USDA

**United States
Department of
Agriculture**

**Animal and Plant
Health Inspection
Service**

Veterinary Services

Epidemiologic and Other Analyses of HPAI/LPAI Affected Poultry Flocks

June 26, 2017 Report

Suggested bibliographic citation for this report:

> USDA-APHIS (2017). Epidemiologic and other analyses of HPAI/LPAI affected poultry flocks: June 26, 2017 Report. USDA:APHIS:VS:STAS:Center for Epidemiology and Animal Health. Fort Collins, CO. June 2017. Doc #391.0317 V2. 39 pgs.

For more information, contact:

> Bruce A. Wagner, PhD
>
> Director, Center for Epidemiology and Animal Health
>
> USDA:APHIS:Veterinary Services
>
> Science, Technology, and Analysis Services
>
> Center for Epidemiology and Animal Health
>
> 2150 Centre Avenue, Bldg. B-2E7, Fort Collins, CO, 80526-8117
>
> Phone (970) 494-7256 | Mobile (970) 237-0857 | Fax (970) 494-7228
>
> Email: Bruce.A.Wagner@aphis.usda.gov
>
> Web: http://www.aphis.usda.gov/animal-health/ceah

NOTE: Red text in a Heading indicates content updates have been made to that section only. (Version 2; June 26, 2017)

Contents

List of Figures and Tables

I.   EXECUTIVE SUMMARY

A combined outbreak of highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) started in early March 2017. The outbreak was first suspected by an increase in mortality on a commercial breeder farm in Tennessee and was confirmed as H7N9 highly pathogenic avian influenza (HPAI) of North American wild bird lineage (AM H7N9 HPAI) via laboratory testing. After initial efforts to control the disease, a series of epidemiologic, genetic, and wildlife investigations were started. This is a report of the findings available to-date, and these analyses are intended to assist in understanding disease introduction and transmission pathways. These studies were undertaken collaboratively with the poultry industry, State agriculture personnel, and the Animal and Plant Health Inspection Service.

The outbreak area lies primarily in south central Tennessee and northern Alabama, but cases have occurred in southern Kentucky and western Georgia. This area is bounded by dense areas of poultry production, including broiler, table-egg layer, and turkey operations.

Sampling and testing of wildlife was implemented near a commercial premises infected with AM H7N9 Influenza A virus (IAV). Initial results have not confirmed the presence of H7N9 IAV from wild bird samples; however, limited evidence suggests that one or more animals sampled may have been previously exposed to an IAV.

Genetic analyses determined that all H7N9 viruses detected from this event are of North American wild bird lineage, and the HPAI and LPAI viruses are highly similar across all eight genes, excluding the multi-basic amino acid insertion at the cleavage site in the HPAI virus.

Initial genetic and epidemiologic evidence suggest the possibility of more than a single introduction with limited lateral transmission. These viruses were most likely introduced in late February based on available barn-level egg production, mortality, and testing information. H7 detections have occurred during summer, fall, and winter and throughout the Mississippi and Atlantic Flyways. Annually, H7 detections tend to peak in early winter. This pattern may have contributed to the timing of this outbreak in the southeastern United States.

The six commercial AM H7N9 LPAI farms involved five different integrated poultry complexes suggesting unique sources of feed, pullets, males, egg transport trucks, and crews for most of the cases. Potential routes of lateral spread between farms, related to potential concerns such as the biosecurity of egg pickup, trucks, visitors, equipment and disposal activities, did not appear to be risk factors during this outbreak. However, factors such as the presence of rodents and other wild mammals and waterfowl near barns, the condition of the housing, and breaches in biosecurity protocols were identified as risk factors that could bring viruses into the barns from the environment. The fact that backyard operations have also been impacted adds credence to the theory that this outbreak was governed in large part by exposure to environmental sources of virus.

Broiler flocks located in the area were unaffected during this outbreak. Results from the expert elicitation highlighted differences in housing type, age of birds, and length of the production cycle, which could help explain why broiler flocks were not infected.

II. INTRODUCTION

In response to the H7N9 avian influenza outbreaks in commercial and backyard poultry in AL, GA, KY, and TN, USDA-APHIS-Veterinary Services, APHIS Wildlife Services, and the affected states have initiated epidemiologic, genetic, and wildlife investigations. These investigations will help provide a better understanding of factors associated with avian influenza virus transmission and its introduction into poultry flocks.

These investigations include the following:

- A field-based study of cases using data collected through site visits and interviews with farm personnel

- Analysis of barn-level egg production and mortality records

- An expert elicitation study

- Virus phylogenetic analyses

- Analysis of waterfowl surveillance

- On-farm sampling of wildlife.

This report includes the results from these investigations, in an effort to provide producers, industry, and other stakeholders with current epidemiologic information.

Description of Outbreak

On March 2, 2017, an increase in mortality was noted on a commercial broiler breeder farm in Lincoln County, TN. The United States Department of Agriculture (USDA) National Veterinary Services Laboratories (NVSL) confirmed H7 highly pathogenic avian influenza (HPAI) of North American wild bird lineage on March 4, 2017 and N9 on March 6, 2017 (AM H7N9 HPAI). Surveillance in the area was immediately initiated to determine the status of other farms. A second commercial broiler breeder farm was confirmed positive for AM H7N9 HPAI on March 15, 2017. The second HPAI-positive farm also was located in Lincoln County, TN. In addition to the two HPAI-positive farms, an additional six commercial broiler breeder operations and six backyard flocks were found positive with H7 and H7N9 low pathogenic avian influenza North American wild bird lineage. The eight commercial broiler breeder farms affected in this outbreak were distributed across six companies and four States (Figure 1, Table 1). The timeline of detections is shown in Figure 2 with dates of confirmation shown in Table 1. In total, approximately 252,000 birds died from disease or were depopulated to control the outbreak.
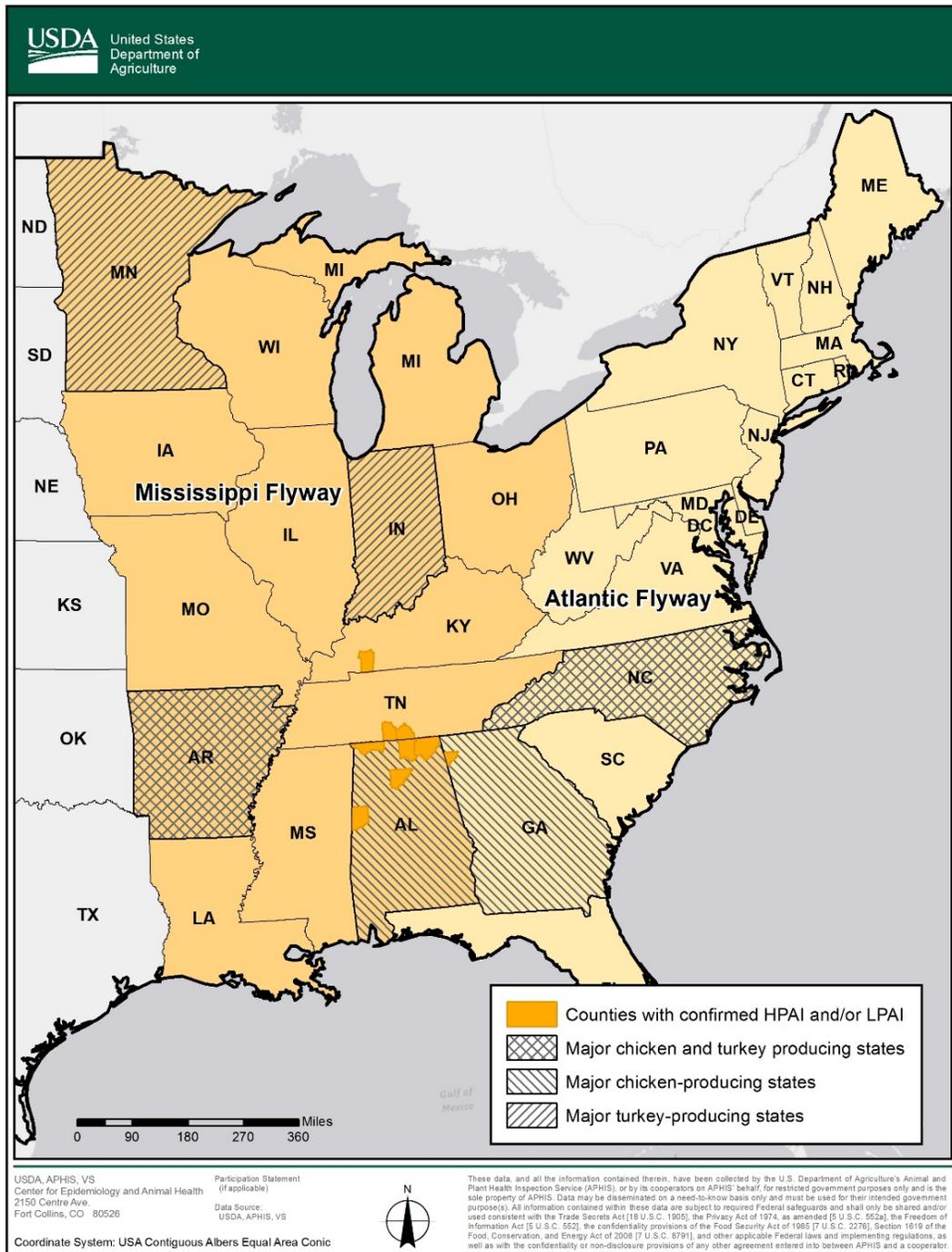
Figure 1. Counties with confirmed findings of HPAI or LPAI H7 or H7N9 since March 1, 2017 and their locations relative to major chicken and turkey-producing States in the United States.

The top four chicken-producing and turkey-producing States are highlighted based on the National Agricultural Statistics Service's Poultry – Production and Value 2015 Summary report.[1]

---

[1] http://usda.mannlib.cornell.edu/usda/nass/PoulProdVa//2010s/2016/PoulProdVa-04-28-2016.pdf
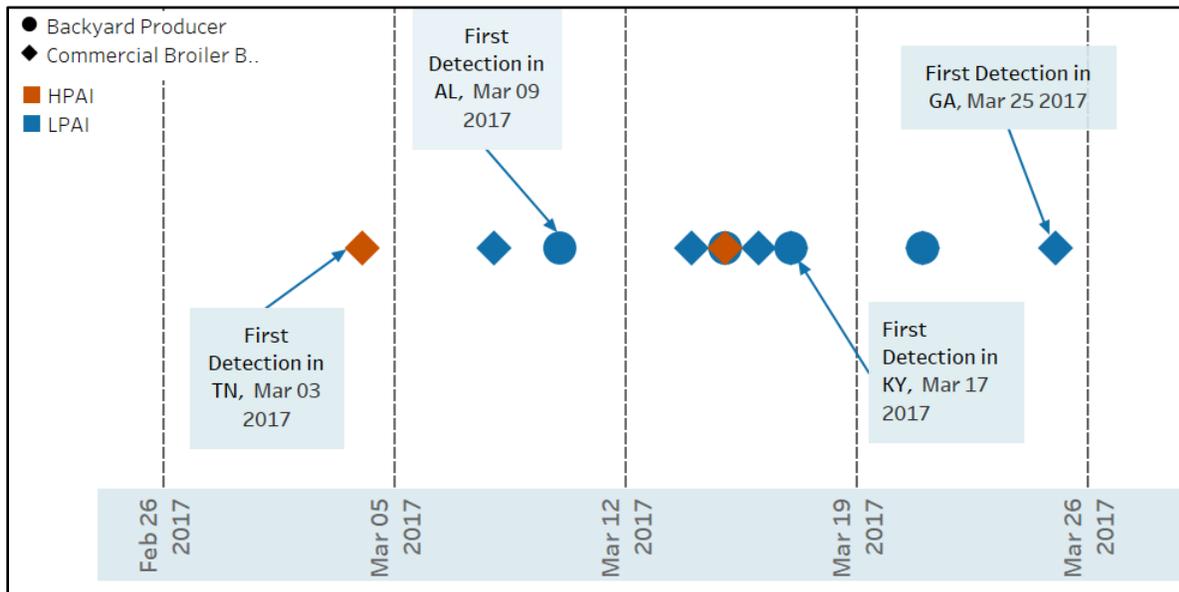
Figure 2. Timeline of detections of HPAI and LPAI infected flocks by flock type and pathogenicity of virus detected. First detections in each State are highlighted.

