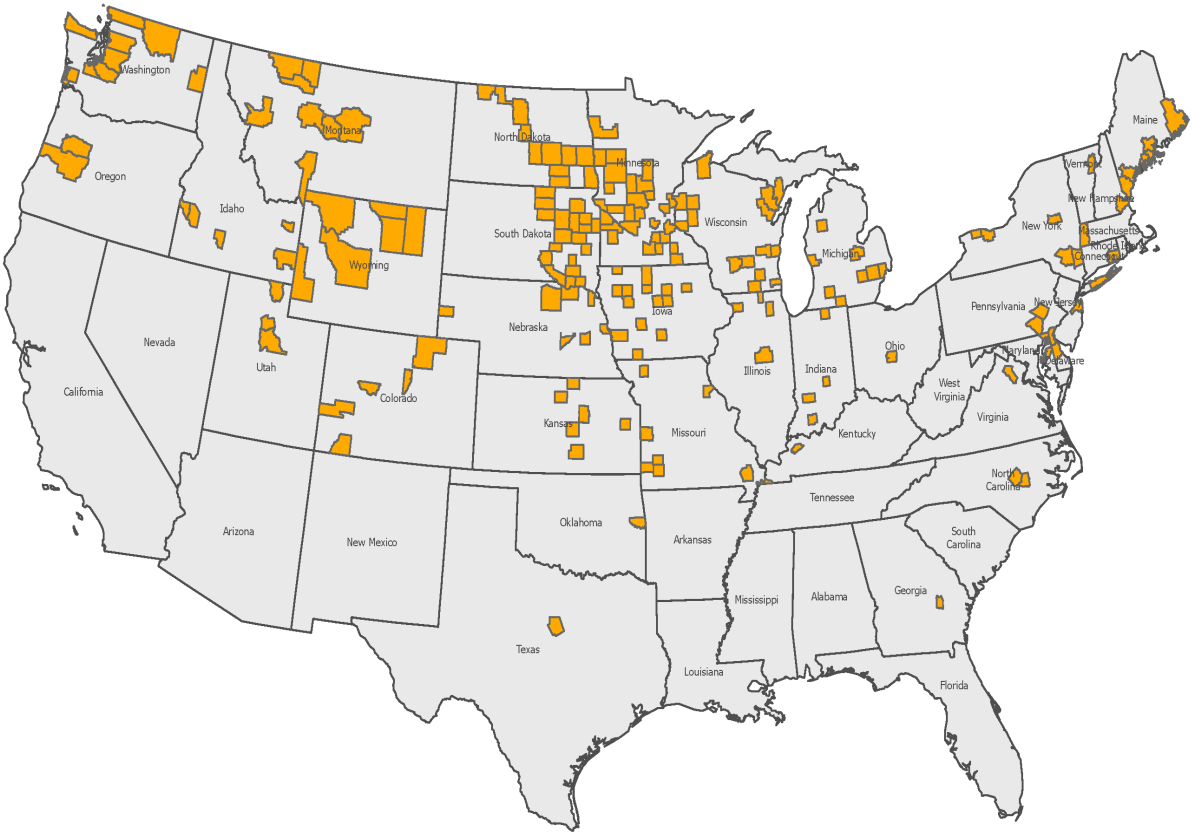
USDA Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Epidemiologic and Other Analyses of HPAI-affected Poultry Flocks
29 February 2024 Interim Report



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EXECUTIVE SUMMARY

In January 2022, the USDA's Animal and Plant Health Inspection Service (APHIS) identified clade 2.3.4.4b H5N1 highly pathogenic avian influenza (HPAI) in a wild bird sample from Colleton County, South Carolina. This detection initiated what is currently the largest avian influenza outbreak in U.S. history, involving many wild bird species and virus reassortments, with spillover into poultry, wild and captive mammals, and outdoor domestic cats. By 29 February 2024, the National Veterinary Services Laboratories at the National Centers for Animal Health, Ames, Iowa (NVSL-NCAH) confirmed HPAI detections in poultry and other domestic birds in 48 States, including 472 WOAH (World Organisation for Animal Health) poultry[1] [commercial],[2] 144 WOAH poultry [backyard],[3] 480 WOAH non-poultry,[4] and 12 WOAH poultry [live bird market] premises. Migratory wild bird movements continue to be the primary driver for the spatial extent of the 2022–2024 HPAI outbreak, with premises traditionally considered WOAH poultry [backyard] and WOAH non-poultry still comprising the largest proportion of detections. Note that this report includes data through February 2024 and does not include genotype B3.13 detections from March 2024 onward associated with livestock, peridomestic animals, and poultry.

Phylogenetic analysis has contributed to the understanding of the movement and evolution of HPAI viruses. Many genotypes identified appear in both wild bird and poultry detections, continuing to highlight the critical role of wild bird-related spread. Although there is currently no active nationwide influenza A virus (IAV) surveillance effort in apparently healthy wild mammals, as of 29 February 2024, there were 212 H5N1 HPAI detections from sick or dead animals in 20 wild mammal species across 28 States. In the United States, 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.

Since the last interim report in June 2023, investigations into risk factors for introduction of HPAI on WOAH poultry [commercial] turkey farms and WOAH poultry [commercial] table egg farms were published (Green et al., 2023; Patyk et al., 2023). Following the published investigations, APHIS posted an info sheet on challenges in implementing biosecurity practices for WOAH poultry [commercial] turkey and table egg producers. These results are reviewed in this report. For turkey producers, hiring and retaining trained personnel were found to be the most significant challenges, while communicating the importance of biosecurity to personnel and enforcing daily biosecurity measures were somewhat less challenging, and keeping shared vehicles cleaned and disinfected was not very challenging. For table egg producers, hiring and retaining trained personnel were also found to be notable challenges, while communicating the importance of biosecurity to personnel, enforcing daily biosecurity measures, and keeping shared vehicles cleaned and disinfected were somewhat less challenging.

In the turkey case-control study, a section of the questionnaire asked about biosecurity investments. The initial results from analyzing this section are summarized in this report and the full report will be available in a peer-reviewed publication. Control farms had statistically significant higher monthly

[1] 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
[2] https://www.ecfr.gov/current/title-9/part-56#p-56.3(b)(1)(ii)
[3] Premises with the [backyard] designation are those that do not meet the CFR definition for [commercial].
[4] Premises designated as WOAH non-poultry are those that do not meet the WOAH definition for poultry.

biosecurity costs than case farms and 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. Logistic regression analyses show that farms making temporary improvements significantly decreased the risk of contracting HPAI, particularly if they introduced temporary wild bird mitigation structures or infrastructure. For permanent investments, older barns that invested in permanent improvements, such as Danish entry, barn ventilation systems, and feed bins, reduced the likelihood of contracting HPAI. Farms with livestock access were less likely to invest in permanent biosecurity and less likely to have ongoing monthly biosecurity expenses, perhaps indicating that farm diversification may signal trends in the livestock market that influence decisions around biosecurity investments.

