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# Using geospatial methods to measure the risk of environmental persistence of avian influenza virus in South Carolina

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ABSTRACT

Avian influenza (AIV) is a highly contagious virus that can infect both wild birds and domestic poultry. This study aimed to define areas within the state of South Carolina (SC) at heightened risk for environmental persistence of AIV using geospatial methods. Environmental factors known to influence AIV survival were identified through the published literature and using a multi-criteria decision analysis with GIS was performed. Risk was defined using five categories following the World Organization for Animal Health Risk Assessment Guidelines. Less than 1% of 1km grid cells in SC showed a high risk of AIV persistence. Approximately 2% - 17% of counties with high or very high environmental risk also had medium to very high numbers of commercial poultry operations. Results can be used to improve surveillance activities and to inform biosecurity practices and emergency preparedness efforts.



## 1. Introduction

Avian influenza viruses (AIVs) have a wide host range and are capable of infecting many species of both wild and domestic birds (Webster et al., 1992). AIVs have been detected in over 100 species of free-living birds, with the highest prevalences reported in the orders Charadriiformes (i.e., shorebirds) and Anseriformes (i.e., waterfowl) (Swayne, 2008). Although wild birds typically do not show clinical signs of infection with AIVs, circulation of AIVs in wild birds may lead to viral introductions to domestic poultry (Alexander, 2000; van den Brand et al., 2018; Li et al., 2018; Hill et al., 2015; Ramey et al., 2018). AIVs are generally classified as either low pathogenicity (LPAI) or high pathogenicity (HPAI) viruses depending on their virulence in inoculated chickens (OIE, 2017a). AIVs are further classified into subtypes, all of which have been isolated from wild birds that serve as natural reservoirs; however, only the H5 and H7 AIV subtypes have been known to cause HPAI infections in domestic poultry (Alexander and Brown, 2009; Lee, et al, 2004). H5 or H7 LPAI viruses from wild birds can be introduced to domestic poultry and mutate to HPAI strains, or a HPAI strain can be introduced directly from wild birds to domestic poultry (e.g., Berhane et al, 2009; Ip et al., 2015). Once introduced, virus can spread or be transmitted to other poultry operations through the movement of birds, people, and equipment, or via airborne or local area spread (Halvorson, 2009). HPAI outbreaks in domestic poultry can have severe local, regional, and national-level economic impacts (Yang et al., 2017; Killian et al., 2016; Alexander and Brown, 2009; Hagerman and Marsh, 2016). For example, approximately 48 million domestic poultry either died from infection or were depopulated during the 2014-2015 H5N8 and H5N2 HPAI outbreak in the United States, incurring outbreak response costs of USD $879 million and economic impacts to producers of USD $1.043 billion (Johnson et al., 2016; Seitzinger and Paarlberg, 2016).

Previous AIV explanatory modeling studies have used outbreak data to determine human, environmental, and wild bird factors that may influence the location of future outbreaks (e.g., Si et al., 2013; Alkhamis et al., 2016; Bouwstra et al., 2017; Humphreys et al., 2020). These factors have also been used to model AIV infection and carriage in wild bird species (e.g., Farnsworth et al., 2012; Fuller et al., 2010; Herrick et al., 2013). Although environmental factors (e.g., water presence, distances to lakes and wetlands, annual temperature, precipitation, type of land

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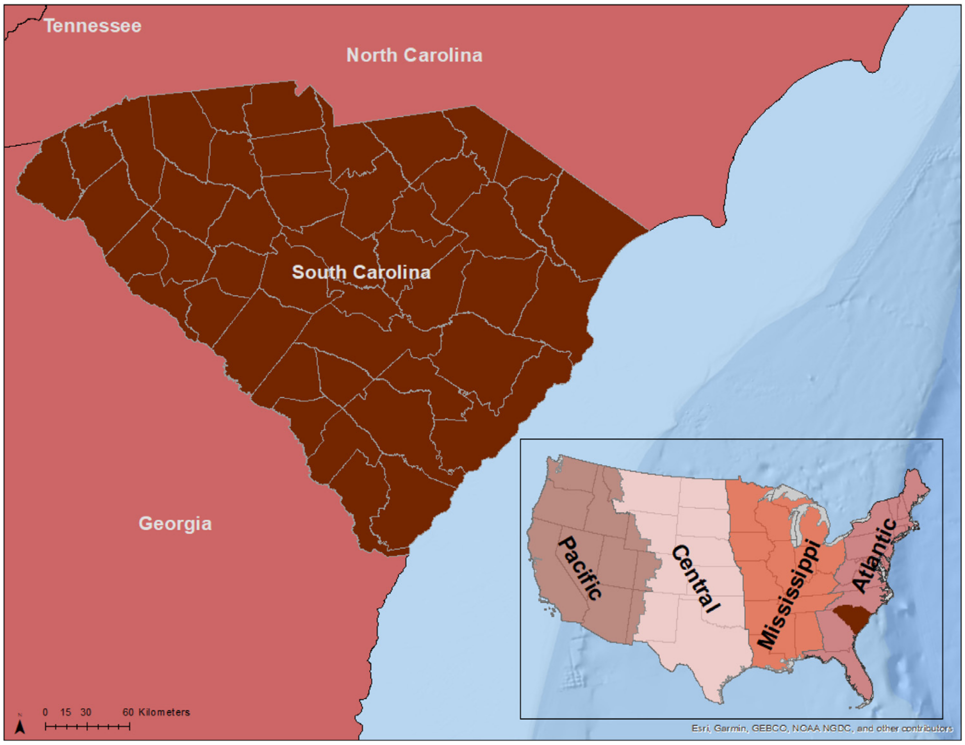


