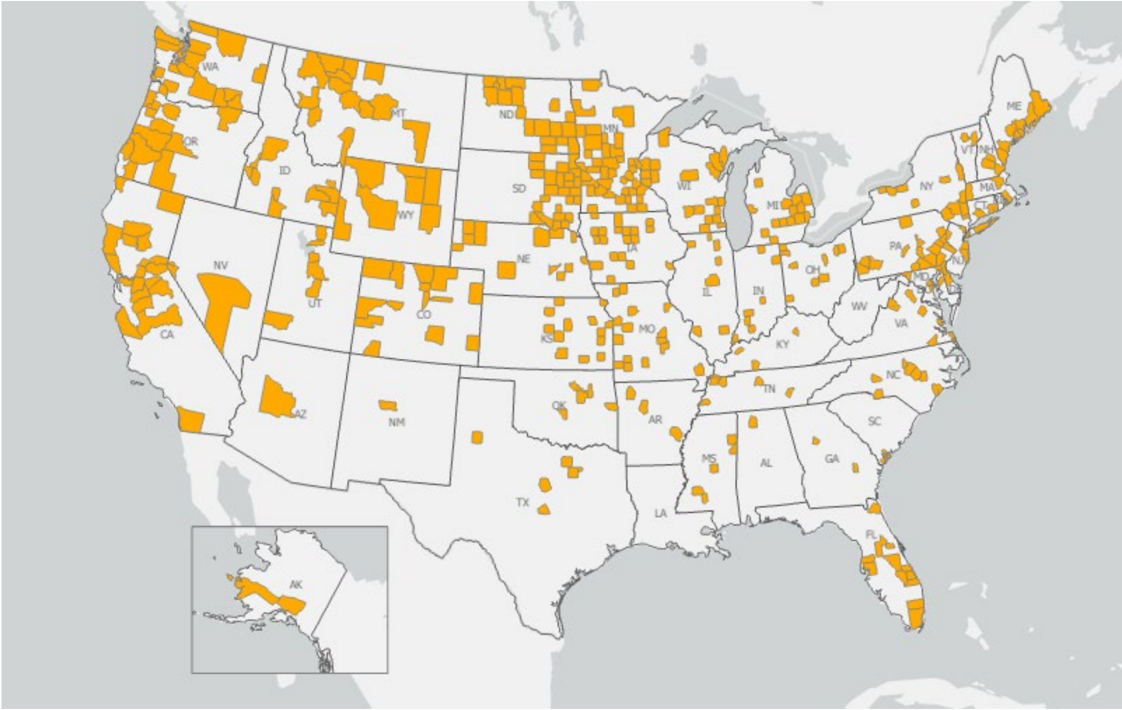
Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Epidemiologic and Other Analyses of HPAI-Affected Poultry Flocks

1 June 2023 Interim Report



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For more information, contact:

Amy Delgado, MS DVM PhD
Director, Center for Epidemiology and Animal Health
USDA: APHIS: Veterinary Services
Center for Epidemiology and Animal Health
2150 Centre Avenue, Bldg. B-2E7, Fort Collins, CO, 80526-8117
Phone (970) 494-7302
Email: Amy.H.Delgado@usda.gov
Web: http://www.aphis.usda.gov/animal-health/ceah

USDA–APHIS–VS–S&P–CEAH
NRRC Building B, M.S. 2W4
2150 Centre Avenue
Fort Collins, CO 80526-8117
(970) 494-7000

Email: vs.ceah@aphis.usda.gov
http://www.aphis.usda.gov/about_aphis/programs_offices/veterinary_services/ceah.shtml

(June 2023)

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EXECUTIVE SUMMARY

In January 2022, clade 2.3.4.4b H5N1 highly pathogenic avian influenza (HPAI) was reported in a wild bird sample from Colleton County, South Carolina. This heralded what is currently the largest avian influenza outbreak in U.S. history, involving many wild bird species and virus reassortments, with spillover into poultry, as well as wild and captive wild mammals and outdoor domestic cats. By March 31, 2023, HPAI was confirmed in 47 States including 323 WOAH[1] poultry [commercial], 120 WOAH poultry [backyard], 370 WOAH non-poultry, and 4 WOAH poultry [live bird market] premises. The primary driver for the spatial extent of the 2022–2023 HPAI outbreak has been migratory wild bird movements, with premises traditionally considered WOAH poultry [backyard] and WOAH non-poultry premises comprising the highest proportion of detections.

Phylogenetic analysis of viruses from this outbreak has highlighted many of the unique viral characteristics influencing this outbreak. Examination of the available wild bird, domestic bird, and poultry sequences determined that at least 83 percent of U.S. detections in domestic birds and poultry are consistent with independent point source (wild bird origin) introductions. Many genotypes have been identified, with the major genotypes appearing in both wild bird and poultry detections, highlighting the critical role of wild bird-related spread in this outbreak. And as of 31 March 2023, 154 cases of infected wild mammals across 17 species, as well as outdoor cats from Nebraska, Oregon, and Wyoming, have been reported. In the U.S., nearly all viruses characterized from mammals are Eurasian/North American reassortants and are often representative of the predominant circulating genotype at the time of detection. To date, there has been no conclusive evidence of sustained transmission between mammals in the U.S.

To explore factors associated with spillover infection from wild birds to domestic poultry, case-control studies for H5N1 HPAI were conducted among WOAH poultry [commercial] table egg layer, pullet, and breeder bird farms, as well as WOAH poultry [commercial] turkey farms. Location within an existing control zone was a significant farm-level risk factor for HPAI on both WOAH poultry [commercial] table egg and turkey farms, highlighting the need for increased biosecurity and surveillance vigilance once a detection occurs in an area. Other important risk factors for table egg layer farms included the presence/sighting of wild waterfowl in surrounding areas, having feed or feed ingredients accessible to wild birds, the absence of a farm gate, and lack of personnel assigned to specific barns. For turkey farms, other factors associated with increased odds of H5N1 HPAI infection included having both brooder and grower turkey production on the farm, having toms as the sex market type, seeing wild waterfowl or shorebirds in the closest field, and the use of rendering for dead bird disposal. Factors with a protective effect included workers entering the selected barn using a shower at least some of the time and having a restroom facility available to crews who visit the farm. The economic analysis found that there are differences between case and control turkey farms in terms of on-farm biosecurity and investments. Control farms had statistically significant higher monthly biosecurity costs than case farms, and control farms also spent more on temporary biosecurity measures, such as gates, parking areas, temporary wild bird mitigations, temporary air intake inlet covers, or temporary vehicle wash stations. Importantly, case farms were 87.7 percent more likely to have plans to make permanent changes to biosecurity. Weather variables, such as precipitation, temperature, and

[1] World Organisation for Animal Health

average wind speed have also been identified as potential risk factors of interest, and further analysis of weather-related variables is ongoing.

The United States Department of Agriculture Animal and Plant Health Inspection Service (USDA–APHIS) used the HPAI National Model to support budget and resource planning, as well as to evaluate alternative control strategies and options. As the outbreak continued, disease spread and control modeling were used to help inform data-driven response strategies and resource allocation. Other modeling approaches were also used to inform the response and improve our understanding of disease transmission. Time of introduction models use diagnostic testing, daily mortality, and water consumption data to predict the time of virus entry into a flock. Analysis of data from 53 WOAH poultry [commercial] premises found that time to first positive sample varied by production type, introduction route, and reason for testing (median of 6 days for farms under surveillance and 8 days for farms passively reported due to clinical signs). The average adequate contact rate (see Appendix E, Table E1 for more information) across all premises was 4.9 contacts per day. In addition, the average number of secondary infections caused by a typical infectious individual over its entire infectious period when introduced into a completely susceptible population, as described by the overall mean basic reproductive number (R0) value, was 13.5 (range 2–62), which would ensure rapid spread through a barn. This work highlighted the value of closely monitoring mortality, water consumption, and egg production to quickly identify disease issues in the flock, while recognizing that these factors may vary, so understanding the trends within each production setting is important.

The U.S. National Surveillance Plan for Highly Pathogenic Avian Influenza in Wild Birds was developed to maximize our ability to detect influenza A virus (IAV) in wild waterfowl. Between 30 December 2021 and 31 March 2023, over 40,000 apparently healthy wild waterfowl were sampled and tested for IAV using real-time reverse transcriptase polymerase chain reaction (rRT-PCR). Overall, targeted surveillance and morbidity/mortality investigations of sick or dead birds have resulted in 6,086 detections of H5N1 HPAI lineage virus in over 130 wild bird species across 49 States, plus Washington, D.C.

To better understand the risk of virus spillover from wild to domestic birds, USDA–APHIS collaborated with the University of Maryland and the U.S. Geological Survey, Eastern Ecological Science Center to model spatio-temporal trends in transmission between wild waterfowl and domestic poultry. Data from the current outbreak were used to evaluate preliminary model performance. Using data through January 2023, we showed that the model performed well at predicting county-level avian influenza virus spillover risk. Model results are now available online for poultry owner use to better understand their own risk context.

Other valuable tools for understanding disease risk are eBird and BirdCast migration data. BirdCast migration maps show real-time intensities of nocturnal bird migration between local sunset to sunrise, as detected by the U.S. weather surveillance radar network. eBird is a database of species-specific, crowd-sourced observational data by scientists and birding enthusiasts. Intense periods of bird migration, as seen by BirdCast maps, were correlated with outbreaks in domestic poultry, suggesting that this tool can be used to increase awareness of heightened HPAI risk due to wild bird movements. Using eBird data, we estimated that HPAI-positive premises were more likely to be detected within the first seven days of heavy wild bird observation within a 50 km spatial window.

Further information on the epidemiologic features of this outbreak and additional analyses will be provided in subsequent reports and peer-reviewed scientific manuscripts.

INTRODUCTION

In response to the Eurasian clade 2.3.4.4b H5N1 HPAI outbreaks in WOAH poultry[2] [commercial][3] and WOAH poultry [backyard] across the U.S., USDA–APHIS–Veterinary Services (VS), APHIS Wildlife Services (WS), 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 introduction into poultry flocks.

These investigations include the following:

- Virus phylogenetic analyses;
- A case-control study in turkey farms examining epidemiologic and economic factors affecting HPAI risk;
- A case control study in table egg layer farms examining risk factors for HPAI;
- A case-crossover study to examine associations between weather variables (e.g., wind speed, relative humidity, precipitation, temperature) and HPAI infection on commercial poultry farms;
- Analysis of barn-level egg production and mortality records;
- Analysis of waterfowl surveillance;
- Modeling the risk of avian influenza virus transmission from wild waterfowl to domestic poultry across the contiguous United States; and
- Analysis of the utility of publicly available data on wild bird migration for predicting increased risk of HPAI spillover to domestic poultry.

To provide producers, industry, and other stakeholders with relevant epidemiologic information, this report includes the results from these investigations.

A. Description of Outbreak

USDA–APHIS identified the Eurasian clade 2.3.4.4b H5N1 HPAI on 13 January 2022 in a wild bird in Colleton County, South Carolina (Animal and Plant Health Inspection Service [APHIS], 2022). This detection was the first Eurasian H5 HPAI detected in the U.S. since December 2016, and followed ongoing reports of clade 2.3.4.4b H5N1 HPAI in Europe (Freath et al., 2022) starting 27 October 2021 for the migration season (note that ancestors of clade 2.3.4.4b have been circulating along Eurasian flyways since 2017) and in Canada (WAHIS, 2023) starting on 4 November 2021. For Europe, Canada, and the U.S., wild bird detections have preceded detections in domestic poultry. Figure 1 describes the temporospatial detections of clade 2.3.4.4b H5N1 HPAI virus in domestic poultry in the U.S. (see Phylogenetic Analysis and Diagnostics section for more details).

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[2] https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=glossaire.htm#terme_volailles

[3] https://www.ecfr.gov/current/title-9/chapter-I/subchapter-B/part-56#:~:text=The%20poultry%20are%20from%3A,with%20at%20least%205%2C000%20birds.

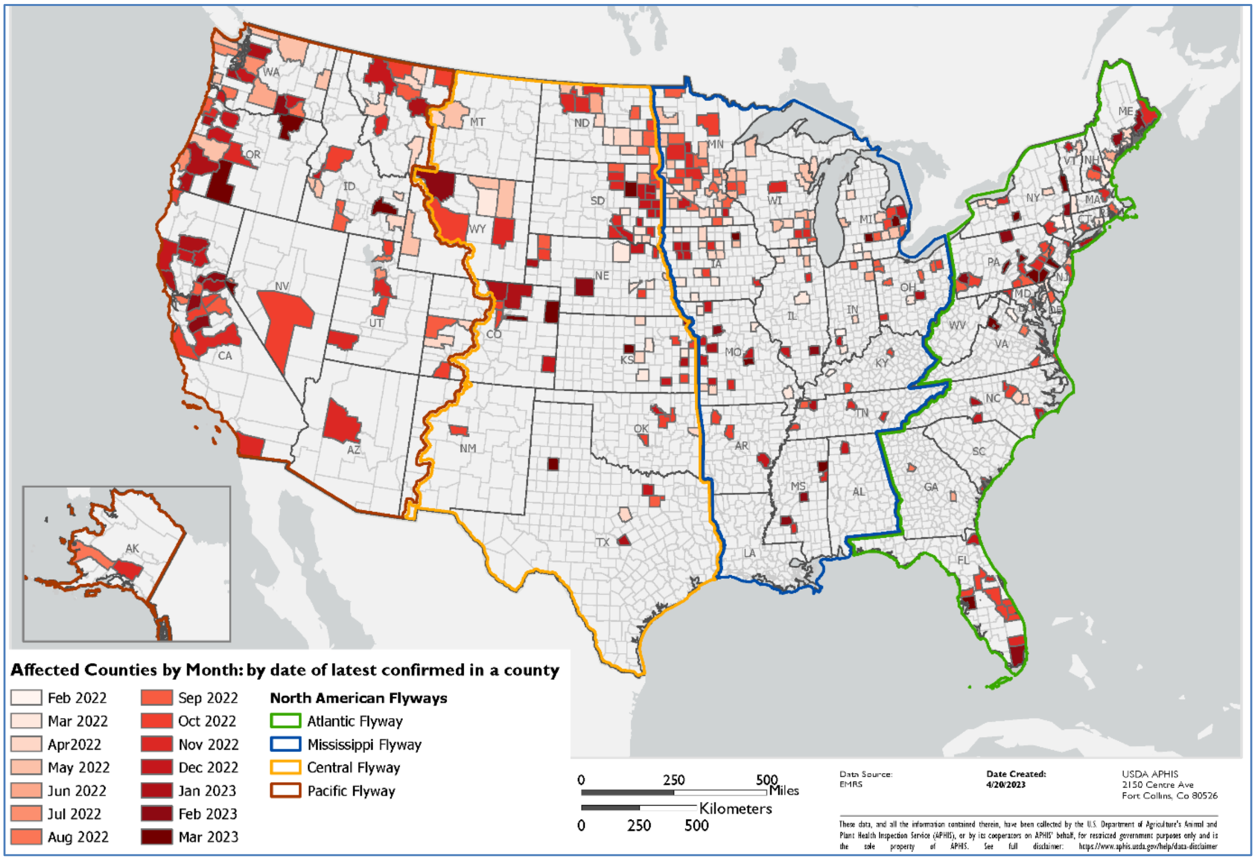

Figure 1. Counties with highly pathogenic avian influenza detections in poultry by month and by flyway as of 31 March 2023.

The first detection of HPAI in a domestic poultry premises occurred on 7 February 2022 on a commercial meat turkey bird operation in Dubois County, Indiana, and 14 additional detections occurred later that same month. These cases represented the beginning of a wave of detections in the U.S. (Figure 2) that corresponded with wild bird migration (see Analysis of BirdCast and e-Bird Migration Data: Implications for Disease Introduction, Spread, and Prevention section for more details). In March 2022, the number of cases rapidly increased to 87 detections confirmed by the National Veterinary Services Laboratory (NVSL). In April 2022, the NVSL confirmed 166 cases of HPAI; this was the highest number of cases confirmed in a single month. In May 2022, a relative decline was observed with 89 cases confirmed by the NVSL. This was the first month in which the combined number of detections among premises considered WOAH poultry [backyard] and WOAH non-poultry exceeded the number of WOAH poultry [commercial] detections.

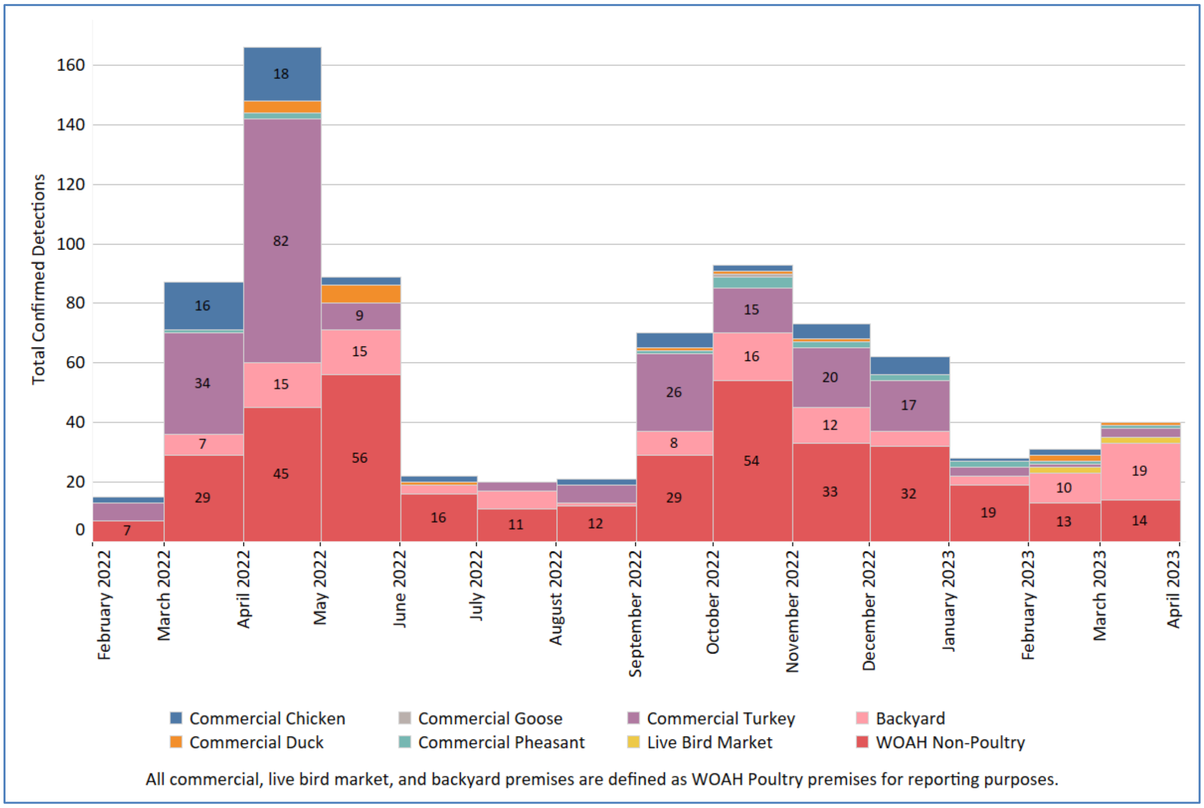

Figure 2. Monthly highly pathogenic avian influenza detections by premises type as of 31 March 2023.

Over the summer months, the number of confirmed cases sharply declined (Figure 2). The NVSL confirmed an average of 21 (range: 20–22) cases in June, July, and August 2022. Most of these cases occurred among WOAH poultry and WOAH non-poultry premises along the Pacific Flyway.

From September 2022 through December 2022, the U.S. observed an increase in the number of confirmed HPAI cases per month (average: 75 cases, range: 62–93; Figure 2). These detections were likely associated with the fall wild bird migration (see Analysis of BirdCast and e-Bird Migration Data: Implications for Disease Introduction, Spread, and Prevention section for more details). Fall cases peaked in October 2022 with 93 confirmed detections. Although the number of WOAH poultry [commercial] detections increased during the fall wave, the number of combined detections among WOAH poultry [backyard] and WOAH non-poultry premises continued to exceed the number of WOAH poultry [commercial] detections.

In January 2023, the NVSL confirmed 28 HPAI detections, predominantly among WOAH non-poultry premises. While this reflects a decline from the number of cases observed during the fall wave, the number of cases soon began to increase again with 31 detections confirmed in February 2023 and 40 detections confirmed in March 2023 (Figure 2). These detections in February and March also included detections within WOAH poultry [live bird market] premises.

Table 1. Confirmed detections of highly pathogenic avian influenza by production type and World Organisation for Animal Health (WOAH) reportable species as of 31 March 2023.

Production Type	Chicken	Turkey	Duck	Pheasant	Goose	Other*
WOAH Poultry						
Commercial Broiler Production	14					
Commercial Broiler Breeder Pullets	2					
Commercial Broiler Breeder	11					
Commercial Table Egg Layer	30					
Commercial Table Egg Pullets	4					
Commercial Table Egg Breeder	3					
Commercial Turkey Meat Bird		209				
Commercial Turkey Breeder Hens		11				
Commercial Turkey Replacement Hens		2				
Commercial Turkey Poult Supplier		1				
Commercial Turkey Breeder Toms		2				
Commercial Duck Meat Bird			7			
Commercial Duck Breeder			10			
Commercial Upland Gamebird Producer				16		
Commercial Breeder Operation					1	
Live Bird Market	2					2
Backyard	49	2	15	5	2	47
WOAH Non-Poultry	250	2	31	3	10	74
Total	365	228	63	24	13	124

*Other species includes assorted pet birds, chukars, ratites, multiple poultry species, and “other poultry” designations.

As of 31 March 2023, the NVSL has confirmed HPAI detections in 47 States. These NVSL-confirmed detections included 323 WOAH poultry [commercial], 120 WOAH poultry, 370 WOAH non-poultry, and 4 WOAH poultry [live bird market] premises. WOAH poultry [commercial] detections included 225 turkey, 37 table egg, 27 broiler, 17 duck, 16 upland gamebird, and 1 goose premises (Table 1). Split by wild bird migratory flyways, detections included 180 premises in the Atlantic Flyway, 267 premises in the Mississippi Flyway, 175 premises in the Central Flyway, and 195 premises in the Pacific Flyway. The contribution of WOAH poultry [commercial] premises to the total number of detections was higher for the inland flyways than coastal flyways (Figure 3). Along the Mississippi and Central Flyways, WOAH poultry [commercial] premises accounted for 55 percent (147 out of 267) and 50 percent (87 out of 175) of detections, respectively. In contrast, WOAH poultry [commercial] premises only accounted for 28 percent (51 out of 180) of detections in the Atlantic Flyway and 20 percent (38 out of 195) of detections in the Pacific Flyway.

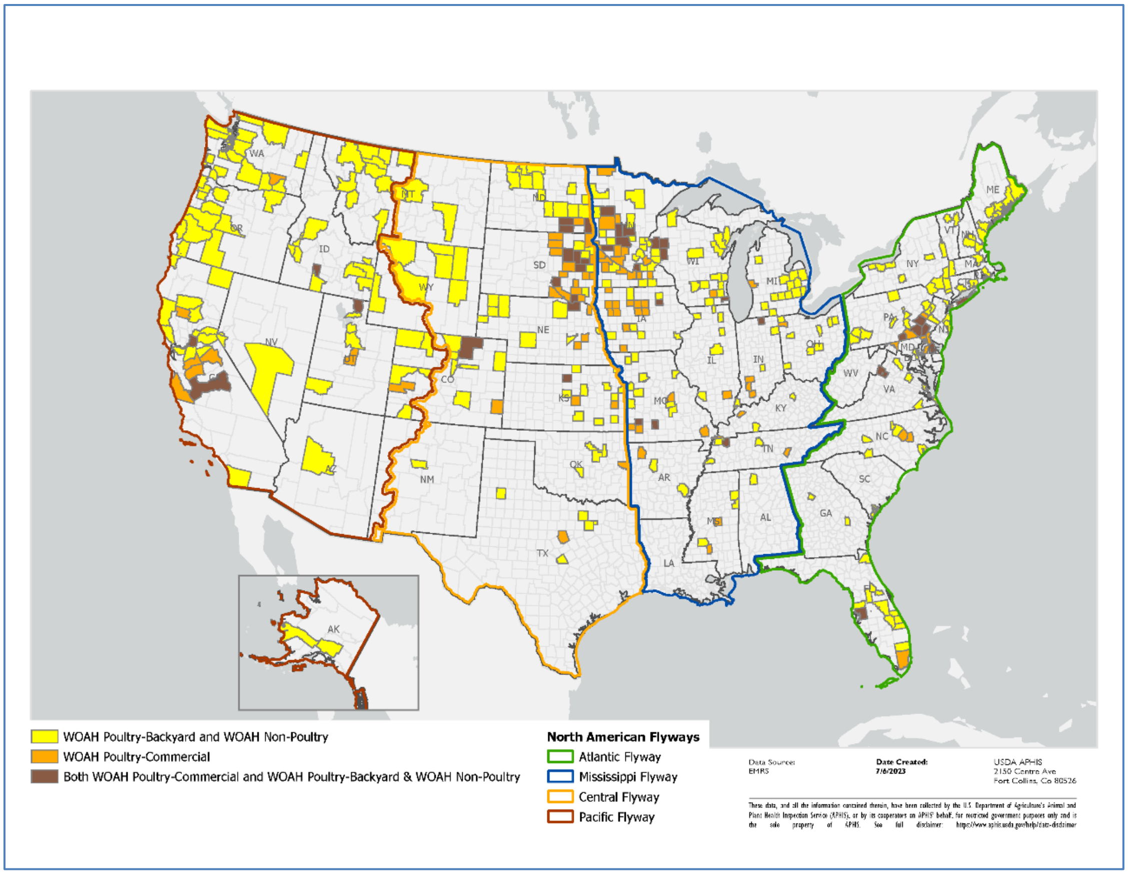

Figure 3. Bird map of HPAI-affected counties by North American flyway and premises type as of 31 March 2023.

Phylogenetic analysis indicates most detections are the result of independent wild bird introductions (see Phylogenetic Analysis and Diagnostics section for more details). Premises traditionally considered as WOAH poultry [backyard] and WOAH non-poultry comprise the highest proportion of detections; these premises generally have lower biosecurity practices, with increased risk of exposure to wild birds. While WOAH poultry [commercial] premises continue to be at risk, with clusters of lateral spread observed following an independent wild bird introduction*,* transmission from WOAH non-poultry premises to WOAH poultry [commercial] premises has not been documented.

A more detailed outbreak overview chronologically describing detections by wild bird migratory flyways, States, and production types is provided in Appendix A: Detailed Outbreak Overview by Flyway.

B. Comparison of 2022–2023 HPAI Outbreak to 2015 HPAI Outbreak

The primary driver for the spatial extent of the 2022–2023 HPAI outbreak has been migratory wild bird movements. The first poultry detection was in the Mississippi Flyway, which followed earlier detections in wild birds along the Atlantic Flyway. The virus then moved through the migration patterns in and out of Canada through the Atlantic, Central, and Pacific Flyways into Alaska. While the 2015 HPAI outbreak was initially preceded by migratory wild bird movements, disease spread was more heavily influenced by lateral transmission between farms after virus introduction to the Midwest, with farm-to-farm spread associated with the movement of people, equipment, and materials between premises. Unlike the 2022–2023 HPAI outbreak, in the 2015 outbreak, the virus was introduced into the Pacific Flyway, moved eastward as far as Indiana, and there were no detections in the Atlantic Flyway.

