed on the population structure of *Escherichia coli* after fecal deposition. *Appl. Environ. Microbiol.* 77:211-219.

4. Bergstra, J., and Y. Bengio. 2012. Random search for hyper-parameter optimization. *J. Mach. Learn. Res.* 13:281-305.

5. Berrang, M. E., R. J. Buhr, J. A. Cason, and J. A. Dickens. 2001. Broiler carcass contamination with *Campylobacter* from feces during defeathering. *J. Food Prot.* 64:2063-2066.

6. Berrang, M. E., R. J. Meinersmann, S. R. Ladely, and N. A. Cox. 2017. *Campylobacter* detection in broiler ceca at processing: A three-year, 211-flock survey. *J. Appl. Poult. Res.* 26:154-158.

7. Bradley, A. P. 1997. The use of the area under the ROC curve in the evaluation of machine learning algorithms. *Pattern Recogn.* 30:1145-1159.

8. Breiman, L. 2001. Random forests. *Mach. Learn.* 45:5-32.

9. Centers for Disease Control and Prevention. 2020. National Outbreak Reporting System (NORS), Outbreaks per state, *Campylobacter*, poultry. Available at: https://wwwn.cdc.gov/norsdashboard/. Accessed 6 February 2020.

10. Chawla, N. V., K. W. Bowyer, L. O. Hall, and W. P. Kegelmeyer. 2002. SMOTE: Synthetic minority over-sampling technique. *J. Artif. Intell. Res.* 16:321-357.

11. Doyle, M. P. 1984. Association of *Campylobacter jejuni* with laying hens and eggs. *Appl. Environ. Microbiol.* 47:533-536.

12. Epps, S., R. Harvey, M. Hume, T. Phillips, R. Anderson, and D. Nisbet. 2013. Foodborne *Campylobacter*: Infections, metabolism, pathogenesis and reservoirs. *Int. J. Environ. Res. Public Health*. 10:6292-6304.

13. Golden, C. E., M. J. Rothrock Jr, and A. Mishra. 2019. Comparison between random forest and gradient boosting machine methods for predicting *Listeria* spp. prevalence in the environment of pastured poultry farms. *Food Res. Int.* 122:47-55.

14. Golden, C. E., M. J. Rothrock Jr, and A. Mishra. 2019. Using farm practice variables as predictors of *Listeria* spp. prevalence in pastured poultry farms. *Front. Sustain. Food Syst.* 3:15.

15. Guerin, M. T., S. W. Martin, J. Reiersen, O. Berke, S. A. McEwen, V. Friðriksdóttir, J. Bisaillon, and R. Lowman. 2008. Temperature-related risk factors associated with the colonization of broiler-chicken flocks with *Campylobacter* spp. in Iceland, 2001–2004. *Prev. Vet. Med.* 86:14-29.

16. Hanning, I., D. Biswas, P. Herrera, M. Roesler, and S. C. Ricke. 2010. Prevalence and characterization of *Campylobacter jejuni* isolated from pasture flock poultry. *J. Food Sci.* 75:M496-502.

17. Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* 6:65-70.

18. Humphery, T. J., A. Henley, and D. G. Lanning. 1993. The colonization of broiler chickens with *Campylobacter jejuni*: Some epidemiological investigations. *Epidemiol. Infect.* 110:601-607.

19. Hwang, D., M. J. Rothrock Jr, H. Pang, M. Guo, and A. Mishra. 2020. Predicting *Salmonella* prevalence associated with meteorological factors in pastured poultry farms in southeastern United States. *Sci. Total Environ.* 713:136359.

20. Ivanek, R., Y. T. Gröhn, M. T. Wells, A. J. Lembo, B. D. Sauders, and M. Wiedmann. 2009. Modeling of spatially referenced environmental and meteorological factors influencing the probability of *Listeria* species isolation from natural environments. *Appl. Environ. Microbiol.* 75:5893-5909.

21. Kaakoush, N. O., N. Castaño-Rodríguez, H. M. Mitchell, and S. M. Man. 2015. Global epidemiology of *Campylobacter* infection. *Clin. Microbiol. Rev.* 28:687-720.

22. Kim, S., Z. Yu, R. M. Kil, and M. Lee. 2015. Deep learning of support vector machines with class probability output networks. *Neural Netw.* 64:19-28.

23. Luangtongkum, T., T. Y. Morishita, L. Martin, I. Choi, O. Sahin, and Q. Zhang. 2008. Prevalence of tetracycline-resistant *Campylobacter* in organic broilers during a production cycle. *Avian Dis.* 52:487-490.

24. Maciorowski, K. G., F. T. Jones, S. D. Pillai, and S. C. Ricke. 2004. Incidence, sources, and control of food-borne *Salmonella* spp. in poultry feeds. *Worlds Poult. Sci. J.* 60:446-457.

25. Matsuki, K., V. Kuperman, and J. A. Van Dyke. 2016. The Random Forests statistical technique: An examination of its value for the study of reading. *Sci. Stud. Read.* 20:20-33.

26. Meldrum, R. J., J. K. Griffiths, R. M. M. Smith, and M. R. Evans. 2005. The seasonality of human *Campylobacter* infection and *Campylobacter* isolates from fresh, retail chicken in Wales. *Epidemiol. Infect.* 133:49-52.

27. Newell, D. G., and C. Fearnley. 2003. Sources of *Campylobacter* colonization in broiler chickens. *Appl. Environ. Microbiol.* 69:4343-4351.

28. Pang, H., R. McEgan, A. Mishra, S. A. Micallef, and A. K. Pradhan. 2017. Identifying and modeling meteorological risk factors associated with pre-harvest contamination of *Listeria* species in a mixed produce and dairy farm. *Food Res. Int.* 102:355-363.

29. Patriarchi, A., A. Fox, B. Maunsell, S. Fanning, and D. Bolton. 2011. Molecular characterization and environmental mapping of *Campylobacter* isolates in a subset of intensive poultry flocks in Ireland. *Foodborne Pathog. Dis.* 8:99-108.

30. Patrick, M. E., L. E. Christiansen, M. Wainø, S. Ethelberg, H. Madsen, and H. C. Wegener. 2004. Effects of climate on incidence of *Campylobacter* spp. in humans and prevalence in broiler flocks in Denmark. *Appl. Environ. Microbiol.* 70:7474-7480.

31. Pedregosa, F., G. Varoquaux, A. Gramfort, V. Michel, B. Thirion, O. Grisel, M. Blondel, P. Prettenhofer, R. Weiss, and V. Dubourg. 2011. Scikit-learn: Machine learning in Python. *J. Mach. Learn. Res.* 12:2825-2830.

32. Rothrock, M. J., and A. Locatelli. 2019. Importance of farm environment to shape poultry-related microbiomes throughout the farm-to-fork continuum of pasture-raised broiler flocks. *Front. Sust. Food Syst.* 3:1-15.

33. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—Major pathogens. *Emerg. Infect. Dis.* 17:7-15.

34. Schets, F. M., W. F. Jacobs-Reitsma, R. Q. van der Plaats, L. K. Heer, A. H. van Hoek, R. A. Hamidjaja, A. M. de Roda Husman, and H. Blaak. 2017. Prevalence and types of *Campylobacter* on poultry farms and in their direct environment. *J. Water Health*. 15:849-862.

35. Schroeder, M. W., J. D. Eifert, M. A. Ponder, and D. G. Schmale. 2014. Association of *Campylobacter* spp. levels between chicken grow-out environmental samples and processed carcasses. *Poult. Sci.* 93:734-741.

36. Semenov, A. V., E. Franz, L. van Overbeek, A. J. Termorshuizen, and A. H. C. van Bruggen. 2008. Estimating the stability of *Escherichia coli* O157:H7 survival in manure-amended soils with different management histories. *Environ. Microbiol.* 10:1450-1459.

37. Shreeve, J. E., M. Toszeghy, M. Pattison, and D. G. Newell. 2000. Sequential spread of *Campylobacter* infection in a multipen broiler house. *Avian Dis.* 44:983-988.

38. Smith, A., B. Sterba-Boatwright, and J. Mott. 2010. Novel application of a statistical technique, Random Forests, in a bacterial source tracking study. *Water Res.* 44:4067-4076.

39. Soneja, S., C. Jiang, C. R. Upperman, R. Murtugudde, C. S. Mitchell, D. Blythe, A. R. Sapkota, and A. Sapkota. 2016. Extreme precipitation events and increased risk of campylobacteriosis in Maryland, USA. *Environ. Res.* 149:216-221.

40. Stern, N. J., B. Wojton, and K. Kwiatek. 1992. A differential-selective medium and dry ice-generated atmosphere for recovery of *Campylobacter jejuni*. *J. Food Prot.* 55:514-517.

41. Strawn, L. K., E. D. Fortes, E. A. Bihn, K. K. Nightingale, Y. T. Gröhn, R. W. Worobo, M. Wiedmann, and P. W. Bergholz. 2013. Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. *Appl. Environ. Microbiol.* 79:588-600.

42. Thakur, S., J. Brake, S. Keelara, M. Zou, and E. Susick. 2013. Farm and environmental distribution of *Campylobacter* and *Salmonella* in broiler flocks. *Res. Vet. Sci.* 94:33-42.

43. Trimble, L. M., W. Q. Alali, K. E. Gibson, S. C. Ricke, P. Crandall, D. Jaroni, M. Berrang, and M. Y. Habteselassie. 2013. Prevalence and concentration of *Salmonella* and *Campylobacter* in the processing environment of small-scale pastured broiler farms. *Poult. Sci.* 92:3060-3066.

44. van Immerseel, F., L. De Zutter, K. Houf, F. Pasmans, F. Haesebrouck, and R. Ducatelle. 2009. Strategies to control *Salmonella* in the broiler production chain. *Worlds Poult. Sci. J.* 65:367-392.

45. Volkova, V. V., R. H. Bailey, and R. W. Wills. 2009. *Salmonella* in broiler litter and properties of soil at farm location. *PLOS ONE*. 4:e6403.

46. Wallace, J. S., K. N. Stanley, J. E. Currie, P. J. Diggle, and K. Jones. 1997. Seasonality of thermophilic *Campylobacter* populations in chickens. *J. Appl. Microbiol.* 82:219-224.

47. Williams, M. S., N. J. Golden, E. D. Ebel, E. T. Crarey, and H. P. Tate. 2015. Temporal patterns of *Campylobacter* contamination on chicken and their relationship to campylobacteriosis cases in the United States. *Int. J. Food Microbiol.* 208:114-121.

48. Willis, W. L., and C. Murray. 1997. *Campylobacter jejuni* seasonal recovery observations of retail market broilers. *Poult. Sci.* 76:314-317.

Table 5.1 *Campylobacter* prevalence in fecal and soil samples collected from United States pastured poultry farms that were used for initial random forest model training and testing and forecast validation

Model stage	Sample type	No. of samples	No. (%) positive samples[a]
Training and testing	Feces	774	466 (60.2) A
	Soil	772	181 (23.4) B
Forecast validation	Feces	90	49 (54.4) A
	Soil	70	42 (60.0) A
Total		1,546	647 (41.8)

[a] Different capital letters represent statistically significant different values within a model stage ($p < 0.05$ as determined by Fisher's exact test)

Table 5.2 *Campylobacter* prevalence by season for samples in fecal and soil samples collected from United States pastured poultry farms that were used for initial random forest model training and testing

Sample type	**Season**	**No. of samples**	**No. (%) positive samples**[a]
Feces	Spring	187	107 (57.2) A
	Summer	422	254 (60.2) A
	Fall	150	95 (63.3) A
	Winter	15	10 (66.7) A
Soil	Spring	186	72 (38.7) A
	Summer	426	86 (20.2) B
	Fall	150	20 (13.3) B
	Winter	10	3 (30.0) AB

[a] Different letters within a sample type represent statistically significant different values (Holm-Bonferroni corrected p values < 0.05 as determined by Fisher's exact test)

Table 5.3 Predictive performance of the generated random forest models

Model predictions		Observed		Sensitivity	Specificity	AUC[a]
		Positive	**Negative**			
Feces model	Positive	89	13	0.957	0.790	0.959
	Negative	4	49			
Soil model	Positive	26	13	0.722	0.891	0.886
	Negative	10	106			

[a] Area under the receiver operating characteristic curve (AUC)

Table 5.4 Forecasting capabilities of the generated random forest models

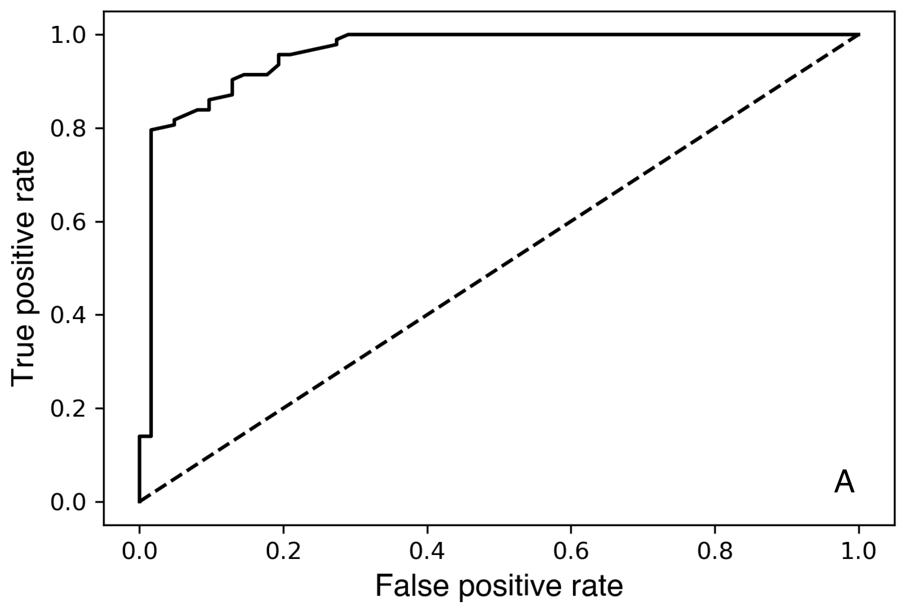
Forecast	Feces model		Soil model	
	MAD[a]	Bias %	MAD	Bias %
1-day	0.292	106.4	0.355	75.8
2-day	0.311	95.7	0.350	69.0
3-day	0.335	92.2	0.324	65.9
4-day	0.324	97.4	0.308	66.5
5-day	0.320	106.1	0.321	67.2
6-day	0.358	87.3	0.337	66.0
7-day	0.345	88.0	0.336	66.6

[a] Mean absolute deviation (MAD)

Days before sampling day

Sampling day	1	2	3	4	5	6	7
Sampling day	1	2	3	4	5	6	7
Sampling day	1	2	3	4	5	6	7
Sampling day	1	2	3	4	5	6	7
Sampling day	1	2	3	4	5	6	7
Sampling day	1	2	3	4	5	6	7
Sampling day	1	2	3	4	5	6	7

Figure 5.1 A schematic detailing predictions made to determine model accuracy based on forecasted weather. Underlined numbers represent the number of days before the sampling day the predictions were made. Boxed in days are days in which forecasted weather data were used, while days not boxed in represent days in which historical data were used for a given forecast day.



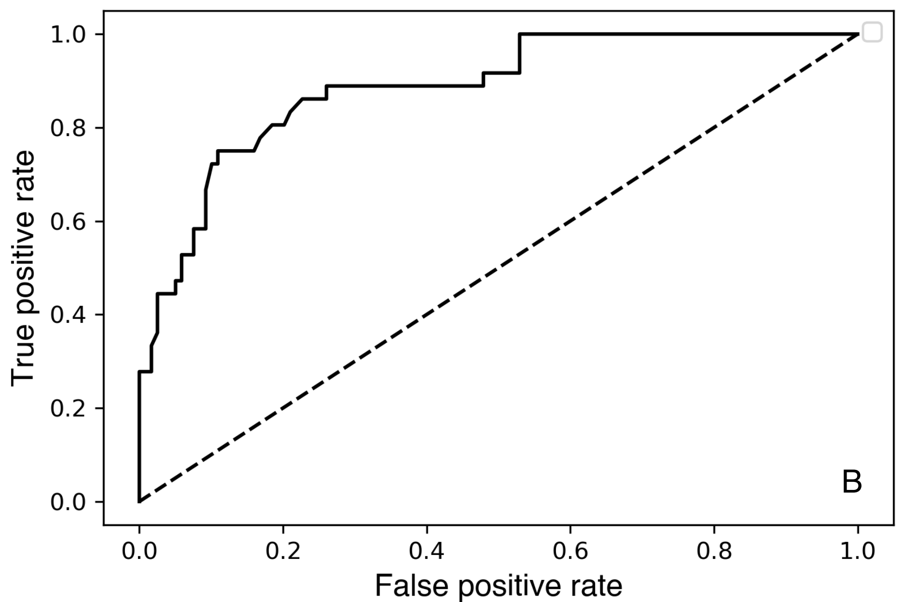


Figure 5.2 Receiver operating characteristic (ROC) curves for the (a) feces and (b) soil random forest models. The dotted line represents the line of no discrimination, or the expectation of a random guess.

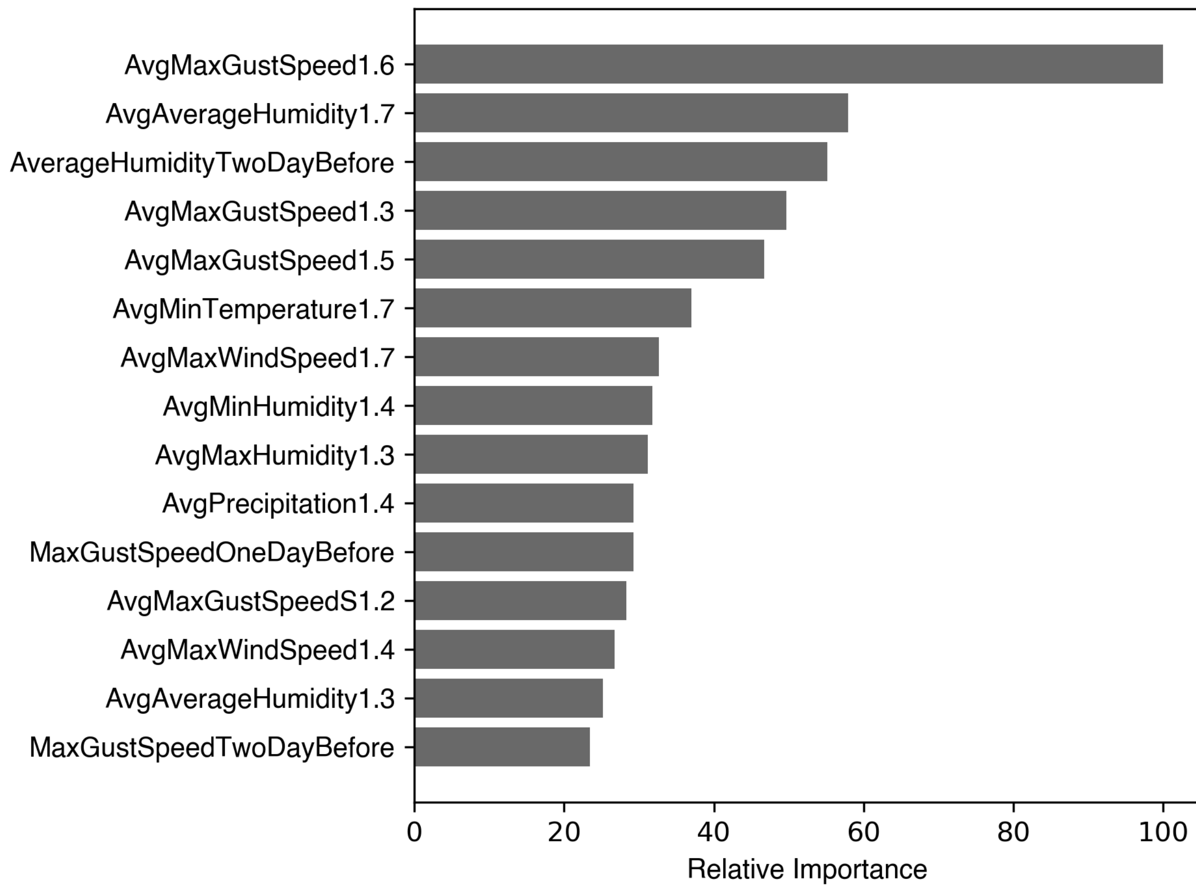


Figure 5.3 Variable importance plot for the feces random forest model. Variables were assigned a relative importance value on a scale of 0 to 100, where a higher importance score means higher relative importance. Variable names with a decimal are the average of that variable from 1 day before sampling to X days before sampling, where X is the number of days before the sampling day. Variable names containing 'Before' represent variable values collected X days before sampling.

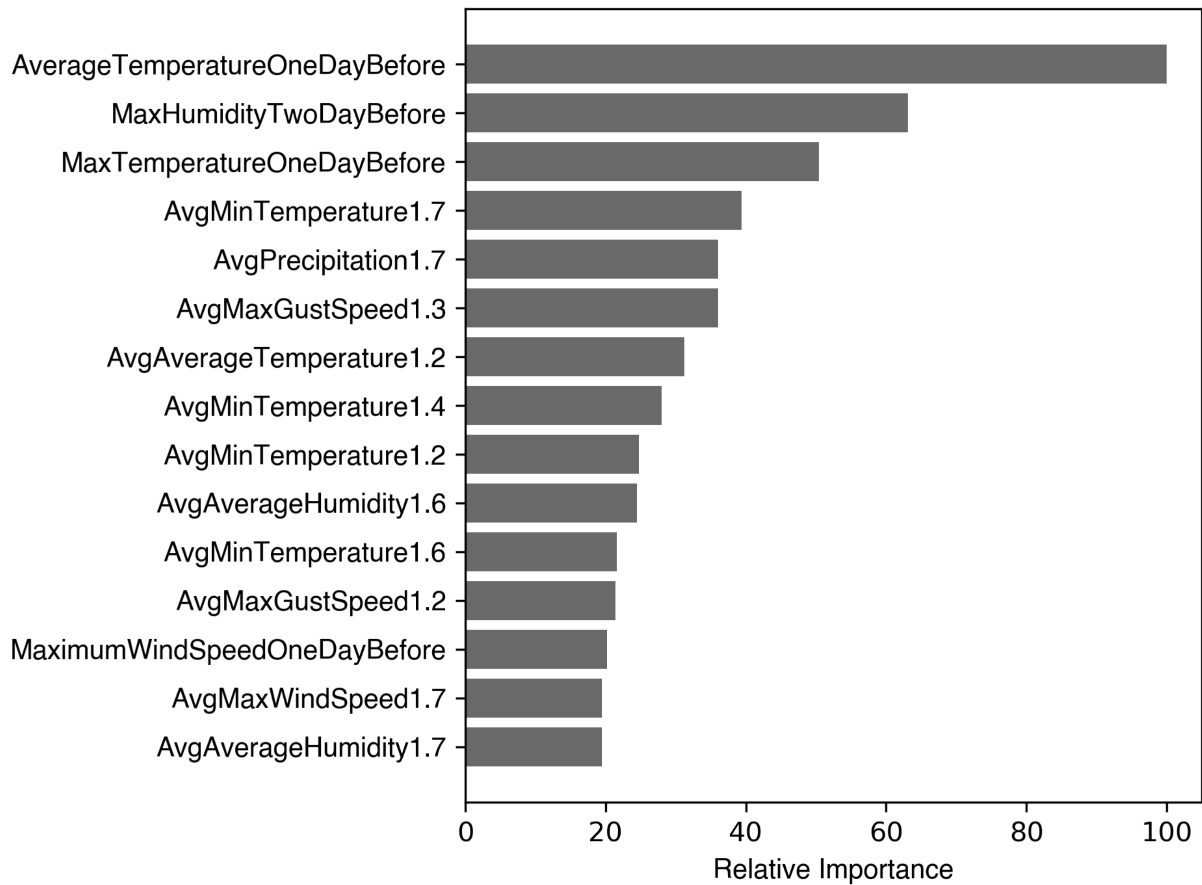


Figure 5.4 Variable importance plot for the soil random forest model. Variables were assigned a relative importance value on a scale of 0 to 100, where a higher importance score means higher relative importance. Variable names with a decimal are the average of that variable from 1 day before sampling to X days before sampling, where X is the number of days before the sampling day. Variable names containing 'Before' represent variable values collected X days before sampling.