Table 1. Location, production type, pathogenicity, and confirmation date of flocks infected during the H7N9 HPAI/LPAI outbreak.

State	County	Production Type	Pathogenicity	Confirmation Date
Tennessee	Lincoln	Commercial Broiler Breeder	HPAI	3/4/2017
Tennessee	Giles	Commercial Broiler Breeder	LPAI	3/8/2017
Alabama	Madison	Backyard Producer	LPAI	3/10/2017
Alabama	Lauderdale	Commercial Broiler Breeder	LPAI	3/14/2017
Alabama	Jackson	Backyard Producer Guinea Fowl	LPAI	3/15/2017
Tennessee	Lincoln	Commercial Broiler Breeder	HPAI	3/15/2017
Tennessee	Lincoln	Backyard Producer	LPAI	3/15/2017
Tennessee	Lincoln	Backyard Producer	LPAI	3/15/2017
Alabama	Pickens	Commercial Broiler Breeder	LPAI	3/16/2017
Alabama	Madison	Backyard Producer	LPAI	3/17/2017
Kentucky	Christian	Commercial Broiler Breeder	LPAI	3/17/2017
Alabama	Cullman	Commercial Broiler Breeder	LPAI	3/21/2017
Kentucky	Christian	Backyard Producer	LPAI	3/21/2017
Georgia	Chattooga	Commercial Broiler Breeder	LPAI	3/25/2017

III. EPIDEMIOLOGIC STUDIES TO INVESTIGATE H7N9 VIRUS IN POULTRY IN TENNESSEE, ALABAMA, KENTUCKY, AND GEORGIA

A. Case Series

USDA-APHIS conducted a case-series study among H7N9 affected commercial broiler-breeder poultry operations. The purpose of this study was to generate hypotheses for potential risk factors for infection with H7N9 avian influenza virus. Additionally, study respondents provided mortality data; egg production data; a copy of biosecurity manuals, if available; and a farm diagram highlighting the locations of key areas, such as ponds. Questions focused on the 3 weeks prior to the earliest indication of infection (clinical signs, drop in production, or positive test) (Appendix A: Case Series Questionnaire).

Commercial Breeder Farms

Completed case-series questionnaires were available from 8 commercial broiler breeder farms (6 multiplier farms and 2 primary breeders), which represented 2 HPAI-confirmed farms and 6 LPAI-confirmed or presumptive farms. Results of the questionnaires are summarized in Table 2.

Results

- Four of the eight farms reported respiratory signs in poultry prior to the positive AI test.

- Ponds (5 farms) and streams (5 farms) were the most common types of water bodies located within 350 yards of the farms. Wild waterfowl were not observed outside the barns on any of the farms and other wild birds were rarely observed. Chickens did not have outdoor access on any farms.

- Workers did not work on other farms or own poultry, and were required to stay off the farm if they had exposure to poultry. A clean/dirty line was established on all eight farms. The barns' hard surface entry way was cleaned and disinfected on three of the farms. Only one farm used different personnel for different barns. Footwear was not scrubbed on any of the farms although change of shoes was required on five farms.

- Feed trucks, egg removal trucks, egg flats/racks/pallets were commonly shared with other farms. Feed, egg, and company personnel vehicles entered the farm and most drove up near the barns. Vehicles were not washed at every entry or exit to the farms during the 3-week risk period (6 farms). All 8 farms had visits from their company service person during the 21-day risk period and the company service person entered the barn on 7 of the farms.

- The most common method of dead bird disposal was composting (6 farms), and the carcasses were covered with soil or manure usually after 2 or more days. The dead bird disposal area was located within 40 yards of the barn on 6 farms.

Summary

Although the results demonstrate common practices across case farms, without information on control farms, we cannot say that these are necessarily risk factors. Therefore, these results need to be interpreted cautiously. However, some of the common practices have been shown to be risk factors in other studies. These results should be considered in context with the findings of the expert elicitation study, which explored the implementation of biosecurity protocols on these farms in comparison to unaffected farms (Section C).

Table 2. Characteristics of poultry farms testing positive for avian influenza H7N9

Characteristic	Level	n	Comments
Farm level factors:			
Bird type	• Multiplier	5	
	• Primary breeder	2	
Clinical signs		4	4 farms reported respiratory signs prior to positive test, 3 farms reported decreased egg production
Outdoor access	• no	8/8	
Water body within 350 yds	• Pond	5/8	
	• stream	5/7	
Other poultry	• No	8/8	
Water source	• municipal	7/8	
Wild birds on the farm outside of barn	• Water fowl	0/8	1 dead buzzard
	• Small perching birds	6/8	
Wild birds in the barns		0/8	
Workers	• Establish clean/dirty line	8/8	
	• Shower	2/8	
	• Change clothes	4/8	
	• Change shoes	5/7	
	• Scrub footwear	0/5	
	• Different personnel for different barns	1/7	
	• Work on other farms/own poultry	0/8	
	• Required to stay off farm after exposure to poultry	8/8	Most 72 hours
Visitors	• Company service person	8/8	
	• Feed delivery personnel	6/8	
	• Egg truck personnel	6/8	
Vehicles shared with other farms	• Feed truck	8/8	
	• Egg removal	8/8	
	• Stored outside uncovered	6/6	

Characteristic	Level	n	Comments
Equipment shared with other farms	• Egg racks/pallets	6/7	
	• Egg flats	6/7	
Vehicles enter the farm	• Feed delivery	8/8	Most reported these vehicles drive up near the barns
	• Company personnel	6/8	
	• Egg truck	8/8	
Vehicles washed prior to or upon leaving farm		2/8	
Dead bird disposal method	• Composting	6	Covered with soil or manure every 2 or more days
	• Incineration	1	
	• Rendering	1	
Wild birds, mammals or domestic animals seen around the dead bird disposal area		2/8	
Barn level factors:			
Health concern		2	Respiratory illness
Ventilation type	• Curtain	2	
	• Tunnel	5	
	• Ceiling/eaves	1	
Fans located at end of barn		7/8	
Hard surface entry cleaned and disinfected		3/8	
Service room for entry		6/8	
Footbath		varies	
Last full cleanout	• Prior to this flock	8/8	
Wild birds (live or dead) in barn		0/8	
Dead bird disposal area within 40 yds		6/8	
Visitors entering the barn	• Company service person	7/8	No other visitor type frequently reported

Backyard Flocks

Questionnaires were completed for five AI-positive backyard operations. Three of these operations were identified by area surveillance and two by routine flea market testing. None of the operations were aware of a health concern during the 3-week risk period prior to testing positive.

Results/Summary:

All 5 operations had chickens; 4 operations had ducks and other poultry. Poultry had outdoor access on all 5 operations. Flock size for respondents ranged from 15 to 340 birds. Dead birds were disposed of by burial (3 operations) or landfill (1 operation). Footbaths were not used by any of the operations. The nearest body of water was within 100 yards of the poultry barn for 3

operations. Water bodies within 350 yards of the premises included ponds (2 flocks) and wetlands or swamp (2 flocks). Wild birds were seen on all 5 operations including wild waterfowl on 2 operations. Wild birds were seen in the barns on all 5 premises. All 5 operations had dogs on the premises. Additional notes on the surveys indicated no known connection could be identified to commercial poultry or other backyard flocks. Also, some flocks had free access to the entire property while others were enclosed in pens. The occurrences of exposure to risk factors and the lack of connection to other commercial or backyard flocks seems to indicate that lateral transmission was not an important route of infection for these flocks.

B. Egg Production and Mortality Data

Egg production and mortality data were obtained for five case farms (farms that tested positive for AI via PCR) and four barns from two non-infected farms. The non-infected farms came from within the control zone in Tennessee. Dates for the last negative serologic test were available for four of the case farms and dates of a positive serologic test for one farm; however, we were unable to link the test results back to an individual barn on these farms. Figure 3 shows eggs per hen per day from September through March for each barn for each LPAI case farm. Figure 4 shows mortality (percent hens that died) for these same barns for the same time period. Figure 5 shows egg production and mortality for the control barns during the same time period.