**Fig. 1.** South Carolina study area with an inset of the United States and the four North American migratory bird flyways.

cover, freeze/thaw dates) have been shown to impact both the level of infection in reservoir birds and the propagation of AIV during outbreaks (Gilbert and Pfeiffer 2012; Herrick et al., 2013; Fuller et al., 2010; Si et al., 2013; Alkhamis et al., 2016), modeling efforts have yet to specifically focus on how these factors impact environmental virus survivability. In laboratory-based studies, viral decay under simulated environmental conditions shows that AIVs retain viability for extended periods once outside of hosts (Dalziel et al., 2016; Brown et al., 2009; Keeler et al., 2014). The environment plays a role in the transmission of virus among and between reservoir species and also in serving as a temporary storage reservoir for AIV (Torremorell et al., 2016; Dalziel et al., 2016; Rohani et al., 2009; Breban et al., 2009). It has been suggested that AIV surveillance sensitivity may be increased in the future by environmental sampling, in addition to the sampling of reservoir species (Lickfett et al., 2018; Densmore et al., 2017; Poulson et al., 2017).

Geospatial models that measure the risk of AIV persistence in the environment are currently absent from the literature. To address this knowledge gap, the primary objective of this study was to use geospatial methods to determine AIV environmental persistence risk in South Carolina. A second objective was to infer the risk of AIV introduction to domestic poultry flocks.

## 2. Materials and methods

### 2.1. Study location

South Carolina (SC) resides in the Atlantic Flyway (Fig. 1) and is the home to migratory and resident waterfowl species in which AIVs have been previously detected (Gordon et al., 1998; Bevins et al., 2014). The commercial broiler, layer, turkey, quail, and duck/goose industries in SC are important segments of the state's agricultural economy (NASS, 2014). Sales of eggs and poultry in SC totaled nearly $1.5 billion in 2012, making it the 8$^{th}$ highest U.S. state for this category (NASS, 2014).

### 2.2. Season

This study evaluated the environmental persistence of AIV across four seasons based on known wild bird migratory patterns: 1) fall migration, defined as September 1 – November 30, (i.e., migratory birds travel to SC from the north), 2) winter, defined as December 1 – February 15 (i.e., birds overwinter in SC), 3) spring migration, defined as February 16 – March 31, (i.e., birds move from SC to northern breeding grounds), and 4) breeding, defined as April 1 – August 31, (i.e., birds typically breed in northern locales outside of SC) (John Stanton, personal communication). Wild bird populations within the state are therefore highest during winter, moderate during migration periods, and low during breeding periods.

### 2.3. Environmental factors

Factors known to influence AIV introduction and survival in the ambient environment were identified through an iterative evaluation of the peer-reviewed literature. For the initial review, three databases, PubMed, Scopus, and Google Scholar, were searched for articles focused on avian influenza and environmental persistence published through 2016 and printed in English. Articles concerned with persistence or survival of AIV in the environment, that were experimental laboratory or field based, and presented quantitative results, were retained. Studies concerning vaccines or pharmaceuticals, humans, non-avian species, methods development focused, or outbreak/situation reports, were excluded. Additional sources that met the inclusion criteria were added through backward citation. This process identified 21 potential covariates, and each of these was further evaluated through the literature focusing on H5 and H7 subtypes, and reviewed with subject matter experts. This information was combined with an assessment of data availability and a total of six environmental factors were selected as inputs to develop seasonal risk models. These factors, corresponding data sources, trends relative to AIV survival, rationale for model inclu-

**Table 1**
Environmental factors selected as model inputs, data sources, rationale for model inclusion, trends with AIV survival, and supporting citations

