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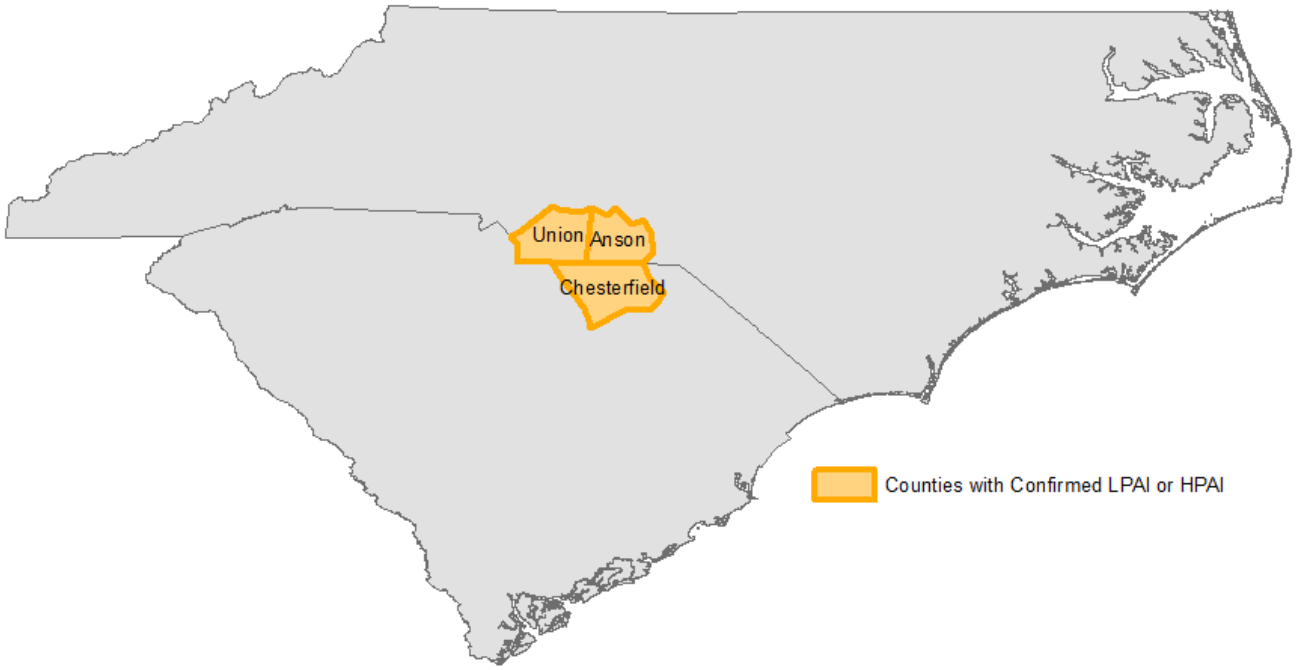
U.S. DEPARTMENT OF AGRICULTURE

Epidemiologic and Other Analyses of Avian Influenza Affected Poultry Flocks

September 2020 Report



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EXECUTIVE SUMMARY

An outbreak of low pathogenic avian influenza (LPAI) was detected in March 2020, in the States of North Carolina and South Carolina. A commercial breeder turkey operation in Anson County, North Carolina noted a slight drop in egg production. Samples were collected on 9 March and tested in accordance with National Poultry Improvement Program (NPIP) requirements. Two additional meat-type turkey flocks in Union County, North Carolina submitted pre-slaughter surveillance samples on 10 March. All three flocks were confirmed with H7N3 LPAI of North American wild bird lineage on 12 March. A total of 12 operations were confirmed with H7N3 LPAI, and one operation in South Carolina was confirmed with H7N3 highly pathogenic avian influenza (HPAI) over the course of this outbreak. Depopulation and disposal were completed for poultry immediately on each farm with the final infected premises being completed by 15 May. A total of 337,362 turkeys were depopulated in response to this outbreak.

Following initial response activities, a series of epidemiologic, genetic and environmental investigations were initiated to better understand virus introduction and transmission. These investigations were a collaboration between the poultry industry, North Carolina Department of Agriculture and Consumer Services (NCDA&CS), State officials from Clemson University's Livestock Poultry Health (CULPH), and the USDA-APHIS-VS.

Analysis of epidemiologic surveys conducted at case and control premises identified a higher number of turkey housing structures and having a common gathering place for workers on the premises as risk factors for avian influenza in this commercial turkey population. However, the results of the case control study should be interpreted with caution since both cases and controls were associated with a single integrator. Results may not be generalizable to the entire poultry industry in the Carolinas. There were extensive overlapping networks of connections identified among the infected premises; however, the level of information provided about network relationships was not sufficient to evaluate the risk from particular movements or connections.

Using diagnostic testing, mortality, and egg production data, a range of dates of possible virus introduction were estimated for six of the infected premises. The earliest most likely date of introduction among the six premises was 6 February 2020. The latest most likely date of introduction, to the premises that was infected with HPAI in one barn and LPAI in four other barns, was 28 March 2020. However, no birds were sent to slaughter from these flocks prior to testing and the subsequent detection of avian influenza. Though NPIP recommended biosecurity measures were in place, the overlap of ranges of detection dates among the premises support the possibility of lateral spread among the infected premises, including the HPAI infected premises.

Phylogenetic analysis of viruses from these premises support a single introduction of North American wild bird lineage H7N3 LPAI into turkey farms in North Carolina followed by lateral spread to other farms, with a single mutation event resulting in HPAI in a single barn on a commercial turkey operation in South Carolina. Analysis suggests the precursor virus likely emerged in wild waterfowl from the Mississippi flyway. The 2020 H7N3 virus is distinguishable from other recent H7 poultry detections (2016 H7N8, 2017 H7N9, 2018 H7N1, and 2018-2019 H7N3) across the entire genome. Although no recent single wild bird origin precursor with all 8 gene segments was identified, highly similar progenitor genes were identified from wild bird surveillance

efforts, particularly from the Mississippi flyway. Analysis also suggests that this H7 HA clade may represent a repetitive threat to poultry.

Introduction of avian influenza virus (AIV) into domestic poultry can be initiated by exposures to infectious wild birds or to virus surviving in the environment. A temporal spatial analysis of environmental factors known to influence AIV environmental persistence in North Carolina and South Carolina was used to create risk maps of AIV persistence from January 2020 through June 2020. The analysis identified an increasing level of weekly relative environmental risk from the beginning of the study period through the week ending with April 7th. From the week beginning April 8th through the end of the study period, weekly relative risk remained similar. Small areas with high and very high risk persisted in all weeks of the study period.

The epidemiologic investigation focused on factors and management practices which have been implicated as risk factors for infection in previous avian influenza outbreaks. Phylogenetic analysis supports a single introduction followed by subsequent lateral spread. The affected premises were part of a highly connected network and transmission through the movement of fomites such as people, vehicles, and equipment between farms, were likely; however data collected on potential epidemiologic links during the window of introduction did not reveal a clear mechanism of spread. Additionally, breaches in biosecurity, for example, contact with wild birds, may have had a role in introduction and transmission. These results can be used to improve surveillance activities and to inform biosecurity practices and emergency preparedness efforts within the Carolinas.

INTRODUCTION

In response to the H7N3 LPAI/HPAI outbreak in commercial turkey operations in Anson and Union counties in North Carolina and Chesterfield county in South Carolina, the North Carolina Department of Agriculture and Consumer Services (NCDA&CS), State officials from Clemson University's Livestock Poultry Health (CULPH), and the USDA-APHIS-VS initiated epidemiologic and genetic investigations to understand factors associated with introduction and transmission of avian influenza virus into and between poultry flocks.

These investigations included:

- A field-based study of infected farms using data collected through site visits and interviews with farm personnel
- A case-control study of infected and non-infected premises
- Analysis of barn-level mortality, egg production, and diagnostic test data from multiple infected farms to estimate the dates of virus introduction into this population
- Virus phylogenetic analysis
- Geospatial estimation of the relative risks of environmental persistence of avian influenza viruses in the environment in North Carolina and South Carolina.

This report includes the results from these investigations, to provide producers, industry, and other stakeholders with epidemiologic data from this outbreak. This is a final report of findings resulting from these investigations.

A. Description of Outbreak

Detection of H7N3 LPAI

On 9 March 2020, a commercial breeder turkey operation in Anson County, North Carolina (**Error! Reference source not found.**), submitted samples for testing as a part of the National Poultry Improvement Program (NPIP) requirements after the flock manager noted a slight drop in egg production. On 10 March 2020, two commercial meat-type turkey flocks in Union County, North Carolina submitted samples to the Rollins Animal Diagnostic Laboratory for testing as part of routine pre-slaughter surveillance. When the samples from all three of these premises were determined by Rollins Animal Disease Diagnostic Laboratory to be non-negative for avian influenza on 11 March 2020, oropharyngeal (OP) swab samples were collected and tested H7 positive at the Rollins Animal Disease Diagnostic Laboratory. Samples were received at the National Veterinary Services Laboratories (NVSL) on 12 March 2020, and H7 LPAI was confirmed the same day for all three flocks. NVSL confirmed North American wild bird lineage H7N3 LPAI on all farms based upon partial Hemagglutinin/Neuraminidase (HA/NA) sequencing.

On 12 March, a North Carolina incident command team began surveillance for commercial flocks in control zones established around all three positive premises. Surveillance was immediately initiated for commercial poultry premises located within 10km zones around all infected farms and for epidemiologically linked premises. Between 12 March 2020 and 2 April 2020, a total of twelve commercial turkey flocks, two breeder

and ten meat-type flocks, were confirmed by NVSL as infected in Anson and Union counties in North Carolina and Chesterfield county South Carolina (Table 1). The owners of confirmed flocks reported no respiratory disease or increased mortality. The only clinical sign reported was a decrease in egg production in the breeder flock confirmed on 12 March.

Following initial detection, NCDA&CS and CULPH, with assistance from USDA-APHIS-VS, immediately began two rounds of active surveillance testing on commercial poultry premises located within 10km zones around all infected farms and for epidemiologically linked premises. In North Carolina, 1,645 Influenza-A tests were conducted on an estimated 18,095 birds tested as a part of the surveillance efforts. In South Carolina, 89 Influenza-A tests were conducted on an estimated 890 birds as a part of the surveillance efforts. A total of 190 facilities were tested in the 10km zones around the infected farms in North Carolina. A total of 16 facilities were tested in the 10km zones around the infected farms in South Carolina. The lead State agencies and Veterinary Services agreed that poultry on the infected premises would be depopulated in accordance with State Response and Containment Plans and USDA-APHIS-VS guidance documents. Depopulation was completed immediately on each farm with the final LPAI infected flock being completed by 2 April 2020. Testing in surveillance zones was completed by 15 April 2020.

Detection of Highly Pathogenic Avian Influenza in South Carolina

On 6 April 2020, CULPH was contacted after an increase in mortality and respiratory signs (snicking) was noted in one of five barns at a commercial meat-type turkey flock located in Chesterfield County, South Carolina. This flock had an epidemiologic link to another LPAI-infected flock in Chesterfield County but had tested negative in three previous rounds of testing, with most recent test negative samples collected on 31 March 2020. On 6 April 2020, the company flock supervisor collected and submitted samples to CULPH for PCR and ELISA testing. CULPH Diagnostic lab reported PCR presumptive positive results for H7 avian influenza for this flock on 7 April 2020. On 8 April 2020, the results were confirmed at the NVSL as H7N3 HPAI from the house that had turkeys showing clinical signs**.** On 9 April 2020, H7 LPAI was confirmed in 3 houses that had turkeys without clinical signs based on partial sequence results. The flock of 33,000 commercial meat-type turkeys was depopulated on 8 April 2020 by State personnel.

Following the confirmation of the H7N3 HPAI, four rounds of testing occurred on all commercial flocks located in a 10km radius control area around the infected premises. Ten of the thirteen confirmed LPAI/HPAI positive facilities were part of a single integrator. As a result, multiple rounds of targeted integrator-based network surveillance were performed on premises located outside of the control area. Testing was completed by 29 April 2020 with all results negative. Routine surveillance for NPIP avian influenza programs is ongoing statewide in both North Carolina and South Carolina.

Table 1. Location, production type, and confirmation date of flocks infected by HPAI/LPAI H7N3.

