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Epidemiologic and Other Analyses of Avian Influenza Affected Poultry Flocks

May 25, 2018 Report

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I. Executive Summary

An outbreak of low pathogenic avian influenza (LPAI) was detected in early March, 2018. The outbreak was first detected through routine pre-movement testing of a commercial meat-type turkey farm in southern Missouri; H7N1 low pathogenicity avian influenza (LPAI) of North American wild bird lineage was confirmed via laboratory testing. Subsequently H7N1 LPAI was confirmed in a commercial broiler breeder farm in northeast Texas and a backyard chicken breeder farm in southern Missouri. Following response activities, a series of epidemiological and genetic investigations were initiated to better understand these findings across two states, three counties, and in apparently unrelated facilities. 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 agricultural personnel, and the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS).

Genetic analysis determined that all H7N1 viruses detected from this event are of North American wild bird lineage and the viruses are 99% similar across all eight genes. Initial genetic and epidemiologic evidence suggest the possibility of three independent introductions. Analysis of mortality records and diagnostic test results suggest that H7N1 was most likely introduced into the first infected farm in early February. Based upon the previous and current yearly Interagency Wild Bird Surveillance data in the U.S., H7 detections in wild birds tend to rise in early winter as the H5 detections decrease. Other wild bird lineage H7 outbreaks in poultry have been detected during the first three months of the year (January – March).

The two commercial H7N1 LPAI farms involved different production types and different integrated poultry companies suggesting unique sources of feed, birds, transport trucks and crews. The companies did not share epidemiologic links such as common conveyances, visitors, equipment, or disposal activities. Risk factors for virus introduction into barns from the environment that have been identified in past outbreaks such as the presence of rodents or other wild mammals and waterfowl near barns, the condition of the housing, and breaches in biosecurity protocols were likely. The fact that a backyard operation was among the impacted farms adds weight to the evidence that this outbreak was governed in large part by exposure to environmental sources of virus.

II. Introduction

In response to the H7N1 LPAI outbreaks in commercial and backyard poultry in MO and TX, USDA-APHIS and the affected states have initiated epidemiologic and genetic 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 the commercial-case farms using data collected through site visits and interviews with farm personnel
- Analysis of barn-level mortality and diagnostic test data from the earliest affected commercial farm to estimate the date of introduction of LPAI
- Virus phylogenetic analysis

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

Description of Outbreak

On 2 March 2018, the USDA-APHIS was notified that a pre-slaughter sample from a commercial meat-type turkey farm in Jasper County, MO tested positive by H7 PCR at the Missouri Department of Agriculture Veterinary Diagnostic Laboratory. The National Veterinary Services Laboratories (NVSL) confirmed H7 on 3 March 2018 and N1 was confirmed on 5 March 2018. A few days later in Texas, H7N1 LPAI was confirmed in commercial broiler breeders on 7 March 2018 in Hopkins County. Back in MO, a third case of H7N1 LPAI was confirmed in a backyard chicken breeder farm on 15 March 2018 in Webster County (Figure 1, Table 1). All three farms were detected through routine pre-movement testing of birds or eggs. Clinical signs were not apparent. Whole genome sequencing of virus isolated from all three of the farms by NVSL identified North American wild bird lineage H7N1 LPAI (AM H7N1 2018) that are >99% similar across the entire genome. Surveillance in the areas around the three farms to determine the status of other farms was immediately initiated. A total of 10 commercial and 55 backyard premises in TX and MO were tested through surveillance with no additional detected farms.

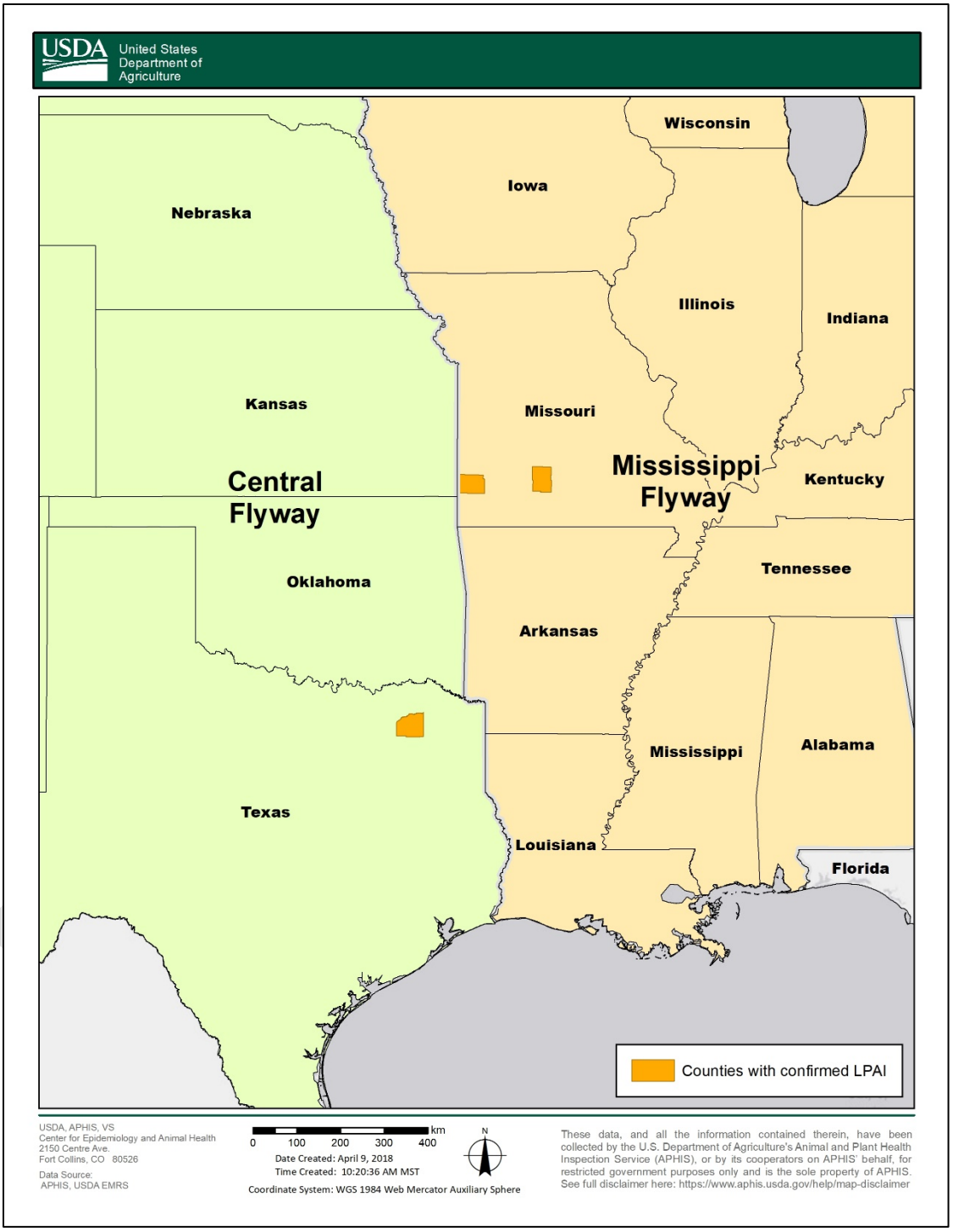


Figure 1. Counties with confirmed findings of H7N1 LPAI since 1 March 2018 and administrative flyway designation of affected and surrounding states.

Table 1. Location, production type, and confirmation date of flocks infected by LPAI H7N1.

State	County	Production Type	Confirmation Date
Missouri	Jasper	Commercial Turkey	3 March 2018
Texas	Hopkins	Commercial Broiler Breeder	7 March 2018
Missouri	Webster	Backyard Chicken Breeder	15 March 2018

III. Epidemiologic Study to Investigate the H7N1 Virus in Commercial Poultry in Texas and Missouri

A. Case Series

USDA-APHIS conducted a case-series study of the H7N1 affected commercial poultry operations. The purpose of this study was to generate hypotheses for potential risk factors for infection with the H7N1 avian influenza virus. Additionally, the study respondents provided mortality and egg production data. Questions focused on the 3 weeks prior to the positive test result (Appendix A: Case Series Questionnaire). Results of the questionnaires are summarized in Table 2.