| Factor | Data Source | Trends with AIV | Rationale | Citations |
|---|---|---|---|---|
| Water Presence | USGS Gap Analysis Program | *AIV particles survive well in water compared to air or other dry media* | 1) AIV survives in water more prominently than on dry land<br>2) AIV is likely to be deposited in water sources by reservoir birds<br>3) Water sources may be used for irrigation/drinking and function in multiple routes of AIV propagation | Brown et al., 2007; Nielsen et al., 2013 |
| Water Salinity | USGS Gap Analysis Program | *Inverse association between AIV persistence and increasing salinity: optimal salinity is fresh water, sub-optimal is brackish* | 1) Salinity of water sources greatly impacts the rate of AIV survival<br>2) In the South Carolina study area, salinity of water sources varies based on proximity to the coast | Keeler et al., 2014; Brown et al., 2009; Nazir et al., 2010; Shoham et al., 2012; Stallknecht et al., 1990a |
| Water Temperature | USGS Moderate Resolution Imaging Spectroradiometer (MODIS) remote sensing data | *Inverse association between AIV persistence and increasing temperature: optimal temperatures are near freezing and sub-optimal temperatures are 17-28 °C* | 1) Temperature of water sources greatly impacts the rate of survival of AIV | Lang et al., 2008; Nazir et al., 2010; Zhang et al., 2006; Brown et al., 2009; Keeler et al., 2012; Stallknecht et al., 1990a; 1990b |
| Wild Birds | eBird; Cornell University | *Wild birds are a primary reservoir for AIV, and introduce the virus to their surrounding environment* | 1) When carrying the virus, wild birds deposit viral particles into water and other habitat locations<br>2) Historically, some AI outbreaks have been associated with wild bird propagation of viral particles | Wood et al., 2011; Bevins et al., 2014; Ramey et al. 2018 |
| Wetland Cover | United States Fish and Wildlife Service | *Wetland cover provides ideal habitat for migratory birds, and contains aquatic zones where AIV can thrive for long periods* | 1) Previous studies show that wetland areas are associated with AIV presence<br>2) Wetland areas in South Carolina represent distinct locations not recognized during simple identification of water bodies | Belkhiria et al., 2016; Hanson et al., 2008; Fuller et al., 2010; Iglesias et al., 2010 |
| Wildlife Refuges, Wildlife Management Units | United States Fish and Wildlife Service | *Wildlife refuges are preservation zones of wild bird habitat* | 1) South Carolina wildlife refuges provide ideal wintering and nesting habitat for wild birds<br>2) Within wildlife refuges in South Carolina, waterfowl management units function to hold water at levels ideal for migratory birds | USFWS, 2016; Keeler et al., 2012; John Stanton, personal communication 2016 |

sion, and references are summarized in Table 1. A detailed description of data processing for each factor follows.

*Water presence and salinity.* AIV has been shown to have improved survival in water compared to dry land (Brown et al., 2009; USGS, 2011), with an inverse relationship between water salinity and survival (Stallknecht et al., 1990a, 1990b; Nazir et al., 2010). United States Geological Survey (USGS) Gap Analysis Project hydrography data collected between 1994 and 2004, derived from satellite imagery at a 30m resolution, were downloaded and reclassified into three categories of surface water based on suitability for AIV survival: presence of fresh water (high suitability), presence of brackish water (moderate suitability), or no water present (low suitability) (USGS, 2011). The surface water presence and salinity layer was aggregated from the original 30m grid cell size (Gap) to a one-kilometer (1km) resolution. This was accomplished by defining each 1km area (hereafter referred to as grid cells) by the value given to a majority of original (30m) grid cells.

*Water temperature.* Water temperature is inversely associated with the rate of AIV survival (Brown et al., 2009; Keeler et al., 2012; Lang et al., 2008; Stallknecht et al., 1990a; 1990b). USGS Moderate Resolution Imaging Spectroradiometer (MODIS)-derived 8-day land surface and emissivity scenes were downloaded for 2006-2015. Using R version 3.3.3, individual scenes were masked by quality indicators (i.e., cloud cover) and recombined to create summary temperature surfaces by season, at a spatial resolution of 1km (Grim and Knievel, 2013; Ke and Song, 2014; NASA, 2012). R code is available upon request from the study authors. Refined MODIS data were then masked with the water presence and salinity layer to reflect locations only where surface water was present, and reclassified to represent high suitability ($<$ 10°C), moderate suitability ($\geq$ 10°C and $<$ 20°C), or low suitability ($\geq$ 20°C) for AIV survival (Brown et al., 2007; Brown et al., 2009; Keeler et al., 2014; Nazir et al., 2010).

*Wetlands, wildlife refuges, and waterfowl management units.* U.S. Fish and Wildlife Service (USFWS) National Wetland Inventory data were used to identify locations of wetlands and wildlife refuges, which are considered favorable for AIV persistence (USFWS, 2016; Keeler et al., 2012; Belkhiria, et al., 2016; Iglesias et al., 2010; Fuller et al., 2010). Locations classified as 'freshwater emergent wetland' and 'freshwater forested/shrub wetland' were extracted. Data were reclassified based on presence or absence of either wetland type and resampled to 1km. For wildlife refuges, USFWS Cadastral data were obtained and reclassified at a 1km resolution based on presence or absence of National Wildlife Refuge (NWR) land. The locations of wetlands, NWRs, and Waterfowl Management Units can be viewed in Supplemental Figure 1.

*Bird presence.* Data for wild bird sightings were obtained from Cornell University's eBird portal (https://ebird.org/home), a citizen scientist application that allows users to categorize birds by species, count, date, and location (Wood et al., 2011). Data were filtered to observations of 11 wild bird species made between 2006 and 2016 in SC (Table 2). The wild bird species selected for this analysis tested positive for AIV by rRT-PCR using the matrix (M) gene primer in SC as part of the Interagency Wild Bird Surveillance Program between 2007 and 2011 (Bevins et al., 2014; Bevins, 2016).