State	County	Production Type	Confirmation Date
North Carolina	Anson	Commercial Breeder Turkey	12 March 2020
North Carolina	Union	Commercial Meat Turkey	12 March 2020
North Carolina	Union	Commercial Meat Turkey	12 March 2020
North Carolina	Union	Commercial Meat Turkey	17 March 2020
North Carolina	Anson	Commercial Meat Turkey	17 March 2020
South Carolina	Chesterfield	Commercial Meat Turkey	17 March 2020
North Carolina	Union	Commercial Meat Turkey	17 March 2020
North Carolina	Union	Commercial Breeder Turkey	17 March 2020
North Carolina	Union	Commercial Meat Turkey	17 March 2020
North Carolina	Union	Commercial Meat Turkey	18 March 2020
North Carolina	Union	Commercial Meat Turkey	21 March 2020
North Carolina	Union	Commercial Meat Turkey	2 April 2020
South Carolina	Chesterfield	Commercial Meat Turkey	8 April 2020

Figure 1. Counties with confirmed findings of H7N3 LPAI or HPAI in March and April 2020.

EPIDEMIOLOGIC STUDY TO INVESTIGATE THE H7N3 LPAI AND HPAI VIRUSES IN COMMERCIAL POULTRY IN NORTH CAROLINA AND SOUTH CAROLINA

A. Case Control Study

In collaboration with the State Animal Health Officials, USDA-APHIS-VS conducted a case-control study of the H7N3 infected commercial turkey farms and some non-infected commercial turkey farms located in North Carolina and South Carolina. A questionnaire was administered to individual(s) on each farm most familiar with the farm's management and operations. Questions focused on the time period beginning three weeks before initial detection of avian influenza in the population up to the date the survey was administered. The purpose of the study was to generate hypotheses for potential risk factors for LPAI/HPAI infection based on a statistical comparison of farm characteristics and management practices of case (infected) farms and control (non-infected) farms.

Methods

An online questionnaire was developed using the ArcGIS Survey 123 platform. The questionnaire was adapted from similar documents previously developed for a case-control study conducted during the 2014-2015 HPAI outbreak in the Midwest, and from a case-series questionnaire developed specifically for turkey operations during the 2019 LPAI outbreak in Minnesota (Garber et al., 2016, USDA-APHIS, 2019). The questionnaire was adapted to meet the requirements of the online survey platform. Transmission of virus in previous outbreaks of avian influenza in the United States has been attributed to the movement of live birds, transportation of manure, equipment sharing, and contaminated feed trucks, vehicles, water, and people (McQuiston et al., 2005, Halvorson, 2009, Garber et al., 2016). The online questionnaire focused on these categories of risk factors and consisted of approximately 100 questions (yes/no or multiple-choice answer format).

Participating turkey operations were recruited with the help of NCDA&CS and CULPH. Five commercial turkey companies operating in North Carolina and South Carolina were invited to participate, including the three that were affected by the outbreak. Due to challenges caused by the COVID-19 pandemic, the two unaffected companies were unable to participate. The three remaining companies provided contact information for 45 operations, including the 13 LPAI/HPAI infected premises. Invitation letters were sent to the potential participants, which included: a description of the study, the questionnaire URL, and a unique PIN to access the online survey.

Odds ratios, and p-values for each question were estimated by univariate logistic regression. To identify significant risk factors while controlling for possible confounding variables, a multivariable logistic regression analysis was performed using all variables that were statistically significant (p-values <0.1) in the univariate analysis. Backward, stepwise elimination was used to obtain the final model. The number (n) of cases and controls that responded to questions, odds ratios, and p-values for variables that were significant at the p<0.1 level are provided in

.

Results

A total of 36 participants (10 cases and 26 controls) completed the questionnaire. All completed questionnaires were submitted by operations belonging to one integrator company.

Premises characteristics

All farms that responded were single-age, meat-turkey grower operations. Most farms (n=34, including all case farms) reported raising toms, while only 2 reported raising hens. Risk factors that were statistically significant at the $p<0.1$ level are summarized below and in

. Additional tabulation of the variables that had p-values>0.1 are provided in ***Error! Reference source not found.*** in Appendix A.

Risk Factors – Univariate Analysis

- Size

  Infected (case) farms were larger than non-infected (control) farms. Although the odds of being infected was only slightly higher for operations with more birds, the odds ratio was statistically significant (OR=1.0001, p=0.038). Likewise, the odds of being infected was greater for farms with a higher number of poultry houses on site (OR=2.4, p=0.015).

- Waterfowl presence

  The odds of being infected were higher for farms which reported having seen waterfowl on the farm (OR=10.7, p=0.025) and for those that had a pond on their property (OR 4.2, p=0.065).

- Potential for workers to act as fomites

  The odds of being infected were also greater for farms that reported that workers mingled in a common gathering place on farm (OR = 6.8, p = 0.036) and that workers mingled off farm with workers from other poultry premises (OR = 10.7, p = 0.054).

- Disposal of daily mortality

  The odds of being infected were much greater for farms which reported on-farm disposal by incinerator (OR = 8, p = 0.019). Other methods of daily mortality disposal (burial, composting, rendering, landfill) were not statistically significant. The odds of being infected were also greater for farms that reported workers performed off-farm disposal versus the growers (OR=6.3, p=0.017).

- Cleanout

  The odds of being infected were greater when cleanout was performed by the grower versus a contractor (OR=4.1, p=0.64).

Table 2. Significant[1] results from univariate logistic regression by characteristics of H7N3 LPAI/HPAI infected premises and control premises.

Characteristic	Level	Case Farms	Control Farms	Odds Ratio	p-value
Number of birds in 1000's Median (range)		35.9 (20.7-75.2)	26.8 (14.0-50.0)	1.0	0.038
Number of houses Median (range)		5.5 (4-10)	4 (2-8)	2.4	0.015
Pond on the property	Yes	5/10	5/26	4.2	0.065
Workers gather/mingle in centralized location on farm	Yes	8/10	10/26	6.8	0.036
Workers ever interact with workers from other poultry premises	Yes	3/10	1/26	10.7	0.054
Grower does cleanout vs Contractor	Grower	6/10	7/26	4.1	0.064
On farm disposal by incinerator	Yes	4/10	2/26	8.0	0.019
Worker does off farm disposal vs Grower	Worker	6/10	5/26	6.3	0.017
Waterfowl seen on farm	Yes	3/10	1/26	10.7	0.054

[1] Univariate results should be interpreted with caution. For a tabulation of variables with p>0.1 see Appendix A, Table 9.

Risk Factors - Multivariate analysis

Some of the risk factors described above are related, which can made univariate odds ratios misleading. A multivariate analysis was performed in order to provide adjusted odds ratios for risk factors, while considering interrelationships among these farm characteristics. Adjusting for all of the significant factors identified in the univariate analysis, a higher number of poultry houses and having workers gather and mingle in a common area remained significant risk factors for infection (

).

Table 3. Significant results from multivariate analysis including adjusted odds ratios (OR) for significant risk factors.

Characteristic	Level	Case Farms	Control Farms	Adj. OR	P-value
Number of houses Median (range)		5.5 (4-10)	4 (2-8)	3.1	0.011
Workers gather/mingle in centralized location on farm	Yes	8/10	10/26	15.6	0.04

Discussion

These results suggest that some factors and management practices were shared across infected farms; however, the sample size of this study was small, reducing the statistical power of the analysis and making interpretation of the significance of many variables difficult. There was also a potential for selection bias, as all case premises that participated in this study belonged to one integrator and all controls were a subset of the remaining premises belonging to the same integrator. Therefore, results may not be representative of the entire population of domestic turkey or other poultry production in North and South Carolina. The multivariate analysis indicated that variables representing the size of the operation and comingling of employees were significantly associated with infection which suggests that biosecurity may have played a role in the spread of the virus, although NPIP recommended biosecurity measures were in place. When considered in conjunction with additional analyses described in other sections of this report, this information may provide insights into management practices in this study population and illuminate opportunities to implement additional mitigations to reduce the risk of avian influenza infection in the future.

B. Contact network among case premises

Methods and Results

In order to quickly collect relevant data to assist with contact tracing, owners of the infected farms were asked to also complete detailed paper questionnaires that included open ended questions about facility

characteristics and biosecurity practices, as well as services and personnel who visited the premises in the 3 weeks prior to detection of LPAI/HPAI. Twelve of 13 case farms completed the questionnaire. Information collected from those questionnaires and follow-up interviews were used to identify a network of common connections between case premises (

Figure 2). Eight categories of connections were identified in the data. The most common connection was feed source followed by litter source, renderer, garbage hauler, flock supervisor, propane delivery, and manure hauler. Two premises were managed by the same grower. Although seven premises identified a common renderer, several of those premises indicated that no renderer pickups occurred during the three weeks prior to detection of virus because daily mortalities are disposed of on farm until the birds reach 12 weeks of age.

As indicated in **Error! Reference source not found.**, there is an extensive network of connections among the infected premises, which is not surprising given their company relationships and geographic proximity. Insufficient information was provided about network relationships to provide strong evidence of a particular mechanism of spread or the temporal progression of infection.

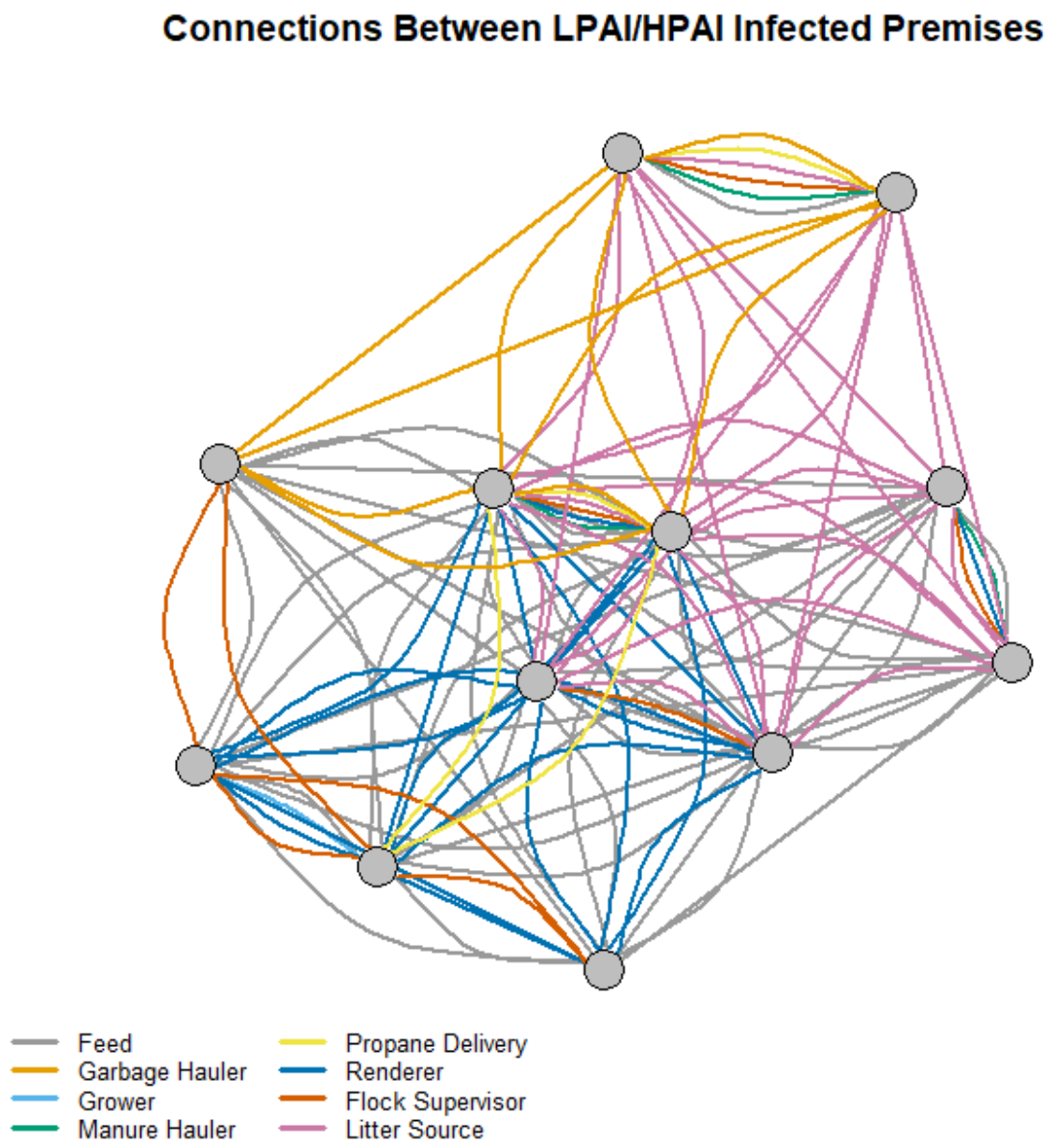

Figure 2. Categories of connections between LPAI/HPAI infected premises (grey dots) in North Carolina and South Carolina that were identified in epidemiologic surveys and/or interviews with premises owners[2].