CHAPTER 6

PREVALENCE OF *SALMONELLA* SPP. AND *CAMPYLOBACTER* SPP. IN ALTERNATIVE AND CONVENTIONALLY PRODUCED CHICKEN IN THE UNITED STATES: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Abstract

The burden of foodborne illness linked to the consumption of contaminated broiler meat is high in the United States. With the increase in popularity of alternative poultry rearing and production systems, it is important to determine if there is a difference in food safety risks presented by alternative systems when compared to conventional methods. While many studies have been conducted surveying foodborne pathogen prevalence along the broiler supply chain, a systematic overview of all of the available results is lacking. In the current study, a systematic review and meta-analysis was conducted to quantify the differences in *Salmonella* spp. and *Campylobacter* spp. prevalence in farming environment, rehang, prechill, postchill, and retail samples between conventional and alternative production systems. A systematic search of Web of Science and PubMed databases was conducted to identify eligible studies. Studies were then evaluated by inclusion criteria, and included studies were qualitatively and quantitatively analyzed. In total, 137 trials from 72 studies were used in the final meta-analysis. Meta-analysis models were individually constructed for subgroups that were determined by sample type, pathogen, and production type. All subgroups possessed high amounts of heterogeneity ($I^2 >$ 75%). For environmental sample subgroups, *Campylobacter* prevalence was estimated to be 15.8 and 52.8% for conventional and alternative samples, respectively. Similar prevalence estimates for both production types were observed for *Salmonella* environmental samples and all retail samples. For conventional samples, *Campylobacter* and *Salmonella* prevalence was highest in samples of prechill carcasses followed by carcasses at rehang and postchill, respectively. The presented results will be of use in future quantitative microbial risk assessments to characterize the differences in foodborne illness risks presented by different broiler production systems.

6.1 Introduction

Foodborne pathogens such as *Campylobacter* spp. and *Salmonella* spp. have presented a major problem for the microbial safety of broiler chicken and chicken products in the United States supply chain. From 1998-2017, there were 298 chicken-related salmonellosis outbreaks resulting in 7,881 illnesses, 905 hospitalizations, and 4 deaths *(16)*. *Campylobacter* spp. are estimated to cause over 800,000 domestically acquired foodborne illnesses annually *(92)*. Additionally, poultry products were implicated in 15 campylobacteriosis outbreaks in the United States from 2004-2012 *(33)*. While much of the efforts are put into controlling *Salmonella* and *Campylobacter* in fresh poultry, *Listeria* spp. have been identified as an emerging poultry-related pathogen *(86)*.

In recent years, the demand for organic and alternatively produced food products has increased; retail sales of organic food products increased from $3.6 billion to $21.1 billion from 1997 to 2008 *(25)*. This trend has also impacted the poultry industry, with organic, free-range, antibiotic-free, and pastured poultry operations becoming more desired. As popularity of these products increases, the need to understand the food safety hazards around these products becomes imperative. Many consumers believe these types of products to be safer due to the reduced use of pesticides and antibiotics, but scientific evidence to support this hypothesis is lacking *(48, 100, 115)*.

Currently, most of the effort in quantifying the differences between conventional and alternative poultry production has gone into microbiological surveying at various points along the poultry supply chain. A useful tool in helping to quantify efforts of similar studies is through systematic review and meta-analysis. Meta-analysis is used to aggregate the results of individual studies, quantify the estimated effect of each study, and provide an overall estimate on the effect

and variability of an intervention or treatment or overall prevalence of an outcome *(34)*. While widely used in the field of medicine, meta-analyses have just started gaining popularity in the food safety literature, with recommendations to utilize the tool in a food safety context by Gonzales-Barron and Butler *(36)* and Sargeant et al. *(91)*. Meta-analysis results are important tools in quantitative microbial risk assessments (QMRAs); they provide an estimate of pathogen prevalence or reduction at certain stages of a product's supply chain, potentially providing a more accurate number than an estimate based on one study or on expert judgement *(36)*.

Recent poultry-related systematic reviews and meta-analyses have been conducted to estimate the prevalence of foodborne pathogens in poultry samples and the effectiveness of various interventions in reducing foodborne pathogen load in poultry *(14, 35, 53, 108, 118, 124)*. A meta-analysis conducted by Young et al. *(124)* worked to address differences in foodborne pathogen numbers between organic and conventional poultry samples but was conducted with international data. There have been numerous recent surveys published on foodborne pathogen prevalence in conventional and alternative broiler chicken samples, showing the need for an updated systematic review and meta-analysis. To our knowledge, there are no current meta-analysis studies that were used to estimate the prevalence of foodborne pathogens in United States broiler chicken samples in conventional and alternative poultry systems.

The purpose of the current study was to use a systematic review and meta-analysis approach to quantify the prevalence numbers of problematic foodborne pathogens in broiler chicken farming, processing, and retail samples in the United States. The results of this study will aid in the construction of QMRAs related to the differences between conventional and alternative broiler chicken production systems and their impact on domestic foodborne illness.

6.2 Materials and methods

6.2.1 Literature search strategy

A systematic review process was adapted from Sargeant et al. *(90)* to address a detailed research question: What are the differences in foodborne pathogen prevalence in farm environment, processing, and retail samples from commercial alternative and conventional broiler chicken production systems in the United States? Foodborne pathogens of interest included *Campylobacter* spp., *Salmonella* spp., and *Listeria* spp. To address this question, a detailed literature search was performed. On September 26, 2019, the Web of Science (www.webofknowledge.com) and PubMed online databases were searched with the following search terms aimed at addressing the aforementioned research question: ("*Salmonella*" OR "*S. enterica*" OR "*Salmonella enterica*" OR "*Campylobacter*" OR "*C. jejuni*" OR "*Campylobacter jejuni*" OR "*Listeria*" OR "*L. monocytogenes*" OR "*Listeria monocytogenes*") AND ("poultry" OR "chicken") AND ("incidence" OR "prevalence" OR "isolation" OR "survey" OR "detection" OR "occurrence") AND ("United States" OR "name of any individual state"). For this step, there were no language or year limitations. With these criteria, the search totaled 2,356 studies. Additional studies (n=7) were identified by hand-searching review articles and article reference lists. Studies included peer-reviewed journal articles and governmental agency reports. All references were managed with the EndNote citation manager (EndNote X8, Clarivate Analytics; Philadelphia, PA). After import to EndNote, duplicate studies were removed manually.

6.2.2 Inclusion criteria

Abstracts of articles were first screened to determine if they were appropriate to address the proposed research question. For articles to pass this stage, the articles needed to be United States prevalence studies of bacterial foodborne pathogens in commercial broiler farm

environments, commercial broiler processing samples, or broiler chicken retail samples. Challenge studies or studies in which flocks of broilers were inoculated with pathogens were excluded.

Following abstract screening, full-text articles were obtained for all remaining studies (n=262) and analyzed for potential inclusion in the final study. Each article was further assessed with the aforementioned screening criteria, as well as additional criteria. If it was not reported that the study was conducted in the United States, inference was made based off the authors' locations and language throughout the text of the manuscript. This meta-analysis is intended to get current prevalence data to best estimate the risk of foodborne illness from consumption of broiler chicken. Thus, the first additional criterion was that articles needed to be published after January 1, 2000. Secondly, the studies needed to report the sample size and the prevalence and/or number of positive samples. The third criterion was that the studies needed to involve the foodborne pathogens of interest, *Salmonella* spp., *Campylobacter* spp., and *Listeria* spp. Studies that involved targeted sampling for pathogen-positive broiler flocks were excluded because these studies could potentially overestimate the prevalence of pathogens. Additionally, studies that did not provide species-level results were excluded. Studies that were questionable for inclusion were discussed by both authors until a consensus was reached.

6.2.3 Data extraction

Articles that were deemed eligible through screening were then included in quantitative and qualitative analysis (n=80). At this step, quantitative and qualitative data were extracted and analyzed. Due to the problematic nature of incorporating study quality scores as factors in meta-analyses, scores were not assigned to individual studies *(44, 51)*. Study quality was determined by the presence of reputable, replicable microbiological methods.

Collected quantitative variables included number of positive samples and sample size. If the number of positive samples was not reported, but the prevalence was reported, the number of positive samples was estimated by multiplying the prevalence by the sample size and rounding accordingly. Studies that contained inconsistent results throughout the text/figures of the study were excluded.

Qualitative data included the pathogen studied, state performed (if presented), type of poultry production (if presented), sample type, and detection method. *Salmonella* serotype prevalence for included samples was also extracted if presented in the study. Only one *Listeria* study was found, so it was not included in the final meta-analysis. For type of poultry production, if nothing was specified in the study or if the study stated that conventional systems were sampled, the production or retail system was inferred to be conventional. Otherwise, the system was marked as alternative. Alternative production and retail systems included organic, pasture-raised, antibiotic-free, farmer's market, or free-range systems. For one included study *(6)*, a conventional production system utilizing antibiotic-free birds was surveyed. Both authors agreed to include this in the alternative production category. Sample type was categorized as farm environmental, processing, or retail. Environmental samples included any samples collected from the environment of broiler farms. Sample types included boot sock, air, feces, soil, litter, water, feed, grass, insect traps, and wild bird droppings. Processing samples included carcass samples collected throughout the processing supply chain. Samples were classified in the manner in which they were referred to in the study (i.e. rehang, prechill, postchill, etc.). For the majority of processing samples, whole carcass rinses (WCR) were collected. A small number of studies also included results for whole carcass enrichment (WCE) and neck skin maceration (NSM) sampling methods. Retail samples included ground chicken, WCR, and rinses of various chicken

parts (e.g. breasts, thighs) that were purchased from a retail establishment or obtained at the end of the production line at a processing establishment. If different types of samples in the same category from a study were collected, those samples were combined for prevalence calculations if the same detection method was used on them. For consistency, prevalence numbers needed to be stated for the sample in the way it was purchased. For example, if a broiler chicken carcass was purchased from a retail store and cut into parts, positive numbers of carcasses needed to be reported. Detection methods were also noted for each study. If a study utilized multiple detection methods on the same samples, the highest prevalence or the number of true positives among the different methods was utilized as a fail-safe measure. If different methods were used on different samples, the study was considered as separate trials and data points.

After all data were extracted, data were grouped by sample type, pathogen, and production type. To be considered for meta-analysis, each subgroup needed to have at least two independent studies. Data from farm environmental, rehang, prechill, postchill, and retail samples were utilized. All data were stored in Microsoft Excel (Version 16.28, Microsoft Corporation; Redmond, WA).

6.2.4 Data analysis

All data analysis was performed using R (Version 3.6.1) *(80)*. Meta-analyses and forest plot generation were conducted using the 'meta' and 'metafor' packages *(95, 116)*.

A generalized linear mixed model (GLMM) approach *(26, 105)* combined with the logit transformation has been recommended by various studies *(96, 120)*, and was used in the current study. For each included study, prevalence values were calculated by dividing the sample size by the number of positive samples. Due to the presence of proportions equal to 0 or 1, values were first transformed using the logit transformation *(56)*:

$$logit\ p = \ln\left(\frac{p}{1-p}\right) \tag{1}$$

with variance:

$$var(logit\ p) = \frac{1}{Np} + \frac{1}{(1-Np)} \tag{2}$$

where p is the prevalence of pathogen reported in a study and N is the sample size of that study.

Following transformation, data for each pathogen and sample type combination were partitioned based on production type (i.e. conventional/alternative) to allow for subgroup analysis *(45)*. If data were only available for conventional productions systems for a given pathogen/sample type combination, these data were modeled alone. A random intercept logistic regression model was then fitted to each subgroup to estimate the population prevalence (PP) and its 95% confidence interval (CI), as well as values to describe the between-study variance (τ^2) and heterogeneity (I^2) present *(12, 47)*. As suggested by Higgins et al. *(47)*, I^2 values of 25%, 50%, and 75% were considered as low, medium, and high measures of heterogeneity, respectively. Additionally, Cochran's *Q*-test with alpha 0.10 was performed to compare the effect sizes yielded by each subgroup meta-analysis model *(37, 38)*. If high amounts of heterogeneity were observed during subgroup analysis, a meta-analysis model with various moderating variables was fitted to the entire population of each pathogen/sample type combination to attempt to describe the between-study heterogeneity *(35)*. The moderating variables included production system, detection method, and year for all sample types, and type of chicken sample for retail samples. Within each population, each moderating variable had to have at least 2 levels to be included. If multiple detection methods or sample types were used in a study, a value of 'multiple' was applied to variable. p values were obtained for all moderating variables and the amount of between-study variability explained (R^2) by the moderating variables

was calculated. Model code can be found on GitHub (https://github.com/cgolden1993/Dissertation).

6.3 Results

6.3.1 Study results

The complete systematic review process is outlined in Fig. 6.1. In total, 137 trials from 72 studies were included in final meta-analysis models. This included 14,735 environmental, 9,200 rehang, 1,270 prechill, 63,306 postchill, and 24,355 retail samples, for a total of 112,866 samples (Tables 6.1-6.3). Between all sample types, there were a total of 82,671 and 30,195 *Salmonella* and *Campylobacter* samples, respectively. There were a significantly greater number of conventional samples than alternative samples, with 108,873 and 3,993 total samples, respectively.

Table 6.4 presents the prevalence of various *Salmonella* serotypes in the different types of samples studied. It has been identified that some serotypes of *Salmonella* have greater public health risk than others, with *Salmonella* Typhimurium, Enteritidis, Newport, and Heidelberg being identified as a few of particular concern in the United States *(30)*. In the present study, *Salmonella* Kentucky was identified as the most common serotype collected from various sample types at some points along along the chicken supply chain.

6.3.2 Meta-analysis results of data from environmental sampling studies

Meta-analysis models were constructed for each combination of pathogen and production type from the environmental sampling data (Table 6.5). For *Campylobacter*, the predicted population prevalence was significantly different ($p < 0.10$) for conventional (15.8%) and alternative (52.8%) environmental samples (Table 6.5, Fig. 6.2a). The predicted population prevalence for *Salmonella* samples was 22.9% (95% CI: 14.5-34.2%) and 19.9% (95% CI: 7.1-

44.8%) for conventional and alternative environmental samples, respectively (Fig. 6.2b). These values were not significantly different. Heterogeneity was very high for all 4 populations, with $I^2 \geq 94.9\%$ for all sample sets. For the combined *Campylobacter* model, 32.8% of the between-study variability was explained by the type of production system, detection method, and year (Table 6.6). Production system and year accounted for 15.31% of the between-study variability for the *Salmonella* population.

6.3.3 Meta-analysis results of data from processing sampling studies

Meta-analysis models were separately created for rehang, prechill, and postchill processing samples to represent three prevalence benchmarks in the broiler chicken production chain (Table 6.5, Figs. 6.3-6.5). If possible, each sample type was evaluated for each combination of pathogen and production type. Among conventional samples, *Campylobacter* prevalence was highest in prechill samples (97.9%; 95% CI: 6.4-100%), followed by rehang (84.9%; 95% CI: 52.4-96.6%) and postchill (60.9%; 95% CI: 41.3-77.6) samples. The same trend was observed for *Salmonella* among conventional processing samples, with prevalence values of 68.6 (95% CI: 20.1-95.0%), 42.9 (95% CI: 24.3-63.8%), and 14.3% (95% CI: 6.3-29.2%) for prechill, rehang, and postchill samples, respectively. The only alternative subgroup to have sufficient number of independent studies to be considered for meta-analysis was the postchill *Campylobacter* group. An estimated population prevalence of 34.3% (95% CI: 8.4-74.8%) was calculated for this group and this value was not significantly different than the conventional estimated PP. Heterogeneity was high for all study groups, and between-study variance (τ^2) was largest for prechill samples. Year was a significant moderator variable for *Campylobacter* rehang and postchill models and *Salmonella* rehang and prechill samples. For all

but the *Salmonella* rehang model, it was estimated that prevalence has decreased over time (Table 6.6).

6.3.4 Meta-analysis results of data from retail sampling studies.

Retail study groups contained the greatest number of studies when compared to other sample types. Meta-analysis models were constructed for each combination of pathogen and production type (Table 6.5; Fig. 6.6). Predicted *Campylobacter* prevalence in retail broiler chicken for both production types were similar with 59.2% (95% CI: 47.6-69.8%) and 55.4% (95% CI: 34.5-74.6%) for conventional and alternative samples, respectively. A similar trend was observed with *Salmonella* prevalence. The random-effects model predicted a population prevalence of 19.0% (95% CI: 12.2-28.3%) for conventional samples and 23.0% (95% CI: 14.8-34.0%) for alternative samples. For both sets of subgroups, there was no significant difference in estimated effect size between alternative and conventional production systems. Again, high heterogeneity ($I^2 \geq 91.8\%$) was observed among each study group. In the combined *Campylobacter* model, production system, detection method, year, and sample type accounted for 50.14% of the between-study variability. Production system, detection method, year, and type of chicken sample accounted for 45.81% of the between-study variability in the *Salmonella* combined model, with production system, detection method, and type of chicken all being significant moderators ($p < 0.10$).

6.4 Discussion

From 1998-2012, poultry caused the greatest number of foodborne illness outbreaks (279), illnesses (9,760), and hospitalizations (565) in the United States *(17)*. As such, it is vitally important to understand the prevalence and transmission of foodborne pathogens throughout the poultry supply chain and the risk posed by various interventions and production methods. It is

unclear whether alternative poultry production practices present more risk than conventional practices. The current meta-analysis attempted to help quantify the differences in *Salmonella* and *Campylobacter* prevalence throughout conventional and alternative broiler chicken supply chains, utilizing data from the food safety literature. While *Listeria* has been identified as a foodborne pathogen of concern to the poultry industry, more studies need to be conducted on the prevalence of the organism throughout the poultry supply chain for inclusion in later meta-analyses *(86)*.

6.4.1 Pathogen prevalence in the environment of broiler farms

In the current study, random-effect models were generated for each sample group of interest, due to the existence of high heterogeneity in each group *(23)*. The high heterogeneity present in groups could be due to variation in types of environmental samples that were collected. Poultry litter, feed, and drinking water have all been identified as potential contamination risk factors for broilers; contamination can result from factors such as contaminated feces and soil, small animals, such as rodents and insects, and poor worker hygiene *(60, 114, 117)*. This highlights the importance of characterizing the prevalence of foodborne pathogens in a wide variety of environmental samples. To our knowledge, this is the first meta-analysis to estimate pathogen prevalence in the preharvest environment of poultry farms.

The estimated environmental prevalence of *Campylobacter* was higher for alternative poultry farms than conventional. Further research needs to be conducted to determine the cause of the increased prevalence of *Campylobacter* in alternative poultry farms. One possible reason could be due to the effect of climate and seasonality on *Campylobacter* prevalence *(97)*. Alternative poultry production methods often provide more outside access to broilers than

conventional methods, where broilers are more likely to be introduced to environmental pathogens.

Estimated *Salmonella* prevalence numbers were similar for both production types. Two included studies compared the prevalence of *Salmonella* among environmental samples from conventional and alternative farms. Alali et al. *(1)* isolated *Salmonella* from 28.8 and 4.3% of feces, feed, and water samples collected from conventional and organic broiler farms, respectively. Siemon et al. *(98)* found a similar trend with *Salmonella* isolated from 29.8 and 16.2% of feces samples collected from conventional and pasture poultry farms, respectively.

6.4.2 Pathogen prevalence in broiler processing samples

During broiler processing, interventions are put into place to attempt to control for bacterial pathogen contamination, so it is important to understand the difference in pathogen prevalence at various steps in the processing chain *(9)*. As part of a systematic review, Guerin et al. *(39)* found that *Campylobacter* prevalence on broiler carcasses was high throughout the entire processing chain, but generally found that prevalence decreased after chilling. These results are similar to those found in the current study. *Campylobacter* prevalence was high in conventional rehang and prechill samples with 84.9 and 97.9% positive samples, respectively, but lowered after chilling to 60.9%. It's important to note that the prechill model only contained 2 studies, where one study found *Campylobacter* in all collected samples *(101)*. Similar trends have also been noted for *Salmonella* prevalence in the processing supply chain. Rivera-Perez et al. *(84)* found that chilling of carcasses effectively reduced *Salmonella* numbers in broiler samples. In the presented models, estimated *Salmonella* prevalence reduced from 68.6 to 14.3%.

Sufficient processing carcass data for alternative production systems were only available for the postchill *Campylobacter* study group. The group consisted of 3 trials from two studies.

Campylobacter prevalence was lower for alternative samples when compared to conventional samples, but more studies need to be conducted to allow for direct comparison.

6.4.3 Pathogen prevalence in retail broiler meat

Retail samples of broiler meat are important, as they are the types of samples that are available for consumers' direct use. While raw chicken should be cooked before consumption, it is important for pathogen prevalence to be controlled to prevent cross-contamination in the kitchen and risk due to undercooking *(59)*.

No discernible difference was observed between pathogen prevalence of conventional and alternative retail meat samples. This refutes consumers' belief that alternatively produced chicken is safer than conventionally produced chicken *(115)*. Various studies directly compared products from the different production groups. Lestari et al. *(54)* did not identify significant differences in *Salmonella* prevalence between conventional and organic retail broiler carcasses. Cui et al. *(22)* found that *Campylobacter* prevalence on organic and conventional retail carcasses was similar, but *Salmonella* prevalence was slightly higher in organic samples. Mollenkopf et al. *(62)* found that retail chicken breast samples were routinely contaminated with bacterial pathogens, but that production status did not seem to play a role, which agrees with the results in the current study. While more studies were included in the conventional random-effects models for retail samples, a significant amount of research has been conducted on pathogen prevalence in alternative retail broiler meat allowing for better comparison of the two methods.

6.4.4 Use of meta-analysis in future QMRAs

One useful method for risk estimation is quantitative microbial risk assessment *(119)*. Quantitative microbial risk assessments utilize qualitative and quantitative data and probability distributions to estimate the foodborne illness risk of the pathogen of interest. Depending on the

question at hand, QMRAs can include detail on the entire farm-to-fork continuum or focus on a certain area of interest *(113)*. Quantitative microbial risk assessments depend on the quality of data at hand, and sometimes have to rely on expert elicitation to fill in gaps of knowledge *(36)*. As such, systematic methods, such as meta-analyses, are important tools in QMRAs. Meta-analyses provide data-driven estimates that can be used to estimate the prevalence of foodborne pathogens at stages along the continuum and the effects that interventions have on pathogen prevalence.

In recent years, QMRAs have been conducted to estimate the annual burden of salmonellosis and campylobacteriosis due to consumption of poultry *(18, 68, 81)*, but comprehensive QMRAs analyzing the differences in risk between conventional and alternative poultry production practices are still lacking. Rosenquist et al. *(85)* found that the risk of *Campylobacter* infection was 1.7 times higher in organically produced broiler meat than conventional, but this study was limited to Danish produced meat. Similar studies analyzing the effects of conventional and alternative poultry production methods on foodborne pathogen risk in the United States need to be conducted. The meta-analysis provided in the current study should aid in the production of future QMRAs addressing these needs.

Normal distributions can be used to fit results generated by meta-analysis when there are low amounts of heterogeneity present *(27)*. As discussed previously, all study groups included high levels of between-study heterogeneity. As such, it is suggested that a beta-PERT distribution is used to describe the information found in the meta-analysis, using both ends of the 95% CI as the minimum and maximum parameters and the observed mean as the most probable value *(15, 27)*.

6.4.5 Potential limitations and advantages

While considering the results of meta-analyses, it is important to take note of the inherent limitations associated with this method. One of these limitations is the potential existence of publication bias, or the tendency to publish studies that contain “positive” results *(102)*. Many methods exist for evaluating the potential for publication bias in a meta-analysis, including funnel plot evaluation and statistical tests such as Egger’s regression and Begg’s adjusted rank correlation tests *(7, 28)*. While these evaluation tools are recommended in many cases, it has been shown that results can be misleading from meta-analyses with fewer than 10 studies or high amounts of between-study heterogeneity *(104)*. It is suggested that it is very difficult to evaluate the true results of statistically significant publication bias tests, especially in the presence of high heterogeneity. In the current meta-analysis, 6 of 15 evaluated subgroups contained > 10 studies; high levels of between-study heterogeneity existed. As such, funnel plots were not constructed for any of the study groups. To address this concern, as high levels of heterogeneity were expected, the systematic review portion of the analysis did not exclude unpublished or non-peer-reviewed studies. For example, two governmental reports *(111, 112)* were included in the final meta-analysis for various study groups. Additionally, since included studies were meant to survey foodborne pathogen prevalence throughout the broiler chicken supply chain, it was not anticipated that the lack of pathogen-positive samples would cause reports not to be published. In fact, prevalence numbers ranged from 0-100% in included studies.

Some potential limitations exist for the current meta-analysis. The first is that many included studies did not include information on whether collected samples came from conventional or alternative production chains. We believed that the best way to handle these instances was to include these studies in the conventional study category, as this was the most

likely event. Conversely, studies included in the alternative production category clearly stated the production type of the sample population. This should give the most accurate estimation of each group's pathogen prevalence without having to discard any studies with valuable data. In addition, random-effects models were generated for both conventional and alternative studies combined for each pathogen/sample type combination, when available. Combined random-effects models were provided for *Salmonella* and *Campylobacter* environmental and retail study groups, as well as for the *Campylobacter* postchill study group. It is important to take into consideration that some study groups contained as few as 2 studies. These results, nevertheless, should provide a better estimate of population behavior than a single study, which is especially important when developing a risk assessment model *(36, 106)*.