Barn-level egg production patterns were similar on case and non-infected farms in that there was a period of approximately 3 to 4 weeks after placement that egg production rapidly increased, then plateaued and gradually decreased over several months.
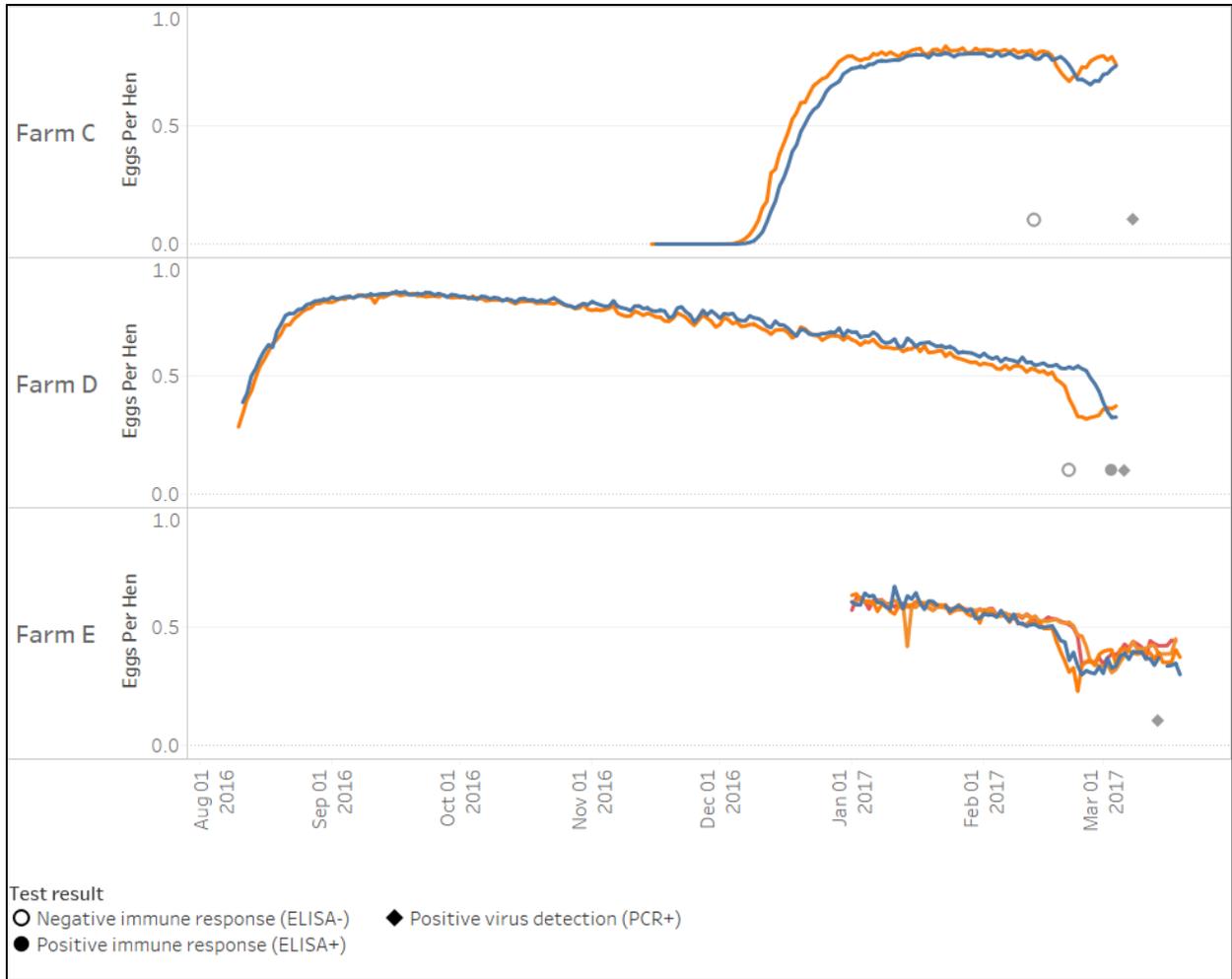
Figure 3. Egg production (eggs per hen) for LPAI case farms from August 2016 through March 2017 by individual barn (colored lines). Most recent ELISA and PCR test results are shown for each farm.
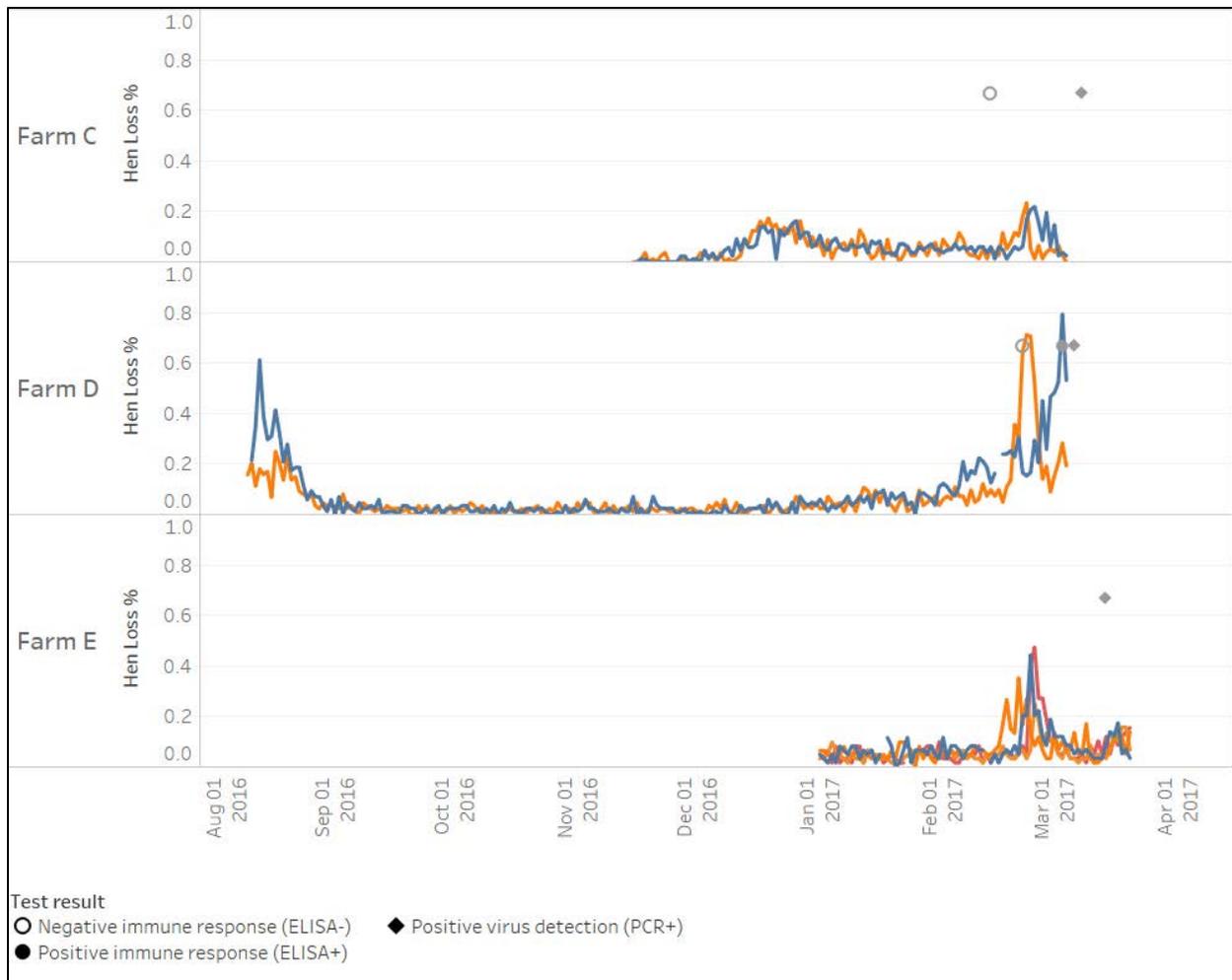
Figure 4. Hen mortality (percent daily mortality) for LPAI case farms from August 2016 to March 2017 by individual barn (colored lines). Most recent ELISA and PCR test results are shown for each farm.
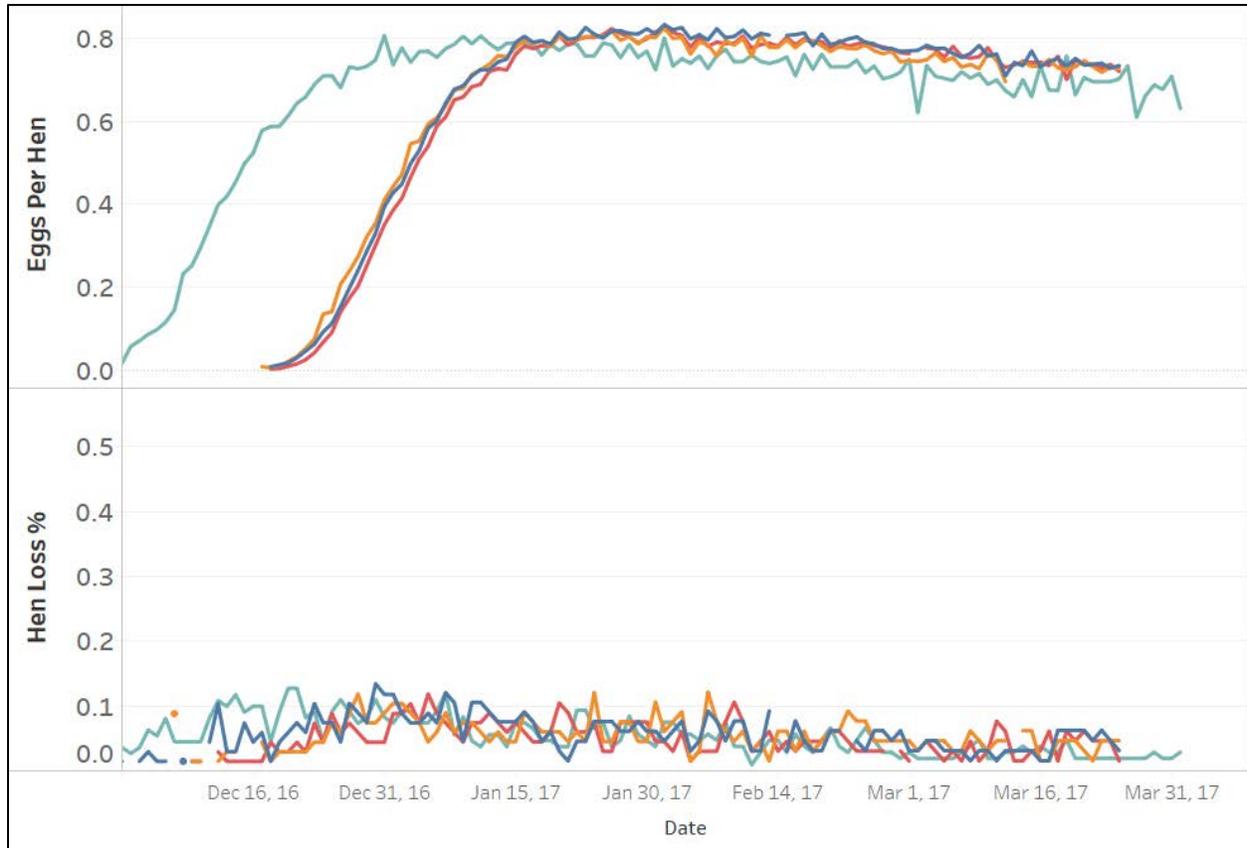
Figure 5. Egg production (egg per hen) and hen mortality (daily percent mortality) for four non-infected barns from December 2016 through March 2017 by individual barn (colored lines).

Results/Summary

Unlike the uninfected barns, all five case farms had drops in egg production for a short period of time in specific barns. A production drop for the two HPAI farms (data not shown) occurred several months in single barns before testing positive for AI. These farms had negative serologic tests long after these egg production drops had resolved, and therefore, it is likely that a problem unrelated to AI was responsible for these early drops in production. Both of the HPAI farms experienced a sudden marked spike in mortality immediately prior to testing positive, with mortality rates reaching 1-4%. There was an approximately 2-week gap between the mortality events on the two HPAI farms. Farms C and D had drops in egg production that occurred after a previous negative serologic test for AI and prior to a positive PCR test. These drops in egg production coincide with a slight increase in mortality. Farm E also had a notable drop in egg production with a coinciding increase in mortality prior to testing positive for AI. (Information regarding the last negative test was not available for Farm E.)

Given standard incubation times for AI viruses in poultry, these findings suggest that the virus was likely introduced into these flocks in late February except for the second HPAI infected farm, which appears to be due to lateral transmission in mid-March. It is possible that these less severe drops in production and increased mortality on farms C, D, and E may be related to infection with a LPAI virus. However, the impacts of LPAI virus introduction are subtle and

difficult to distinguish from regular variability in egg production or mortality, as shown in the data from the uninfected barns.

C. Expert Elicitation Study

An expert opinion elicitation was conducted to rapidly characterize risk factors and possible routes of exposure for commercial premises involved in the 2017 AM H7N9 HPAI/LPAI outbreak in the four State region of Tennessee, Alabama, Kentucky and Georgia. The expert panel included 6 company veterinarians, 1 laboratory clinician, and 1 government poultry health specialist—all with first-hand knowledge of the outbreak. The elicitation involved a series of questions about known (real) cases and controls, as well as a hypothetical component in which experts envisioned the likely distribution of risk factors among farms involved in an imagined outbreak that was similar to, but larger than the real outbreak. Survey questions and introductions are available in Appendix B. The inquiry method was scripted to estimate likelihood ratios (LR, measures of association akin to odds ratios) for risk factors of potential influence in this outbreak.

In individual interviews, experts responded to a series of risk factor questions about the case broiler breeder farm(s) with which they were most familiar. They also responded to the same series of questions about a control (non-affected) broiler breeder farm for each case. Controls were selected by the experts under the direction that each should match a case site in production type, timing, and region and their level of familiarity. The identity of neither cases nor controls were provided to the analysts; so, in order to avoid site duplication, only the six company veterinarians' (one per company) responses were used in the matched (real) case-control component. For the case control segment, the goal was to allow a rapid characterization of risk factors among known sites in lieu of a formal but more intensive epidemiologic field study. All eight experts participated in the hypothetical scenario. Here, experts were asked to assign the probable rates of occurrence of risk factors among affected versus unaffected farms in an imagined outbreak of the same etiology, timing, and type as the existing outbreak, but scaled up in size. The goal of this segment was to encourage structured speculation on key factors most relevant to prevention and control of AM H7N9 IAV and similar viruses in this industry.

Results/Summary

Results suggest a limited role for farm-to-farm (lateral) transmission, and a likely greater role for environmental sources of exposure, in this outbreak. While lateral spread was implicated between two HPAI sites based on genetic homology between the isolates, close geographic proximity, and company ties between the two farms, direct farm-to-farm transfer routes among the AM H7N9 LPAI commercial broiler breeder sites were not apparent. The six commercial LPAI farms involved five different integrated poultry complexes suggesting unique sources of feed, pullets, males, egg transport trucks, and crews for most of the cases. Furthermore, factors representing potential for lateral spread were evenly balanced in abundance between cases and controls. Thus, questions about the biosecurity of egg pickup, trucks, visitors, equipment and disposal activities, held no predictive strength in this analysis. In contrast, several

environmental risk factors, present in a preponderance of cases over controls, did show predictive strength. These included disturbance by mesopredators or rodents, enclosure defects that could jeopardize pest exclusion or events that might result in local attraction of these animals. Density of poultry operations in the area was also predictive. Interestingly, proximity to large congregations of waterfowl was cited in only one case, and wetland environments were no more prevalent in cases than controls.

The hypothetical scenario similarly pointed to environmental factors such as rodents, waterfowl (rather than mesopredators), and the condition of the housing. However, in the hypothetical scenario, biosecurity of equipment, workers and visitors entering the chicken houses, as well as biosecurity of vehicles entering the farm, ranked high in predictive strength. Egg carts rolled from truck to barn across outdoor concrete pads, workroom doors held open for cooling that might allow an alternative to the formal entrance with foot bath, improper use (e.g., without first removing organics) and maintenance of foot baths, or inadequate worker instruction in biosecurity, were potential gaps cited. The focus of expert discussion was on exposure to virus present in the surrounding environment, though many of the same mitigations would also reduce opportunities for lateral spread. The relevance of biosecurity measures ranking high in the hypothetical component, but not in the case control component, emphasizes concerns that breaches in standard protocol could be the avenue for virus introduction onto these farms. The fact that backyard operations have also been impacted adds credence to the theory that this outbreak was governed in large part by exposure to environmental sources of virus.