[2] Connections were included in the figure if the data indicated common business or personnel relationships; however not all types of contacts identified in the figure occurred during the risk window for LPAI/HPAI infection (e.g., renderer pickup). The spatial arrangement of the premises in the figure does not represent true geographic relationships between premises.

ESTIMATING THE TIME OF H7N3 LPAI/HPAI INTRODUCTION INTO COMMERCIAL TURKEY FLOCKS USING DIAGNOSTIC TEST RESULTS AND PRODUCTION DATA

A. Summary

Determining the time of LPAI or HPAI virus introduction in a flock is an important part of outbreak investigations. By narrowing the time window of possible virus introduction, we can better identify the potential routes of virus introduction and enhance our understanding of the pattern of disease spread. In this analysis, diagnostic testing, egg production data or daily mortality data (where applicable) was used to estimate the time of introduction in five premises in North Carolina and South Carolina: two LPAI infected turkey breeder flocks, two LPAI infected meat turkey premises, and one HPAI/LPAI infected meat turkey premises.

The analysis was performed using a simulation-based method in which the likelihood of observing the data was estimated from a within-house disease transmission model for various candidate times of exposure. Two Bayesian estimation approaches were used depending on the types of premises data available. For premises that had a drop in egg production or daily mortality above 3 birds per 1,000 on multiple days within two weeks of the time of detection, a within-house disease transmission model along with approximate Bayesian computation (ABC) was used. For premises that did not have a drop in egg production or mortality above 3 birds per 1,000, only diagnostic data were used for estimation. The limited number of diagnostic tests made it difficult to estimate the contact rate for these latter premises, which is the parameter that determines the rate of disease spread. Therefore, the analysis was performed for slow and fast contact rate scenarios. In general, slower rates of disease spread are associated with earlier virus introduction times. As such, it is important to consider slow rates of spread specifically in order to get an indication of how long the virus could have been circulating in a house prior to detection.

Dates of introduction were estimated for Anson 1, Union 6, Union 7, Union 8, and Chesterfield 2 (**Error! Reference source not found.**). Union 7 had the earliest most likely date of introduction among the premises evaluated (6 February 2020; 95% C.I., 29 January 2020-8 March 2020 under the slow spread scenario). Anson 1 was estimated to have the subsequent most likely date of introduction (26 February 2020; 95% C.I., 23 February-28 February). Union 6 and Union 8 had similar estimates for the most likely date of introduction (4 March 2020 under the slow spread scenario and 8 or 9 March 2020 under the fast spread scenario). Chesterfield 2 Barn 2 had an estimated LPAI introduction date of 23 March 2020 (95% C.I., 9 March-29 March) under the slow spread scenario. Barn 3 of Chesterfield 2 had an estimated virus introduction date of 28 March 2020 (95% C.I., 24 March-29 March).

Overall, the time of virus introduction estimates help inform epidemiological investigations together with other epidemiological and phylogenetic analyses. For example, the results were used to evaluate whether it was conceivable for LPAI to have been introduced to Chesterfield 2 from other infected premises in mid-March and have a sequence of negative test results prior to detection on April 6th.

Table 4.The most likely time of virus introduction with 95% CI and, when applicable, the most likely contact rate[3] with the 95% CI for barns infected during the 2020 LPAI/HPAI H7N3 outbreak.

Premises/Barn	Data Sources	Estimated time of virus introduction (95% CI)	Contact Rate; (95% CI)
Anson 1/ Barn2	Diagnostic test results, egg production, daily mortality	2/26 (2/23-2/28)	4.6 (1.6-5.9)
Union 6/ Barn 2	Diagnostic test results	Slow spread: 3/4 (2/28-3/7) Fast spread: 3/8 (3/2-3/10)	NA
Union 7/ Positive Barn	Diagnostic test results	Slow spread: 2/6 (1/29-3/8) Fast spread: 2/13 (2/4-3/8)	NA
Union 8/ Barn 1	Diagnostic test results	Slow spread: 3/4 (2/10-3/10) Fast spread: 3/9 (2/17-3/13)	NA
Chesterfield 2/ Barn 2 (LPAI)	Diagnostic test results	Slow spread: 3/23 (3/9-3/29) Fast spread: 3/28 (3/14-3/31)	NA
Chesterfield 2/ Barn 3 (HPAI)	Diagnostic test results, daily mortality	3/28 (3/24-3/29)	2.8 (1.7-3.9)

B. Methods

The data used in the analysis included the sequence of available rRT-PCR test results, egg production data or daily mortality data. The analysis was based on rRT-PCR test results alone for those barns where the mortality levels within 3 weeks prior to detection did not exceed a threshold of 3 per 1,000 birds on multiple days. The 3 per 1,000 bird threshold was used as a criterion for normal daily mortality in the Secure Turkey Supply plan (University of Minnesota, Secure Food Systems Team, USDA-APHIS-VS-Center for Epidemiology and Animal Health, 2018). This threshold was developed based on an analysis of mortality data from 116 turkey tom houses and 48 turkey hen houses, which indicated that this mortality is exceeded on less than 2% of days, resulting in a low frequency of false-positive triggers.

[3] For barns where the contact rate could not be estimated, a slow rate of spread scenario and a fast rate of spread scenario were used to estimate the dates of introduction.

For premises that had daily mortality that exceeded 3 per 1,000 birds on 2 or more days within 3 weeks of detecting LPAI on the premises, mortality data and diagnostic data were both used to estimate the dates of introduction. LPAI viruses cause milder disease relative to HPAI and most infected premises in this outbreak reported that no clinical signs were observed; however, LPAI viruses can contribute to clinical signs and mortality due to concurrent bacterial infections or environmental stress (Beltrán-Alcrudo et al., 2009, Halvorson et al., 2003, Mutinelli et al., 2003). In an experimental inoculation study of 12 North American H7 LPAI strains, 3 strains did not cause any disease mortality while 10-60 percent of the inoculated birds died when infected with the other 9 LPAI strains (Spackman et al., 2010). In contrast, there was no mortality among experimentally inoculated turkeys with the 2016 LPAI H7N8 strain (Pantin-Jackwood et al., 2017, Garber et al., 2019). Some of the secondary infections that can cause disease mortality together with LPAI include *E*. *coli*, *P*. m*ulticoda, Alcaligenes faecalis*, *Aspergillus fumigatus*, *Riemerella anatipestifer, Ornithobacterium rhinotracheale*, *Staphylococcus aureus* and *Mycoplasma gallisepticum* (Umar et al., 2018, Umar et al., 2017). An experimental study of LPAI H6N1 and *E. coli* showed marked synergistic or additive effects, where the mortality and clinical score with coinfection was much higher than due to either disease alone. Under field conditions, the mortality in LPAI infected flocks may vary significantly due to factors such as age, co-infection, or environmental factors such as ventilation and litter condition (Halvorson et al., 2003).

C. Results

The mortality patterns for the flocks involved in the current outbreak were quite variable (**Error! Reference source not found.** and **Error! Reference source not found.**). There were mortality patterns observed above 3 per 1,000 birds where LPAI was discounted as a contributing factor. Several premises had mortality above that threshold as early as January 2020 (Union 4, Union 7, Union 2, Union 8). Based on previous epidemiological analyses for a LPAI H5N2 outbreak and other LPAI studies in the literature, it is unlikely that a flock would still be shedding at the time of detection in March if LPAI infection was contributing to mortality as early as January (USDA-APHIS, 2019). Therefore, mortality data above the 3 per 1,000 threshold that occurred in December and January were not used in the analysis. Similarly, in some cases, mortality data was excluded because some barns with mortality above 3 per 1,000 on multiple days within 3 weeks of detection also tested negative close to the time of detection (e.g., barns on Union 3, Union 7). For example, the peak mortality in barn 3 on Union 3 occurred one day prior to a negative rRT-PCR test result. This result would be unlikely if the mortality was due to LPAI since high mortality would be expected to be associated with high prevalence in the flock. High prevalence in turn would mean a high likelihood of detection. Likewise, 3 out of 4 barns in Union 7 tested negative on March 13, 14 and 17. Three barns in Union 7 had peak mortality on March 7, 9 and 10 with mortality above 3 per 1,000 birds. Overall, it is unlikely for these barns to have tested negative if the mortality was due to LPAI.

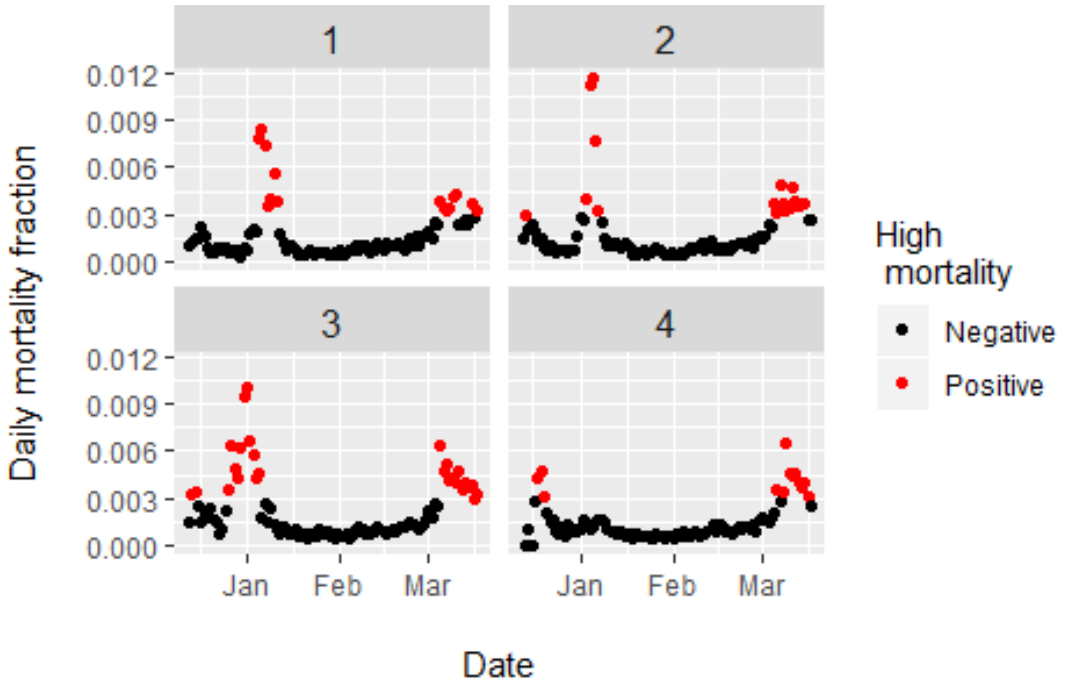

Figure 3. Variation in daily mortality fraction among 4 barns of Union 7. The data points with mortality above 3 per 1000 birds are marked in red.

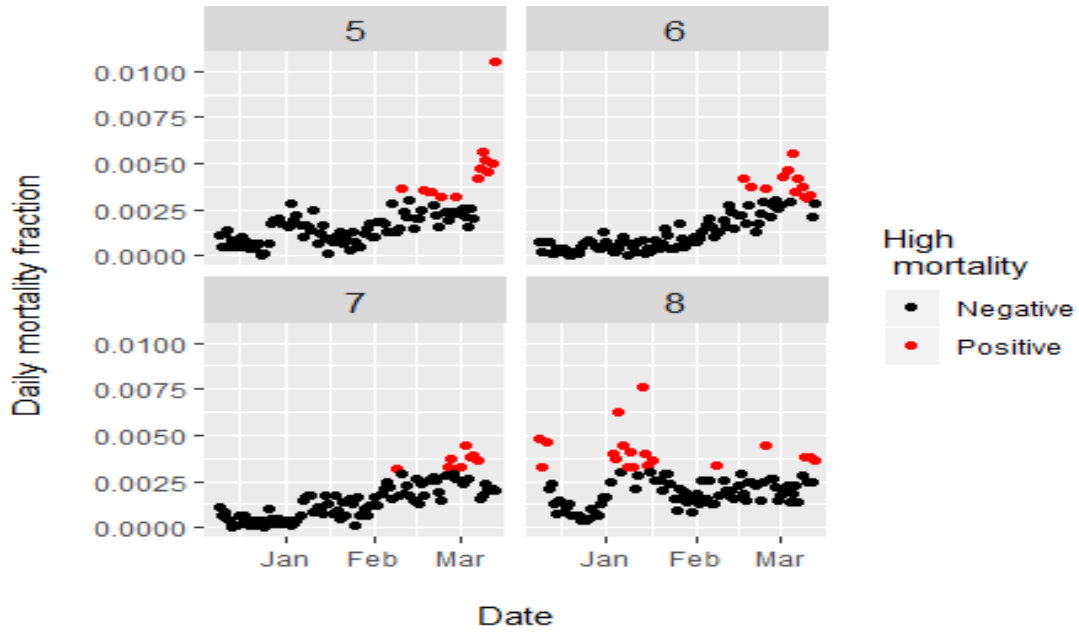

Figure 4.Variation in daily mortality fraction among 4 barns of Union 2. The data points with mortality above 3 per 1000 birds are marked in red.