Due to a high potential for sampling bias in the eBird data (Gu and Swihart, 2004; Hefley et al., 2013), observations for each wild bird species of interest were classified to reflect either presence or non-detection and aggregated to a 1km resolution by season. If a 1km grid cell contained at least one detection, the grid cell was considered eBird positive for that species and season. Although these processing steps lowered the geographic precision

**Table 2**
Common and scientific names of wild bird species that were included in the model

| Common Name | Scientific Name |
|---|---|
| Ruddy duck | *Oxyura jamaicensis* |
| Northern shoveler | *Anas clypeata* |
| Blue-winged teal | *Anas discors* |
| American green-winged teal | *Anas carolinensis* |
| Northern pintail | *Anas acuta* |
| Ring-necked duck | *Aythya collaris* |
| American wigeon | *Anas americana* |
| Mottled duck | *Anas fulvigula* |
| Gadwall | *Anas strepera* |
| Mallard | *Anas platyrhynchos* |
| Wood duck | *Aix sponsa* |

of our analysis, spatial aggregation helped reduce sampling bias (Dormann et al., 2007; Phillips et al., 2009) and the spatial autocorrelation known to be present in eBird records (Humphreys et al., 2019). Given that eBird data indicates bird presence only, areas of non-detection were assumed to represent available or potential habitat and not confirmed bird absences. Likewise, results are interpreted in the context of relative suitability (not occurrence probability) to ensure that our input data and inferences are correctly linked (Guillera-Arroita et al., 2015) The final seasonal bird layers reflected the presence or non-detection of the 11 species of interest within each grid cell.

*Poultry operation count.* County-level numbers of commercial poultry (broiler, layer, turkey, duck/goose) operations were downloaded from the NASS 2012 Census of Agriculture. The number of operations by county were reclassified into quintiles as very low, low, medium, high, or very high (Supplemental Figure 2).

Data were prepared using tools within ArcGIS 10.4, and final layers were projected to Universal Transverse Mercator (UTM) Zone 17N in the datum World Geodetic System 1984 (WGS1984).

### 2.4. Model execution

Risk of environmental AIV persistence was determined using a multi-criteria decision analysis (MCDA) (Pfeiffer et al., 2008). A simple additive expression was built with environmental factors weighted based on their relative contribution to the persistence of AIV, as determined through subject matter expert (SME) consultation and the strength of the literature (Table 3). We consulted with 10 SMEs on this MCDA: four veterinarian epidemiologists (DVM), two wildlife biologists, three ecologists and one entomologist. All SMEs are government-employed scientists at the federal level or the state level with expertise in the following areas: wild birds and migratory pathways, avian influenza, climate data, geographic information systems, and poultry production. Two of the SMEs are coauthors on the manuscript (AJ and KAP). A separate query expression was created for each season of interest, using data layers created during model preparation. After executing each seasonal expression, values acquired from the MCDA (maps) were characterized into risk categories using quintiles and the World Organization for Animal Health (OIE) Risk Assessment Guidelines: negligible/very low, low, moderate, high, and extremely high risk (OIE, 2017b). Reclassification followed the scheme outlined in Table 4. The MCDA was implemented using the Esri Predictive Analysis (PA) Tools Add-In within ArcGIS 10.4 (PA Add-In: http://appsforms.esri.com/products/download/). The PA Add-In is a collection of tools that builds models to predict the location of suitable sites based on input factors; in this study, environmental or wild bird conditions. The 'Query Expression Editor' within the PA Add-In was used to build mathematical expressions using Boolean operators, and calculated new values where input variables overlap.

**Table 3**
Environmental factors, layer values, and PA add-in query expression weights

| Factor | Layer Values | Weighted PA Values |
|---|---|---|
| Wild Birds | 1 = Presence | 3 |
| | 0 = Non-detection | 0 |
| Water Temperature | 1 = < 10°C | 3 |
| | 2 = 10°C ≤ > 20°C | 2 |
| | 3 = ≥ 20°C | 1 |
| Water Presence / Salinity | 2 = Fresh water | 3 |
| | 1 = Brackish water | 1 |
| | 0 = No water | 0 |
| Wetlands | 1 = Presence | 2 |
| | 0 = Absence | 0 |
| Wildlife Refuges | 1 = Presence | 3 |
| | 0 = Absence | 0 |

**Table 4**
PA model values and reclassification into OIE risk categories

| Original model value | Risk category (value) |
|---|---|
| 0-1 | *Negligible/Very low risk (0)* |
| 2-4 | *Low risk (1)* |
| 5-7 | *Moderate risk (2)* |
| 8-10 | *High risk (3)* |
| 11-13 | *Very high risk (4)* |

The risk of environmental persistence of AIV derived from the 1km resolution models was then aggregated to a county level. Each county was classified according to one of the five OIE risk categories based on the maximum risk value identified for any one grid cell within the county. For each season, the number of commercial poultry operations (NASS) was overlaid with counties having high or very high risk.