6.5 Conclusions

The current study provided random-effects meta-analysis models as a means to estimate the prevalence of *Campylobacter* spp. and *Salmonella* spp. at various points throughout the broiler chicken supply chain. The presented results will be of use in the construction of future quantitative microbial risk assessments and as a means to help characterize the differences in risk between conventional and alternative broiler chicken production methods.

6.6 References

1. Alali, W. Q., S. Thakur, R. D. Berghaus, M. P. Martin, and W. A. Gebreyes. 2010. Prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. *Foodborne Pathog. Dis.* 7:1363-1371.

2. Bailey, J. S., and D. E. Cosby. 2003. Detection of *Salmonella* from chicken rinses and chicken hot dogs with the automated BAX PCR system. *J. Food Prot.* 66:2138-2140.

3. Bailey, J. S., and D. E. Cosby. 2005. *Salmonella* prevalence in free-range and certified organic chickens. *J. Food Prot.* 68:2451-2453.

4. Bailey, J. S., N. A. Cox, S. E. Craven, and D. E. Cosby. 2002. Serotype tracking of *Salmonella* through integrated broiler chicken operations. *J. Food Prot.* 65:742-745.

5. Bailey, J. S., N. J. Stern, P. Fedorka-Cray, S. E. Craven, N. A. Cox, D. E. Cosby, S. Ladely, and M. T. Musgrove. 2001. Sources and movement of *Salmonella* through integrated poultry operations: A multistate epidemiological investigation. *J. Food Prot.* 64:1690-1697.

6. Bailey, M. A., R. M. Taylor, J. S. Brar, S. C. Corkran, C. Velasquez, E. Novoa Rama, H. F. Oliver, and M. Singh. 2019. Prevalence and antimicrobial resistance of *Campylobacter* from antibiotic-free broilers during organic and conventional processing. *Poult. Sci.* 98:1447-1454.

7. Begg, C. B., and J. A. Berlin. 1988. Publication bias: A problem in interpreting medical data. *J. R. Stat. Soc. A Stat.* 151:419-445.

8. Berghaus, R. D., S. G. Thayer, B. F. Law, R. M. Mild, C. L. Hofacre, and R. S. Singer. 2013. Enumeration of *Salmonella* and *Campylobacter* spp. in environmental farm samples and processing plant carcass rinses from commercial broiler chicken flocks. *Appl. Environ. Microbiol.* 79:4106-4114.

9. Berrang, M. E., J. S. Bailey, S. F. Altekruse, B. Patel, W. K. Shaw, R. J. Meinersmann, and P. J. Fedorka-Cray. 2007. Prevalence and numbers of *Campylobacter* on broiler carcasses collected at rehang and postchill in 20 US processing plants. *J. Food Prot.* 70:1556-1560.

10. Berrang, M. E., J. S. Bailey, S. F. Altekruse, and W. K. Shaw. 2008. Presence and numbers of *Campylobacter*, *Escherichia coli*, and *Salmonella* determined in broiler carcass rinses from United States processing plants in the hazard analysis and critical control point-based inspection models project. *J. Appl. Poult. Res.* 17:354-360.

11. Berrang, M. E., R. J. Meinersmann, N. A. Cox, and T. M. Thompson. 2018. Multilocus sequence subtypes of *Campylobacter* detected on the surface and from internal tissues of retail chicken livers. *J. Food Prot.* 81:1535-1539.

12. Borenstein, M., L. Hedges, and H. Rothstein. 2007. Meta-analysis: Fixed effect vs. random effects. Available at: https://www.meta-analysis.com/downloads/M-a_f_e_v_r_e_sv.pdf. Accessed 6 November 2019.

13. Brichta-Harhay, D. M., T. M. Arthur, and M. Koohmaraie. 2008. Enumeration of *Salmonella* from poultry carcass rinses via direct plating methods. *Lett. Appl. Microbiol.* 46:186-191.

14. Bucher, O., A. M. Farrar, S. C. Totton, W. Wilkins, L. A. Waddell, B. J. Wilhelm, S. A. McEwen, A. Fazil, and A. Rajić. 2012. A systematic review-meta-analysis of chilling interventions and a meta-regression of various processing interventions for *Salmonella* contamination of chicken. *Prev. Vet. Med.* 103:1-15.

15. Buczinski, S., and J. M. Vandeweerd. 2016. Diagnostic accuracy of refractometry for assessing bovine colostrum quality: A systematic review and meta-analysis. *J. Dairy Sci.* 99:7381-7394.

16. Centers for Disease Control and Prevention. 2020. National Outbreak Reporting System (NORS), Outbreaks per state, *Salmonella*, chicken. Available at: https://wwwn.cdc.gov/norsdashboard/. Accessed 17 January 2020.

17. Chai, S. J., D. Cole, A. Nisler, and B. E. Mahon. 2017. Poultry: The most common food in outbreaks with known pathogens, United States, 1998–2012. *Epidemiol. Infect.* 145:316-325.

18. Chapman, B., A. Otten, A. Fazil, N. Ernst, and B. A. Smith. 2016. A review of quantitative microbial risk assessment and consumer process models for *Campylobacter* in broiler chickens. *Microb. Risk Anal.* 2:3-15.

19. Cox, N. A., R. J. Buhr, D. P. Smith, J. A. Cason, L. L. Rigsby, D. V. Bourassa, P. J. Fedorka-Cray, and D. E. Cosby. 2014. Sampling naturally contaminated broiler carcasses for *Salmonella* by three different methods. *J. Food Prot.* 77:493-495.

20. Cox, N. A., L. J. Richardson, J. A. Cason, R. J. Buhr, Y. Vizzier-Thaxton, D. P. Smith, P. J. Fedorka-Cray, C. P. Romanenghi, L. V. B. Pereira, and M. P. Doyle. 2010. Comparison of

neck skin excision and whole carcass rinse sampling methods for microbiological evaluation of broiler carcasses before and after immersion chilling. *J. Food Prot.* 73:976-980.

21. Craven, S. E., N. J. Stern, E. Line, J. S. Bailey, N. A. Cox, and P. J. Fedorka-Cray. 2000. Determination of the incidence of *Salmonella* spp., *Campylobacter jejuni*, and *Clostridium perfringens* in wild birds near broiler chicken houses by sampling intestinal droppings. *Avian Dis*. 44:715-720.

22. Cui, S., B. Ge, J. Zheng, and J. Meng. 2005. Prevalence and antimicrobial resistance of *Campylobacter* spp. and *Salmonella* serovars in organic chickens from Maryland retail stores. *Appl. Environ. Microbiol.* 71:4108-4111.

23. DerSimonian, R., and R. Kacker. 2007. Random-effects model for meta-analysis of clinical trials: An update. *Contemp. Clin. Trials*. 28:105-114.

24. Dickins, M. A., S. Franklin, R. Stefanova, G. E. Schutze, K. D. Eisenach, I. Wesley, and M. D. Cave. 2002. Diversity of *Campylobacter* isolates from retail poultry carcasses and from humans as demonstrated by pulsed-field gel electrophoresis. *J. Food Prot.* 65:957-962.

25. Dimitri, C., and L. Oberholtzer. 2009. Marketing US organic foods: Recent trends from farms to consumers. Economic Information Bulletin No. 58. United States Department of Agriculture, Economic Research Service.

26. Dobson, A. J., and A. G. Barnett. 2008. An introduction to generalized linear models. Chapman and Hall/CRC, New York, NY.

27. Dogan, O. B., J. Clarke, F. Mattos, and B. Wang. 2019. A quantitative microbial risk assessment model of *Campylobacter* in broiler chickens: Evaluating processing interventions. *Food Control*. 100:97-110.

28. Egger, M., G. D. Smith, M. Schneider, and C. Minder. 1997. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 315:629-634.

29. Erickson, A. K., D. L. Murray, L. A. Ruesch, M. Thomas, Z. Lau, and J. Scaria. 2018. Genotypic and phenotypic characterization of *Salmonella* isolated from fresh ground meats obtained from retail grocery stores in the Brookings, South Dakota, area. *J. Food Prot.* 81:1526-1534.

30. Foley, S. L., R. Nayak, I. B. Hanning, T. J. Johnson, J. Han, and S. C. Ricke. 2011. Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. *Appl. Environ. Microbiol.* 77:4273-4279.

31. Fratamico, P. M. 2003. Comparison of culture, polymerase chain reaction (PCR), TaqMan *Salmonella*, and Transia Card *Salmonella* assays for detection of *Salmonella* spp. in naturally-contaminated ground chicken, ground turkey, and ground beef. *Mol. Cell. Probes*. 17:215-221.

32. Gad, A. H., U. H. Abo-Shama, K. K. Harclerode, and M. K. Fakhr. 2018. Prevalence, serotyping, molecular typing, and antimicrobial resistance of *Salmonella* isolated from conventional and organic retail ground poultry. *Front. Microbiol.* 9:2653.

33. Geissler, A. L., F. Bustos Carrillo, K. Swanson, M. E. Patrick, K. E. Fullerton, C. Bennett, K. Barrett, and B. E. Mahon. 2017. Increasing *Campylobacter* infections, outbreaks, and antimicrobial resistance in the United States, 2004-2012. *Clin. Infect. Dis.* 65:1624-1631.

34. Glass, G. V. 1976. Primary, secondary, and meta-analysis of research. *Educ. Res.* 5:3-8.

35. Goncalves-Tenorio, A., B. Silva, V. Rodrigues, V. Cadavez, and U. Gonzales-Barron. 2018. Prevalence of pathogens in poultry meat: A meta-analysis of European published surveys. *Foods*. 7:69.

36. Gonzales-Barron, U., and F. Butler. 2011. The use of meta-analytical tools in risk assessment for food safety. *Food Microbiol.* 28:823-827.

37. Gonzales-Barron, U., A. Gonçalves-Tenório, V. Rodrigues, and V. Cadavez. 2017. Foodborne pathogens in raw milk and cheese of sheep and goat origin: A meta-analysis approach. *Curr. Opin. Food Sci.* 18:7-13.

38. Gonzales-Barron, U., A. Thébault, P. Kooh, L. Watier, M. Sanaa, and V. Cadavez. 2019. Strategy for systematic review of observational studies and meta-analysis modelling of risk factors for sporadic foodborne diseases. *Microb. Risk Anal.*: https://doi.org/10.1016/j.mran.2019.07.003.

39. Guerin, M. T., C. Sir, J. M. Sargeant, L. Waddell, A. M. O'Connor, R. W. Wills, R. H. Bailey, and J. A. Byrd. 2010. The change in prevalence of *Campylobacter* on chicken carcasses during processing: A systematic review. *Poult. Sci.* 89:1070-1084.

40. Guran, H. S., D. Mann, and W. Q. Alali. 2017. *Salmonella* prevalence associated with chicken parts with and without skin from retail establishments in Atlanta metropolitan area, Georgia. *Food Control*. 73:462-467.

41. Han, F., S. I. Lestari, S. Pu, and B. Ge. 2009. Prevalence and antimicrobial resistance among *Campylobacter* spp. in Louisiana retail chickens after the enrofloxacin ban. *Foodborne Pathog. Dis.* 6:163-171.

42. Hanning, I., D. Biswas, P. Herrera, M. Roesler, and S. C. Ricke. 2010. Prevalence and characterization of *Campylobacter jejuni* isolated from pasture flock poultry. *J. Food Sci.* 75:M496-502.

43. Hardy, B., N. Crilly, S. Pendleton, A. Andino, A. Wallis, N. Zhang, and I. Hanning. 2013. Impact of rearing conditions on the microbiological quality of raw retail poultry meat. *J. Food Sci.* 78:M1232-1235.

44. Herbison, P., J. Hay-Smith, and W. J. Gillespie. 2006. Adjustment of meta-analyses on the basis of quality scores should be abandoned. *J. Clin. Epidemiol.* 59:1249-1256.

45. Higgins, J., J. Thomas, J. Chandler, M. Cumpston, T. Li, M. Page, and V. Welch. 2019. Cochrane handbook for systematic reviews of interventions, 2nd edition. John Wiley & Son, Chichester, UK.

46. Higgins, J. P., R. L. Andreatti Filho, S. E. Higgins, A. D. Wolfenden, G. Tellez, and B. M. Hargis. 2008. Evaluation of *Salmonella*-lytic properties of bacteriophages isolated from commercial broiler houses. *Avian Dis.* 52:139-142.

47. Higgins, J. P., S. G. Thompson, J. J. Deeks, and D. G. Altman. 2003. Measuring inconsistency in meta-analyses. *BMJ*. 327:557-560.

48. Hughner, R. S., P. McDonagh, A. Prothero, C. J. Shultz, and J. Stanton. 2007. Who are organic food consumers? A compilation and review of why people purchase organic food. *J. Consumer Behav.* 6:94-110.

49. Jain, S., and J. Chen. 2006. Antibiotic resistance profiles and cell surface components of Salmonellae. *J. Food Prot.* 69:1017-1023.

50. Jung, Y., A. C. S. Porto-Fett, B. A. Shoyer, E. Henry, L. E. Shane, M. Osoria, and J. B. Luchansky. 2019. Prevalence, levels, and viability of *Salmonella* in and on raw chicken livers. *J. Food Prot.* 82:834-843.

51. Jüni, P., A. Witschi, R. Bloch, and M. Egger. 1999. The hazards of scoring the quality of clinical trials for meta-analysis. *Jama*. 282:1054-1060.

52. Kegode, R. B., D. K. Doetkott, M. L. Khaitsa, and I. V. Wesley. 2008. Occurrence of *Campylobacter* species, *Salmonella* species and generic *Escherichia coli* in meat products from retail outlets in the Fargo metropolitan area. *J. Food Saf.* 28:111-125.

53. Kerr, A. K., A. M. Farrar, L. A. Waddell, W. Wilkins, B. J. Wilhelm, O. Bucher, R. W. Wills, R. H. Bailey, C. Varga, and S. A. McEwen. 2013. A systematic review-meta-analysis and meta-regression on the effect of selected competitive exclusion products on *Salmonella* spp. prevalence and concentration in broiler chickens. *Prev. Vet. Med.* 111:112-125.

54. Lestari, S. I., F. F. Han, W. Fei, and B. L. Ge. 2009. Prevalence and antimicrobial resistance of *Salmonella* serovars in conventional and organic chickens from Louisiana retail stores. *J. Food Prot.* 72:1165-1172.

55. Line, J. E., N. J. Stern, C. P. Lattuada, and S. T. Benson. 2001. Comparison of methods for recovery and enumeration of *Campylobacter* from freshly processed broilers. *J. Food Prot.* 64:982-986.

56. Lipsey, M. W., and D. B. Wilson. 2001. Practical meta-analysis. Sage Publications, Inc, Thousand Oaks, CA.

57. Liu, L., S. K. Hussain, R. S. Miller, and O. A. Oyarzabal. 2009. Efficacy of mini VIDAS for the detection of *Campylobacter* spp. from retail broiler meat enriched in Bolton broth, with or without the supplementation of blood. *J. Food Prot.* 72:2428-2432.

58. Luangtongkum, T., T. Y. Morishita, L. Martin, I. Choi, O. Sahin, and Q. Zhang. 2008. Prevalence of tetracycline-resistant *Campylobacter* in organic broilers during a production cycle. *Avian Dis.* 52:487-490.

59. Luber, P. 2009. Cross-contamination versus undercooking of poultry meat or eggs—Which risks need to be managed first? *Int. J. Food Microbiol.* 134:21-28.

60. Maciorowski, K. G., F. T. Jones, S. D. Pillai, and S. C. Ricke. 2004. Incidence, sources, and control of food-borne *Salmonella* spp. in poultry feeds. *Worlds Poult. Sci. J.* 60:446-457.

61. Melendez, S. N., I. Hanning, J. Han, R. Nayak, A. R. Clement, A. Wooming, P. Hererra, F. T. Jones, S. L. Foley, and S. C. Ricke. 2010. *Salmonella enterica* isolates from pasture-raised poultry exhibit antimicrobial resistance and class I integrons. *J. Appl. Microbiol.* 109:1957-1966.

62. Mollenkopf, D. F., J. K. Cenera, E. M. Bryant, C. A. King, I. Kashoma, A. Kumar, J. A. Funk, G. Rajashekara, and T. E. Wittum. 2014. Organic or antibiotic-free labeling does not impact the recovery of enteric pathogens and antimicrobial-resistant *Escherichia coli* from fresh retail chicken. *Foodborne Pathog. Dis.* 11:920-929.

63. Mollenkopf, D. F., B. De Wolf, S. M. Feicht, J. K. Cenera, C. A. King, J. C. van Balen, and T. E. Wittum. 2018. *Salmonella* spp. and extended-spectrum cephalosporin-resistant *Escherichia coli* frequently contaminate broiler chicken transport cages of an organic production company. *Foodborne Pathog. Dis.* 15:583-588.

64. Musgrove, M. T., N. A. Cox, M. E. Berrang, and M. A. Harrison. 2003. Comparison of weep and carcass rinses for recovery of *Campylobacter* from retail broiler carcasses. *J. Food Prot.* 66:1720-1723.

65. Myint, M. S., Y. J. Johnson, N. L. Tablante, and R. A. Heckert. 2006. The effect of pre-enrichment protocol on the sensitivity and specificity of PCR for detection of naturally contaminated *Salmonella* in raw poultry compared to conventional culture. *Food Microbiol.* 23:599-604.

66. Nannapaneni, R., I. Hanning, K. C. Wiggins, R. P. Story, S. C. Ricke, and M. G. Johnson. 2009. Ciprofloxacin-resistant *Campylobacter* persists in raw retail chicken after the fluoroquinolone ban. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* 26:1348-1353.

67. Nannapaneni, R., R. Story, K. C. Wiggins, and M. G. Johnson. 2005. Concurrent quantitation of total *Campylobacter* and total ciprofloxacin-resistant campylobacter loads in rinses from retail raw chicken carcasses from 2001 to 2003 by direct plating at 42 degrees C. *Appl. Environ. Microbiol.* 71:4510-4515.

68. Nauta, M., A. Hill, H. Rosenquist, S. Brynestad, A. Fetsch, P. van der Logt, A. Fazil, B. Christensen, E. Katsma, and B. Borck. 2009. A comparison of risk assessments on *Campylobacter* in broiler meat. *Int. J. Food Microbiol.* 129:107-123.

69. Noormohamed, A., and M. K. Fakhr. 2012. Incidence and antimicrobial resistance profiling of *Campylobacter* in retail chicken livers and gizzards. *Foodborne Pathog. Dis.* 9:617-624.

70. Noormohamed, A., and M. K. Fakhr. 2014. Prevalence and antimicrobial susceptibility of *Campylobacter* spp. in Oklahoma conventional and organic retail poultry. *Open Microbiol. J.* 8:130-137.

71. Oscar, T. P. 2013. Initial contamination of chicken parts with *Salmonella* at retail and cross-contamination of cooked chicken with *Salmonella* from raw chicken during meal preparation. *J. Food Prot.* 76:33-39.

72. Oscar, T. P. 2014. Use of enrichment real-time PCR to enumerate *Salmonella* on chicken parts. *J. Food Prot.* 77:1086-1092.

73. Oscar, T. P. 2019. Process risk model for *Salmonella* and ground chicken. *J. Appl. Microbiol.* 127:1236-1245.

74. Oscar, T. P., G. K. Rutto, J. B. Ludwig, and S. Parveen. 2010. Qualitative map of *Salmonella* contamination on young chicken carcasses. *J. Food Prot.* 73:1596-1603.

75. Oyarzabal, O. A., K. S. Macklin, J. M. Barbaree, and R. S. Miller. 2005. Evaluation of agar plates for direct enumeration of *Campylobacter* spp. from poultry carcass rinses. *Appl. Environ. Microbiol.* 71:3351-3354.

76. Oyarzabal, O. A., A. Williams, P. Zhou, and M. Samadpour. 2013. Improved protocol for isolation of *Campylobacter* spp. from retail broiler meat and use of pulsed field gel electrophoresis for the typing of isolates. *J. Microbiol. Methods*. 95:76-83.

77. Parveen, S., M. Taabodi, J. G. Schwarz, T. P. Oscar, J. Harter-Dennis, and D. G. White. 2007. Prevalence and antimicrobial resistance of *Salmonella* recovered from processed poultry. *J. Food Prot.* 70:2466-2472.

78. Price, L. B., E. Johnson, R. Vailes, and E. Silbergeld. 2005. Fluoroquinolone-resistant *Campylobacter* isolates from conventional and antibiotic-free chicken products. *Environ. Health Perspect.* 113:557-560.

79. Price, L. B., L. G. Lackey, R. Vailes, and E. Silbergeld. 2007. The persistence of fluoroquinolone-resistant *Campylobacter* in poultry production. *Environ. Health Perspect.* 115:1035-1039.

80. R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

81. Rajan, K., Z. Shi, and S. C. Ricke. 2017. Current aspects of *Salmonella* contamination in the US poultry production chain and the potential application of risk strategies in understanding emerging hazards. *Crit. Rev. Microbiol.* 43:370-392.

82. Ramirez-Hernandez, A., M. Bugarel, S. Kumar, H. Thippareddi, M. M. Brashears, and M. X. Sanchez-Plata. 2019. Phenotypic and genotypic characterization of antimicrobial resistance in *Salmonella* strains isolated from chicken carcasses and parts collected at different stages during processing. *J. Food Prot.* 82:1793-1801.

83. Richardson, L. J., N. A. Cox, J. S. Bailey, M. E. Berrang, J. M. Cox, R. J. Buhr, P. J. Fedorka-Cray, and M. A. Harrison. 2009. Evaluation of TECRA broth, Bolton broth, and direct plating for recovery of *Campylobacter* spp. from broiler carcass rinsates from commercial processing plants. *J. Food Prot.* 72:972-977.

84. Rivera-Perez, W., E. Barquero-Calvo, and R. Zamora-Sanabria. 2014. *Salmonella* contamination risk points in broiler carcasses during slaughter line processing. *J. Food Prot.* 77:2031-2034.

85. Rosenquist, H., L. Boysen, A. L. Krogh, A. N. Jensen, and M. Nauta. 2013. *Campylobacter* contamination and the relative risk of illness from organic broiler meat in comparison with conventional broiler meat. *Int. J. Food Microbiol.* 162:226-230.

86. Rothrock Jr, M. J., M. L. Davis, A. Locatelli, A. Bodie, T. G. McIntosh, J. R. Donaldson, and S. C. Ricke. 2017. *Listeria* occurrence in poultry flocks: Detection and potential implications. *Front. Vet. Sci.* 4:125.

87. Roy, P., A. S. Dhillon, L. H. Lauerman, D. M. Schaberg, D. Bandli, and S. Johnson. 2002. Results of *Salmonella* isolation from poultry products, poultry, poultry environment, and other characteristics. *Avian Dis.* 46:17-24.

88. Salaheen, S., M. Peng, and D. Biswas. 2016. Ecological dynamics of *Campylobacter* in integrated mixed crop-livestock farms and its prevalence and survival ability in post-harvest products. *Zoonoses Public Health*. 63:641-650.

89. Sanchez, M. X., W. M. Fluckey, M. M. Brashears, and S. R. McKee. 2002. Microbial profile and antibiotic susceptibility of *Campylobacter* spp. and *Salmonella* spp. in broilers processed in air-chilled and immersion-chilled environments. *J. Food Prot.* 65:948-956.

90. Sargeant, J. M., M. D. R. Amezcua, A. Rajic, and L. Waddell. 2005. A guide to conducting systematic reviews in agri-food public health. Available at: http://publications.gc.ca/Collection/HP5-9-2005E.pdf. Accessed 13 October 2019.

91. Sargeant, J. M., A. Rajic, S. Read, and A. Ohlsson. 2006. The process of systematic review and its application in agri-food public-health. *Prev. Vet. Med.* 75:141-151.

92. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—Major pathogens. *Emerg. Infect. Dis.* 17:7-15.

93. Scheinberg, J., S. Doores, and C. N. Cutter. 2013. A microbiological comparison of poultry products obtained from farmer's markets and supermarkets in Pennsylvania *J. Food Saf.* 33:259-264.

94. Schroeder, M. W., J. D. Eifert, M. A. Ponder, and D. G. Schmale. 2014. Association of *Campylobacter* spp. levels between chicken grow-out environmental samples and processed carcasses. *Poult. Sci.* 93:734-741.

95. Schwarzer, G. 2007. meta: An R package for meta-analysis. *R News*. 7:40-45.

96. Schwarzer, G., H. Chemaitelly, L. J. Abu-Raddad, and G. Rücker. 2019. Seriously misleading results using inverse of Freeman-Tukey double arcsine transformation in meta-analysis of single proportions. *Res. Synth. Methods*. 10:476-483.

97. Sibanda, N., A. McKenna, A. Richmond, S. C. Ricke, T. Callaway, A. C. Stratakos, O. Gundogdu, and N. Corcionivoschi. 2018. A review of the effect of management practices on *Campylobacter* prevalence in poultry farms. *Front. Microbiol.* 9:2002.