Various modeling approaches have been used throughout this HPAI outbreak to inform the response and improve 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, which can help to identify potential transmission routes and enhance understanding of the pattern of disease spread. Analysis of data from 69 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 9 days for farms passively reported due to clinical signs). The average adequate contact rate (see Appendix A, Table A1 for more information) across all premises was 5.3 contacts per day (range 0.5–19.8) and was similar across all production types except for broiler premises (2.3 contacts per day, range 0.6–7.5). 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 15 (range 2–62), which would ensure rapid spread through a barn. This work adds additional premises to previous analyses and is generally consistent with earlier findings. It continues to highlight the value of closely monitored production data to quickly identify disease in a flock, while recognizing that there is variation in different production settings.

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. Between 30 December 2021 and 29 February 2024, over 82,000 apparently healthy wild birds were sampled and tested for IAV using real-time reverse transcriptase polymerase chain reaction (rRT-PCR). Overall, targeted surveillance of apparently healthy wild birds and morbidity/mortality investigations of sick or dead birds have resulted in 10,142 detections of H5Nx HPAI lineage virus in at least 166 wild bird species across 49 States and Washington, D.C. For wild birds, H5Nx detections are reported as opposed to H5N1 because there are occasionally reassortants in wild birds that result in other N detections, and because there are a number of HPAI positives where the N-specific assay is not successful.

Further information on the epidemiologic features of this outbreak is available in the two previous epidemiology reports, 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 [commercial] and WOAH poultry [backyard] across the United States, USDA–APHIS–Veterinary Services (VS), APHIS Wildlife Services (WS), and the affected States have initiated epidemiologic, genetic, and wildlife investigations. These investigations help provide a better understanding of factors associated with avian influenza virus transmission and introduction into poultry flocks.

Since the previous interim epidemiological report, these investigations include the addition of the following:

- updated virus phylogenetic analyses
- investigating challenges to implementation of biosecurity practices on turkey farms and table egg farms
- analyzing biosecurity investments related to HPAI risk on commercial meat turkey operations
- using barn-level egg production and mortality records to perform time of introduction analyses
- an updated description of wild bird and mammal surveillance

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 United States since December 2016. It followed ongoing reports of clade 2.3.4.4b H5N1 HPAI in Europe (Freath et al., 2022) that started 27 October 2021 for the migration season. Ancestors of clade 2.3.4.4b have been circulating along Eurasian flyways since 2017 and in Canada (World Animal Health Information System [WAHIS], 2023), starting on 4 November 2021. For Europe, Canada, and the United States, 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 United States (see Phylogenetic Analysis and Diagnostics section for more details).

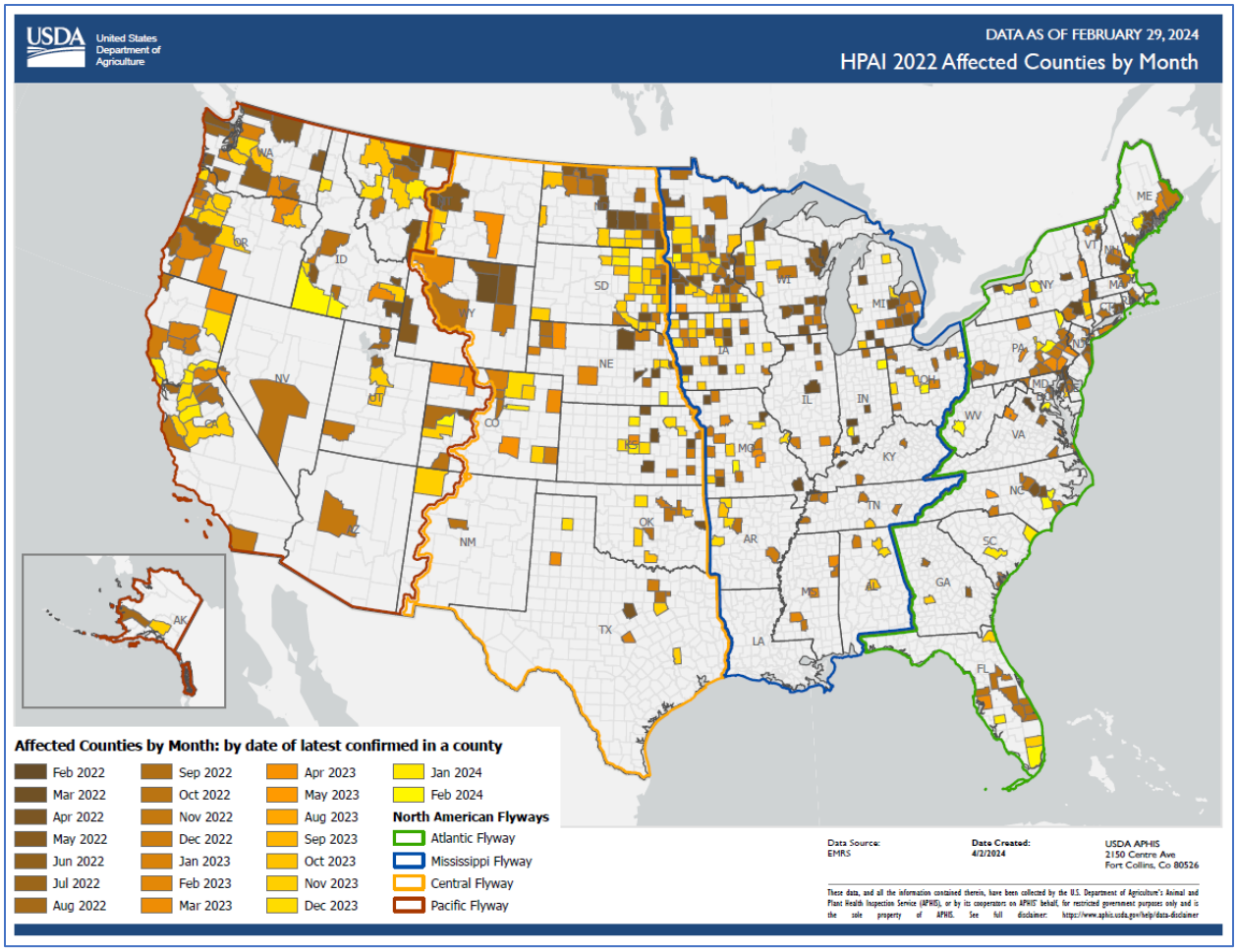


Figure 1. Counties with HPAI detections in poultry by month and by flyway as of 29 February 2024.

The first detection of HPAI in a domestic poultry premises occurred on 7 February 2022 on a commercial meat turkey operation in Dubois County, Indiana, and 14 additional detections occurred later that same month. These cases, confirmed at the NVSL, represented the beginning of seasonal waves of detections in the United States and continued through the drafting of this report (Figure 2). Seasonal detections identified by the NVSL as likely caused by independent wild bird introductions based on phylogenetic analysis were correlated with wild bird migrations (noted in the Analysis of eBird and BirdCast Migration Data: Implications for Disease Introduction, Spread, and Prevention sections of the Epidemiologic and Other Analysis of HPAI-Affected Poultry Flocks in the July 2022 and June 2023 interim reports).