- Flocks appeared normal at testing. Retrospective records review revealed either minor respiratory symptoms or minor production losses at both farms prior to the positive AI test.
- Neither farm had water bodies within 350 yards of the houses; however, there was a stream approximately 500 yards from one and small ponds approximately 500 yards from the other.
- Workers did not visit other farms with poultry or live with workers from other poultry facilities.
- Feed truck deliveries were reported as the most frequent type of visitor at both farms, followed by company service personnel. Other visitors included propane delivery and a company veterinarian.
- The method of dead bird disposal was reported as composting on site for both, neither farm used renderers.
- Each farm is associated with a different parent company. No epidemiological links between the two farms were identified.

Results suggest that while the two farms exhibited some common practices and features, the significance is difficult to interpret with just two cases and in the absence of control farm data.

Table 2. Characteristics of H7N1 LPAI infected commercial poultry farms.

Characteristic	Level	n	Comments
Bird type	Meat-type turkey Broiler breeder	1 1	
Clinical signs		2/2	Minor respiratory symptoms or production losses
Water body within 350 yards	Pond Stream	0/2 0/2	Stream approximately 500 yards from 1 farm, surface water ponds approximately 500 yards from 1 farm
Other Poultry	no	2/2	
Visitors	Company service personnel Feed delivery personnel Egg truck personnel	2/2 2/2 1/1	
Equipment shared with other farms	no	2/2	
Vehicles enter the farm	Feed Delivery Company personnel Egg truck	2/2 2/2 1/1	
Dead bird disposal method	Composting Incineration Rendering	2/2	
Ventilation type	Curtain Tunnel Ceiling/eaves	2/2	
Fans located at end of barn		2/2	
Birds introduced onto premises	no	2/2	
Manure from another premises brought onto premises	no	2/2	
Workers visited other premises with poultry	no	2/2	
Crews (e.g., catch or vaccination) visited premises	no	2/2	

IV. Estimating the Time of H7N1 LPAI Infection for the Commercial Meat-type Turkey Flock in Missouri using Diagnostic Test Results and Flock Mortality Data

Summary

Determining the time of LPAI virus introduction in a flock is an important part of outbreak investigations intended to identify the factors that likely contributed to virus introduction. By narrowing the time window of possible introduction, we can better isolate the potential routes of virus introduction and enhance our understanding of the pattern of disease spread. Consequently, we used the diagnostic test results and flock mortality data from each of the 4 barns on the premises of the commercial meat-type turkey operation in Missouri to estimate the most likely time of introduction of H7N1 LPAI virus onto the farm, and the most likely barn first infected. Similar data were not available for the layer flock at the time of this analysis.

Using a disease transmission model, we simulated LPAI spread within a turkey barn and estimated the daily percentage of infectious and seropositive birds in the barn over time. Based on the results of this model, we estimated the time of LPAI introduction, given the observed diagnostic test results.

Based on the results of our model and the observed diagnostic test results, the time of introduction of LPAI onto the premises in Barn 2 was estimated to be around 7 February, with a range of possible introduction dates extending from 31 January to 10 February. Barn 2 was likely to have been the first barn to become infected, considering the higher proportion of diagnostic samples with high hemagglutination inhibition (HI) titers. LPAI was estimated to have been introduced later into Barn 4, on 12 February, with a range of possible dates between 8 February and 14 February; and this barn continued to test positive via rRT-PCR even on later sampling dates.

As with all analytic models, this analysis is subject to some data limitations. Information on the characteristics of disease spread of the specific LPAI virus from the recent outbreak were not available, so data from published studies of other H7N1 viruses were used in our disease transmission model. Strain variability and species susceptibility may impact virus transmission. Additional studies of the LPAI Missouri virus isolate will help improve future estimates of farm infection dates.

Methods Overview

Summary of LPAI outbreak data

Outbreak data available for this analysis consisted of rRT-PCR, virus isolation (VI), and serological test results, including ELISA and hemagglutination inhibition (HI) tests. A summary of test results is provided in Table 3. Additional diagnostic test details including rRT-PCR Ct values and HI titers are provided in Table 4 and Table 5, respectively. Daily flock mortality data for each of the 4 barns were also used in the analysis.

Table 3. Summary of surveillance protocols and test results from the epidemiological investigation of a LPAI outbreak in a commercial meat-type turkey flock in Missouri, date the sample was collected, purpose of the surveillance sample, sampling protocol, and qualitative interpretation of results.

Date collected	Purpose	Sample	Results
26 February	National Poultry Improvement Program (NPIP)	1 pool of 6 swabs[a]	rRT-PCR positive, VI negative
2 March	Foreign Animal Disease (FAD) investigation	1 pool of 11 swabs per-Barn[b]	All Barns rRT-PCR positive, all VI negative
		10 serum samples per-Barn	All positive by ELISA and by HI
8 March	Controlled marketing	3 pools of 11 swabs per-Barn	2 pools from Barn 4 rRT-PCR positive, remainder negative, 1 of 2 VI positive
14 March	Controlled marketing	3 pools of 11 swabs per-Barn	2 pools from Barn 4 rRT-PCR positive, remainder negative, both VI negative

[a] *NPIP pre-slaughter surveillance protocol*
[b] *4 Barns on the premises*

Table 4. Test results (Ct values[a]) reported for various rRT-PCR tests conducted on samples from the 4 barns of the commercial meat-type turkey flock in Missouri.

rRT-PCR test event	Ct value
26 February NPIP - 1 pool tested at the NAHLN laboratory and at NVSL	Ct 34.8
2 March FAD investigation - 4 pooled samples tested at NVSL (sera collected at the same time reported in Table 5)	Barn 1 at Ct 37.8; Barn 2 at Ct 39.3; Barn 3 at Ct 38.8; Barn 4 at Ct 39.7
8 March Controlled marketing - 12 pooled samples tested at NVSL	Barn 4 sample 1 at Ct 36.4; Barn 4 sample 2 at Ct 39.7
14 March Controlled marketing - 12 pooled samples tested at NAHLN – 2 samples to NVSL for VI only	Barn 4 (front) Ct 39.2; Barn 4 (center) 39.3

[a] *Ct (cycle threshold) values are inversely proportional to the amount of virus nucleic acid in the sample. So the lower the Ct value, the greater the amount of virus genetic material in the sample.*

Table 5. H7 hemagglutination inhibition (HI) assay titers from serum samples collected on 2 March 2018. HI titers for all Barns suggest that Barn 2 may have been the site of the original introduction, followed by Barns 1 and 3, with Barn 4 affected last. Titers >=1:8 are suggestive of exposure.

Barn 1	Barn 2	Barn 3	Barn 4
≥ 1:32	**≥ 1:32**	**≥ 1:32**	**≥ 1:32**
≥ 1:32	**≥ 1:32**	**≥ 1:32**	1:16
≥ 1:32	**≥ 1:32**	**≥ 1:32**	1:16
≥ 1:32	**≥ 1:32**	**≥ 1:32**	1:16
≥ 1:32	**≥ 1:32**	**≥ 1:32**	1:16
≥ 1:32	**≥ 1:32**	**≥ 1:32**	1:8
1:16	**≥ 1:32**	1:16	1:8
1:16	**≥ 1:32**	1:16	1:8
1:16	1:16	1:8	1:8
1:8	1:16	1:8	1:8

Overview of modeling approach

To estimate the possible dates of LPAI introduction into a barn, we used a disease transmission model to predict the percentages of infectious and seropositive birds in a barn over time. Because data on the transmission characteristics for the recently isolated North American H7 LPAI virus in turkeys were not available for this analysis, we estimated virus characteristics using data from a review of published literature. Specifically, we used studies describing the characteristics of the 1999 Italian H7N1 LPAI virus strain.

The results of this model were used to estimate the time of LPAI introduction, based on the observed diagnostic test results. Specifically, the likelihood of observing each set of diagnostic test results was calculated for different possible dates of LPAI introduction (from 4 to 45 days prior to 2 March 2018). The average likelihood possible introduction dates given all test results was calculated by running the model 10,000 times.