98. Siemon, C. E., P. B. Bahnson, and W. A. Gebreyes. 2007. Comparative investigations of prevalence and antimicrobial resistance of *Salmonella* between pasture and conventionally reared poultry. *Avian Dis.* 51:112-117.

99. Simmons, M., D. L. Fletcher, J. A. Cason, and M. E. Berrang. 2003. Recovery of *Salmonella* from retail broilers by a whole-carcass enrichment procedure. *J. Food Prot.* 66:446-450.

100. Sofos, J. N. 2008. Challenges to meat safety in the 21st century. *Meat Sci.* 78:3-13.

101. Son, I., M. D. Englen, M. E. Berrang, P. J. Fedorka-Cray, and M. A. Harrison. 2007. Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. *Int. J. Food Microbiol.* 113:16-22.

102. Spector, T. D., and S. G. Thompson. 1991. The potential and limitations of meta-analysis. *J. Epidemiol. Community Health*. 45:89-92.

103. Stern, N. J., and S. Pretanik. 2006. Counts of *Campylobacter* spp. on U.S. broiler carcasses. *J. Food Prot.* 69:1034-1039.

104. Sterne, J. A., A. J. Sutton, J. P. Ioannidis, N. Terrin, D. R. Jones, J. Lau, J. Carpenter, G. Rücker, R. M. Harbord, and C. H. Schmid. 2011. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ*. 343:d4002.

105. Stijnen, T., T. H. Hamza, and P. Özdemir. 2010. Random effects meta-analysis of event outcome in the framework of the generalized linear mixed model with applications in sparse data. *Stat. Med.* 29:3046-3067.

106. Sutton, A. J., K. R. Abrams, and D. R. Jones. 2001. An illustrated guide to the methods of meta-analysis. *J. Eval. Clin. Pract.* 7:135-148.

107. Thakur, S., J. Brake, S. Keelara, M. Zou, and E. Susick. 2013. Farm and environmental distribution of *Campylobacter* and *Salmonella* in broiler flocks. *Res. Vet. Sci.* 94:33-42.

108. Totton, S. C., A. M. Farrar, W. Wilkins, O. Bucher, L. A. Waddell, B. J. Wilhelm, S. A. McEwen, and A. Rajić. 2012. A systematic review and meta-analysis of the effectiveness of

biosecurity and vaccination in reducing *Salmonella* spp. in broiler chickens. *Food Res. Int.* 45:617-627.

109. Trimble, L. M., W. Q. Alali, K. E. Gibson, S. C. Ricke, P. Crandall, D. Jaroni, and M. Berrang. 2013. *Salmonella* and *Campylobacter* prevalence and concentration on pasture-raised broilers processed on-farm, in a Mobile Processing Unit, and at small USDA-inspected facilities. *Food Control*. 34:177-182.

110. Trimble, L. M., W. Q. Alali, K. E. Gibson, S. C. Ricke, P. Crandall, D. Jaroni, M. Berrang, and M. Y. Habteselassie. 2013. Prevalence and concentration of *Salmonella* and *Campylobacter* in the processing environment of small-scale pastured broiler farms. *Poult. Sci.* 92:3060-3066.

111. United States Department of Agriculture. 2009. The nationwide microbiological baseline data collection program: Young chicken survey. Available at: https://www.fsis.usda.gov/shared/PDF/Baseline_Data_Young_Chicken_2007-2008.pdf?redirecthttp=true. Accessed 5 October 2019.

112. United States Department of Agriculture. 2012. The nationwide microbiological baseline data collection program: Raw chicken parts survey. Available at: https://www.fsis.usda.gov/shared/PDF/Baseline_Data_Raw_Chicken_Parts.pdf. Accessed 5 October 2019.

113. van Gerwen, S. J., and M. H. Zwietering. 1998. Growth and inactivation models to be used in quantitative risk assessments. *J. Food Prot.* 61:1541-1549.

114. van Immerseel, F., L. De Zutter, K. Houf, F. Pasmans, F. Haesebrouck, and R. Ducatelle. 2009. Strategies to control *Salmonella* in the broiler production chain. *Worlds Poult. Sci. J.* 65:367-392.

115. van Loo, E. J., V. Caputo, R. M. Nayga Jr, J.-F. Meullenet, and S. C. Ricke. 2011. Consumers' willingness to pay for organic chicken breast: Evidence from choice experiment. *Food Qual. Prefer.* 22:603-613.

116. Viechtbauer, W. 2010. Conducting meta-analyses in R with the metafor package. *J. Stat. Softw.* 36:1-48.

117. Volkova, V. V., R. H. Bailey, and R. W. Wills. 2009. *Salmonella* in broiler litter and properties of soil at farm location. *PloS One*. 4:e6403.

118. Voss-Rech, D., L. Potter, C. S. L. Vaz, D. I. B. Pereira, L. A. Sangioni, A. C. Vargas, and S. de Avila Botton. 2017. Antimicrobial resistance in nontyphoidal *Salmonella* isolated from human and poultry-related samples in Brazil: 20-year meta-analysis. *Foodborne Pathog. Dis.* 14:116-124.

119. Walls, I., and V. N. Scott. 1997. Use of predictive microbiology in microbial food safety risk assessment. *Int. J. Food Microbiol.* 36:97-102.

120. Warton, D. I., and F. K. Hui. 2011. The arcsine is asinine: The analysis of proportions in ecology. *Ecology*. 92:3-10.

121. White, D. G., S. Zhao, R. Sudler, S. Ayers, S. Friedman, S. Chen, P. F. McDermott, S. McDermott, D. D. Wagner, and J. Meng. 2001. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. *N. Engl. J. Med.* 345:1147-1154.

122. White, P. L., A. L. Naugle, C. R. Jackson, P. J. Fedorka-Cray, B. E. Rose, K. M. Pritchard, P. Levine, P. K. Saini, C. M. Schroeder, M. S. Dreyfuss, R. Tan, K. G. Holt, J. Harman, and S. Buchanan. 2007. *Salmonella* Enteritidis in meat, poultry, and pasteurized egg products regulated by the US food safety and inspection service, 1998 through 2003. *J. Food Prot.* 70:582-591.

123. Williams, A., and O. A. Oyarzabal. 2012. Prevalence of *Campylobacter* spp. in skinless, boneless retail broiler meat from 2005 through 2011 in Alabama, USA. *BMC Microbiol.* 12:184.

124. Young, I., A. Rajić, B. Wilhelm, L. Waddell, S. Parker, and S. McEwen. 2009. Comparison of the prevalence of bacterial enteropathogens, potentially zoonotic bacteria and bacterial resistance to antimicrobials in organic and conventional poultry, swine and beef production: A systematic review and meta-analysis. *Epidemiol. Infect.* 137:1217-1232.

125. Zhang, J. Y., A. Massow, M. Stanley, M. Papariella, X. Chen, B. Kraft, and P. Ebner. 2011. Contamination rates and antimicrobial resistance in *Enterococcus* spp., *Escherichia coli*, and *Salmonella* isolated from “no antibiotics added’’-labeled chicken products. *Foodborne Pathog. Dis.* 8:1147-1152.

126. Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D. G. White, D. Wagner, and J. Meng. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. *Appl. Environ. Microbiol.* 67:5431-5436.

127. Zhao, S., P. F. McDermott, S. Friedman, J. Abbott, S. Ayers, A. Glenn, E. Hall-Robinson, S. K. Hubert, H. Harbottle, and R. D. Walker. 2006. Antimicrobial resistance and genetic relatedness among *Salmonella* from retail foods of animal origin: NARMS retail meat surveillance. *Foodborne Pathog. Dis.* 3:106-117.

128. Zhao, S., S. R. Young, E. Tong, J. W. Abbott, N. Womack, S. L. Friedman, and P. F. McDermott. 2010. Antimicrobial resistance of *Campylobacter* isolates from retail meat in the United States between 2002 and 2007. *Appl. Environ. Microbiol.* 76:7949-7956.

Table 6.1 Summary of broiler farm environmental sampling studies used in meta-analysis

Pathogen	Production	State/Region	Sample type	Detection method	No. positive samples	Sample size	Citation
Campylobacter	NR[a]	GA	Boot sock, drag swab, feces, litter	SE[b]	262	549	*(8)*
Campylobacter	NR	VA	Air, feces/litter, feed pans, water lines	SE	32	120	*(94)*
Campylobacter	NR	NC	Feed, litter, grass, inside/outside swabs	SE	4	500	*(107)*
Campylobacter	NR	NC	Feces	DP[c]	118	400	*(107)*
Campylobacter	Pasture	NR	Feed, water, drag swabs, insect traps	SE	53	178	*(42)*
Campylobacter	Organic	OH	Feces, feed, grass, litter, water	SE	152	215	*(58)*
Campylobacter	Pasture	Southeast	Soil, compost, processing wastewater	SE	73[d]	127	*(110)*
Salmonella	Conv.	NC	Feces, feed, water	SE	115	400	*(1)*
Salmonella	NR	NR	Feces	SE	108	349	*(4)*
Salmonella	NR	AL, AR, CA, GA	Various environ. samples	SE	860	8739	*(5)*
Salmonella	NR	GA	Boot sock, drag swab, feces, litter	SE	177	549	*(8)*
Salmonella	NR	GA	Wild bird droppings near farm	SE	10[d]	119	*(21)*
Salmonella	NR	Southcentral	Environ. drag swabs	SE	37	68	*(46)*
Salmonella	NR	NC	Feed, litter, grass, inside/outside swabs	SE	112	900	*(107)*
Salmonella	Conv.	NC, SC, VA, WI	Feces	SE	125	419	*(98)*
Salmonella	Organic	NC	Feces, feed, water	SE	13	300	*(1)*

Salmonella	Pasture	AR	Feed, water, drag swabs, insect traps	SE	41	164	*(61)*
Salmonella	Pasture	NC, SC, VA, WI	Feces	SE	83	512	*(98)*
Salmonella	Pasture	Southeast	Soil, compost, processing wastewater	SE	72[d]	127	*(110)*

[a] Not reported (NR)
[b] Selective enrichment (SE)
[c] Direct plating (DP)
[d] Estimated from reported prevalence and sample size.

Table 6.2 Summary of broiler processing sampling studies used in meta-analysis

Category	Pathogen	Production	State/Region	Sampling method	Detection method	No. positive samples	Sample size	Citation
Rehang	*Campylobacter*	NR[a]	GA	WCR[c]	SE[h]	205	330	*(8)*
	Campylobacter	NR	AL, AR, CA, DE, GA, IN, MO, NC, SC, TN, TX, VA, WV	WCR	DP[i]	596	800	*(9)*
	Campylobacter	NR	Nationwide	WCR	SE	139	140	*(83)*
	Campylobacter	NR	Nationwide	WCR	DP	2337	3275	*(111)*
	Salmonella	NR	NR	WCR	SE	25	150	*(4)*
	Salmonella	NR	GA	WCR	SE	142	330	*(8)*
	Salmonella	NR	AL, AR, CA, DE, GA, IN, MO, NC, SC, TN, TX, VA, WV	WCR	BAX/SE	633[k]	900	*(10)*
	Salmonella	NR	Nationwide	WCR	BAX/MPN	1500	3275	*(111)*
Prechill	*Campylobacter*	NR	GA	WCR	SE	194	330	*(8)*
	Campylobacter	NR	NR	WCR	SE	75	75	*(101)*
	Salmonella	NR	GA	WCR	SE	60	330	*(8)*
	Salmonella	NR	NR	WCR	SE	60	60	*(13)*
	Salmonella	NR	NR	WCR/NSM[d]/WCE[ef]	SE	32	40	*(19)*
	Salmonella	NR	NR	WCR/NSM[g]	SE	93	180	*(20)*
	Salmonella	NR	Midatlantic	WCR	BAX/SE	212	240	*(77)*
	Salmonella	NR	GA	WCR	BAX/SE	1	15	*(82)*
Postchill	*Campylobacter*	NR	GA	WCR	SE	144	330	*(8)*
	Campylobacter	NR	AL, AR, CA, DE, GA, IN, MO, NC,	WCR	DP	279	800	*(9)*

		SC, TN, TX, VA, WV					
Campylobacter	NR	GA	WCR	DP[f]	34	36	*(55)*
Campylobacter	NR	GA	WCR	SE[g]	24	24	*(55)*
Campylobacter	NR	NR	WCR	DP[g]	152	240	*(75)*
Campylobacter	NR	Nationwide	WCR	SE	118	140	*(83)*
Campylobacter	NR	MO, NE	WCR	SE	132[k]	300	*(89)*
Campylobacter	NR	VA	WCR	SE	45	120	*(94)*
Campylobacter	NR	NR	WCR	SE	65	125	*(101)*
Campylobacter	NR	Nationwide	WCR	DP	1094	4200	*(103)*
Campylobacter	NR	Nationwide	WCR	SE	1526	3275	*(111)*
Campylobacter	Conv. AF[b]	Midwest	WCR	SE	19[k]	80	*(6)*
Campylobacter	Organic	Midwest	WCR	SE	8[k]	80	*(6)*
Campylobacter	Pasture	Southeast	WCR	SE	216	270	*(109)*
Salmonella	NR	NR	WCR	BAX/SE[f]	2	61	*(2)*
Salmonella	NR	AL, AR, CA, GA	WCR	SE	49	798	*(5)*
Salmonella	NR	GA	WCR	SE	8	330	*(8)*
Salmonella	NR	AL, AR, CA, DE, GA, IN, MO, NC, SC, TN, TX, VA, WV	WCR	BAX/SE	172[k]	900	*(10)*
Salmonella	NR	NR	WCR	SE	25	60	*(13)*
Salmonella	NR	NR	WCR/NSM[g]	SE	17	177	*(20)*
Salmonella	NR	NR	WCR/NSM/WCE[f]	SE	19	40	*(19)*
Salmonella	NR	Midatlantic	WCR	BAX/SE	202	240	*(77)*
Salmonella	NR	GA	WCR	BAX/SE	0	15	*(82)*
Salmonella	NR	MO, NE	WCR	SE	65[k]	300	*(89)*
Salmonella	NR	Nationwide	WCR	BAX/MPN	267	3275	*(111)*
Salmonella	NR	Nationwide	WCR	Enrichment, screening[j]	5251	47090	*(122)*

[a] Not reported (NR)
[b] Antibiotic-free (AF)
[c] Whole carcass rinse (WCR)
[d] Neck skin maceration (NSE)
[e] Whole carcass enrichment (WCE)
[f] Multiple sampling/detection methods used. Highest no. of positive samples among reported methods used.
[g] Multiple sampling/detection methods used. The number of true positives among all methods used as no. of positive samples.
[h] Selective enrichment (SE)
[I] Direct plating (DP)
[j] Standard screening method transitioned from immunoassay to BAX PCR at the end of the study, so both methods were used as screening tools in the study.
[k] Estimated from reported prevalence and sample size.

Table 6.3 Summary of retail broiler sampling studies used in meta-analysis

Pathogen	Production	State/Region	Sample type	Detection method	No. positive samples	Sample size	Citation
Campylobacter	NR	GA	Outside liver swab	SE/DP	29	45	*(11)*
Campylobacter	NR	GA	Inside liver swab	SE/DP	14	45	*(11)*
Campylobacter	NR	GA	Blended liver	SE/DP	30	45	*(11)*
Campylobacter	Conv.	MD	WCR[b]	SE	45	61	*(22)*
Campylobacter	NR	AR	WCR	DP	59	72	*(24)*
Campylobacter	Conv.	LA	WCR	SE	61	141	*(41)*
Campylobacter	NR	ND	PR[c]	SE	11	123	*(52)*
Campylobacter	NR	NR	PE[d]	SE[e]	36	55	*(57)*
Campylobacter	Conv.	MI, OH, PA	PR	SE	12	95	*(62)*
Campylobacter	NR	NR	WCR/Weep[e]	SE	39	60	*(64)*
Campylobacter	NR	NR	WCR/Weep[e]	SE/DP[e]	36	40	*(64)*
Campylobacter	NR	AR	WCR	DP	332[k]	420	*(67)*
Campylobacter	NR	AR	WCR	DP	548	744	*(66)*
Campylobacter	NR	OK	Liver/gizzard rinse	SE	136	202	*(69)*
Campylobacter	Conv.	OK	Chicken rinse	SE	32	85	*(70)*
Campylobacter	NR	AL, WA	PR/PE[f]	SE	70	120	*(76)*
Campylobacter	NR	AL, WA	PR/PE[f]	SE	103	120	*(76)*
Campylobacter	NR	MD	PE	SE	43	45	*(78)*
Campylobacter	Conv.	MD	PE	SE	64	80	*(79)*
Campylobacter	Conv.	MD	PR[g]	SE	12	36	*(88)*
Campylobacter	Conv.	PA	WCR	SE	26	50	*(93)*
Campylobacter	NR	Nationwide	PR	SE	534	2496	*(112)*
Campylobacter	NR	AL	PE	SE	308	755	*(123)*

Campylobacter	NR	MD	WCR	SE	130	184	*(126)*
Campylobacter	NR	CT, GA, MD, MN, OR, TN	PR	SE	3064	6138	*(128)*
Campylobacter	Organic	MD	WCR	SE	150	198	*(22)*
Campylobacter	Organic	LA	WCR	SE	23	53	*(41)*
Campylobacter	Pasture	NR	WCR	DP	36	48	*(42)*
Campylobacter	Organic	MI, OH, PA	PR	SE	2	40	*(62)*
Campylobacter	AF[a]	MI, OH, PA	PR	SE	11	96	*(62)*
Campylobacter	Organic	OK	Chicken rinse	SE	21	71	*(70)*
Campylobacter	AF	MD	PE	SE	33	45	*(78)*
Campylobacter	AF	MD	PE	SE	88	118	*(79)*
Campylobacter	Organic	MD	PR[g]	SE	20	28	*(88)*
Campylobacter	Farmer's market	MD	PR[g]	SE	28	32	*(88)*
Campylobacter	Farmer's market	PA	WCR	SE	90	100	*(93)*
Campylobacter	Organic	PA	WCR	SE	14	50	*(93)*
Salmonella	Conv.	MD	WCR	SE	27	61	*(22)*
Salmonella	NR	SD	GCE[h]	BAX/SE	6	42	*(29)*
Salmonella	NR	NR	GCE	SE, PCR, ELISA[f]	61[k]	172	*(31)*
Salmonella	NR	GA	PE	SE	170	525	*(40)*
Salmonella	Conv.	TN	WCR	SE	0	50	*(43)*
Salmonella	NR	GA	PE	SE	16	100	*(49)*
Salmonella	NR	DE, NJ, PA	Liver enrichment	BAX/SE	148	249	*(50)*
Salmonella	NR	ND	PR	SE	5	123	*(52)*
Salmonella	Conv.	LA	WCR	SE	31	141	*(54)*
Salmonella	Conv.	MI, OH, PA	PR	SE	24	95	*(62)*
Salmonella	NR	NR	GCE	SE	2	72	*(63)*
Salmonella	NR	MD	PE	SE, PCR[f]	25	90	*(65)*

Salmonella	NR	NR	PE[g]	SE	40	70	*(74)*
Salmonella	NR	MD	PE[g]	SE	2	31	*(71)*
Salmonella	NR	NR	PE[g]	SE	7	10	*(72)*
Salmonella	NR	MD	GCE	SE	19	100	*(73)*
Salmonella	NR	GA	PR	BAX	12	15	*(82)*
Salmonella	NR	Nationwide	GCE	SE	153	519	*(87)*
Salmonella	NR	Nationwide	WCR	SE	99	1197	*(87)*
Salmonella	Conv.	PA	WCR	SE	4	50	*(93)*
Salmonella	NR	GA	WCE[i]	SE	85	251	*(99)*
Salmonella	NR	Nationwide	PR	BAX/MPN	657	2496	*(112)*
Salmonella	NR	DC	GCE	SE	18[k]	51	*(121)*
Salmonella	NR	Nationwide	GCE	Enrichment, screening[j]	387	1722	*(122)*
Salmonella	Conv.	IN	PR	SE	3[k]	201	*(125)*
Salmonella	NR	MD	WCR	SE	9	212	*(126)*
Salmonella	NR	CA, CT, GA, MD, MN, NY, OR, TN	PR	SE	142[k]	1513	*(127)*
Salmonella	Free-range	NR	WCR	SE	42	135	*(3)*
Salmonella	All-natural	NR	WCR	SE	13	53	*(3)*
Salmonella	Organic	MD	WCR	SE	121	198	*(22)*
Salmonella	Organic	OK	GCE	SE	13	49	*(32)*
Salmonella	Organic	TN	WCR	SE	2	50	*(43)*
Salmonella	Organic	LA	WCR	SE	11	53	*(54)*
Salmonella	Pasture	AR	WCR	SE	18	36	*(61)*
Salmonella	Organic	MI, OH, PA	PR	SE	7	40	*(62)*
Salmonella	AF	MI, OH, PA	PR	SE	25	96	*(62)*
Salmonella	Farmer's market	PA	WCR	SE	28	100	*(93)*
Salmonella	Organic	PA	WCR	SE	10	50	*(93)*
Salmonella	AF	IN	PR	SE	10[k]	201	*(125)*

[a] Antibiotic-free (AF)

[b] Whole carcass rinse (WCR)
[c] Chicken parts rinse (PR)
[d] Chicken parts whole sample enrichment (PE)
[e] Multiple sampling/detection methods used. The number of true positives among all methods used as no. of positive samples.
[f] Multiple sampling/detection methods used. Highest no. of positive samples among reported methods used.
[g] Parts were cut from a carcass and sampled. The number of carcasses with at least one positive chicken part is reported.
[h] Ground chicken enrichment (GCE)
[i] Whole carcass enrichment (WCE)
[j] Standard screening method transitioned from immunoassay to BAX PCR at the end of the study, so both methods were used as screening tools in the study.
[k] Estimated from reported prevalence and sample size.

Table 6.4 *Salmonella* serotype prevalence in various sample types collected along the chicken supply chain

***Environmental* (n = 255):**			
Alachua (0.4%)	Bareilly (2.4%)	Cubana (0.4%)	Enteritidis (3.9%)
Havana (0.4%)	Heidelberg (3.9%)	Kentucky (10.6%)	Mbandaka (7.8%)
Newport (0.4%)	Ohio (10.6%)	Schwarzengrund (4.3%)	Senftenberg (9.8%)
Tennessee (1.2%)	Typhimurium (43.9%)		
***Rehang* (n = 25):**			
Alachua (4.0%)	Mbandaka (4.0%)	Ohio (16.0%)	Schwarzengrund (24.0%)
Senftenberg (52.0%)			
***Prechill* (n = 201):**			
Heidelberg (0.5%)	Kentucky (54.2%)	Litchfield (9.0%)	Mbandaka (5.0%)
Rough (4.5%)	Schwarzengrund (2.5%)	Senftenberg (1.0%)	Thompson (0.5%)
Typhimurium (21.9%)	Virchow (0.5%)	Worthington (0.5%	
***Postchill* (n = 198):**			
Kentucky (68.7%)	Litchfield (3.5%)	Mbandaka (6.1%)	Newington (0.5%)
Rough (3.0%)	Schwarzengrund (2.5%)	Typhimurium (15.7%)	
***Retail* (n = 1290):**			
4,[5],12:i:- (1.2%)	4,12:Nonmotile (0.2%)	4:i:- (0.1%)	6,7:-:1,5 (0.1%)
6,7:-:e,n,z15 (0.1%)	8,20:-:z6 (0.3%)	8,20:2:z6 (0.1%)	8,20:i:- (0.1%)
Agona (0.3%)	Blockley (0.1%)	Braenderup (0.5%)	Brandenburg (0.1%)
Duisburg (0.1%)	Enteritidis (15.3%)	Give (0.1%)	Glostrup (0.3%)
Hadar (0.6%)	Heidelberg (12.5%)	Infantis (2.3%)	Istanbul (1.2%)
Johannesburg (0.2%)	Kentucky (29.1%)	Kiambu (0.1%)	Mbandaka (0.2%)
Montevideo (0.9%)	Muenchen (0.1%)	Newport (0.3%)	Ohio (0.3%)
Orion (0.1%)	Ouakam (0.1%)	Rough_O:f,g,s:- (0.1%)	Schwarzengrund (1.3%)
Senftenberg (0.3%)	Thompson (2.9%)	Typhimurium (28.5%)	Uganda (0.1%)
Worthington (0.1%)			

Table 6.5 Meta-analysis results for pathogen prevalence at different stages throughout the chicken supply chain subgrouped by type of production system

Pathogen	Sample type	Production	No. of trials	Population prevalence (95% CI)[c]	$\tau^{2\,d}$	$I^{2\,e}$ (%)
Campylobacter	Environmental	Conventional	4	0.158 (0.030-0.528) A	3.25	99.4
		Alternative	3	0.528 (0.328-0.718) B	0.51	94.9
		Combined[a]	7	0.289 (0.105-0.585)	2.77	99.2
	Rehang	Conventional[b]	4	0.849 (0.524-0.966)	2.58	99.7
	Prechill	Conventional[b]	2	0.979 (0.064-1.000)	14.52	93.5
	Postchill	Conventional	11	0.609 (0.413-0.776) A	1.67	99.6
		Alternative	3	0.343 (0.084-0.748) A	2.28	97.3
		Combined	14	0.556 (0.367-0.731)	2.04	99.6
	Retail	Conventional	25	0.592 (0.476-0.698) A	1.35	99.0
		Alternative	12	0.554 (0.345-0.746) A	2.18	96.1
		Combined	37	0.581 (0.477-0.678)	1.61	98.8
Salmonella	Environmental	Conventional	8	0.229 (0.145-0.342) A	0.63	98.5
		Alternative	4	0.199 (0.071-0.448) A	1.41	97.8
		Combined[a]	12	0.219 (0.140-0.326)	0.89	98.7
	Rehang	Conventional[b]	4	0.429 (0.243-0.638)	0.74	99.1
	Prechill	Conventional[b]	6	0.686 (0.201-0.950)	6.70	99.1

Postchill	Conventional[b]	12	0.143 (0.063-0.292)	2.37	99.6
Retail	Conventional	27	0.190 (0.122-0.283) A	1.76	98.9
	Alternative	12	0.230 (0.148-0.340) A	0.81	91.8
	Combined	39	0.202 (0.146-0.272)	1.44	98.3

[a] Represents the meta-analysis model for both production methods combined.
[b] Data were only available for the conventional production method.
[c] Values with different superscript letters represent significantly different ($p < 0.10$) estimated pooled prevalence values for conventional and alternative production systems.
[d] Between study variability (τ^2)
[e] Heterogeneity (I^2)

Table 6.6 The heterogeneity explained by various moderator variables in the combined meta-analysis models for each sample type and pathogen combination

Organism	Combined model	Moderator variable *P*-values				Heterogeneity explained	
		Production system	Detection method	Year (est.)[a]	Type of chicken sample	τ^2	R^2 (%)
Campylobacter	Environmental	0.213	0.517	0.986	–[b]	1.87	32.28
	Rehang	–	0.002	0.002 (-0.788)	–	0.37	85.85
	Prechill	–	–	–	–	–	–
	Postchill	0.285	0.169	0.003 (-0.252)	–	1.08	47.19
	Retail	0.410	0.389	0.339	0.437	0.80	50.14
Salmonella	Environmental	0.443	–	0.154	–	0.75	15.31
	Rehang	–	0.007	0.054 (0.111)	–	0.17	76.69
	Prechill	–	0.567	0.009 (-0.478)	–	2.40	64.18
	Postchill	–	0.926	0.633	–	2.40	0.00
	Retail	0.008	0.001	0.145	0.096	0.78	45.81

[a] If statistically significant ($p < 0.10$), the estimated change in logit-transformed prevalence per one unit increase in year is shown.
[b] –, not applicable.