Given the expanded geographic range and extent of viral shedding in wild birds, it is not surprising that at least 83 percent of premises affected in the 2022–2023 HPAI outbreak have been consistent with independent wild bird introductions, shaping the spatial distribution of cases seen at the county level in Figure 3. Although many more counties have been impacted by HPAI in 2022 and 2023, compared to 2015, many of these counties represent a very small number of cases with no further spread between farms. This shift may suggest that improvements in biosecurity on farms and increased messaging around the importance of proactive measures to reduce the spread between locations have had a positive impact on limiting lateral transmission of virus.

The broad geographic extent of this outbreak has had serious impacts on resource requirements for response. USDA–APHIS personnel began deploying to the first HPAI detection in February 2022 and have continued to deploy through drafting this report. More than 892 USDA–APHIS personnel and contractors have been deployed in support of the response, representing over 2,399 deployments as of 31 March 2023.

The distribution of farm types impacted over the course of the past outbreak has also differed. In comparison to the 2015 HPAI outbreak, which only had 21 WOAH poultry [backyard] and WOAH non-poultry flocks impacted, the 2022–2023 HPAI outbreak has confirmed 490 WOAH poultry [backyard] and WOAH non-poultry flocks as of 31 March 2023. This likely reflects an increase in the prevalence of the virus in wild birds across all migratory flyways. To promote awareness of HPAI, the USDA expanded messaging on the importance of reporting disease among WOAH poultry [backyard] and WOAH non-poultry flocks and distributed information through social media outlets. During the COVID-19 pandemic, the number of individuals engaged in rearing chickens as a hobby and egg source increased exponentially (Lesley, 2021). Infections of WOAH poultry [backyard] and WOAH non-poultry flocks often serve as indicators of the presence of virus among wild birds, but in this outbreak, as with the 2015 outbreak, they do not seem to play a role in the epidemiology of the outbreak overall. There has been no evidence to support transmission of virus spread from WOAH poultry [backyard] or WOAH non-poultry flocks to WOAH poultry [commercial] based on epidemiologic and phylogenetic analysis (see Phylogenetic Analysis and Diagnostics section for more details).

Figure 4 shows a comparison of the epidemiologic curves and number of birds lost or depopulated due to disease between the two outbreaks. A higher number of cases, in addition to an increase in the number of birds affected in the 2022–2023 HPAI outbreak, are reflective of increased detections of HPAI in WOAH poultry [backyard] or WOAH non-poultry flocks, as previously noted. As the current outbreak continues to evolve, additional comparisons and lessons learned between the two outbreaks are underway. USDA–APHIS has made numerous changes to response processes in a concerted effort to improve efficiency in control activities, indemnity and virus elimination payments, and repopulation processes and timelines. Initial estimates suggest that significant improvements have been made in all aforementioned areas, and more detailed information will be provided in the final epidemiologic report for this outbreak.

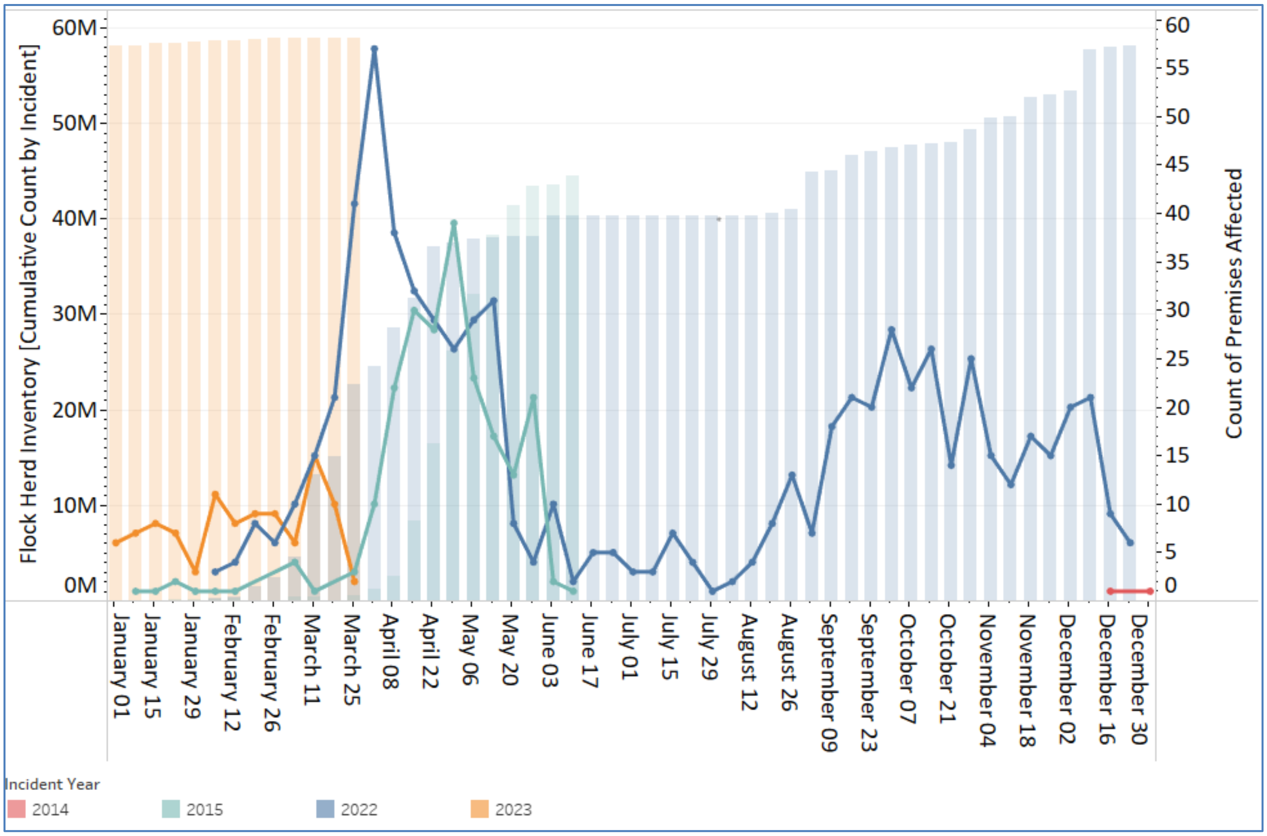

Figure 4. Epidemiological curve of flock/herd inventory [cumulative count by incident year] and count of premises affected by week of confirmed diagnosis date as of 31 March 2023.

PHYLOGENETIC ANALYSIS AND DIAGNOSTICS

A. Phylogenetic Analyses

The 2022–2023 H5N1 clade 2.3.4.4b HPAI outbreak has surpassed the 2014–2015 H5 clade 2.3.4.4c outbreak as the largest animal health emergency in U.S. history (Figure 4) (Animal and Plant Health Inspection Service [APHIS], 2023), involving many wild bird species and virus reassortments (Animal and Plant Health Inspection Service [APHIS], n.d.), with spillover into poultry, as well as wild and captive wild mammals and feral domestic cats. Ongoing phylogenetic and biologic analysis has been conducted by the NVSL (Ames, IA; national and international reference laboratory for influenza A virus in animals) in close collaboration with USDA–APHIS–WS and the USDA Agricultural Research Services Southeast Poultry Research Laboratory (ARS–SEPRL; international collaboration center for influenza A virus). High throughput, near real-time full genome sequence analysis was leveraged by sequencing samples directly; the data pipeline includes RAxML to generate phylogenetic trees and generating tables of single nucleotide polymorphisms (SNPs) created using the vSNP pipeline.[4] Analysis of the available wild bird, domestic bird, and poultry sequences determined that at least 83 percent of analyzed U.S. detections in domestic birds and poultry are consistent with independent point source (wild-bird origin) introductions.

The H5N1 clade 2.3.4.4b genotype A1 was first identified in wild birds collected in December 2021. Genotype A1 spread across all four flyways and reassortants with North American (AM) wild bird avian influenza viruses first identified in February 2022. Reassortant viruses account for at least 87 percent of viruses with several genotypes (Youk et al., 2023) spilling over into domestic birds and poultry; however, reassortment of the neuraminidase gene has been rare and has not been sustained to date. Several fully Eurasian H5N1 clade 2.3.4.4b introductions have since been identified following initial detection of genotype A1 in December 2021—genotype A2 was detected in February 2022 in the northeastern US and A3 was likely introduced via the Pacific Flyway in April 2022. Three additional introductions have since been identified in wild bird samples collected from October 2022 through December 2022—A4 via the Pacific flyway in wild birds in Alaska and A5 and A6 via Atlantic flyway (of note, A6 is a fully Eurasian virus with a reassorted neuraminidase (H5N5)). Reports in wild birds, domestic birds, poultry, and mammals extend into Central and South America as of fall 2022.

In May 2022, HPAI was confirmed in a red fox in Rock County, Wisconsin, and by the end of March 2023, 154 wild mammals across 17 species (Animal and Plant Health Inspection Service [APHIS], 2023a), as well as outdoor cats in Nebraska, Oregon, and Wyoming, had been reported. In the U.S., nearly all HPAI viruses characterized from mammals are Eurasian/North American reassortants and are often representative of the predominant circulating genotype at the time of detection. To date, there has been no conclusive evidence of sustained transmission between mammals in the U.S. E627K, a molecular marker in the PB2 gene previously associated with adaptation in mammals has been identified in seven foxes, two raccoons, three skunks, a grizzly bear, a black bear, and a harbor seal (only one of several characterized), from different States and genotypes as of 30 March 2023. The change from E to K in position 627 of the PB2 segment happens during virus replication in a mammalian species. While mammals are largely considered dead-end hosts, transmission from mammal to bird cannot be ruled out when E627K is present. The E627K marker has

[4] https://github.com/USDA-VS/vSNP

been identified in two wild birds as of 30 March 2023—a red-tailed hawk and a turkey vulture, both species likely to predate or scavenge on small mammals. The mutation has also been identified in eight WOAH non-poultry flocks (one of which has a matching virus from a skunk found on the property) and one WOAH poultry [commercial] turkey flock. Other mammalian-associated mutations of interest (T271A, D701N) have been detected in a handful of viruses, largely from mammals and raptor species. Representative poultry and mammal sequences have been uploaded to a public database.[5] Major genotypes (Youk et al., 2023) and their geographic lineage (Eurasian or North American) are listed in Table 2. Their prevalence in wild birds and WOAH poultry [commercial] premises is shown in Figure 5.

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

Table 2. Major genotypes and gene constellation (ea = Eurasian segment, am = North American segment).[6]

	Segment Group							
Genotype	PB2	PB1	PA	HA	NP	NA	M	NS
A1	ea1	ea1	ea1	ea1	ea1	ea1	ea1	ea1
A2	ea2	ea2	ea2	ea2	ea2	ea2	ea2	ea2
A3	ea3	ea3	ea3	ea3	ea3	ea3	ea3	ea3
B1.1	am1.1	am1.1	ea1	ea1	am1.2	ea1	ea1	ea1
B1.2	am1.1	am1.1	am1	ea1	am1.2	ea1	ea1	ea1
B1.3	am1.3	am1.3	am1.2	ea1	am1.2	ea1	ea1	ea1
B2.1	am1.2	ea1	ea1	ea1	am1.1	ea1	ea1	ea1
B2.2	am1.2	ea1	ea1	ea1	am1.1	ea1	ea1	am1.2
B3.1	am2.1	ea1	ea1	ea1	am1.4.1	ea1	ea1	ea1
B3.2	am2.1	am1.2	ea1	ea1	am1.4.1	ea1	ea1	am1.1
B4.1	am2.2	ea1	ea1	ea1	am1.3	ea1	ea1	ea1

[5] https://gisaid.org/

[6] Youk et al., 2023

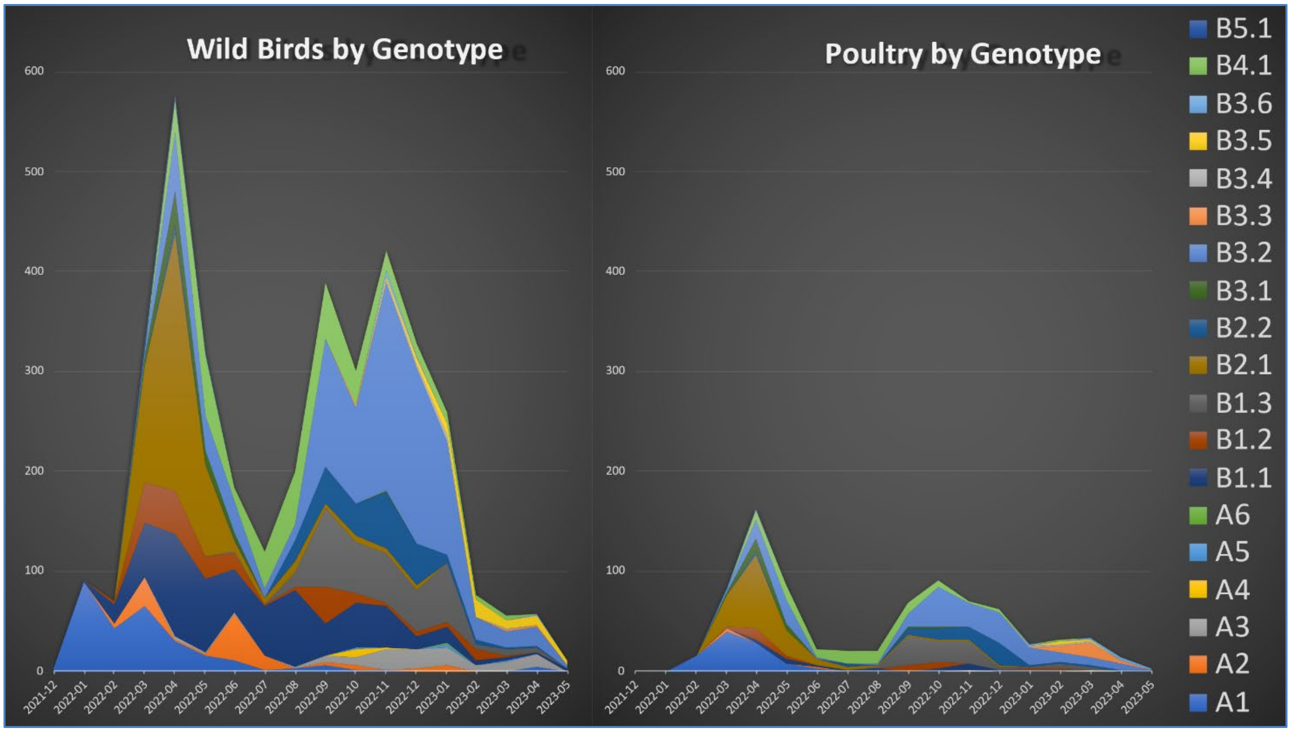

Figure 5. Genotype distribution as (A) total counts and (B) proportion of all detections from December 2021 to 31 March 2023 in wild birds and mammals, left, and commercial poultry, right.

B. Public Health Aspects

The NVSL rapidly shares genetic and biological materials in collaboration with the Influenza Division of the Centers for Disease Control and Prevention (CDC), USDA–APHIS–WS, USDA–ARS–SEPRL, and other key partners. Whole genome sequence data shared by NVSL is 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.

To date, there is one report of clade 2.3.4.4b H5N1 HPAI detection in humans in the U.S. The U.S. case involved bird depopulation at an affected site in Colorado and tested positive by PCR only on the day of depopulation; all other testing was negative. The CDC continues to monitor the situation closely for signs that the risk to human health has changed (World Health Organization, 2022). The health of response workers and on-farm personnel is monitored at the State level in cooperation with USDA–APHIS and the CDC.

C. General Influenza A Diagnostics and Characterization

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 pathogenicity (LPAI) 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 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 it provides a semi-quantitative result. The NAHLN tests samples first by the IAV-M test and further by the NAHLN H5 and H7 tests when IAV is detected. Genomic data also confirms that diagnostic assays are fit for purpose. *In silico* analysis via computational modeling approaches confirms high similarity between the H5N1 virus sequences and the relevant primers and probes used for the IAV and H5 diagnostic rRT-PCR tests.

All poultry samples with a non-negative test result for IAV by either serology or PCR are forwarded to the NVSL for confirmatory testing. The NVSL uses molecular tools to determine the subtype and pathotype (LPAI vs. HPAI) directly from swab or tissue samples. Whole genome sequencing is conducted directly from samples and on all isolated viruses and select viruses may be further characterized by pathotype assay in specific pathogen-free chickens. Major genotypes and their lineage (Eurasian or North American) are listed in Table 2. Their prevalence in wild birds and WOAH poultry [commercial] premises is shown in Figure 5.

EPIDEMIOLOGIC STUDIES TO INVESTIGATE THE H5N1 VIRUS IN WOAH POULTRY [COMMERCIAL] AND WOAH POULTRY [BACKYARD] IN THE UNITED STATES

A. Table Egg Production Case-Control Study

BACKGROUND

The commercial table egg sector has had the highest percentage of affected birds from WOAH poultry [commercial] operations in the 2022 U.S. HPAI H5N1 outbreak, with over 75 percent of affected birds from WOAH poultry [commercial] operations part of the commercial table egg production sector.

Wild bird introductions were identified as the primary means of spread in the 2022–2023 outbreak, and to explore factors associated with spillover infection from wild birds to domestic poultry, a case-control study for H5N1 HPAI was conducted among WOAH poultry [commercial] table egg layer, pullet, and breeder bird farms in Delaware, Iowa, Maryland, Minnesota, Nebraska, Ohio, Pennsylvania, and Utah. This study was conducted by USDA–APHIS in collaboration with State partners, academia, and national poultry organizations. The goals of this study included identifying risk factors for HPAI and biosecurity challenges on WOAH poultry [commercial] table egg farms and refining biosecurity recommendations to support prevention of HPAI. This information will improve understanding of risk factors associated with HPAI on table egg farms in the U.S. and support science-based guidance on farm-level preventive measures.

DATA COLLECTION

Eligible case farms (infected farms) included those WOAH poultry [commercial] table egg layer, pullet, and breeder farms in the States listed above, with confirmed infection and reported onset of clinical signs from 22 February 2022 through 31 August 2022. A total of 22 farms met the inclusion criteria. While confirmed infections also occurred in several other States, these were not included due to resource constraints or lack of eligible control premises. Eligible control farms (uninfected farms) were any WOAH poultry [commercial] table egg layer, pullet, or breeder farms selected from the same States as case farms, using the USDA–APHIS–VS Emergency Management Response System. Randomized lists of 10 potential controls per case were shared with interviewers in each participating State, with a goal of enrolling up to two control farms per case farm. Potential controls were contacted by interviewers via phone or email to confirm eligibility and interest in participation. To be eligible, control farms needed to have 50,000 or more birds, as well as birds on-site for a minimum two-week window of risk within the State-specific high-risk timeframe. High-risk timeframes were determined according to reported onset of clinical signs for confirmed infections within the States. Interviewers were asked to match risk windows for cases and controls as closely as possible.

Between 26 September 2022 and 28 December 2022, questionnaires were administered by Federal or State veterinary medical officers via telephone. The fillable pdf forms were then uploaded to a secure USDA–APHIS location. Interviewers in each State only had access to their State's data, and all data was treated as confidential business information. Producer participation was voluntary.

DATA ENTRY AND MANAGEMENT

Questionnaires were completed for 18 case farms and 22 control farms across 8 States (Figure 6). Survey data were entered into a SAS dataset using SAS version 9.4 (SAS Institute Inc., Cary, NC). Survey responses were validated by USDA–APHIS–VS National Animal Health Monitoring System (NAHMS) staff prior to analysis. Two primary approaches to multiple logistic regression were taken to glean the most information from this small dataset. The top findings are presented here.

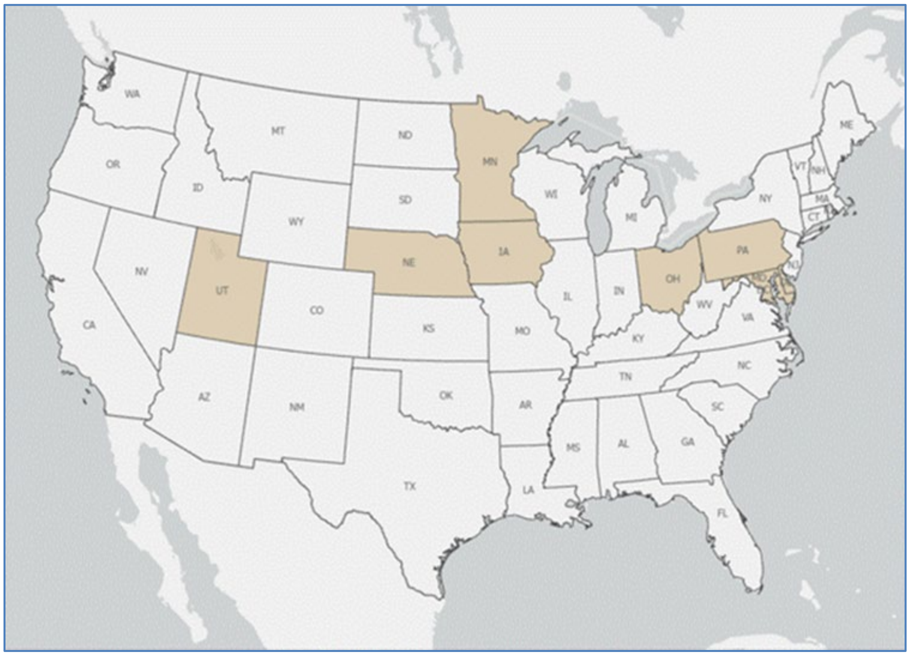

Figure 6. U.S. States with farms that participated in the 2022 HPAI H5N1 WOAH poultry [commercial] table egg case-control study.

Additionally, using the results of whole genome sequencing from the NVSL, case farms that had virus sequences consistent with independent wild bird introduction of HPAI were further examined via univariate analyses.

MULTIVARIABLE ANALYSIS

The multivariable modeling results are summarized in Table 3 and Table 4 using two different modeling methods (using the best fit exact multiple logistic regression model and using Bayesian model averaging, which averages effects across all top models, respectfully). Findings from the modeling approaches are summarized below.

Table 3. Estimated odds ratios, and their 95% confidence intervals, of an operation being positive for each of the variables included in the best fitting exact multiple logistic regression model. Also included are the p-values for the tests that the effect size is non-zero.

Variable	Level	Exact conditional test *p*-value	Odds ratio (95% confidence interval)
Flock size (number of birds on the farm on the reference date)	Large (500,000)	0.59	2.6 (0.3 – 39.5)
	Small (<500,000)		(referent)
Farm in an existing control zone on the reference date	Yes	0.09	10.3 (0.8 – 377.0)
	No		(referent)
Wild waterfowl or shorebirds in closest crop field during the 14-day reference period	Yes	0.12	5.8 (0.7 – 79.4)
	No		(referent)
Gate to the farm entrance present	Yes	0.21	(referent)
	No		3.8 (0.6 – 31.5)
Personnel assigned to specific barns (dedicated barn personnel)	Yes	0.34	(referent)
	No		6.2 (0.3 – 427.5)

Table 4. Estimated odds ratios, and their 95% confidence intervals, of an operation being positive for each of the variables included in Bayesian model averaging. Also included are the estimated probabilities that the effect size is non-zero.

Variable	Probability the variable effect size is non-zero	Odds ratio (95% confidence interval)
Control zone	0.55	10.3 (1.1 – 100.5)
No farm entrance gate	0.53	7.0 (1.1 – 43.7)
Waterfowl presence	0.40	6.2 (1.1 – 39.6)
Wild bird access to feed	0.25	5.0 (0.8 – 30.8)
Flock size	0.22	5.9 (0.8 – 44.3)
Offsite disposal	0.17	4.1 (0.7 – 25.5)
No specific barn personnel	0.14	6.4 (0.4 – 97.1)
At least some rodent problems	0.11	3.1 (0.6 – 15.3)
Change of clothing not always required for workers	0.10	4.5 (0.4 – 48.4)
Sharing company trucks/trailers	0.07	3.1 (0.4 – 23.5)
Mowing less than 4 times/month	0.07	2.8 (0.4 – 18.6)
Lower level of vehicle washing	0.07	2.7 (0.4 – 20.0)

The most significant farm-level risk factor for HPAI on WOAH poultry [commercial] table egg farms was being located within an existing control zone. Farms that are located near an infected farm must be particularly diligent about biosecurity-related practices to protect flock health. Study findings confirm the need for both biosecurity and surveillance on poultry farms near an infected farm, to prevent infection and ensure rapid detection, whether the virus is likely spreading by wild birds or laterally between farms.

Sightings of wild waterfowl or shorebirds were also associated with increased risk. While this result may be due in part to recall bias by producers on case farms, producers seeking to decrease risk for HPAI may wish to work with a wildlife management specialist to develop a wild bird management plan. Having feed or feed ingredients accessible to wild birds was also a risk factor, with 50 percent of case farms and only 27 percent of control farms reporting this access. In addition, although not statistically significant, only 40 percent of farms that had a protocol to clean spilled feed immediately were classified as cases, while 60 percent of farms that had no protocol listed or a protocol to clean spilled feed less frequently were classified as cases. This further supports the need to include regular inspection of feed housing and prompt cleanup of feed spills in an overall flock management and wild bird management plan.

The presence of a farm gate was found to be protective. Gates were much more commonly reported on control operations than on case operations (64 percent vs. 22 percent). Having a gate may be a proxy variable for other biosecurity practices and could even be associated with a highly proactive approach to biosecurity, i.e., gates improve control of traffic onto farms and may increase the likelihood that visitors will see posted signage and follow requested biosecurity procedures. Workers assigned to specific barns was found to be protective as movement of employees between barns is a known biosecurity risk. Having sufficient time and personnel can affect the degree to which workers are able to carry out good biosecurity practices. Flock size was non-significantly associated with increased risk, with larger operations tending to be more at risk than smaller operations in terms of number of birds on the operation. This may be a finding associated with selection bias because smaller control producers may have been more likely to participate in the study.

A structural windbreak, such as a hill, was one of the factors univariately associated with decreased risk of wild bird introduction of HPAI at $p \leq 0.20$; 0 percent of case farms and 30 percent of control farms reported having an on-farm structural windbreak. Reducing pooling of water around the farm environment is important in minimizing areas around the farm where wild birds may congregate and having a drainage ditch visible or within 350 yards of the farm was a risk factor; 64 percent of case farms had this feature, while 35 percent of control farms did. Having seen wild waterfowl or shorebirds in the closest field during the reference period was also a risk factor; 36 percent of case farms reported having seen wild waterfowl or shorebirds in the closest field during this timeframe, while only 5 percent of control farms did. A higher percentage of case farms reported having any rodent problem, with 73 percent compared to 40 percent of control farms. A higher percentage of case farms also reported wild bird access to feed or feed ingredients at least sometimes, with 73 percent compared to 30 percent of control farms. Feed accessible to wild birds could act as a congregation point for wild birds on the farm and could increase risk of exposure to virus shed by affected wild birds. Cleaning up feed spills immediately was more common among control farms, with 80 percent compared to 50 percent of case farms.

CONCLUSIONS AND NEXT STEPS

This study compared management and biosecurity factors on case and control WOAH poultry [commercial] table egg farms in the U.S. during the 2022 HPAI H5N1 outbreak. Information on risk factors for infection has become increasingly important as this outbreak continues into 2023 and as additional infections in domestic poultry flocks, wild birds, and wildlife species are detected. Study results identified key risk factors associated with HPAI infection on farms and provided information that can be directly applied to support science-based updates to prevention and control recommendations to safeguard table egg farms in the U.S.