In some of the barns, there was an observed mild increase in mortality for only a few days within one month of the 2 March rRT-PCR positive test result. To improve our estimates of the day of introduction, we combined the results of our model using the diagnostic test results with the observed mortality data. When the observed timing of the peak in bird mortality, estimated by the disease transmission model, most closely matched the observed date of peak mortality, we considered the estimated date of introduction from the disease transmission model to be the most likely date.

Results

The pattern of diagnostic test results was the same for Barns 1, 2 and 3 (ELISA and rRT-PCR positive on 2 March, and rRT-PCR test negative on 8 March). As expected, the estimated date of LPAI introduction based on these results was also similar for these barns. LPAI may have been first introduced into Barn 2 given the greater proportion of samples with higher HI titers for this barn on 2 March (Table 5). The likelihood of observing the diagnostic test results from birds in Barn 2 on each test date, and the combined likelihood of observing all three sets of test results (ELISA on 2 March, rRT-PCR on 2 March and rRT-PCR on 8 March) are provided in Figure 2. The top graph in Figure 2 shows the likelihood of observing each set of diagnostic test results for a range of possible LPAI introduction dates. By taking into account:

a) available serology results which are impacted by the birds immune response,

b) positive rRT-PCR results which are impacted by presence of the virus, and

c) negative test results seen on 8 March (bottom graph of Figure 2),

the likely time of introduction was estimated around 7 February with a range from 31 January to 10 February.

Table 6. Results for the estimated date of LPAI introduction for Barns 1, 2, and 3.

	Based only on observed diagnostic test results		Based on observed diagnostic test results and the timing of peak mortality	
Barn	**Mean (90% C.I.)**	**Most likely**	**Mean (90% C.I.)**	**Most likely**
Barn 2	6 February (31 Jan-10 Feb)	7 February	8 February (4 Feb-11 Feb)	8 February
Barn 3	6 February (31 Jan-10 Feb)	7 February	9 February (5 Feb-11 Feb)	9 February
Barn 1	6 February (1 Feb-11 Feb)	7 February	NA	NA

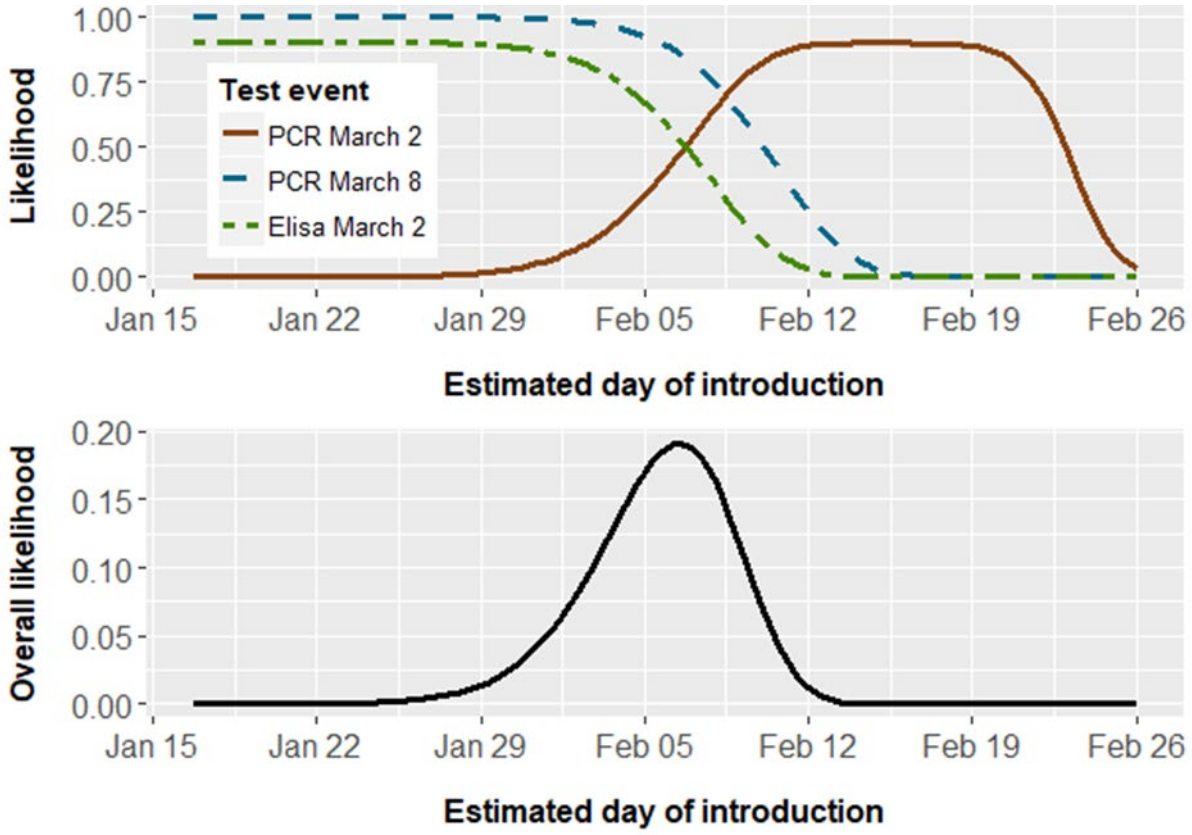


Figure 2. The estimated likelihood of LPAI introduction into Barn 2 on various days based on the results from each diagnostic test event (top) and the combined likelihood for all the test events (bottom). The diagnostic test events include all tests conducted on birds in Barn 2 (Table 3).

As shown in Figure 3**,** observed peak mortality for Barn 2 occurred on 26 February when the mortality on the last day of growing was excluded (mortality on this day was likely related to culling before the flock was processed). When the timing of the peak mortality was taken into account, the estimated most likely introduction date shifted slightly (**Figure** 4) from 7 February to 8 February (range of dates between 4 February to 11 February). A similar approach was used for Barn 1, 2, and 3 and the most likely introduction dates are shown in Table 6.

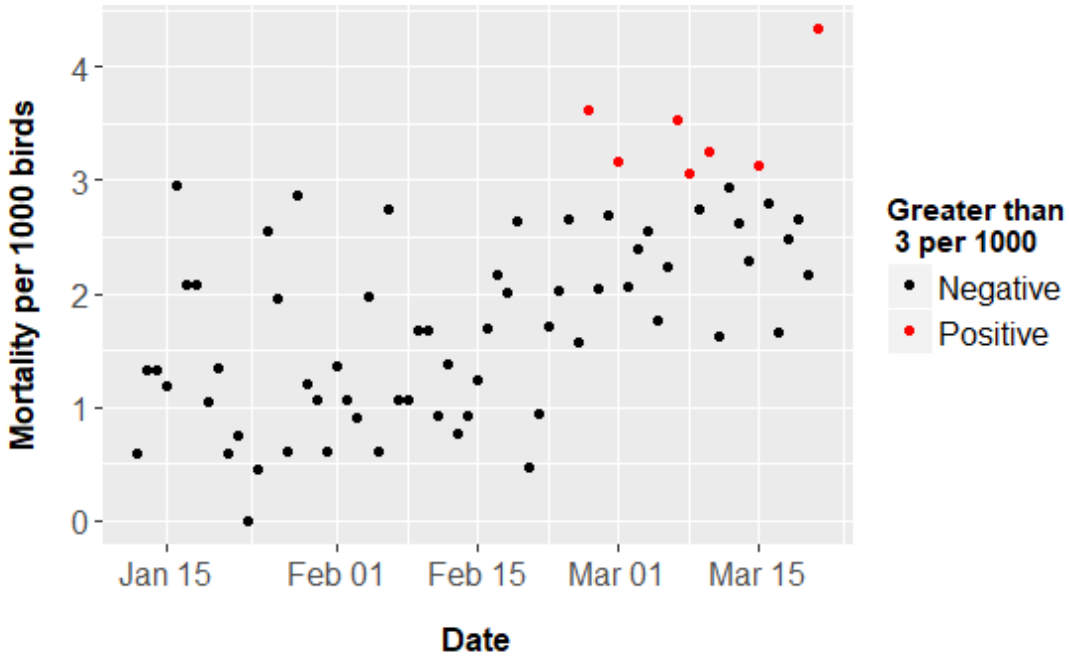


Figure 3. Daily mortality per 1000 birds in Barn 2.