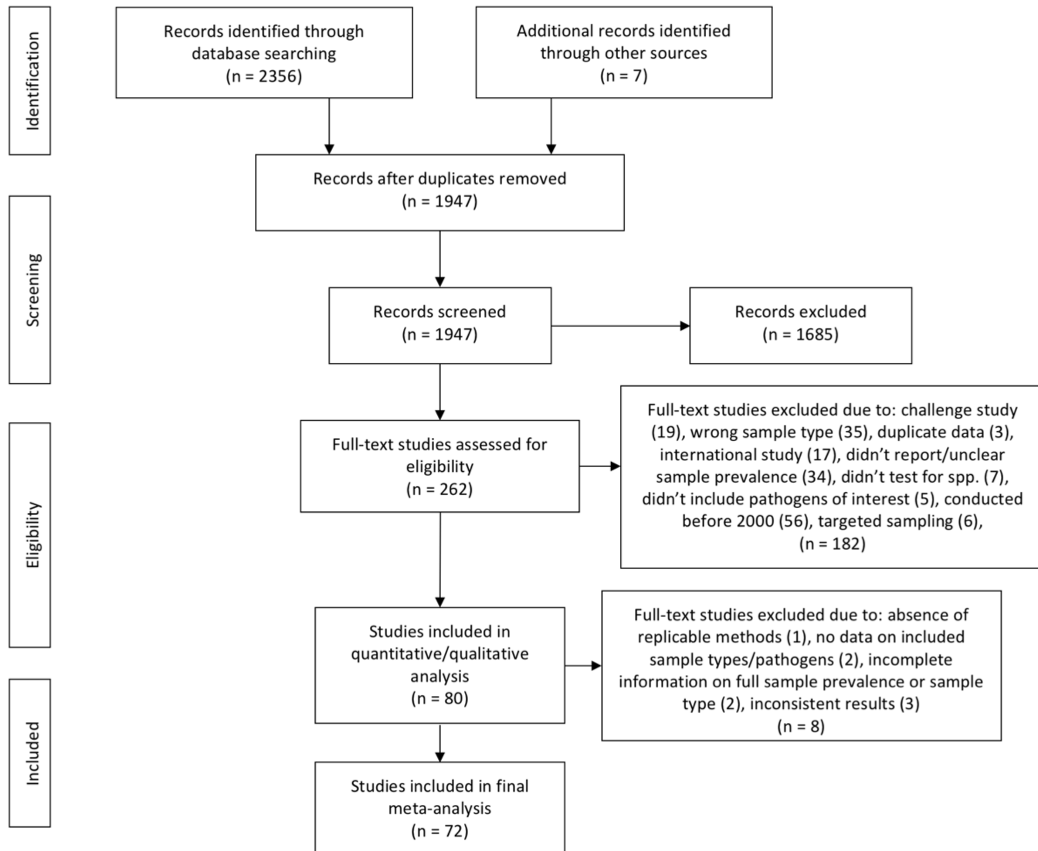
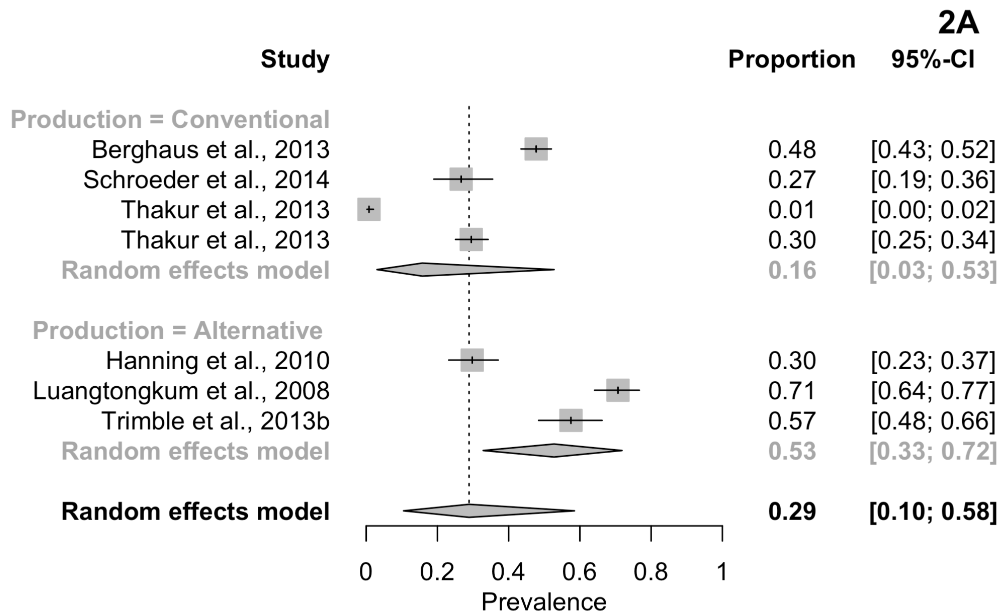


Figure 6.1 Flow diagram of the systematic review process.



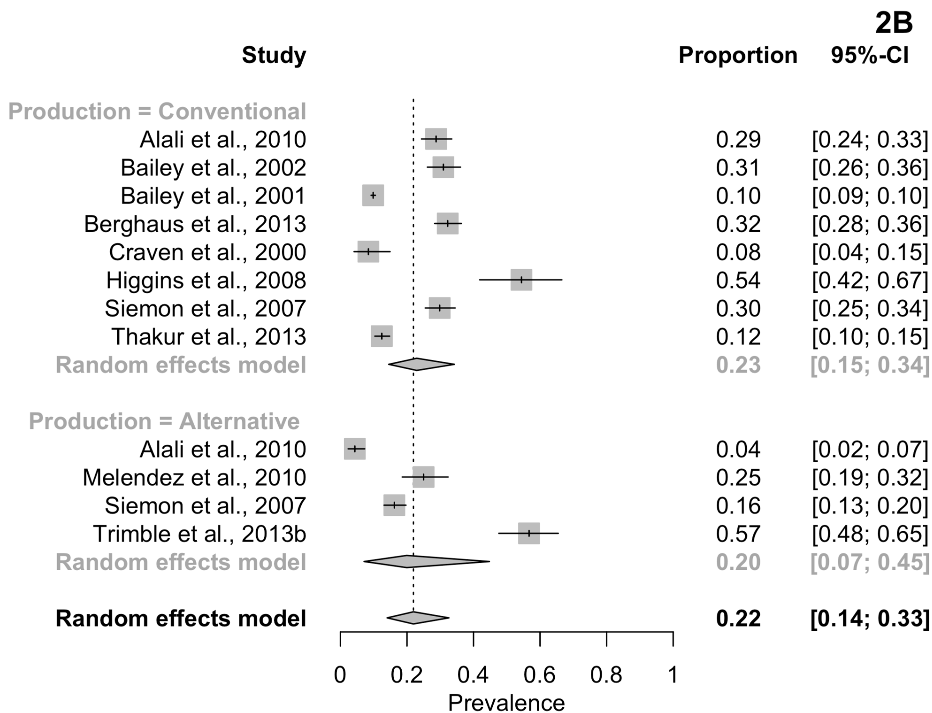
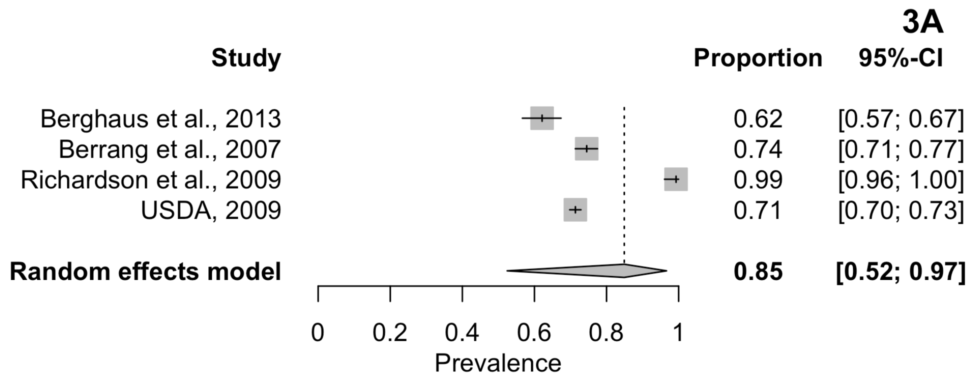


Figure 6.2 Forest plots for the (a) *Campylobacter* spp. and (b) *Salmonella* spp. environmental sample subgroups.



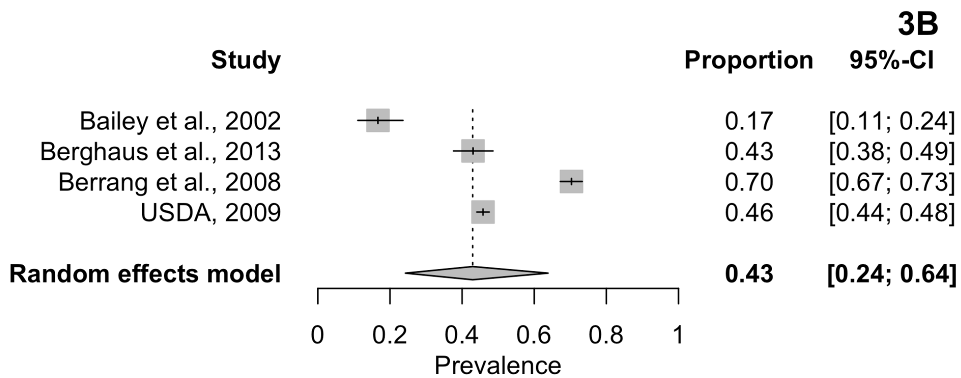
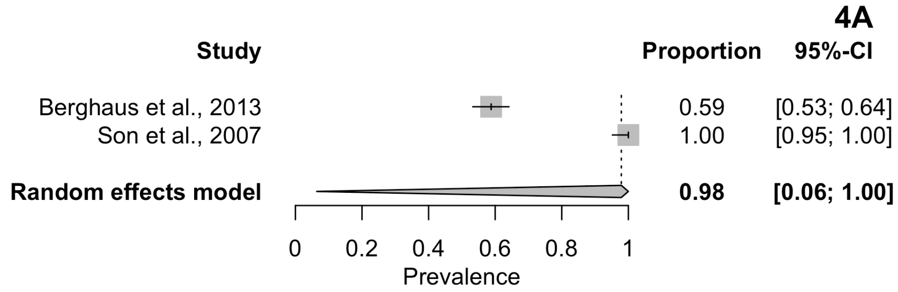


Figure 6.3 Forest plots for the (a) *Campylobacter* spp. and (b) *Salmonella* spp. rehang sample subgroups.



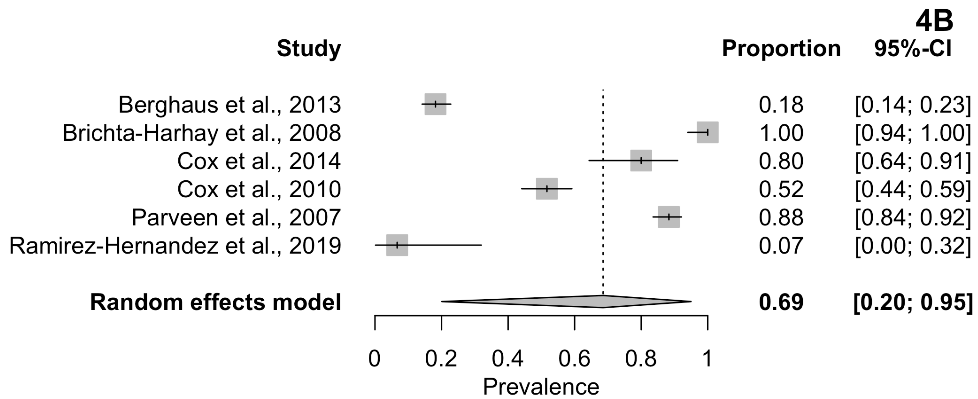
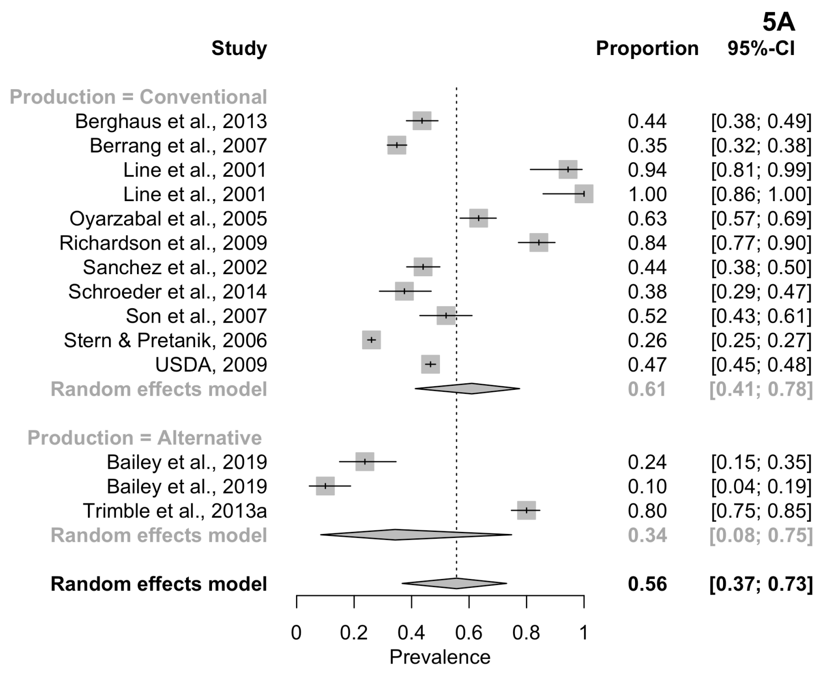


Figure 6.4 Forest plots for the (a) *Campylobacter* spp. and (b) *Salmonella* spp. prechill sample subgroups.



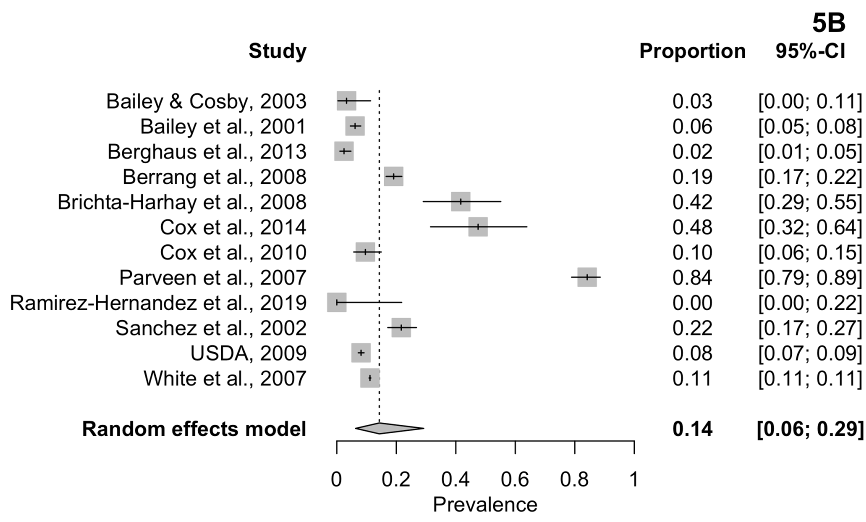


Figure 6.5 Forest plots for the (a) *Campylobacter* spp. and (b) *Salmonella* spp. postchill sample subgroups.

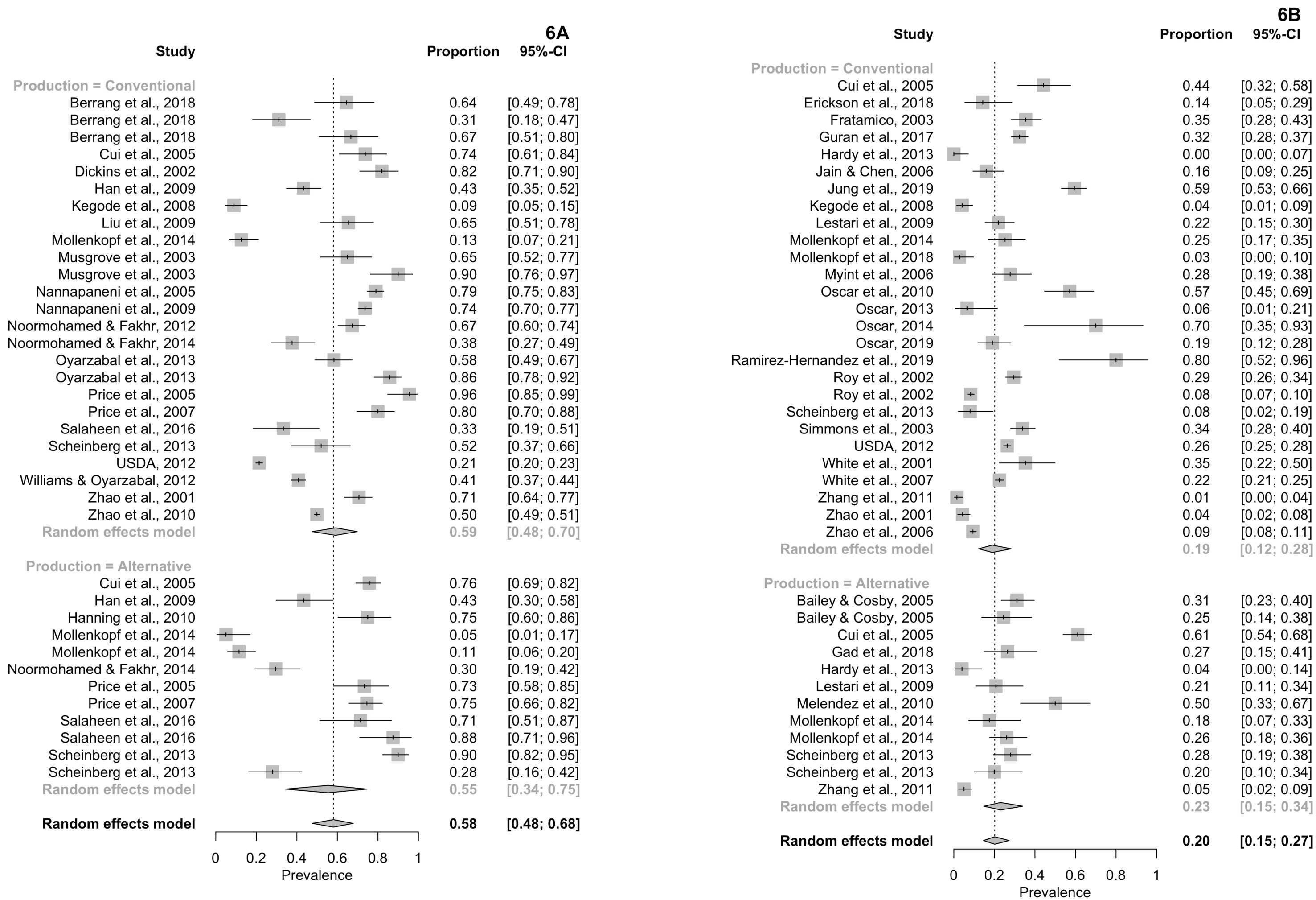


Figure 6.6 Forest plots for the (a) *Campylobacter* spp. and (b) *Salmonella* spp. retail sample subgroups.

CHAPTER 7

ASSESSING THE RISK OF SALMONELLOSIS FROM CONSUMPTION OF CONVENTIONALLY AND ALTERNATIVELY PRODUCED BROILER MEAT PREPARED IN-HOME IN THE UNITED STATES

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[1] Golden, C.E. and A. Mishra. Submitted to *Microbial Risk Analysis*, 3/2/2020.

Abstract

Salmonella spp. have long presented a major problem related to the food safety of broiler meat. As the popularity of alternatively produced (*e.g.* organic) broiler meat increases, an understanding of the food safety risks associated with these types of products is needed. The purpose of this study was to develop a retail-to-consumption quantitative microbial risk assessment model that could be used to estimate the differences in risk of salmonellosis acquired from consumption of conventionally and alternatively produced broiler meat annually in the United States. Data were extracted and used to define distributions to estimate *Salmonella* growth during retail storage, transportation, and home storage, as well as concentration changes during preparation or due to cross-contamination. A Monte Carlo simulation with 100,000 iterations was performed to estimate the total risk per serving and total number of illnesses in the United States annually from both meat types. Sensitivity analyses were performed to determine factors that were highly correlated with increased risk of salmonellosis. Conventionally produced chicken meat was estimated to have a median risk per serving of 7.62×10^{-8} and cause a median of 1,695 illnesses annually compared with a median risk per serving of 9.23×10^{-8} and 280 estimated illnesses for alternatively produced chicken. The sensitivity analysis identified cross-contamination of hands during meal preparation as the most important factor linked to risk. The 'what-if' scenario analysis estimated that using antimicrobial soap during hand washing after handling raw chicken can reduce risk considerably. The developed risk assessment model provides information on the public health risk of conventionally and alternatively produced broiler meat. These results will be useful in determining the key intervention strategies to mitigate the food safety risks associated with the consumption of contaminated chicken products.

7.1 Introduction

Salmonella enterica presents an annual food safety problem for the poultry industry. According to the Centers for Disease Control and Prevention (CDC), from 1998-2017 there were 298 salmonellosis outbreaks in the United States that were linked to consumption of chicken, accounting for 7,881 illnesses and 4 deaths *(11)*. The gastrointestinal tract of broilers has been shown to be a reservoir for *Salmonella*, potentially signifying why the organism is so often linked to chicken-borne salmonellosis *(10)*. Live broilers and broiler carcasses can be contaminated with *Salmonella* spp. during many points of the supply chain, such as pre-slaughter contamination due to poor environmental conditions, poor worker hygiene, contaminated transport crates, and leakage of fecal material during processing *(1, 7, 27, 58)*. An effort has been made to quantify the prevalence of *Salmonella* on broiler carcasses and broiler meat throughout the supply chain, including its United States retail prevalence *(22)*.

Recently, alternative types of poultry production, such as organic, pastured, and free-range, have increased in popularity due to consumers' desire for sustainable food products and products they see as "natural" *(15, 56)*. Researchers have shown that consumers are willing to pay more for organic chicken meat and that consumers view organic foods as safer and healthier options than conventionally produced food products due to the lack of pesticides and antibiotics in organic farming *(29, 62, 70)*. An understanding of the food safety risks presented by alternatively produced broiler meat is still needed, due to different production and environmental conditions *(23, 24, 30)*.