A full description of this study, including methods and results, will be available in an open access peer-reviewed publication.

Two additional topics were included in the case-control questionnaire but were not reported here. One section of the questionnaire related to barn-level factors on case and control farms. These data will be analyzed and reported separately. The questionnaire also included challenge-level questions asking for producers' opinions on the level of challenge of certain topics, including biosecurity-, personnel-, and equipment-related issues.

B. Turkey Case Control Study

BACKGROUND

WOAH poultry [commercial] turkey farms comprised the highest percentage of affected WOAH poultry [commercial] farms in the 2022–2023 HPAI outbreak in the U.S.; over 70 percent of all affected WOAH poultry [commercial] farms in the U.S. in 2022 were turkey farms. Wild bird introductions were identified as the primary mechanism of spread in this outbreak. In comparison, the 2015 outbreak was heavily influenced by lateral transmission of virus between farms. Several studies conducted during the 2015 HPAI outbreak explored potential risk factors for transmission of virus between farms (e.g., Dargatz et al, 2016; Garber et al., 2016; Wells et al., 2017). The differences in spread mechanism, as well as the larger geographic scope of the 2022–2023 outbreak as compared to the 2015 outbreak, necessitated further examination into transmission risk and biosecurity practices on turkey farms.

At the request of and with support from State and national poultry organizations, USDA–APHIS conducted a case control study among WOAH poultry [commercial] meat turkey operations to investigate potential risk factors for introduction of HPAI virus onto farms. The objectives of the study included: 1) identify risk factors for infection with HPAI; 2) identify biosecurity challenges on turkey farms; 3) refine biosecurity recommendations to support prevention of infection on farms; and 4) identify priority areas for investment in biosecurity measures to reduce the risk of HPAI infection.

DATA COLLECTION

WOAH poultry [commercial] turkey farms that raised meat turkeys were eligible to participate in the study, whereas WOAH poultry [commercial] turkey breeder farms and WOAH poultry [backyard] farms with turkeys were excluded. Case farms were defined as farms that were confirmed to be positive for HPAI H5N1 by the NVSL between January 2022 and October 2022. Control farms were defined as farms that did not have HPAI in the same time period and were in the same State as case farms. Contact information for case and control farms was obtained from the USDA–APHIS–VS Emergency Management Response System, Thomson Reuters® CLEAR software, State databases where available, and poultry company representatives. At the start of the study, there were 161 HPAI-affected WOAH poultry [commercial] meat turkey farms in 13 States. A total of 153 case farms from 13 States were contacted for participation, and 8 case farms were excluded due to a lack of contact information availability within the study timelines.

A 24-page questionnaire (Appendix C: Commercial Turkey Case Control Survey) was administered to farm managers or supervisors on each participating farm via telephone by National Agricultural Statistics Service (NASS) enumerators, USDA–APHIS epidemiologists, or by mail. The questions focused on farm characteristics, wild birds, wildlife, biosecurity, personnel, visitors, vehicles and equipment, and management practices for the 14 days prior to detection of infection on a case farm and a comparable 14-day reference period on control farms. Some questions asked about practices for the entire farm, and some asked about practices for a "selected barn." The selected barn on case farms was the first barn on the farm to be confirmed HPAI positive, and for control farms, respondents were asked to identify a single barn at random to be designated as the selected barn. Data collection took place between 7 November 2022 and 27 February 2023.

STATISTICAL ANALYSIS

Survey responses were validated for error detection prior to analysis with SAS software. Data were analyzed to identify statistical associations between infected status (case vs. control) and farm or selected barn characteristics, such as management practices. The percentages of case and control farms having each characteristic were calculated. Univariate analyses were performed to identify variables potentially associated with the presence of HPAI. Variables with $p \leq 0.20$ that were also biologically plausible for risk of HPAI infection were considered for entry into candidate multivariable models. Multivariable logistic regression models were fit using forward, backward, and stepwise selection procedures.

Case farms were subset by likely route of virus introduction (common source/lateral transmission or independent wild bird introduction) using the results of whole genome sequencing from NVSL. Subsets of farms that had either common source/lateral transmission or independent wild bird introduction were evaluated via univariate analyses, while a multivariable model was only created for wild bird introduction due to the low number of cases associated with common source/lateral transmission exposure between farms.

MULTIVARIABLE ANALYSIS

Questionnaires were completed for 67 case farms and 61 control farms across 12 States. One case and one control questionnaire were excluded because the farms had only breeder turkeys on-site during the 14-day reference period. One case farm completed both a case and a control questionnaire and the control questionnaire was subsequently excluded from this analysis. After excluding the farms without meat turkeys and adjusting for case-control status, 66 case farms and 59 control farms across 12 States completed questionnaires (Figure 7). The sample included 30 company farms, 50 contract farms (including lessees), and 44 independent farms; 1 farm had a missing response for this question.

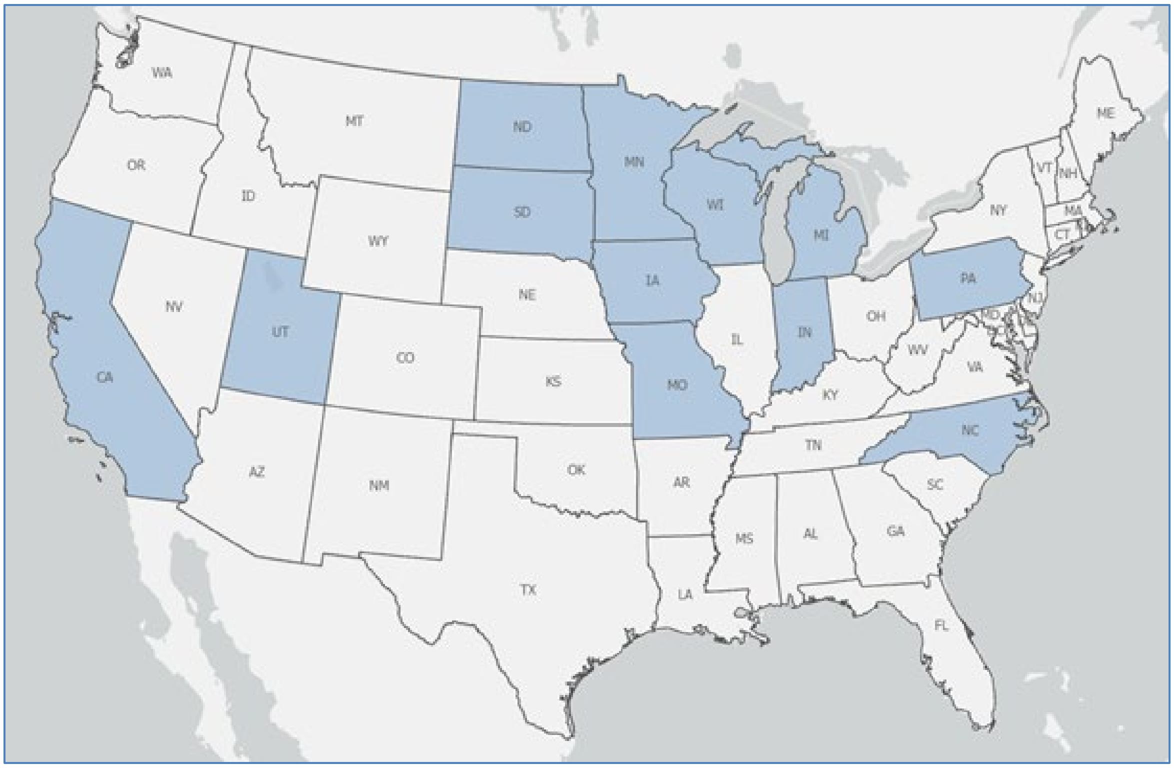

Figure 7. U.S. States with farms that participated in the 2022 HPAI H5N1 WOAH poultry [commercial] meat turkey case-control study.

Seven variables remained in the final multivariable model (Table 5). Farms within an existing control zone had increased odds of being a case (odds ratio [OR] = 3.68, 95% confidence interval [CI] = 1.06–12.74). Other factors associated with increased odds of H5N1 HPAI infection included having both brooder and grower turkey production on the farm (OR = 7.35, CI = 2.51–21.54) and having toms as the sex market type on the farm (OR = 6.86, CI = 1.83–25.79). Seeing wild waterfowl or shorebirds in the closest field was also associated with increased odds of infection (OR = 6.02, CI = 1.83–19.78). The use of rendering for dead bird disposal during the 14-day reference period was associated with increased odds of infection (OR = 8.26, CI = 2.25–30.34). Factors found to have a protective effect included workers entering the selected barn using a shower during the 14-day reference period at least some of the time (OR = 0.29, CI = 0.09–0.98) and having a restroom facility available to crews who visit the farm (OR = 0.32, CI = 0.10–1.05).

Table 5. Results of multivariable logistic regression analysis of factors associated with HPAI H5N1 infection on U.S. WOAH poultry [commercial] meat turkey farms.

Characteristic	% Case farms	% Control farms	Odds ratio (95% CI)	*p*-value
In an existing control zone	31.8	11.9	3.68 (1.06 – 12.74)	0.04
Both brooder and grower stages on farm	51.5	27.1	7.35 (2.51 – 21.54)	<0.01
Sex: toms	86.4	67.8	6.86 (1.83 – 25.79)	<0.01
Waterfowl/shorebirds seen in closest field	30.3	11.9	6.02 (1.83 – 19.78)	<0.01
Worker biosecurity includes shower before entering barn[A]	10.6	27.1	0.29 (0.09 – 0.98)	0.05
Restroom facility available to crews visiting farm	45.5	69.5	0.32 (0.10 – 1.05)[B]	0.05
Render dead birds	30.3	13.6	8.26 (2.25 – 30.34)	<0.01

[A] Workers always, most of the time, or sometimes used the practice before entering the barn vs. never or not available. This question was asked specifically for the selected barn, and for the 14-day reference period.

[B] Odds ratio is for comparison between always/sometimes available vs. never available.

A multivariate model based on data from the subset of farms linked to wild bird introductions was similar to the risk factors identified from the farm-level model described above in Table 5, other than the control zone becoming non-significant (data not shown).

CONCLUSIONS AND NEXT STEPS

This study compared management and biosecurity factors on case and control meat turkey farms in the U.S. during the HPAI H5N1 outbreak in 2022–2023. Knowledge of risk factors for infection has become increasingly important as this outbreak continues into 2023 and as additional infections in domestic poultry flocks, wild birds, and wildlife species are detected. Study results identified key risk factors associated with HPAI infection on farms and provided information that can be directly applied to support science-based updates to prevention and control recommendations to safeguard turkey farms in the U.S.

A full description of this study, including methods and results, will be available in a peer-reviewed publication.

Future work may help further improve our understanding of the complex epidemiology of avian influenza transmission between wild birds and domestic poultry. Two additional topics were included in the case control questionnaire but were not reported here. One section of the questionnaire was related to biosecurity investments, including questions regarding ongoing biosecurity expenses and permanent and temporary improvements made since 2015 that impact farm biosecurity. These data will be analyzed and reported separately to identify priority areas for investment in biosecurity measures to reduce risk for HPAI. The questionnaire also included challenge-level questions asking for producers' opinions on the level of challenge of certain topics, including biosecurity-, personnel-, and equipment-related issues. Finally, weather conditions and patterns related to avian influenza virus transmission have been examined previously and could have played a role in the outbreak in 2022–2023 (Chen et al., 2022; Liu et al., 2007; Si et al., 2013). Future work could expand upon the case control study presented here to incorporate weather variables, such as temperature, relative humidity, precipitation, and wind speed in the time preceding detection to investigate the role of weather on risk of HPAI infection.

C. Turkey Case-Control Study Economic Analysis

PRELIMINARY RESULTS

Initial results from the turkey case-control study show that there is heterogeneity between case and control farms in on-farm biosecurity and investments. Comparing mean values of data collected for control and case farms, we find that case farms were slightly larger, having a mean barn count of 5.24, compared to 4.47 for control farms (statistically significant difference in means at $p < 0.05$). Control farms had statistically significant ($p < 0.01$) higher monthly biosecurity costs than case farms, $1,572 and $950, respectively. Control farms also spent more on temporary biosecurity measures ($27,657 vs. $21,159; $p < 0.01$), such as gates, parking areas, temporary wild bird migration, temporary air intake inlet covers, or temporary vehicle wash stations.

Logistic regression analyses show that there are heterogenous factors driving investments in biosecurity. National Poultry Improvement Plan (NPIP) participation increased the likelihood of investing in additional temporary biosecurity measures by 26.9 percent ($p < 0.10$). Dual sex farms were 0.2 percent more likely ($p < 0.10$) to spend more each month on biosecurity costs than single sex farms. This may be related to added precautions for birds, or the type of biosecurity system that requires more monthly outlays. Surprisingly, dual sex farms were 48.6 percent less likely ($p < 0.01$) to have invested in temporary biosecurity over the previous two years. This may reflect a more modernized infrastructure or an inclination for permanent investments over temporary for this production type.

Farms that had permanently invested in improvements or renovations in the last year were less likely to have plans for future permanent investments. For example, a farmer who invested in a room that separates the “outside area” from the “inside area,” such as a Dutch entrance, was estimated to be 42.7 percent less likely to have permanent biosecurity investment plans. This may imply that those making the investment in the prior two years may have no economically feasible investments left to make. Importantly, case farms were 87.7 percent ($p < 0.001$) more likely to have plans to make permanent changes to biosecurity. This could signal ongoing and continued improvements in biosecurity to help mitigate HPAI incursions.

The results presented here are preliminary. Analysis of the biosecurity investment data collected as part of the turkey case-control study is ongoing. Final results will be presented in a future report and in peer-reviewed publication.

D. Turkey and Table Egg Study Weather Analysis

BACKGROUND

During the 2022–2023 HPAI outbreak in the U.S., several industry members expressed an interest in the role of weather in HPAI infections. Previous research has investigated the effects of temperature, humidity, and other weather variables on risk of avian influenza transmission (e.g., Chen et al. 2022; Liu et. al. 2018; Si et al., 2013).

USDA–APHIS is conducting a case-crossover study to examine associations between weather variables, such as wind speed, relative humidity, precipitation, and temperature, and HPAI infection on WOAH poultry [commercial] farms during the 2022 HPAI outbreak. Preliminary results are presented in this report, and this report only covers farms that were infected during 2022.

DATA COLLECTION AND STATISTICAL ANALYSIS

For this study, we included all WOAH poultry [commercial] turkey and table egg farms that were infected with HPAI in 2022, based on Emergency Management Response System (EMRS) data. This included turkey breeder, turkey meat production, layer breeder, layer pullet, and table egg layer farms. Each HPAI-affected farm served as its own control in a case-crossover study design. The hazard period was defined as the two weeks before onset of HPAI, and the control period was the two weeks before the hazard period (Figure 8). The hazard period was selected based on results from time of introduction analysis presented in this report. The control period was selected to be the same duration and close in time to the hazard period.

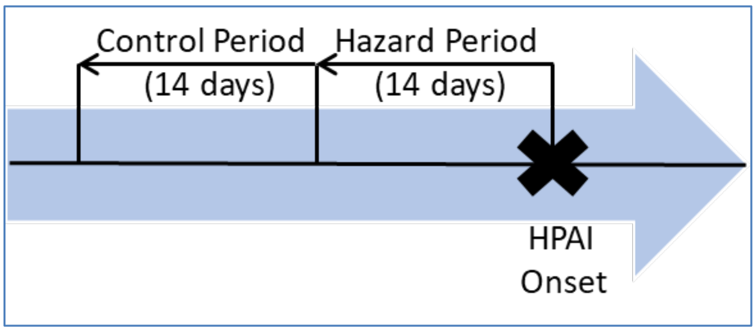

Figure 8. Definition of hazard and control periods used in the WOAH poultry [commercial] turkey and table egg study weather analyses.

Weather data for the hazard and control periods was obtained from gridMET (2023), a database containing daily weather information in 4 km^2 grids across the U.S. Weather variables were averaged for each 14-day period of interest, and weather was compared between the hazard and control periods using conditional logistic regression models, which are appropriate for matched case-control data. The following weather variables were evaluated for a univariate association with HPAI infection risk on-farm: daily maximum temperature, daily minimum temperature, daily precipitation, daily average wind velocity, daily maximum relative humidity, daily minimum relative humidity, daily specific humidity, and downward shortwave radiation.

PRELIMINARY RESULTS

A total of 218 HPAI events on turkey farms and 37 HPAI events on layer farms were included in the analysis. The univariate analysis for turkey farms showed that increases in precipitation, minimum temperature, and wind speed were associated with increased risk of HPAI (Table 6). The univariate analysis for layer farms showed that increases in precipitation were associated with increased risk of HPAI (

Table 7).

Table 6. Results of univariate conditional logistic regression analysis of weather factors associated with HPAI H5N1 infection on U.S. WOAH poultry [commercial] turkey farms.

Variable (14-day average)	Median Hazard Period	Median Control Period	Odds ratio (95% CI)	*p*-value
Precipitation (mm)	1.43	0.78	1.28 (1.08 – 1.52)	0.004
Minimum temperature (°C)	-2.87	-3.68	1.08 (1.02 – 1.14)	0.006
Average wind speed (m/s)	5.10	4.47	4.25 (2.77 – 6.52)	<0.001

Table 7. Results of univariate conditional logistic regression analysis of weather factors associated with HPAI H5N1 infection on U.S. WOAH poultry [commercial] layer farms.

Variable (14-day average)	Median Hazard Period	Median Control Period	Odds ratio (95% CI)	*p*-value
Precipitation (mm)	1.44	0.84	1.52 (1.02 – 2.25)	0.038

Most HPAI infections on turkey farms occurred in the spring. The higher HPAI risk with increasing temperature may not be a direct effect from temperature; instead, it may be an indirect effect from increasing wild bird activity as temperature increases in the spring. The results presented here are preliminary and should be interpreted cautiously since weather variables often have complex interactions. Multivariable modeling is needed before drawing final conclusions about associations between weather and HPAI risk.

CONCLUSIONS AND NEXT STEPS

Weather conditions, including wind, precipitation, and temperature may play a role in HPAI infection on WOAH poultry [commercial] farms. The next step for this study is to assess weather variables collectively using multivariable logistic regression modeling. Since many weather variables are related, multivariable modeling will allow the best understanding of how weather affects HPAI risk.

ESTIMATING THE TIME OF H5N1 HPAI INTRODUCTION INTO WOAH POULTRY [COMMERCIAL] FLOCKS USING DIAGNOSTIC TEST RESULTS AND PRODUCTION DATA

A. Summary

Determining the time of HPAI virus introduction in a flock is an important part of outbreak investigations. By narrowing the time window of possible virus introduction, we can better identify the potential transmission routes and enhance our understanding of the pattern of disease spread. In collaboration with researchers at the University of Minnesota, time of introduction (TOI) analysis was conducted on a subset of premises at the request of field responders and was dependent on the willingness of producers to provide the necessary data. Additionally, premises initially thought to be involved with lateral spread clusters were prioritized. Although most premises have more than one house of birds, TOI analysis was only performed on the house speculated to be the index case for the premises. Data utilized in the analysis included diagnostic testing, daily mortality, and water consumption data (where applicable). A total of 53 WOAH poultry [commercial] premises were analyzed, including 37 WOAH poultry [commercial] meat turkey, 4 broiler chicken, 8 table egg layer, 1 table egg layer pullet, 3 duck breeder, and 1 duck meat bird flocks. Detailed modeling methodology can be found in Appendix E: Time of Introduction Modeling Methods.

B. Results

The analyzed premises were grouped into anonymized State and phylogenetic clusters and results are presented by a cluster-specific, relative timeline (i.e., Day 1 for each cluster is a different calendar date than Day 1 for the other clusters; Figure 9). For each premises analyzed, a most likely day of introduction was estimated, as well as a 95 percent credibility interval, which represents a window of possible virus introduction for each premises. The day of presumptive diagnosis is also noted in (Figure 9) to provide an indication of the period of likely infectiousness for each premises. The source of introduction, also indicated in Figure 9, is based on phylogenetic analysis (see Phylogenetic Analysis and Diagnostics section for details) that is supportive of either independent wild bird introduction (IWBI) or common source/lateral spread (CS LT). Phylogenetic evidence is valuable in identifying and supporting potential sources of introduction but cannot be considered definitive proof and must be evaluated in conjunction with available epidemiological data. A few premises were classified as independent wild bird introduction with genotypic similarities (IWBI*), which means viruses isolated from these premises were either phylogenetically similar to those at other premises but had no plausible epidemiological links or were genetically similar to another premises' virus but had more viral sequence mutations than those used to define CS LT cases. Among the premises included in this analysis, the phylogenetic data suggested 17 of the analyzed premises were IWBI, 6 premises were IWBI*, and 30 were CS LT. One premises included in this analysis was unable to be sequenced.

Each cluster presents a unique pattern, that in combination with phylogenetic evidence, can be suggestive of the timing and routes of introduction to help narrow the focus of epidemiologic investigations.

State A had four premises analyzed as part of a regional cluster of six premises. Phylogenetic analysis suggested three of these premises were the result of an IWBI. One of the IWBI premises was phylogenetically linked to three premises thought to be infected via a common source or lateral spread. Time of introduction analysis was only performed on one of the linked premises and the estimated window of likely virus introduction occurred after the detection of the initially infected premises. One of the premises not analyzed was detected in between these two premises, which suggests it may have played a role in the transmission between the cluster of farms; however, field investigations did not identify direct epidemiological links between the four phylogenetically-linked premises.

The analyzed premises in State B and State C are geographically related and part of a cluster of seven premises detected in a one-month period initially speculated to be a phylogenetic cluster. Field investigators only identified epidemiological links between two premises in State B, and both premises belonged to the same corporate producer. A notable observation in these clusters is the wide time range of possible introduction and the time to detection for the broiler premises in State B. Time of introduction analysis relies heavily on baseline mortality data. Prior to detection, this premises had an increase in mortality associated with another pathogen that resolved prior to the rapid increase in HPAI-associated mortality. In addition, diagnostic samples were collected, and the premises was quarantined on a Friday, but the samples were not analyzed until the following Monday, delaying the initial diagnosis.

The two analyzed premises in State D were in a phylogenetic cluster of seven infected premises attributed to common source or lateral spread transmission. While the definitive causes of spread between all premises were not determined, field investigation identified company affiliations, shared farm personnel, equipment, vehicles, and contracted rendering services as potential routes of transmission.

State E had several phylogenetic clusters during the outbreak. Time of introduction analysis was only performed on a subset of these premises due to data and resource availability. Several of the premises analyzed were initially thought to be a part of lateral spread clusters based on their geographic proximity to other infected premises. For example, two turkey meat bird premises in the independent cluster were initially assumed to be associated with the premises in cluster 4 due to their relative locations; however, genetic sequencing indicated these premises were not linked phylogenetically. Rather, the premises in cluster 4 was linked to a separate premises that was not analyzed. Results for seven out of nine premises in cluster 5 are shown in Figure 9. Although two premises were not analyzed, the windows of introduction for the premises potentially infected by a common source or lateral spread are after the day of presumptive diagnosis for the clusters index case. Analytical epidemiologists investigated the potential for windborne transmission during the depopulation and disposal of the index premises, based on the shared geographic relationship between index premises and the cluster premises subsequently detected.

Results are presented for 10 out of 16 premises in a phylogenetic cluster in State F. Time of introduction results for the WOAH poultry [commercial] duck premises should be cautiously interpreted due to limited information to inform modeling priors and the unique structure of WOAH poultry [commercial] duck production facilities. Potential sources of lateral spread within this cluster included employees with common living arrangements, egg movements, and shared company ownership.

State G is the last analyzed State with phylogenetic clusters. This State had 2 temporal clusters of HPAI cases—originally a cluster of 3 premises, subsequently followed by a cluster of 15 premises. In the first cluster, a rendering truck was speculated to be involved in lateral transmission; however, a unique genotypic mutation assumed to occur in a wild bird suggested the third infected premises was the result of a separate wild bird introduction. Given the relative proximity of these premises to each other, it is plausible that the three barns were infected from a common wild bird source. In the second cluster, premovement tests failed to capture an infected premises that led to the infection of three other premises; definitive sources of spread for the remaining 12 premises in the cluster were not identified, though potential sources included company affiliation, spatial proximity, feed delivery, and the movement of people.

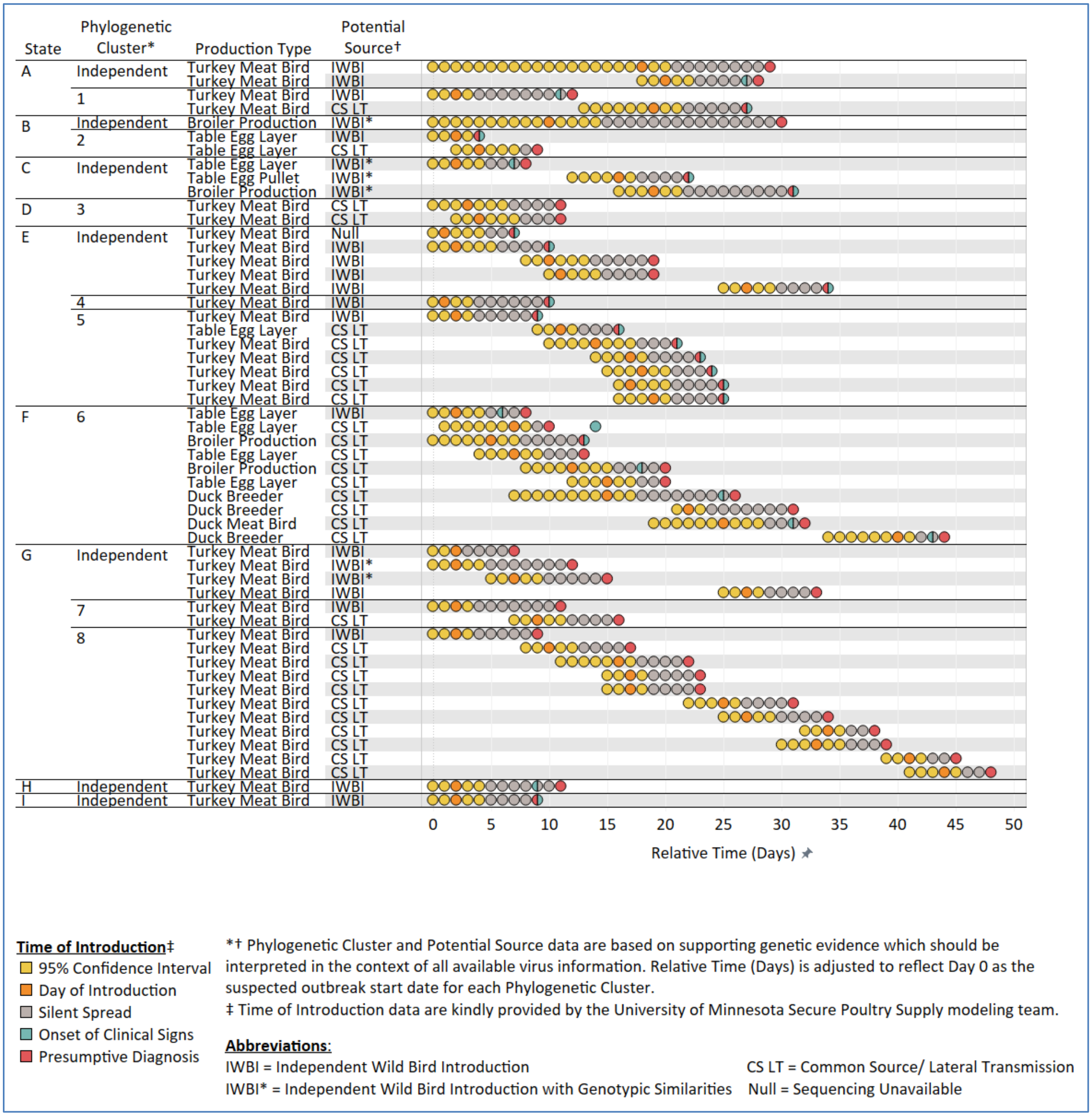

Figure 9. Relative timeline for time of introduction analysis by State, phylogenetic cluster, production type, and likely source of introduction.