Ultimately, we included mortality data in the main analysis for two premises, Anson 1-barn 2 and Chesterfield 2-barn 3. Anson 1-barn 2 had a pattern of mortality above 3 per 1,000 with a simultaneous severe drop in egg production and with a low rRT-PCR CT (~22) value for samples collected on 9 March 2020. Chesterfield 2-barn 3 was a HPAI barn with marked, exponentially increasing disease mortality.

Estimating the time of LPAI introduction for Anson 1

On this turkey breeder premises, only barn 2 tested positive via 2 pooled samples tested by rRT-PCR with samples collected on 9 March and 12 March 2020. There was a severe drop in egg production and increased mortality in this barn. The model fits for the increased mortality and drop in egg production are shown in **Error! Reference source not found.** and **Error! Reference source not found.**. The model fit indicated a significant delay of 6.53 days (95% C.I., 4.96-6.96 days) between when birds were infected and when the drop in egg production occurred. Other studies in the literature also indicated a similar interval from the LPAI infection of turkeys to when drops in egg production occurred (Pillai et al., 2010, Samadieh et al., 1970).

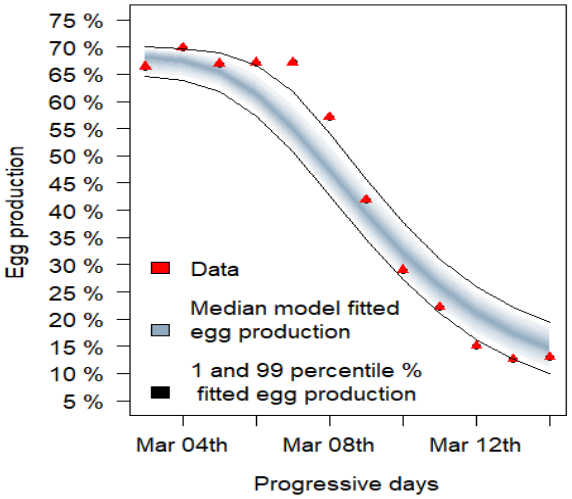

Figure 5. **Observed egg production and model fitted egg production for Barn 2, Anson 1**

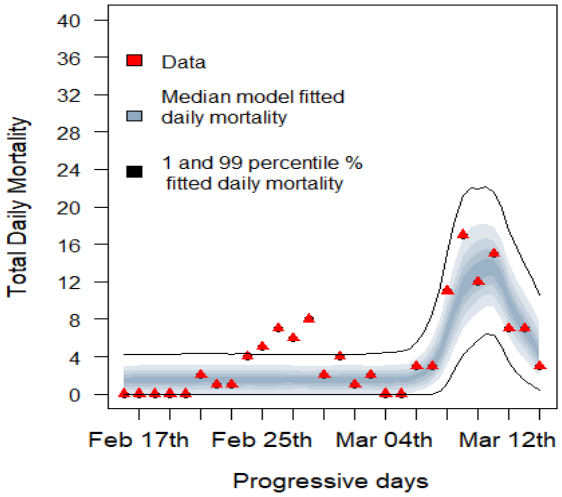

Figure 6.Observed daily mortality and model fitted daily mortality for Barn 2, Anson 1.

Estimating the time of LPAI introduction for Union 6, Union 7, and Union 8

Union 6 was a meat type turkey premises with three barns. Barn 2 on Union 6 first tested positive by rRT-PCR performed on a pooled sample of 11 swabs taken on 15 March 2020. A pooled sample taken 17 March from barn 2 also tested positive. The remaining test samples (a 13 March sample from barn 2, 13 March and 15 March samples from barn 1, and 14 March and 15 March samples from barn 3) all tested negative. Due to low daily mortality in barn 2, only testing data was considered in the estimation of the time of virus introduction. Furthermore, in order to reduce uncertainty in the estimates for barn 2, the test results from all barns on the premises were included in the estimation procedure (**Error! Reference source not found.**).

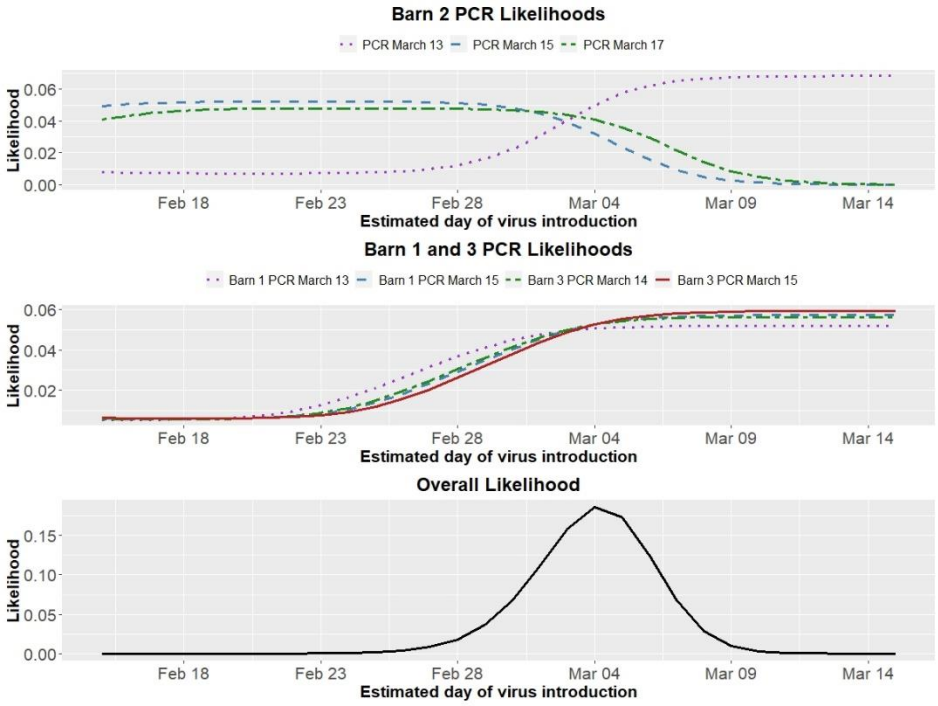

Figure 7. The likelihood of observing individual and combined test results assuming different times of LPAI virus introduction for Union 6 under the slow rate of spread scenario.

Union 7 was a meat turkey premises with five barns. The barns were tested by rRT-PCR on 13 March with one pooled sample of 11 swabs per barn, 14 March with two pooled samples of 10 swabs per barn, and on 17 March also with two pooled samples of 10 swabs per barn. One of the samples from an unspecified barn tested positive on 14 March, all other samples tested negative. All barns were assumed in the analysis to have been equally likely to have been the barn with the positive test result. Daily mortality was low in the barns on this premises, leading to only the diagnostic test results being used to estimate the time of virus introduction (**Error! Reference source not found.**.

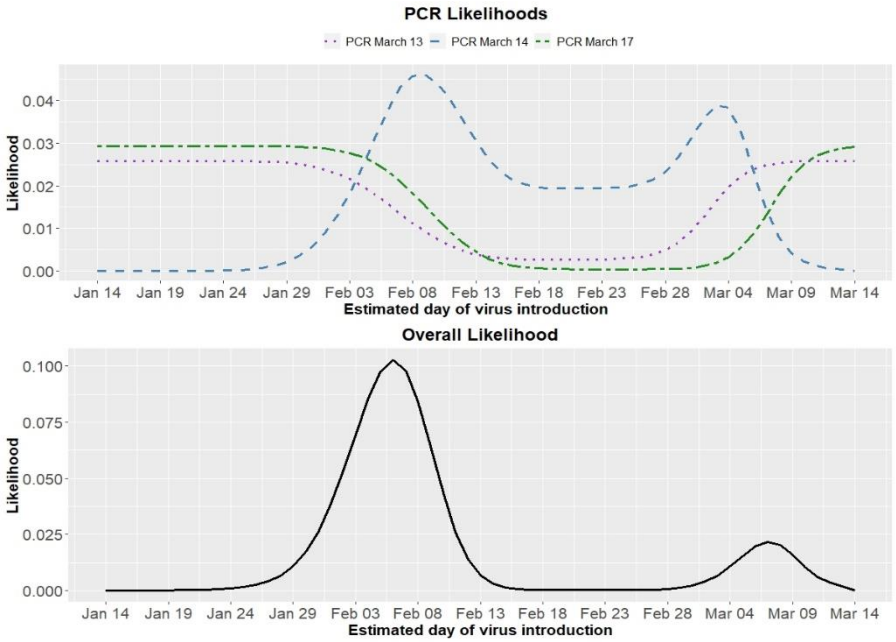

Figure 8. **The likelihood of observing individual and combined test results assuming different times of LPAI virus introduction for Union 7.**

The six barns on Union 8, also a meat type turkey premises, all tested negative on 13 March and then all tested positive on 19 March by rRT-PCR performed on a pooled sample of 11 swabs. The barns on Union 8 had low daily mortality during the observation period, leading to only testing data being used in the analysis. As all barns on Union 8 had the same test results and similar flock sizes, the estimated time of virus introduction would be expected to be nearly identical for each barn (**Error! Reference source not found.**). There was substantial uncertainty in the estimates for barn 1, Union 8 due to the limited amount of information present in the data for this premises.

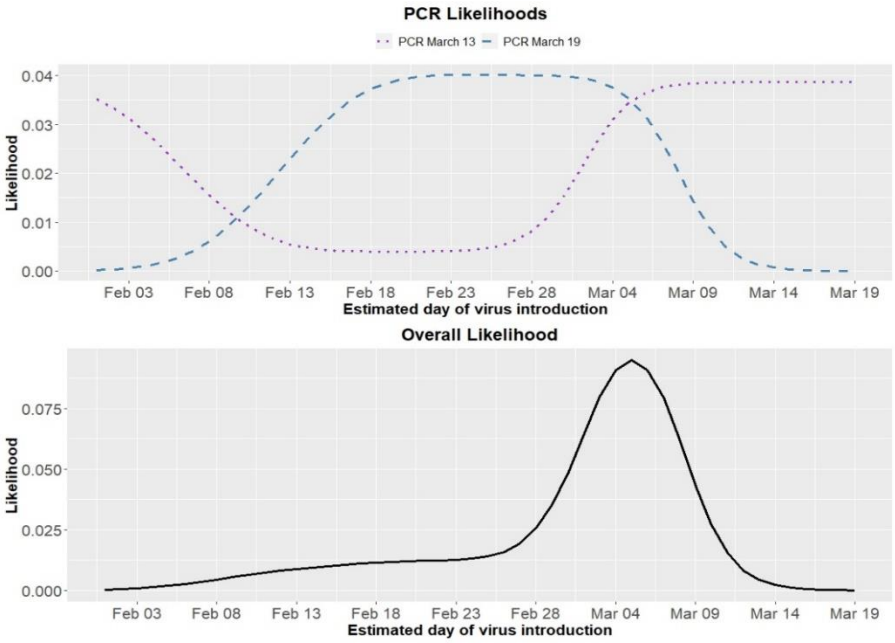

Figure 9. **The likelihood of observing individual and combined test results assuming different times of LPAI virus introduction for barn 1, Union 8**

Estimating the time of HPAI and LPAI introduction for Chesterfield 2

Each of the five barns on Chesterfield 2 were negative on testing by pooled rRT-PCR on 15 March, 20 March, and 1 April before testing positive on 6 April. Only in barn 3 was heightened mortality observed (**Error! Reference source not found.**). In this analysis, the most likely dates of introduction were estimated for HPAI in barn 3 and LPAI in barn 2. Barns 1, 4, and 5 would be expected to have nearly identical results to barn 2 given similarities in mortality and test results. Due to the low levels of mortality in barn 2, only testing data was used to estimate the time of virus introduction in this barn.

Under the slow spread scenario, LPAI was introduced into barn 2 as late as 23 March 2020 and possibly as early as 9 March 2020 (**Error! Reference source not found.**). The range of dates of introduction for barn 2 overlap with the detection dates of earlier LPAI infected farms in the current outbreak suggesting that lateral spread from one of those premises to Chesterfield 2 was possible.