Finally, experts were asked for their hypotheses explaining the exclusion of broilers and table egg layers in this outbreak. For table egg layers, the answer most often cited was their lack of abundance in the affected region. For broilers, responses included their younger age, shorter production cycle, fewer opportunities for testing prior to market, and possible reduced susceptibility to the virus. Experts observed that most broiler farms' barns are solid wall sided and temperature controlled, while many broiler breeder farms' barns are curtain sided and lack the need for tight temperature control. Broiler operations sustain less foot traffic than broiler breeders which require frequent worker entry for egg, feed, and animal oversight. Additionally, broiler breeder barns include roosting/feeding sections raised up on slats above ground, which could potentially offer safe harborage underneath for rodents or other pests. Thus, the two systems vary in conditions that could impact virus exposure and persistence, as well as host susceptibility and detection.

The small sample size and subjective nature of the data limit the conclusive strength of this study. However, results can help bridge decision support until further research is conducted. Further exploration might consider the role of mesopredators and rodents, the importance of housing type and condition (on virus exposure, bird susceptibility, or viral persistence), conditions or periods predictive of greatest risk of introduction from the environment, and design of enhanced biosecurity protocols to balance periods of elevated risk. Finally, experts

noted that adjusting routine testing to include PCR, in addition to serology, could improve speed of detection of HPAI/LPAI viruses during high-risk periods.

D. Route of Lateral Spread between HPAI-Infected Premises

Epidemiologic and genomic evidence (Section IV) suggest there was farm-to-farm (lateral) spread of virus between the two HPAI-positive commercial broiler breeder farms. Epidemiologic investigation of the movements of eggs and workers for the two farms did not clearly identify the linkage that could have resulted in viral introduction to the second farm. Individuals involved with the outbreak hypothesized that the virus was spread farm-to-farm via wind, although other methods of spread were also possible. In order to explore this hypothesis, wind conditions were obtained for the period of time between the two HPAI detections. Both HPAI-positive farms were part of the same poultry company and the first HPAI-infected farm was located approximately one mile southeast of the second HPAI-infected farm.

Results/Summary

Wind direction during the period of interest was predominately blowing from the south, which supports the wind spread hypothesis (Figure 6). Anecdotal evidence obtained during the expert opinion elicitation also supports this hypothesis. An individual on the second farm reported the wind was blowing from the direction of the first farm during the disposal period, and that they were able to smell odors from disposal activities. Additionally, the first barn positive on the second farm was the barn closest to the first farm. Of special note, there was a broiler farm between these two farms that did not become infected, which is inconsistent with the wind spread hypothesis. Possible reasons for this lack of infection of the broiler farm that was in proximity to two AM H7N9 HPAI infected farms are discussed in the Expert Opinion Elicitation (Section C). There are differences in housing and ventilation structure that could result in a broiler farm not receiving the same amount of exposure to wind-related materials.

Although these results are not conclusive, detection of AIV in air has been documented (Torremorell, 2016) and standard operating procedures should be followed when managing an HPAI-infected flock in order to reduce opportunities for wind-related spread.

E. References:

Montserrat Torremorell, Carmen Alonso, Peter R. Davies, Peter C. Raynor, Devi Patnayak, Mia Torchetti, and Brian McCluskey (*2016*). Investigation into the Airborne Dissemination of H5N2 Highly Pathogenic Avian Influenza Virus during the 2015 Spring Outbreaks in the Midwestern United States. Avian Diseases: September 2016, Vol. 60, No. 3, pp. 637-643.
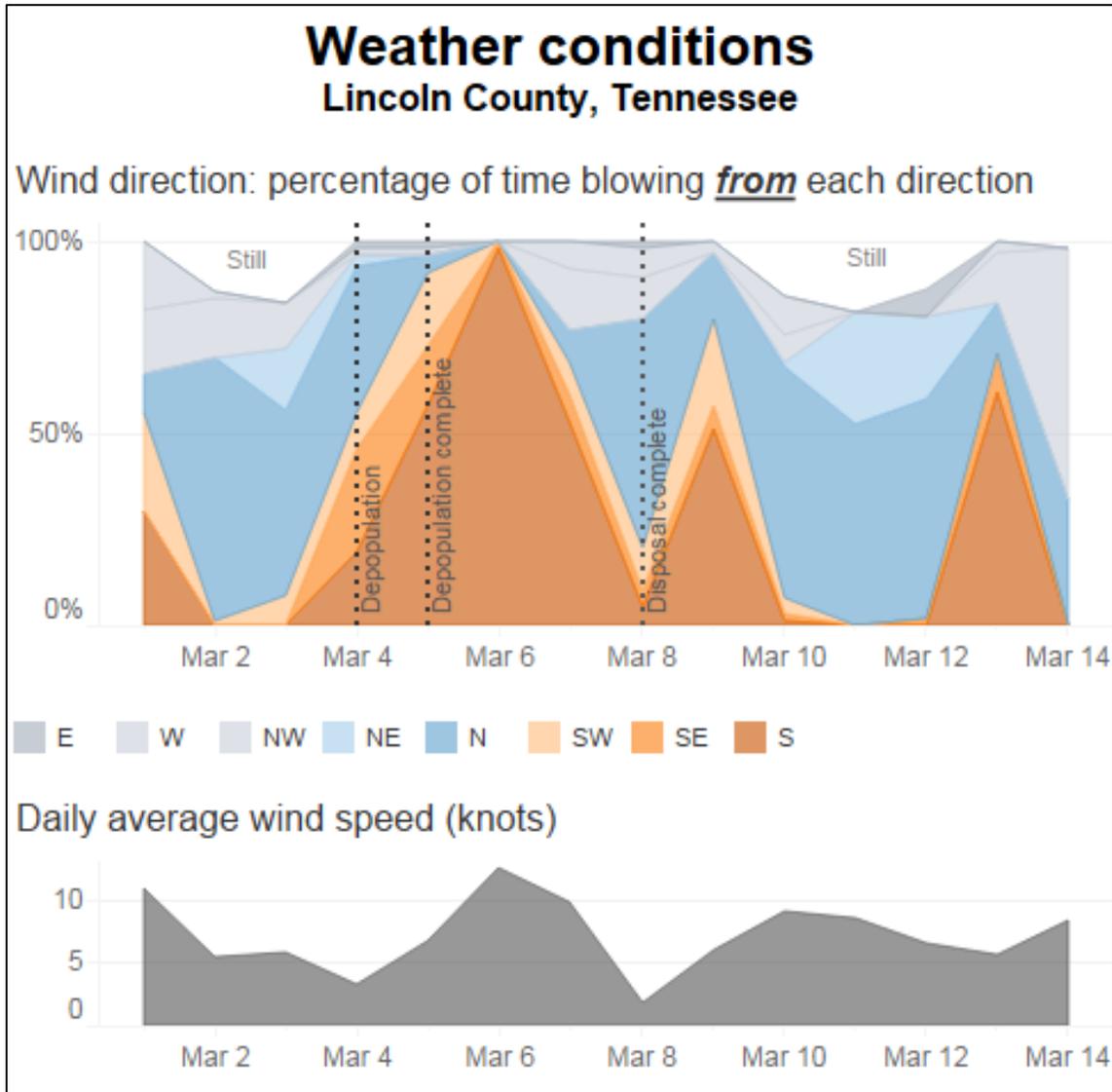
Figure 6. Wind-related weather conditions for Lincoln County, TN during the first two weeks of March

IV. PHYLOGENETIC ANALYSIS AND DIAGNOSTICS

A. North American H7N9 HPAI and LPAI from poultry (AM H7N9 2017)

This section describes AM H7N9 HPAI and LPAI from poultry confirmed by the National Veterinary Services Laboratories (NVSL) during March 2017.[2] These viruses are different from the Asian H7N9 virus from China that emerged in 2013 and continues to impact poultry and humans in Asia.

Two broiler breeder flocks were confirmed with AM H7N9 HPAI within 10 days of one another in Lincoln County, Tennessee. The insertion at the cleavage site responsible for the highly pathogenic phenotype of the AM H7N9 HPAI is likely derived from chicken host 28S ribosomal RNA, suggesting that AM H7N9 LPAI virus was introduced to the first flock and mutated to the highly pathogenic form during virus replication in poultry (Figure 7). Similar insertion events of chicken ribosomal RNA have contributed to the emergence of HPAI in poultry from LPAI from this and other poultry-related H7 IAV.

During the time between the two HPAI detections, AM H7N9 LPAI was confirmed in a broiler breeder flock in Giles County, Tennessee. Additional H7 (and N9, where confirmed) LPAI and presumptive LPAI were detected through zone and routine surveillance and extended into AL, KY, and GA, respectively.

The second flock affected by HPAI was in the control zone and therefore undergoing weekly testing by real-time reverse transcription polymerase reaction (rRT-PCR) for permitting purposes. Just prior to the onset of clinical signs, one of 18 samples (6 pooled samples per house) collected 3/13/2017 and tested at the NAHLN lab had a high Ct by IAV-M PCR, suggesting the presence of a low amount of viral RNA (the subtype tests were negative).[3] While the high Ct was not repeatable at NVSL, HPAI virus was recovered from this sample during a second passage. Clinical signs were reported in the flock on 3/14/2017, and samples collected from sick and dead birds in 3/14/2017 confirmed the presence of HPAI in the same house as the high Ct sample from 3/13/2017. The high similarity between the HPAI viruses from the first and second site, the timing of detection, and the available epidemiological data, indicates the potential for secondary spread of HPAI from the first site to the second site.

---

[2] Virus availability announcements: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/veterinary-biologics/biologics-regulations-and-guidance/ct_vb_notices

[3] Samples with a high Ct by IAV-M, which is the primary surveillance tool, may be negative by the subtype tests that target a different gene.

The AM H7N9 HPAI and LPAI viruses are highly similar across the entire genome (Figure 7; excluding the multibasic amino acid insertion at the cleavage site responsible for the mutation to HPAI) and are of North American wild bird lineage based upon the full genome sequence[4] (North American Cluster III viruses; blue box in Figure 8). [5]

North American H7N9 LPAI has previously been detected in wild birds in the U.S., and a closely related H7N9 LPAI virus (Figure 7; >99.1-99.8% similar across all eight gene segments) was recovered from a Wildlife Services Wild Bird Surveillance sample. This sample was collected from a blue-winged teal in Wyoming as part of a live-bird banding effort in September 2016. Other highly similar gene segments have been identified from North American H7N9, N3, N7, and H11 LPAI in wild birds from 2015-2017 (Figure 9).
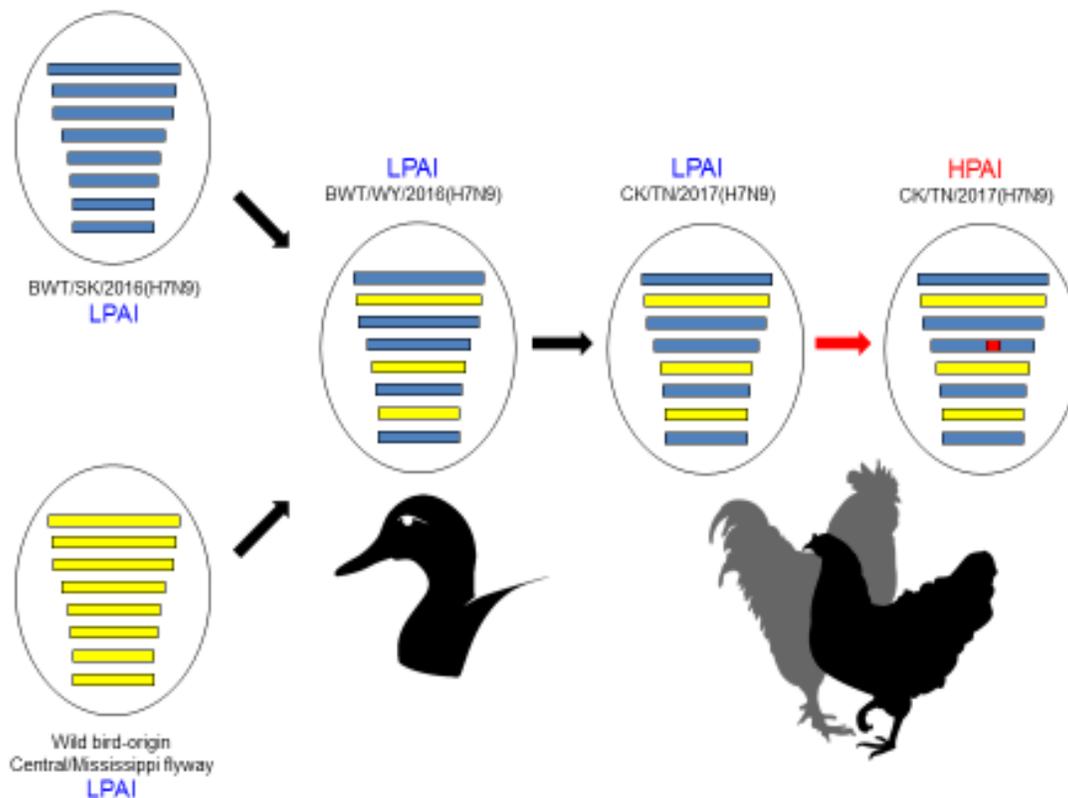
Figure 7. Nearest wild bird precursor viruses contributing to the AM H7N9 gene constellation.