In addition to contributing to epidemiological investigations, time of introduction analysis can provide insight into how rapidly cases were detected following virus introduction and to within-house virus-spread dynamics. To assess the timeline of case detection, we examined the time to first positive sample (TFPS), which was the time difference between the estimated time of flock exposure and when the first rRT-PCR positive samples were collected. The observed differences in TFPS among the production types are seen in Figure 10. Table egg layers or pullet premises had the shortest TFPS, with a range of two to six days. Broiler production premises had the longest TFPS, with a range of 8 to 20 days. Turkey meat bird and duck premises were in between with a range of 4 to 11 days from the most likely day of virus exposure to disease detection.

The shorter TFPS for table egg layers may be because seven out of nine table egg layer premises included in the analysis were under active surveillance as part of Control Area or Surveillance Zone protocols. These premises were frequently submitting diagnostic tests for permitted egg movements. Overall, when premises are grouped by reason for testing, the estimated median TFPS was six days for premises under ongoing surveillance testing (e.g., testing within a Control Area or Surveillance Zone or for movement permits), and eight days when testing was requested based on observing HPAI clinical signs in the flock. When grouped by the likely source of introduction, the estimated median TFPS was six days for common source or lateral spread introductions and eight days for independent wild bird introductions. This pattern held constant across production types. A possible explanation for the earlier detection of common source or lateral spread infections is the local area surveillance activities that occur after a detection.

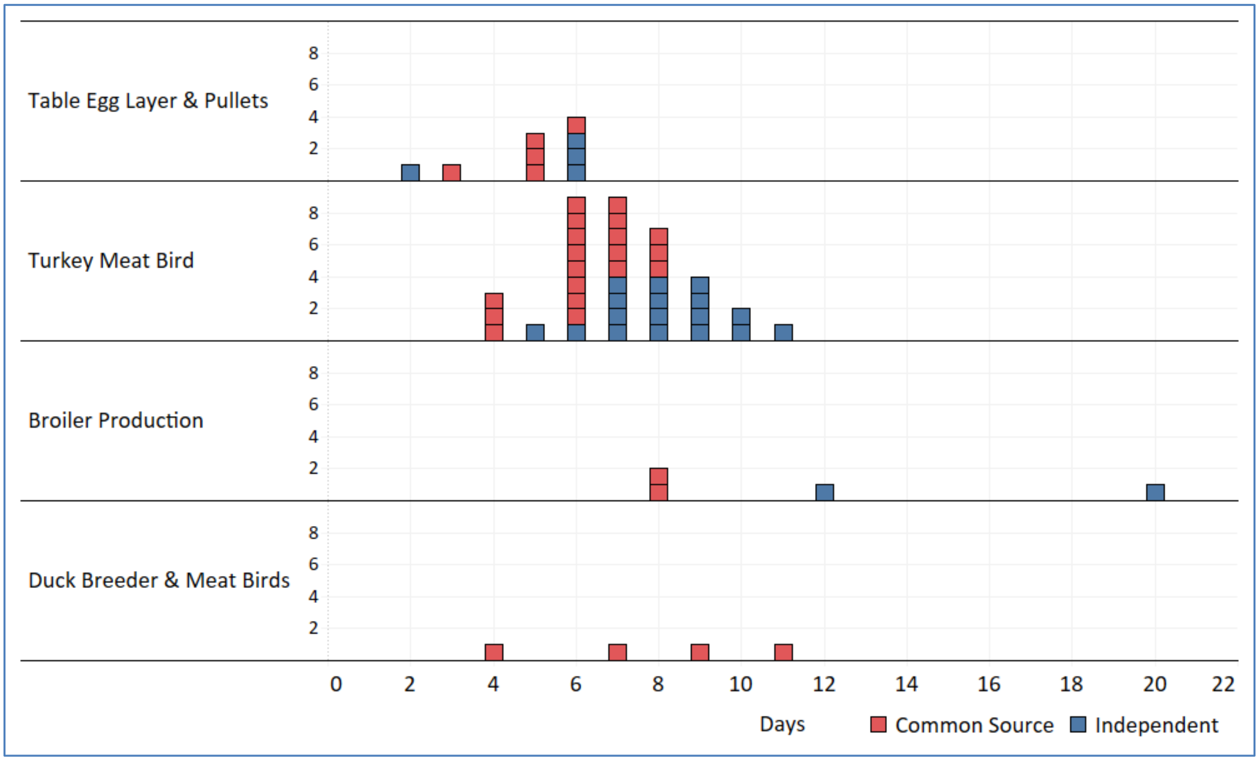

Figure 10. Time from estimated HPAI introduction to detection by production type and likely source of introduction.

The estimated adequate contact rate, or transmission parameter, is the number of contacts per day a bird has with other birds that would be sufficient to result in infection. This is the parameter that determines the rate of virus spread within the flock. The mean of the most likely value for the adequate contact rate from all premises was 4.9 contacts per day (range 0.5–18.7 contacts per day; Figure 11). Contact rates were similar across all production types, except for broiler production premises. The mean contact rate was 5.0 contacts per day (range 0.5–14.1) for turkey premises, 5.8 contacts per day (range 1.1–18.7) for table egg layer and pullet premises, 5.3 contacts per day (range 1.1–8.7) for duck premises, and 1.7 contacts per day (range 0.6–2.7) for broiler premises. The reason for the difference between broiler production premises and the other production types is unknown but may be a combination of factors related to species, housing, and other management practices. For example, turkey meat bird and broiler production premises are both floor-raised production types, where birds have the opportunity to interact with any other bird in the house; however, past studies have shown turkeys to be more susceptible to avian influenza viruses than chickens, which may result in a higher rate of spread in turkey houses (Pillai, Pantin-Jackwood, Yassine, Saif, & Lee, 2010). The difference between broilers and table egg layers may be related to differences in housing type, ventilation, foot traffic, or other production practices that could increase the rate of spread in table egg layer houses compared to broilers. When grouped by the source of introduction, the estimated mean contact rate was 4.8 for CS LT and 5.1 for IWBI.

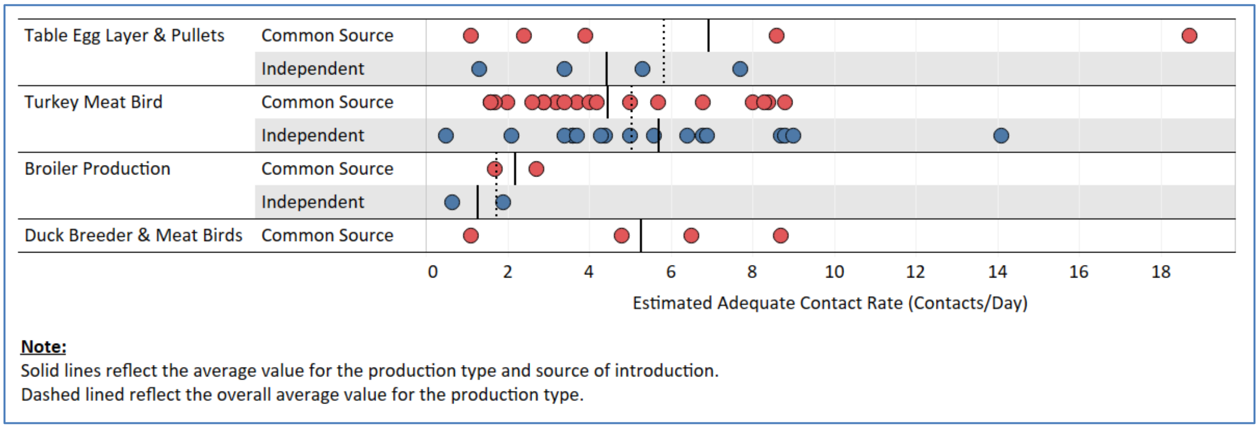

Figure 11. Estimated adequate contact rate by production type and likely source of introduction, estimated by time of introduction analysis.

The following is a similar breakdown by production type for the estimated basic reproduction number (R0; Figure 12). The overall mean R0 value was 13.5 (range 2–62). For turkeys, the mean R0 was 16.3 (range 3–47). For table egg layer and pullet premises, the mean R0 was 11.9 (range 2–37). For the broilers, the mean R0 was 5.6 (range 2.4–8). For ducks, the mean R0 was 36.75 (range 9–62). The basic reproduction number is a function of the rate of transmission and the duration of infectiousness; therefore, the difference in R0 between turkeys and table egg layers that was not observed in their contact rates could be due to differences in the duration of infectiousness. As mentioned previously in relation to TFPS, the difference in duration of infectiousness may be related to faster detection in table egg layers than turkeys because of a greater intensity of active surveillance and/or premovement testing applied to the table egg layers in this analysis. The H5N1 virus responsible for these infections is particularly adapted to waterfowl and may explain why WOAH poultry [commercial] ducks had higher reproduction numbers. Grouped by source of introduction, the average basic reproduction number was 16.7 for CS LT and 15.8 for IWBI.

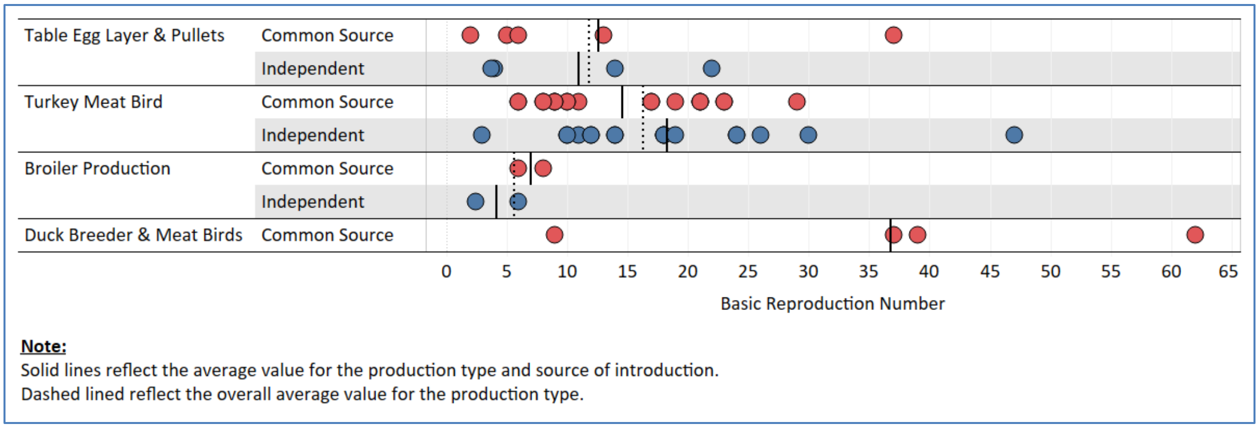

Figure 12. Basic reproduction number (R0) by production type and likely source of introduction, estimated by time of introduction analysis.

C. Discussion

Estimating the time of HPAI virus introduction provides a valuable piece of information for epidemiologic investigations and outbreak response. In this analysis, we estimated the time window for HPAI introduction and transmission parameters for 53 infected barns using diagnostic test results and production data. The analysis was used to narrow the time window of possible virus introduction to help identify routes of transmission.

This work is dependent on information on the progression of disease mortality and clinical signs from production records and regular laboratory diagnostic testing. Access to different categories of detailed, high quality production data, such as daily mortality, egg production, and water consumption helps to provide more robust estimates of the time of introduction and reduce the uncertainty. For example, the estimated time of introduction 95 percent credible interval was narrower where both daily mortality and water consumption data were incorporated into the analysis. Conversely, the estimated intervals for time of introduction were wider for premises without elevated mortality and with fewer days of diagnostic testing. This work also highlights the value of closely monitoring mortality, water consumption, and egg production to quickly identify disease issues in the flock. These factors may vary across flocks and between barns, so understanding the trends within each production setting is important. It should also be acknowledged that model results rely on input parameters from HPAI experimental studies and may vary as data from newer studies are considered; however, preliminary sensitivity analysis suggests that time of introduction estimates are relatively robust, and changes are not anticipated to be substantial.

AVIAN INFLUENZA SURVEILLANCE IN WILD BIRDS

A. Background

Waterfowl are natural reservoir hosts for influenza A viruses (IAV; subtypes H1-H16), but not usually highly pathogenic avian influenza. 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 13). 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.

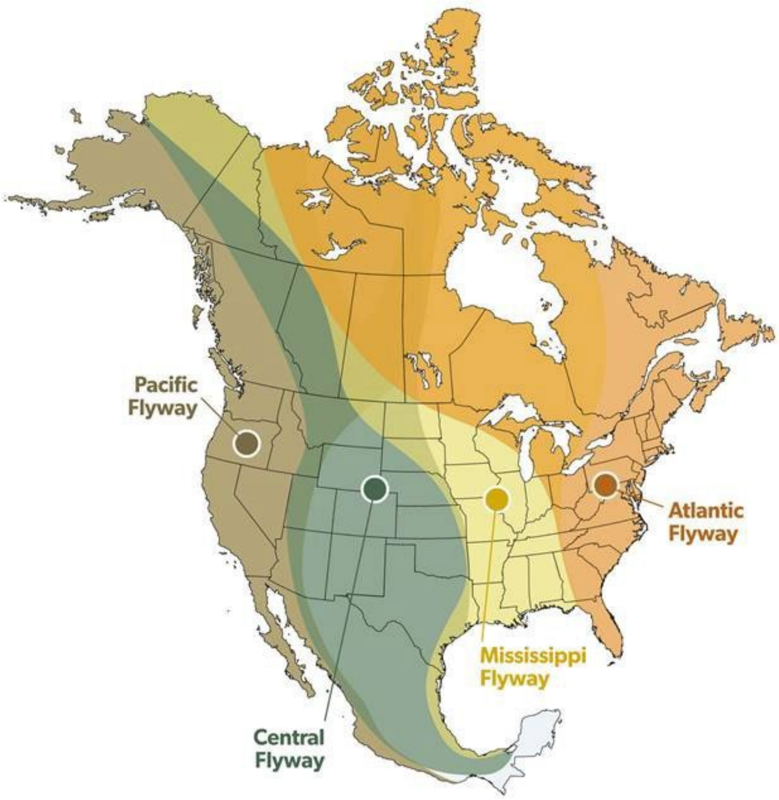

Figure 13. Map depicting the four primary North American waterfowl flyways.[7]

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. During migratory movement, wild birds have the potential of dispersing pathogens, such as IAV, across wide geographic distances.

The first detection of Eurasian strain (EA) H5N1 HPAI in North America occurred in a great black-backed gull in December 2021 in Newfoundland and Labrador, Canada. The bird was showing neurologic signs and was part of a large mortality event. The first subsequent detection of H5N1 HPAI in the U.S. was reported in January 2022 in a dabbling duck from South Carolina. The bird was exhibiting no neurologic signs and was an apparently healthy bird collected during hunter harvest.

B. Wild Bird Surveillance Program

The U.S. National Surveillance Plan for Highly Pathogenic Avian Influenza in Wild Birds was developed to maximize our ability to detect IAV in wild waterfowl. Surveillance helps to 1) understand how IAV is distributed in the U.S.; 2) detect the spread of IAV to new areas of concern; and 3) monitor wild dabbling duck populations for introductions of novel viruses (e.g., Eurasian lineage H5 and H7). Targeted surveillance focuses on sampling apparently healthy dabbling duck species from areas with extensive mixing of wild bird populations and a history of IAV detection.

Between 30 December 2021 and 31 March 2023, over 40,000 apparently healthy wild waterfowl were sampled and tested by rRT-PCR for IAV. 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 samples where viral RNA is detected. H5 and H7 positive samples are forwarded to NVSL for confirmatory testing. The number of H5N1 lineage virus detections is based on viruses confirmed at NVSL from H5N1 presumptive samples forwarded by NAHLN laboratories. Overall, targeted wild bird surveillance conducted by USDA–APHIS–WS has resulted in 2,264 detections of H5N1 HPAI lineage virus across all four administrative flyways (Table 8).

Table 8. Number of H5N1 HPAI detections from apparently healthy wild birds sampled by USDA–APHIS–WS between 1 June 2022 and 31 March 2023.

Flyway	# Birds Sampled	# H5N1 HPAI Detections
Atlantic	12,920	667
Mississippi	10,569	584
Central	5,899	443
Pacific	10,717	570

[7] Ducks Unlimited Canada. (n.d.). Flyways of North America [Map]. Retrieved June 21, 2022, from https://www.multivu.com/players/English/7804651-ducks-unlimited-migration

Total	40,105	2,264

C. Morbidity/Mortality Sampling

The investigation of morbidity/mortality events is another important strategy for detection of HPAI in wild birds. During morbidity/mortality events, sick or dead birds may be submitted for testing and cause-of-death determination, and a subset of birds may be sampled for IAV testing. Investigations related to morbidity/mortality events are conducted regardless of the time of year or species involved, and morbidity/mortality events may involve one bird or hundreds of birds (although a small subset of birds are typically sampled at large-scale die offs). Morbidity/mortality samples are collected by a wide variety of entities, including but not limited to USDA–APHIS–WS, State wildlife agencies, the U.S. Fish and Wildlife Service, USGS National Wildlife Health Center, and universities.

Between 30 December 2021 and 31 March 2023, morbidity/mortality investigations resulted in 3,639 detections of H5N1 HPAI lineage virus in sick or dead birds across all four Flyways: Atlantic – 1,033; Mississippi – 990; Central – 752; and Pacific – 864. Altogether, targeted surveillance samples collected by USDA–APHIS–WS and other agencies, and morbidity/mortality investigations of sick or dead birds during this time period have resulted in 6,086 detections of H5N1 HPAI lineage virus in over 130 wild bird species across 49 States, plus Washington, D.C. (Figure 14 and Table D1).

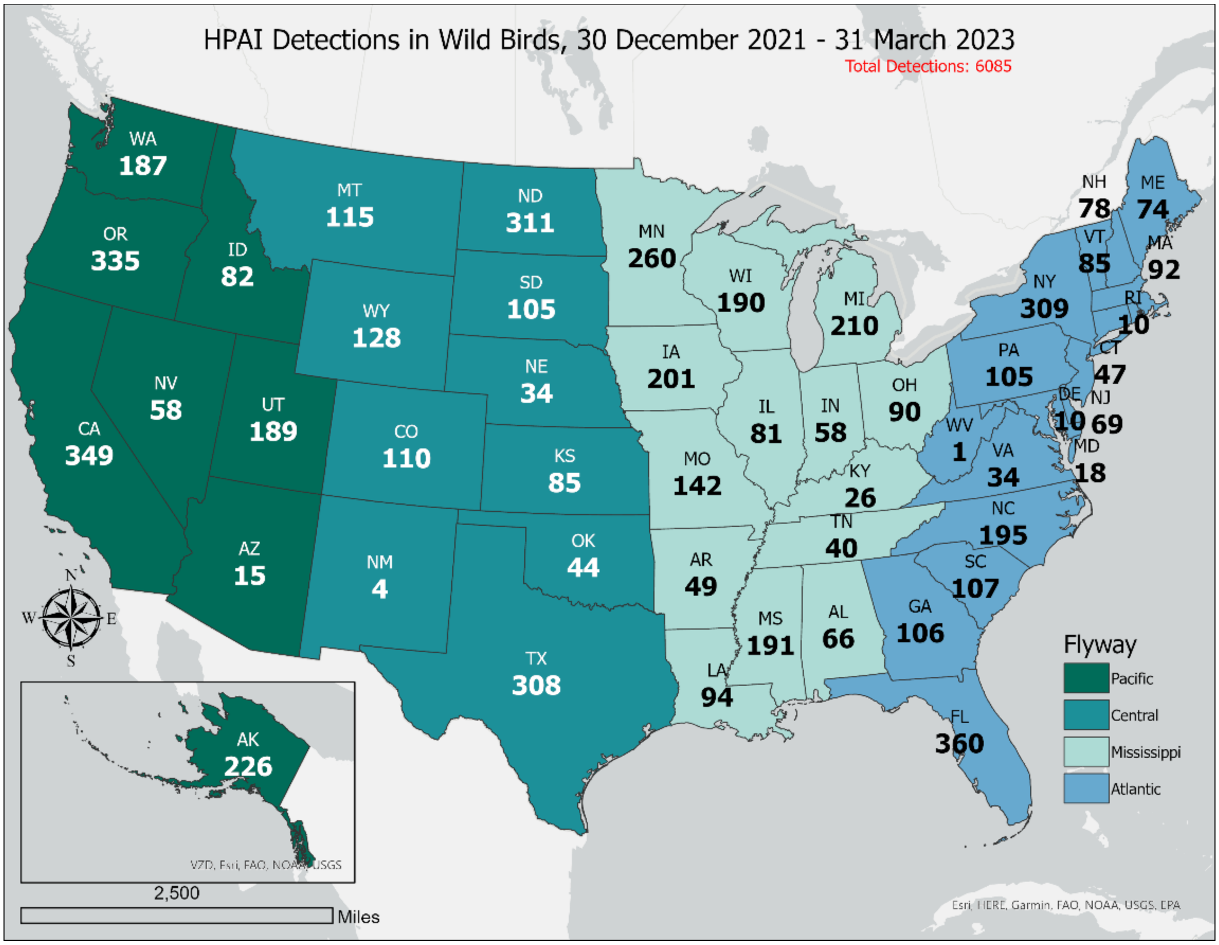

Figure 14. Number of H5N1 HPAI detections in wild bird species tested between 1 June 2022 and 31 March 2023. State totals include detections from both apparently healthy birds and sick/dead birds sampled as part of morbidity/mortality investigations.

D. HPAI Detections in Mammals

Although IAVs primarily affect poultry and wild birds, these viruses can occasionally be transmitted to mammals. A rising number of H5N1 HPAI cases have been reported in several terrestrial and aquatic mammalian animals across the U.S. (Figure 15). Infection may cause illness, including severe disease and death in some cases. As of 31 March 2023, there have been 154 H5N1 HPAI detections in at least 17 wild mammal species in 23 States since the start of the 2022 - 2023 HPAI outbreak. All the H5N1 HPAI detections in mammals have been from sick or dead animals. There is currently no nationwide active HPAI surveillance effort in apparently healthy wild mammals.

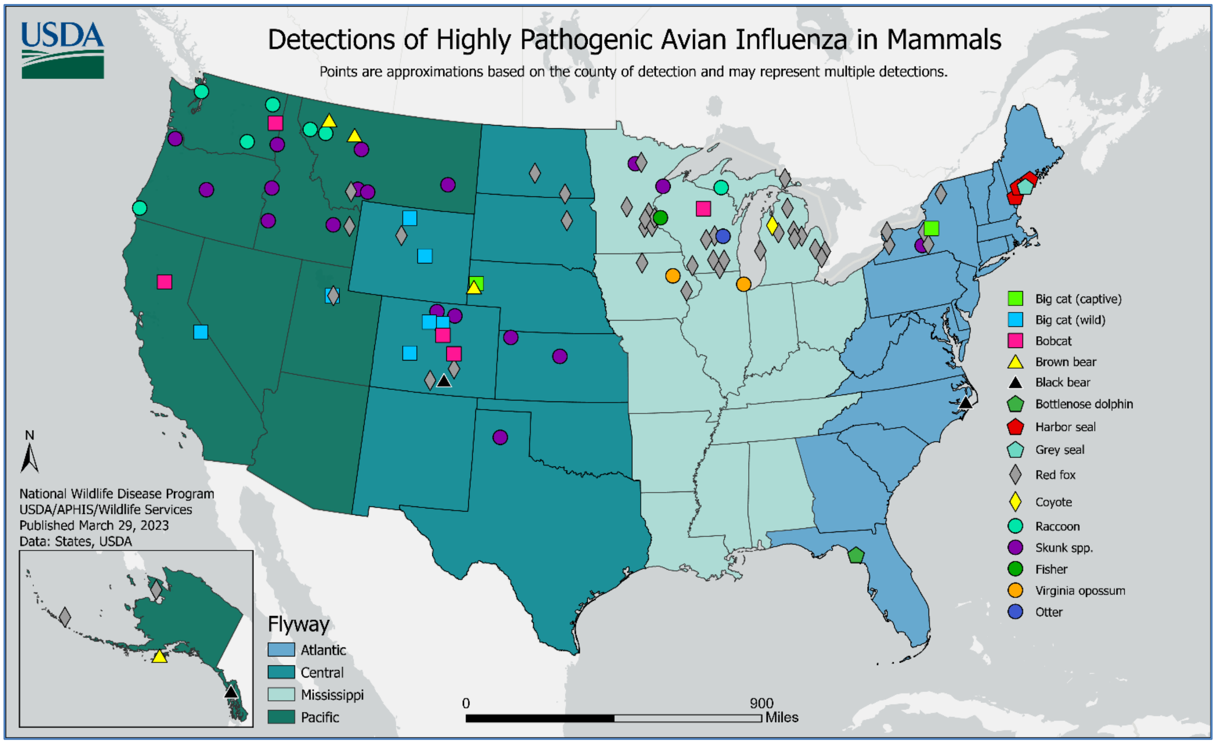

Figure 15. Detections of HPAI in mammals as of 31 March 2023.

MODELING AVIAN INFLUENZA TRANSMISSION AT THE INTERFACE OF WILD BIRDS AND DOMESTIC POULTRY

A. Background

With support from USDA–APHIS, collaborators from the United States Geological Survey (USGS) and the University of Maryland are currently modeling the risk of avian influenza virus transmission from wild waterfowl to domestic poultry across the contiguous U.S. The objectives of the study include the creation of fine scale spatio-temporal transmission risk estimates of spillover of virus from wild birds to domestic poultry for the contiguous U.S., and identification of factors influencing changes in relative risk of spillover across the landscape through time.

B. Methods

MODELING FRAMEWORK

Our objective is to model variation in both the spatial and temporal risk of an avian influenza spillover event from wild reservoir species into domestic poultry operations. Spillover events are generally not reported in the U.S. for viruses of low-pathogenicity, and incursion of highly pathogenic viruses has historically been rare; therefore, little data have been available to predict the risk of spillover from wild birds to domestic poultry.

The risk of a wild to domestic spillover event (hereafter, spillover event) can be modeled as the transmission risk (R) between wild birds and domestic poultry operations using the following equations:

$$R = W_i \times P_r$$

where W_i is the estimated number of infected waterfowl present at a given time and place and P_r is the number of poultry facilities in that location weighted by their risk of a spillover event occurring given facility type. This equation can be further broken down to:

$$R = \sum_s (A_s \times P_s) \times \sum_t (N_t \times R_t)$$

where A_s is the abundance of waterfowl of species s, P_s is the percentage of those waterfowl positive with avian influenza virus, N_t is the number of poultry operations of a given type t, and R_t is the relative risk associated with each operation type.

Estimates of waterfowl abundance (A_s) were taken from the predicted relative abundance models generated for eBird Status & Trends (Fink et al., 2020). We included estimates for 30 common waterfowl species. The percentage of infected waterfowl (P_s) of each species at each weekly interval comes from a previous effort to model avian influenza prevalence in wild waterfowl (Kent et al., 2022), and the number and type of poultry operations (N_t) come from a previously developed model (Patyk et al., 2020). Finally, as there is known variation in the relative risk of different farm types, we developed a relative risk scaler for each poultry operation type.

