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Using Farm Practice Variables as Predictors of *Listeria* spp. Prevalence in Pastured Poultry Farms

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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. Feces, 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 feces 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 RF model and final sample RF model 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.

Keywords: *Listeria* spp., food safety, predictive microbiology, random forest, alternative poultry production, pastured poultry, machine learning

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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 (Martín et al., 2014; Sasaki et al., 2014; Ahmed et al., 2015). *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 (Carpentier and Cerf, 2011). *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 (Frye et al., 2002; Olsen et al., 2005; Zhu et al., 2005; Gottlieb et al., 2006; Dev Kumar et al., 2016). From 2010–2016, there were 18 multistate foodborne illness outbreaks attributed

to *L. monocytogenes*, resulting in 324 illnesses and 65 deaths (Centers for Disease Control Prevention, 2018).

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. (Rothrock et al., 2017). 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 (Ivanek et al., 2009). Additionally, studies have shown that the occurrence of *L. monocytogenes* in food products is mainly contributed to cross contamination from processing and retail environments, emphasizing the importance of eliminating the transfer of the organism into a processing facility from the outside environment (Lianou and Sofos, 2007; Ferreira et al., 2014). *Listeria* can colonize the intestines of poultry and spread into the litter and environment through poultry feces (Njagi et al., 2004; Dhama et al., 2013). This marks 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 for *Listeria* spp. prevalence in the environment and postharvest product of pastured poultry farms.

In the recent years, pastured poultry and other similar alternative poultry products have increased in interest in the United States (Hilimire, 2012). Pastured poultry farms are characterized by poultry flocks that are reared in open-air, movable pens (Siemon et al., 2007). Pens are often rotated to fresh pasture to encourage forage intake by the birds (Salatin, 1993). Flock sizes vary, but often contain <3,000 birds (Elkhoraibi et al., 2017). While 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 (Van Loo et al., 2011; O'Bryan et al., 2017). Compared to conventional broiler farms, research on the food safety of pastured poultry farms is very limited. An understanding on 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.

The current study evaluated preharvest environmental and processed broiler samples collected from pastured poultry farms from 2014 to 2017 and constructed random forest (RF) models to predict *Listeria* spp. prevalence based on various farm practice variables, like 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.

MATERIALS AND METHODS

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 1**. Data were collected for 40 major farm practice variables (**Table 2**) over a flock's lifecycle and all samples were evaluated for the presence of *Listeria* spp.

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 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, feces, 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 (Semenov et al., 2008; Bergholz et al., 2011).

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 3**). This step monitors prevalence at the point closest to the carcass being available at the consumer level. 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 to the lab, no further preparation was performed with the WCR samples.

At each farm, the respective farmers managed all of the flocks on farm, and the processing was performed by the farmers or the processing facility workers, not the researchers or the technicians. The preharvest samples were not collected directly from live birds and all postharvest samples were taken post mortem, therefore, no ethics review process was required for the current study.

Listeria spp. Enrichment and Isolation

Listeria spp. enrichment and isolation followed a modified version of the USDA-FSIS MLG 8.10 method (United States Department of Agriculture-Food Safety Inspection Service, 2017). To prepare the feces and soil samples for pre-enrichment,

TABLE 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)

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 homogenized 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.

Listeria spp. Characterization by PCR

Speciation of *Listeria* was carried out using the procedures described by Huang et al. (2007) and Locatelli et al. (2017). 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. (2004). 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).

Statistical Analyses

All statistical analyses were performed in R (Version 3.4.0; R Foundation for Statistical Computing, Vienna, Austria). The Fisher's exact test was used to compare *Listeria* spp. prevalence among different sample types. $P < 0.05$ were considered statistically significant. The *caret* package was used for model training and analysis (Kuhn, 2008).