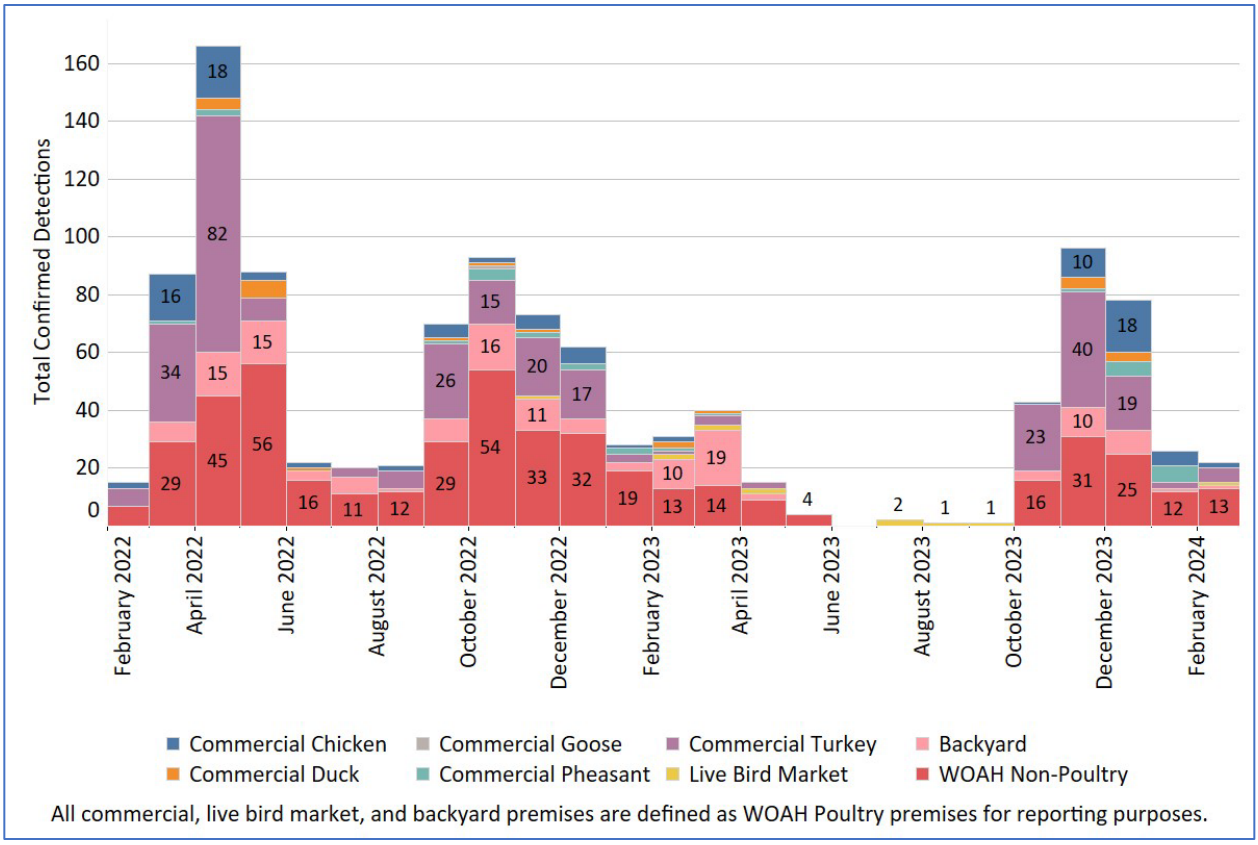


Figure 2. Monthly HPAI detections by premises type as of 29 February 2024.

During March, April, and May of 2022, the NVSL confirmed 342 cases of HPAI. The same months in 2023 confirmed 59 cases, with peaks occurring in April 2022 (166 confirmed cases) and March 2023 (40 confirmed cases). The number of confirmed cases declined sharply over the course of June, July, and August 2022 as the NVSL confirmed 63 cases of HPAI. During those same months in 2023, they confirmed 2 cases of HPAI, with an average of 21 (range: 20–22) cases per month during these months in 2022 and an average of 1 (range: 0–1) case per month during the same time in 2023. During September, October, November, and December of 2022 and 2023, the number of confirmed cases increased with the first confirmed detection in a live bird market in November 2022. For these months in 2022, the NVSL confirmed 298 cases of HPAI, and in 2023, 221 cases, with peaks occurring in October 2022 (93 confirmed cases) and November 2023 (98 confirmed cases). Compared to December 2022 (62 confirmed cases), case numbers dropped in January 2023 (28 confirmed cases) and February 2023 (31 cases) and were even lower in January 2024 (26 cases) and February 2024 (22 cases). Over the course of the outbreak, the Pacific flyway showed increased activity in the summer of 2022 and the Atlantic flyway showed increased activity in the spring of 2023.

Table 1. Confirmed detections of HPAI by production type and WOAH reportable species as of 29 February 2024.

Production Type	Chicken	Turkey	Duck	Pheasant	Goose	Other*
WOAH Poultry						
Commercial Broiler Production	26					
Commercial Broiler Breeder	13					
Commercial Broiler Breeder Pullets	4					
Commercial Table Egg Layer	46					1
Commercial Table Egg Pullets	8					
Commercial Table Egg Breeder	3					
Commercial Turkey Meat Bird		285				1
Commercial Turkey Breeder Hens		18				
Commercial Breeder Operation		4			1	
Commercial Turkey Breeder Replacement Hens		4				
Commercial Turkey Breeder Toms		3				
Commercial Turkey Poult Supplier		1				
Commercial Duck Breeder			14			
Commercial Duck Meat Bird			9			
Commercial Raised for Release Waterfowl			1			
Commercial Raised for Release Upland Game Bird				3		
Commercial Upland Gamebird Producer				25		1
Commercial Breeder (Multiple Bird Species)						1
Live Bird Market	4					8
Backyard	63	2	18	6	2	53
WOAH Non-Poultry	322	2	37	3	13	103
Total	489	319	79	37	16	168

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

As of 29 February 2024, the NVSL confirmed HPAI detections in 48 States. These NVSL-confirmed detections included 472 WOAH poultry [commercial], 144 WOAH poultry [backyard], 480 WOAH non-poultry, and 12 WOAH poultry [live bird market] premises. WOAH poultry [commercial] detections included 316 turkey, 58 table egg, 43 broiler, 23 duck, 30 upland gamebird, 1 goose, and 1 multiple bird species premises (Table 1). Split by wild bird migratory flyways, detections included 212 premises in the Atlantic flyway, 369 in the Mississippi flyway, 263 in the Central flyway, and 264 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), when compared over the course of the entire outbreak. Along the Mississippi and Central flyways, WOAH poultry [commercial] premises accounted for 59.1 percent (218 out of 369) and 51.7 percent (136 out of 263) of detections, respectively. In contrast, WOAH poultry [commercial] premises only accounted for 27.4 percent (58 out of 212) of detections in the Atlantic flyway and 25.8 percent (68 out of 264) of detections in the Pacific flyway.