MODEL VALIDATION

In general, these models are designed to estimate the risk of spillover of low-pathogenicity endemic viruses into domestic poultry operations, but validation data for such spillover events are generally not available in the U.S. We instead used data from the current U.S. HPAI outbreak for model validation.

Model validation took place at the county level by summing predicted risk within each county for each weekly step. Inference on model validation was based on the area under the receiver operating characteristic curve (AUC) to understand the model's ability to correctly identify counties that experienced a spillover event. This value was based on whether a county experienced a spillover event in each week and the rank-relative risk for that county during that week.

C. Preliminary Results

In general, we see the predicted risk of spillover events shifting north and south with waterfowl migration, with the overall level of risk rising and falling with avian influenza prevalence, reaching its lowest levels in late spring and early summer. Additionally, we see some areas of particularly high poultry production that remain at an elevated risk year-round. Results from selected weeks can be viewed in Figure 16.

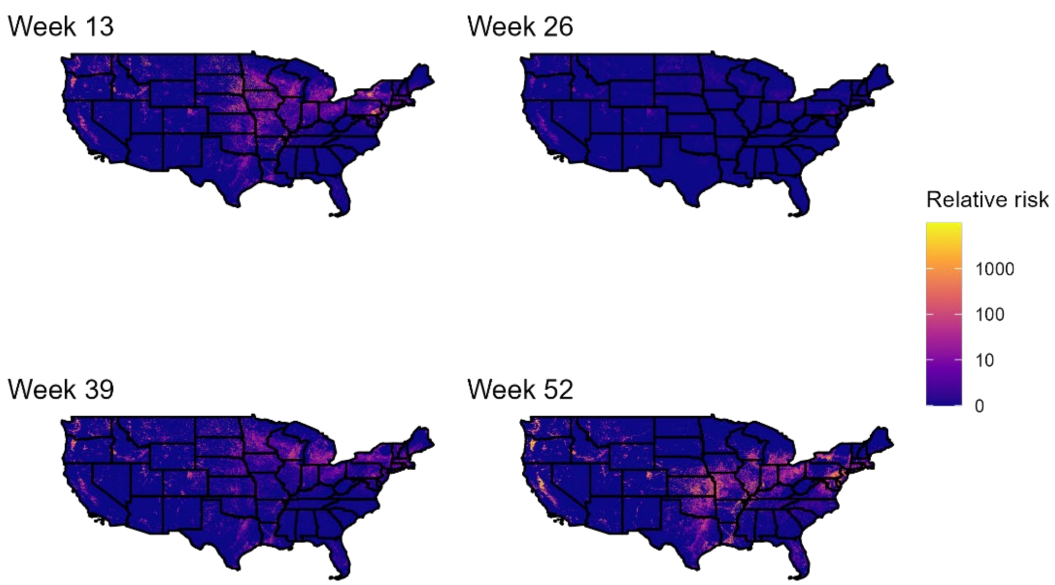

Figure 16. Model-predicted risk of avian influenza spillover from wild waterfowl to domestic poultry across the contiguous United States during four selected weeks of the year.

Data through January 2023 of the current outbreak were used to evaluate model performance. Outbreaks overwhelmingly occurred in counties with greater than average risk (Figure 17), and overall, the model appears to have performed well at predicting spillover events during the current HPAI outbreak. In counties with above-average risk, 244 spillover events occurred, while only 20 occurred in counties with a below-average risk. Only 5 five spillover events occurred in counties categorized as "low risk" by the interface model, compared to 195 spillover events in "high-risk" counties. Model results are now available online for poultry owner use to better understand their own risk context.[8]

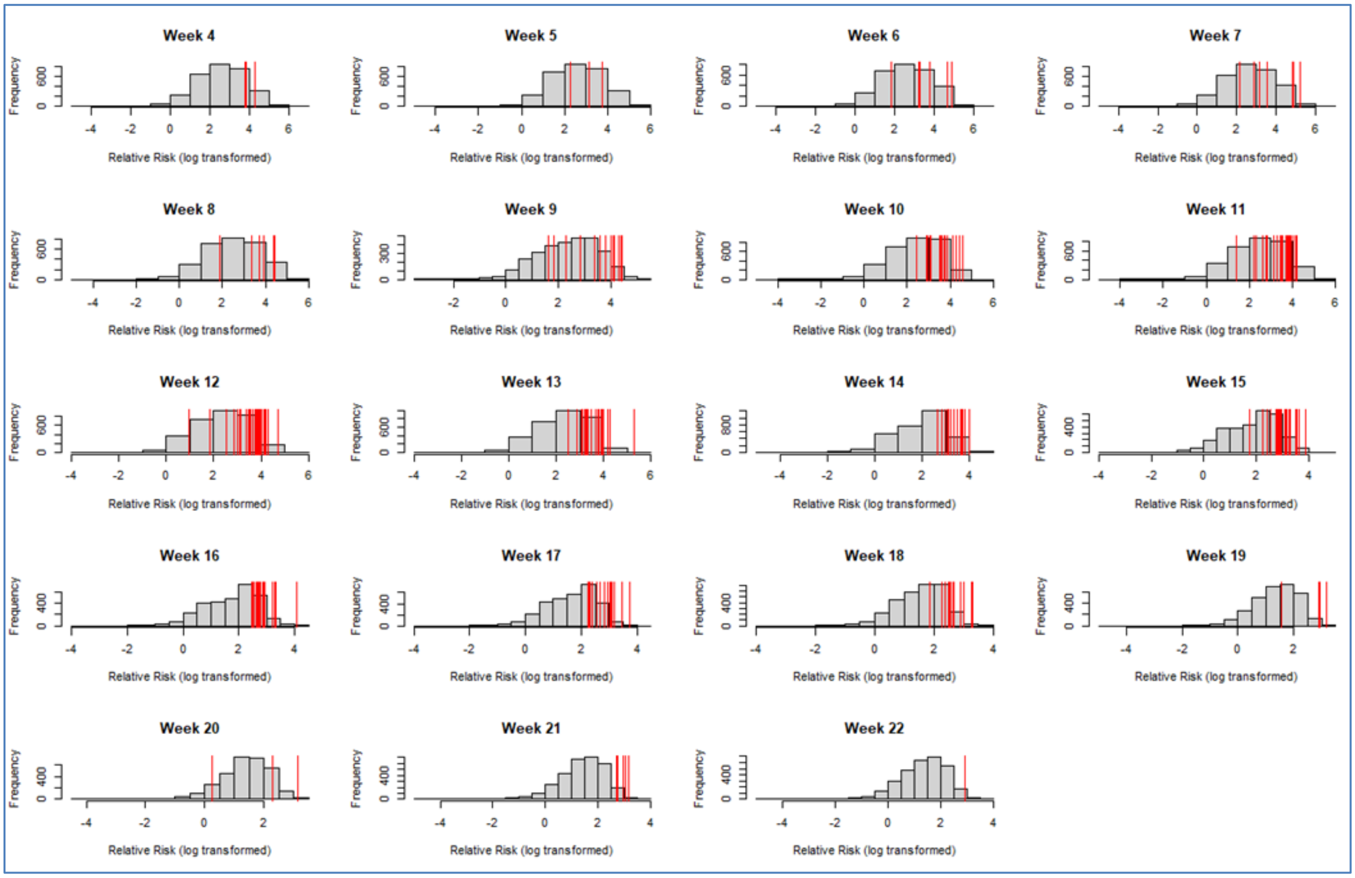

Figure 17. Distribution of risk values across all counties (grey bars) for each week of the year in which spillover events occurred. Red lines indicate the risk level for counties that actually had spillover events.

D. Next Steps

The data presented here represent preliminary results, and models are still being finalized. Once models are finalized and validated, they will be published and results shared publicly for use by interested managers, policy makers, and researchers.

It is important to note that these models are not meant to be static elements without the inclusion of new data. Concurrent with the finalization of these initial models, we are beginning a regression analysis on HPAI outbreak locations and associated covariates, ranging from production type to surrounding habitat features. Lessons learned from this analysis could then be integrated into future iterations of the interface models, further refining elements, such as the relative risk scaler based on poultry operation type.

[8] https://www.pwrc.usgs.gov/ai/

ANALYSIS OF BIRDCAST AND E-BIRD MIGRATION DATA: IMPLICATIONS FOR DISEASE INTRODUCTION, SPREAD, AND PREVENTION

A. Potential Use of BirdCast Data for Awareness to Enhance Prevention Measures

As a prevention tool following initial detections of HPAI in wild birds in the U.S., BirdCast notes dates, times, and areas of more intense wild bird migration can be used to increase awareness of potential increased risk to poultry producers. The following 22 September 2022 22:10 ET BirdCast live bird migration map, with a height of 813.2 million of birds in flight in a point in time over a 24-hour period, depicts strong pink-orange to yellow-white colors indicating areas of more intense density of bird migration in all or parts of a State, including Wisconsin, Massachusetts, Michigan, eastern Minnesota, New Hampshire, Ohio, western Pennsylvania, and Tennessee, among others (Figure 18).

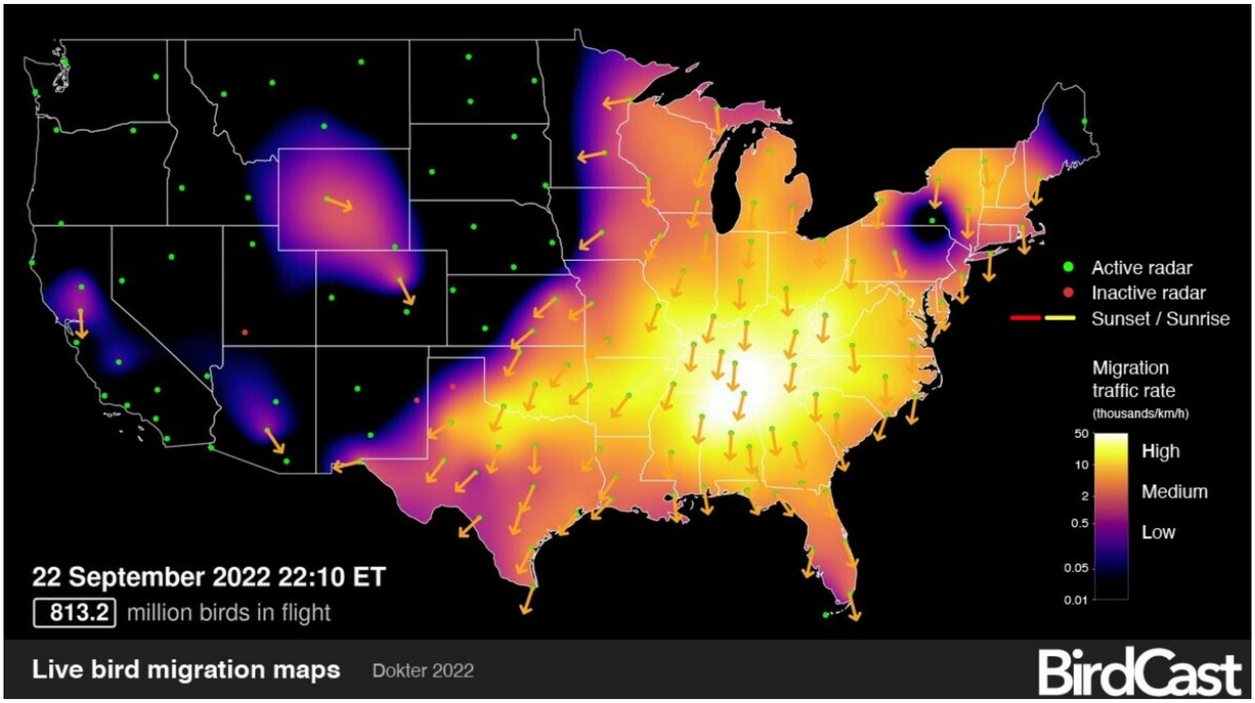

Figure 18. BirdCast Live Bird Migration Map of 22 September 2022 at 22:10 ET.

The NVSL presented phylogenetic analyses on 26 September 2022 and 5 October 2022 of samples collected from WOAH poultry [commercial] and WOAH poultry [backyard] premises from the aforementioned States. They identified all as most likely caused by independent wild bird introduction (IWBI) by looking at just one of the genotypes, H5N1 HPAI genotype B1.2, circulating since 4 April 2022, though increasing in prevalence in the samples in September 2022 (Figure 19).

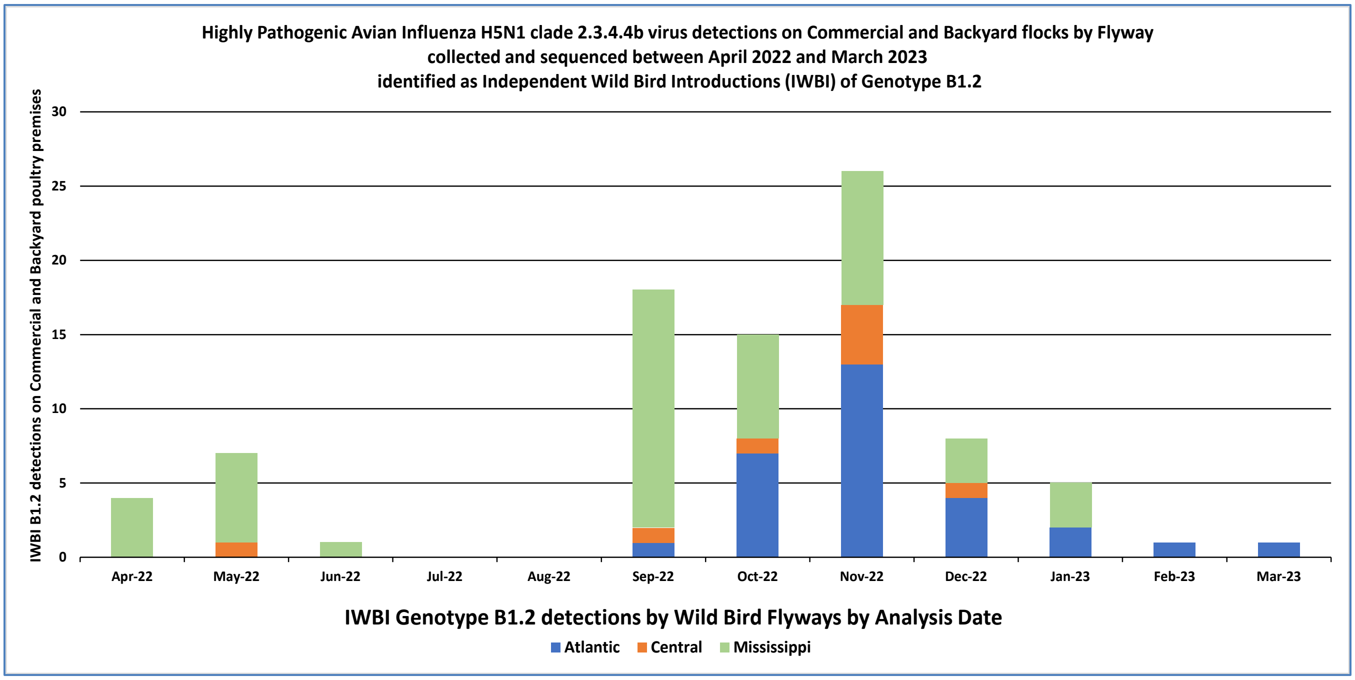

Figure 19. H5N1 HPAI clade 2.3.4.4b virus of genotype B1.2 detections in WOAH poultry [commercial] and WOAH poultry [backyard] from April 2022 to March 2023 identified as IWBI.

Notable differences between numbers of birds in flight from 15 July 2021 through 31 March 2022 and 15 July 2022 through 31 March 2023 are likely explained by weather impacts, which may influence risk of HPAI exposure on WOAH poultry [commercial] premises. The increase in density of millions of birds in flight preceded increases in numbers of HPAI-positive wild birds and poultry premises, particularly in September and October 2022. Similarly, decreases in positive poultry premises followed observed decreases in BirdCast's migration density, as tracked by millions of birds in flight per evening (Figure 20).

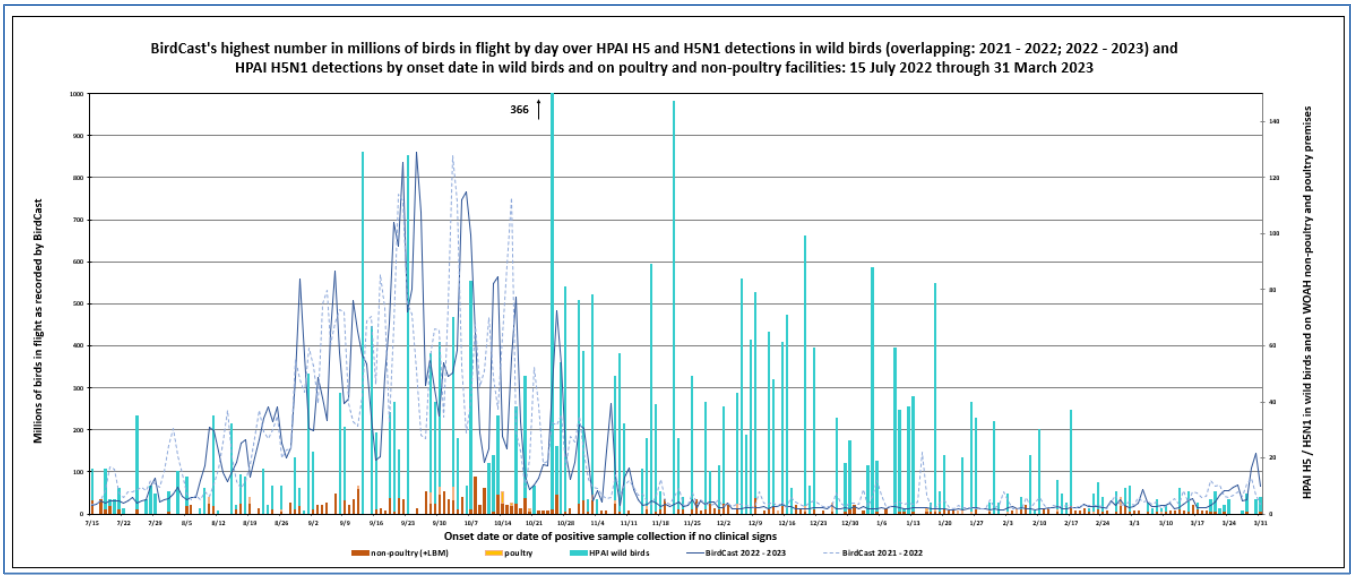

Figure 20. Overlapping 15 July 2021 through 31 March 2022 and 15 July 2022 through 31 March 2023, BirdCast's highest daily number of millions of birds in flight by day is shown over daily counts of H5 and H5N1 HPAI detections in wild birds, WOAH non-poultry, and WOAH poultry premises between 15 July 2022 and 31 March 2023.

B. eBird Output of Relative Abundance of Migrating Birds

eBird outputs provide additional information on potential indicators of risk for HPAI transmission from specific wild bird species in flight and opportunities for prevention. Relative abundances recorded in the eBird dashboard are counts of individual birds of a given species detected by an expert. User skill, hourly weather conditions, specific for a given region, season, and species are considered to maximize detection rates. For each species, relative abundance is estimated for all 52 weeks of the year across a regular spatial grid with a density of one location per 2.96 km x 2.96 km. Estimates at each location and date are made based on the local habitat, elevation, and topography (Figure 21).

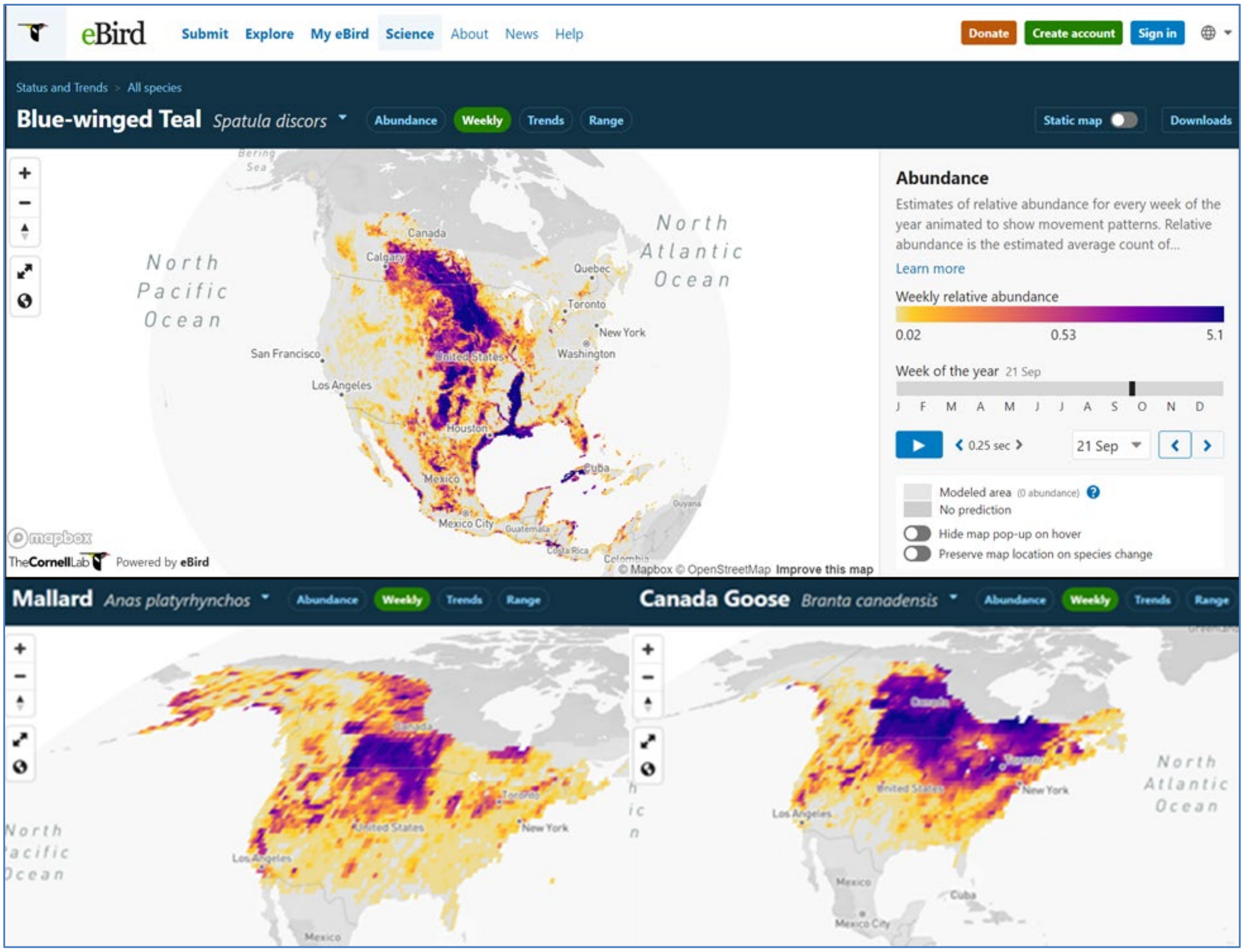

Figure 21. Maps pulled from eBird Status and Trends in the U.S.[9] for the week of 21 September 2022, focused on the relative abundance of blue-winged teal, Canada goose, and mallard species.

[9] https://science.ebird.org/en/status-and-trends/species/wooduc/abundance-map-weekly?week=38

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APPENDIX A: DETAILED OUTBREAK OVERVIEW BY FLYWAY

MISSISSIPPI FLYWAY February – March 2022

On 7 February 2022, one WOAH poultry [commercial] turkey meat bird operation in Dubois County, Indiana, submitted diagnostic test samples to the Indiana Animal Disease Diagnostic Laboratory (a member of the NAHLN) after observing signs of lethargy, increased mortality, and decreased water consumption in one tom turkey production house. The samples tested non-negative for H5 and were forwarded to the NVSL. On 8 February 2022, the NVSL received samples from the affected premises and confirmed clade 2.3.4.4b H5N1 HPAI.

Continuing in the Mississippi Flyway, the next States affected were Kentucky and Virginia. On 11 February 2022, one WOAH poultry [commercial] broiler production operation in Fulton County, Kentucky, submitted samples to the Murray State University Breathitt Veterinary Center laboratory (a member of the NAHLN) after observing decreased water consumption and increasing mortality in one house. The NAHLN laboratory detected H5 and forwarded the samples; NVSL confirmed H5N1 HPAI on 12 February 2022. Also on 11 February 2022, oropharyngeal swabs from dead chickens and turkeys belonging to a mixed-species WOAH poultry [backyard] flock in Fauquier County, Virginia, tested non-negative for H5 at the Virginia Department of Agriculture and Consumer Services Harrisonburg diagnostic laboratory (a member of the NAHLN). The samples were transported to NVSL on 12 February 2022, where H5 HPAI was confirmed. Subsequently, between 8 February 2022 and 2 March 2022, H5N1 HPAI was confirmed in seven WOAH poultry [commercial] meat bird turkey premises and one WOAH poultry [commercial] broiler production premises in two counties in southern Indiana and two counties in western Kentucky (Figure 1).

ATLANTIC FLYWAY February – March 2022

Moving to the Atlantic Flyway, on 18 February 2022, the NVSL confirmed clade 2.3.4.4b H5N1 HPAI in a non-poultry premises in Suffolk County, New York. This detection was the first of a series of detections among WOAH poultry [backyard] premises in Atlantic Flyway States along the Northeastern Atlantic coastline (Figure 1); impacted States included Maine, Connecticut, and Massachusetts (Table 1). On 20 February 2022, one WOAH poultry [commercial] layer hen facility in New Castle County, Delaware, observed increased mortality in a single house. Samples from the affected house were collected and submitted to the Allen Laboratory at the University of Delaware (a member of the NAHLN) on 21 February 2022, and H5 was detected the same day. NVSL confirmed HPAI on 22 February 2022. On 3 March 2022, a table egg layer premises located in Cecil County, Maryland, and within the established New Castle Control Area, observed increased mortality. Diagnostic samples were submitted, and the NAHLN laboratory detected H5 the same day. The NVSL confirmed H5N1 HPAI the following day. Subsequently, six more premises on the Delmarva Peninsula were confirmed between 8 March 2022 and 18 March 2022. Affected premises types included table egg layer, table egg processing, table egg pullet, and broiler production facilities.

MISSISSIPPI FLYWAY February – May 2022

Back in the Mississippi Flyway, on 23 February 2022, the NVSL confirmed clade 2.3.4.4b H5N1 HPAI in a mixed species WOAH non-poultry flock in Kalamazoo County, Michigan. Subsequently, from 24 March

2022 to 10 May 2022, 12 additional premises in Michigan were confirmed; these included 9 WOAH non-poultry, 2 WOAH poultry [backyard] chicken flocks, and 1 WOAH poultry [commercial] turkey meat bird premises. From 1 March 2022 to 18 May 2022, HPAI was confirmed in additional Midwest States located in the Mississippi Flyway, including Iowa, Missouri, Illinois, Wisconsin, Minnesota, and Ohio (Table 1; Figure 1). HPAI detections in Iowa and Missouri occurred predominately among WOAH poultry [commercial] premises. In Iowa, affected premises included nine WOAH poultry [commercial] turkey, six WOAH poultry [commercial] table egg layer, and four WOAH non-poultry premises. In Missouri, detections included four WOAH poultry [commercial] turkey, one WOAH poultry [commercial] broiler, and three non-poultry premises. In Wisconsin, confirmed premises included 7 WOAH poultry [commercial] turkey, 1 WOAH poultry [commercial] table egg layer, 1 WOAH poultry [backyard], and 13 WOAH non-poultry premises. As of 31 May 2022, a total of 80 detections occurred in Minnesota, accounting for 22.4 percent of the total cases. Confirmed premises included 57 WOAH poultry [commercial] turkey, 1 WOAH poultry [commercial] table egg layer, 1 WOAH poultry [commercial] broiler, and 21 WOAH non-poultry producers. Backyard premises in Minnesota included 19 WOAH non-poultry and 2 WOAH poultry [backyard] premises. HPAI detections in Illinois and Ohio were limited to WOAH non-poultry premises. From 8 April 2022 to 19 May 2022, additional confirmations in Indiana were reported for three WOAH poultry [commercial] duck premises and two WOAH non-poultry premises.