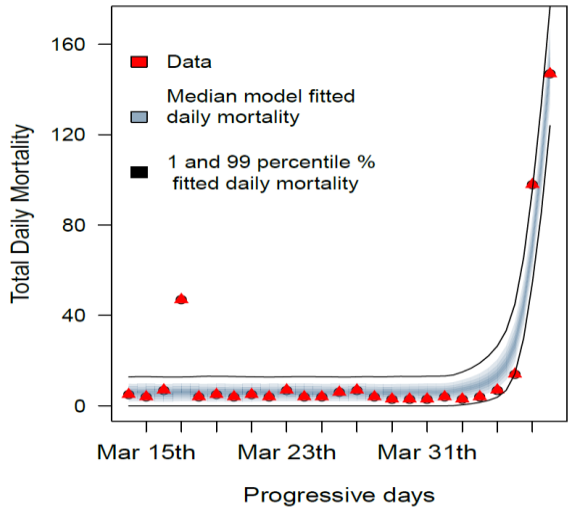

Figure 10. **Observed daily mortality and model fitted daily mortality for barn 3, Chesterfield 2.**

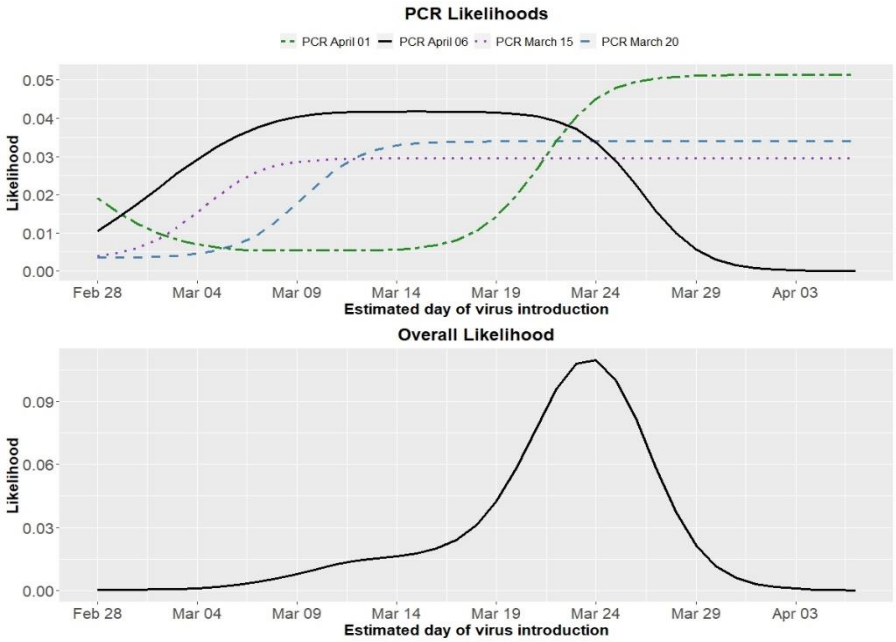

Figure 11.**The likelihood of observing individual and combined test results assuming different times of LPAI virus introduction for barn 2, Chesterfield 2 under the slow rate of spread scenario.**

Estimating the time of LPAI introduction for Union 2 and Union 3

The results for Union 2 and Union 3 are presented here for exploratory purposes but were not included with the other premises in **Error! Reference source not found.** due to a higher degree of uncertainty in the estimates (**Error! Reference source not found.**). Mortality exceeding the 3 per 1,000 bird threshold was observed in barns on both of these premises. However, it cannot be ruled out that this mortality was due primarily to another pathogen, in which case it would not be representative of the transmission of LPAI but rather the other pathogen.

Table 5. The most likely time of virus introduction with the 95% C.I. and the most likely contact rate with the 95% C.I. for barns infected on Union 2 and Union 3 during the 2020 LPAI/HPAI H7N3 outbreak.[4]

Premises	Barn	Data sources	Estimated Day of Virus Introduction (95% C.I.)	Contact rate (95% C.I.)
Union 2	Barn 6	Diagnostic test results daily mortality	21 January 2020 (15 Jan-1 Feb)	0.35 (0.27-0.46)
	Barn 5	Diagnostic test results daily mortality	20 February 2020 (13 Feb-23 Feb)	1.04 (0.63-1.94)
Union 3	Barn 4	Diagnostic test results daily mortality	12 February 2020 (7 Feb-16 Feb)	0.7 (0.5-2.76)
	Barn 3	Diagnostic test results daily mortality	21 February 2020 (19 Feb-25 Feb)	3.6 (1.1-6.2)

Union 2 was a 10-barn commercial LPAI infected meat turkey premises. The premises was sampled on 10 March (5 houses) and 12 March 2020 (9 houses) and all the samples were positive by IAV Matrix gene rRT-PCR. For Union 2, data to link test results to individual barns were not available. We considered partial information from the test results from samples collected on 12 March by calculating the likelihood of a barn being sampled (9/10 barns were sampled). The magnitude of daily mortality exceeding the 3 bird per 1,000 threshold was quite variable in this premises with some barns showing a rapid increase in mortality (**Error! Reference source not found.**), while other barns had a more gradual increase (**Error! Reference source not found.**).

[4] **.** Note that the results should be viewed cautiously due to uncertainty that the daily mortality exceeding 3 birds per 1,000 in these barns was due to LPAI.

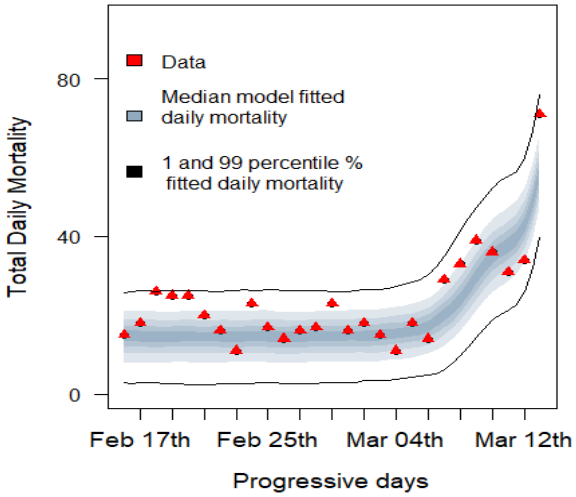

Figure 12. Observed daily mortality and model fitted daily mortality for Union 2, barn 5.

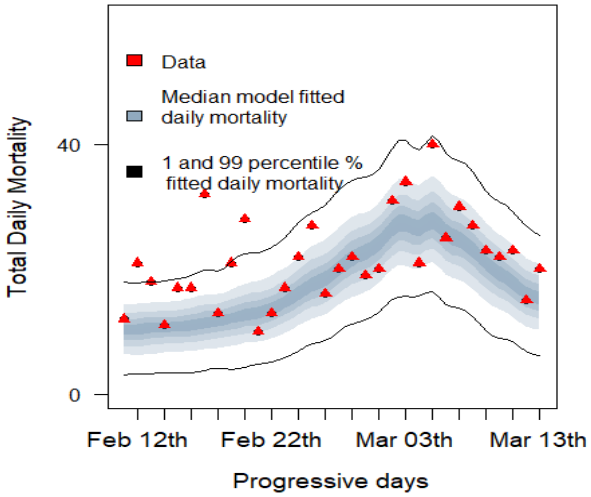

Figure 13. Observed daily mortality and model fitted daily mortality for Union 2, barn 6.

Union 3 was a meat turkey premises with birds in two out of six barns at the time of detection. Barn 4 tested positive via samples taken on 13 March while barn 3 tested negative. The daily mortality observed in the barns with a model fit is given in **Error! Reference source not found.** and **Error! Reference source not found.**. Both barns experienced elevated mortality; however, the peak mortality in barn 3, which exceeded the 3 bird per 1,000 threshold, occurred one day prior to the negative test result on 13 March (**Error! Reference source not found.** and **Error! Reference source not found.**). This result would be unlikely if the mortality was due to LPAI. Furthermore, the CT value of the positive rRT-PCR result in barn 4 was quite high. In the 2018 LPAI H5N2 outbreak in Minnesota CT values close to 40 were generally observed when the flock was close to recovered (USDA-APHIS, 2019). Based on this observation, introduction of the virus into barn 4 could have occurred

much earlier than the heightened mortality, in which case the mortality exceeding the threshold could be due to a pathogen other than LPAI.

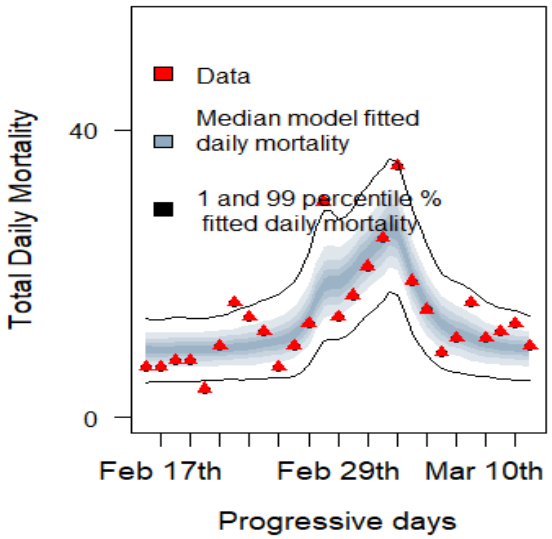

Figure 14. Observed daily mortality and model fitted daily mortality for Union 3, barn 4.

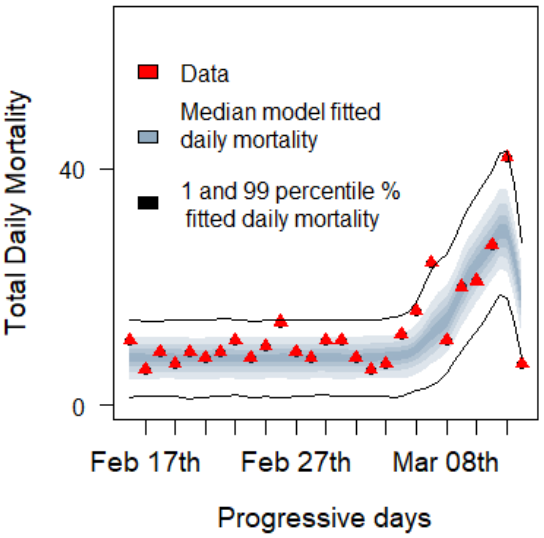

Figure 15. Observed daily mortality and model fitted daily mortality for Union 3, barn 3.

Data Limitations/Sources of Uncertainty

There were several data limitations contributing to uncertainty in the analysis. First, information linking the rRT-PCR pooled samples to individual barns were not available for all the premises. While the premises-level status is most important during an outbreak, the barn-level test results can be very helpful in improving understanding of the outbreak progression. In addition, serology tests were not performed in the current

outbreak on infected premises. Serology tests performed after a rRT-PCR positive result can provide information on the recovery status of the flock and therefore on how long the infection has been circulating, reducing uncertainty in epidemiological investigations.

There is considerable uncertainty in the transmission model parameters for the current LPAI H7N3 outbreak strain. Transmission model inputs, such as the length of the latent and infectious period, while estimated from data on other North American H7 LPAI virus strains, may not capture the behavior of the H7N3 strain involved in the current outbreak. Data from transmission experiments performed with the current outbreak strain, such as those being done at the USDA ARS Southeast Poultry Research Laboratory (SEPRL), can help inform these parameters.

Finally, in some premises (e.g., Union 2 and Union 3), there was uncertainty in the nature of the daily mortality exceeding the threshold. For example, a coinfection could have occurred when a flock was close to recovering from LPAI. In this scenario, mortality patterns that look characteristic of LPAI could be indicative of another bird health issue. Information regarding disease investigations for other concurrent pathogens may help reduce this uncertainty in some cases.

Discussion

Estimating the time of virus introduction into barns provides a valuable piece of information for epidemiologic investigations and outbreak response. The estimated parameters can improve understanding of disease spread patterns together with other epidemiological and phylogenetic analyses. For example, the results from this analysis were used to evaluate whether it was conceivable for LPAIV to have been introduced onto the HPAI premises from other infected premises in mid-March. The overlap in the estimated dates of LPAIV introduction onto the HPAI premises with the interval where other flocks are likely infectious supports the possibility of lateral spread among infected premises despite biosecurity measures. Of note, the NPIP program requires all commercial flocks to be tested prior to slaughter. Although the virus may have been circulating in these flocks for a time prior to detection, no infected birds were sent to slaughter.

Some of the premises had wide intervals for the LPAIV introduction date given the minimal disease mortality and rRT-PCR test results available. Additional diagnostic testing performed after an rRT-PCR positive result (e.g., serology tests) can provide information on the recovery status of the flock, reducing uncertainty in epidemiological investigations.