The random forest (RF) model is an ensemble, tree-based, machine learning (ML) model that was introduced by Breiman (2001). This method utilizes a large number of unpruned classification and regression trees (Breiman, 1984) and two sources of randomization during construction (Prasad et al., 2006; Philibert et al., 2011). During construction of each tree, data are randomly chosen using the non-parametric bootstrap sampling method (Efron and Tibshirani, 1994), 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 (Breiman, 1984). 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 (Philibert et al., 2011). 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 (Breiman, 2001). 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 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

TABLE 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 1 year	Numeric
AvgNumFlocks	Average number of flocks that the farm handled in 1 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 (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 (W); corn, soy, oats (CSO); corn, cotton seed mill, wheat (CMW); peas, corn, oats (PCO)
PaGMOFree	Was the brooding feed GMO free?	2 levels: yes (Y), no (N)
PaSoyFree	Was the brooding 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.*

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

TABLE 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.*

as described by Breiman (2001) and Breiman (2002). 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 (Greenwell, 2017).

For the 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 (Chawla et al., 2002). The SMOTE method utilizes a mixture of over-sampling the minority class (positives) and under-sampling the majority class (negatives) 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) (Bradley, 1997), sensitivity, and specificity.

RESULTS

The prevalence of *Listeria* spp. in all samples collected in the current study was 17.4% (**Table 4**). Out of the 1,867 total samples, only 37 (2.0%) of the samples 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 illustrated in **Table 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.

TABLE 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	1,867	324 (17.4)	37 (2.0)

[a]*Whole carcass rinse (WCR).*

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 **Figure 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 (**Figure 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 (**Figure 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 (**Figure 2B**).

The variable importance plot for the WCR RF model is shown in **Figure 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 (**Figure 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 (**Figure 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

TABLE 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	1,637	273 (16.7)	230	51 (22.2)

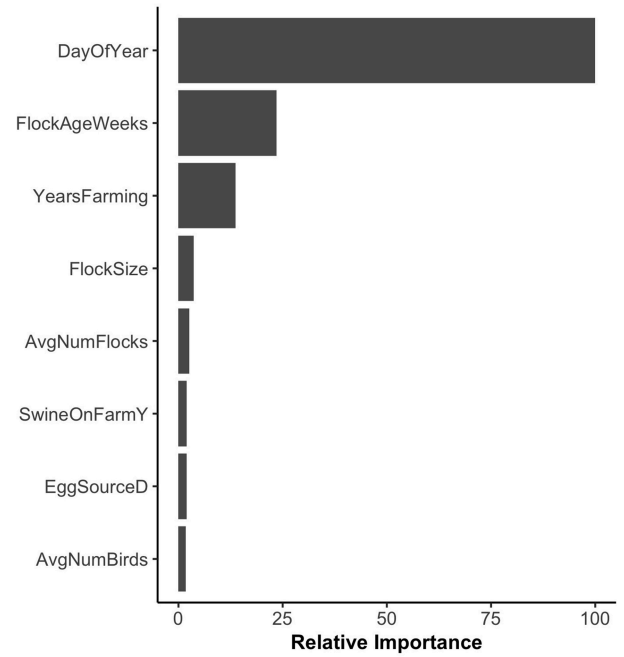

FIGURE 1 | Variable importance plot for the preharvest sample random forest model. Relative importance is determined by average mean decrease in Gini and scaled from 0 to 100, where 100 is most important. SwineOnFarmY refers to farms that had swine present at the farm. EggSourceD refers to farms that received broiler eggs from Company D.

to illustrate the comparison of model predictions and observed outcomes (**Table 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 (**Figure 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 (**Table 6**).

DISCUSSION

Currently, there is a lack of understanding on the impact and prevalence of *Listeria* spp. in pastured poultry farming environments and products (Rothrock et al., 2017). 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 2**) that are associated with a higher risk of *Listeria* spp. presence, as predicted by the models.

The Risk of *Listeria* to Pastured Poultry Farmers

While there have been no known chicken-related listeriosis outbreaks in the United States, there have been several foodborne illness outbreaks due to *Listeria monocytogenes* contamination of deli turkey and other types of RTE meats (Olsen et al., 2005). 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* (Ivanek et al., 2009). This means that an environment that is contaminated with