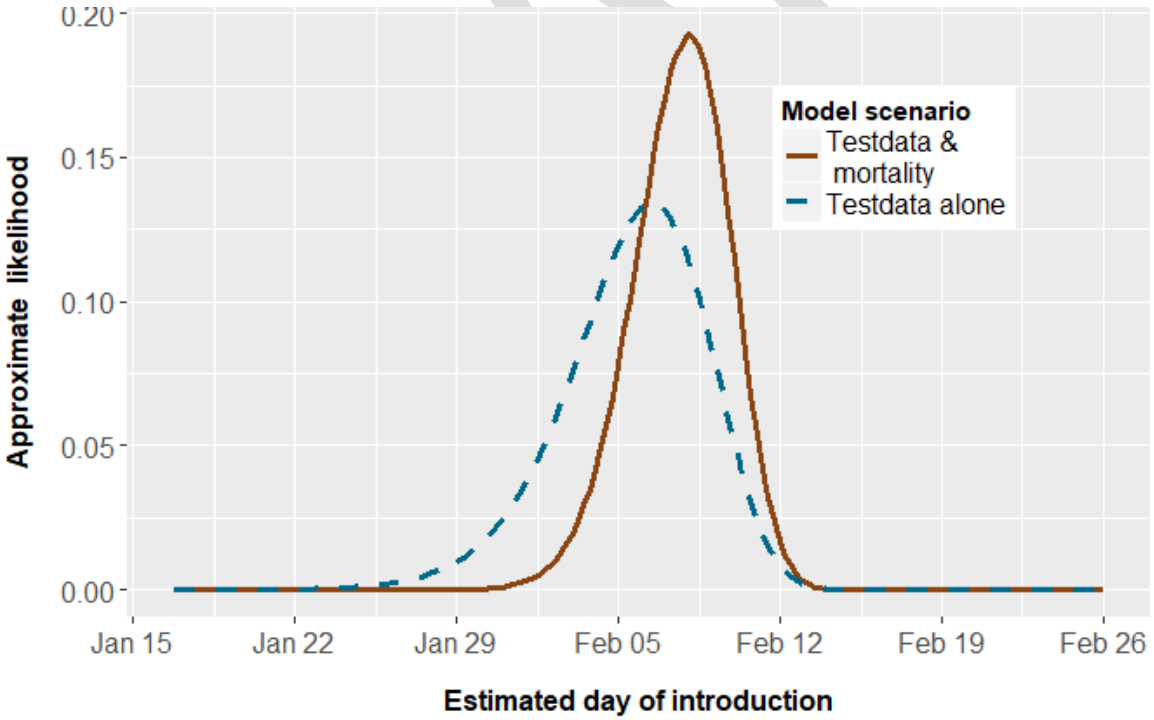


Figure 4. The approximate estimated likelihood of LPAI introduction into Barn 2 on various days based only on diagnostic test results (blue dashed line), and considering diagnostic tests as well as the timing of peak mortality (red solid line)

The diagnostic test results were different for Barn 4. In this case, LPAI was estimated to be introduced later relative to the other barns as shown in Figure 6, based on the observed positive rRT-PCR results on later sampling dates, as well as the lower HI titers from samples collected on 2 March. The date of introduction estimated from test results alone was 12 February (range of dates between 8 February to 14 February), and 13 February (range of dates 10 February to 15 February) when diagnostic test results and the observed peak mortality were considered together (Figure 7). Note that as shown in Figure 5, the peak mortality occurred a few days later (4 March) for this barn relative to Barn 2, potentially indicating a later time of introduction.

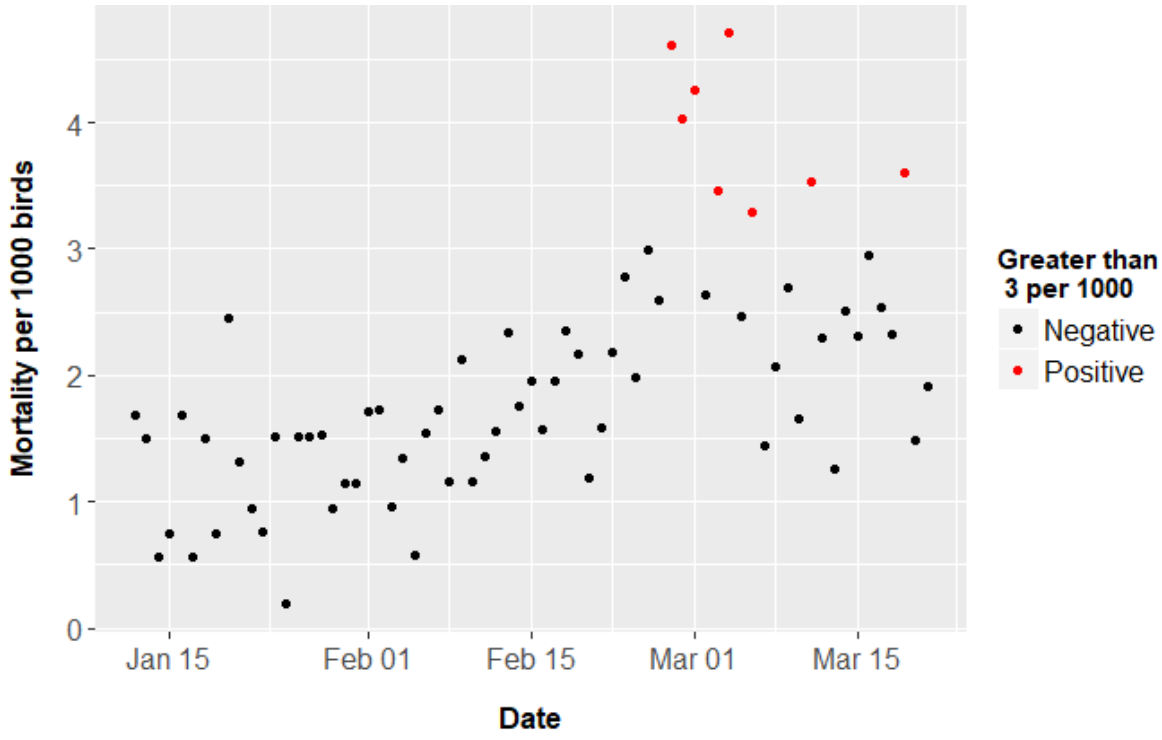


Figure 5. Daily mortality per 1000 birds in Barn 4.

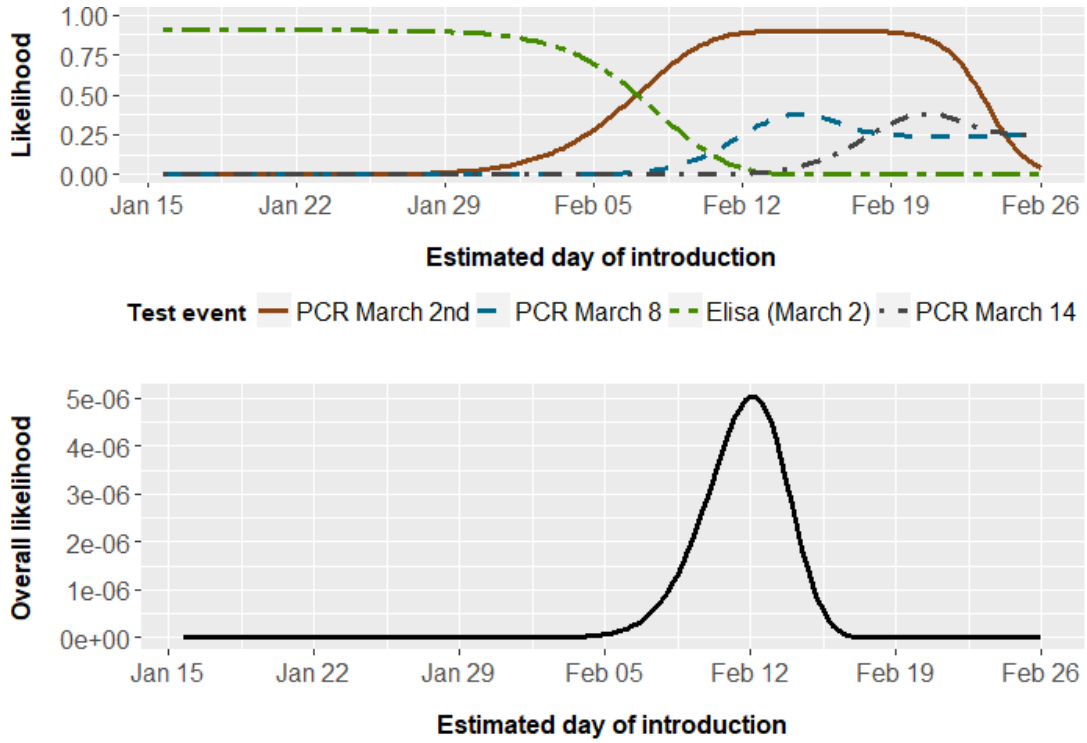


Figure 6. The estimated likelihood of LPAI introduction into Barn 4 on various days based on individual diagnostic test events (top) and the combined likelihood for all the test events (bottom). The diagnostic test events considered include all diagnostic tests conducted on birds in Barn 4 (Table 3).