This work is dependent on the quality of production records and access to laboratory diagnostic results. Adding serologic testing to PCR testing can provide additional information to improve the precision of time of introduction estimates. Quickly sharing this information early in an outbreak can help target response resources for traceability and improve our understanding of how avian influenza viruses may spread between flocks. This work also highlights the value of closely monitoring egg production, mortality, and/or feed and water consumption to quickly identify disease issues in the flock. These factors may vary across flocks and between barns, so understanding the trends within each production setting is important. The presence of other illness in the flock can make it difficult to determine whether the observed mortality is related to LPAI.

PHYLOGENETIC ANALYSIS AND DIAGNOSTICS

A. North American H7N3 HPAI and LPAI from poultry (AM H7N3 2020)

This section of the report describes AM H7N3 HPAI and LPAI from poultry confirmed by the National Veterinary Services Laboratories (NVSL) during March and April 2020. AM H7N3 LPAI was confirmed in two commercial meat-type turkey operations in Union County, North Carolina and one commercial breeder turkey operation in Anson County, North Carolina. Samples were collected from the breeder turkey operation on 09 March 2020 (Anson County) due to a drop in egg production. Samples were collected on 10 March 2020 from the two meat turkey operations (Union County) for routine pre-slaughter surveillance. Enhanced surveillance identified ten additional H7N3 LPAI-infected premises in North Carolina and one premises in South Carolina, followed by confirmation of H7N3 HPAI in a single barn of a second premises in South Carolina on 08 April 2020 (the LPAI virus was detected in all other houses). Intravenous pathogenicity index (IVPI) was 0.0 for LPAI virus isolates (n = 5), and 2.47 for the HPAI virus.

Phylogenetic analysis of viruses from these events support a single introduction of North American wild bird lineage H7N3 LPAI into turkey farms in North Carolina followed by lateral spread to other farms, with a single mutation event resulting in HPAI in a single barn on a commercial turkey operation in South Carolina (Youk et al., 2020). Analysis suggests the precursor virus likely emerged in wild waterfowl from the Mississippi flyway.

The insertion at the cleavage site responsible for the mutation to HPAI likely originated from host 28S rRNA; this 27-nucleotide sequence is highly conserved across many avian and mammalian species. An identical insertion occurred during the 2017 AM H7N9 LPAI, HPAI event, demonstrating a repetitive pathway by which H7s may acquire insertion at the cleavage site to mutate to HPAI.

Detailed information on the evolution and clustering of the H7N3 viruses involved in this outbreak is available online[5].

NOTE: The outcomes of phylogenetic analysis should be interpreted in context of all available virus and epidemiologic information and should not be used directly to infer transmission.

[5] Youk S, Lee D, Killian ML, et al. Highly Pathogenic Avian Influenza A(H7N3) Virus in Poultry, United States, 2020. *Emerging Infectious Diseases*. 2020;26(12):2966-2969. doi:10.3201/eid2612.202790.

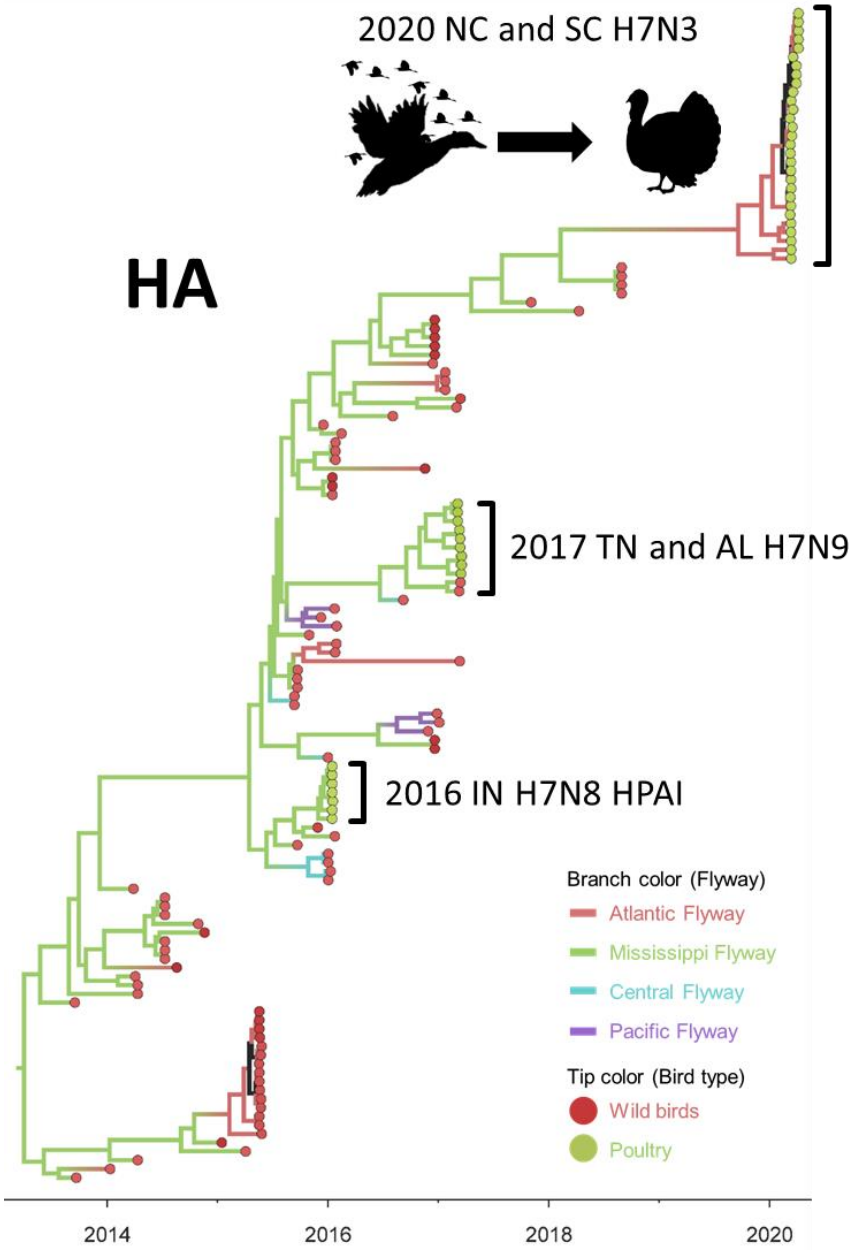

Figure 16. Phylogenetic analysis using a maximum-likelihood tree data suggests that the LPAI introduction was of wild bird origin, and that the H7N3 is distinct from other recent H7 viruses from poultry, including 2016 IN H7N8, 2017 TN H7N9, and Mexico H7N3 HPAI.[6]

Comparison to Other Viruses/Lineages

The 2020 H7N3 virus is distinguishable from other recent H7 poultry detections (2016 H7N8, 2017 H7N9, 2018 H7N1, and 2018-2019 H7N3) across the entire genome (**Error! Reference source not found.**). 2020 H7N3 was also genetically distinct from H7N3 HPAI viruses present in Mexico since 2012, as well as the Anhui lineage

[6] **Slide courtesy of ML Killian NVSL and colleagues at USDA ARS Southeast Poultry Research Laboratory.**

H7N9 LPAI/HPAI originating in China which has infected poultry since 2013 with transmission to humans. Although no recent single wild bird origin precursor with all 8 gene segments was identified, highly similar progenitor genes were identified from wild bird surveillance efforts, particularly from the Mississippi flyway. Analysis also suggests that this H7 HA clade may represent a repetitive threat to poultry.

B. Public Health Aspects

There were no reports of the 2020 H7N3 virus infection in humans. State-level efforts to monitor the health of response workers and on-farm personnel were conducted.

The virus sequences have been shared with CDC for analysis which indicated that the viruses lack key amino acid substitutions associated with human-like receptor binding or substitutions in the polymerase or other internal genes associated with increased virulence and transmission in mammals; no known markers of neuraminidase inhibitor (Oseltamivir) resistance have been identified.

C. Diagnostics and Characterization for Influenza A Viruses

The NVSL rapidly shares genetic and biological materials in collaboration with the Southeast Poultry Research Laboratory, the Influenza Division of the Centers for Disease Control and Prevention, Wildlife Services, as well as other key partners. Consensus data from whole genome sequencing are used to monitor virus evolution and assess the risk to veterinary and public health based upon the presence/absence of specific amino acid substitutions or protein motifs. Analysis of sequence data includes phylogeny of all eight segments and determination of amino acid substitutions across the HA1 protein. Genetic data are also used to confirm that diagnostic assays are fit for purpose. *In silico* analysis confirmed high identity between the H7N3 virus sequences and the primers and probes used for the IAV and H7 diagnostic rRT-PCR tests.

General Information

Avian influenza subtypes H5 and H7 are reportable worldwide because of their potential for mutation to high pathogenicity during replication in poultry. The presence of basic amino acids at the cleavage site contribute to the mutation from low to high pathogenicity. Mechanisms by which H5/H7 mutate from LPAI to HPAI include the gradual accumulation of basic amino acids (AA), insertion of repeated basic AA, and insertion of non-homologous genetic material (only reported for H7 viruses).

Molecular diagnostic tests for influenza A virus (IAV) are used across the U.S. National Animal Health Laboratory Network (NAHLN). The most sensitive and specific tool for influenza A detection is the Type A-specific rRT-PCR, which targets at least the matrix gene (IAV-M); this is the primary surveillance tool used and provides a semi-quantitative result. The NAHLN tests samples first by the IAV-M test and further by the NAHLN H5 and H7 tests where IAV is detected.

All poultry samples with a non-negative test result for IAV (serology or PCR) are forwarded to NVSL for confirmatory testing. The NVSL uses Sanger sequencing protocols to generate partial HA/NA gene sequence directly from the sample for subtype and pathotype determination, when sufficient viral RNA is present. Whole genome sequencing is conducted on all isolated viruses, and select viruses are further characterized by pathotype assay in specific pathogen-free chickens.

NVSL confirms the virus HA and NA subtype through molecular sequencing and/or antibody subtyping, and the pathotype (LPAI vs HPAI); if no virus is recovered nor sequence obtained directly from the sample, the pathotype is determined by the clinical presentation of the flock compared to the USDA HPAI case definition.

USING GEOSPATIAL METHODS TO MEASURE THE RELATIVE RISK OF ENVIRONMENTAL PERSISTENCE OF AVIAN INFLUENZA VIRUS IN NORTH CAROLINA AND SOUTH CAROLINA

A. Summary

Introduction of avian influenza virus (AIV) into domestic poultry can be initiated by exposures to infectious wild birds or to virus surviving in the environment. This analysis aimed to evaluate the risk of AIV environmental persistence in North Carolina and South Carolina both spatially and temporally. Environmental factors known to influence AIV survival were identified through a review of the published literature. Using Esri GIS software, temporal and static spatial data were weighted based on their influence on virus survivability and then combined to produce weekly results at a 1km resolution from 29 January to 30 June 2020. This period encompasses the avian influenza outbreak in domestic poultry in these States, 11 March to 6 April 2020. Five categories defined in the World Organization for Animal Health (OIE) Risk Assessment Guidelines were assigned to each 1km grid cell ranging from very low/negligible to very high. Maps and charts showing the relative risk of AIV persistence in the North Carolina and South Carolina environment were created from these results. Maps were also produced showing the numbers of commercial poultry premises in relation to areas at risk. These results can be used to improve surveillance activities and to inform biosecurity practices and emergency preparedness efforts within the Carolinas.

B. Model Inputs and Development

Factors known to influence AIV introduction and survival in the ambient environment, focusing on H5 and H7 subtypes, were identified through an evaluation of the peer-reviewed literature. This information was combined with an assessment of data availability and five factors (water presence, water temperature, wetland cover, presence of wildlife refuges, and presence of wild birds) were selected as inputs to develop weekly risk models. These factors, their corresponding data sources, trends relative to AIV survival, and rationale for model inclusion are summarized in **Error! Reference source not found.**. Detailed descriptions of data sources and processing for each factor are available in Appendix B.

Table 6. Environmental factors selected as model inputs, data sources, trends relative to AIV survival, and rationale for model inclusion.