Quantitative microbial risk assessment (QMRA) has been a widely used tool in the food industry to estimate the risk of biological hazards to food consumers; in the late 1990s Whiting

and Buchanan *(72)* used QMRA to estimate the salmonellosis risk due to consumption of liquid eggs. Risk assessment is characterized by four main steps as defined by Codex Alementarius Comission *(14)*: i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization. Quantitative microbial risk assessment models help to quantify the risk due to specified hazards, but also help to point out data gaps in the literature and usefulness of intervention strategies in reducing risk *(9, 19)*.

To our knowledge, there has only been one QMRA estimating the food safety risk of alternatively produced broiler meat, where Rosenquist et al. *(57)* found campylobacteriosis risk to be 1.7 times higher in Danish organic poultry meat when compared to conventional meat. The goal of the current study was to construct a retail-to-consumption QMRA model that could be used to estimate the number of annual illnesses caused by consumption of conventionally and alternatively produced broiler meat prepared in-home in the United States and how different intervention strategies could mitigate those risks.

7.2 Materials and methods

7.2.1 QMRA overview

A process flow of the time from when broiler meat is packaged to the time when consumers consume the meat was developed. Areas of the flow included retail storage, transportation to consumer home, home storage, preparation, and consumption. A literature search was conducted to identify distributions that could be used to describe parameters in each of these areas, as well as the growth and inactivation kinetics of *Salmonella* at various temperatures. A list of all variables is shown in Table 7.1.

7.2.2 *Salmonella* growth kinetics

To capture the growth behavior of *Salmonella* at the wide variety of temperatures it could encounter during the retail-to-consumption chain, a comprehensive understanding of *Salmonella* growth rates on various types of broiler meat was needed. As such, Combase (https://www.combase.cc/index.php/en/) was searched for primary growth data using *Salmonella* spp. as the "organism" search parameter and chicken as the "food name" parameter. A similar method was used by Dominguez and Schaffner *(17)*, but only data obtained from *Salmonella* growth on chicken were considered in the current study. In the search, various sources of primary growth data were identified *(3, 8, 31, 47, 49-52)* (Table 7.2). For each study, primary growth data were extracted and the three-phase linear model was fitted to growth data to determine growth rate, or μ, using the following equations *(5, 32)*:

$$y_t = y_0 \text{ for } t \leq t_{lag} \quad (1)$$

$$y_t = y_0 + \mu(t - t_{lag}) \text{ for } t_{lag} < t < t_{max} \quad (2)$$

$$y_t = y_{max} \text{ for } t \geq t_{max} \quad (3)$$

where y_t = the number of cells at time *t* (log CFU/g); y_0 = the initial number of cells (Log CFU/g); y_{max} = the maximum number of cells supported by the environment (log CFU/g); μ = growth rate (log CFU/h); *t* = elapsed time (h); t_{lag} = lag time (h); and t_{max} = time when y_{max} is reached (h). When only two phases were present in the growth data, the biphasic model was fitted instead. Primary models were fitted using the United States Department of Agriculture (USDA) Integrated Pathogen Modeling Program (IPMP; Version 2013) *(28)*.

After obtaining μ from each set of primary data, the Ratkowsky model was fitted to growth rates, characterized by the following equation *(55)*:

$$\sqrt{\mu} = b(T - T_{min}) \quad (4)$$

where T = temperature (°C); T_{min} = the theoretical minimum temperature for growth (°C); and b is a growth constant. It has been shown that the Ratkowsky model should be applied for temperatures between the minimum and optimum growth temperatures of an organism, so only growth rates from temperatures between 5.9 and 37°C were used *(17, 54)*. As such, if simulated temperatures were below 5.9°C in the QMRA, a growth rate of 0 was used to represent no growth. Secondary modeling was performed using the MATLAB Curve Fitting Toolbox (Version R2019b; Mathworks, Natick, MA), and estimates were obtained for b and T_{min}. A PERT distribution was defined for each parameter, where the Ratkowsky model estimate was treated as the most likely value, and the 95% confidence intervals for the estimates were used to describe the 2.5 and 97.5% percentiles of the distribution.

7.2.3 Product temperature change

Newton's law of heating has been used to describe the change of temperature of a food product when it enters a warmer ambient environment as a function of the starting temperature of the product, the ambient temperature, and the time it is in the ambient temperature *(42)*. It can be described by the following equation:

$$T = T_a - (T_a - T_0)e^{-Bt} \quad (5)$$

where T = final product temperature (°C); T_a = ambient temperature (°C); T_0 = starting product temperature (°C); t = time in ambient temperature (h); and B is a constant (h^{-1}). Using this eq. 5, eq. 4 can be re-written as:

$$\sqrt{\mu} = b((T_a - (T_a - T_0)e^{-Bt}) - T_{min}) \quad (6)$$

to describe the growth rate of *Salmonella* when chicken enters a warmer ambient temperature (e.g. when it leaves refrigerated storage during transportation home), given that the starting meat temperature, ambient temperature, time the meat is exposed to the new temperature, and the B constant are known.

Since the value of B is different for every type of food product, experimental data were needed to estimate its value for broiler meat. Raw boneless/skinless thighs and breasts and ground chicken were procured from a local Athens, GA supermarket and stored at approximately 5°C. Portions of 100-150 g of each type of chicken were placed in 20 and 30°C incubators for three hours, and temperature values were obtained from K-type probes attached to data loggers (EL-USB-TC-LCD; Lascar Electronics, Eerie, PA) placed approximately 1 cm beneath the surface of the meat. Temperature values were recorded every 10 s for 3 h; 2 replications were performed for each temperature/meat combination (N=12). The value of B was estimated for each replication using MATLAB Curve Fitting Toolbox (Version R2019b; Mathworks, Natick, MA).

For the QMRA model, a PERT distribution was used to describe B, where the experimental mean was considered the most likely value, and the observed minimum and maximum experimental value for B were used as the minimum and maximum values in the distribution, respectively. Equation 6 was used to estimate *Salmonella* growth rate when a change from cold ambient temperature to a warmer ambient temperature was expected, otherwise eq. 4 was used.

7.2.4 Retail storage and prevalence

To determine *Salmonella* prevalence on retail broiler meat, Golden and Mishra *(22)* conducted a meta-analysis utilizing reported literature prevalence values and provided estimates on the prevalence values for both conventionally and alternatively produced broiler meat. A PERT distribution was defined for each type of meat, with the estimated population prevalence used as the most likely value and the 95% confidence interval used to define the 2.5 and 97.5% boundaries of the distribution. The PERT distribution was chosen due to high amounts of observed heterogeneity in the meta-analysis *(6, 16)*.

Starting *Salmonella* concentration was based off a nationwide USDA survey conducted in 2012, where chicken samples were collected at the end of the production line, and *Salmonella* was quantified from positive samples (MPN/mL; Table 7.3) *(65)*. These values were then converted to MPN/g and used as the starting concentration in terms of CFU/g. While these units are not equal, it has been shown in the literature that the MPN from a sample is often higher than the CFU from a sample *(13, 39)*, so this can be considered a fail-safe choice.

To model retail storage temperature, distributions were fit to retail refrigerator temperatures observed in a study by Ecosure *(18)*. A Laplace distribution was the best fitting distribution. To eliminate abnormally high values during simulation, the distribution was truncated at the minimum and maximum observed temperatures *(18)*. A correlation coefficient of -0.75 was applied to storage time and temperature, as meat that is stored at a high temperature for a long time is unlikely to reach a consumer before it is spoiled *(73)*.

7.2.5 Transportation and home storage

Distributions were constructed to estimate growth conditions for *Salmonella* on chicken during the time from when meat leaves retail refrigerated storage until it reaches home refrigeration. Growth was estimated using eq. 6, where the ambient temperature and time spent at the ambient temperature were chosen from distributions constructed from the Ecosure *(18)* data, and starting temperature was determined by the retail storage temperature.

In the baseline model, scenarios were constructed for whether or not a consumer chose to freeze the purchased chicken meat or not. Consumer freezing behavior was obtained in a study by Mazengia et al. *(38)*, who found that 40% of surveyed individuals froze chicken meat before consumption. If a consumer did not freeze their chicken, *Salmonella* growth during home storage was calculated using home refrigerator data and time of refrigerated storage *(4, 38)*. If a consumer did choose to freeze their chicken meat, the same amount of refrigerated storage was used to simulate the time it took for a consumer to place their chicken into frozen storage. It was assumed that no growth took place while in frozen storage, as representative freezer temperatures would not allow for the growth of *Salmonella (64)*. Following frozen storage, 4 different thawing methods were considered: refrigerated, running water, microwave, and room temperature thawing. The behavior chosen by a consumer was based off the Mazengia et al. *(38)* study. Growth rates were determined by thawing time and temperatures encountered during various thawing methods. Thawing times were based on USDA recommendations and personal communication with an expert *(66)*. For the running water thawing method, a correlation coefficient of -0.75 was used, as a higher running water temperature was assumed to lead to a shorter thawing time. For the microwave thawing method, a correlation coefficient of 0.75 was

used to represent that longer microwave times were likely to lead to higher meat temperatures. For all methods, it was assumed that following thawing consumers immediately prepared their chicken.

7.2.6 Cross-contamination during preparation

Different types of cross-contamination scenarios were assessed in the baseline QMRA model: raw chicken to hands transfer, raw chicken to utensils (e.g. knives, cutting boards, etc.), hands to cooked chicken, and contaminated utensils to cooked chicken. Various studies were identified as part of a literature search that reported transfer rates relating to the types of cross-contamination considered in the current study (Table 7.4) *(12, 36, 43, 71)*. While transfer rates from raw chicken to hands and utensils were available, no identified studies presented data on transfer rate from contaminated hands and utensils to cooked chicken. Instead, surrogate food products, such as bread, lettuce, and cucumber were used.

For each study, transfer rates were extracted from the articles and assigned to one of the four types of cross-contamination. Transfer rates were then log transformed and distributions were fit to the data, as recommended by Chen et al. *(12)*. These distributions were sampled during simulation to represent a variety of transfer rates that could occur during each cross-contamination step. *Salmonella* concentration on hands after handling raw chicken could be reduced if the consumer washed their hands. In the baseline model, it was assumed that regular soap was being used by the consumer and the probability of the consumer washing their hands was determined by a poultry handling study conducted by Kosa et al. *(34)*. A beta distribution with parameters (3.01, 1.91, -3.0, 0.6) was recommended by Montville et al. *(44)* to represent

bacterial reduction (log CFU) after hand washing with regular soap. A transfer rate of 0% was assumed if a consumer used different utensils than what were used to handle the raw chicken.

7.2.7 Cooking

Undercooking of chicken allows the opportunity for *Salmonella* survival in consumed product. For the current study, undercooking was defined as cooking chicken to less than 165°F (73.9°C), the recommended temperature by USDA *(67)*. To determine the probability of a consumer undercooking their chicken, a weighted average of the reported number of undercooked samples from Bruhn *(4)* and Ecosure *(18)* was calculated. If chicken was properly cooked, it was assumed that 0 CFU/g *Salmonella* would be present in the cooked product. If chicken was undercooked, the D-value was calculated based on cooking time and temperature using an inactivation model proposed by Smadi et al. *(61)*, and subtracted from the concentration prior to cooking. Because the inactivation model only utilized inactivation data from 55-70°C, only simulated cooking temperatures within this range were considered. A PERT distribution was used for cooking temperature, with the most likely value being the mean temperature of undercooked chicken samples collected by Bruhn *(4)*. A correlation coefficient of -0.75 was used to describe cooking time and temperature, as higher temperatures are likely to lead to shorter cooking times *(60, 73)*.

7.2.8 Dose-response modeling and risk characterization

Probability of illness was determined using a beta-Poisson model represented by the following equation *(26)*:

$$P = (1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha} \qquad (7)$$

where P = probability of illness; D = ingested dose (CFU); and α and β represent model parameters. A triangular distribution was used to describe α and β during simulation *(20, 73)*.

The total risk per serving of chicken was then determined by multiplying the probability of illness by the retail prevalence for both conventionally and alternatively produced chicken meat *(53)*. Total number of salmonellosis cases caused annually by each type of meat was then determined by multiplying the total risk per serving by the estimated annual number of servings consumed by the United States population.

7.2.9 "What-if" scenarios

A variety of best- and worst-case alternate scenarios to the baseline QMRA model were evaluated, and the total number of estimated salmonellosis cases from each scenario was compared to the baseline result. First, the effect of handwashing was considered by running simulations where all and no consumers washed their hands after handling raw chicken. Another simulation was run to see the effect of all consumers using an antimicrobial soap, compared to the baseline QMRA model where all consumers used a regular soap. A beta distribution with parameters (4.19, 2.99, -4.50, 1.50) was used to model the log CFU reduction on hands after washing with antimicrobial soap *(44)*. Next, the effect of cooking chicken to the USDA recommended 165°F (73.9°C) was considered by running simulations where all consumers cooked chicken to the recommended temperature and another simulation where all consumers cooked chicken to a temperature between 55-70°C. Finally, the effect of using new dishes or utensils after handling raw chicken was determined by running simulations where all and no consumers used new dishes/utensils after handling raw chicken.

7.2.10 Risk modeling

All distribution fitting and simulations were performed using @Risk software (Version 7.6.1; Palisade, Ithaca, NY). All Monte Carlo simulations were performed using 100,000 iterations and Latin hypercube distribution sampling. Spearman's correlation coefficients were used in sensitivity analysis to determine the most impactful factors in determining total risk per serving and total number of illness cases per year.

7.3 Results and discussion

Primary *Salmonella* growth rates were estimated at various temperatures from 0-45°C (Table 7.2). These growth rates are graphically displayed in Fig. 7.1a. As mentioned previously, the Ratkowsky model was only fitted to growth rates from temperature profiles that were in the range of 5.9-37°C *(17)* (Fig 7.2b). The Ratkowsky model provided parameter estimates of b = 0.031 (95% CI: 0.030-0.032) and T_{min} = 6.30°C (95% CI: 5.67-6.94°C), with an adjusted-R^2 = 0.933. It is important to note that the T_{min} value estimated by the Ratkowsky model is a theoretical value, different than what is observed in practice *(40)*. For *Salmonella* growth to occur in the current QMRA model, temperatures needed to be above 5.9°C, as recommended by Dominguez and Schaffner *(17)*.

Newton's law of heating was applied to various types of chicken to account for time it takes for chicken to reach ambient temperature when it enters a warmer environment. From the experimental data, the average value for Newton's heating constant, B, was 2.26 h^{-1} (SD: 0.54 h^{-1}). This value is intended to help estimate the surface temperature of chicken after a known time in a known ambient temperature. This is vitally important when estimating pathogen growth during transportation to a consumer's home from retail refrigerated storage, where a rise in

product temperature is often observed *(41)*. Kim et al. *(33)* found that fresh meat reached up to 30°C after storage in a car trunk exposed to sunlight for 90 min. In a study by Ecosure *(18)*, the average temperature of refrigerated products when they were placed in a consumer's home after retail purchase was 8.39°C. To account for this in the baseline QMRA model, distributions were fit to estimate the ambient temperature during transportation and the time a consumer takes to place retail purchased chicken in their home refrigerator (Table 7.1).

To account for the uncertainty in how consumers handle chicken meat once it is in the home, various potential pathways were included in the QMRA model. Specifically, consumer freezing and thawing behavior was modeled. In a United States survey, Mazengia et al. *(38)* found that 40% of respondents stored their raw poultry in the freezer. As such, a Bernoulli distribution with $p = 0.40$ was used in each model iteration to indicate whether a consumer froze their chicken meat or not. In the same survey it was identified that, on average, consumers stored poultry meat in the refrigerator for 1 to 7 days before use *(38)*. If a consumer did not freeze their meat, it was assumed that it was cooked directly after refrigerated storage. A Weibull distribution was used to model the time meat was stored in the refrigerator before either cooking or freezing.

Similarly, uncertainty in the way that different consumers thaw their frozen meat needed to be accounted for. Data were extracted from a survey conducted by Mazengia et al. *(38)* and used to determine the probabilities that a consumer would thaw by refrigeration, microwave, running water, or room temperature thawing methods. All methods besides room temperature thawing are recommended by USDA for safe defrosting of meat *(66)*. Thawing times for refrigeration and running water methods were based off USDA recommendations, and microwave thawing times were based off typical home microwave "defrost" settings (25-30%

power) *(63, 66)*. For each thawing method, it was assumed that a consumer would cook the meat directly after defrosting. Before-cooking *Salmonella* concentration was estimated for each iteration, dependent on consumer handling (Table 7.1).

After considering cooking and cross-contamination events within the home kitchen, final *Salmonella* concentration was calculated in log CFU/g chicken. These results are displayed graphically in Fig. 7.2. The final concentration numbers were approximately normally distributed with a mean of -5.74 log CFU/g (SD: 1.66 log CFU/g) and maximum of 6.37 log CFU/g. These numbers are representative of chicken servings which were contaminated with *Salmonella* at retail purchase. *Salmonella* prevalence at retail for both production systems was used to calculate the risk per serving of chicken meat.

For both production systems, the total salmonellosis risk per chicken serving and total number of illnesses due to consumption of chicken in United States homes were calculated (Table 7.5). Due to the presence of outliers, median and interquartile range (IQR) statistics were generated from simulation results so as not to be distorted by the skewed data *(37)*. Median total risk per serving was slightly higher for alternatively produced chicken (9.2×10^{-8}, IQR: 9.7×10^{-7}) when compared to conventionally produced chicken (7.6×10^{-8}, IQR: 1.2×10^{-6}) (Fig. 7.3). These values reflect the trend seen in retail *Salmonella* prevalence, where prevalence estimates for alternative chicken were slightly higher than conventional chicken, but differences were not significant *(22)*.

To determine the number of illnesses caused by each production type annually in the United States, the total number of servings for each production type was estimated from publicly available data, using the reference amount customarily consumed per eating occasion for poultry

meat of 85 g (9 CFR §381.412) *(25, 45, 46, 69)*. This resulted in estimates of 2.2×10^{10} and 3.0×10^{9} of total number of annual servings for conventionally and alternatively produced chicken meat cooked in-home, respectively (Table 7.1). The estimated median number of illnesses caused by consumption of conventionally and alternatively produced chicken in the baseline QMRA model were approximately 1,695 (IQR: 21,693.4) and 280 (IQR: 3,577.7), respectively.

The baseline QMRA model predicted a median number of 1,985 cases of salmonellosis cases annually when combing both production systems (Table 7.6). From 1998-2017, according to the National Outbreak Reporting System (NORS) from the CDC, there were 7,881 cases of salmonellosis linked to the consumption of chicken *(11)*. Of these, there were 2,194 cases where chicken was the only identified source of illness, resulting in 109.7 illness cases per year. This estimate is best to consider, as the current model did not account for cross-contamination from other ingredients. Scallan et al. *(59)* recommended a multiplier of 29.3 to account for the underdiagnosis of non-typhoidal salmonellosis, which would result in 3,214.2 annual cases. This number is higher than the number predicted by the baseline QMRA model, the number accounting for underdiagnosis includes illnesses acquired from consumption of chicken in restaurants and other out-of-home scenarios, while the QMRA model only accounts for chicken prepared in-home.

Sensitivity analysis showed that cross-contamination events were the three most important QMRA variables in determining total *Salmonella* risk per serving, followed by *Salmonella* concentration at purchase and ambient temperature during transportation (Fig. 7.4). The current model specifically identified handling cooked chicken with hands that had handled

raw meat as the most significant risk indicator. Studies have shown that even when hands are properly washed, significant numbers of bacteria can remain *(44)*. Interestingly, no variables linked to cooking steps during chicken preparation were identified in the top 8 most important variables. These results are concurrent with other risk assessment studies that have shown that cross-contamination events are the primary route of exposure for *Salmonella* and *Campylobacter* infection during poultry consumption *(35, 60)*.

Various "what-if" scenarios were considered and the total number of estimated illnesses for each scenario was compared to the baseline model (Table 7.6). Alternate handwashing scenarios were considered first. Consumers always washing their hands with regular soap after handling raw chicken resulted in 19.4% reduction in total illnesses, compared with a 347.1% increase when hands were never washed, further emphasizing the importance of handwashing after handling raw chicken. The effect of hand soap was also observed by running a simulation where all consumers used an antimicrobial soap during handwashing. This resulted in a 26.1% decrease in estimated number of illnesses from the baseline model. As handwashing reduction was identified as an important variable in determining risk (Fig. 7.4), and antimicrobial soap has been shown to increase the bacterial reduction during handwashing *(44)*, this was expected. While this can be viewed as a measure to reduce the risk of illness due to chicken consumption, the risk of bacterial antimicrobial resistance to chemicals found in antimicrobial soaps, such as triclosan, should also be considered *(21)*. Additionally, when consumers always washed dishes/utensils or used new dishes/utensils after handling raw chicken, estimated number of illnesses decreased by 11.6% compared to the baseline model. Again, undercooking of chicken

had very little impact in the model. Simulations where chicken was always undercooked by consumers only resulted in an increase of 5.2% estimated number of illnesses.

The current QMRA model was constructed to best reflect the current knowledge and practices of the retail-to-consumption broiler meat supply chain. In doing so, several assumptions were made in the model and knowledge gaps were identified. First, it was assumed that consumers did not use refrigerated storage during the transportation of meat from retail to home. It is likely that other purchased products would impact the temperature change in the chicken, but data were too limited to specifically address this point. Next, assumptions had to be made in thawing times during the considered thawing methods to best reflect the behavior that is most often performed by United States consumers. Data on thawing method tendencies were available, but information on the exact procedures carried out during these methods were lacking, so USDA guidance and personal communication with an expert had to be relied on *(66)*. It was also assumed that bacterial transfer rates for *Salmonella* are similar to the surrogates used in the included cross-contamination studies (Table 7.4), as relevant transfer rate data for *Salmonella* were lacking. Finally, other recognized types of cross-contamination events, such as chicken washing or cross-contamination from other types of food items, were not included in the QMRA model *(68)*. For chicken washing, data on transfer rate of chicken to various surfaces in the kitchen were not able to be identified. Cross-contamination from other foods was not included, as the current model was only interested in determining the number of annual illnesses caused specifically by broiler meat.

7.4 Conclusions

In conclusion, the current QMRA model estimated the number of annual cases of salmonellosis caused by consumption of alternatively and conventionally produced chicken meat prepared in-home in the United States. The total risk per serving presented by both production methods was similar. The model specifically identified cross-contamination events to be the most important predictors of risk. The presented model provides a framework of chicken consumption from purchase at retail to consumption in-home and identified various data gaps that could be addressed to better fill out the framework, as well as strategies to employ to reduce the risk of salmonellosis due to chicken meat consumption in the United States.

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7.6 References

1. Baggesen, D. L., J. Olsen, and M. Bisgaard. 1992. Plasmid profiles and phage types of *Salmonella typhimurium* isolated from successive flocks of chickens on three parent stock farms. *Avian Pathol.* 21:569-579.

2. Booten, C., J. Robertson, D. Chritensen, M. Heaney, D. Brown, P. Norton, and C. Smith. 2017. Residential indoor temperature study. Available at: https://www.nrel.gov/docs/fy17osti/68019.pdf. Accessed 3 January 2020.

3. Bovill, R., J. Bew, N. Cook, M. D'agostino, N. Wilkinson, and J. Baranyi. 2000. Predictions of growth for *Listeria monocytogenes* and *Salmonella* during fluctuating temperature. *Int. J. Food Microbiol.* 59:157-165.

4. Bruhn, C. M. 2014. Chicken preparation in the home: An observational study. *Food Prot. Trends*. 34:318-330.

5. Buchanan, R. L., R. C. Whiting, and W. C. Damert. 1997. When is simple good enough: A comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. *Food Microbiol.* 14:313-326.

6. Buczinski, S., and J. M. Vandeweerd. 2016. Diagnostic accuracy of refractometry for assessing bovine colostrum quality: A systematic review and meta-analysis. *J. Dairy Sci.* 99:7381-7394.

7. Bull, S. A., V. M. Allen, G. Domingue, F. Jørgensen, J. A. Frost, R. Ure, R. Whyte, D. Tinker, J. E. L. Corry, and J. T. J. H. Gillard-King. 2006. Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. *Appl. Environ. Microbiol.* 72:645-652.

8. Callaghan, K., G. Betts, L. Everis, and A. Parish. 2003. Growth of *Salmonella* on chicken at chilled storage temperatures. Available at: https://www.cbpremium.org/images/help/Callaghan_2003_Ss_chicken_thigh.pdf. Accessed 2 December 2019.

9. Campagnollo, F. B., U. Gonzales-Barron, V. A. P. Cadavez, A. S. Sant'Ana, and D. W. Schaffner. 2018. Quantitative risk assessment of *Listeria monocytogenes* in traditional Minas cheeses: The cases of artisanal semi-hard and fresh soft cheeses. *Food Control*. 92:370-379.