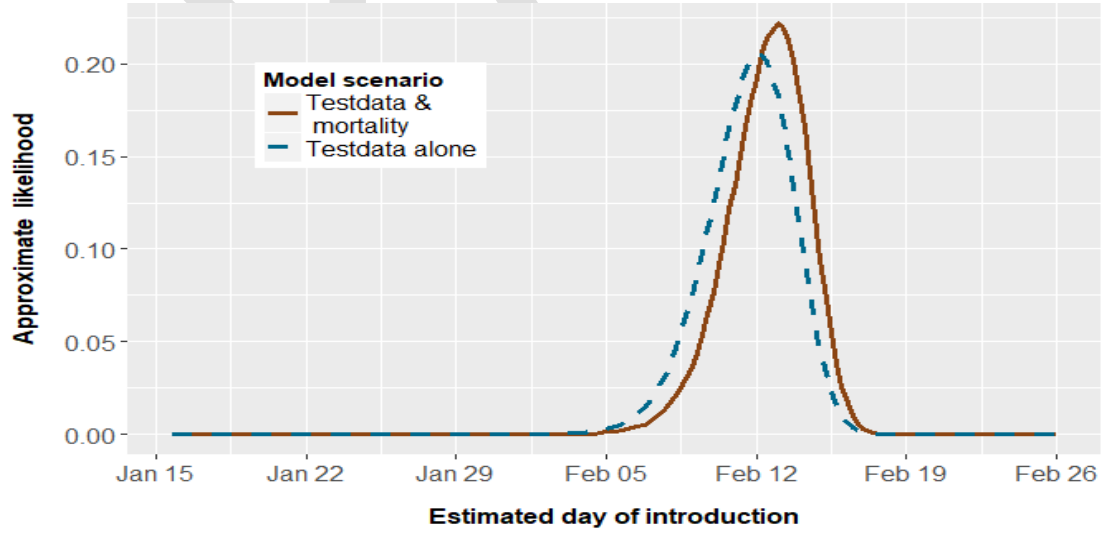


Figure 7. The approximate estimated likelihood LPAI introduction into Barn 4 on various days based only on diagnostic test results (blue dashed line), and considering diagnostic tests as well as the timing of peak mortality (red solid line).

Discussion

Diagnostic test results and flock mortality data can be used to estimate the stage of infection of a LPAI infected flock, as well as the potential day of virus introduction. We estimated an approximate range for the time of LPAI introduction into barns on a commercial meat-type turkey operation in Missouri using a stochastic disease transmission model and other analytic methods. The most likely timing of LPAI introduction onto the premises was estimated to be 8 February, with a 90% Confidence Interval between 31 January to 10 February. Barn 2 was also likely to have been the first barn to become infected. LPAI was estimated to have been introduced later into Barn 4 in the interval from 8 February to 14 February (90% C.I.) consistent with the presence of viral RNA based upon rRT-PCR on later sampling dates.

The estimated time of introduction based on our analyses is an approximation for a variety of reasons including that data from only one farm was available to develop the model, the characteristics of the LPAI virus infecting the farm were largely unknown, and the number and frequency of diagnostic tests were limited. The majority of experimental data used to estimate virus characteristics were based on the 1999 Italian H7N1 LPAI virus strain. Differences in disease transmission between the Italian H7N1 LPAI virus and the current Missouri H7N1 LPAI outbreak strain may have affected the accuracy of the predictions for the date of introduction. Similarly, most experimental studies estimating seroconversion in LPAI infected turkeys do not collect samples daily, making it a challenge to estimate the date of seroconversion following exposure to the virus. Therefore, we assumed that the time to seroconversion is similar to the time when birds are shedding virus. This assumption was based on similar work in broiler breeders where more data are available.

Overall, the current analysis demonstrates that by evaluating the results of tests detecting virus (rRT-PCR) and tests detecting immune reaction in infected birds (ELISA and HI), as well as barn-level bird mortality data, we can estimate the date of LPAI introduction into an infected flock. As more data become available, these types of analyses may improve our ability to determine routes of virus introduction to farms, better understand seasonal and environmental risk, and to characterize subsequent disease spread.

V. Phylogenetic Analysis and Diagnostics

Phylogenetic Analysis and Diagnostics

North American H7N1 LPAI from poultry (AM H7N1 2018)

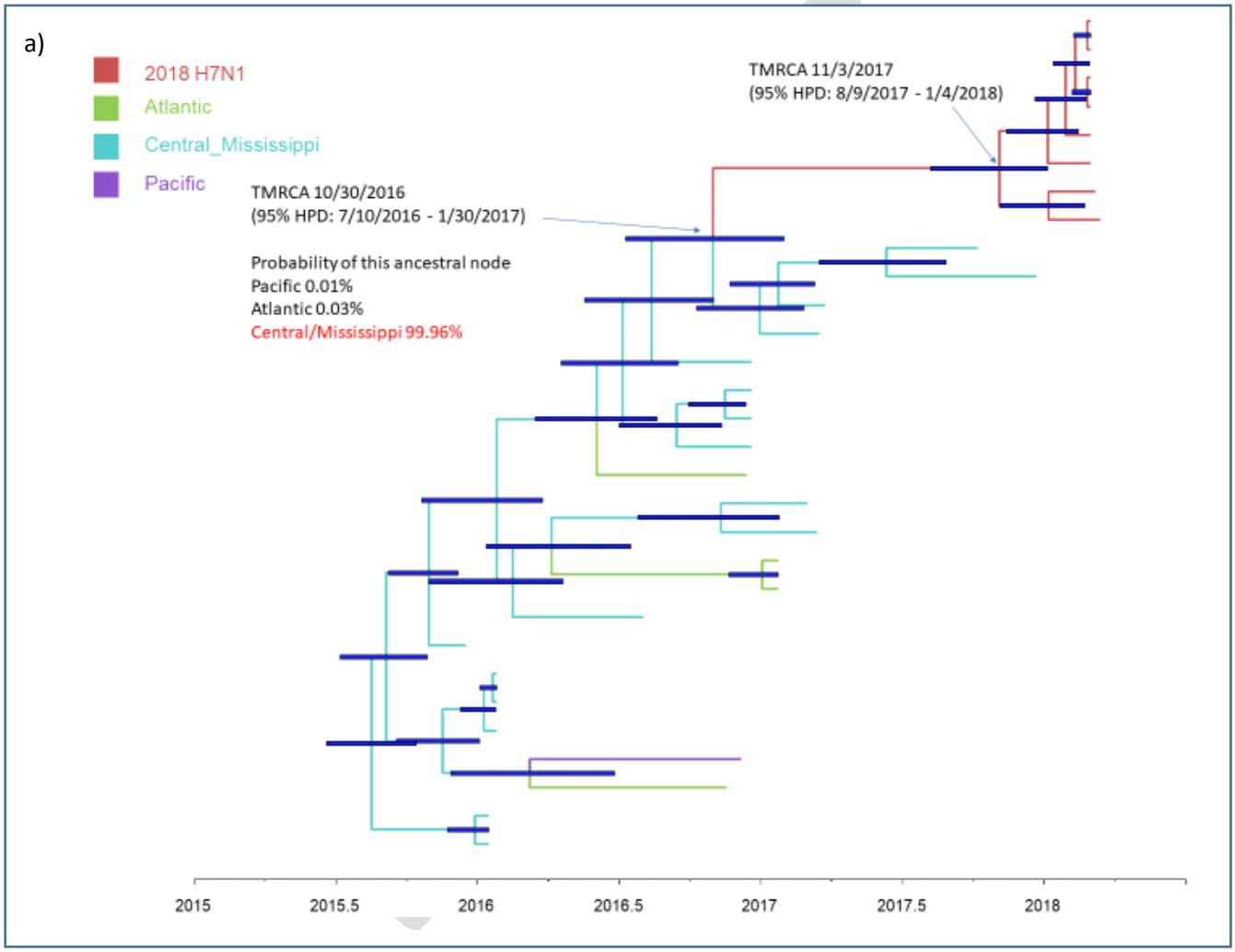
This section describes H7N1 LPAI from poultry confirmed by the National Veterinary Services Laboratories (NVSL) during February and March 2018. The first detection was from a routine pre-slaughter commercial turkey flock sample collected 26 February 2018 in Jasper County, Missouri (MO). Virus was recovered and characterized from Barn 4 as North American wild bird lineage H7N1 LPAI with an intravenous pathogenicity index (IVPI) of zero as defined by OIE.