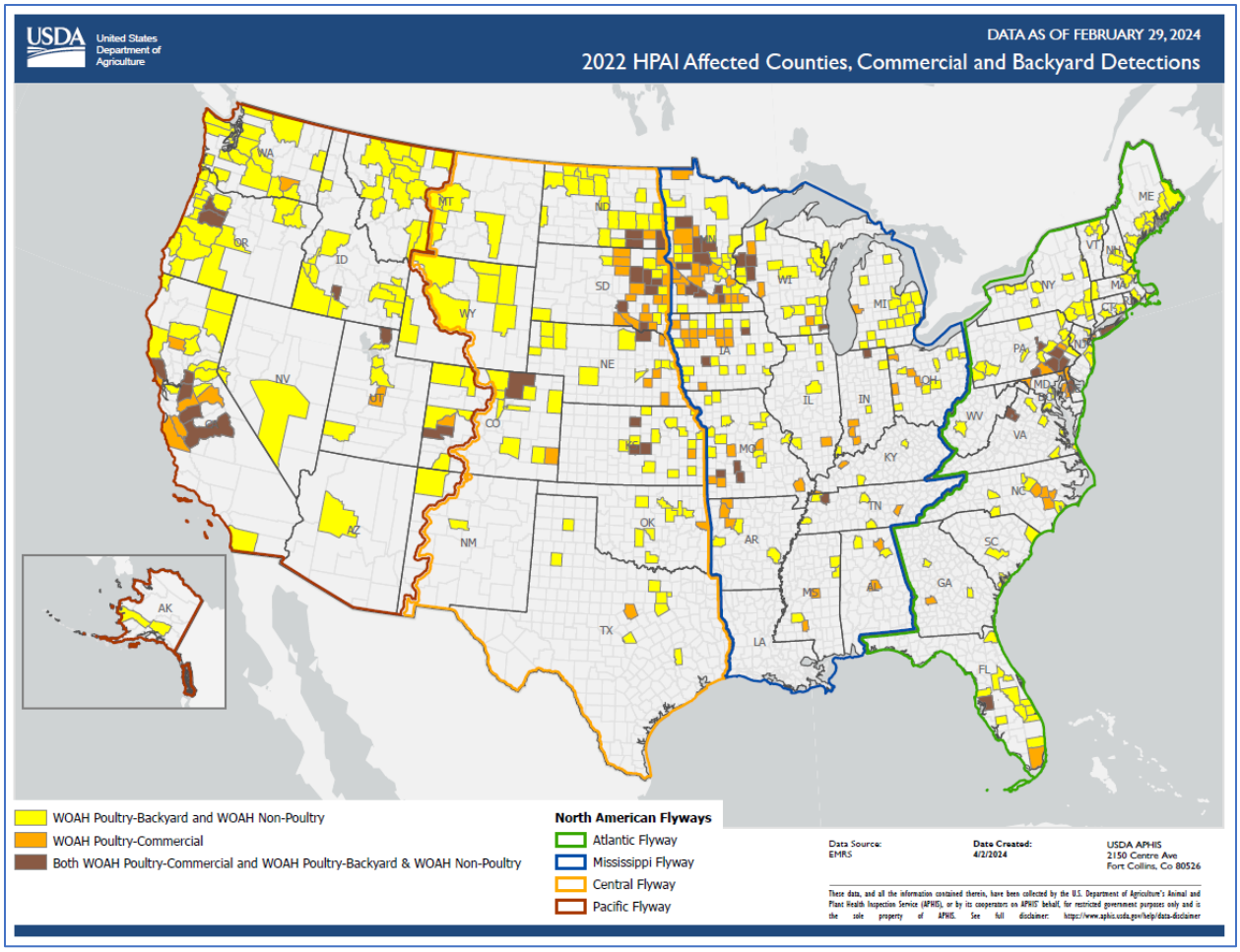


Figure 3. Map of HPAI-affected counties by North American flyway and premises type as of 29 February 2024.

Phylogenetic analysis indicates most detections are the result of independent wild bird introductions. Premises considered 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.

B. Comparison of the 2022–2024 HPAI Outbreak to the 2015 HPAI Outbreak

The primary driver of the 2022–2024 HPAI outbreak has continued to be migratory wild bird movements. The 2015 HPAI outbreak also began with migratory wild bird movements but was more heavily influenced by lateral transmission between farms following virus introduction in the Midwest. During the current HPAI outbreak, at least 82 percent of affected premises have had findings consistent with independent wild bird introductions, seen in the spatial distribution of cases at the county level in Figure 3. Although more counties have been impacted by HPAI in 2022–2024 when compared to 2015, many of these counties represent a 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 the virus. This difference may also reflect that a larger proportion of affected premises are WOAH non-poultry and WOAH poultry [backyard] in 2022–2024 (56.4 percent; 628 out of 1113) than in 2015 (9.1 percent; 21 out of 232), which given their typical activities, may have less opportunity to transmit virus to other premises.

The extended duration and broad geographic range of this outbreak has seriously impacted resource requirements for response. USDA–APHIS personnel began deploying to the first HPAI detection in February 2022 and have continued to deploy through drafting of this report. More than 1,090 USDA–APHIS personnel have been deployed in support of the response, representing over 3,150 deployments as of 29 February 2024.

Figure 4 shows a comparison of the epidemiologic curves and number of birds lost or depopulated due to disease between the two outbreaks. 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 an epidemiologic report at the conclusion of this outbreak.