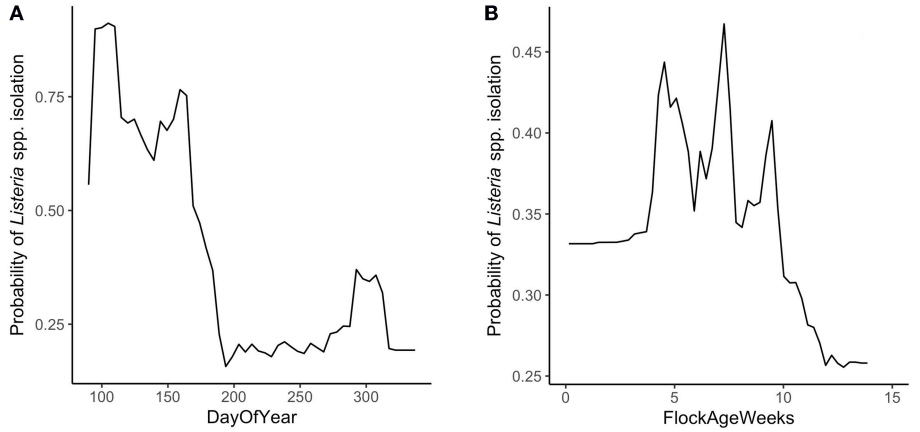

FIGURE 2 | Partial dependency plots (PDPs) for the two most important predictors in the preharvest sample random forest model. **(A)** DayOfYear represents the day of the year that samples were collected, numerically from 1 to 365. **(B)** FlockAgeWeeks represents the age of the flock in weeks at the time of sampling.

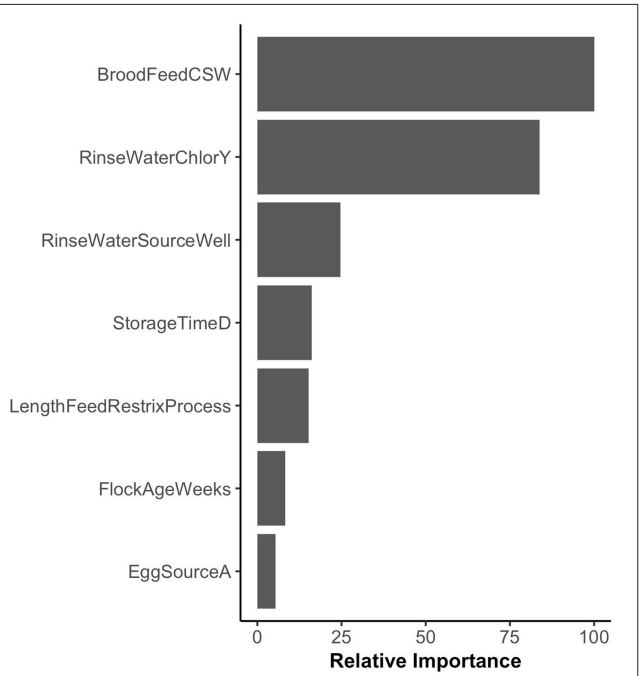

FIGURE 3 | Variable importance plot for the whole carcass rinse (WCR) final product random forest model. Relative importance is determined by average mean decrease in Gini and scaled from 0 to 100, where 100 is most important. BroodFeedCSW refers to brood feed with top 3 sources of protein as corn, soy, 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.

Listeria could be a source of contamination for downstream processing areas (Ivanek et al., 2006). Furthermore, poultry can act as reservoirs for the organism (Njagi et al., 2004; Dhama et al., 2013). 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.

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 collected 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 (**Figure 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 (Guerini et al., 2007). 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 survive at temperatures as low as 1°C, whereas enteric foodborne pathogens are not (Doyle, 1989). Strawn et al. (2013) 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 analyze 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