Shortly after the Missouri detection, samples from a broiler breeder flock in Texas (TX) collected 2 March 2018 were confirmed by H7 and N1 antibody from sera, and H7 LPAI based upon direct sequence attempt from a swab sample. An H7N1 LPAI virus was characterized from these samples and found to have >99% identity with the MO H7N1 virus. The third detection was from

a backyard flock sample collected 14 March 2018 in MO; this virus also had >99% similarity to the other MO and TX H7N1 viruses across the entire genome.

The current phylogenetic analysis of viruses from these three events support independent introductions from a common source, with a >99.9% probability that the introductions arose from wild birds in the Central/Mississippi flyway (Figure 8). The lack of epidemiologic links further supports this finding (refer to Section III.).

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.



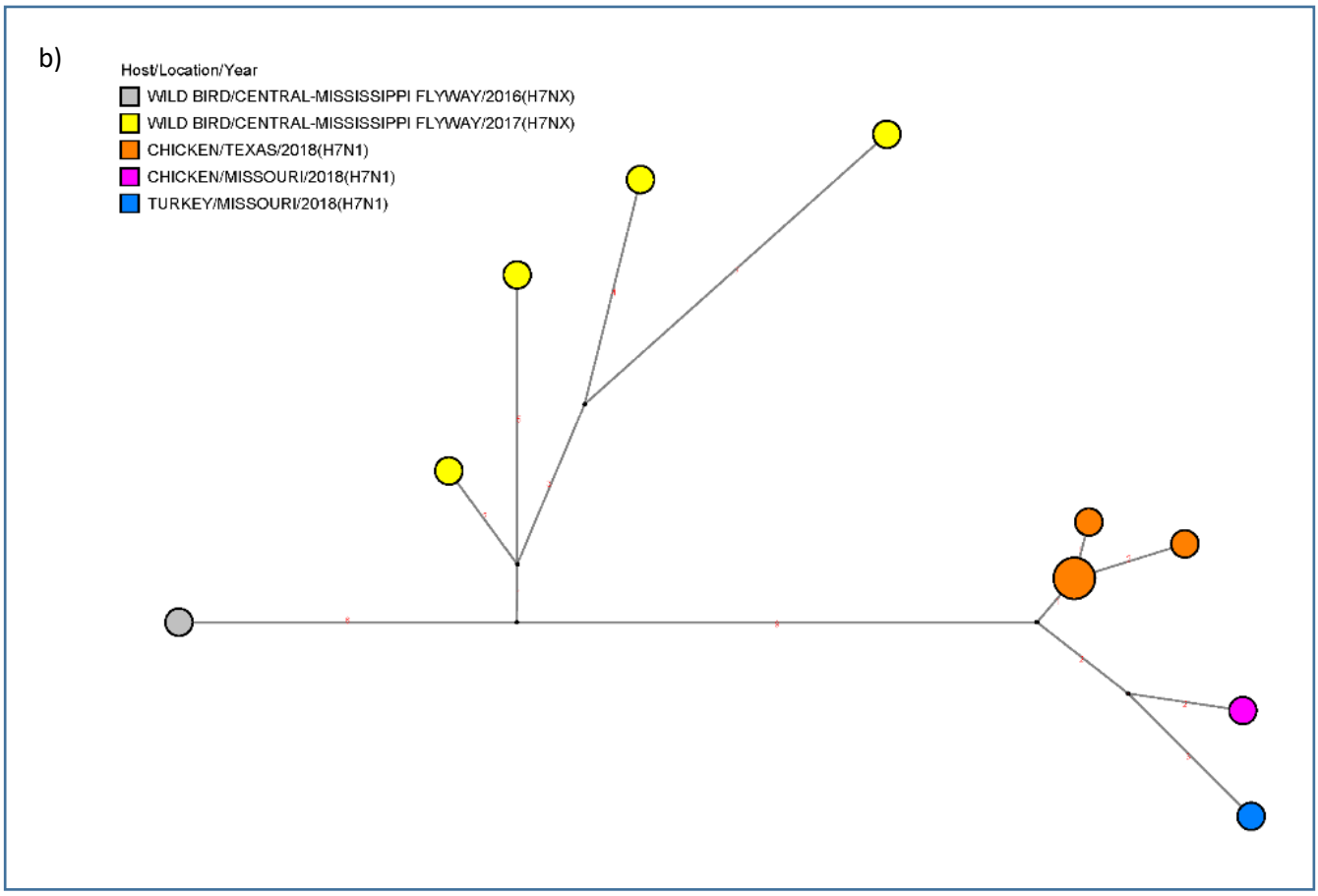


Figure 8. Phylogenetic analysis of viruses from these three events support independent introductions from a common source: a) indicates >99.9% probability that the introductions arose from wild birds in the Central/Mississippi flyway (abbreviations: TMRCA = estimated time to mean common ancestor; HPD=highest posterior density); b) network analysis of the HA gene. Courtesy of Interagency wild bird surveillance data, ML Killian NVSL, and DH Lee USDA-ARS Southeast Poultry Research Laboratory.

Comparison to Other Viruses/Lineages

The H7N1 virus clusters with recent wild bird viruses and apart from other recent H7 poultry detections (H7N8 2016 and H7N9 2017) across the entire genome. The neuraminidase gene of the H7N1 was also compared to a recent H6N1 virus detected in Arkansas broiler breeders late February 2018. The neuraminidase sequences did not cluster together, but similarity was noted for one of the internal genes (PB1) between the H7N1 and H6N1 viruses. Although highly similar gene segments were identified from the recent Interagency wild bird surveillance, particularly from the Central/Mississippi flyway, no single wild bird virus ancestor was identified for the H7N1 identified in MO and TX poultry. This result suggests that reassortment event(s) occurred prior to introduction to poultry.

Public Health Aspects

To date, there have been no reports of H7N1 LPAI 2018 virus infection in humans. The health of response workers and on-farm personnel was monitored at the state level. The virus sequences have been shared with CDC 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.

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, USDA-APHIS 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 H7N1 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); where no virus can be recovered nor sequence obtained directly from sample(s), the pathotype is determined by the clinical presentation of the flock compared to the USDA-APHIS HPAI case definition.

VI. 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 9). 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.

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.

Figure 9. The four primary North American waterfowl flyways

Since January 1, 2016, nearly 80,000 wild waterfowl have been sampled and tested by rRT-PCR for IAV, with H7 detected in over 550 samples across all four flyways. Of the H7 detections, nearly 250 were from the Central and Mississippi Flyways. H7 detections have occurred during

summer, fall, and winter and throughout the Central and Mississippi Flyways (Figure 10). Annually, H7 detections in wild waterfowl tend to peak in early winter. This pattern may have contributed to the timing of the recent events in Missouri and Texas.

The graph for influenza A detections is based upon PCR testing at NAHLN laboratory by the Type A-specific (IAV-M) and H7 tests, regardless of the virus recovery status.

- 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 where IAV 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 isolation in subsequent testing of samples.