### 2.5. Model validation

Population models for wild bird species previously shown to be AIV positive within SC were created to validate the risk models. Season specific relative abundance of wild birds was first determined at a 1km resolution across the state. This result was then compared to the modeled environmental persistence risk, to 1) determine if abundance was associated with environmental risk, and 2) determine if estimated risk categories were significantly different within each season. Wild bird relative abundance was estimated using a log-Gaussian Cox process (LGCP) under assumed preferential sampling (Supplemental Figure 3). To implement the LGCP model, wild bird presence data for the years 2000 - 2016 was obtained from the eBird portal for each of the species listed in Table 2, aggregated by season, and then run using the R-INLA package (Rue et al., 2009; Lindgren, et al., 2015). Pre-processing and validation information for these abundance models can be found in the data supplement. The correlation between relative abundance of AIV species and environmental persistence risk was measured for each season using Spearman's $\rho$ followed by Kruskal-Wallis tests (Kruskal and Wallis, 1952) and post hoc Dunn's Tests (Dunn, 1961). The Kruskal-Wallis test is an evaluation for statistically significant differences between categories or groups within an independent variable. After the Kruskal-Wallis indicated that significant differences did exist across all risk levels, the nonparametric Dunn's Test was applied to identify precisely which pairwise risk categories were significantly different than each other.

### 2.6. Sensitivity analysis

No low pathogenicity or high pathogenicity avian influenza outbreaks have been detected in domestic poultry in SC in recent

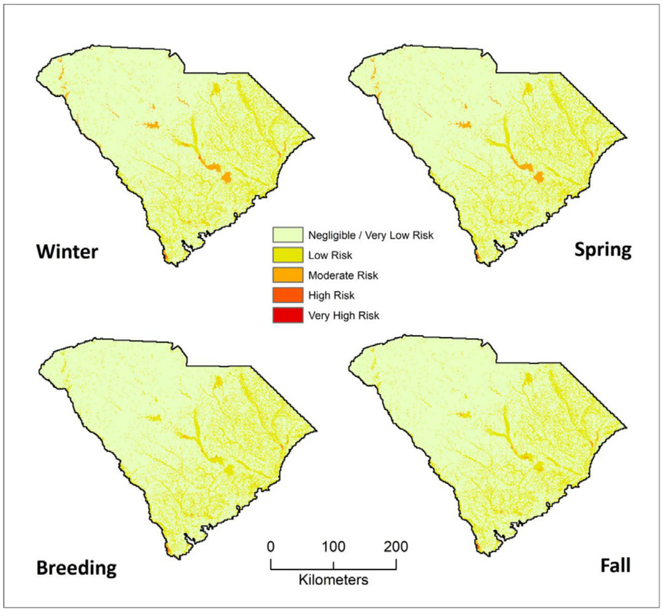


**Fig. 2.** Risk of seasonal AIV environmental persistence in South Carolina.

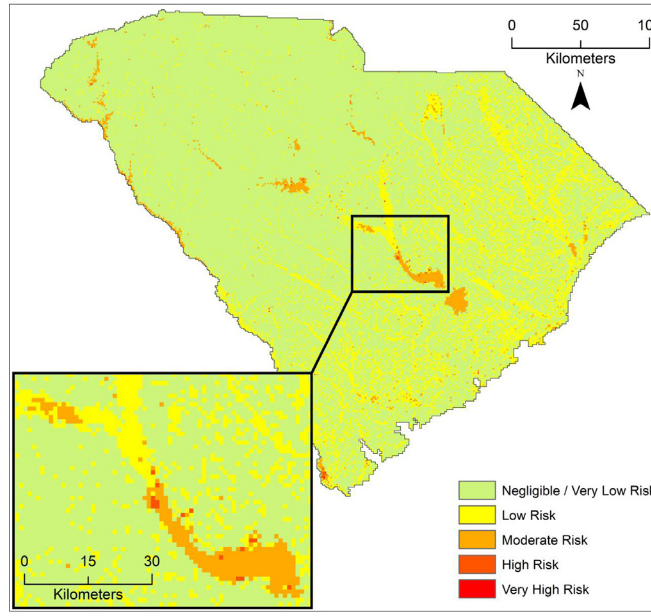


**Fig. 3.** AIV environmental persistence risk in South Carolina during winter (highest risk season).

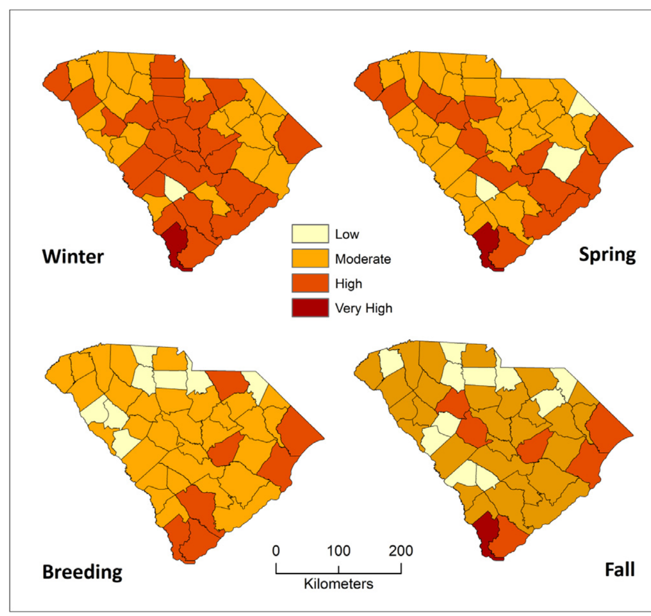


**Fig. 4.** County-level risk of seasonal AIV environmental persistence in South Carolina.

years. Without these data, we could not test the performance of the MCDA output for the study area. Instead we ran the MCDA without weights for each season and compared these results to the weighted results to evaluate differences in spatial extent of factors associated with environmental AIV persistence risk by season.