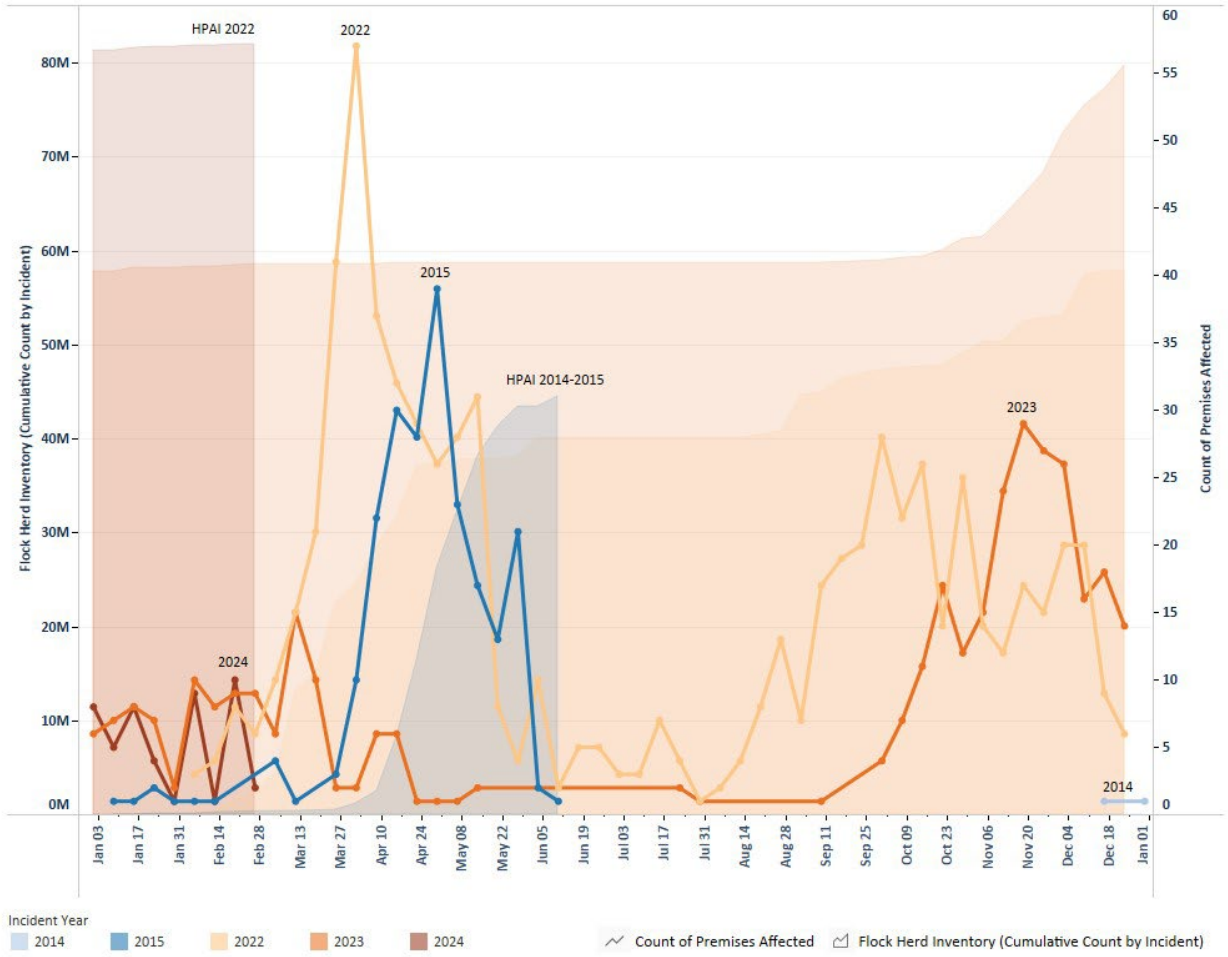


Figure 4. Epidemiological curve of flock/herd inventory (cumulative count by incident year) and count of premises affected by week of confirmed diagnosis date as of 29 February 2024.

PHYLOGENETIC ANALYSIS AND GENOTYPE DISTRIBUTION

Whole genome sequencing for disease tracing and outbreak investigations is routinely required for high consequence diseases. The NVSL-NCAH conducts confirmatory testing, sequencing, and analysis of influenza A viruses from animals. In addition to a robust data pipeline that includes several analytical workflows, the sequences are analyzed by vSNP[5], an accreditation-friendly and robust tool, designed for easy error correction and SNP validation. vSNP rapidly generates annotated SNP tables and corresponding phylogenetic trees that can be easily scaled for reporting purposes. It is able to process large scale datasets, and efficiently accommodates multiple references. This analysis is shared with the field for action as soon as it is available.

Additionally, the GenoFlu tool[6] was developed to classify HPAI H5N1 goose/Guangdong clade 2.3.4.4b viruses detected in North American flyways. This tool considers all eight gene segments and can classify H5 clade 2.3.4.4b viruses that have reassorted with North American low pathogenic viruses. The GenoFlu tool was developed for H5 clade 2.3.4.4.b viruses in North America, utilizing references detected primarily within the United States. The A1 GenoFlu genotype corresponds to the European National Reference Laboratory (EURL) genotype “C,” which is the Eurasian wigeon/Netherlands-like virus that was predominant at the time the A1 virus was initially identified in Newfoundland.

Using GenoFlu, fully Eurasian and distinct introductions of H5 2.3.4.4b virus are denoted by “A” and serial numbering. Genotype A1 Eurasian viruses that have reassorted with North American low pathogenic viruses by their initial introduction are denoted by “B,” and reassortments of the A2 virus introduction are denoted by “C.” To date, other Eurasian introductions (“A” genotypes) into the United States have not been observed to reassort with North American viruses. Reassortants with up to five North American lineage genes have been identified, including genes of the polymerase complex (PA, PB1, PB2), nucleoprotein (NP), and nonstructural gene (NS); only the hemagglutinin (HA), neuraminidase (NA), and matrix (M) genes have not undergone reassortment. The GenoFlu system also ensures that viruses sharing a common lineage can be classified. For example, B3 genotypes include 13 distinct reassortants, denoted B3.1 to B3.13, and each share a common HA/NA phylogeny in addition to shared North American segments. Minor genotypes are assigned serially (and not reused) as novel constellations are identified and may be reassigned with a formal genotype where specified criteria are met. Named genotypes are assigned as they meet the criteria of at least 20 wild bird detections and/or infection of two or more poultry premises. Unusual events, such as an atypical host species, may also prompt establishment of a named genotype.

Distribution of GenoFlu Genotypes

The distribution of the described genotypes over the course of the outbreak through 29 February 2024 is shown in Figure 5 and the genotypes by overall percent, dates of detection, and flyway distribution are shown in Figure 6. Genotype A1 (fully Eurasian [EA]) was first identified in Newfoundland in November 2021 and in wild birds collected December 2021 in the Atlantic flyway. A1 became the predominant unreassorted genotype across all four flyways during 2022, and

5 GitHub - USDA-VS/vSNP: vSNP -- validate SNPs
6 GitHub - USDA-VS/GenoFLU: Influenza data pipeline to automate genotyping assignment

reassortants of A1 with North American (AM) low pathogenic avian influenza viruses created the "B"' genotypes that subsequently predominated this event. Spillover events into poultry have occurred for all major genotypes except A4, A5, and A6.

The first "B" reassortant genotype was collected in late January 2022, and continuing into 2024, detection of several other reassortant genotypes have followed. The B3.2 genotype is a four-gene EA/AM reassortant first detected from samples collected in March 2022 and is the most frequently detected genotype to date in the Americas. By fall of 2022, this genotype had disseminated along flyways into Central and South America, with detections as far south as Antarctica.