CENTRAL FLYWAY March 2022

The first of several poultry premises were affected in the Central Flyway, and on 5 March 2022, the NVSL confirmed HPAI on one WOAH poultry [commercial] turkey meat bird premises in Charles Mix County, South Dakota. From 12 March 2022 to 20 May 2022, additional detections in South Dakota were confirmed for 33 WOAH poultry [commercial] turkey, 1 WOAH poultry [commercial] table egg layer, 1 WOAH poultry [commercial] gamebird, 2 WOAH poultry [backyard] chicken, and 2 WOAH non-poultry premises. Detections in South Dakota accounted for 11.2 percent of all detections in domestic poultry as of 31 May 2022, and predominately occurred on the eastern side of the State. Following initial detections in South Dakota, subsequent detections were confirmed in Kansas starting 11 March 2022, in Nebraska starting 15 March 2022, and in North Dakota starting 29 March 2022. Confirmed detections in Kansas included two WOAH non-poultry, three WOAH poultry [backyard], and one WOAH poultry [commercial] turkey premises. In Nebraska, the detections included four WOAH non-poultry, two WOAH poultry [commercial] broiler, and two WOAH poultry [commercial] table egg layer premises. In North Dakota, HPAI affected predominately WOAH poultry [backyard] and WOAH non-poultry premises but was also found in four WOAH poultry [commercial] turkey premises.

ATLANTIC FLYWAY March – May 2022

While HPAI detections were increasing within the Mississippi and Central Flyways, reports were received from previously unaffected States along the east coast in the Atlantic Flyway. These States included North Carolina, Pennsylvania, Vermont, and New Jersey. On 29 March 2022, the NVSL confirmed H5 HPAI in one WOAH poultry [commercial] turkey premises in Johnston County, North Carolina. Active surveillance testing for the infection zone identified two additional WOAH poultry [commercial] turkey premises. Between 1 April 2022 and 11 April 2022, HPAI was confirmed in three WOAH poultry [commercial] turkey and three WOAH poultry [commercial] broiler bird premises in Wayne County, North Carolina. A few days later in Pennsylvania, on 15 April 2022, HPAI was confirmed in one WOAH poultry [commercial] table egg layer premises in Lancaster County. The NVSL later confirmed HPAI in four additional table egg layer and two WOAH poultry [commercial] broiler premises in Lancaster County. On 29 April 2022, the NVSL confirmed HPAI infection in one WOAH poultry [commercial] duck facility, which was the first of eight detections of WOAH poultry [commercial] duck premises in Lancaster and Berks Counties. Infections were also confirmed for two WOAH poultry [commercial] table egg layer premises in Berks County. The confirmed detections in Vermont on 28 April 2022 and in New Jersey on 17 May 2022 occurred in WOAH non-poultry premises.

CENTRAL/PACIFIC FLYWAYS March – May 2022

Continuing in the Central Flyway and moving into the Pacific Flyway, on 28 March 2022, the NVSL confirmed the detection of H5N1 HPAI in one WOAH non-poultry premises in Johnson County, Wyoming. This detection represented movement of the outbreak into the Mountain West, which is split by the Central and Pacific Flyways (Figure 1). States with confirmed detections included Texas, Montana, Colorado, Idaho, Utah, and Oklahoma (Table 1). All confirmations in Wyoming, Montana, and Idaho occurred among WOAH poultry [backyard] and WOAH non-poultry premises. In Wyoming, all eight detections were WOAH non-poultry premises. In Montana, the detections were among seven WOAH non-poultry and two WOAH poultry [backyard] premises. The 25 detections in Idaho included 15 WOAH non-poultry, 9 WOAH poultry [backyard], and 1 WOAH poultry [backyard] duck premises. NVSL-confirmed detections in Colorado and Utah included both WOAH non-poultry and WOAH poultry premises. In Colorado, two WOAH poultry [backyard], one WOAH poultry [commercial] broiler, one WOAH poultry [commercial] table egg layer, and one WOAH non-poultry premises were detected. The four detections in Utah included three WOAH non-poultry and one WOAH poultry [commercial] table egg layer premises. Detection of HPAI in Texas and Oklahoma was limited to one WOAH poultry [commercial] upland game bird and one WOAH poultry [commercial] broiler breeder premises, respectively.

Moving to the west coast, on 29 April 2022, the NVSL confirmed the detection of H5N1 HPAI in one WOAH non-poultry premises in Matanuska Susitna, Alaska. This detection represented the first detection along the coastline of the Pacific Flyway. On 5 May 2022, HPAI was confirmed in one WOAH non-poultry premises in Linn County, Oregon, and one WOAH poultry [backyard] chicken premises in Pacific County, Washington. As of 31 May 2022, additional detections occurred in one WOAH poultry [backyard] premises in Oregon.

PACIFIC FLYWAY June 2022 – December 2022

The number of premises confirmed infected with HPAI decreased during the summer months of June, July, and August with an average of 21 (range: 20–22) premises detected per month. Most of these detections (48 out of 63) occurred in States within the Pacific Flyway, including Alaska, California, Colorado, Montana, Nevada, Oregon, Utah, Washington, and Wyoming (Figure 1). Premises in Washington accounted for a third of the summer detections (21 out of 63) and included 11 WOAH non-poultry and 1 poultry premises in June, 3 WOAH non-poultry and 2 poultry premises in July, and 4 WOAH non-poultry premises in August. Detections across the U.S. increased during the fall months of September, October, and November. However, from September to December, only five additional detections occurred in Washington. These detections included one WOAH poultry [commercial] table egg layer, three WOAH non-poultry, and one WOAH poultry [backyard] premises. Confirmed on 14 December 2022, the table egg layer premises was the first WOAH poultry [commercial] premises detected in Washington. Detections in Oregon accounted for almost 15 percent (9 out of 63) of summer detections and included one WOAH non-poultry premises detected in June and four WOAH non-poultry and four WOAH poultry [backyard] premises detected in July. Subsequently, from 28 September 2022 to 21 December 2022, the NVSL confirmed detections for 10 WOAH non-poultry and 2 WOAH poultry [backyard] premises in Oregon.

Utah accounted for 10 percent (6 out of 63) of the summer detections. These detections included two WOAH non-poultry premises in June, three WOAH poultry [commercial] turkey meat bird premises in July, and one WOAH non-poultry premises in August. The 3 WOAH poultry [commercial] turkey meat bird premises confirmed infected between 14 July 2022 and 26 July 2022 were the first in a series of 18 detections in San Pete County, Utah, with the additional 15 premises detected between 13 September 2022 and 25 October 2022. Between 3 October 2022 and 28 November 2022, the NVSL confirmed infections in an additional two WOAH non-poultry and one WOAH poultry [backyard] premises in Utah.

On 7 July 2022, the NVSL confirmed the first detection in Nevada on a small WOAH non-poultry premises in Carson City County, followed by a second detection on 17 October 2022 on one WOAH poultry [backyard] premises. The second detection in Alaska, and the first detection since April, was confirmed by the NVSL for one WOAH non-poultry premises on 2 August 2022. Subsequent detections in Alaska, between 5 October 2022 and 25 November 2022, occurred on an additional four premises, including two WOAH poultry [backyard] and two WOAH non-poultry premises.

Montana, Wyoming, Colorado, and New Mexico are split by the Pacific and Central Flyways. In Montana, the NVSL confirmed the detection of one WOAH non-poultry premises within the Pacific Flyway on 26 July 2022. From 15 September 2022 to 16 December 2022, additional detections in this portion of Montana included two WOAH poultry [backyard] and three WOAH non-poultry premises. In the Pacific Flyway portion of Colorado, the NVSL confirmed three detections of WOAH non-poultry premises between 29 September 2022 to 26 November 2022. No additional detections occurred during the summer or fall along the Pacific Flyway in Wyoming or New Mexico.

On 10 August 2022, the NVSL confirmed the first detection in California on one WOAH non-poultry premises located in Sacramento County. This was the first of nine premises in California confirmed infected in August, which included two more WOAH non-poultry premises, two WOAH poultry [commercial] broiler breeder, and four turkey meat bird premises. From 1 September 2022 to 22 December 2022, California had an additional 17 premises confirmed infected. These premises included three additional WOAH poultry [commercial] broiler breeder, three turkey meat bird, two duck breeder, one upland gamebird producer, two WOAH poultry [backyard], and six WOAH non-poultry premises.

The remaining States in the Pacific Flyway had a combined total of six detections between 12 September 2022 and 20 December 2022. These included three WOAH non-poultry premises in Idaho confirmed infected on 12 September 2022, 20 October 2022, and 20 December 2022, and one WOAH poultry [commercial] upland gamebird producer in Idaho confirmed on 21 September 2022. Arizona had two WOAH non-poultry premises confirmed infected on 2 November 2022 and 3 November 2022, respectively.

CENTRAL FLYWAY June 2022 – December 2022

During the summer months, detections in the Central Flyway included one WOAH non-poultry premises in North Dakota confirmed on 6 June 2022 and two WOAH poultry [commercial] table egg premises in Weld County, Colorado confirmed on 7 June 2022 and 9 June 2022, respectively. In the fall, additional confirmed detections in North Dakota occurred between 1 September 2022 and 10 November 2022, including one WOAH poultry [commercial] turkey meat bird, four WOAH poultry [backyard], and four WOAH non-poultry premises. In the Central Flyway portion of Colorado, subsequent detections occurred from 21 September 2022 to 20 December 2022. These detections included three WOAH poultry [commercial] table egg layer, one WOAH poultry [commercial] upland gamebird, two WOAH poultry [backyard], and three WOAH non-poultry premises.

Additional WOAH poultry [commercial] premises detections in the Central Flyway during the fall months occurred in Nebraska and South Dakota. From 19 September 2022 to 14 December 2022, Nebraska had a total of seven premises confirmed infected. These premises included two WOAH poultry [commercial] upland gamebird, one WOAH poultry [commercial] table egg layer, one WOAH poultry [backyard], and three WOAH non-poultry premises. Meanwhile, South Dakota had a total of 35 premises confirmed infected between 20 September 2022 and 20 December 2022. The detected premises included 20 WOAH poultry [commercial] turkey meat bird, 3 upland gamebirds, 1 table egg layer, 1 goose breeder, 1 WOAH poultry [backyard], and 9 WOAH non-poultry premises.

The NVSL confirmed infections on an additional 11 WOAH non-poultry and 3 WOAH poultry [backyard] premises in the Central Flyway, located in Kansas, New Mexico, Oklahoma, Texas, and Wyoming. Detections in Texas occurred on three WOAH non-poultry premises between 24 September 2022 and 6 December 2022. On 4 October 2022, the first and only confirmed detection in New Mexico was on one WOAH non-poultry premises in Bernalillo County. In Kansas, detections included three WOAH poultry [backyard] premises confirmed between 7 October 2022 and 19 October 2022 and one WOAH non-poultry premises on 28 December 2022. The detections in the Central Flyway portion of Wyoming included two WOAH non-poultry premises and occurred on 12 October 2022 and 7 November 2022, respectively. In Oklahoma, the NVSL confirmed three premises between 14 October 2022 and 21 October 2022 and a fourth premises on 6 December 2022.

MISSISSIPPI FLYWAY June 2022 – December 2022

Along the Mississippi Flyway, detections in June were limited to one WOAH poultry [backyard] and one WOAH non-poultry premises in Allen County, Indiana. In July, no detections in domestic poultry occurred in the Mississippi Flyway. The only detections in August occurred on two WOAH poultry [commercial] turkey meat bird premises in Meeker County, Minnesota. Between 1 September 2022 and 14 December 2022, the NVSL confirmed infection on an additional 28 premises in Minnesota. These premises included 18 WOAH poultry [commercial] turkey meat bird, 2 WOAH poultry [commercial] turkey breeder hen, and 8 WOAH non-poultry premises. The NVSL confirmed subsequent infections in Indiana on 1 September 2022 in one WOAH poultry [backyard] premises and on 13 December 2022 in one WOAH poultry [commercial] turkey meat bird premises. Epidemiological investigation identified a second WOAH poultry [commercial] turkey meat bird premises in Indiana as a dangerous contact, and therefore, this premises was also depopulated.

In addition to Indiana and Minnesota, from September 2022 to December 2022, the NVSL confirmed detections in 11 of the 12 remaining States in the Mississippi Flyway. Between 2 September 2022 and 7 November 2022, the NVSL confirmed seven detections in Wisconsin. These detections included three WOAH non-poultry, one WOAH poultry [backyard], one WOAH poultry [commercial] duck meat bird, one WOAH poultry [commercial] turkey meat bird, and one WOAH poultry [commercial] upland gamebird premises. On 3 September 2022, the NVSL confirmed the first WOAH poultry [commercial] detection in Ohio on a table egg layer premises in Defiance County. This detection was the beginning of a series of first detections along the Mississippi Flyway during the fall months, including the first domestic detection in Tennessee on 15 September 2022 in one WOAH poultry [backyard] premises in Obion County, the first domestic detection in Arkansas in one WOAH poultry [commercial] broiler breeder pullet premises on 7 October 2022 in Madison County, the first domestic detection in Mississippi on 4 November 2022 in one WOAH poultry [commercial] broiler breeder in Lawrence County, and the first detection in Alabama on 5 December 2022 in one WOAH non-poultry premises in Lawrence County.

Following the first WOAH poultry [commercial] detection in Ohio, the NVSL confirmed infections in four WOAH non-poultry premises between 3 September 2022 and 15 September 2022. Additional detections in Ohio occurred on 3 October 2022 in one WOAH non-poultry premises and on 7 November 2022 in one WOAH poultry [backyard] premises. In Tennessee, seven additional confirmed detections occurred between 31 October 2022 and 28 December 2022. These premises included two WOAH poultry [backyard], two WOAH non-poultry, and three WOAH poultry [commercial] broiler breeder premises. Subsequent detections in Arkansas included two WOAH non-poultry premises confirmed on 14 October 2022 and 1 December 2022, respectively. Mississippi had one additional WOAH non-poultry premises detected on 4 November 2022.

Fall detections in Michigan, Kentucky, and Illinois occurred on WOAH poultry [backyard] and WOAH non-poultry premises. From 13 September 2022 to 12 October 2022, Michigan had four WOAH poultry [backyard] and two WOAH non-poultry premises confirmed infected, followed by one WOAH poultry [backyard] detection on 10 November 2022 and one WOAH non-poultry detection on 30 December 2022. One additional WOAH poultry [backyard] premises was a dangerous contact and was depopulated. The NVSL confirmed the first detections in Kentucky since the initial detections at the beginning of the outbreak on 6 October 2022 in one WOAH poultry [backyard] premises and 11 October 2022 in one WOAH non-poultry premises. The two confirmed detections in Illinois during the fall occurred on 30 November 2022 in one WOAH poultry [backyard] premises and 21 December 2022 in one WOAH non-poultry premises.

The detections in Missouri and Iowa included a mix of WOAH poultry [commercial], WOAH poultry [backyard], and WOAH non-poultry premises. On 18 October 2022, the NVSL confirmed the first fall detection in Missouri on one WOAH non-poultry premises. Seven subsequent detections occurred between 22 November 2022 and 21 December 2022 and included four WOAH non-poultry, two WOAH poultry [commercial] turkey meat bird, and one WOAH poultry [backyard] premises. The NVSL confirmed infected a total of 11 premises in Iowa between 20 October 2022 and 12 December 2022. From 20 October 2022 to 8 November 2022, confirmed detections included two WOAH non-poultry and two WOAH poultry [commercial] table egg premises. From 5 December 2022 to 12 December 2022, the detections included seven WOAH poultry [commercial] turkey meat bird premises.

ATLANTIC FLYWAY June 2022 – December 2022

During the summer months of June, July, and August, the NVSL confirmed infected a total of eight premises along the Atlantic Flyway (Figure 1). These detections included two WOAH non-poultry premises in Georgia, one WOAH poultry [commercial] duck and one WOAH poultry [backyard] premise in Pennsylvania, one WOAH non-poultry premises in Maine, two WOAH non-poultry premises in Florida, and one WOAH non-poultry premises in Virginia. The confirmed infection of one WOAH non-poultry premises on 1 June 2022 in Toombs County was the first detection in Georgia during this outbreak; the second confirmed infection occurred on 22 August 2022 in Henry County. In Pennsylvania, one WOAH poultry [commercial] duck premises confirmed infected on 2 June 2022 was the last premises in a cluster of WOAH poultry [commercial] duck premises in Berks County. The WOAH non-poultry premises in Virginia confirmed infected on 27 August 2022 was the first detection in Virginia since the detection at the beginning of the outbreak.

From September 2022 to December 2022, the NVSL confirmed an additional 65 premises infected along the Atlantic Flyway (Figure 1). Between 18 September 2022 and 17 November 2022, Pennsylvania had an additional 17 premises confirmed infected. These detections included eight WOAH poultry [commercial] turkey, four WOAH poultry [backyard], and seven WOAH non-poultry premises. Two additional WOAH poultry [backyard] premises were dangerous contacts and depopulated. Among the eight WOAH poultry [commercial] turkey premises was a cluster of six premises confirmed between 1 November 2022 and 9 November 2022. In Maine, the confirmed detection of one additional WOAH non-poultry premises occurred on 21 November 2022. Subsequent detections in Florida accounted for 32 percent (21 out of 65) of infections along the Atlantic Flyway. From 11 October 2022 to 16 December 2022, the NVSL confirmed detections on 3 WOAH poultry [backyard] and 18 WOAH non-poultry premises. In Virginia, four additional confirmed infections of WOAH non-poultry premises happened between 5 October 2022 and 4 November 2022.

Aside from the detections in Maine, the NVSL confirmed an additional 16 fall detections in the northeastern portion of the Atlantic Flyway. These detections included 1 WOAH poultry [commercial], 13 WOAH non-poultry, and 2 WOAH poultry [backyard] premises. In Massachusetts, detections of two WOAH non-poultry premises were confirmed on 15 September 2022 and 3 November 2022, respectively. Maryland's detections included one WOAH non-poultry premises on 22 September 2022 and one WOAH poultry [commercial] table egg breeder on 29 November 2022. New Hampshire had one WOAH poultry [backyard] premises confirmed infected on 23 September 2022. Delaware had two WOAH non-poultry premises confirmed on 24 September 2022. Confirmed infections of WOAH non-poultry premises occurred in Connecticut on 5 October 2022, in Rhode Island on 20 October 2022, and in Vermont on 6 December 2022. The NVSL also confirmed the infections of three WOAH non-poultry premises in New Jersey between 13 October 2022 and 2 November 2022. Detections in New York included two WOAH non-poultry premises confirmed on 1 November 2022 and 4 November 2022 and one WOAH poultry [backyard] premises confirmed on 12 November 2022.

An additional six detections occurred in the southern portion of the Atlantic Flyway in North Carolina and South Carolina. Between 21 October 2022 and 9 December 2022, the NVSL confirmed the infection of five WOAH non-poultry premises in North Carolina. On 3 November 2022, the NVSL confirmed the first and only domestic detection in South Carolina in one WOAH non-poultry premises in Beaufort County.

PACIFIC FLYWAY January 2023 – March 2023

Following the increase in confirmed HPAI detections during the fall months, the number of infections dropped at the beginning of 2023. The NVSL confirmed 99 cases between January and the end of March, including 28 infections in January, 31 infections in February, and 40 infections in March (Figure 2). The Pacific Flyway had a total of 21 confirmed infections, including 19 WOAH non-poultry, 1 WOAH poultry [backyard], and 1 WOAH poultry [commercial] duck breeder premises (Figure 1). The 19 WOAH non-poultry premises included 7 in California, 5 in Oregon, 3 in Washington, and 1 premises each in Montana, Colorado, Nevada, and Idaho. The one WOAH poultry [backyard] and one WOAH poultry [commercial] duck premises detections occurred in California on 12 January 2023 and 6 February 2023, respectively.

CENTRAL FLYWAY January 2023 – March 2023

In the Central Flyway, the NVSL confirmed 16 detections between January and March 2023 (Figure 1). These detections included eight WOAH non-poultry, four WOAH poultry [backyard], and four WOAH poultry [commercial] upland gamebird premises located in Kansas, South Dakota, Colorado, Nebraska, and Texas. The detections in Kansas included three WOAH poultry [commercial] upland gamebird premises confirmed between 4 January 2023 and 8 February 2023, and two WOAH non-poultry premises confirmed on 13 February 2023 and 24 March 2023, respectively. South Dakota had one WOAH poultry [backyard] premises confirmed on 5 January 2023 and one WOAH poultry [commercial] upland gamebird premises confirmed infected on 22 March 2023. In the Central Flyway portion of Colorado, the NVSL confirmed two WOAH non-poultry premises in January 2023 and one WOAH non-poultry and one WOAH poultry [backyard] premises in March 2023. In Nebraska, two confirmed detections included one WOAH non-poultry premises on 9 January 2023 and one WOAH poultry [backyard] premises on 21 February 2023. Texas also had two confirmed detections, one WOAH poultry [backyard] premises on 18 January 2023 and one WOAH non-poultry premises on 22 March 2023. The detection confirmed in the Central Flyway portion of Wyoming occurred on 6 February 2023 on one WOAH non-poultry premises.

MISSISSIPPI FLYWAY January 2023 – March 2023

From January 2023 to March 2023, the NVSL confirmed 13 cases of HPAI along the Mississippi Flyway (Figure 1). These premises included two WOAH poultry [commercial] broiler, two WOAH poultry [commercial] turkey meat bird, seven WOAH non-poultry, and two WOAH poultry [backyard] premises. Detections in Missouri and Ohio were limited to WOAH non-poultry premises. Missouri had three WOAH non-poultry premises confirmed on 4 January 2023, 13 February 2023, and 16 March 2023. Ohio had one premises confirmed on 18 January 2023. In Tennessee, the NVSL confirmed one WOAH poultry [commercial] broiler production premises on 20 January 2023. While this premises was geographically close to the broiler breeder premises detected at the end of December 2022 in Kentucky, phylogenetic sequencing and lack of epidemiological evidence suggested it was an independent wild-bird introduction. The two NVSL-confirmed WOAH poultry [commercial] turkey premises were independent cases and occurred in Iowa on 25 January 2023 and in Illinois on 24 February 2023. In Iowa, the NVSL also confirmed the infection of one WOAH non-poultry premises on 15 March 2023. Premises confirmed in Mississippi included one WOAH poultry [commercial] broiler production on 7 February 2023, one WOAH poultry [backyard] premises on 17 February 2023, and one WOAH non-poultry premises on 16 March 2023. One additional WOAH poultry [commercial] broiler premises in Mississippi was depopulated as a dangerous contact. Confirmed detections in Michigan included one WOAH non-poultry premises on 14 March 2023 and one WOAH poultry [backyard] premises on 23 March 2023.

ATLANTIC FLYWAY January 2023 – March 2023

NVSL-confirmed detections along the Atlantic Flyway accounted for 49 percent (49 out of 99) of the infections between January 2023 and March 2023 (Figure 1). Detections occurred in Virginia, New Hampshire, North Carolina, New York, Maine, Pennsylvania, and Florida and included 25 WOAH poultry [backyard], 12 WOAH non-poultry, 4 WOAH poultry [live bird market], 5 WOAH poultry [commercial] turkey, 2 WOAH poultry [commercial] duck, and 1 WOAH poultry [commercial] broiler premises. In Virginia, infected premises included two WOAH poultry [commercial] turkey meat bird premises confirmed on 19 January 2023 and 25 January 2023, respectively, and one WOAH non-poultry premises confirmed on 6 March 2023 in Rockingham County. Confirmed infections of one WOAH non-poultry premises occurred in New Hampshire on 20 January 2023 and in North Carolina on 23 January 2023. In Maine, the NVSL confirmed the infections of three additional WOAH non-poultry premises between 26 January 2023 and 1 February 2023. Confirmed detections in New York included one WOAH non-poultry premises on 25 January 2023, one WOAH poultry [backyard] premises on 15 February 2023, and one WOAH poultry [backyard] game farm on 22 March 2023.

The majority of the detections (32 out of 49) occurred in Pennsylvania, and included 23 WOAH poultry [backyard], 3 WOAH non-poultry, 3 WOAH poultry [commercial] turkey, 2 WOAH poultry [commercial] duck, and 1 WOAH poultry [commercial] broiler premises. Lancaster County had 23 of these detections, Chester County had 4, and the remaining 5 were individual county detections. The localized outbreak was largely attributed to lateral spread associated with the sale of infected birds through the live bird market (LBM) and the delayed reporting of sick birds. To get the outbreak under control, the Pennsylvania Department of Agriculture temporarily shut down live bird markets and later issued a general quarantine order mandating weekly testing for LBM-associated premises in specific townships. This response led to an immediate decline in the number of new detections within the LBM system and effectively prevented further lateral spread between markets. Spread through the LBM was also responsible for the infection of an LBM premises in Virginia confirmed on 23 February 2023 and an LBM premises in New York confirmed on 22 March 2023. The remaining three confirmed premises were located in Florida and included one WOAH non-poultry premises confirmed on 14 February 2023 and two LBM premises confirmed on 28 February 2023 and 21 March 2023. Phylogenetics determined the viral genotype responsible for the infection of these LBM premises was a different genotype than the other LBM premises.