## 3. Results

Seasonal models of environmental AIV persistence are shown in Fig. 2. SC encompasses a total of 80,644 km$^2$, and during all seasons a majority (81%) of grid cells were considered to be negligible/very low risk for AIV persistence in the environment. A moderate percentage (17.1–18.6%, depending on season) of the landmass was classified as low risk. The winter and spring migration seasons trended toward higher risk values when compared to the breeding and fall migration seasons (Figs. 2 and 3). Areas with moderate risk encompassed 1.8% and 1.7% of the total area during the winter and spring migration, respectively. This same moderate risk level represented only 0.5% of total area in both the fall migration and breeding seasons. Similarly, few areas in any season were classified as high risk. High risk areas encompassed 0.8% and 0.4% of the total area in winter and spring migration, and only 0.2% and 0.3% of the total area in breeding and fall migration seasons. Notably, only a single grid cell (located in the southeastern portion of the state) in the fall migration, spring migration, and winter seasons, was considered to be very high risk.

When aggregating grid cells to the county level for poultry operation comparison, no counties had negligible/very low risk of AIV environmental persistence and few (2%–24%, depending on season) had low risk (Fig. 4). Counties with low risk in more than one season tended to be inland. A majority of counties had moderate risk, 46% - 67%, by season. Several counties were high risk, a greater percentage of which were seen in the winter (50%) and spring migration (28%) seasons, compared to the breeding (15%) and fall migration (13%) seasons. Three counties were categorized as high risk in all seasons, including the characteristically lower-risk fall migration and breeding seasons. These counties tended to be either centrally located or coastal. One southern county was designated as very high risk in the fall migration, spring migration, and winter seasons, likely due to the presence of wetlands and wildlife refuge locations within the county.

Overall, the winter and spring migration seasons had a greater number of counties where high environmental AIV persistence risks co-occurred with higher numbers of domestic poultry operations (Fig. 5). During winter and spring migration seasons, 7% of the counties had co-occurring high risk of AIV persistence and a very high number of commercial poultry operations. During the fall migration season there was just a single county fitting these same criteria and no counties met these criteria in the breeding season. During the spring migration, fall migration, and breeding seasons, 8 – 13% of SC counties had both high/very high risk of AIV persistence where the number of commercial poultry operations was very low or low. During these same seasons, 2 – 9% of counties with medium numbers of commercial poultry operations had high/very high risk of AIV persistence. Overall, the winter season

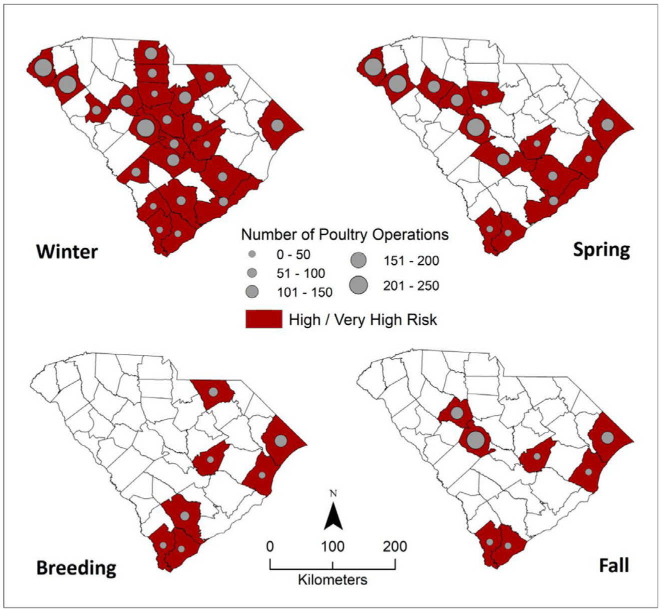


**Fig. 5.** Numbers of commercial poultry operations, South Carolina counties with high or very high AIV environmental persistence risk, by season.

had the greatest number of counties with higher AIV persistence risks co-occurring with a varying range of poultry operation numbers. A total of 33% of counties in the winter had high/very high AIV persistence risk combined with very low/low number of poultry operations. This dropped to 11% of counties classified as high risk for AIV persistence where a medium number of commercial poultry operations existed. These counties tended to be dispersed throughout the state.