Two wild bird viruses were collected from blue-winged teal: one in Saskatchewan, Canada during August 2016 (BWT/SK/2016; blue bars representing the eight gene segments), and the other in Wyoming during September 2016 (BWT/WY/2016; blue and yellow bars – NOTE: the pictogram with the yellow bars is a theoretical precursor virus based upon phylogenetic analysis). The BWT/WY/2016 and the virus from chickens in TN (CK/TN/2017) are greater than 99% similar across the entire genome.
Courtesy of D.H. Lee, USDA ARS Southeast Poultry Research Laboratory and Y. Berhane, National Centre for Foreign Animal Diseases - Avian Diseases Unit, Canada.

---

[4] Genbank IDs: AM H7N9 HPAI KY818808-818815 and KY818832-818839; AM H7N9 LPAI KY818816-818823

[5] Xu Y, Bailey E, Spackman E, et al. 2016. Contemporary H7 Avian-Origin Influenza A Viruses from North America (Scientific Reports, 6: 20688)

This virus is NOT the same as the Asian H7N9 virus that emerged in China during 2013 and continues to impact poultry and humans. All AM H7N9 2017 viruses detected from this event are of North American wild bird lineage.

Further phylogenetic analyses of the full genome from HPAI viruses from two sites in TN, and LPAI viruses from one commercial flock each in TN and AL, and two AL backyard flocks was conducted. In summary:

1) The AM H7N9 HPAI and LPAI viruses are highly similar across the entire genome (excluding the multibasic amino acid insertion at the cleavage site responsible for the mutation to HPAI) and are of North American wild bird lineage based upon the full genome sequence (Figure 7);

2) The insertion at the cleavage site responsible for the highly pathogenic phenotype of the AM H7N9 HPAI is likely derived from chicken host 28S ribosomal RNA;

3) The virus from the Wyoming blue-winged teal likely represents a precursor to the AM H7N9 2017 viruses found in poultry (Figure 7 and Figure 8); and

4) Molecular, epidemiologic, and serologic (antibody) data suggest the AM H7N9 virus circulated in the area undetected in poultry prior to the initial HPAI detection.

5) Molecular and epidemiologic support exists for a) secondary spread from the first HPAI site to the second, and b) more than one independent introduction of AM H7N9 LPAI, for example the available data show that LPAI/HPAI viruses from TN appear to have a common source and cluster separately from the AL viruses.

Figure 8. Schematic phylogenetic tree of the HA1 nucleotide sequences of H7 AIVs.

The tree was constructed based on the phylogeny inferred by using the maximum-likelihood method. Boxes represent the three major genetic clusters; the Eurasian lineage (EU) is represented by the large black triangle. Am = North American lineage.

The AM H7N9 2017 viruses are located in Cluster III.

Courtesy of Xi-Feng Wan et al, Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University
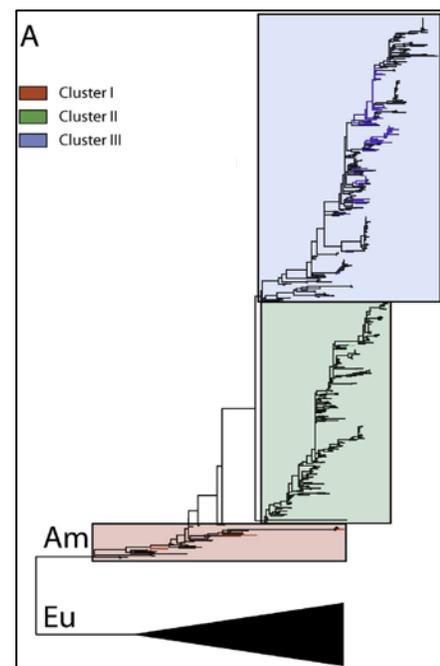
NOTE: The outcomes of phylogenetic analysis should be interpreted in context of all available virus and epidemiologic information and should not be used to infer transmission. Studies to determine virus infectivity and transmission are underway at the USDA ARS Southeast Poultry Research Laboratory.

B. Comparison to Other Viruses/Lineages

Before the current events in 2017, the most recent H7 HPAI event occurred in 2016, affecting turkeys in Indiana. Both the AM H7N9 2017 and Indiana H7N8 2016 events were identified when an LPAI virus mutated to HPAI and samples were collected in response to clinical signs observed in the birds. The distribution of cases and the presence of antibody-positive flocks for the AM H7N9 suggest that detection occurred later during the course of infection as compared to the H7N8 2016, where infection was detected shortly after virus introduction based upon active virus shedding in both HPAI and LPAI affected barns. The H7N8 2016 and AM H7N9 2017 viruses are somewhat related across five (PB2, PB1, HA, NP, MP) of eight genes but are clearly different in the other three genes (PA, NA, NS).
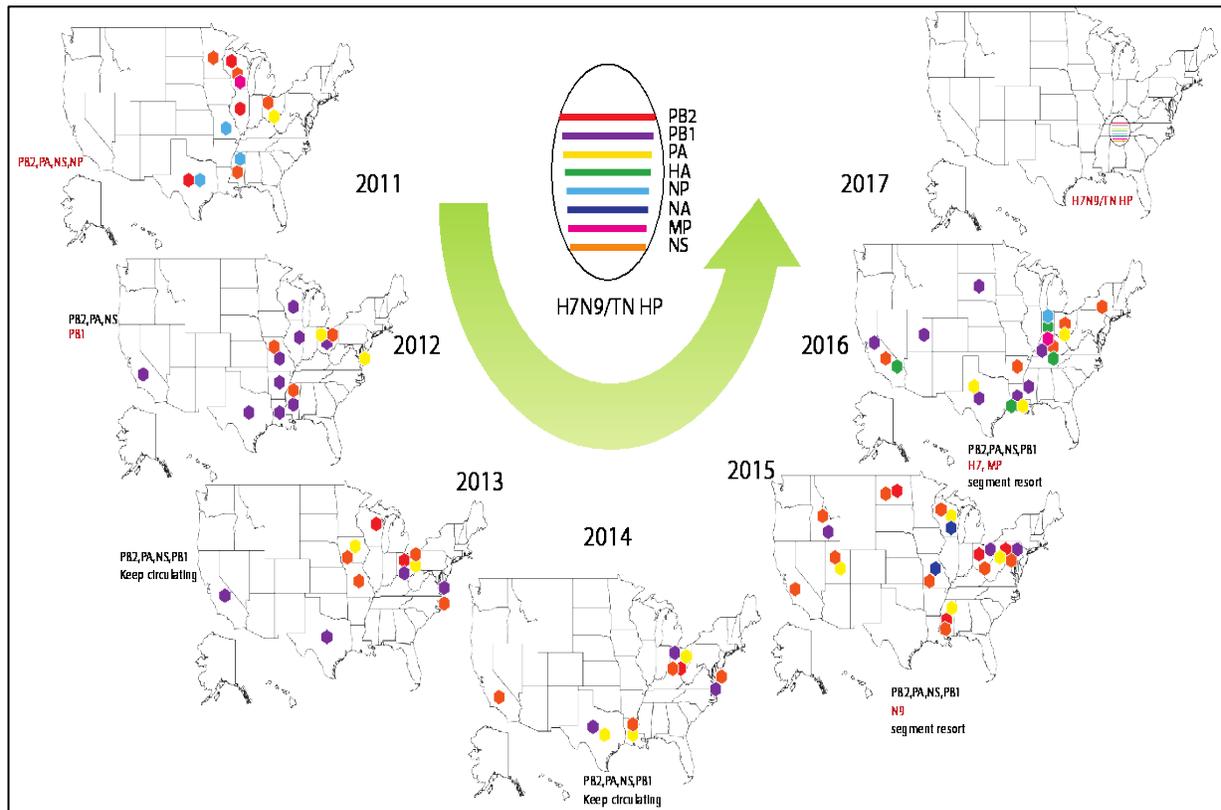
**Figure 9. Distribution and flow of related gene segments in wild bird viruses that contribute to the AM H7N9 2017.**

The colors correspond to different gene segments; the maps show the temporal and geographic distribution of the detection of related gene segments in other wild bird viruses.

Courtesy of Lei Li et al, Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University

There have been other reports of North American H7N9, most recently in 2009 and 2011. Compared to the AM H7N9 2017, both the H7N9 2011 and 2009 viruses show some relatedness across three genes (2011: HA, NA, MP; 2009: PB1, NA, MP), but are different across the other five genes. This finding was expected as IAV reassorts frequently in wild birds; Figure 9 demonstrates the detection of related gene segments in wild bird viruses over time that contribute to the AM H7N9 2017 constellation. Additionally, the H7 of the AM H7N9 2017 belongs to a different lineage compared to the H7N3 HPAI from Mexico reported in the public database.

C. Public Health Aspects

To date, no cases of AM H7N9 2017 virus infection have been reported in humans. Efforts to monitor the health of response workers and on-farm personnel continue.

The virus and sequence have been shared with CDC (refer to this link) for analysis which indicated that the viruses to date 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.

D. 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 the 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 AM H7N9 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 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.

Confirmation of the virus involves determination of the HA and NA subtype, and the pathotype (LPAI vs HPAI). Until NVSL results are available (usually less than 24 hours from sample receipt for H5 and H7), the HA-subtype remains '*presumptive*.' The pathotype is also '*presumptive'* based upon the clinical presentation of the flock compared to the USDA HPAI case definition. NVSL results '*confirm'* the HA and NA subtype (by molecular and/or antibody testing) and the pathotype (LPAI or HPAI by molecular testing); however, where virus is not recovered and sequence cannot be obtained directly from the sample, the pathotype will remain '*presumptive*.'

V.   WATERFOWL SURVEILLANCE

Waterfowl are natural reservoir hosts for influenza A viruses (IAV; subtypes H1-H16), but not usually HPAI. Influenza A viruses in wild birds tend to circulate seasonally within migratory flyways, and subtype prevalence can wax/wane in multiyear cycles. Areas where birds from different flyways congregate provide opportunities for viruses to mix across flyways.

Waterfowl migration in North America generally consists of north-south seasonal movements between breeding grounds and wintering areas. There are four major flyways in North America (Figure 10). These flyways are broadly defined corridors where the migratory paths of many species of interest tend to converge and are associated with major topographical features in North America, which also tend to be aligned along a north-south axis. The four North American flyways have areas of overlap and convergence, particularly at the north and south ends. Flyway boundaries are defined administratively, and are not biologically fixed or sharply defined.

Pacific Flyway
Central Flyway
Mississippi Flyway
Atlantic Flyway

Figure 10. The four primary North American waterfowl flyways

North American flyways represent the predominant pathways of migratory bird movements within broad geographic areas. Many migratory bird species use specific flyways during spring and fall; however, many species migrate across flyways. The Pacific Flyway is most likely the area of introduction for the HPAI viruses detected in Canada and the western United States in December 2014.