10. Cason, J. A., N. A. Cox, and J. S. Bailey. 1994. Transmission of *Salmonella typhimurium* during hatching of broiler chicks. *Avian Dis.*:583-588.

11. Centers for Disease Control and Prevention. 2020. National Outbreak Reporting System (NORS), Outbreaks per state, *Salmonella*, chicken. Available at: https://wwwn.cdc.gov/norsdashboard/. Accessed 17 January 2020.

12. Chen, Y., K. M. Jackson, F. P. Chea, and D. W. Schaffner. 2001. Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. *J. Food Prot.* 64:72-80.

13. Cho, K. H., D. Han, Y. Park, S. W. Lee, S. M. Cha, J.-H. Kang, and J. H. Kim. 2010. Evaluation of the relationship between two different methods for enumeration fecal indicator bacteria: Colony-forming unit and most probable number. *J. Environ. Sci.* 22:846-850.

14. Codex Alementarius Comission. 2015. Procedural manual. Available at: http://www.fao.org/3/a-i5079e.pdf. Accessed 1 January 2020.

15. Dimitri, C., and L. Oberholtzer. 2009. Marketing US organic foods: Recent trends from farms to consumers. Economic Information Bulletin No. 58. United States Department of Agriculture, Economic Research Service.

16. Dogan, O. B., J. Clarke, F. Mattos, and B. Wang. 2019. A quantitative microbial risk assessment model of *Campylobacter* in broiler chickens: Evaluating processing interventions. *Food Control*. 100:97-110.

17. Dominguez, S. A., and D. W. Schaffner. 2008. Modeling the growth of *Salmonella* in raw poultry stored under aerobic conditions. *J. Food Prot.* 71:2429-2435.

18. Ecosure. 2008. 2007 U.S. cold temperature evaluation design and summary pages. Available at: http://old.foodrisk.org/default/assets/File/EcoSure%202007%20Cold%20Temperature%20Report.pdf. Accessed 3 January 2020.

19. Farber, J. M., W. H. Ross, and J. Harwig. 1996. Health risk assessment of *Listeria monocytogenes* in Canada. *Int. J. Food Microbiol.* 30:145-156.

20. Giacometti, F., P. Bonilauri, S. Albonetti, S. Amatiste, N. Arrigoni, M. Bianchi, B. Bertasi, S. Bilei, G. Bolzoni, and G. Cascone. 2015. Quantitative risk assessment of human salmonellosis and listeriosis related to the consumption of raw milk in Italy. *J. Food Prot.* 78:13-21.

21. Giuliano, C. A., and M. J. Rybak. 2015. Efficacy of triclosan as an antimicrobial hand soap and its potential impact on antimicrobial resistance: A focused review. *Pharmacotherapy*. 35:328-336.

22. Golden, C. E., and A. Mishra. 2020. Prevalence of *Salmonella* spp. and *Campylobacter* spp. in alternative and conventionally produced chicken in the United States: A systematic review and meta-analysis. *J. Food Prot.*:(In Press, DOI: 10.4315/JFP-19-538).

23. Golden, C. E., M. J. Rothrock Jr, and A. Mishra. 2019. Comparison between random forest and gradient boosting machine methods for predicting *Listeria* spp. prevalence in the environment of pastured poultry farms. *Food Res. Int.* 122:47-55.

24. Golden, C. E., M. J. Rothrock Jr, and A. Mishra. 2019. Using farm practice variables as predictors of *Listeria* spp. prevalence in pastured poultry farms. *Front. Sustain. Food Syst.* 3:15.

25. Greene, C., G. Ferreira, A. Carlson, B. Cooke, and C. Hitaj. 2017. Growing organic demand provides high-value opportunities for many types of producers. Available at: https://www.ers.usda.gov/amber-waves/2017/januaryfebruary/growing-organic-demand-provides-high-value-opportunities-for-many-types-of-producers/. Accessed 16 December 2019.

26. Haas, C. N. 2002. Conditional dose-response relationships for microorganisms: Development and application. *Risk Anal.* 22:455-463.

27. Heyndrickx, M., D. Vandekerchove, L. Herman, I. Rollier, K. Grijspeerdt, and L. De Zutter. 2002. Routes for *Salmonella* contamination of poultry meat: Epidemiological study from hatchery to slaughterhouse. *Epidemiol. Infect.* 129:253-265.

28. Huang, L. 2014. IPMP 2013—A comprehensive data analysis tool for predictive microbiology. *Int. J. Food Microbiol.* 171:100-107.

29. Hughner, R. S., P. McDonagh, A. Prothero, C. J. Shultz, and J. Stanton. 2007. Who are organic food consumers? A compilation and review of why people purchase organic food. *J. Consum. Behav.* 6:94-110.

30. Hwang, D., M. J. Rothrock Jr, H. Pang, M. Guo, and A. Mishra. 2020. Predicting *Salmonella* prevalence associated with meteorological factors in pastured poultry farms in southeastern United States. *Sci. Total Environ.* 713:136359.

31. Juneja, V. K., M. V. Melendres, L. Huang, V. Gumudavelli, J. Subbiah, and H. Thippareddi. 2007. Modeling the effect of temperature on growth of *Salmonella* in chicken. *Food Microbiol.* 24:328-335.

32. Juneja, V. K., A. Mishra, and A. K. Pradhan. 2017. Dynamic predictive model for growth of *Bacillus cereus* from spores in cooked beans. *J. Food Prot.* 81:308-315.

33. Kim, S. A., S. J. Yun, S. H. Lee, I. G. Hwang, and M.-S. Rhee. 2013. Temperature increase of foods in car trunk and the potential hazard for microbial growth. *Food Control*. 29:66-70.

34. Kosa, K. M., S. C. Cates, S. Bradley, E. Chambers IV, and S. Godwin. 2015. Consumer-reported handling of raw poultry products at home: Results from a national survey. *J. Food Prot.* 78:180-186.

35. Luber, P. 2009. Cross-contamination versus undercooking of poultry meat or eggs—Which risks need to be managed first? *Int. J. Food Microbiol.* 134:21-28.

36. Luber, P., S. Brynestad, D. Topsch, K. Scherer, and E. Bartelt. 2006. Quantification of *Campylobacter* species cross-contamination during handling of contaminated fresh chicken parts in kitchens. *Appl. Environ. Microbiol.* 72:66-70.

37. Manikandan, S. 2011. Measures of central tendency: Median and mode. *J. Pharmacol. Pharmacother.* 2:214.

38. Mazengia, E., C. Fisk, G. Liao, H. Huang, and J. Meschke. 2015. Direct observational study of the risk of cross-contamination during raw poultry handling: Practices in private homes. *Food Prot. Trends*. 35:8-23.

39. McCarthy, J. A., H. A. Thomas Jr, and J. E. Delaney. 1958. Evaluation of the reliability of coliform density tests. *Am. J. Public Health Nations Health*. 48:1628-1635.

40. McKellar, R. C., and P. Delaquis. 2011. Development of a dynamic growth-death model for *Escherichia coli* O157:H7 in minimally processed leafy green vegetables. *Int. J. Food Microbiol.* 151:7-14.

41. Mercier, S., S. Villeneuve, M. Mondor, and I. Uysal. 2017. Time-temperature management along the food cold chain: A review of recent developments. *Compr. Rev. Food Sci. Food Saf.* 16:647-667.

42. Mishra, A., M. Guo, R. L. Buchanan, D. W. Schaffner, and A. K. Pradhan. 2016. Prediction of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* growth in leafy greens without temperature control. *J. Food Prot.* 80:68-73.

43. Montville, R., Y. Chen, and D. W. Schaffner. 2001. Glove barriers to bacterial cross-contamination between hands to food. *J. Food Prot.* 64:845-849.

44. Montville, R., Y. Chen, and D. W. Schaffner. 2002. Risk assessment of hand washing efficacy using literature and experimental data. *Int. J. Food Microbiol.* 73:305-313.

45. National Chicken Council. 2014. Chicken usage summary. Available at: https://www.nationalchickencouncil.org/wp-content/uploads/2014/07/Consumer-Research-2014-Presentation-Final-0717141-1.pdf. Accessed 13 January 2020.

46. National Chicken Council. 2015. How broilers are marketed. Available at: https://www.nationalchickencouncil.org/about-the-industry/statistics/how-broilers-are-marketed/. Accessed 19 February 2019.

47. Nissen, H., T. Maugesten, and P. Lea. 2001. Survival and growth of *Escherichia coli* O157:H7, *Yersinia enterocolitica* and *Salmonella enteritidis* on decontaminated and untreated meat. *Meat Sci.* 57:291-298.

48. Oscar, T. P. 2004. A quantitative risk assessment model for *Salmonella* and whole chickens. *Int. J. Food Microbiol.* 93:231-247.

49. Oscar, T. P. 2006. Validation of a tertiary model for predicting variation of *Salmonella* Typhimurium DT104 (ATCC 700408) growth from a low initial density on ground chicken breast meat with a competitive microflora. *J. Food Prot.* 69:2048-2057.

50. Oscar, T. P. 2007. Predictive models for growth of *Salmonella typhimurium* DT104 from low and high initial density on ground chicken with a natural microflora. *Food Microbiol.* 24:640-651.

51. Oscar, T. P. 2011. Extrapolation of a predictive model for growth of a low inoculum size of *Salmonella* Typhimurium DT104 on chicken skin to higher inoculum sizes. *J. Food Prot.* 74:1630-1638.

52. Oscar, T. P. 2012. Growth of *Salmonella* Typhimurium DT104 at 30 C is not affected by anatomical location on the chicken carcass. *J. Food Prot.* 75:164-168.

53. Pang, H., E. Lambertini, R. L. Buchanan, D. W. Schaffner, and A. K. Pradhan. 2017. Quantitative microbial risk assessment for *Escherichia coli* O157:H7 in fresh-cut lettuce. *J. Food Prot.* 80:302-311.

54. Pooni, G. S., and G. C. Mead. 1984. Prospective use of temperature function integration for predicting the shelf-life of non-frozen poultry-meat products. *Food Microbiol.* 1:67-78.

55. Ratkowsky, D. A., J. Olley, T. A. McMeekin, and A. Ball. 1982. Relationship between temperature and growth rate of bacterial cultures. *J. Bacteriol.* 149:1-5.

56. Reisch, L., U. Eberle, and S. Lorek. 2013. Sustainable food consumption: An overview of contemporary issues and policies. *Sci. Pract. Pol.* 9:7-25.

57. Rosenquist, H., L. Boysen, A. L. Krogh, A. N. Jensen, and M. Nauta. 2013. *Campylobacter* contamination and the relative risk of illness from organic broiler meat in comparison with conventional broiler meat. *Int. J. Food Microbiol.* 162:226-230.

58. Sarlin, L. L., E. T. Barnhart, D. J. Caldwell, R. W. Moore, J. A. Byrd, D. Y. Caldwell, D. E. Corrier, J. R. Deloach, and B. M. Hargis. 1998. Evaluation of alternative sampling methods for *Salmonella* critical control point determination at broiler processing. *Poult. Sci.* 77:1253-1257.

59. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—Major pathogens. *Emerg. Infect. Dis.* 17:7-15.

60. Smadi, H., and J. M. Sargeant. 2013. Quantitative risk assessment of human salmonellosis in Canadian broiler chicken breast from retail to consumption. *Risk Anal.* 33:232-248.

61. Smadi, H., J. M. Sargeant, H. S. Shannon, and P. Raina. 2012. Growth and inactivation of *Salmonella* at low refrigerated storage temperatures and thermal inactivation on raw chicken meat and laboratory media: Mixed effect meta-analysis. *J. Epidemiol. Glob. Health*. 2:165-179.

62. Sofos, J. N. 2008. Challenges to meat safety in the 21st century. *Meat Sci.* 78:3-13.

63. Taher, B. J., and M. M. Farid. 2001. Cyclic microwave thawing of frozen meat: Experimental and theoretical investigation. *Chem. Eng. Process.* 40:379-389.

64. Towns, R. E., R. W. Cullen, J. A. Memken, and N. E. Nnakwe. 2006. Food safety-related refrigeration and freezer practices and attitudes of consumers in Peoria and surrounding counties. *J. Food Prot.* 69:1640-1645.

65. United States Department of Agriculture. 2012. The nationwide microbiological baseline data collection program: Raw chicken parts survey. Available at: https://www.fsis.usda.gov/shared/PDF/Baseline_Data_Raw_Chicken_Parts.pdf. Accessed 5 October 2019.

66. United States Department of Agriculture. 2013. The big thaw — Safe defrosting methods for consumers. Available at: https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/the-big-thaw-safe-defrosting-methods-for-consumers. Accessed 29 January 2020.

67. United States Department of Agriculture. 2019. Chicken from farm to table. Available at: https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/poultry-preparation/chicken-from-farm-to-table/ct_index#:~:text=The%20USDA%20recommends%20cooking%20whole,thickest%20part%20of%20the%20breast. Accessed 9 March 2020.

68. United States Department of Agriculture. 2019. Food safety consumer research project: Meal preparation experiment related to poultry washing. Available at: https://www.fsis.usda.gov/wps/wcm/connect/9bb3a252-e12e-40e5-b76b-cb46a2322c3f/FSCRP_Year+2_Final_Aug2019.pdf?MOD=AJPERES. Accessed 31 January 2020.

69. United States Department of Agriculture/Economic Research Service. 2019. Food availability (per capita) data system. Available at: https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/. Accessed 9 January 2020.

70. van Loo, E. J., V. Caputo, R. M. Nayga Jr, J. Meullenet, and S. C. Ricke. 2011. Consumers' willingness to pay for organic chicken breast: Evidence from choice experiment. *Food Qual. Prefer.* 22:603-613.

71. Verhoeff-Bakkenes, L., R. R. Beumer, R. De Jonge, F. M. Van Leusden, and A. E. I. De Jong. 2008. Quantification of *Campylobacter jejuni* cross-contamination via hands, cutlery, and cutting board during preparation of a chicken fruit salad. *J. Food Prot.* 71:1018-1022.

72. Whiting, R. C., and R. L. Buchanan. 1997. Development of a quantitative risk assessment model for *Salmonella enteritidis* in pasteurized liquid eggs. *Int. J. Food Microbiol.* 36:111-125.

73. World Health Organization/Food and Agriculture Organization of the United Nations. 2002. Risk assessments of *Salmonella* in eggs and broiler chickens. Available at: http://www.fao.org/3/a-y4392e.pdf. Accessed 10 January 2020.

Table 7.1 Description of QMRA inputs

Description	Cell	Logic	Unit	Reference
Growth parameters:				
Growth model, b	B4	=RiskPertAlt(2.5%,0.030077,50%,0.031168,97.5%,0.032259)	Constant	Current study
Growth model, T_{min}	B5	=RiskPertAlt(2.5%,5.667,50%6.304,97.5%,6.941)	°C	Current study
Observed T_{min}	B6	=5.9	°C	*(17)*
Newton heating constant, B	B7	=RiskPert(1.703,2.261,3.014)	h^{-1}	Current study
Retail:				
Retail *Salmonella* prevalence, conventional	B10	=RiskPertAlt(2.5%,0.12,50%,0.19,97.5%,0.28)	%	*(23)*
Retail *Salmonella* prevalence, alternative	B11	=RiskPertAlt(2.5%,0.15,50%,0.23,97.5%,0.34)	%	*(23)*
Salmonella concentration at purchase	B12	=LOG(RiskKumaraswamy(0.15141,2.343,0.0066037,3.6434))	log CFU/g	*(65)*
Retail storage time	B13	=RiskPert(24,204,336)	h	Expert opinion
Retail storage temperature	B14	=RiskLaplace(3.6642,2.4903,RiskTruncate(-6.67,19.4))	°C	*(18)*
Growth rate during retail storage	B15	=IF(B14<B6,0,(B4*(B14-B5))^2)	log CFU/h	Calculated
Change during retail storage	B16	=B15*B13	log CFU/g	Calculated
Conc. at point of purchase	B17	=B16+B12	log CFU/g	Calculated
Transportation:				
Ambient temperature during transportation	B20	=RiskKumaraswamy(0.99019,1.0419,12.778,33.333)	°C	*(18)*
Transportation time	B21	=RiskPert(0.3,1.167,3.7)	h	*(18)*
Transportation growth rate	B22	=IF(B20<B6,0,(B4*((B20-(EXP(-B6*B21)*(B20-B14)))-B5))^2)	log CFU/h	Calculated

Change during transportation	B23	=B21*B22	log CFU/g	Calculated
Concentration after transportation	B24	=B23+B17	log CFU/g	Calculated
Home storage:				
Does chicken get frozen?[a]	B27	=RiskBernoulli(0.4)	No units	*(38)*
If frozen:				
Time until frozen	B30	=RiskWeibull(1.0859,1.9476,RiskTruncate(,7)RiskShift(0.98163))*24	h	*(38)*
Home refrigerator temperature	B31	=RiskLaplace(4.4638,2.1715,RiskTruncate(-0.56,15.56))	°C	*(4)*
Growth rate in refrigerator	B32	=IF(B31<B6,0,(B4*(B31-B5))^2)	log CFU/h	Calculated
Change before frozen	B33	=B32*B20	log CFU/g	Calculated
Concentration before frozen	B34	=B33+B24	log CFU/g	Calculated
Freezer temperature	B35	=RiskPert(-23.33,-18.39,-6.67)	°C	*(64)*
Thawing method[b]	B36	=RiskDiscrete((1,2,3,4),(0.48,0.14,0.24,0.14))	No units	*(38)*
If thaw method = 1:				
Thaw time	B38	=RiskTriang(2,24,72)	h	Expert opinion
Growth rate during refrigerated thawing	B39	=IF(B31<B6,0,(B4*((B31-(EXP(-B6*B38)*(B31-B35)))-B5))^2)	log CFU/h	Calculated
Change during refrigerated thawing	B40	=IF(B36=1,B39*B38,0)	log CFU/g	Calculated
If thaw method = 2:				
Running water temperature	B42	=RiskTriang(10,20,35)	°C	Expert opinion
Thaw time	B43	=RiskTriang(0.25,1,2)	h	Expert opinion
Growth rate during running water thawing	B44	=(B4*((B42-(EXP(-B6*B43)*(B42-B35)))-B5))^2	log CFU/h	Calculated

Change during running water thawing	B45	=IF(B36=2,B44*B43,0)	log CFU/g	Calculated
If thaw method = 3:				
Temperature of meat during microwave thawing	B47	=RiskPert(-8,-4,8)	°C	*(63)*
Thaw time	B48	=RiskUniform(8,20)	h	Expert opinion
Growth rate during microwave thawing	B49	=IF(B47<B6,0,(B4*((B47-(EXP(-B6*B48)*(B47-B35)))-B5))^2)	log CFU/h	Calculated
Change during microwave thawing	B50	=IF(B36=3,B49*B48,0)	log CFU/g	Calculated
If thaw method = 4:				
Ambient room temperature	B52	=RiskPert(13.89,22.52,30.55)	°C	*(2)*
Thaw time	B53	=RiskUniform(1,10)	h	Expert opinion
Growth during room temperature thawing	B54	=(B4*((B52-(EXP(-B6*B53)*(B52-B35)))-B5))^2	log CFU/h	Calculated
Change during room temperature thawing	B55	=IF(B36=4,B54*B53,0)	log CFU/g	Calculated
Concentration after thawing	B56	=IF(B27=1,B34+B40+B45+B50+B55,0)	log CFU/g	Calculated
If not frozen:				
Time, refrigerated storage	B59	=B30	h	*(38)*
Growth rate during refrigerated storage	B60	=IF(B31<B6,0,(B4*(B31-B5))^2)	log CFU/h	Calculated
Change during refrigerated storage	B61	=B60*B59	log CFU/g	Calculated
Concentration after refrigerated storage	B62	=IF(B27=1,0,B61+B24)	log CFU/g	Calculated
Preparation:				
Concentration before preparation	B65	=B56+B62	log CFU/g	Calculated

Raw chicken handling:				
Transfer rate from raw chicken to hands	B67	=(10^RiskNormal(0.40615,0.64307,RiskTruncate(,2)))*0.01	%	*(12, 36, 43, 70)*
Concentration on hands after handling	B68	=LOG(B67*(10^B65))	log CFU	Calculated
Concentration left on chicken	B69	=IF(10^B65-10^B68=0,0,LOG(10^B65-10^B68))	log CFU/g	Calculated
Transfer rate from raw chicken to utensils	B70	=(10^RiskTriang(-2.2106,-0.69897,1.9863))*0.01	%	*(12, 36, 70)*
Concentration on utensils after handling	B71	=LOG((10^B69)*B70)	log CFU	Calculated
Concentration on chicken	B72	=LOG(10^B69-10^B71)	log CFU/g	Calculated
Cooking:				
Is chicken undercooked?[a]	B74	=RiskBernoulli(0.399)	No units	*(4, 18)*
Cooking time	B75	=RiskPert(15,30,45)	min	*(50)*
Cooking temperature	B76	=RiskPert(55,65.83,70)	°C	*(4)*
D-value	B77	=10^(6.0157-(0.0429*B76)-(0.00075*B76^2))	min	*(61)*
Change during undercooking	B78	=B75/B77	log CFU/g	Calculated
Concentration after undercooking	B79	=B72-B78	log CFU/g	Calculated
Cooked product handling:				
Are hands washed?[a]	B81	=RiskBernoulli(0.883)	No units	*(34)*
Hand washing reduction	B82	=RiskBetaGeneral(3.01,1.91,-3,0.6)	log CFU	*(44)*
Concentration on hands after washing	B83	=B82+B68	log CFU	Calculated
Transfer rate to cooked chicken by hands	B84	=(10^RiskNormal(-0.29639,1.0115,RiskTruncate(,2)))*0.01	%	*(12, 36)*

Concentration after handling cooked chicken with hands	B85	=LOG(IF(B81=0,(10^B68)*B84,(10^B83)*B84)+IF(B74=0,0, 10^B79))	log CFU/g	Calculated
Are different dishes or utensils used?[a]	B86	=RiskBernoulli(0.959)	No units	*(34)*
Transfer rate to cooked chicken from dirty utensils	B87	=(10^RiskExtvalueMin(1.0356,0.73594))*0.01	%	*(12, 36)*
Final concentration	B88	=LOG(10^B85+IF(B86=0,B87*(10^B71),0))	log CFU/g	Calculated
Dose-response and illnesses:				
Serving size	B91	=85	g	9 CFR §381.412
Concentration per serving	B92	=(10^B88)*B91	CFU	Calculated
Dose-response model, α	B93	=RiskTriang(0.0763,0.1324,0.2274)	Constant	*(20, 72)*
Dose-response model, β	B94	=RiskTriang(38.49,51.45,57.96)	Constant	*(20, 72)*
Probability of illness	B95	=1-(1+(B92/B94))^-B93		Calculated
Total risk per serving, conventional	B96	=B95*B10		Calculated
Total risk per serving, alternative	B97	=B95*B11		Calculated
Total per capita poultry availability	B98	=41004.75	g	*(68)*
Total used in raw chicken preparation	B99	=B98*0.5	g	*(46)*
U.S. population	B100	=325186237	People	*(68)*
Number of consumers who purchased chicken from grocery/supermarket	B101	=B100*0.83	People	*(45)*
Servings per capita per year, total	B102	=93.6	Servings	*(45)*

Servings per capita per year, conventional	B103	=B102-B104	Servings	Calculated
Servings per capita per year, alternative	B104	=B102*0.12	Servings	*(25)*
Total conventional servings consumed per year	B105	=B103*B101	Servings	Calculated
Total alternative servings consumed per year	B106	=B104*B101	Servings	Calculated
No. of annual illnesses, conventional	B107	=B105*B96	Illnesses	Calculated
No. of annual illnesses, alternative	B108	=B106*B97	Illnesses	Calculated
No. of annual illnesses, total	B109	=B107+B108	Illnesses	Calculated

[a] (0 = no, 1 = yes)
[b] (1 = refrigerator, 2 = running water, 3 = microwave, 4 = room temperature

Table 7.2 Studies containing primary *Salmonella* growth data on chicken used in secondary growth model fitting

Study	Type of chicken used	Temperature(s) used (°C)	No. of data points	Reference
Bovill et al., 2000	Minced chicken	10.7, 30	2	*(3)*
Callaghan et al., 2003	Chicken thigh	0, 4, 6, 8, 10, 12	60	*(8)*
Juneja et al., 2007	Ground chicken	10, 15, 20, 25, 28, 32, 35, 37, 42, 45	20	*(31)*
Nissen et al., 2001	Chicken breast	10	2	*(47)*
Oscar, 2006	Ground chicken	10, 11, 12, 14, 18, 22, 26, 30, 34, 40	30	*(48)*
Oscar, 2007	Ground chicken	10, 11, 12, 14, 18, 22, 26, 30, 34, 40	85	*(49)*
Oscar, 2011	Chicken skin	20, 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, 42.5, 45	47	*(51)*
Oscar, 2012	Chicken parts	30	47	*(52)*