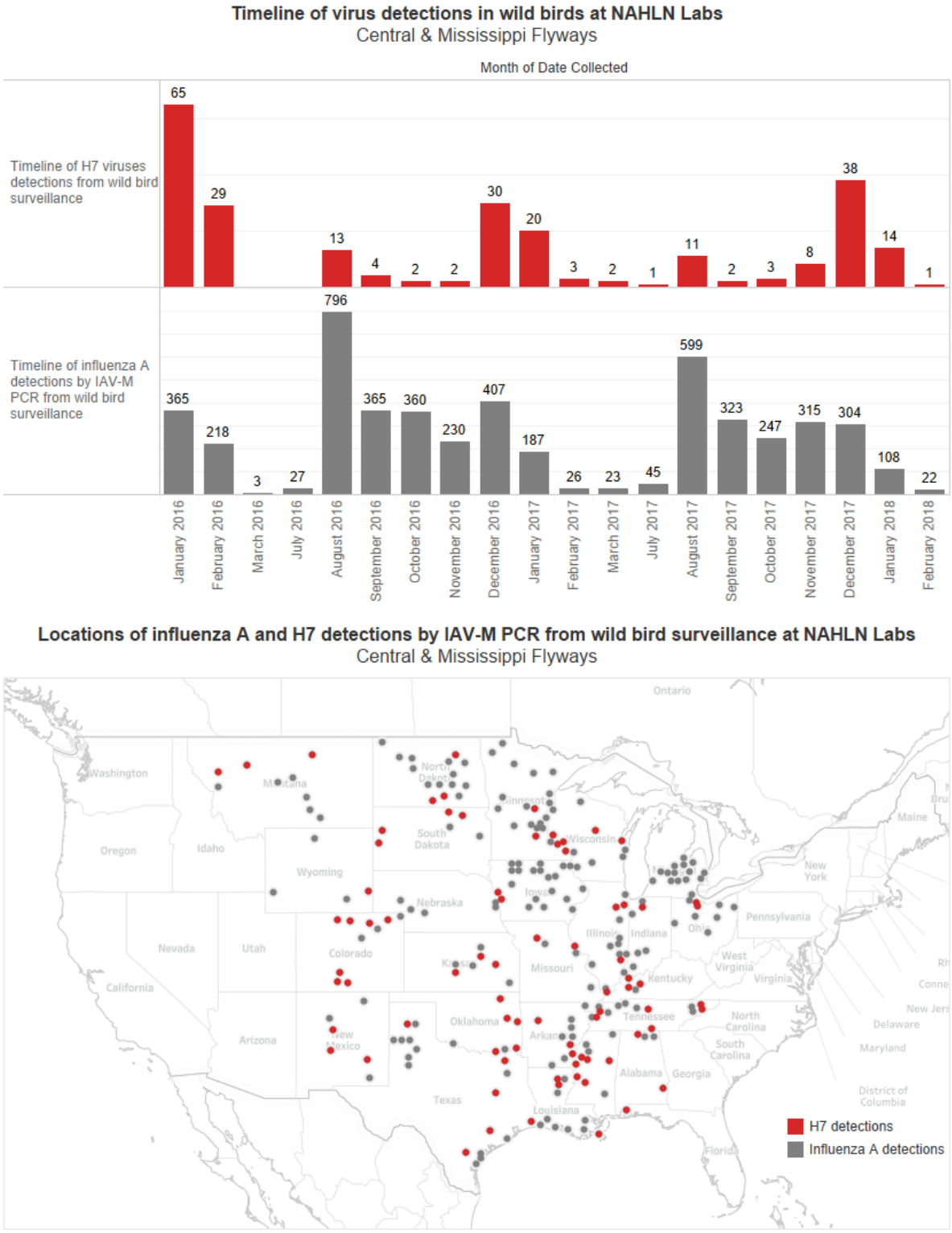


Figure 10. Timeline of virus detections in wild birds (upper panel) and locations of influenza A detections by IAV-M PCR from wild bird Surveillance (lower panel)

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.

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, 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 United States Department of Agriculture

HPAI Response
Initial Contact
Epidemiological (Epi) Report
June 27, 2016

I. Premises Information

Premises Identification Number: _____

Name of Premises: _____

Owner of Premises: _____

Address of Premises: _____

County of Premises: _____

Premises Owner Phone: _____

Premises Owner Email: _____

Premises Entrance Latitude: _____

Premises Entrance Longitude: _____

II. Owner Information

Owner of Animals: _____

Address of Animal Owner: _____

Animal Owner Phone: _____

Animal Owner Email: _____

III. Interview Contact Information

Name of person administering questionnaire: _____

Name of person answering questionnaire: _____

Phone: _____

Position (e.g., owner, manager, veterinarian, etc.): _____

Date of interview: _____

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IV. Flock Information

Clinical signs (*brief* description)	
Baseline daily mortality rate: (insert rate from farm records)	
Daily mortality rate (# of dead birds/bird population on date of initial sampling)	
Date first clinical signs were noted	
Date initial samples were collected	
Laboratory to which initial samples were submitted	
Results of any AI tests in past 21 days	
Date premises quarantine or hold order was issued	

House ID	Type of Birds	Number of Birds	Age of Birds	House Dimensions	Ceiling Height	Ventilation Type	Date of Onset of Clinical Signs

Do you have a veterinarian who regularly advises you on disease prevention?

☐ Yes ☐ No

If yes, name of veterinarian: _____

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Do you have a pre-arranged depopulation plan for this flock? ☐ Yes ☐ No

If yes, briefly describe the pre-arranged depopulation method: _____

_____

Have you exercised or used this method previously? ☐ Yes ☐ No

V. Trace-in and Trace-Out Questionnaire

Name of person administering questionnaire: _____

Name of person answering questionnaire: _____

Phone: _____

Position (e.g., owner, manager, veterinarian, etc.): _____

1. How are dead birds (daily mortality) disposed of on this farm (please circle one or more)? Also specify if disposal occurs on or off this premises.

   a. Composting
   b. Burial
   c. Incineration
   d. Rendering
   e. Landfill

   Other (specify): _____

   If disposal occurs at another premises:

Name and Location (company name)	Transported by

2. List any locations that **accept manure/litter** from this premises during the last 21 days.

Name and location (company name)	Date (mm/dd/yy)	Intended use

Name and location (company name)	Date (mm/dd/yy)	Intended use

3. Was manure or animal material from another premises brought onto this premises during the last 21 days?

☐ Yes ☐ No If yes:

Product	Source	Date (mm/dd/yy)

4. Have you or any of your employees (including any contractors or volunteers) visited any other premises with poultry or any processors of eggs or poultry products during the last 21 days (e.g., farm, slaughter, processing, market, residence with poultry)?

☐ Yes ☐ No If yes:

Premises/processor name	Person/title	Date (mm/dd/yy)

5. Is there a community living situation where farm workers from this premises interact with workers from other poultry facilities?

☐ Yes ☐ No

If Yes, describe: _____

6. Did any crews (e.g., catch crews, load-out, vaccination, insemination) enter the premises during the last 21 days?

☐ Yes ☐ No If yes:

Date (mm/dd/yy)	Crew type	Name/company

Date (mm/dd/yy)	Crew type	Name/company

7. Did any of the following visit the premises during the last 21 days?
If Yes, give date and name or company information.