The U.S. National Surveillance Plan for Highly Pathogenic Avian Influenza in Wild Birds was implemented in 2015 to maximize our ability to detect IAV in wild waterfowl. Surveillance helps to: 1) understand how IAV is distributed in the United States, 2) detect the spread of IAV to new areas of concern, 3) monitor wild dabbling duck populations for introductions of novel viruses, and 4) estimate the apparent prevalence of IAVs of concern (e.g., Eurasian lineage H5 and H7). The surveillance plan targets areas with extensive mixing of wild bird populations and a history of IAV detection.

Since July 1, 2015, over 80,000 wild waterfowl have been sampled and tested by rRT-PCR for IAV. Overall, the wild waterfowl surveillance has resulted in detection of about 500 H7 viruses across all four flyways. Of these detections, 215 were from the Atlantic and Mississippi Flyways. H7 detections have occurred during summer, fall, and winter and throughout the Mississippi and Atlantic Flyways (Figure 11). Annually, H7 detections tend to peak in early winter. This pattern may have contributed to the timing of the recent outbreak in the southeastern United States.

The H7 graph is based on viruses recovered at NVSL from H7 presumptive samples forwarded by NAHLN laboratories.

- Wild bird surveillance testing follows the NAHLN testing algorithm: samples are first tested by a Type A-specific test (IAV-M) and further tested by the H5/H7 subtype tests in which viral RNA is detected. H5 and H7 samples are forwarded to NVSL, as genetic sequencing is the most reliable test for determining virus subtype(s) in wild birds.

- Wild birds are often exposed to IAV and mount an antibody response that can kill the virus, leading to unsuccessful virus isolations.

The graph for influenza A detections is based upon NAHLN laboratory detections using the Type A-specific test (IAV-M), regardless of the virus recovery status.
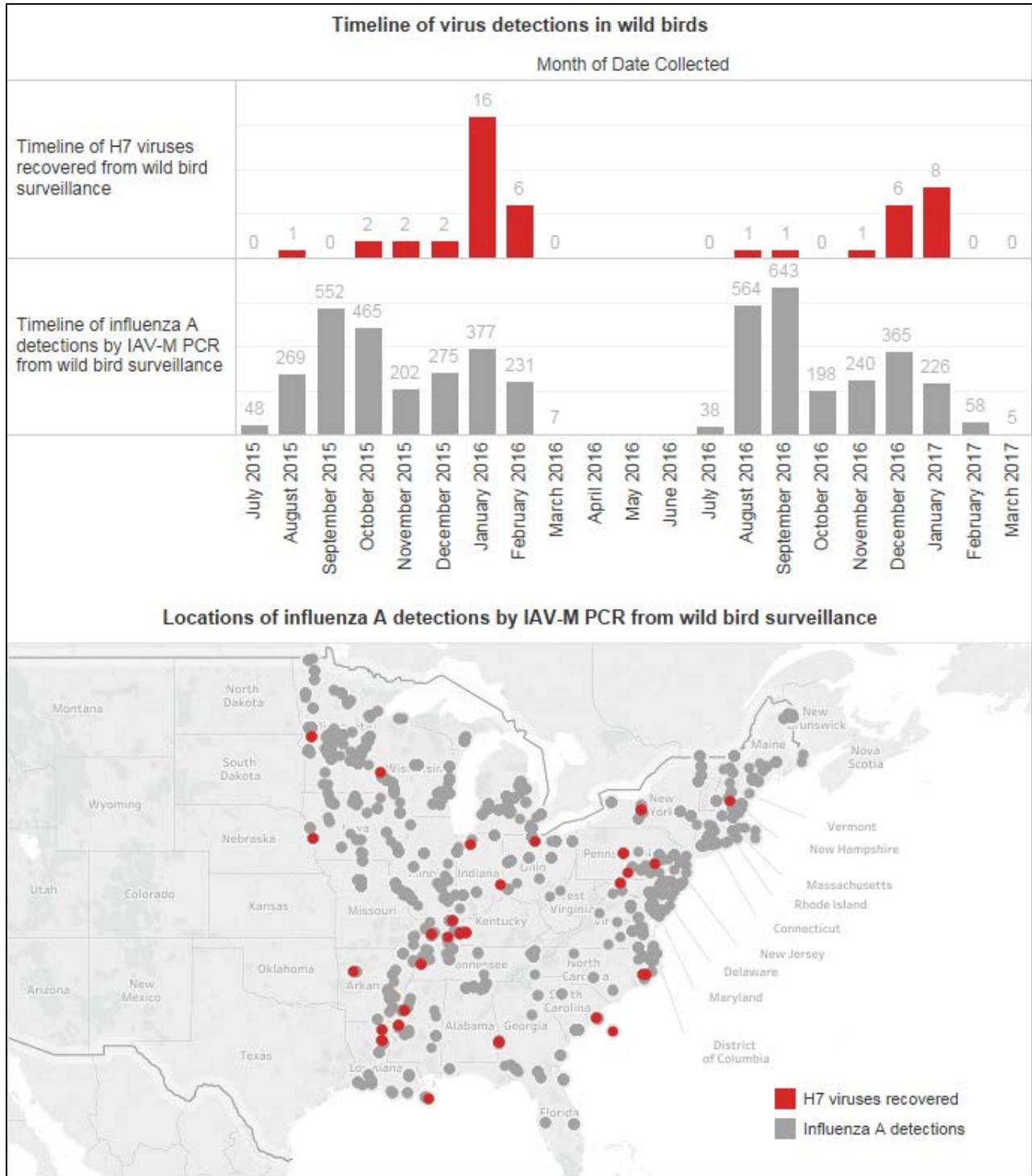
Figure 11. Timeline of virus detections in wild birds (A) and locations of influenza A detections by IAV-M PCR from wild bird surveillance from July 2015 through March 2017 (B)

VI. WILDLIFE ON-FARM SAMPLING

A. Sampling for Avian Influenza Virus in Synanthropic Wildlife at an Infected Premises

Objective

To evaluate the potential of synanthropic wildlife associated with commercial chicken (*Gallus gallus domesticus*) flocks becoming exposed or infected with North American (AM) H7N9 IAV, we sampled peridomestic birds and mammals on a single farm where chickens were infected with AM H7N9 IAV. The timeframe for sampling additional farms for wildlife did not allow collection on other affected premises.

Wildlife sampling at the premises was initiated within 6 six days after virus shedding was confirmed in poultry by the NVSL. The flock was depopulated 4 to 5 days (dependent on barn number) before sampling was initiated. We sampled throughout the farm, but focused in and around the barn with confirmed AM H7N9 IAV infected chickens prior to depopulation.

B. Procedures

Sampling Procedures

Wild birds and wild mammals were captured on the infected farm, primarily around farm structures, with an emphasis (e.g., more traps and nets) on sampling at the barn on this property in which chickens were infected with AM H7N9 IAV. Wild birds were captured using mist nets and baited funnel traps. Wild mammals were trapped using baited collapsible Sherman traps and baited Tomahawk traps. To test for evidence of AM H7N9 IAV infection, captured animals were sampled via swab collection, nasal washes (mammals), and tissues (from some animals). Evidence of prior exposures was investigated by testing serum for the presence of antibodies.

From birds, we collected oral swabs, cloacal swabs, and blood samples (when possible). From mammals, we collected oral swabs, nasal swabs/washes, and blood samples. We also collected lung-tissue samples from targeted species (e.g., house mice and house sparrows). Swabs, washes, and tissue samples were placed in 1-3mL of viral transport media and stored chilled. Blood was collected into serum separator tubes, allowed to clot, and centrifuged. Field personnel shipped samples overnight on ice packs to testing laboratories during the week or stored them in a refrigerator and drove them to laboratories during weekends.

Laboratory Procedures

All samples were screened for influenza A virus (IAV) matrix (M) gene RNA via real-time reverse transcriptase polymerase chain reaction (rRT-PCR). The Veterinary Diagnostic Laboratory at Colorado State University conducted IAV-M rRT-PCR testing of avian oral and cloacal swabs, while the National Wildlife Research Center Virology Laboratory conducted all other IAV-M testing. Samples with Ct>0 by IAV-M were submitted to the NVSL for confirmatory testing. Detections by the IAV-M PCR were tested for presence of the H5 and H7 subtypes, and

genetic sequencing when possible; virus isolation in embryonated chicken eggs was conducted in parallel. All serum samples with sufficient volume and from selected species were screened for antibodies to an IAV using the IDEXX AI Multi-S Screen Ab test, which is a multispecies blocking enzyme linked immunosorbent assay (ELISA) targeting an epitope of the nucleoprotein. Samples were also forwarded to NVSL for hemagglutinin inhibition (HI) testing.

C. Test Results

Wildlife Sampling Results

We sampled 118 individual animals over a 4-day period in March 2017. In all, 61 mammals represented by four species (mice and mesocarnivores) and 57 birds represented by 19 species (primarily passerines) were sampled (Table 3). The most common mammal captured was the house mouse (*Mus musculus),* and the most common bird captured was the American robin (*Turdus migratorius*). Overall, we collected 109 serum samples, 118 oral swabs, 57 cloacal swabs, 61 nasal swab/wash samples, and 57 lung-tissue samples (Table 4).

Table 3. Number of wildlife species sampled at a farm with chickens infected with AM H7N9 IAV, Tennessee, 2017, by species sampled.

Sampled species	Scientific Name	Number Sampled
House mouse	*Mus musculus*	53
Virginia opossum	*Didelphis virginiana*	4
White-footed mouse	*Peromyscus leucopus*	3
Raccoon	*Procyon lotor*	1
	Total Mammals:	**61**
American robin	*Turdus migratorius*	14
White-throated sparrow	*Zonotrichia albicollis*	8
Savannah sparrow	*Passerculus sandwichensis*	6
Northern cardinal	*Cardinalis cardinalis*	5
Song sparrow	*Melospiza melodia*	4
Northern mockingbird	*Mimus polyglottos*	3
Eastern phoebe	*Sayornis phoebe*	3
House finch	*Haemorhous mexicans*	3
House sparrow	*Passer domesticus*	1
Eastern towhee	*Pipilo erythrophthalmus*	1
Blue jay	*Cyanocitta cristata*	1
Vesper sparrow	*Pooecetes gramineus*	1
Dark-eyed junco	*Junco hyemalis*	1
Brown thrasher	*Toxostoma rufum*	1
Carolina wren	*Thryothorus ludovicianus*	1
Yellow-rumped warbler	*Setophaga coronata*	1
Carolina chickadee	*Poecile carolinensis*	1
Eastern bluebird	*Sialia sialis*	1
Mourning dove	*Zenaida macroua*	1
	Total Birds:	**57**
	Total Combined:	**118**

Table 4. Number of samples collected from birds and mammals at a farm with chickens infected with AM H7N9 IAV in Tennessee, 2017, by sample type.

Sample type	Total number collected	Number Collected from Birds	Number Collected from Mammals
Serum	109	49	60
Oral Swab	118	57	61
Cloacal Swab	57	57	---- [a]
Nasal Swab/Wash	61	----[a]	61
Lung Tissue	57	1	56
[a]*Samples not collected.*			

Laboratory Results

All avian swab samples were screened by IAV-M rRT-PCR. A very low level of matrix gene RNA was detected in one (1) of 57 combined oral/cloacal swab samples (represented by a high Ct/Cq value of 39.45). This sample, collected from a savannah sparrow, tested negative by H5 and H7 assays at the original testing laboratory, which was not unexpected as the low level of viral RNA detected approaches the limit of detection for the assay. The sample was forwarded to NVSL where the high Ct was not repeatable (also not unexpected). Virus isolation was not successful on the sample, which was not unexpected. Of interest, the bird was captured adjacent to the barn (approximately 5 meters) that housed AM H7N9 HPAI infected chickens prior to depopulation.

ELISAs were conducted on avian serum samples with sufficient volume and on select mammalian samples. Two of the 42 samples screened showed potential evidence of antibodies reactive to influenza A virus. These results were associated with an American robin (average sample-to-negative [S/N] ratio of 0.525) and a Virginia opossum (Average S/N ratio of 0.665). The S/N ratio of the American robin is consistent with that of one from an American robin sampled in Iowa during 2015, which was confirmed positive for antibodies to icA-H5 by HI (Shriner et al. 2016). To our knowledge, this ELISA has not been previously validated with Virginia opossum sera. The S/N ratio of the suspect positive Virginia opossum (0.665) was moderately lower than the average S/N ratio of the three other opossum serum samples (0.820). Serum samples were forwarded to the NVSL for HI tests. Serum samples that were not prescreened with ELISA tests (all mesocarnivores except the Virginia opossum mentioned above) and with sufficient volume were assayed with an HI for H7 antibodies. None tested positive by HI. In addition, 13 avian samples, primarily those with insufficient volumes for ELISA tests and the single American robin with an S/N ratio of 0.525, tested negative for H7 antibodies by HI. Because the remaining avian serum samples were prescreened by ELISA tests, additional HIs were not conducted.