Factor	Data Source	Trends with AIV	Rationale
Water Presence	USGS Gap Analysis Program	AIV particles survive well in water compared to air or other dry media	AIV survives in water more prominently than dry land
Weekly Water Temperature	USGS Moderate Resolution Imaging Spectroradiometer (MODIS) remote sensing data	Inverse association between persistence and increasing temperature: optimal temperatures are near freezing and sub-optimal temperatures are 17-28°C	Temperature of water sources greatly impacts the rate of survival of AIV
Weekly Wild Bird Presence	Dabbling Duck Occurrence and Abundance model (USGS Patuxent Wildlife Research Center)	Wild birds are a main reservoir for AIV, and introduce the virus to their surrounding environment	When carrying the virus, wild birds deposit viral particles into water and other habitat locations
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	Previous studies show that wetland areas are associated with AIV presence
Wildlife Refuges	United States Fish and Wildlife Service	Wildlife refuges are preservation zones of wild bird habitat	Refuges provide ideal habitat for AIV reservoir birds

C. Model Execution

Risk of environmental AIV persistence was calculated from the inputs using the Esri Spatial Analyst toolset within ArcGIS 10.6. Weights were determined based on their relative contribution to the persistence of AIV, based upon subject matter expert consultation and literature review (**Error! Reference source not found.**). Weighted grids for each environmental factor and week were created using spatial analyst tools and then summed using a simple raster calculator additive expression.

Table 7. Environmental factors, spatial layer values, and weights used to develop the final predictive model for the persistence of avian influenza virus in the environment.

Factor	Spatial Layer Values	Weighted Values
Wild Birds	1 = Presence	3
	0 = Absence	0
Water Temperature	1 = < 10°C	3
	2 = 10°C ≤ > 20°C	2
	3 = ≥20°C	1
Water Presence	1 = Fresh water	3
	0 = No water	0
Wetlands	1 = Presence	2
	0 = Absence	0
Wildlife Refuges	1 = Presence	3
	0 = Absence	0

D. Results and Discussion

The raw model output values (ranging from 0 – 14) were reclassified to illustrate relative risk (OIE categories ranging from 0 – 5). The five risk categories displayed correspond to the OIE Risk Assessment Guidelines for describing risk: negligible/very low, low, moderate, high, and extremely high risk (OIE, 2017). Reclassification followed the scheme outlined in **Error! Reference source not found.**. The results by week for each State and North Carolina and South Carolina combined are illustrated in **Error! Reference source not found.**. The results are also displayed for the three affected counties graphically (**Error! Reference source not found.**) and spatially (**Error! Reference source not found.**).

Table 8. **Raw model output values (0-14) and reclassification into OIE risk categories.**

Original model value	Risk category (value)
0	*Negligible/Very low risk (0)*
1-2	*Low risk (1)*
3-4	*Moderate risk (2)*
5	*High risk (3)*
6-14	*Very high risk (4)*

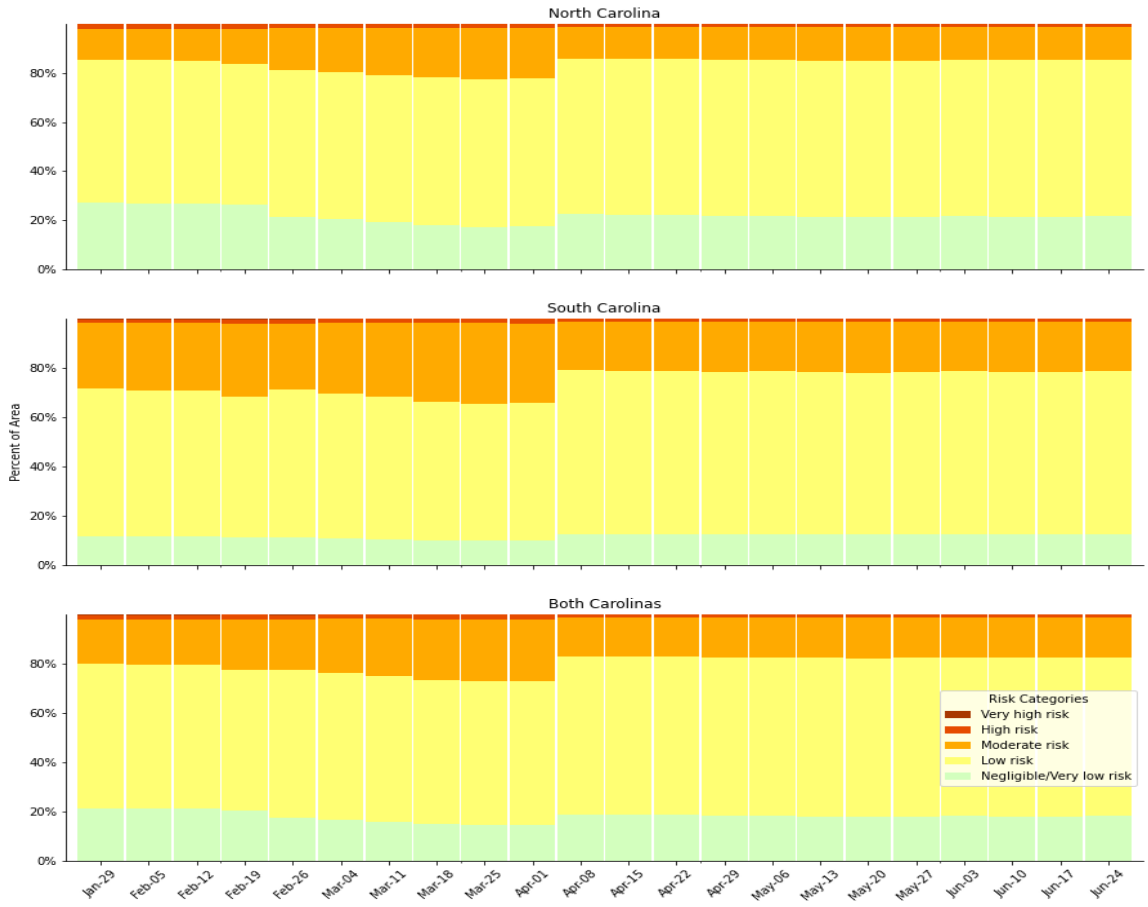

Figure 17. Percent of geographic area in each risk category by week (1 km resolution) in North Carolina, South Carolina, and both North and South Carolina (29 January – 30 June 2020).

Figure 18. Percent of geographic area in each risk category by week (1 km resolution) in HPAI and LPAI affected counties (29 January 29 – 30 June 2020).

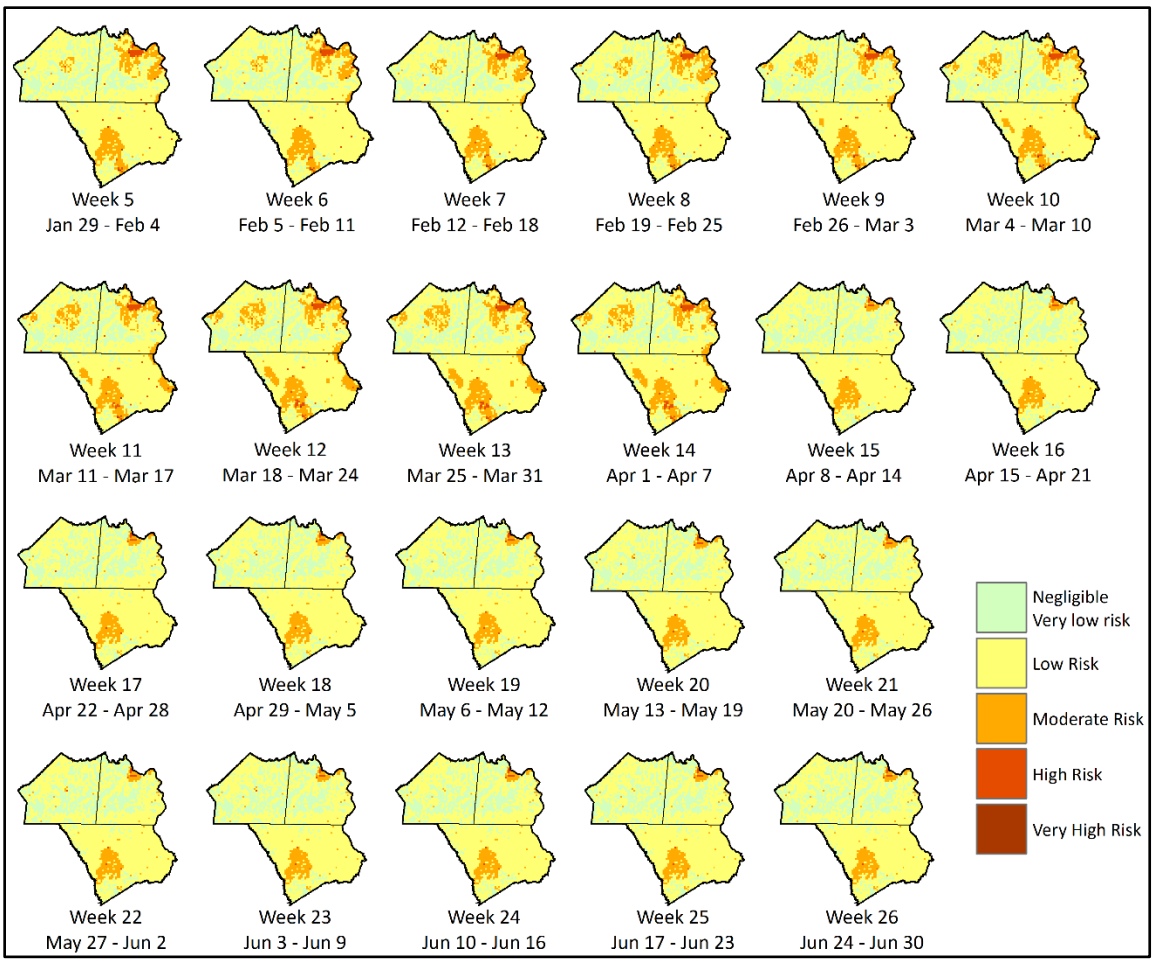

Figure 19. **Weekly risk (1 km resolution) of environmental persistence of AIV in counties affected by AI in spring 2020 in North Carolina and South Carolina (29 January – 30 June 2020).**

Figures 17, 18, and 19 illustrate an increasing level of weekly relative environmental risk from the beginning of the study period through the week ending with April 7th. From the week beginning April 8th through the end of the study period, weekly relative risk remains similar. This result may be related to observed warmer water temperatures not supportive of AIV persistence in the later part of the study period and annual migration of dabbling duck species out of the study area. Small areas with High and Very High risk persisted in all weeks of the study period.

Error! Reference source not found. illustrates the typical variation in risk categories within a small area in the three affected counties. This result shows that a wide diversity of risk can exist within a small geographic area even within states or counties that are generally low risk. Individual poultry operations may be in close proximity to moderate and/or high-risk geographic areas at 1km spatial resolution.

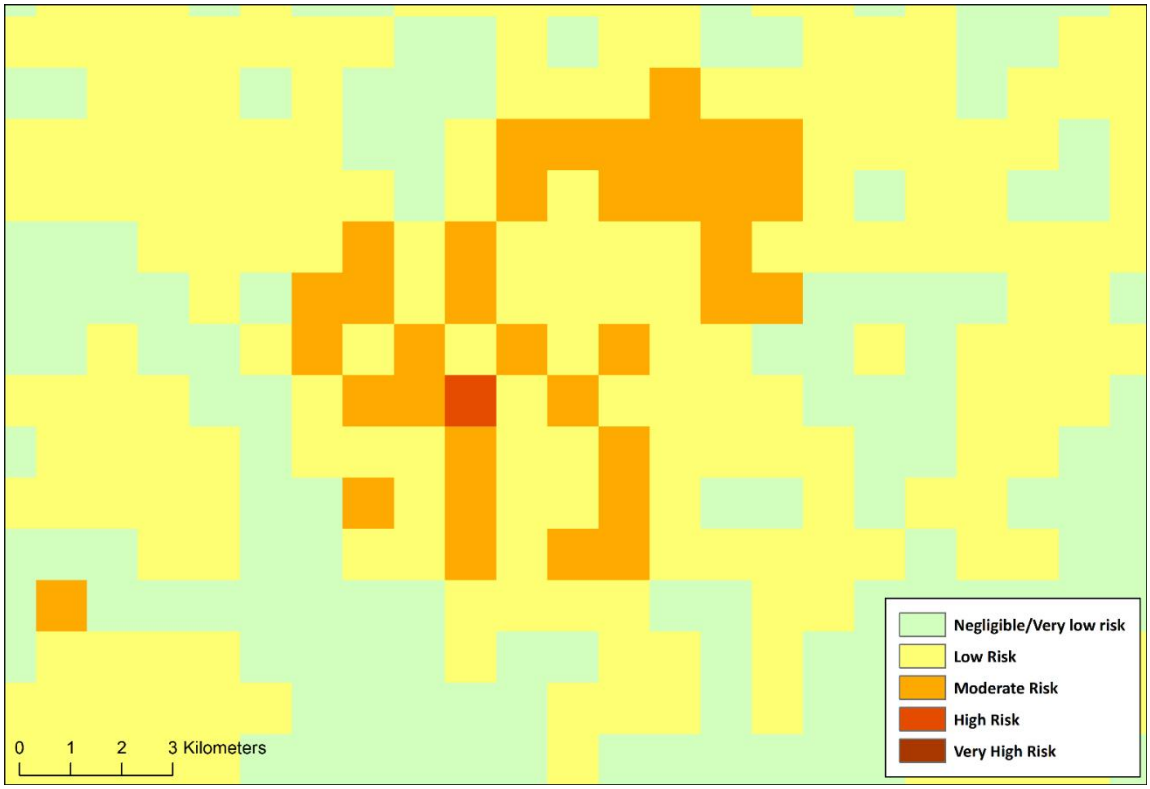

Figure 20. **Relative risk (1 km resolution) of environmental persistence of AIV in an undisclosed area within an AI-affected county.**

Error! Reference source not found. illustrates the number and location of commercial poultry facilities in combination with relative risk for AIV persistence, with model results for the week of February 26th to March 3rd chosen by way of example. Counties in the central portion of the two-state area including those affected by the outbreak generally have lower risk of AIV persistence than other areas such as the coast plains of North Carolina and the river valleys in South Carolina. Counties in higher risk areas such as the coastal plains in North Carolina have relatively low numbers of commercial poultry operations.