Other fully Eurasian genotypes were also introduced into the Atlantic flyway (A2, A5, A6) and the Pacific flyway (A3, A4). Poultry have been affected by genotypes A1, A2, and A3. The A2 genotype was first detected in February 2022 in the northeastern United States and persisted in the Atlantic flyway for much of 2022 and early 2023. This genotype was associated with sea bird and marine mammal mortality events in the northeastern United States. The genotype spread to the Mississippi flyway by the fall of 2023 and reassorted with AM wild bird avian influenza viruses, including a neuraminidase reassortment (H5N6), giving rise to "C" and other minor genotypes.

Genotype B3.3 was first detected in natural hosts in the Mississippi flyway during October 2022. There were only a few detections among wild species, yet in February 2023, this genotype became predominant among northeastern U.S. live bird markets with spillovers into layer flocks by early spring 2023. This genotype was subsequently stamped out in the live bird markets.

Genotype B4.1 is an EA/AM reassortant with AM PB2 and NP genes. This genotype was first detected in the Central flyway in April 2022 and moved westward and southward into the Pacific flyway, including Alaska. In October 2023, the first reassortants with the A2 genotype were detected. Genotype C1.1 was detected in a single poultry premises in Minnesota and genotype C2.1 was first detected in the Atlantic flyway in late October 2023. This genotype has been steadily detected throughout the winter of 2023–2024 and continuing into February 2024.

While genotype B3.2 detections continue sporadically into 2024, the B3 lineage has continued to evolve and reassort. Genotypes B3.5 and B3.6 emerged in November 2022 in the Mississippi and Central flyways, respectively. B3.5 remained primarily in the Central and Mississippi flyways, with several mammalian detections in the northeastern United States during spring 2023, and sporadic detections continued into 2024. Genotype B3.6 emerged in November 2022 and has become the second most common migratory wild bird genotype extending across all four flyways.

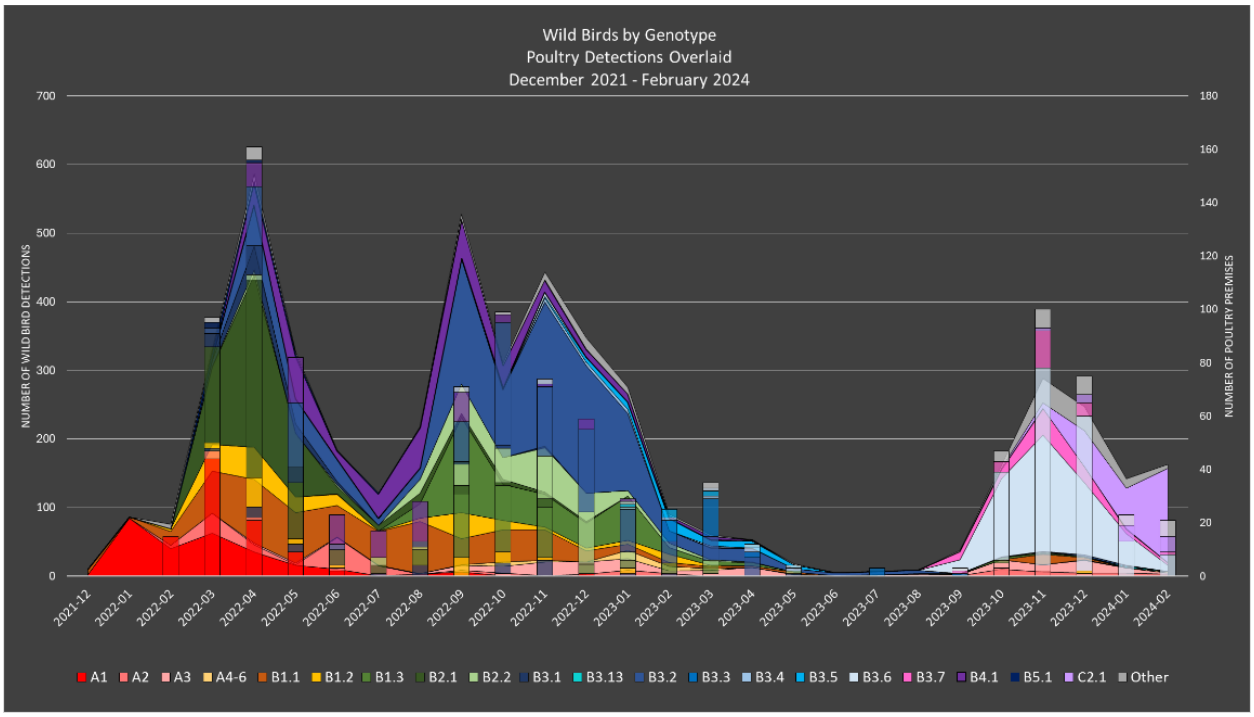


Figure 5. Genotype distribution of wild bird detections from December 2021 to 29 February 2024 in wild birds, with poultry detections overlaid.

Genotype	First detected	Last detected	Flyway distribution (initial detection in bold)	Overall percent (n=6440)
A1	Dec-21	Dec-23	**ATL**>MISS CEN PAC	5.4
B1.1	Jan-22	Dec-23	**ATL**>MISS CEN	9.7
A2	Feb-22	Feb-24	**ATL**>MISS	2.8
B1.2	Feb-22	Dec-23	**ATL**>MISS CEN	4.0
B2.1	Mar-22	Sep-23	**MISS**>CEN PAC	10.2
B2.2	Mar-22	Nov-23	**MISS**>CEN PAC ATL	4.6
B3.1	Mar-22	Nov-23	**MISS**>CEN PAC southern ATL	1.5
B3.2	Mar-22	Jan-24	**MISS**>CEN PAC ATL	20.4
B5.1	Mar-22	Jul-22	**CEN** MISS	0.3
A3	Apr-22	Feb-24	**PAC**	2.9
B4.1	Apr-22	Apr-23	**CEN**>PAC	6.4
B1.3	Jun-22	Dec-23	**ATL**>MISS CEN	7.6
‡A4	Sep-22	Nov-22	**northern PAC**	0.1
‡A5	Oct-22	Mar-23	**northern ATL**	0.2
B3.3	Oct-22	Sep-23	**MISS** CEN*	0.5
B3.4	Nov-22	Apr-23	**MISS**>CEN	0.4
B3.5	Nov-22	May-23	**MISS** CEN>northern ATL	1.3
B3.6	Nov-22	Feb-24	**CEN** MISS>PAC ATL	10.8
‡A6	Jan-23	Feb-24	**northern ATL**	0.2
B3.7	Sep-23	Feb-24	**PAC**>CEN MISS ATL	1.8
B3.10	Oct-23	Jan-24	**northern PAC**	0.1
B3.11	Oct-23	Dec-23	**CEN**	<0.1
C1.1	Oct-23	Oct-23	**MISS**	<0.1
C2.1	Oct-23	Feb-24	**ATL**>MISS CEN	5.1
B3.8	Nov-23	Dec-23	**CEN**	0.1
B3.9	Nov-23	Dec-23	**ATL**>MISS CEN	0.2
B3.12	Nov-23	Feb-24	**CEN** MISS	0.1
Minors	n/a	n/a	n/a	3.1

‡ wild birds only * genotype only detected in LBM flocks during 2023

Figure 6. Migratory bird GenoFlu genotypes by overall percent, dates of detection, and flyway distribution as of 29 February 2024. Includes detections in wild migratory birds, poultry species, and non-dairy mammals; only one sequence per poultry premises was included to represent the premises. For dates, shading is white for oldest and green for more recent dates in the column. Note: This figure excludes genotype B3.13, first detected in the Central flyway and for which only four early viruses are available prior to the event in dairy cattle.