Wildlife Observational Results

A relatively high rodent burden was noted on the infected farm. Of interest, the barns farthest from the infected barn had the highest densities of mice, many of which were observed primarily utilizing exterior walls of the barns for cover.

Overall, the farm had very small populations (< 10 per species) of invasive birds (house sparrows [*Passer domesticus*] and European starlings [*Sturnus vulgaris*]) during the sampling period. This small population may be indicative of the lack of spilled feed associated with grain hoppers at this premises.

A pond located on the farm's property, likely attracts some wildlife species for water resources. Although we did not directly observe any ducks or geese using this pond during the wildlife sampling period, we did observe wood ducks (*Aix sponsa*) and Canada geese (*Branta canadensis*) flying over the infected premises during the evening on two sequential days. In addition, that same morning a great blue heron (*Ardea herodias*) was observed foraging in the pond.

Anecdotal evidence of other wildlife on the property (those not captured) included potential signs of skunks (*Mephitis* sp.), nine-banded armadillos (*Dasypus novemcinctus*), and coyotes (*Canis latrans*). Regarding the latter, the property owner reported recently observing coyotes on his farm, and the wildlife epidemiology team observed one coyote within 1 mile of the infected premises during the sampling period. Direct observations of groundhogs (*Marmota monax*) on the east side of the farm were also made on multiple days. Some additional avian species observed on the perimeter edge of the farm but not captured included cedar waxwings (*Bombycilla cedrorum*), an unidentified tanager (*Piranga* sp.), white-crowned sparrows (*Zonotrichia leucophrys*), and red-winged blackbirds (*Agelaius phoeniceus*).

D. Summary

It is clear from both wildlife captures and observations that a diversity of wildlife used this facility for various reasons. Initial results did not confirm the presence of AM H7N9 IAV from wild bird or mammal samples; however, limited evidence suggests that one or more animals sampled may have been previously exposed to a different IAV.

E. References

Shriner, S.A., J.J. Root, M.W. Lutman, J.M. Kloft, K.K. VanDalen, H.J. Sullivan, T.S. White, M.P. Milleson, J.L. Hairston, S.C. Chandler, P.C. Wolf, C.T. Turnage, B.J. McCluskey, A.L. Vincent, M.K. Torchetti, T. Gidlewski, and T.J. DeLiberto. 2016. Surveillance for highly pathogenic H5 avian influenza virus in synanthropic wildlife associated with poultry farms during an acute outbreak. Scientific Reports 6:36237.

VII. ACKNOWLEDGEMENTS

We greatly appreciate the cooperation and support of the poultry industry and farm owners for allowing us access to their properties, for providing information on their biosecurity and production practices, and for their full cooperation with this investigation.

We also appreciate the poultry health professionals who participated in the expert elicitation study and the valuable time they shared with our team, as well as the many partners who assisted with this study while serving in the incident command for this outbreak.

This report would not have been possible without a large group of researchers, epidemiologists, laboratory staff, and data scientists who participated in the various analyses and drafting and review of this report. We appreciate their dedication and professionalism in providing the best information possible to help producers reduce the risk of IAV introduction into their operations.

APPENDIX A: CASE SERIES QUESTIONNAIRE

USDA	**HPAI/LPAI**	National Animal Health

	HPAI/LPAI	National Animal Health Monitoring System
USDA	**Questionnaire**	2150 Centre Ave., Bldg B Fort Collins, CO 80526
Animal and Plant Health Inspection Service	**Broilers**	Form Approved OMB Number 0579-0376 Approval Expires: 9/30/2017
Veterinary Services		

INSTRUCTIONS

State and local poultry organizations and the U.S. Department of Agriculture APHIS (USDA–APHIS) are collecting data as part of the avian influenza investigation efforts to identify factors that may contribute to transmission of avian influenza virus to poultry.

We are asking you to fill out this survey, which includes questions about things done daily on the farm, facility and premises condition, deliveries to the farm, and ill birds. We ask about a 3-week (21-day) period on the farm starting on a particular date that we will provide. It might be difficult to remember back that far, so please use a pocket calendar or other agenda manager and any feed and other delivery records that might be available to you.

Term	Case definition	Control definition
Premises	Farm location with flocks confirmed to be avian influenza infected by NVSL, including all barns and buildings, even if not all barns and buildings contain infected birds.	Farm location with no infected birds in any barn or building, in close proximity (less than 10 miles) to the case farm. (If case farm is a broiler breeder, select a non-infected broiler breeder as the control.)
Barn	Barn or building that houses avian influenza-infected birds.	On control premises, a barn or building that does not house any avian influenza-infected birds.

The complete survey is available on request.

HPAI case control
questionnaire_broilers

APPENDIX B: EXPERT ELICITATION QUESTIONNAIRE

2017 LPAI/HPAI Expert Opinion Elicitation

Introduction

Thank you for agreeing to participate in this expert opinion elicitation to rapidly characterize risk and protective mechanisms involved in the 2017 outbreak of H7N9 in Tennessee, Alabama, Georgia, and Kentucky. Our goal is to capture, through your opinions, data similar to what are more typically obtained through case control studies. This approach is much faster, allows experts to balance their responses with prior knowledge and context, and removes any references to specific sites. As a subjective process, it can also be swayed by bias, but, to improve objectivity, we ask for counts of events (rather than probabilities), and ground your counts first in real observations. We also stage a forum in which group results can be challenged and discussed, after which experts will have a chance to individually revise their responses. Individual responses will remain confidential; group median scores and variability will be reported. If it looks like a publication may result, we'll ask you to review the draft manuscript and consider co-authorship, if interested.

Below are a series of questions about plausible routes of HPAI/LPAI introduction onto a farm. We ask that you to respond to these questions in two parts. Part one will reference one or two case (HPAI/LPAI positive) farms and one or two control (HPAI/LPAI negative) farms that you know well. Part two will revolve around a set of hypothetical farms. **Focus on the 6-8 week period <u>before</u> detection** in the case farms, and the same period for their controls. We'll walk you through both processes.

Part One: Real Cases and Controls

Select (without naming) one or more H7N9 broiler breeder case farms (HPAI or LPAI) with which you are most familiar. Call these Case A, Case B, etc. Select (again without naming) two broiler breeder control farm for each case. Call these Control A1, Control A2, Control B1, Control B2 ,etc. **Each control farm should match its case farm in production type (primary versus multiplier breeder), timing of production, size of operations, and within same control area if possible.** Otherwise, it should simply be a farm with which you are most familiar. Please answer the following questions about each of these cases and controls.

1. Spiking Male Source   Responses
Did the farm test spiking male source populations by a protocol geared to detect a
recent introduction of LPAI/HPAI?

	Case	Controls

   a. Yes, <u>including</u> PCR in the pre-movement test, targeting sick or dead birds for sampling, and routinely including PCR to investigate clinical signs or drops in production
   b. No, using ACIA or serology or only sampling healthy birds for testing, or using only serology to investigate clinical signs or drops in production

2. Pullet Source
Did the farm source pullets from populations first tested by a protocol geared to detect
a recent introduction of LPAI/HPAI?

	Case	Controls

   a. Yes, <u>including</u> PCR in the pre-movement test, targeting sick or dead birds for sampling, and routinely including PCR to investigate clinical signs or drops in production
   b. No, using ACIA or serology or only sampling healthy birds for testing, or using only serology to investigate clinical signs or drops in production

3. Wind
Was the farm situated downwind (and within a mile or so) of a source of infective
material (e.g., positive farm, disposal location or depopulation/disposal traffic)?

	Case	Controls

   a. Yes
   b. No

4. Habitat Disruption
Did the farm experience conditions that may attract local rodent/wildlife (e.g., sale of a
neighboring farm, disruption of perimeter habitats, or local feed/waste spills)?

	Case	Controls

   a. Yes
   b. No

5. Integrated Poultry Complex
Was the farm part of a poultry complex with other farm(s) subsequently found to be
LPAI/HPAI positive?

	Case	Controls

   a. Yes, the complex included another infected farm
   b. No, the complex does not include other infected farms, but periodically had to externally source feed or pullets
   c. No, the complex did not include other infected farms, and did not have to externally source feed or pullets

6. <u>Vehicle Biosecurity</u>
Which best describes biosecurity measures used for vehicles involved in bird delivery, egg pickup or bird processing or disposal?

     Case   Controls

   a. Minimal biosecurity precautions
   b. Moderate biosecurity precautions (e.g., interior and exterior of vehicles, including tires, are cleaned and disinfected <u>daily</u>)
   c. Strong biosecurity precautions (e.g., interior and exterior of vehicles, including tires, are cleaned and disinfected <u>between</u> farms)

7. <u>Operation Equipment Biosecurity</u>
Which best describes biosecurity used for equipment shared between farms and in contact with birds or eggs (e.g., farm racks, egg flats, carts, egg buggies, bird crates)?

     Case   Controls

   a. Minimal biosecurity precautions
   b. Strong biosecurity precautions (e.g., cleaned and disinfected before entry to farm)

8. <u>Farm Equipment Biosecurity (tillers, skid steers, etc)</u>
Which best describes biosecurity for farm equipment used in chicken houses?

     Case   Controls

   a. Minimal biosecurity precautions
   b. Moderate biosecurity precautions (e.g., cleaned and disinfected before entry to houses; if sharing between farms washed after use)
   c. Strong biosecurity precautions (e.g., cleaned and disinfected before entry to houses, <u>no sharing between farms</u>)

9. <u>Shared Disposal Routes</u>
Did the farm share mortality disposal destinations (renderers, compost piles, burial pits, etc) with other farms subsequently found to be LPAI/HPAI positive?

     Case   Controls

   a. Yes, shared with a farm subsequently found LPAI/HPAI positive
   b. Yes, shared with other farms, but unsure which ones
   c. Either (1) shared but <u>not</u> with farms subsequently found LPAI/HPAI positive, or (2) not shared with other farms

10. <u>Catch Crews and Processing</u>
Did the farm process chicken houses at different times (*not including* depopulation)?

     Case   Controls

   a. Yes, processed barns at different times (split pickup)
   b. Yes, held multiple age classes but no processing during time period of interest
   c. No, processes barns simultaneously (all-in all-out)

11. <u>Outside Personnel and Visitors with Barn Access</u>
Which best describes biosecurity precautions required of external personnel/visitors with access to chicken houses (e.g., company service representatives, health professionals, maintenance, technicians)?

     Case   Controls

   a. Minimal biosecurity precautions
   b. Moderate biosecurity precautions (e.g., clean clothes, hands, footwear; site-specific gear; visitors do not enter barns unless absolutely necessary)
   c. Strong biosecurity precautions (e.g., above plus biosecurity briefing, and signed statement declaring any recent contact with birds)

12. <u>Workers with Barn Access</u>
Which best describes biosecurity precautions taken by workers with access to chicken houses?