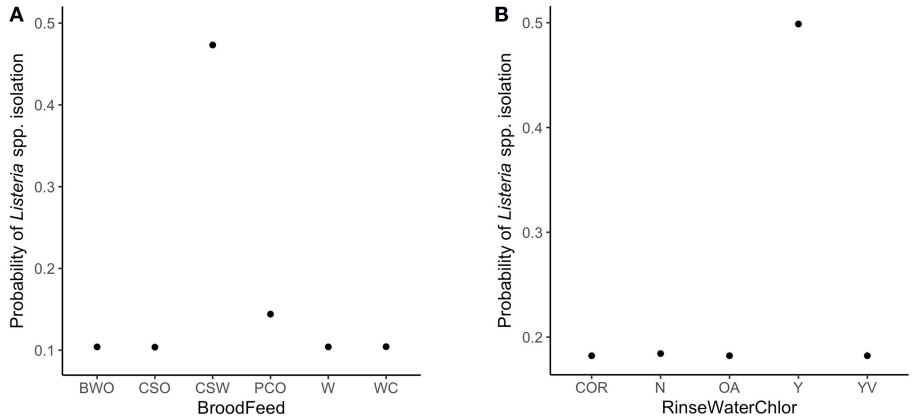

FIGURE 4 | Partial dependency plots (PDPs) for the two most important predictors in the whole carcass rinse (WCR) final product random forest model. **(A)** Brood feed refers to up to the top 3 sources of protein in the brood feed. Ingredients include: barley (B), corn (C), oats (O), wheat (W), soy (S), 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).

TABLE 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.*

southeastern pastured poultry farms. It is most 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 (**Figure 1**). *Listeria* spp. isolation from the environment was predicted to have the highest probability when a flock was between 5 and 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 (Milillo et al., 2012).

Farm Management Practices Can Impact *Listeria* spp. Prevalence in the Final Broiler Product

The presented final WCR RF model identified brood feed as the most important explanatory variable in predicting *Listeria* spp. presence in the final product (**Figure 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 2**). After hatching, the intestinal microbiota of a chicken develops rapidly (Lan et al., 2005; Chambers and Gong, 2011). As such, the type of feed that a chick receives early on its life can have a profound effect on its gut microbiota (Lan et al., 2005). 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 (**Figure 3**). Studies have shown that gram-positive organisms, such as *Listeria monocytogenes*, are more resistant to chlorine than gram-negative bacteria (Virto et al., 2005). 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 (Skrivanová et al., 2006). 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*.

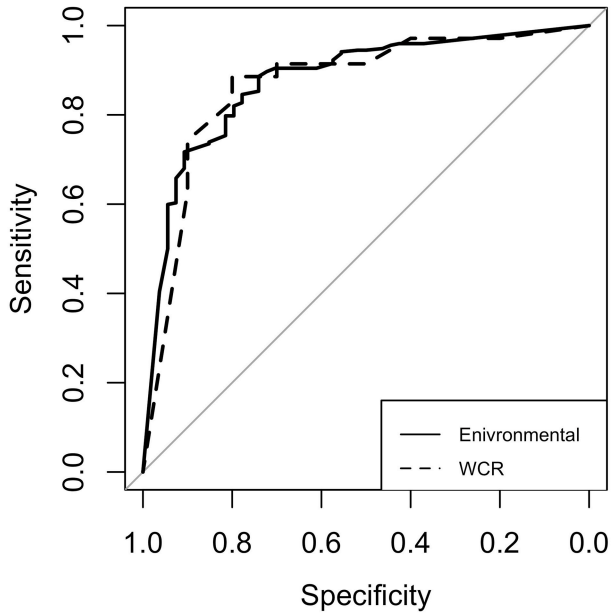

FIGURE 5 | Receiver operating characteristic (ROC) curves for the environmental (solid line) and whole carcass rinse (dashed line) random forest models. The diagonal line represents the line of no discrimination, or the expectation of a random guess. Curves above this line are considered better than random guess. The area under each curve is referred to as AUC.

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. (2013) 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. (2001) 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.

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 (Barco et al., 2012; Gu et al., 2015; Pang et al., 2017) and classification and regression tree models (Mokhtari et al., 2006; Ivanek et al., 2009; Strawn et al., 2013) 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 (Rodriguez-Galiano et al., 2012).

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 (Dupuy and Simon, 2007). Models are then evaluated by prediction performance on the testing set, and evaluated by metrics such as ROC curves and AUC (Bradley, 1997). In the current study, both RF models performed well with respect to AUC, with both scoring >0.85 (**Table 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 (**Figure 3**). Strobl and Zeileis (2008) reported that importance measures from models trained on smaller data sets were not significantly different from those trained on larger data sets.

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 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.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

CG performed all statistical analyses and developed the predictive models. MR performed the data collection and organization. CG drafted the manuscript. MR and AM provided project oversight and assistance in manuscript preparation. All authors approved of the final manuscript draft.

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