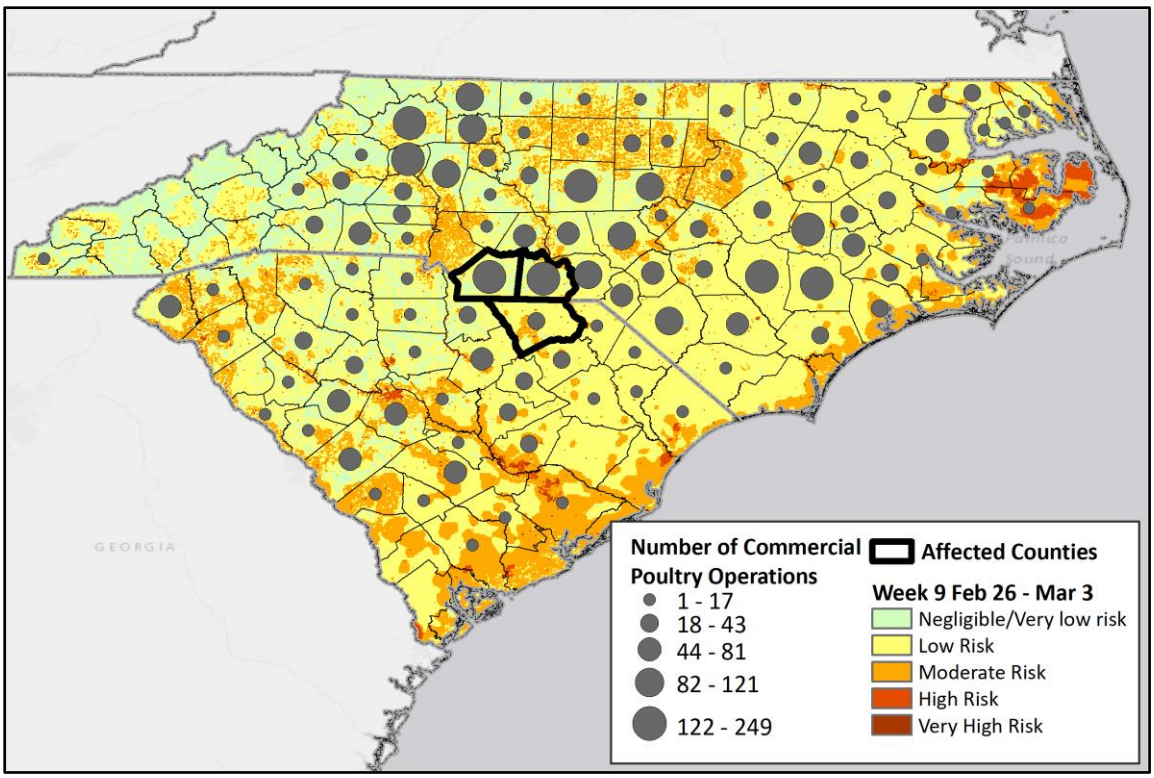

Figure 21. Commercial poultry facilities in relation with relative risk for AIV environmental persistence for the week of 29 February - 3 March 2020.

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APPENDIX A: TABLE OF VARIABLES INCLUDED IN THE CASE-CONTROL QUESTIONNAIRE

Table 9. Variables evaluated in the case-control study. All variables included in the table were not statistically significant at the p<0.1 level by univariate analysis. Variables with p-values<0.1 can be found in Table 2.

Characteristic	Level	n	
		Case	Control
Other animals present on farm			
Beef cattle		1/10	9/26
Horses		0/10	1/26
Prearranged depopulation plan		6/10	20/26
Is the plan exercised		0/6	0/20
House with family on premises		5/10	9/26
Common drive entrance		3/5	7/9
Number of farm entrances	• 1	6/10	20/26
	• 2	3/10	6/26
	• 3	1/10	0/26
Biosecurity signage		10/10	26/26
Farm fenced in		2/10	10/26
Double perimeter fencing		0/2	0/10
Gate to premises		2/2	1/10
Gate locked		1/2	0/10
Are the following vehicle types provided access			
Garbage truck	• Not at all	8/10	20/26
	• Perimeter of farm	1/10	3/10
	• On farm away from barns	1/10	2/10
	• On farm near barns	0/10	1/10
	• Enter barns	0/10	0/10
Propane delivery	• Not at all	8/10	21/26
	• Perimeter of farm	0/10	2/26
	• On farm away from barns	2/10	3/26

	• On farm near barns	0/10	0/10
	• Enter barns	0/10	0/10
Feed delivery	• Not at all	0/10	0/26
	• Perimeter of farm	0/10	0/26
	• On farm away from barns	0/10	0/26
	• On farm near barns	10/10	25/26
	• Enter barns	0/10	1/26
Renderer	• Not at all	8/10	22/26
	• Perimeter of farm	1/10	3/26
	• On farm away from barns	1/10	1/26
	• On farm near barns	0/10	0/26
	• Enter barns	0/10	0/26
Company personnel	• Not at all	0/10	0/26
	• Perimeter of farm	0/10	0/26
	• On farm away from barns	0/10	0/26
	• On farm near barns	0/10	0/26
	• Enter barns	10/10	26/26
Other business visitors	• Not at all	10/10	17/25
	• Perimeter of farm	0/10	1/25
	• On farm away from barns	0/10	3/25
	• On farm near barns	0/10	2/25
	• Enter barns	0/10	2/25
Number of times property mowed in last 6 months	• 6 or fewer	0/10	2/26
	• 7-12	2/10	4/26
	• 13-18	3/10	6/26
	• 19-24	3/10	13/26
	• > 24	2/10	1/26
Facility free of debris		8/10	24/26
Vehicle wash station		5/10	9/26

Located on farm	5/10	9/26
Organic material removed	2/5	7/9
Vehicle exterior washed	5/5	9/9
High pressure spray wash	3/10	6/26
Frame cleaned and disinfected underneath	3/10	9/26
Interior cleaned	0/5	0/9
Designated parking area for workers and visitors	8/10	26/26
Changing area for workers	7/10	17/26
Workers shower at premises before entering barns	0/7	1/17
Workers don dedicated laundered coveralls before entering barns	2/9	7/26
Workers wear rubber boots or covers in poultry houses	10/10	26/26
Barn/pen doors lockable	6/10	20/26
Barn/pen doors routinely locked	0/6	0/20
Feed covers kept closed	10/10	26/26
Foot pans available at barn/pen entrances	2/10	9/26
Dry disinfectant	0/2	3/9
Liquid disinfectant	2/2	6/9
Foot pans in use prior to February 19th	1/2	5/9
Barns/pens have entry areas	8/10	20/26
Biosecurity audits on premises	10/10	26/26
Workers assigned to entire farm	10/10	26/26
Workers employed by other poultry premises	0/10	1/26
Family members employed by other poultry premises	2/10	1/25
Family members employed by other processing plants	0/10	0/26
Biosecurity training per year = 1	10/10	26/26
Hire extra part-time workers or extended family	4/10	9/25
Workers restricted from contact with backyard poultry	10/10	26/26
Communicated verbally	8/10	22/26
Communicated in writing	0/10	0/10
Communicated both ways	2/10	4/26

Grower or workers visited other poultry premises	4/10	0/26
Workers ever interact with workers from other poultry premises	3/10	1/26
Visitor log to sign	2/10	1/26
Visitor log current	2/2	0/1
Outer clothing provided to visitors	2/10	4/26
Service visits after February 19th		
Flock supervisor	10/10	26/26
Feed delivery	7/10	26/26
Processing plant	1/10	18/26
Rendering	1/10	1/26
Cleanout	4/10	0/26
Litter	0/10	3/26
Service visits with access to birds		
Flock supervisor	6/10	17/26
Feed delivery	0/10	1/26
Processing plant	1/10	10/26
Rendering	0/10	0/26
Cleanout	0/10	0/26
Litter	0/10	0/26
Poult delivery	0/10	6/26
Service visit equipment shared/rented/borrowed	2/10	3/26
Shared equipment had contact with birds	2/10	3/26
Equipment used jointly on other premises		
Company vehicles	4/10	2/26
Feed trucks	3/10	2/26
Lawn mowers	4/10	0/26
Live haul loaders	3/10	2/26
Poult trailers	3/10	2/26
Pressure washers/sprayers	2/10	0/26
Skid-steer	2/10	0/26

Tillers	4/10	0/26
Trucks	4/10	0/26
Other equipment	1/10	1/26
Litter type = wood shavings	10/10	26/26
Litter shed present	4/10	16/26
Litter disposal		
Off farm	8/10	25/26
On farm	0/10	1/26
Both	2/10	0/26
How is daily mortality handled		
Rendering	9/10	20/26
Composting	0/10	5/26
Landfill	2/10	1/26
Burial	3/10	6/26
Incinerator	4/10	2/26
If burial or composting pits used, covered with soil daily	4/5	11/13
Carcass bin have a cover	9/9	22/22
Carcass bin routinely kept closed	9/9	22/22
Observe wild birds around farm	8/10	25/26
Types of wild birds observed		
Small perching birds	8/10	25/26
Waterfowl	3/10	1/26
Other birds	0/10	1/26
Fly control used	4/10	13/26
Raccoons, possums, coyotes, foxes, rabbits observed around poultry houses	5/10	6/26

APPENDIX B: DATA FACTORS AND PROCESSING FOR GEOSPATIAL ANALYSIS PREDICTING AVIAN INFLUENZA VIRUS PERSISTENCE IN THE ENVIRONMENT

A. Data Preparation

All data were prepared using tools within ArcGIS 10.6, and final layers were projected to Universal Transverse Mercator (UTM) Zone 17 North in the datum World Geodetic System 1984 (WGS1984).

Predictive Factors and Data Sources

Water Presence

Avian influenza viruses (AIV) have been shown to have improved survival in water compared to dry land (Brown et al., 2009; USGS, 2011). 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 two categories of surface water based on suitability for AIV survival: presence of fresh water (high suitability), or no water present (low suitability). The surface water presence layer was aggregated to a one-kilometer (1km) resolution.

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 2015-2017. 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). Refined MODIS data were then masked with the water presence layer to reflect locations only where surface water was present, and reclassified to represent high suitability (< 10° C), moderate suitability (≥ 10°C and < 20°C), or low suitability (≥ 20°C) for AIV survival (Brown et al., 2007; Brown et al., 2009; Keeler et al., 2014; Nazir et al., 2010).

Wetlands and Wildlife Refuges

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.

Wild Bird Presence

Data for wild bird presence were provided by the USGS Patuxent Wildlife Research Center. These data were derived using a spatio-temporal model estimating seasonal occurrence (presence) and

abundance of 13 dabbling duck species (**Error! Reference source not found.**) throughout the conterminous United States at a 1km resolution (Humphreys, 2019). The occurrence model data for each species was combined then reclassified to create a presence/absence dataset for North Carolina and South Carolina.

Table 10. **Common and scientific names of dabbling duck species that were included in the wild bird model.**

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

Commercial Poultry Operations

Poultry operation data were extracted from the USDA APHIS Emergency Management Response System (EMRS). The data included location information for 4,416 commercial poultry operations. These data were summarized by county to illustrate the number of commercial premises in relation to areas at high risk for AIV introduction from wild birds.