     Case   Controls

    a.   Minimal biosecurity precautions

    b.   Moderate biosecurity precautions (e.g., employees use site-specific gear, employees are not employed at more than one operation)

    c.   Strong biosecurity precautions (e.g., above plus employees do not own or hunt birds; employees receive biosecurity training in a language in which they are fluent)

13. **Foot Baths at Entry to Chicken Houses**

Which best describes foot bath use and maintenance on this farm?   Case   Controls

    a.   Minimal biosecurity precautions (e.g., foot baths, hand wash stations at most entrances)

    b.   Moderate biosecurity precautions (e.g., above plus foot baths at <u>all entrances</u> including garage doors and interior doors, use required at all times, no way to bypass, no pets)

    c.   Strong biosecurity precautions (e.g., above, plus <u>change footbaths daily</u> or more frequently if dirty, pre-wash or cover footwear to <u>remove organics</u> before stepping in footbath)

14. **Egg Room Access**

Does the farm unload and wheel egg carts into egg or work rooms across an outdoor delivery area?   Case   Controls

    a.   Yes, some biosecurity (e.g., carts cleaned at hatchery, concrete pad)

    b.   Yes, strong biosecurity (e.g., above, plus concrete pad cleaned/disinfected prior to use)

    c.   No, <u>fully enclosed</u> loading area, cleaned and disinfected between each use

15. **Enclosure**

Are the chicken houses in need of repair?   Case   Controls

    a.   Yes, tears, cracks, or defects in foundations, doors, walls or curtains

    b.   Normal wear and tear, maintenance planned for end of production cycle

    c.   No visible defects

16. **Water Supply**

Does the farm use any untreated surface water for consumption, cooling or cleaning?   Case   Controls

    a.   Yes

    b.   No

17. **Farming Density**

Is the farm in a region densely populated by poultry farms?   Case   Controls

    a.   Densely populated by poultry farms (> 3 facilities per square mile)

    b.   Moderately populated by poultry farms (1-3 facilities per square mile)

    c.   Sparsely populated by poultry farms (< 1 facility per square mile)

18. **Environment (geology, wetlands, etc.)**

What best describes the environment of the farm?   Case   Controls

    a.   A wetland, stream, or marshy area on the property or adjacent property <u>with</u> large congregations of waterfowl

    b.   A wetland, stream or marshy area on the property or adjacent property <u>without</u> large congregations of waterfowl

    c.   Dry

19. Raccoon, skunk, or similar wildlife
Was there any indication of raccoon, skunk or similar wildlife disturbance (e.g., tracks/scat indoors, under the slats, or at entrances, or evidence of predation)?   Case   Controls
    a.   Yes
    b.   No

20. Wild birds
Was there any evidence of any wild bird disturbance (e.g., nesting in the eaves, or found indoors)   Case   Controls
    a.   Yes
    b.   No

21. Rodents
Was there clear evidence of rodent infestation?   Case   Controls
    a.   Heavy infestation (e.g., rodents seen inside during daylight, or visible damage to grounds or curtains)
    b.   Some infestation (e.g., rodents present, but low numbers)
    c.   None observed

22. Curtain Siding
Does the farm have curtain siding?   Case   Controls
    a.   Yes, curtain siding
    b.   No, solid side walls

Part Two: Hypothetical Scenario

In this section, you'll answer the same series of questions about a hypothetical scenario.
Imagine a hypothetical setting and industry that is very much like what currently exists in the affected region of Tennessee, Alabama, Georgia and Kentucky. Let's also imagine a hypothetical outbreak of H7N9 HPAI/LPAI that is very much like what this region has recently experienced. The only thing that we're going to change is the numbers (and names) of the operations affected: this particular mock outbreak has impacted a larger number of facilities. Assume that all the other defining characteristics of the outbreak (timing, weather, spread patterns, genotype, response, etc.) remain the same.

Next, imagine a random selection of 40 *commercial broiler breeder farms* (belonging to multiple companies) from this mock outbreak. 20 are HPAI or LPAI case farms and 20 are controls. Answer the following questions for this random selection of farms. Your answers are hypothetical, but should reflect your field experience and knowledge of the current TN/AL/KY/GA situation. Focus on the 6-8 week period <u>before</u> detection in the case farms, and the same period of time for their controls. We'll need your responses first for the 20 case farms, and separately for the 20 controls.

1. <u>Spiking Male Source</u>   Responses
How many of the 20 farms tested spiking male source populations by a protocol geared to
detect a recent introduction of LPAI/HPAI?

	Cases	Controls
a. Geared, including PCR in the pre-movement test, targeting sick or dead birds for sampling, and routinely including PCR to investigate clinical signs or drops in production	a.	a.
b. Not geared, using ACIA or serology or only sampling healthy birds for testing, or using only serology to investigate clinical signs or drops in production	b.	b.

2. <u>Pullet Source</u>
How many of the 20 farms sourced pullets from populations first tested by a protocol geared
to detect a recent introduction of LPAI/HPAI?

	Cases	Controls
a. Geared, including PCR in the pre-movement test, targeting sick or dead birds for sampling, and routinely including PCR to investigate clinical signs or drops in production	a.	a.
b. Not geared, using ACIA or serology or only sampling healthy birds for testing, or using only serology to investigate clinical signs or drops in production	b.	b.

3. <u>Wind</u>
How many of the 20 farms were situated downwind (and within a mile or so) of a source of
infective material (e.g., positive farm, disposal location or depopulation/disposal traffic)?

	Cases	Controls
a. Downwind	a.	a.
b. Not downwind	b.	b.

4. <u>Habitat Disruption</u>
How many of the 20 farms experienced conditions that may attract local rodent/wildlife (e.g.,
sale of neighboring farms, disruption of perimeter habitats, or local feed/waste spills)?

	Cases	Controls
a. Attraction	a.	a.
b. No attraction	b.	b.

5. <u>Integrated Poultry Complex</u>

How many of the 20 farms were part of a poultry complex with other farm(s) subsequently found to be LPAI/HPAI positive?

	Cases	Controls
a. Yes, the complex included another infected farm	a.	a.
b. No, the complex did not include other infected farms, but periodically had to externally source feed or pullets	b.	b.
c. No, the complex did not include other infected farms, and did not have to externally source feed or pullets	c.	c.

6. Vehicle Biosecurity

How many of the 20 farms employed biosecurity for vehicles involved in bird delivery, egg pickup or bird processing or disposal using precautions best described as follows?

	Cases	Controls
a. Minimal biosecurity precautions	a.	a.
b. Moderate biosecurity precautions (e.g., interior and exterior of vehicles, including tires, are cleaned and disinfected <u>daily</u>)	b.	b.
c. Strong biosecurity precautions (e.g., interior and exterior of vehicles, including tires, are cleaned and disinfected <u>between</u> farms)	c.	c.

7. Operational Equipment Biosecurity

How many of the 20 farms employed biosecurity for equipment shared between farms and in contact with birds or eggs (e.g., farm racks, egg flats, carts, egg buggies, bird crates) using precautions best described as follows?

	Cases	Controls
a. Minimal biosecurity precautions	a.	a.
b. Strong biosecurity precautions (e.g., cleaned and disinfected before entry to farm)	b.	b.

8. Farm Equipment Biosecurity (tillers, skid steers, etc)

How many of the 20 farms employed biosecurity for farm equipment used in chicken houses following precautions best described below?

	Cases	Controls
a. Minimal biosecurity precautions	a.	a.
b. Moderate biosecurity precautions (e.g., cleaned and disinfected before entry to houses; if shared between farms washed after use)	b.	b.
c. Strong biosecurity precautions (e.g., cleaned and disinfected before entry to houses, <u>no sharing between farms</u>)	c.	c.

9. Shared Disposal Routes

How many of the 20 farms shared mortality disposal destinations (renderers, compost piles, burial pits, etc) with other farms subsequently found to be LPAI/HPAI positive?

	Cases	Controls
a. Shared with a farm subsequently found LPAI/HPAI positive	a.	a.
b. Shared with other farms, but unsure which ones	b.	b.
c. Either (1) shared but <u>not</u> with farms subsequently found LPAI/HPAI positive, or (2) not shared with other farms	c.	c.

10. Catch Crews and Processing

How many farms processed their chicken houses at different times (*not including depopulation*)?

	Cases	Controls
a. Yes, processed barns at different times (split pickup)	a.	a.
b. Yes, held multiple age classes but no processing during time period of interest	b.	b.
c. No, processed barns simultaneously (all-in all-out)	c.	c.

11. Outside Personnel and Visitors with Barn Access

How many of the 20 farms employed biosecurity for external personnel or visitors with access to chicken houses (e.g., company service representatives, health professionals, maintenance, or technicians) using precautions best described as follows?

	Cases	Controls

a. Minimal biosecurity precautions

b. Moderate biosecurity precautions (e.g., clean clothes, hands, footwear; site-specific gear; visitors do not enter barns unless absolutely necessary)

c. Strong biosecurity precautions (e.g., above plus biosecurity briefing, and signed statement declaring any recent contact with birds)

	a.	a.
	b.	b.
	c.	c.

12. <u>Workers with Barn Access</u>

How many of the 20 farms employed biosecurity for workers with access to chicken houses using precautions best described as follows?

a. Minimal biosecurity precautions

b. Moderate biosecurity precautions (e.g., employees use site-specific gear, employees are not employed at more than one operation)

c. Strong biosecurity precautions (e.g., above plus employees do not own or hunt birds; employees receive biosecurity training in a language in which they are fluent)

	Cases	Controls
a.	a.	a.
b.	b.	b.
c.	c.	c.

13. <u>Foot Baths at Entry to Chicken Houses</u>

How many of the 20 farms utilized foot baths following precautions best described as follows?

a. Minimal biosecurity precautions (e.g., foot baths, hand wash stations at most entrances)

b. Moderate biosecurity precautions (e.g., above plus foot baths at <u>all entrances</u> including garage doors and interior doors, use required at all times, no way to bypass, no pets)

c. Strong biosecurity precautions (e.g., above, plus <u>change footbaths daily</u> or more frequently if dirty, pre-wash or cover footwear to <u>remove organics</u> before stepping in footbath)

	Cases	Controls
a.	a.	a.
b.	b.	b.
c.	c.	c.

14. <u>Egg Room Access</u>

How many of the 20 farms unload and wheel egg carts into egg or work rooms across an outdoor delivery area?

a. Yes, some biosecurity (e.g., carts cleaned at hatchery, concrete pad)

b. Yes, strong biosecurity (e.g., above, plus concrete pad cleaned/disinfected prior to use)

c. No, <u>fully enclosed</u> loading area, cleaned and disinfected between each use

	Cases	Controls
a.	a.	a.
b.	b.	b.
c.	c.	c.

15. <u>Enclosure</u>

In how many of the 20 farms are chicken houses in need of repair?

a. Tears, cracks, or defects in foundations, doors, walls, or curtains

b. Normal wear and tear, maintenance planned for end of production cycle

c. No visible defects

	Cases	Controls
a.	a.	a.
b.	b.	b.
c.	c.	c.

16. <u>Water Supply</u>

How many of the farms use untreated surface water for consumption, cooling or cleaning?

a. Untreated

b. Ground or treated water only

	Cases	Controls
a.	a.	a.
b.	b.	b.

17. <u>Farming Density</u>

How many of the 20 farms are in poultry regions best described as follows?

a. Densely populated by poultry farms (> 3 facilities per square mile)

b. Moderately populated by poultry farms (1-3 facilities per square mile)

c. Sparsely populated by poultry farms (< 1 facility per square mile)

	Cases	Controls
a.	a.	a.
b.	b.	b.
c.	c.	c.

18. <u>Environment (geology, wetlands, etc.)</u>
How many of the 20 farms are situated in environments best described as follows?

	Cases	Controls
a. A wetland, stream, or marshy area on the property or adjacent property <u>with</u> large congregations of waterfowl	a.	a.
b. A wetland, stream or marshy area on the property or adjacent property <u>without</u> large congregations of waterfowl	b.	b.
c. Dry	c.	c.

19. <u>Raccoon, skunk, or similar wildlife</u>
How many of the 20 farms have indication of raccoon, skunk or similar wildlife disturbance (e.g., tracks/scat indoors, under the slats, or at entrances, or evidence of predation)?

	Cases	Controls
a. Indication	a.	a.
b. No indication	b.	b.

20. <u>Wild birds</u>
How many of the 20 farms have evidence of any wild bird disturbance (e.g., birds nesting in the eaves or seen indoors)

	Cases	Controls
a. Evidence	a.	a.
b. No evidence	b.	b.

21. <u>Rodents</u>
How many of the 20 farms have clear evidence of rodent infestation?

	Cases	Controls
a. Heavy infestation (e.g., rodents seen inside during daylight, or visible damage to grounds or curtains)	a.	a.
b. Some infestation (e.g., rodents present, but low numbers)	b.	b.
c. None observed	c.	c.

22. <u>Curtain Siding</u>
How many of the 20 farms have curtain siding?

	Cases	Controls
a. Curtain siding	a.	a.
b. Solid side walls	b.	b.

Open Response
1. What do you think is protecting the broilers and table-egg layers?

2. What do you think protected your control farm you selected in the first section?

Epidemiologic and Other Analyses of HPAI/LPAI Affected Poultry Flocks

June 26, 2017 Report