### *3.1. Model validation*

Correlation between the modeled relative density of wild birds and AIV environmental persistence risk was significant ($p = <0.0001$) in each season assessed (winter $\rho = 0.15$; spring migration $\rho = 0.07$; breeding $\rho = 0.13$; fall migration $\rho = 0.13$), indicating that similar patterns were not likely due to chance. Kruskal-Wallis tests showed significant differences in wild bird abundance between risk categories for all seasons (winter (Chi square = 2688.8, df = 4, p-value < 0.001), spring migration (Chi square = 2505.4, df = 4, p-value < 0.001), breeding (Chi square = 954.3, df = 3, p-value < 0.001), and fall migration (Chi square = 963, df = 4, p-value < 0.001)). Post hoc Dunn's tests to examine pairwise differences between risk categories by season suggested that because few locations were classified as very high risk, the category was difficult to distinguish in all seasons. Similarly, a relatively low occurrence of locations assigned to the high-risk category resulted in the high and moderate risk categories not being statistically different from each other. All other combinations of the negligible, low, moderate, and high-risk categories were statistically different with p-values < 0.001 for all seasons. P-values for all pairwise risk categories are provided by season in Supplementary Table 1.

### *3.2. Sensitivity analysis*

The unweighted MCDA resulted in a binary classification (e.g., suitable, not suitable) for the study area; these maps are provided in Supplemental Figure 4. Total suitable area for AIV persistence for South Carolina was the same as for the weighted MCDA when the four OIE risk categories (low, moderate, high and very high risk) were aggregated.

## 4. Discussion

Previous geospatial models have identified factors that are associated with AIV transmission among wild birds and domestic poultry and have estimated the probability of future HPAI outbreaks in different locations (Adhikari et al., 2009; Si et al., 2013; Alkhamis et al., 2016; Prosser et al., 2016). Avian influenza virus surveillance data from migratory wild birds has been used to investigate georeferenced factors that influence the maintenance of AIV among these reservoir species (Farnsworth et al., 2012; Fuller et al., 2010; Herrick et al., 2013). In contrast, this study models the risk of AIV persistence in the environment. Overlaying this model with commercial poultry operations provides insight into areas potentially at higher risk for AIV introductions into domestic poultry populations.

Our results showed the majority of the SC landmass as negligible/very low risk for environmental persistence of AIV across seasons, suggesting that transmission and propagation of AIVs through the environment may be minimal throughout the year. These results are consistent both with previous studies aimed at identifying areas at risk for AIV and with a lack of reported avian influenza outbreaks within SC in recent years (Fuller et al., 2010; Farnsworth et al., 2012; Belkhiria et al., 2016). Despite a majority of low risk areas, there were also grid cells classified as moderate to very high risk within the state. As a general trend, these areas were characterized by a higher number of eBird sightings during the winter and spring migration seasons. Seasonal variation of risk has been previously reported and follows historical avian influenza outbreak patterns in North America where events most commonly occur during cooler seasons (i.e., winter and early spring) (Swayne 2008; Halvorson, 2009; Alkhamis et al., 2016; Si et al, 2013). During these time periods in SC specifically, fresh water had the lowest (most suitable) temperature, and wild bird populations were highest due to over-wintering or the start of a northerly migration. A study of waterfowl habitats on the Delmarva Peninsula, United States, found the presence of AIV in sediment samples to be associated with the total number, density, and specific species of waterfowl present at sampling locations (Densmore et al., 2017). Further, a meta-analysis of environmental factors associated with LPAI virus survival specifically in water showed that temperature had a stronger influence on viral decay than other variables evaluated (e.g., salinity, pH) (Dalziel et al., 2016). Our results support these findings; that the temporal presence of wild bird species and the location of cold, fresh waterways greatly influence the risk of AIV persistence in the environment. Importantly, a large number of wild birds migrating in and out of modeled moderate and high-risk areas may provide opportunities for multiple introductions of AIV into the environment, allowing for maintenance of the virus for a short time at these sites.

Ideally, data used for model validation would have included both environmental and wild bird positive AIV samples in SC. Although not currently available, future AIV surveillance work that includes both the sampling of environmental locations and wild birds (Nazir et al., 2011; Densmore et al., 2017; Lickfett et al., 2018; Poulson et al., 2017) would provide the most suitable data for model validation. Current wild bird AIV surveillance data does not capture the original location of individual bird(s), but instead aggregates diagnostic samples to central collection sites (Bevins et al., 2014). In the absence of individual bird location data, a surrogate dataset, wild bird abundance for species known to carry AIV, was used to assess our confidence in the model. Both the validation data set and the risk model used eBird data; however, the inputs were different as the risk model used presence/non-detection (versus abundance) and utilized records from 2006 – 2016 (versus 2000 – 2016). Because eBird records were collected through opportunistic survey, it was necessary to consider potential sampling biases when constructing both the risk and abundance models. Spatial aggregation was used for the risk models and a preferential sampling protocol was implemented for those used to estimate abundance. Sampling bias is inherent to citizen science data and failing to account for issues like non-detection may lead to unreliable results (Hefley et al., 2013). Another possible mechanism for model validation would be the use of outbreak data; however no low pathogenicity or high pathogenicity avian influenza outbreaks have been detected in SC in recent years. Additionally, using outbreak data from commercial poultry would require differentiation between premises infected by introduction of AIV from the environment / wild birds and those infected due to between farm transmission.