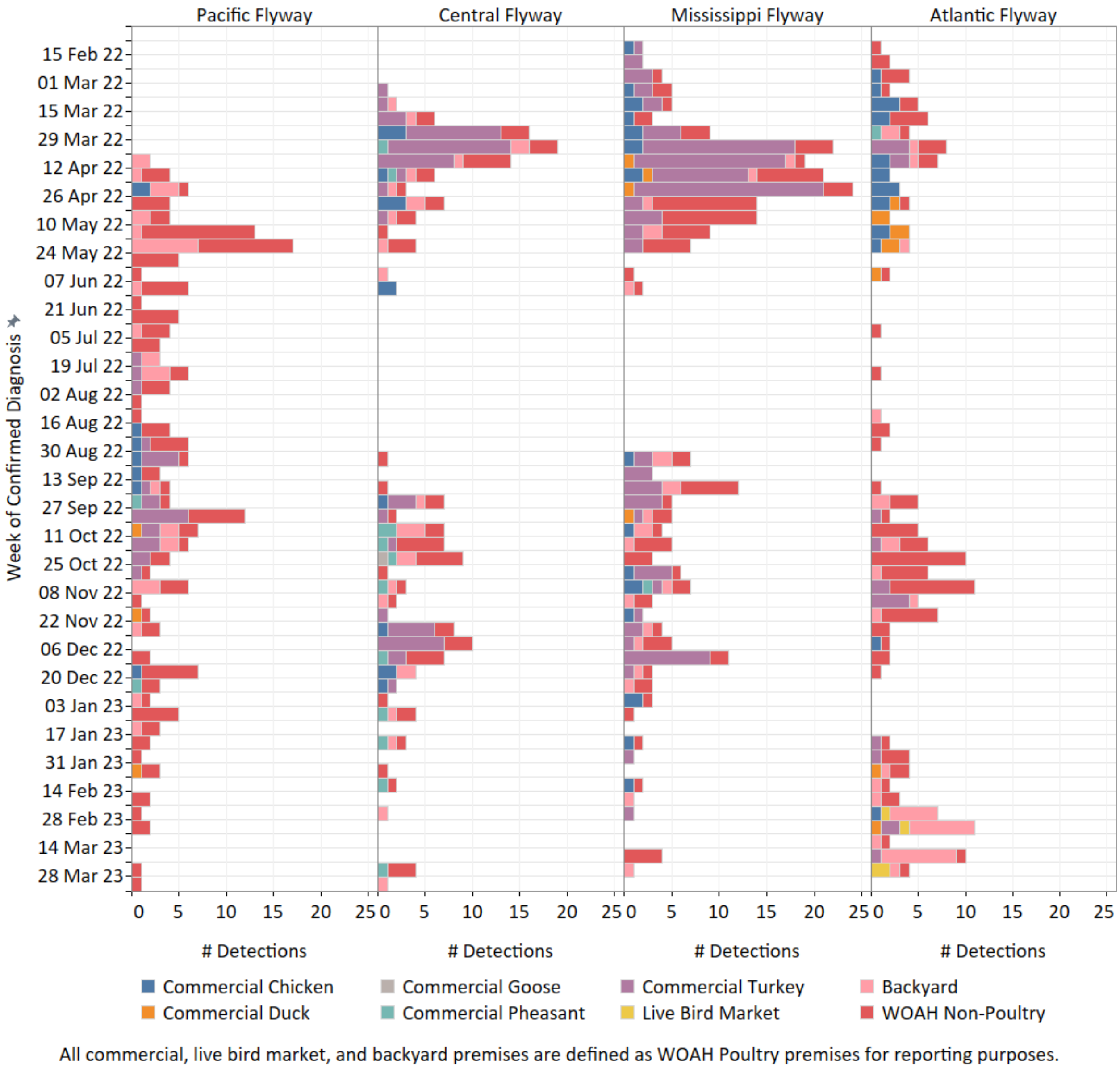

Figure A1. Weekly confirmed detections of highly pathogenic avian influenza by reportable species and wild migratory bird flyways as of 31 March 2023.

Table A1. Confirmed detections of highly pathogenic avian influenza by State as of 31 March 2023.

State	Total Detections	First Detection	Last Detection	Chicken	Turkey	Duck	Pheasant	Goose	Other*
Alabama	1	12/5/2022	12/5/2022						1
Alaska	6	4/29/2022	11/25/2022	5		1			
Arizona	2	11/2/2022	11/3/2022	2					
Arkansas	3	10/7/2022	12/1/2022	2					1
California	35	8/10/2022	2/17/2023	20	7	5	2		1
Colorado	24	4/8/2022	3/28/2023	16	1	1	1		5
Connecticut	2	3/1/2022	10/5/2022	1					1
Delaware	5	2/22/2022	9/24/2022	3					2
Florida	26	7/21/2022	3/21/2023	23		1			2
Georgia	2	6/1/2022	8/22/2022			1			1
Idaho	30	4/14/2022	3/6/2023	20		1	1	1	7
Illinois	7	3/11/2022	2/24/2023	3	1	1	1		1
Indiana	15	2/8/2022	12/13/2022	2	7	3			3
Iowa	32	3/1/2022	3/15/2023	13	17				2
Kansas	15	3/11/2022	3/24/2023	7	1	1	3	1	2
Kentucky	4	2/12/2022	10/11/2022	1	1				2
Maine	17	2/19/2022	2/6/2023	15		1		1	
Maryland	6	3/4/2022	11/29/2022	5		1			
Massachusetts	3	3/29/2022	11/3/2022	1		1			1
Michigan	23	2/23/2022	3/23/2023	16	2	3	2		
Minnesota	110	3/25/2022	12/14/2022	28	79			1	2
Mississippi	5	11/4/2022	3/16/2023	3				1	1
Missouri	20	3/4/2022	3/16/2023	8	7				5
Montana	16	4/7/2022	1/10/2023	4	1				11
Nebraska	17	3/15/2022	2/21/2023	14			2		1
Nevada	3	7/7/2022	3/3/2023	3					
New Hampshire	3	3/16/2022	1/23/2023	3					
New Jersey	4	5/17/2022	11/2/2022			1			3
New Mexico	1	10/4/2022	10/4/2022	1					
New York	16	2/18/2022	3/22/2023	8			2		6
North Carolina	15	3/29/2022	1/25/2023	7	6	1			1
North Dakota	24	3/29/2022	11/10/2022	12	5				7
Ohio	9	3/29/2022	1/18/2023	6			1	2	
Oklahoma	5	4/30/2022	12/6/2022	2		1			2
Oregon	28	5/5/2022	3/30/2023	15		4		1	8
Pennsylvania	67	4/15/2022	3/17/2023	19	12	21		1	14
Rhode Island	1	10/20/2022	10/20/2022			1			
South Carolina	1	11/3/2022	11/3/2022	1					

State	Total Detections	First Detection	Last Detection	Chicken	Turkey	Duck	Pheasant	Goose	Other*
South Dakota	77	3/5/2022	3/22/2023	13	53		5	1	5
Texas	6	4/2/2022	3/22/2023	4		1	1		
Vermont	2	4/28/2022	12/6/2022	1		1			
Washington	42	5/5/2022	2/14/2023	25		8		2	7
Wyoming	11	3/29/2022	2/6/2023	11					

*Other species includes assorted pet birds, chukars, ratites, multiple poultry species, and “other poultry” designations.

APPENDIX B: INITIAL CONTACT EPIDEMIOLOGICAL REPORT

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: ____________________

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: ....

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

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,

Crew type	Date (mm/dd/yy)	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 (mm/dd/yy)	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
		☐ 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 C: COMMERCIAL TURKEY CASE CONTROL SURVEY

UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES 2150 CENTRE AVE, BLDG B FORT COLLINS, CO 80526	**COMMERCIAL TURKEY CASE CONTROL SURVEY**

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0484. The time required to complete this information collection is estimated to average 75 minutes per response, including the time to review instructions, search existing data resources, gather the data needed, and complete and review the information collected.	OMB Approved 0579-0484 EXP: 04/30/2023

The information you provide will be used for statistical purposes only. Your responses will be kept confidential and any person who willfully discloses ANY identifiable information about you or your operation is subject to a jail term, a fine, or both. This survey is conducted in accordance with the Confidential Information Protection and Statistical Efficiency Act of 2018, Title III of Pub. L. No. 115-435, codified in 44 U.S.C. Ch. 35 and other applicable Federal laws. For more information on how we protect your information please visit: https://www.nass.usda.gov/confidentiality. Response is **voluntary**.

Date (mm/dd/yy): ________________date

Section A – Case or Control

1. Is this a case or control farm? **[During the interview, verify the farm name and address printed on the label are correct for this farm.]** t101
   - ☐1 Case farm **[Go to Item 2.]**
   - ☐3 Control farm **[Go to Item 3.]**

2. If this is a **case** farm,
   a. The following is the 14-day reference period for this farm:
   t102/t102a __________________ – ________________ mm/dd/yy – mm/dd/yy

In this questionnaire, we will ask many questions about a 14-day reference period. The "reference period" for your farm is the 14 days between [Insert the dates listed in Item 2a above]. This is the 14 days before the detection of HPAI on this farm.

   b. How many turkeys were on this farm on the last day of the reference period? t103 ______ # turkeys
   c. During the 2022 HPAI outbreak, how many of the barns on this farm were confirmed or were suspected to be infected with HPAI? .... t104 ___________# barns

[Go to Section B.]

3. If this is a **control** farm,
   a. The tentative 14-day reference period for this farm is:
   t105/t105a _________________ – _______________ mm/dd/yy – mm/dd/yy
   b. Did you have turkeys for the entire 14-day period between the dates in Item 3a above? t106
   - ☐1 Yes
   - ☐3 No

If Yes, the "14-day reference period" for your farm is [Insert the dates listed in Item 3a above]. We will refer to this as the "reference period" throughout the questionnaire. *[Proceed to Item 3c.]*
If No, help the producer identify the closest 14-day period to the reference period from Item 3a during which they had turkeys on the farm and enter that period into the fields below. This period must be during 2022.

Enter the selected 14-day period here: t107 Start date _____________ mm/dd/yy
(Finish date = start date + 14 days) t108 Finish date ____________ mm/dd/yy

All questions regarding the "reference period" refer to the 14 days selected above. We will refer to this as the "reference period" throughout the questionnaire.
[If the farm did not have turkeys during 2022, go to Section L.]

   c. How many turkeys were on this farm on the last day of the reference period? t109 ______ # turkeys

Section B – Premises Description

1. What stage(s) of turkey production is on this farm?
   a. Brooder .... t201 ☐1 Yes ☐3 No
   b. Grower .... t202 ☐1 Yes ☐3 No
   c. Breeder .... t203 ☐1 Yes ☐3 No
   d. Other (specify: ______________________) t204oth.......... t204 ☐1 Yes ☐3 No

2. What is the sex of the market type on this farm? *[Check all that apply.]*
   - ☐1 Hens t205
   - ☐1 Toms t206

☐1 Breeder hens t207
☐1 Breeder toms t208

3. Is this farm multiple age or single age? *[Check one only.]* t209
   ☐1 Multiple age
   ☐2 Single age

4. What other type(s) of poultry is present on this farm?
   a. Broiler ....t210 ☐1 Yes ☐3 No
   b. Layer ....t211 ☐1 Yes ☐3 No
   c. Domestic ducks or geese (exclude wild birds) ....t212 ☐1 Yes ☐3 No
   d. Other (specify: ____________________) t213oth....................t213 ☐1 Yes ☐3 No

5. Is this farm certified organic? ....t214 ☐1 Yes ☐3 No

6. Is this facility enrolled in NPIP?....................t215 ☐1 Yes ☐3 No
   a. If yes, is this facility enrolled in an NPIP Avian Influenza Program?..........t216 ☐1 Yes ☐3 No

7. Is this a: *[Check one only.]* t217
   ☐1 Company farm?
   ☐2 Contract farm?
   ☐3 Independent farm?
   ☐4 Other? (specify:______________________________) t217oth

8. How many barns are on this farm? .... t218 ________# barns
   a. In the last year, how many of these barns housed birds? .... t219 ________# barns

For the remainder of the questionnaire, some questions will ask about practices for the entire farm, and other questions will ask about practices for a "selected barn."

INSTRUCTIONS for selecting a barn:

Case farm: Select the **first** barn on this premises that was confirmed to be HPAI positive. If more than one barn was confirmed to be HPAI positive on the same date, choose one barn. **Answer questions for the 14 days prior to the onset of clinical signs or increased mortality (the reference period). [Section A, Item 2a]**

Control farm: Randomly select one barn to be the "**selected barn**." Choose one that had birds during the 14-day reference period [Section A, Item 3]. Use this barn to answer all questions about the "**selected barn**."

9. What is the barn ID or name for the **selected barn**? ....t220 ________________
10. During the 14-day reference period, did any birds on the farm or **selected barn** have access to the outdoors?
    a. Any birds on the farm ....t221 ☐1 Yes ☐3 No
    b. **Selected barn** ....t222 ☐1 Yes ☐3 No

11. Were any livestock, excluding poultry, on the farm, or located within 350 yards of the farm, fed hay or grain in the pasture or in outdoor feed troughs during the 14-day reference period?
    (350 yards is about the length of three football fields.) ....t223 ☐1 Yes ☐3 No ☐4 Don't Know

12. What is the water source for poultry?
    a. Off-site fresh water (for example, municipal, federal, cooperative, community, commercial)....................t224 ☐1 Yes ☐3 No
    b. Well....................t225 ☐1 Yes ☐3 No

c. Surface water (for example, pond) ....t226 ☐1 Yes ☐3 No
d. Other (specify: ______________________) t228oth ....t227 ☐1 Yes ☐3 No

13. Are water treatments such as chlorination used in the drinking water for the poultry on this farm? t228 ☐1 Yes ☐3 No

a. If Yes, are these treatments given: ....t229 ☐1 Continuously? ☐3 Intermittently?

14. Are windbreaks present on this farm?

Windbreak type	Present?	If Yes, distance to closest poultry barn	
a. Evergreen or juniper	☐1 Yes ☐3 No	________ yards	t230/t233
b. Deciduous tree	☐1 Yes ☐3 No	________ yards	t231/t234
c. Structural (for example, hill, natural break)	☐1 Yes ☐3 No	________ yards	t232/t235

15. Are the following water body type(s) visible or within 350 yards (about three football fields) of this farm?
a. Pond ....t236 ☐1 Yes ☐3 No
b. Lake ....t237 ☐1 Yes ☐3 No
c. Stream ....t238 ☐1 Yes ☐3 No
d. River ....t239 ☐1 Yes ☐3 No
e. Wetland or swamp ....t240 ☐1 Yes ☐3 No
f. Wastewater lagoon ....t241 ☐1 Yes ☐3 No
g. Standing water during the 14-day reference period ....t242 ☐1 Yes ☐3 No
h. Drainage ditch or canal...t243 ☐1 Yes ☐3 No
i. Other (specify: ______________________) t245oth.....................................t244 ☐1 Yes ☐3 No

[If Question 15 a through i are all equal to No, skip to Question 17.]

16. For those water bodies, including drainage ditches and lagoons within 350 yards on the farm, approximately how many wild waterfowl or shorebirds (for example, ducks, geese, wading birds, gulls) were seen on the water during the 14-day reference period? *[Check one only.]* t245
☐1 None ☐2 Tens ☐3 Hundreds ☐4 Thousands ☐5 Don't know

17. What is the distance (in yards) of the closest body of water (for example, pond, lake, stream, river, wetland) to this farm? t246 ....yards

18. In the 14-day reference period, approximately how many wild waterfowl or shorebirds (for example, ducks, geese, wading birds, gulls) might have been seen on this body of water at one time? *[Check one only.]* t247
☐1 None ☐2 Tens ☐3 Hundreds ☐4 Thousands ☐5 Don't know

19. What is the approximate distance (in yards) to the closest field where crops or hay are harvested?
t248 ______________yards

20. What crop was last grown in this field? *[Check one only.]* t249
☐1 Corn
☐2 Soybeans
☐3 Alfalfa or grass intended for livestock feed
☐4 Other (specify:_________________________________) t250oth

☐5 Don't know

21. Was this field tilled in:
   a. Fall 2021? .... t250 ☐1 Yes ☐3 No ☐4 Don't know
   b. Spring 2022? .... t251 ☐1 Yes ☐3 No ☐4 Don't know

22. Was this field actively worked (for example, tilled, disked, hay harvested, trees cut, row crops harvested) during the 14-day reference period?......................................t252 ☐1 Yes ☐3 No ☐4 Don't know

23. For this closest field, approximately how many wild waterfowl or shorebirds (for example, ducks, geese, wading birds, gulls) were seen during the 14-day reference period? *[Check one only.]* t253
   ☐1 None ☐2 Tens ☐3 Hundreds ☐4 Thousands ☐5 Don't know

Section C – Wild Birds

1. During the 14-day reference period, how frequently were the following types of wild birds seen on the farm and within 100 yards of the outside of the barns?

Bird type	Often	Sometimes	Never	
a. Waterfowl (for example, ducks, geese)	☐1	☐2	☐3	t301
b. Gulls	☐1	☐2	☐3	t302
c. Small perching birds (for example, sparrows, starlings, swallows)	☐1	☐2	☐3	t303
d. Blackbirds and crows	☐1	☐2	☐3	t304
e. Other water birds (for example, egrets, cormorants)	☐1	☐2	☐3	t305
f. Wild turkeys, pheasants, quail	☐1	☐2	☐3	t306
g. Raptors (for example, eagles, hawks, owls, vultures)	☐1	☐2	☐3	t307
h. Pigeons and doves	☐1	☐2	☐3	t308
i. Other (specify:_________________) t309oth	☐1	☐2	☐3	t309

2. During the 14-day reference period, how frequently were the following types of wild birds seen inside the **selected barn**?

Bird type	Often	Sometimes	Never	
a. Large birds (for example, pigeons, crows)	☐1	☐2	☐3	t310
b. Small birds (for example, finches, sparrows, starlings)	☐1	☐2	☐3	t311
c. Other (specify: ________________) t312oth	☐1	☐2	☐3	t312

3. During the 14-day reference period, did you or other farm workers observe any of the following types of **sick or dead** wild birds **inside** the barns or **outside** of the barns?

Sick/dead bird type	Inside the barns	Outside the barns

a.	Large birds (for example, pigeons, crows)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	t313/t316
b.	Small birds (for example, finches, sparrows, starlings)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	t314/t317
c.	Other (specify: ________________) t315oth	☐1 Yes ☐3 No	☐1 Yes ☐3 No	t315/t318

4. If Yes to Questions 3a, 3b, or 3c, what was done with the sick or dead wild birds?
   a. Left for predators ....t319 ☐1 Yes ☐3 No
   b. Disposal by same method used for daily turkey mortality on farm ....t320 ☐1 Yes ☐3 No
   c. Taken to rehab center, animal control or veterinarian ....t321 ☐1 Yes ☐3 No
   d. Something else (specify: ____________________) t322oth....................t322 ☐1 Yes ☐3 No

Section D – Farm Biosecurity

1. What best describes the road surface on this farm that vehicles coming onto the operation drive on? *[Check one only.]* t401
   ☐1 Hard top/asphalt
   ☐2 Gravel
   ☐3 Dirt
   ☐4 Other (specify: __________________________________) t401oth

2. In general, do the following types of vehicles:

Codes for question 2
1 = come to the perimeter of the farm only
2 = enter the farm but not near the barns
3 = come near the barns
4 = do not come at all

Enter the codes that apply.

   a. Garbage or dumpster pick up ....t402 _______code
   b. Propane delivery ....t403 _______code
   c. Feed delivery ....t404 _______code
   d. Feed ingredient delivery ....t405 _______code
   e. Renderer ....t406 _______code
   f. Company personnel (for example, catch/vaccination crew, barn workers, service person, veterinarian) ....t407 _______code
   g. Other business visitors (for example, meter reader, repairman) ....t408 _______code

3. In general, how many vehicles (including employee vehicles) come to the following locations on a weekly basis?
   a. Perimeter of the farm only .... t409 ________vehicles per week
   b. Enter the farm but not near the barns.................... t410 ________vehicles per week
   c. Come near the barns .... t411 ________vehicles per week

4. Excluding driveways on farm, what is the distance (in yards or miles) from this farm to the nearest public gravel or dirt road? ....t412y __________ yards OR t412m____________ miles

5. How frequently is vegetation mowed and/or bush hogged on the premises?
   (Answer for when vegetation is present, for example, spring and summer)......t413 _______ times/month

6. Was there a wash station or spray area being used for vehicles during the 14-day reference period?
   t414 ☐1 Yes ☐3 No

[If Question 6 = No, SKIP to Question 8.]

7. During the 14-day reference period, was the vehicle wash station or spray area:
   a. Located on the farm?..........t415 ☐1 Yes ☐3 No
   b. Were the vehicle tires washed?..........t416 ☐1 Yes ☐3 No
   c. Was the vehicle exterior washed?..........t417 ☐1 Yes ☐3 No
   d. Was the vehicle interior cleaned (for example, floor mats)..........t418 ☐1 Yes ☐3 No
   e. Were the following vehicles washed?
      i. Worker vehicles..........t419 ☐1 Yes ☐3 No ☐4 NA
      ii. Feed trucks..........t420 ☐1 Yes ☐3 No ☐4 NA
      iii. Vehicles delivering or removing birds..........t421 ☐1 Yes ☐3 No ☐4 NA
      iv. Other vehicles (specify: _________________) t422oth..........t422 ☐1 Yes ☐3 No ☐4 NA
   f. What disinfectant was used?..........t423 _____________________
   g. What was the distance from the vehicle wash station to the **selected barn** in yards?
      t424 _________yards

8. Did workers and visitors always, sometimes, or never park in a restricted area away from the poultry barns during the 14-day reference period?
   a. Workers..........t425 ☐1 Always ☐2 Sometimes ☐3 Never
   b. Visitors..........t426 ☐1 Always ☐2 Sometimes ☐3 Never

9. During the 14-day reference period, were wild mammals, such as raccoons, opossums, skunks, coyotes, or foxes, or evidence of their presence, seen in or around poultry barns?
   t427 ☐1 Yes ☐3 No

10. During the 14-day reference period, which of the following pest and wild bird control measures were used on this farm?
    a. Rat and mouse bait stations..........t428 ☐1 Yes ☐3 No
       i. If Yes, how frequently are they checked per month?..........t429 ________times/month
    b. Beetle control (for example, sprays, baits, boric acid)..........t430 ☐1 Yes ☐3 No
    c. Fly control (for example, baits, larvicide, space sprays/fogger, biological predators)
       ....t431 ☐1 Yes ☐3 No
    d. Netting on barns to prevent wild bird access..........t432 ☐1 Yes ☐3 No

11. How often were rodents observed in the **selected barn** during the 14-day reference period? *[Check one only.]*
    t433
    ☐1 Frequently (for example, daily)
    ☐2 Occasionally (for example, weekly)
    ☐3 Never

12. What was the intensity of beetles observed in the **selected barn** during the 14-day reference period? *[Check one only.]* t434
    ☐1 High

☐2 Medium
☐3 Low
☐4 None

13. What was the intensity of flies observed in the **selected barn** during the 14-day reference period? *[Check one only.]* t435
☐1 High
☐2 Medium
☐3 Low
☐4 None

14. Does the **selected barn** have a hard-surface entry pad (for example, concrete, asphalt)? t436 ☐1 Yes ☐3 No

If Yes,
a. Is the entry pad cleaned? ....t437 ☐1 Yes ☐3 No
i. If Yes, specify frequency t438/t438a ______ times/ ☐1 week ☐2 month OR ☐3 year
b. Is disinfectant used? ....t439 ☐1 Yes ☐3 No

15. During the 14-day reference period, how frequently were wild birds, wild animals, and rodents able to access poultry feed or feed ingredients (for example, feed spillage, open bag, cover left open)?
For this question, “Always” is 100% of the time, “Most of the time” is 51-99% of the time, “Sometimes” is 1-50% of the time, and “Never” is 0% of the time.

Type	Always	Most of the time	Sometimes	Never	
a. Wild birds	☐1	☐2	☐3	☐4	t440
b. Wild animals (such as raccoons, opossums, skunks, coyotes, or foxes)	☐1	☐2	☐3	☐4	t441
c. Rodents	☐1	☐2	☐3	☐4	t442

16. Does this farm have a written wildlife management plan that includes methods to minimize wildlife or wild bird entry and reduce wildlife attractants such as standing water? t443 ☐1 Yes ☐3 No

17. In the 2 years before the 14-day reference period, were any biosecurity audits or assessments (company or third party) conducted on this farm? t444 ☐1 Yes ☐3 No ☐4 Don’t know

18. Considering the following biosecurity topics, how challenging would you say these are for producers to achieve? *[Check one box per row.]*

	Not at all challenging	Slightly challenging	Somewhat challenging	Quite challenging	Extremely challenging	
a. Keeping feed safe from rodents	☐1	☐2	☐3	☐4	☐5	t445
b. Keeping feed safe from wildlife	☐1	☐2	☐3	☐4	☐5	t446

Section E – Biosecurity Investments

1. Over the past year, has this farm had the following ongoing biosecurity expenses?
   If Yes, what was the typical monthly cost of each?

Biosecurity type	Ongoing expenses?	If Yes, what is the typical monthly cost?	
a. Wash station or spray area being used for vehicles	☐1 Yes ☐3 No	$________	t501/t507
b. Foot baths	☐1 Yes ☐3 No	$________	t502/t508
c. Pest and bait stations	☐1 Yes ☐3 No	$________	t503/t509
d. Wash stations for employees (for example, sinks, showers)	☐1 Yes ☐3 No	$________	t504/t510
e. PPE for employees and visitors (for example, gloves, coveralls, boot covers)	☐1 Yes ☐3 No	$________	t505/t511
f. Other (specify:________________) t506oth	☐1 Yes ☐3 No	$________	t506/t512
Total monthly cost		$________	t513

2. Since 2015, has this farm built or made **permanent** improvements or renovations on the following farm structures that impact the farm's biosecurity?
   a. A service room that personnel must enter through that separates "outside area" from "inside area" (for example, Danish entry)........t514 ☐1 Yes ☐3 No
   b. Wash stations for employees (for example, sinks, showers) ....t515 ☐1 Yes ☐3 No
   c. Permanent improvements or renovations to limit wild bird access to barns t516 ☐1 Yes ☐3 No
   d. Barn ventilation system ....t517 ☐1 Yes ☐3 No
   e. Other barn improvements or renovations........t518 ☐1 Yes ☐3 No
   f. Feed bins ....t519 ☐1 Yes ☐3 No
   g. Permanent vehicle wash stations (for example, automated truck wash)....t520 ☐1 Yes ☐3 No
   h. Other (specify: ____________________) t521oth........t521 ☐1 Yes ☐3 No
   i. If Yes to any in 2a through 2h, what was the approximate total cost of **all** of these improvements?
      t522 $

3. Over the next two years, does this farm have plans to build or make **permanent** improvements or renovations on farm structures such as barns, feed bins, or other structures that impact the farm's biosecurity?
   t523 ☐1 Yes ☐3 No

4. Since 2015, has this farm built or installed any of the following **temporary** structures or infrastructure that impact the farm's biosecurity?
   a. Gates ....t524 ☐1 Yes ☐3 No
   b. Parking area ....t525 ☐1 Yes ☐3 No
   c. Temporary wild bird mitigation ....t526 ☐1 Yes ☐3 No
   d. Landscape fabric on air intake inlets or curtains ....t527 ☐1 Yes ☐3 No
   e. Temporary vehicle wash stations (for example, hand sprayer)........t528 ☐1 Yes ☐3 No
   f. Other (specify: ____________________) t529oth........t529 ☐1 Yes ☐3 No

g. If Yes to any in 4a through 4f, what was the approximate total cost of **all** of these improvements?
t530 $

5. Over the next two years, does this farm have plans to build or install any **temporary** structures that impact the farm's biosecurity? ....t531 ☐1 Yes ☐3 No

6. How much did the 2014-2015 HPAI outbreak influence your decisions about biosecurity investments for this farm? t532 ☐1 Not at all ☐2 Slightly ☐3 Somewhat ☐4 Quite a bit ☐5 Extremely

Section F – Farm Help / Workers

Questions in this section refer to persons such as the producer, employees, farm help, crews, etc.

1. What is the total number of employees working on this farm that have access to or directly work with poultry (including family, both paid and unpaid)? ....t601 ____________ #

2. Did this farm use occasional or emergency workers such as family members or part-time help to fill in for any employees during the 14-day reference period? ....t602 ☐1 Yes ☐3 No

3. During the 14-day reference period, how frequently were the following measures used by workers entering the **selected barn**?
For this question, "Always" is 100% of the time, "Most of the time" is 51-99% of the time, "Sometimes" is 1-50% of the time, and "Never" is 0% of the time. N/A=not applicable.