Table 7.3 Cumulative distribution of *Salmonella* concentration in *Salmonella* positive chicken samples taken from the end of the production line

MPN/mL	MPN/g	Cumulative probability (%)
< 0.030	< 0.007	30.59
0.030-0.30	0.007-0.066	78.69
0.301-3.00	0.066-0.661	94.22
3.01-30.00	0.664-6.614	100.00
> 30.01	> 6.616	100.00

Source: United States Department of Agriculture (USDA) *(65)*

Table 7.4 Cross-contamination studies containing bacterial transfer rate data used in this study

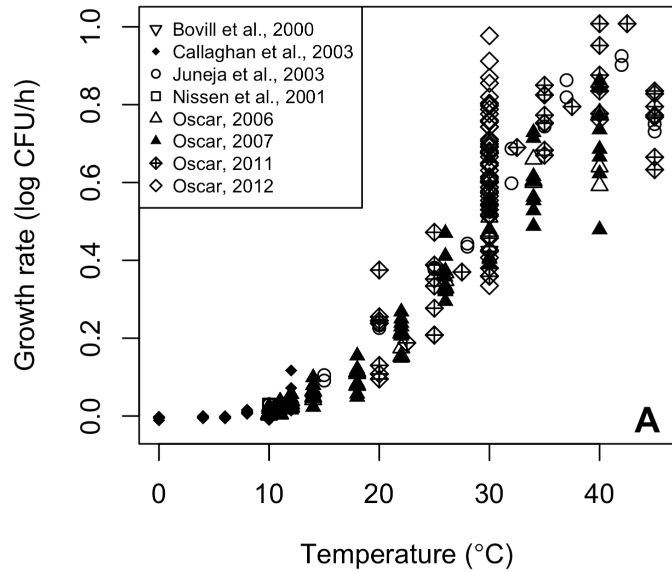
Study	Types of transfer rates (no. data points)	Organism	Reference
Chen et al., 2001	Raw chicken to hand (36), raw chicken to cutting board (8), contaminated cutting board to lettuce (32), contaminated hand to lettuce (30)	*Enterobacter aerogenes*	*(12)*
Luber et al., 2006	Raw chicken to hand (27), contaminated plate to fried sausage (7), raw chicken to cutting board/cutlery/plate (22), contaminated utensils to cucumber (11), contaminated hand to bread (5)	*Campylobacter* spp.	*(36)*
Montville et al., 2001	Raw chicken to hand (30)	*Enterobacter aerogenes*	*(43)*
Verhoeff-Bakkenes et al., 2007	Raw chicken to hand (5), raw chicken to cutting board/cutlery (10)	*Campylobacter jejuni*	*(70)*

Table 7.5 Median, 25th percentile, and 75th percentile estimated total risk per serving and total annual number of illnesses due to consumption of conventionally and alternatively produced broiler meat, as determined by the baseline QMRA model

Production method	**Total risk per serving**			**No. of illnesses**		
	Median	**25%**	**75%**	**Median**	**25%**	**75%**
Conventional	7.6×10^{-8}	6.2×10^{-9}	9.8×10^{-7}	1,694.2	138.8	21,832.2
Alternative	9.2×10^{-8}	7.6×10^{-9}	1.2×10^{-6}	279.9	23.0	3,600.7

Table 7.6 Median, 25th percentile, and 75th percentile estimates of total annual number of illnesses caused by "what-if" scenarios compared to the baseline QMRA model

Scenario	No. of illnesses		
	Median	**25%**	**75%**
Baseline	1,985.0	163.4	25,549.3
Handwashing:			
Always wash hands	1,600.9	132.1	20,619.8
Never wash hands	8,874.0	1,032.3	86,691.3
Antimicrobial soap	1,466.0	87.9	23,037.8
Cooking:			
Always cook to ≥ 165°F	1,940.4	161.5	24,908.5
Always undercook	2,087.7	177.1	26,187.8
Cleaning:			
Always use different dishes/utensils	1,754.7	149.0	21,857.4
Never use different dishes/utensils	34,279.8	4,890.0	284,344.7



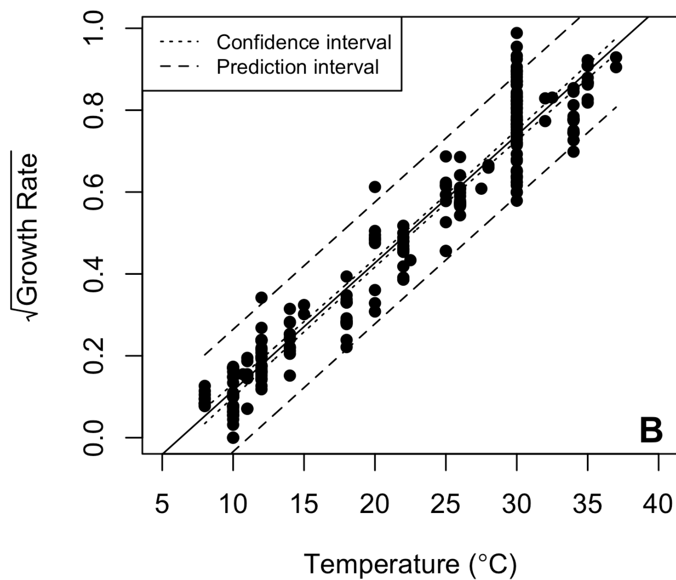


Figure 7.1 Growth rates derived from primary growth data from Combase data sources (a). Secondary modeling of primary growth rates in temperature profiles from 5.9-37°C (b). The dotted line represents the 95% confidence interval and the dashed line represents the prediction interval.

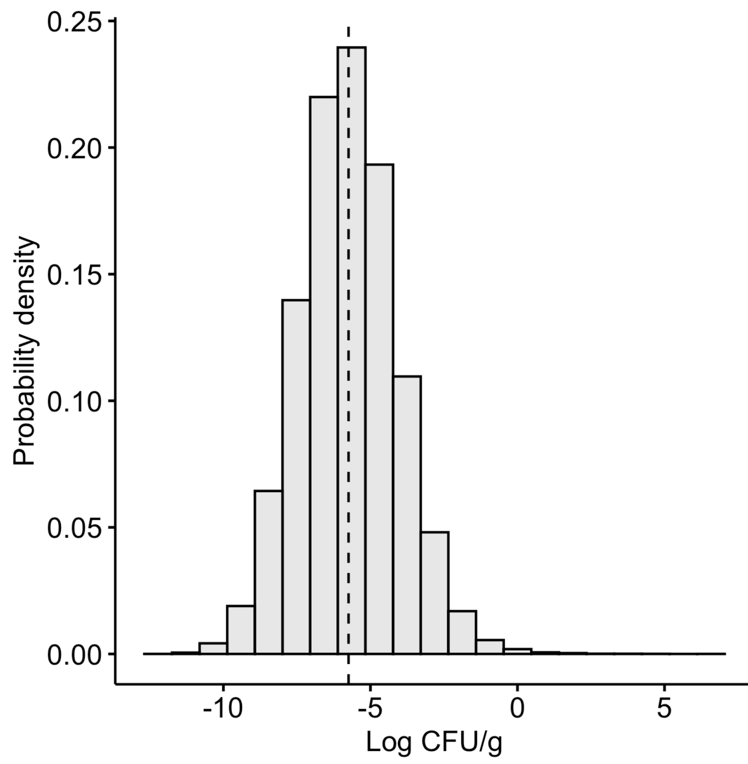


Figure 7.2 The distribution of final *Salmonella* concentration numbers in log CFU/g across all Monte Carlo iterations.

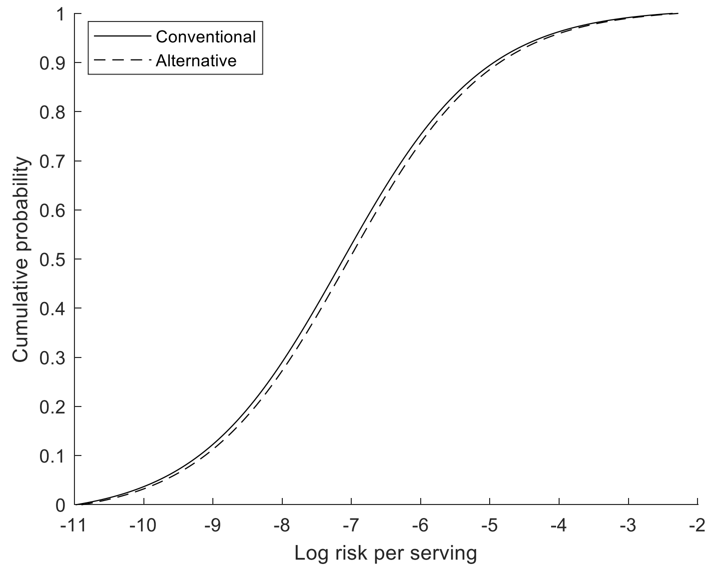


Figure 7.3 Cumulative distribution functions for log *Salmonella* risk per serving for conventionally produced broiler meat (solid line) and alternatively produced broiler meat (dashed line) as predicted by the baseline QMRA model.

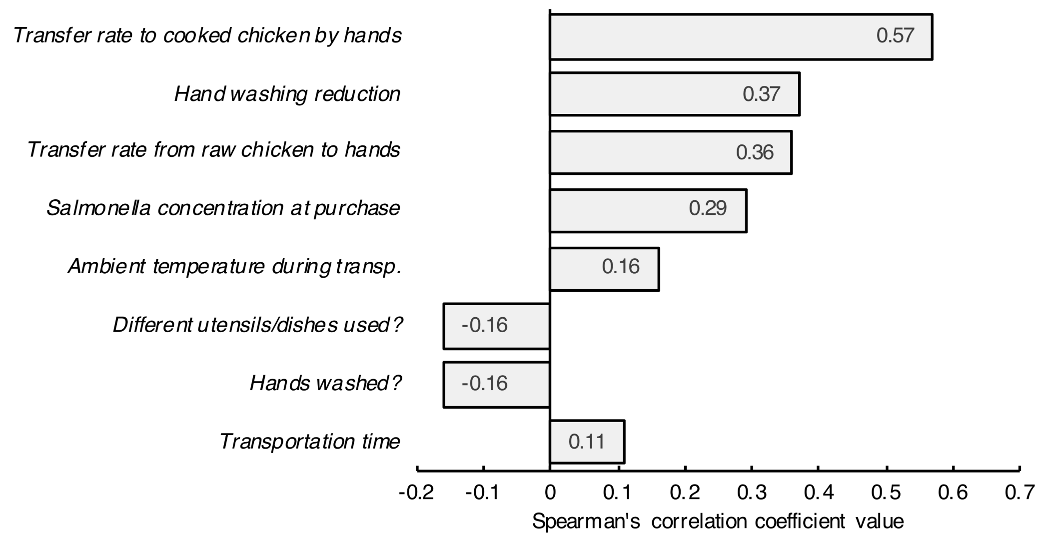


Figure 7.4 Spearman's correlation coefficients showing the 8 most important model parameters in predicting the total *Salmonella* risk per serving of broiler meat.

CHAPTER 8

SUMMARY AND CONCLUSIONS

In Chapter 3, the gradient boosting machine and random forest models were evaluated for their use in predicting *Listeria* spp. in preharvest pastured poultry samples based on recent weather patterns. This was the first study in the food safety literature evaluating the gradient boosting machine model for its efficacy in predicting foodborne pathogen prevalence. It was shown that both models can effectively be used in a food safety context. The models provided information on the most important predictors of *Listeria* spp. contamination of preharvest samples, allowing broiler farmers and producers to be aware of weather conditions that could lead to higher risk of pathogen contamination.

Chapter 4 presented random forest models that were constructed to evaluate major pastured poultry farming practices as potential risk factors for *Listeria* spp. contamination of preharvest and postharvest broiler samples. Both models performed well, with area under the receiver operating characteristic curve (AUC) values of >0.87. The preharvest model identified that days of the year that are commonly associated with colder temperature provided a higher risk of *Listeria* contamination in feces and soil samples. These results are in agreement with the results provided in Chapter 3, where minimum temperature was identified as a significant predictor of *Listeria* prevalence in both sample types.

In Chapter 5, random forest models were constructed to predict *Campylobacter* prevalence in feces and soil samples collected from pastured poultry farms based on recent

weather patterns. Again, both models performed well with AUC values >0.88. Additionally, the models were built into a usable tool that provides *Campylobacter* prevalence probabilities based on forecasted weather for up to seven days ahead. The forecasting ability of the models was validated on additional feces and soil samples collected during the 2019 growing season. The generated models will be useful for pastured poultry farmers and producers in risk-based decision making, such as how often to move to new areas of pasture during farming.

Chapters 6 and 7 were conducted to address the need for a critical understanding of the relative risk of *Campylobacter* and *Salmonella* contamination in alternatively produced broilers, such as organic, free-range, and pastured broilers. In Chapter 6, a systematic review and meta-analysis study was performed to provide estimates of the prevalence of *Campylobacter* and *Salmonella* at various points along the broiler supply chain based off prevalence surveys previously conducted by other researchers. In total, 72 studies were included. Estimated prevalence values were compared for conventional and alternative broiler production systems, when possible. Estimated prevalence values were similar for both productions systems, except for *Campylobacter* prevalence in environmental samples, where estimated prevalence was significantly higher in alternative production. The obtained results provide a systematic estimate of prevalence values along the broiler chain that can be used in quantitative microbial risk assessment (QMRA) studies, as shown in Chapter 7.

To determine the risk that *Salmonella* poses to human consumers in the United States due to in-home preparation and consumption of broiler meat and the differences in risk between conventionally and alternatively produced broilers, a retail-to-consumption QMRA was conducted in Chapter 7. Small differences in probability of illness per serving of chicken

consumed were observed between conventional and alternative production. In total, the model predicted that there were around 2,000 illnesses caused by consumption of chicken meat prepared in home due to *Salmonella* contamination. Post cook-step cross-contamination events were identified as the most important determinants of risk for both production systems. The generated model can be used to identify key intervention steps to mitigate the risk of salmonellosis due to consumption of chicken meat.

This dissertation worked to address knowledge gaps relative to the food safety of alternatively produced broilers, but further research into this area is recommended. For example, gaining a further understanding on the gut microbiota of alternatively produced broilers could provide insight into the nature of contamination of the broiler gastrointestinal tract. Additionally, to provide for even more accurate QMRA studies, a priority needs to be put on conducting consumer behavior studies and surveys to get a better understanding of the range of occurrences that can occur during chicken preparation. For example, hand washing frequency and bacterial transfer during chicken washing are two key parameters that could be included in future broiler QMRA studies to provide better risk estimates. Finally, surveys on *Listeria* prevalence throughout the broiler supply chain should be conducted to address the limited information on *Listeria* contamination of broilers present in the literature

APPENDICES

Appendix A List of the meteorological predictor variables used in the construction of random forest and gradient boosting machine models generated in Chapter 3

Variable	Description (unit)
MaximumWindSpeedSampleDay	Maximum wind speed on the day of sample collection (m/s)
MaximumWindSpeedOneDay	Maximum wind speed 1 day before the day of sample collection (m/s)
MaximumWindSpeedTwoDay	Maximum wind speed 2 days before the day of sample collection (m/s)
AvgMaxWindSpeedSamp.1	Average maximum wind speed between the day of sample collection and 1 day prior (m/s)
AvgMaxWindSpeedSamp.2	Average maximum wind speed between the day of sample collection and 2 days prior (m/s)
AvgMaxWindSpeedSamp.3	Average maximum wind speed between the day of sample collection and 3 days prior (m/s)
AvgMaxWindSpeedSamp.4	Average maximum wind speed between the day of sample collection and 4 days prior (m/s)
AvgMaxWindSpeedSamp.5	Average maximum wind speed between the day of sample collection and 5 days prior (m/s)
AvgMaxWindSpeedSamp.6	Average maximum wind speed between the day of sample collection and 6 days prior (m/s)
AvgMaxWindSpeedSamp.7	Average maximum wind speed between the day of sample collection and 7 days prior (m/s)
AverageWindSpeedSampleDay	Average wind speed on the day of sample collection (m/s)
AverageWindSpeedOneDay	Average wind speed 1 day before the day of sample collection (m/s)
AverageWindSpeedTwoDay	Average wind speed 2 days before the day of sample collection (m/s)

AverageAvgWindSpeedSamp.1	Average wind speed between the day of sample collection and 1 day prior (m/s)
AverageAvgWindSpeedSamp.2	Average wind speed between the day of sample collection and 2 days prior (m/s)
AverageAvgWindSpeedSamp.3	Average wind speed between the day of sample collection and 3 days prior (m/s)
AverageAvgWindSpeedSamp.4	Average wind speed between the day of sample collection and 4 days prior (m/s)
AverageAvgWindSpeedSamp.5	Average wind speed between the day of sample collection and 5 days prior (m/s)
AverageAvgWindSpeedSamp.6	Average wind speed between the day of sample collection and 6 days prior (m/s)
AverageAvgWindSpeedSamp.7	Average wind speed between the day of sample collection and 7 days prior (m/s)
MaxGustSpeedSampleDay	Maximum wind gust speed on the day of sample collection (m/s)
MaxGustSpeedOneDay	Maximum wind gust speed 1 day before the day of sample collection (m/s)
MaxGustSpeedTwoDay	Maximum wind gust speed 2 days before the day of sample collection (m/s)
AvgMaxGustSpeedSamp.1	Average maximum wind gust speed between the day of sample collection and 1 day prior (m/s)
AvgMaxGustSpeedSamp.2	Average maximum wind gust speed between the day of sample collection and 2 days prior (m/s)
AvgMaxGustSpeedSamp.3	Average maximum wind gust speed between the day of sample collection and 3 days prior (m/s)
AvgMaxGustSpeedSamp.4	Average maximum wind gust speed between the day of sample collection and 4 days prior (m/s)
AvgMaxGustSpeedSamp.5	Average maximum wind gust speed between the day of sample collection and 5 days prior (m/s)
AvgMaxGustSpeedSamp.6	Average maximum wind gust speed between the day of sample collection and 6 days prior (m/s)

AvgMaxGustSpeedSamp.7	Average maximum wind gust speed between the day of sample collection and 7 days prior (m/s)
MinHumiditySampleDay	Minimum humidity on the day of sample collection (%)
MinHumidityOneDay	Minimum humidity 1 day before the day of sample collection (%)
MinHumidityTwoDay	Minimum humidity 2 days before the day of sample collection (%)
AvgMinHumiditySamp.1	Average minimum humidity between the day of sample collection and 1 day prior (%)
AvgMinHumiditySamp.2	Average minimum humidity between the day of sample collection and 2 days prior (%)
AvgMinHumiditySamp.3	Average minimum humidity between the day of sample collection and 3 days prior (%)
AvgMinHumiditySamp.4	Average minimum humidity between the day of sample collection and 4 days prior (%)
AvgMinHumiditySamp.5	Average minimum humidity between the day of sample collection and 5 days prior (%)
AvgMinHumiditySamp.6	Average minimum humidity between the day of sample collection and 6 days prior (%)
AvgMinHumiditySamp.7	Average minimum humidity between the day of sample collection and 7 days prior (%)
MaxHumiditySampleDay	Maximum humidity on the day of sample collection (%)
MaxHumidityOneDay	Maximum humidity 1 day before the day of sample collection (%)
MaxHumidityTwoDay	Maximum humidity 2 days before the day of sample collection (%)
AvgMaxHumiditySamp.1	Average maximum humidity between the day of sample collection and 1 day prior (%)
AvgMaxHumiditySamp.2	Average maximum humidity between the day of sample collection and 2 days prior (%)

AvgMaxHumiditySamp.3	Average maximum humidity between the day of sample collection and 3 days prior (%)
AvgMaxHumiditySamp.4	Average maximum humidity between the day of sample collection and 4 days prior (%)
AvgMaxHumiditySamp.5	Average maximum humidity between the day of sample collection and 5 days prior (%)
AvgMaxHumiditySamp.6	Average maximum humidity between the day of sample collection and 6 days prior (%)
AvgMaxHumiditySamp.7	Average maximum humidity between the day of sample collection and 7 days prior (%)
AverageHumiditySampleDay	Average humidity on the day of sample collection (%)
AverageHumidityOneDay	Average humidity 1 day before the day of sample collection (%)
AverageHumidityTwoDay	Average humidity 2 days before the day of sample collection (%)
AvgAverageHumiditySamp.1	Average humidity between the day of sample collection and 1 day prior (%)
AvgAverageHumiditySamp.2	Average humidity between the day of sample collection and 2 days prior (%)
AvgAverageHumiditySamp.3	Average humidity between the day of sample collection and 3 days prior (%)
AvgAverageHumiditySamp.4	Average humidity between the day of sample collection and 4 days prior (%)
AvgAverageHumiditySamp.5	Average humidity between the day of sample collection and 5 days prior (%)
AvgAverageHumiditySamp.6	Average humidity between the day of sample collection and 6 days prior (%)
AvgAverageHumiditySamp.7	Average humidity between the day of sample collection and 7 days prior (%)
MinTemperatureSampleDay	Minimum temperature on the day of sample collection (°C)

MinTemperatureOneDay	Minimum temperature 1 day before the day of sample collection (°C)
MinTemperatureTwoDay	Minimum temperature 2 days before the day of sample collection (°C)
AvgMinTemperatureSamp.1	Average minimum temperature between the day of sample collection and 1 day prior (°C)
AvgMinTemperatureSamp.2	Average minimum temperature between the day of sample collection and 2 days prior (°C)
AvgMinTemperatureSamp.3	Average minimum temperature between the day of sample collection and 3 days prior (°C)
AvgMinTemperatureSamp.4	Average minimum temperature between the day of sample collection and 4 days prior (°C)
AvgMinTemperatureSamp.5	Average minimum temperature between the day of sample collection and 5 days prior (°C)
AvgMinTemperatureSamp.6	Average minimum temperature between the day of sample collection and 6 days prior (°C)
AvgMinTemperatureSamp.7	Average minimum temperature between the day of sample collection and 7 days prior (°C)
MaxTemperatureSampleDay	Maximum temperature on the day of sample collection (°C)
MaxTemperatureOneDay	Maximum temperature 1 day before the day of sample collection (°C)
MaxTemperatureTwoDay	Maximum temperature 2 days before the day of sample collection (°C)
AvgMaxTemperatureSamp.1	Average maximum temperature between the day of sample collection and 1 day prior (°C)
AvgMaxTemperatureSamp.2	Average maximum temperature between the day of sample collection and 2 days prior (°C)
AvgMaxTemperatureSamp.3	Average maximum temperature between the day of sample collection and 3 days prior (°C)
AvgMaxTemperatureSamp.4	Average maximum temperature between the day of sample collection and 4 days prior (°C)

AvgMaxTemperatureSamp.5	Average maximum temperature between the day of sample collection and 5 days prior (°C)
AvgMaxTemperatureSamp.6	Average maximum temperature between the day of sample collection and 6 days prior (°C)
AvgMaxTemperatureSamp.7	Average maximum temperature between the day of sample collection and 7 days prior (°C)
AverageTemperatureSampleDay	Average temperature on the day of sample collection (°C)
AverageTemperatureOneDay	Average temperature 1 day before the day of sample collection (°C)
AverageTemperatureTwoDay	Average temperature 2 days before the day of sample collection (°C)
AvgAverageTemperatureSamp.1	Average temperature between the day of sample collection and 1 day prior (°C)
AvgAverageTemperatureSamp.2	Average temperature between the day of sample collection and 2 days prior (°C)
AvgAverageTemperatureSamp.3	Average temperature between the day of sample collection and 3 days prior (°C)
AvgAverageTemperatureSamp.4	Average temperature between the day of sample collection and 4 days prior (°C)
AvgAverageTemperatureSamp.5	Average temperature between the day of sample collection and 5 days prior (°C)
AvgAverageTemperatureSamp.6	Average temperature between the day of sample collection and 6 days prior (°C)
AvgAverageTemperatureSamp.7	Average temperature between the day of sample collection and 7 days prior (°C)
PrecipitationSampleDay	Average amount of precipitation on the day of sample collection (mm)
PrecipitationOneDay	Average amount of precipitation 1 day before the day of sample collection (mm)
PrecipitationTwoDay	Average amount of precipitation 2 days before the day of sample collection (mm)

AvgPrecipitationSamp.1	Average amount of precipitation between the day of sample collection and 1 day prior (mm)
AvgPrecipitationSamp.2	Average amount of precipitation between the day of sample collection and 2 days prior (mm)
AvgPrecipitationSamp.3	Average amount of precipitation between the day of sample collection and 3 days prior (mm)
AvgPrecipitationSamp.4	Average amount of precipitation between the day of sample collection and 4 days prior (mm)
AvgPrecipitationSamp.5	Average amount of precipitation between the day of sample collection and 5 days prior (mm)
AvgPrecipitationSamp.6	Average amount of precipitation between the day of sample collection and 6 days prior (mm)
AvgPrecipitationSamp.7	Average amount of precipitation between the day of sample collection and 7 days prior (mm)