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

A. Turkey and Table Egg Case-Control Publications

The 2022 HPAI turkey and table egg case-control study results were both published in *Frontiers in Veterinary Science*, in the Veterinary Epidemiology and Economics section, as part of the special research topic, Pathogen Transmission at the Domestic-Wildlife Interface: A Growing Challenge that Requires Integrated Solutions.

Green, A. L., Branan, M. A., Fields, V., Patyk, K. A., Kolar, S. K., Beam, A., Marshall, K. L., McGuigan, R. E., Vuolo, M., Freifeld, A., Torchetti, M. K., Lantz, K., & Delgado, A. H. (2023). Investigation of risk factors for introduction of highly pathogenic avian influenza H5N1 virus onto table egg farms in the United States, 2022: a case-control study. *Frontiers in Veterinary Science,* 10. https://doi.org/10.3389/fvets.2023.1229008

This publication describes the 2022 HPAI table egg case-control study carried out by USDA–APHIS to identify potential risk factors for introduction of HPAI virus onto commercial table egg operations. Data were collected from 18 case farms and 22 control farms in 8 States. Univariate and multivariable analyses were conducted to compare farm characteristics, management, and biosecurity factors on case and control farms. Factors associated with increased risk of infection included being in an existing control zone, sightings of wild waterfowl, mowing or bush hogging vegetation less than four times a month, having an off-site method of daily mortality disposal, and wild bird access to feed/feed ingredients at least some of the time. Protective factors included a high level of vehicle washing for trucks and trailers entering the farm, having designated personnel assigned to specific barns, having a farm entrance gate, and requiring a change of clothing for workers entering poultry barns.

Patyk, K. A., Fields, V., Beam, A., Branan, M. A., McGuigan, R., Green, A., Torchetti, M. K., Lantz, K., Freifeld, A., Marshall, K. L., & Delgado, A. H. (2023). Investigation of risk factors for introduction of highly pathogenic avian influenza H5N1 infection among commercial turkey operations in the United States, 2022: a case-control study. *Frontiers in Veterinary Science,* 10. https://doi.org/10.3389/fvets.2023.1229071

This publication describes the 2022 HPAI turkey case-control study carried out by USDA–APHIS to identify potential risk factors for introduction of HPAI virus onto commercial meat turkey operations. Data were collected from 66 case farms and 59 control farms in 12 States. Univariate and multivariable analyses were conducted to compare management and biosecurity factors on case and control farms. Factors associated with increased risk of infection included being in an existing control zone, having both brooders and growers, having toms, seeing wild waterfowl or shorebirds in the closest field, and using rendering for dead bird disposal. Protective factors included having a restroom facility available to crews that visit the farm and workers having access and using a shower at least some of the time when entering a specified barn. These findings provide a better understanding of risk factors for HPAI infection.

Additional info sheets summarizing findings from both studies can be found here: Avian Influenza | Animal and Plant Health Inspection Service (usda.gov) under Resources for Producers.

B. Turkey and Table Egg Case-Control Studies: Challenges in Implementing Biosecurity Practices for Commercial Turkey and Table Egg Producers

APHIS conducted two studies to investigate potential risk factors for introducing the HPAI virus onto farms in 2022 (Green et al., 2023; Patyk et al., 2023). One study focused on commercial turkey farms raising meat turkeys. The other study focused on commercial table egg farms, including breeder, pullet, and table egg layer farms. For the turkey study, 125 farms from 12 States participated. Case turkey farms were confirmed positive for HPAI between January 2022 and October 2022. For the table egg study, 40 farms from 8 States participated. Case table egg farms were confirmed positive for HPAI between February 2022 and September 2022. For both studies, control farms were selected from the same States as case farms, but controls did not have HPAI during the same period.

APHIS previously reported risk factors for HPAI introduction onto commercial turkey and table egg farms, based on the findings of these studies. Additionally, producers provided information on biosecurity challenges related to feed, personnel, and equipment. A published info sheet, *HPAI: Challenges in Implementing Biosecurity Practices for Commercial Turkey and Table Egg Producers*, reported on these challenges.

To better understand industry perspectives on areas of biosecurity that may be difficult to implement, both studies asked participants how challenging it is for producers to achieve various biosecurity goals. Figure 7 and Figure 8 provide a few examples of the combined results for all participating turkey and table egg farms, both cases and controls. Due to small sample sizes in some of the response categories, the "quite" and "extremely" challenging levels were combined in the analysis.