For all seasons, model validation showed a statistically significant correlation between modeled AIV environmental persistence risk and the spatial location of wild bird species known to carry AIV. This suggests value in using the model to identify and target geographic areas that may have a greater risk of AIV introduction from the environment or via wild birds into domestic poultry operations.

One kilometer resolution risk models are valuable to guide emergency planning and response activities to support an AIV outbreak. Additionally, understanding the risk of introduction of AIV into domestic poultry flocks across a larger area, for example at a county level, is important for reducing future outbreaks. Evaluating risk at the county level was advantageous for this assessment because county is an administrative unit where operation-level biosecurity and management actions are typically implemented and where outreach activities can be more readily communicated to producers. A conservative approach was taken to classify county-level risk, whereby the maximum environmental risk value of any grid cell in the county was assigned to the entire county. Although outcomes may over-represent county-level risk based on this approach, consequences of HPAI detection in a single commercial poultry operation are devastating to the industry as a whole and are costly to the U.S. economy. Given the historical impacts of HPAI events, it is preferable to overestimate rather than underestimate AIV persistence risk at this scale. Although the overall persistence risk across the state was low, certain counties containing a combination of factors that influence AIV viability were deemed as high risk and are locations that may be a focus for improving AIV targeted awareness or communication. When high risk counties were evaluated in combination with commercial poultry operation numbers, seasonal trends similar to those of the higher resolution (1km) risk models were observed. Maps that describe co-occurrence of poultry operations and risk for AIV persistence in the environment provide decision makers with actionable information to set priorities for planning and outreach to minimize risk of AIV introductions.

While the model provides insight into environmental areas with greater AIV persistence risk and potential for AIV introduction to commercial poultry operations, there are several limitations. Model inputs were selected based on an investigation of the current literature describing AIV survivability and consultation with subject matter experts. Although this was the most reasonable approach for determining essential factors for inclusion in the model, there may be undetermined factors influencing environmental survivability of the virus (e.g., weather patterns, human presence near waterfowl congregation sites). In addition, data for some environmental factors known to influence AIV survival and transmission, such as water pH and the presence of bridge hosts, were either not readily available in digital format or for the study area (Shriner et al., 2016; Poulson et al., 2017; Brown et al., 2009; Keeler et al., 2014; Stallknecht et al., 1990a). The inclusion of these additional factors would likely alter model results and potentially improve the validity of outcomes. The weighting model inputs is also vital to the accuracy of output maps, and environmental factors with greater (relative) weights in the current model included the presence of wild birds and fresh water. Although these approaches followed trends in peer-reviewed publications and the suggestions of SMEs, alternative weighting strategies would likely impact seasonal outcomes. The eBird data were the most comprehensive wild bird location data available; however, citizen-scientist observations represent a non-random sample of points. Importantly, when viewed as individual data layers, the eBird data acquired and processed for inclusion in models followed expected trends for wild bird density within the state, by season (data not shown).

The focus for the current study was to evaluate the risk of AIV persistence in the environment. While we did overlay environmental risk maps with maps of county-level counts of commercial poultry operations, an evaluation of introduction of virus into poultry operations was not explicitly measured. Future studies may focus on the potential risk of AIV spillover from both wild birds and environmental sources of the virus, using information gathered here as context. These studies may also concentrate on the relative importance of potential mechanisms for introduction, and the role of operation biosecurity in AIV transmission, which were also outside the scope of the current work.

## 5. Conclusion

The current study demonstrated a methodological approach to determine where AIV may persist in the environment, specifically in South Carolina. We have identified several counties with large numbers of domestic poultry operations and high risk for environmental AIV persistence risk during the winter season. Animal health authorities may use this information for targeted delivery of education and resources, or for example to conduct preparedness exercises. The methods established may be applied in the future at a larger scale (e.g., by migratory flyway, in a different state, across the contiguous United States), and results may also be integrated into models that investigate AIV transmission at the interface of domestic poultry and their surrounding environment. The mechanisms of transmission at the interface between wild birds and domestic poultry, and the ecology of AIV, including the role of the environment in this system, are complex. Improved understanding of potential AIV reservoirs and spillover risks is a valuable contribution to AIV preparedness in the United States.

## Statement of author contributions

C. Stenkamp-Strahm performed primary data analysis, executed the GIS for the project, and wrote the first drafts of the manuscript.

K. Patyk provided project coordination, poultry SME, assisted with results analysis and interpretation, and revised the manuscript.

M. McCool-Eye provided project coordination, assisted with GIS execution and model interpretation, and revised the manuscript.

A. Fox provided GIS SME, and assisted with GIS execution, model interpretation and creation of figures.

J. Humphreys provided statistical SME, developed models for validation, and assisted with the execution of validation procedures.

A. James provided spatial modeling and wildlife SME during project initiation, and assisted with results analysis and interpretation.

D. South assisted with GIS execution and provided feedback during results interpretation.

S. Magzamen provided project leadership and coordination, aided in data gathering, GIS execution, results analysis and interpretation, and revised the manuscript.

## Declaration of Competing Interest

The authors have no competing interests to declare.

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## Supplementary materials

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