Measure	Always	Most of the time	Sometimes	Never	N/A-Not available	
a. An established clean/dirty line	☐1	☐2	☐3	☐4	☐5	t603
b. A service room that personnel must enter through that separates "outside area" from "inside area"	☐1	☐2	☐3	☐4	☐5	t604
c. Shower	☐1	☐2	☐3	☐4	☐5	t605
d. Wash hands or use hand sanitizer before entering the barn	☐1	☐2	☐3	☐4	☐5	t606
e. Wear disposable gloves	☐1	☐2	☐3	☐4	☐5	t607
f. Different personnel for different barns	☐1	☐2	☐3	☐4	☐5	t608
g. Locks on the barn doors	☐1	☐2	☐3	☐4	☐5	t609
h. Wear disposable coveralls	☐1	☐2	☐3	☐4	☐5	t610
i. Change of clothing/coveralls (washable)	☐1	☐2	☐3	☐4	☐5	t611
j. Change of shoes or use of shoe covers	☐1	☐2	☐3	☐4	☐5	t612
k. Scrub footwear (bucket and brush)	☐1	☐2	☐3	☐4	☐5	t613
l. Foot bath (liquid)	☐1	☐2	☐3	☐4	☐5	t614
m. Foot bath (dry, such as powdered or particulate)	☐1	☐2	☐3	☐4	☐5	t615

[If both Question 3 l and m = not available, SKIP to Question 5.]

4. What was the frequency that liquid or dry footbath solutions were changed for the **selected barn** during the 14-day reference period? t616/t616a ________________________ times/ ☐1 week ☐2 month OR ☐3 year
   a. What disinfectant was used in the footbaths? .... t617 ____________________________

5. During a typical month, do any workers on this farm visit another poultry farm?
   t618 ☐1 Yes ☐3 No ☐4 Don't know

6. Are any workers or members of their household employed by other poultry operations, other company farms, rendering plants, or processing plants?
   a. Workers .... t619 ☐1 Yes ☐3 No ☐4 Don't know
   b. Members of household .... t620 ☐1 Yes ☐3 No ☐4 Don't know

7. Do any employees own their own poultry, including small backyard flocks?
   t621 ☐1 Yes ☐3 No ☐4 Don't know

8. Are employees required to stay off farm after exposure to other poultry?............t622 ☐1 Yes ☐3 No
   a. If Yes, for how long (hours)? ....t623 ________ hours

9. In a typical week, how much time is spent by **all** employees on biosecurity activities on the farm?
   t624 ________ hours

10. Considering the following personnel-related topics, how challenging would you say these are for producers to achieve? *[Check one box per row.]*

	Not at all challenging	Slightly challenging	Somewhat challenging	Quite challenging	Extremely challenging	
a. Hiring new personnel	☐1	☐2	☐3	☐4	☐5	t625
b. Retaining trained personnel	☐1	☐2	☐3	☐4	☐5	t626
c. Communicating the importance of biosecurity to personnel	☐1	☐2	☐3	☐4	☐5	t627
d. Enforcing daily biosecurity measures	☐1	☐2	☐3	☐4	☐5	t628
e. Other personnel-related challenges (Specify: t629oth _________)	☐1	☐2	☐3	☐4	☐5	t629

Section G – Farm Visitors

1. How often is a visitor log used to record visitor traffic onto the farm?
   t701 ☐1 Always ☐2 Sometimes ☐3 Never

2. Did any of the following types of people visit the farm during the 14-day reference period?

If Yes, how many times did they visit during the 14-day reference period and did they enter the **selected barn**?

Visitor type	Did they visit the farm?	If Yes, How many times did they visit?	If Yes, Did this visitor enter the selected barn?	
a. Federal/State veterinary or animal health worker	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t702/t725/t748
b. Extension agent or university veterinarian	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t703/t726/t749
c. Private or company veterinarian	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t704/t727/t750
d. Company service person	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t705/t728/t751
e. Nutritionist or feed company consultant	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t706/t729/t752
f. Bird delivery personnel (for example, poult placement, brood to grow move)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t707/t730/t753
g. Vaccination crew	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t708/t731/t754
h. Catch crew (bird removal)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t709/t732/t755
i. Artificial insemination crew (for breeder farms)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t710/t733/t756
j. Feed ingredient delivery person	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t711/t734/t757
k. Feed delivery personnel	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t712/t735/t758
l. Egg truck personnel (for breeder farms)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t713/t736/t759
m. Fresh litter delivery services	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t714/t737/t760
n. Litter removal services (for example, litter broker, litter disposal)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t715/t738/t761
o. Customer (private individual)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t716/t739/t762
p. Wholesaler, buyer, or dealer	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t717/t740/t763
q. Renderer	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t718/t741/t764
r. Dead bird pickup other than by renderer	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t719/t742/t765
s. Rodent control crew	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t720/t743/t766
t. Occasional worker (for example, family member, part-time help over holiday)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t721/t744/t767
u. Construction workers, repair or maintenance personnel	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t722/t745/t768
v. Other business visitors (including other producers,	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t723/t746/t769

meter readers, package delivery (UPS), propane, or similar)				
w. Other nonbusiness visitors (including neighbors, family members, friends, and school field trips)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	t724/t747/t770

3. For those visitors who entered the **selected barn** during the 14-day reference period, did you require the following? *[Check one per row.]*

	Yes, verified at farm	**Yes, visitor responsibility**	**No**	
a. Change of outer clothing/farm specific clothing/coveralls	☐1	☐2	☐3	t771
b. Foot covers or change of footwear	☐1	☐2	☐3	t772
c. Mask	☐1	☐2	☐3	t773
d. Hand sanitizing or handwashing	☐1	☐2	☐3	t774
e. Gloves	☐1	☐2	☐3	t775
f. Not visit multiple farms in the same day	☐1	☐2	☐3	t776
g. Other (specify: _________________) t777oth	☐1	☐2	☐3	t777

4. How often is a restroom facility (including portable) available to crews that visit the farm?
t778 ☐1 Always (24 hours/day) ☐2 Sometimes ☐3 Never

Section H – Farm Vehicles and Equipment

1. Were the following vehicles shared with another farm during the 14-day reference period?
If Yes, how often were they cleaned and disinfected prior to returning to this farm?

Vehicle type	**Shared with another farm in the 14-day reference period?**	**If Yes, how often was it cleaned and disinfected prior to returning to this farm?**	
a. Company trucks or trailers (for example, pickup truck, trailer with supplies, supervisor truck, or similar)	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t801/t810
b. Feed trucks	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t802/t811
c. Feed ingredient truck	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t803/t812

d. Bird delivery vehicles (for example, placing birds)	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t804/t813
e. Bird removal vehicles (for example, moved to slaughter, moved to grow)	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t805/t814
f. Egg removal vehicles (for breeder farms)	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t806/t815
g. Manure/litter hauling	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t807/t816
h. ATV/4-wheeler	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t808/t817
i. Other (specify:________________________) t809oth	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t809/t818

2. Were the following pieces of equipment shared with another farm during the 14-day reference period? If Yes, how often were they cleaned and disinfected prior to returning to this farm?

Equipment type	**Shared with another farm in the 14-day reference period?**	**If Yes, how often was it cleaned and disinfected prior to returning to this farm?**	
a. Gates/panels	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t819/t830
b. Lawn mowers	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t820/t831
c. Live haul loaders	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t821/t832
d. Catch pens	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t822/t833
e. Scales for weighing birds	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t823/t834

f. Vaccination equipment	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t824/t835
g. Pressure sprayers/washers/foamers	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t825/t836
h. Skid-steer loaders	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t826/t837
i. Litter/manure handling	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t827/t838
j. Tillers/de-caking equipment	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t828/t839
k. Other (specify:______________________) t829oth	☐1 Yes ☐3 No	☐1 Always ☐2 Sometimes ☐3 Never	t829/t840

3. Considering the following equipment-related topics, how challenging would you say these are for producers to achieve? *[Check one box per row.]*

	Not at all challenging	Slightly challenging	Somewhat challenging	Quite challenging	Extremely challenging	
a. Keeping shared vehicles cleaned and disinfected	☐1	☐2	☐3	☐4	☐5	t841
b. Keeping shared small equipment (such as catch pens or litter tillers) cleaned and disinfected	☐1	☐2	☐3	☐4	☐5	t842
c. Other equipment or vehicle-related challenges (Specify: t843oth ________________)	☐1	☐2	☐3	☐4	☐5	t843

Section I – Litter Handling

1. Was fresh litter/bedding brought onto the farm during the 14-day reference period?
   t901 ☐1 Yes ☐3 No
   a. If Yes, who brought the fresh litter onto the farm? *[Check one only.]* t902
      ☐1 Company personnel
      ☐2 Litter provider
      ☐3 Other (specify: ____________________) t902oth
2. Is the fresh litter heat treated prior to delivery? t903 ☐1 Yes ☐3 No ☐4 Don't know
3. Prior to use, is fresh litter stored on the farm:
   a. Outside .... t904 ☐1 Yes ☐3 No
      i. If Yes, is it covered? .... t905 ☐1 Yes ☐3 No
   b. In a shed .... t906 ☐1 Yes ☐3 No
      i. If Yes, is the shed closed? .... t907 ☐1 Yes ☐3 No

[If both Questions 3 a and b = No, SKIP to Question 6.]

4. What is the distance (in yards) from the on-site fresh litter storage area to the **selected barn**?
   .... t908 _________ yards
5. Prior to use, is fresh litter accessible to:
   a. Wild birds .... t909 ☐1 Yes ☐3 No
   b. Wild animals (for example, raccoons, opossum, coyotes, foxes) .... t910 ☐1 Yes ☐3 No
   c. Domestic animals (for example, dogs, cats) .... t911 ☐1 Yes ☐3 No
6. What was the date that used litter was last removed from any barn on this farm prior to the end of the 14-day reference period? .... t912 _________ mm/dd/yy
7. How was used litter disposed of prior to or during the 14-day reference period?
   a. Composted on-farm .... t913 ☐1 Yes ☐3 No
      i. If Yes, what is the distance (in yards) to the **selected barn**? .... t914 ________ yards
   b. Stored on-farm .... t915 ☐1 Yes ☐3 No
   c. Applied to land on this farm .... t916 ☐1 Yes ☐3 No
      i. If Yes, what was the date litter was applied to land? .... t917 ________ mm/dd/yy
   d. Taken off-site .... t918 ☐1 Yes ☐3 No
8. Was manure or used litter from other farms brought onto this farm or adjacent farms prior to or during the reference period? t919 ☐1 Yes ☐3 No ☐4 Don't know
9. How many times was fresh litter added to the **selected barn** during the reference period?
   t920 ________ times

These next three questions ask about the litter management practices for the selected barn around the time of the 14-day reference period.

10. Was litter "tilled" after it was placed in the **selected barn**? .... t921 ☐1 Yes ☐3 No
11. Was there a partial clean out of the **selected barn**? .... t922 ☐1 Yes ☐3 No
12. When was the last full clean out of the **selected barn**? *[Check one only.]* t923
    ☐1 Prior to this flock
    ☐2 Two flocks ago

☐3 Three or more flocks ago

Section J – Dead Bird Disposal

1. What is the approximate normal daily mortality on this farm? .... t1001 __________#/day

2. During the 14-day reference period, what were the method(s) of dead bird (daily mortality) disposal on this farm?
   a. Composting..t1002 ☐1 Yes ☐3 No
   b. Burial..t1003 ☐1 Yes ☐3 No
   c. Incineration ....t1004 ☐1 Yes ☐3 No
   d. Rendering ....t1005 ☐1 Yes ☐3 No
   e. Landfill ....t1006 ☐1 Yes ☐3 No
   f. Other (specify: ____________________) t1007oth.....................................t1007 ☐1 Yes ☐3 No

3. If Question 2a (composting) or Question 2b (burial) is Yes, how frequently are carcasses covered with:
   a. Soil?..t1008 ☐1 Daily ☐2 Every 2 or more days ☐3 Never
   b. Manure?...t1009 ☐1 Daily ☐2 Every 2 or more days ☐3 Never

4. If Question 2d (rendering) is Yes,
   a. Is the carcass bin kept covered? ....t1010 ☐1 Yes ☐3 No
   b. Are carcasses: *[Check one only.]* t1011
      ☐1 Taken by the producer or worker to the renderer?
      ☐2 Picked up by the renderer from the farm?
   c. How many times were carcasses moved to the renderer during the 14-day reference period? t1012 _________ # times

5. Does this farm have an alternative mortality disposal plan if the typical method is disrupted, and carcasses cannot be moved off farm? .... t1013 ☐1 Yes ☐3 No

6. Were any wild birds or wild mammals observed around the dead bird collection area (such as burial, compost pile, rendering bin, or similar) during the 14-day reference period?
   a. Wild birds .... t1014 ☐1 Yes ☐3 No
   b. Wild mammals .... t1015 ☐1 Yes ☐3 No

7. During the 14-day reference period, did this farm use a shared collection point for dead bird disposal? *[Check one only.]* t1016 ☐1 Yes – located on this farm ☐2 Yes – located off this farm ☐3 No

8. How far is the **selected barn** (in yards) from the dead bird disposal/holding area including carcass bin for rendering? t1017.. yards

Section K – Selected Barn Characteristics

Answer this entire section for the selected barn that was chosen in Section B. **Answer questions for the 14-day reference period.**

1. Which best describes the ground surface immediately surrounding (within 1 yard) this barn (excluding vehicle approach and loading area)? *[Check one only.]* t1101
   ☐1 Gravel or hard surface
   ☐2 Dirt
   ☐3 Short grass

☐4 Tall grass or brush

2. Were the following type(s) of poultry present in this barn during the 14-day reference period?
   a. Brooder .... t1102 ☐1 Yes ☐3 No
   b. Grower toms .... t1103 ☐1 Yes ☐3 No
   c. Grower hens .... t1104 ☐1 Yes ☐3 No
   d. Breeders .... t1105 ☐1 Yes ☐3 No
   e. Other (specify: ____________________) t1106oth........ t1106 ☐1 Yes ☐3 No

3. For the flock that was present during the 14-day reference period, how many birds were placed in this barn?
   t1107 .... # birds

4. What was the date of placement in this barn? .... t1108 _________ mm/dd/yy

5. How old were birds when placed in this barn?..t1109d ___________ days OR t1109w _____________ weeks

6. Were different stages of production (for example, brooders and growers) present in this barn at the same time during the 14-day reference period? .... t1110 ☐1 Yes ☐3 No

7. Was there a partial load-out of this barn during the reference period? .... t1111 ☐1 Yes ☐3 No

8. Was there another health concern in this flock during the reference period? .... t1112 ☐1 Yes ☐3 No
   a. If Yes, specify condition: t1113 ______________________________

9. Was this flock being treated for a condition or health concern during the reference period?
   t1114 ☐1 Yes ☐3 No
   a. If Yes, specify treatment: t1115______________________________

10. How old is this barn structure? .... t1116 ________years

11. How long has it been since the last remodel of the barn structure? .... t1117 ________years
   t1117a ☐1 NA – Never remodeled

12. How well has the barn structure been maintained? *[Check one only.]* t1118
   ☐1 Well – For example, walls, curtains, and mud boards do not have holes, no visible daylight, the barn is tight and well insulated
   ☐2 Moderate – For example, barn could have rust or small holes, mud boards may be damaged, curtains may be torn or not in good repair, curtains may not close all the way, insulation may not be in good repair, the poly may be hanging from the ceiling
   ☐3 Poor – For example, holes in walls and mud boards are apparent, tin is rusted, may have leaks in roof, there might be some holes large enough for wild birds to enter, multiple areas with daylight visible, insulation may be hanging from the ceiling

13. Is this barn bird proof? .... t1119 ☐1 Yes ☐3 No

14. During the 14-day reference period, did you notice any water seepage into the barn (for example, water entering the barn from snowmelt or rainwater)? .... t1120 ☐1 Yes ☐3 No ☐4 Don't know

15. What type of ventilation was used for this barn during the 14-day reference period? *[Check one only.]* t1121
   ☐1 Curtain ventilated
   ☐2 Environmental control/tunnel ventilation
   ☐3 Side doors (such as tip outs)
   ☐4 Other (specify: ____________________) t1121oth

16. During the 14-day reference period,
    a. What percentage of time were the curtains open? t1122/t1133 ________ % time ☐4 Don't know
    b. How many days were the curtains open or partially open? t1123 /t1134 ______ # days ☐4 Don't know
17. Was intake air filtered during the 14-day reference period? .... t1124 ☐1 Yes ☐3 No
    a. If Yes, specify type of filter: t1125 ____________________________________
18. During the 14-day reference period, was landscape fabric in place on either air intake inlets or along curtains on the barn?
    a. On air intake inlets .... t1126 ☐1 Yes ☐3 No
    b. Along curtains .... t1127 ☐1 Yes ☐3 No

[If both Question 18a and 18b = No, SKIP to Question 21.]

19. During the 14-day reference period, was any of this landscape fabric installed or replaced on either air intake inlets or along curtains? .... t1128 ☐1 Yes ☐3 No
20. During the 14-day reference period, was any of this landscape fabric sprayed with disinfectant on either air intake inlets or along curtains?.. t1129 ☐1 Yes ☐3 No
    a. If Yes, how often was it sprayed: .... t1130/t1130a _____times / ☐1 day OR ☐2 week
21. How frequently were the following used in this barn during the 14-day reference period? *[Check one per row.]*

	Used regularly	Not used regularly	Not available	
a. Cool cell pads	☐1	☐2	☐3	t1131
b. Misters	☐1	☐2	☐3	t1132

Section L – Office Use Only

1. Interview response code. *[Check only one.]* t1201
   - ☐1 Survey completed
   - ☐2 Refused
   - ☐3 Out of business
   - ☐4 No turkeys present during 2022
   - ☐5 Inaccessible
   - ☐6 Other (specify: ____________________) t1201oth

Comment Section

Please use this section for anything else that you would like to add. For example, how do you think HPAI was/is spreading within your geographic area? t1301

APPENDIX D: H5N1 HPAI BIRD DETECTIONS

Table D1. Number of H5N1 HPAI detections in apparently healthy and sick/dead birds from 30 December 2021 to 31 March 2023.

Species	# HPAI Detections in Apparently Healthy Birds	# HPAI Detections in Sick/Dead Birds
American black duck	42	0
American coot	0	2
American crow	0	58
American green-winged teal	392*	3
American kestrel	0	1
American robin	0	1
American white pelican	1	90
American wigeon	232*	2
American wood stork	0	2
Arctic tern	0	2
Bald eagle	0	344
Barn owl	0	1
Barred owl	0	10
Black skimmer	0	2
Black turnstone	0	1
Black vulture	0	546
Black-billed magpie	0	3
Black-crowned night heron	0	5
Black-legged kittiwake	0	1
Blue-winged teal	385*	7
Boat-tailed grackle	0	1
Bonaparte's gull	0	1
Brant	0	7
Brown pelican	0	12
Bufflehead	1	8
Cackling goose	0	17
California condor	0	1
California gull	0	2
Canada goose	6	556
Caspian tern	0	13
Cinnamon teal	17*	2
Common eider	0	28
Common goldeneye	2	4
Common grackle	0	2
Common loon	0	8
Common merganser	0	2
Common raven	0	32
Common tern	0	11
Cooper's hawk	0	16
Cormorant (unidentified)	0	8
Crested caracara	0	1
Crow (unidentified)	0	4
Double-crested cormorant	0	26
Duck (unidentified)	0	12
Dunlin	0	3

Species	# HPAI Detections in Apparently Healthy Birds	# HPAI Detections in Sick/Dead Birds
Eagle (unidentified)	0	1
Eared grebe	0	13
Eastern screech owl	0	3
Eider (unidentified)	0	1
Ferruginous hawk	0	1
Fish crow	0	5
Forster's tern	0	1
Fulvous whistling duck	1	0
Gadwall	186*	11
Gannet	0	1
Glaucous gull	0	12
Glossy ibis	0	1
Golden eagle	0	3
Goose (unidentified)	0	79
Great black-backed gull	0	28
Great blue heron	0	9
Great egret	0	3
Great horned owl	0	192
Greater sage grouse	0	1
Greater scaup	0	1
Greater white-fronted goose	0	16
Great-tailed grackle	0	3
Green heron	0	1
Gull (unidentified)	0	24
Harris hawk	0	1
Hawk (unidentified)	0	21
Heron (unidentified)	0	1
Herring gull	0	36
Hooded merganser	1	22
Horned grebe	0	2
House sparrow	0	1
Laughing gull	0	1
Lesser scaup	5*	27
Long-eared owl	0	1
Magpie (unidentified)	0	2
Mallard	750*	69
Merganser (unidentified)	0	6
Merlin	0	1
Mottled duck	1	0
Muscovy duck	0	31
Mute swan	1	18
Neotropic cormorant	0	3
Northern fulmar	0	1
Northern gannet	0	2
Northern harrier	0	2
Northern pintail	51*	4
Northern shoveler	82	0
Osprey	0	2
Owl (unidentified)	0	11
Pacific loon	0	1

Species	# HPAI Detections in Apparently Healthy Birds	# HPAI Detections in Sick/Dead Birds
Parasitic jaeger	0	2
Pelican (unidentified)	0	9
Peregrine falcon	0	38
Pheasant (unidentified)	0	3
Pied-billed grebe	0	1
Prairie falcon	0	1
Redhead duck	3	9
Red-necked grebe	0	1
Red-necked phalarope	0	1
Red-shouldered hawk	0	8
Red-tailed hawk	0	221
Red-winged blackbird	0	1
Ring-billed gull	0	5
Ring-necked duck	2	8
Roseate spoonbill	0	1
Ross's goose	17	85
Rough-legged hawk	0	5
Royal tern	0	3
Ruddy turnstone	0	1
Ruffed grouse	0	1
Sabine's gull	0	3
Sanderling	0	17
Sandhill crane	0	10
Sandwich tern	0	1
Sharp-shinned hawk	0	5
Short-billed gull	0	1
Short-eared owl	0	1
Snow goose	93*	419
Snowy egret	0	2
Snowy owl	0	15
Snowy plover	0	4
Swainson's hawk	0	6
Swan (unidentified)	0	11
Thayer's gull	0	1
Tree swallow	0	1
Trumpeter swan	0	18
Tundra swan	1	9
Turkey vulture	0	99
Vulture (unidentified)	0	2
Western grebe	0	1
Western gull	0	7
Western screech owl	0	2
White-faced ibis	0	1
White-winged scoter	0	1
Wild turkey	0	17
Willet	0	1
Wood duck	174	37
Grand Total	2,446*	3,639

*Includes apparently healthy bird samples collected by other agencies

APPENDIX E: TIME OF INTRODUCTION MODELING METHODS

We used approximate Bayesian computation (ABC) to estimate the likely time of virus introduction and key model parameters, such as the adequate contact rate—a parameter which regulates the rate of within-flock disease spread—from the available production and test data.

A stochastic individual-based simulation model was first used to simulate the disease mortality, infection prevalence over time, and water consumption (where applicable) over a wide range of values for model parameters, such as the adequate contact rate, times of disease introduction, and bird-level latent and infectious period distributions (i.e., prior distributions).

In the next step, the sum of the squared distance between the model-predicted daily mortality and water consumption (where applicable) and the observed data, and the difference between observed and simulated diagnostic test results, was calculated as a measure of deviation between the model output and data (ψ). The parameters in model iterations where the metric ψ was sufficiently small, indicating a good fit to the data, were then accepted to estimate the distribution of the time of introduction and other model parameters.

We used wide priors for input variables based on published literature and estimates from previous SEPRL challenge studies. Preliminary data from SEPRL challenge studies in turkeys and chickens with a current outbreak isolate (A/American Widgeon/SC/22-000345-001/2022 (H5N1) HPAIV) were made available in May 2022. We estimated the disease state durations from the challenge study data using Markov chain Monte Carlo algorithms. The estimated disease state durations were then used to update the prior distributions for the latent and infectious periods. The updated prior distributions used in the analysis for WOAH poultry [commercial] meat turkey and table egg layer flocks based on SEPRL data and other published studies are summarized in Table E1 and Table E2, respectively. We also performed a sensitivity analysis for the impact of the mean infectious period prior for selected premises given the uncertainty in this parameter.

Table E1. Input prior distribution parameters used in the ABC approach to estimate the adequate contact rate and time of virus introduction for WOAH poultry [commercial] meat turkey flocks.

Parameter Name	Description	Distribution
Adequate Contact Rate	Daily average number of contacts a bird has with other birds that are sufficient to transmit infection	Uniform (min = 0.2, max = 7) per day
Latent Period Length Distribution	Length of the interval when a bird is latently infected and is not infectious	Gamma (shape = 4.037, scale = 0.1809); mean = 0.64 days; variance = 0.67
Mean Infectious Period	Prior distribution for the mean infectious period	Uniform (1.9 – 6.3 days)
Shape Parameter for Infectious Period	Prior distribution for the shape parameter of gamma distributed infectious period	Uniform (1 – 20)

Table E2. Input prior distribution parameters used in the ABC approach to estimate the adequate contact rate and time of virus introduction for WOAH poultry [commercial] table egg layer flocks.

Parameter Name	Description	Distribution
Adequate Contact Rate	Daily average number of contacts a bird has with other birds that are sufficient to transmit infection	Uniform (min = 0.5, max = 9) per day
Latent Period Length Distribution	Length of the interval when a bird is latently infected and is not infectious	Gamma (shape = 2.54, scale = 0.33); mean = 0.8 4 days; variance = 0.67
Mean Infectious Period	Prior distribution for the mean infectious period	Uniform (0.74 – 4 days)
Shape Parameter for Infectious Period	Prior distribution for the shape parameter of gamma distributed infectious period	Uniform (1 – 20)

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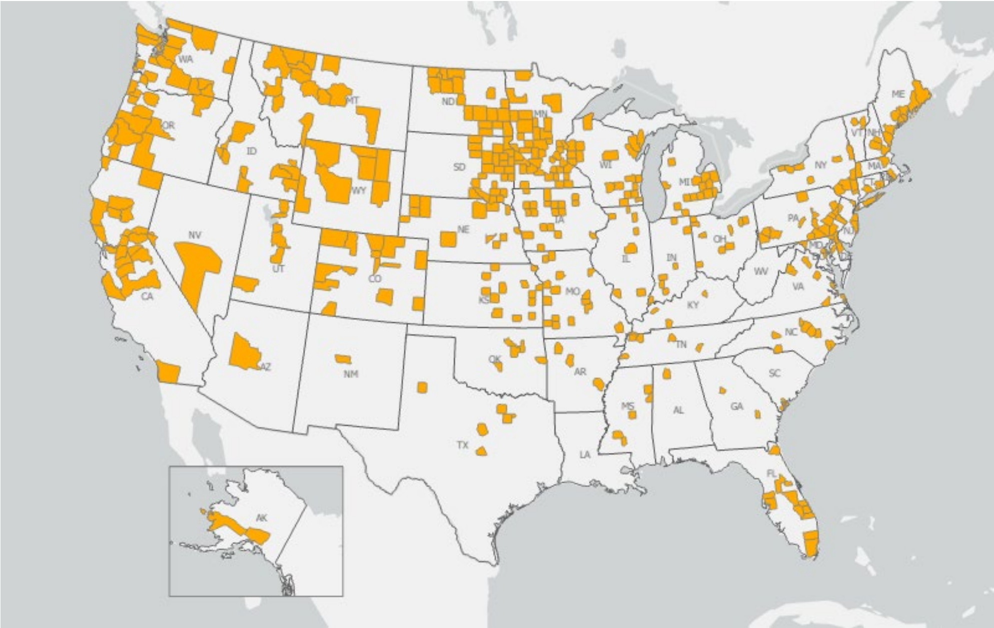

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Center for Epidemiology and Animal Health

2150 Centre Avenue, Bldg. B, Fort Collins, CO, 80526-8117

Web: http://www.aphis.usda.gov/animal-health/ceah