Visitor type	Date(s) of visit	Name/company
a. Federal/State veterinary or animal health worker		
b. Extension agent or university veterinarian		
c. Private or company veterinarian		
d. Company service person		
e. Nutritionist or feed company consultant		
f. Inspector (e.g., FDA, NOP, biosecurity auditor, etc.)		
g. Feed delivery		
h. Egg truck		
i. Litter/bedding delivery		
j. Litter removal		
k. Renderer/dead bird pick up		
l. Pest/rodent control		
m. Manure truck		
n. Trash pick up		
o. Occasional worker (e.g., family member, part-time help over holiday)		
p. Wholesaler, buyer, or dealer		
q. Customer/consumer (private individual)		
r. Other		

8. Specify if any equipment was shared with another premises during the last 21 days, whether you received or loaned the equipment, and the location and name of the companies or premises the equipment was shared with:

Vehicle	Received/loaned	Specify (name, company, location)
ATV/4-wheeler	☐ Rec'd ☐ Loaned	
Tractor	☐ Rec'd ☐ Loaned	
Gates/panels	☐ Rec'd ☐ Loaned	
Skid-steer loaders	☐ Rec'd ☐ Loaned	
Egg flats	☐ Rec'd ☐ Loaned	
Egg racks	☐ Rec'd ☐ Loaned	
Pallets	☐ Rec'd ☐ Loaned	
Dead bird containers	☐ Rec'd ☐ Loaned	
Manure/litter handling equipment	☐ Rec'd ☐ Loaned	
Pressure sprayers/ washers/foamers	☐ Rec'd ☐ Loaned	
Other cleaning equipment	☐ Rec'd ☐ Loaned	
Vaccination equipment	☐ Rec'd ☐ Loaned	
Bird catching equipment	☐ Rec'd ☐ Loaned	
Live haul loader	☐ Rec'd ☐ Loaned	
Other (specify: _____ _____)	☐ Rec'd ☐ Loaned	

9. Were any birds introduced onto the premises during the last 21 days?

☐ Yes ☐ No If yes:

Date (mm/dd/yy)	Bird type (e.g., chicks, poults, spiking roosters, layers, breeders, etc.)	Source	Transported by

10. Have any birds moved off the premises during the last 21 days?

☐ Yes ☐ No If yes:

Date (mm/dd/yy)	Bird type (e.g., chicks, poults, spiking roosters, layers, breeders, etc.)	Destination	Transported by

11. Were any birds moved within the premises during the last 21 days? (e.g., from one barn to another on the same premises)

☐ Yes ☐ No

If Yes,

a. Was a contract crew used?

☐ Yes ☐ No

If Yes, specify company/crew name: _____

b. Was farm specific equipment used?

☐ Yes ☐ No

If No, describe: _____

12. Were any eggs moved onto the premises during the last 21 days?

☐ Yes ☐ No

If Yes,

a. List source (name and location) for eggs **coming onto** this premises during the last 21 days, the dates eggs were received, and whether the eggs were intended for hatching, or were processed or unprocessed from source.

Source name and location (company name)	Date (mm/dd/yy)	Intended for hatching?	Processed?*
		☐ Yes ☐ No	☐ Yes ☐ No
		☐ Yes ☐ No	☐ Yes ☐ No
		☐ Yes ☐ No	☐ Yes ☐ No

Source name and location (company name)	Date (mm/dd/yy)	Intended for hatching?	Processed?*
		☐ Yes ☐ No	☐ Yes ☐ No
		☐ Yes ☐ No	☐ Yes ☐ No
*Method of processing: _____			

13. Were any eggs moved off the premises during the last 21 days?

☐ Yes ☐ No

If Yes,

a. List source (name and location) for eggs **moving off** this premises during the last 21 days, the dates eggs left, and whether the eggs were intended for hatching, or were processed or unprocessed from source.

Source name and location (company name)	Date (mm/dd/yy)	Intended for hatching?	Processed?*
		☐ Yes ☐ No	☐ Yes ☐ No
		☐ Yes ☐ No	☐ Yes ☐ No
		☐ Yes ☐ No	☐ Yes ☐ No
		☐ Yes ☐ No	☐ Yes ☐ No
		☐ Yes ☐ No	☐ Yes ☐ No
*Method of processing: _____			

14. Is there any additional or important information that we need to know at this time regarding the disease on your farm?

☐ Yes ☐ No

If Yes, describe: _____

Appendix B: Estimation of Model Parameters for Within-Flock Spread of H7N1 LPAI Within a Turkey Barn

Estimation of the latency and infectious period distributions

In an experimental inoculation study, three groups of 10 turkeys were infected with H7N1 LPAI virus (Italy/1279) at nominal doses of 10^2, 10^4, or 10^6 EID_{50}, and the infection status of inoculated and contact turkeys was monitored at 24 hour intervals over 6 days using buccal and cloacal swabs tested for the presence of virus antigen by RRT-PCR (Iqbal et al., 2012).

In a second study, three-week old broad-breasted turkeys were inoculated with 10^2, 10^4, or 10^6 EID_{50} of LPAI H7N8 (turkey/IN/16-001571-6/2016) (Pantin-Jackwood et al., 2017). Infection status was monitored in directly inoculated turkeys at 12 hour intervals, and contact birds at 24 hour intervals, using oropharyngeal and cloacal swabs tested for the presence of virus antigen by quantitative RRT-PCR (Pantin-Jackwood et al., 2017). Turkeys directly inoculated at 10^4 and 10^6 EID_{50} and turkeys contact exposed to these turkeys became infected (Pantin-Jackwood et al., 2017).

Saenz *et al* (2012), inoculated a three week old turkey with 10^6 EID_{50} H7N1 LPAI (A/chicken/Italy/1279/99, LP) virus and exposed 40 or 41 contact turkeys in two large scale transmission studies (Saenz et al., 2012). Buccal swabs were taken daily mostly) to monitor infection status (Saenz et al., 2012).

Comin *et al* (2011), used data from a previously conducted vaccine study, where 18 twelve-week old turkeys had been inoculated with two LPAI strains (Comin et al., 2011). Nine birds were challenged with H5N2 LPAI virus (A/TK/IT/80) and 9 birds with H7N3 LPAI virus (A/TK/IT/8000/02) at an infective dose of 10^4 EID_{50} (Comin et al., 2011). Cloacal swabs were taken at day 3, 5, 7, 10, 12, 15 and 20 post inoculation and tested using a real-time RT-PCR assay and virus isolation in SPF fertile eggs (Comin et al., 2011).

Three week old turkeys were inoculated with 10^7 EID_{50} H7N2 LPAI A/turkey/VA/SEP/67/2002, and oropharyngeal swabs were collected from all birds from 1 to 4 dpi, and at 6, 8, and 10 (Costa-Hurtado et al., 2014). Results for 10 inoculated turkeys from Figure 1 of the publication were used in this analysis.

To estimate the latent period distribution, we used buccal swab data from 10 turkeys inoculated with $10^{3.8}$ EID_{50} (turkeys 81-90) from (Iqbal et al., 2012); and 10 turkeys inoculated with $10^{5.2}$ EID_{50} (turkeys 21-30; Supplement Table S1) (Iqbal et al., 2012). We also used data from oropharyngeal swabs from 17 turkeys inoculated with 10^6 EID_{50} from (Pantin-Jackwood et al., 2017).

To estimate the infectious period distribution, we used data from 81 turkeys from two LPAI transmission studies from Saenz et al (2012), Table 5 and Table 6 from the published paper (Saenz et al., 2012). We also used data from 20 turkeys from Table 8 from (Iqbal et al., 2012), data from 8 turkeys (Table 3, K10 to K18), and data from (Comin et al., 2011).

The gamma distribution parameters for the latent and infectious periods were estimated via maximum likelihood methods. Details are provided in Table 7.

Table 7. Parameter estimates for the LPAI transmission model for turkey barns.

Parameter name	Parameter description	Distribution/Value
Contact rate (transmission parameter)	The number of direct or indirect contacts a bird has that are sufficient to transmit infection per unit time	Pert distribution (min 1.1, mode 2.01, max 3.5 and lambda 4.0) based on estimates from Saenz et al., 2012.
Latent period distribution	Length of the latent period	Gamma distribution (shape 2.4404741, scale 0.2607466; mean 0.6363 days)
Infectious period distribution	Length of the infectious period	Gamma distribution (shape 6.490312, scale 1.290292; mean 8.37 days)
Time to seroconversion	Time to seroconvert post-infection	Gamma distribution (shape 6.490312, scale 1.29029; mean 8.37 days)
Proportion seroconverting	Proportion of LPAI infected turkeys that seroconvert	0.99

Proportion of turkeys that seroconvert to LPAI

In an experimental inoculation study comparing the pathogenesis of twelve H7 LPAI virus isolates from North America, nearly all (88/89 or 99%) surviving turkeys had detectable antibody by day 18 to 21 post inoculation (Spackman et al., 2010). For comparison, ~ 95 % (110/116) of surviving chickens seroconverted in the same study (Spackman et al., 2010). Based on this study, we estimated that 99% of turkeys would seroconvert following infection with LPAI virus.

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