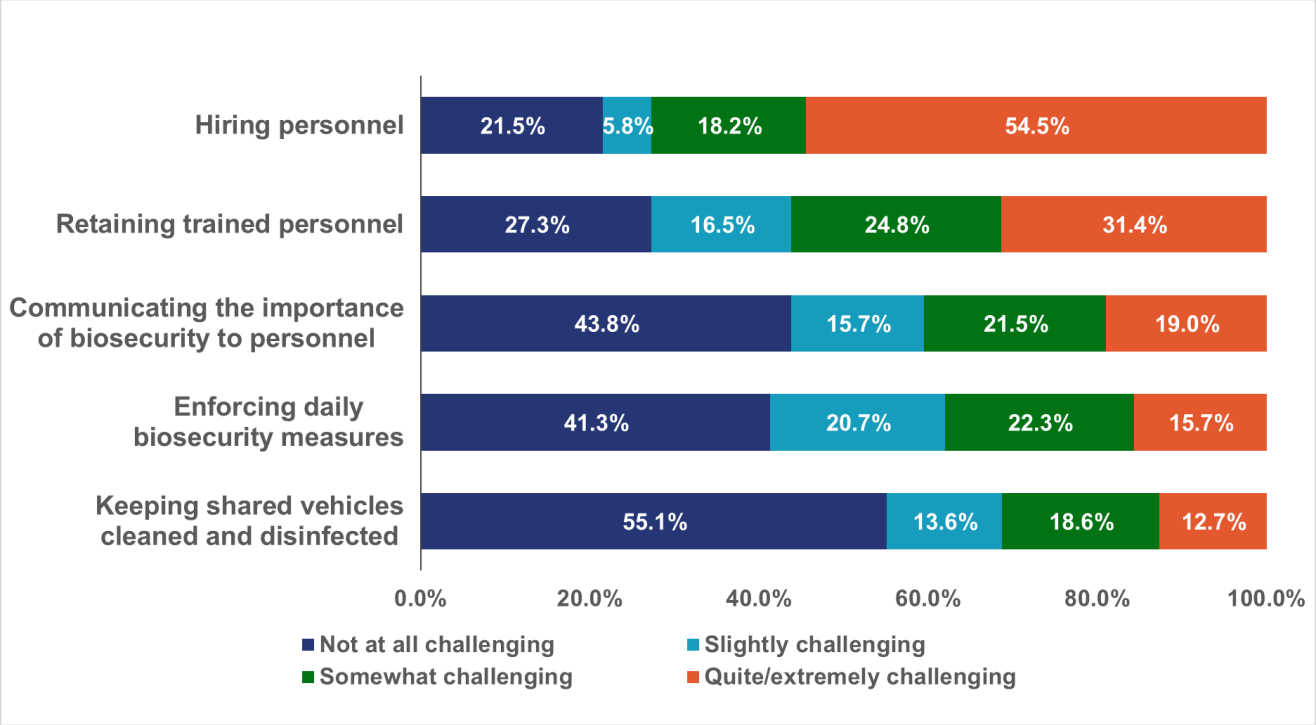


Figure 7. Biosecurity challenges for turkey farms.

Figure 7 shows a few notable opinion results for the turkey case-control study. Hiring and retaining trained personnel were some of the more significant challenges faced by producers. Over half of all respondents (54.5 percent) reported that hiring personnel is quite/extremely challenging. Communicating the importance of biosecurity to personnel and enforcing daily biosecurity measures were at least slightly challenging for almost 60 percent of respondents. Overall, both case and control producers responded that keeping shared vehicles cleaned and disinfected was not at all challenging (55.1 percent).

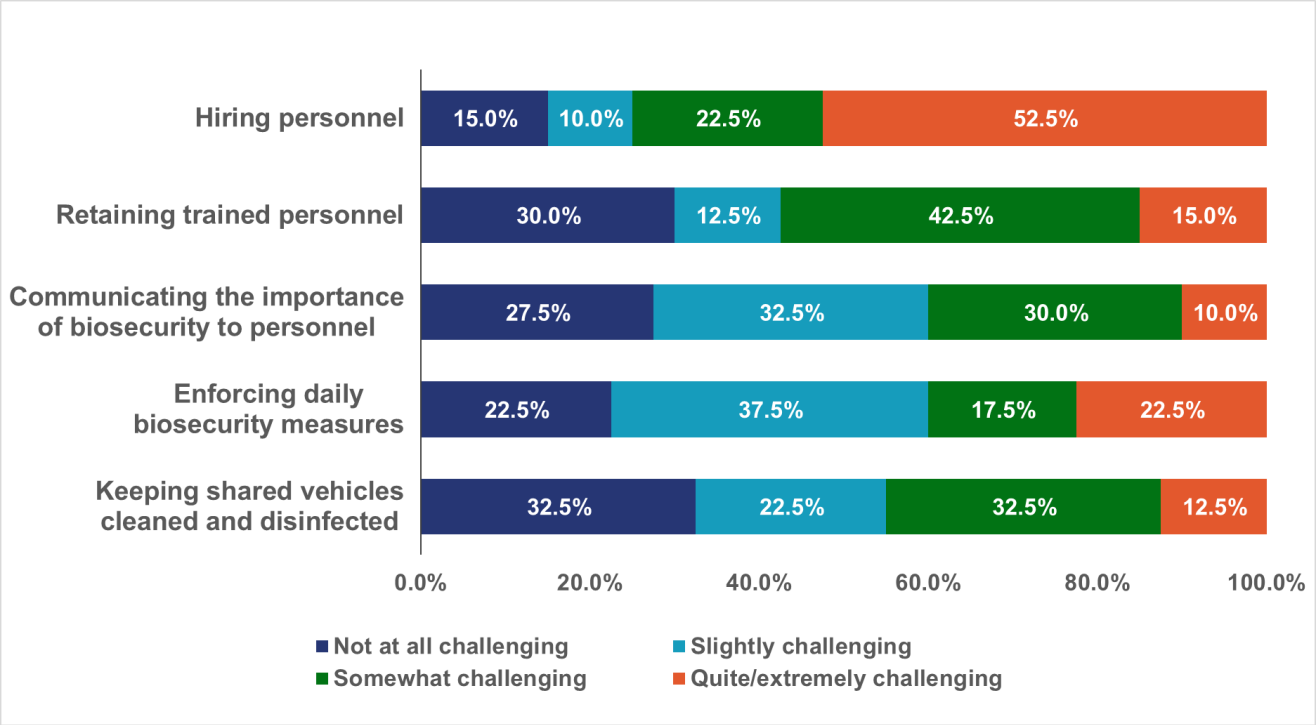


Figure 8. Biosecurity challenges for table egg farms.

Figure 8 shows results for the table egg case-control study, highlighting the same challenge-level categories as Figure 7. Hiring and retaining trained personnel were some of the more notable challenges faced by producers on table egg farms. Over half of all respondents (52.5 percent) reported that hiring personnel is quite/extremely challenging. Communicating the importance of biosecurity to personnel and enforcing daily biosecurity measures was at least slightly challenging for about 75 percent of respondents. Overall, about two-thirds of case and control producers (67.5 percent) responded that keeping shared vehicles cleaned and disinfected was at least slightly challenging.

The full results of the challenge-level questions and a few science-based actions that could be implemented on-farm are found at the end of the info sheet. The results of these studies may also be used by industry to help support and address the most frequently reported challenges that producers identified on their farms.

C. Turkey Case-Control Study Economic Analysis

APHIS conducted a case-control study to identify potential risk factors for introduction of HPAI virus onto commercial meat turkey operations in 2022. Data were collected from 66 case farms and 59 control farms in 12 States. Factors associated with the risk of infection of HPAI were reported in a previous APHIS report and in the published literature (Patyk et al., 2023). 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. Data collected from this section are being analyzed separately, and initial results

are presented here. A full description of the biosecurity investments section of the turkey case-control study, including methods and results, will be available in a peer-reviewed publication.

Results from the turkey case-control study show that there is heterogeneity between case and control farms for on-farm biosecurity and investments. Comparing mean values of data collected for control and case farms, we find that case farms were slightly larger, with a mean barn count of 5.24, compared to 4.47 for control farms ($p < 0.05$). 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 mitigations, temporary air intake inlet covers, or temporary vehicle wash stations.

Logistic regression analyses show that there are heterogenous factors driving investments in biosecurity. Farms making temporary improvements decreased the risk of contracting HPAI significantly (61.7 percent; $p < 0.05$) for investments, including gates, parking, temporary wild bird mitigation, temporary air intake inlet covers, and temporary vehicle wash stations. Specifically, temporary wild bird mitigation structures or infrastructure had the highest statistically significant reduction in HPAI cases with a 70.1 percent ($p < 0.05$) reduction in risk. Given that independent wild bird introductions were the predominant route of introduction of virus onto U.S. turkey farms in 2022 (Youk et al., 2023), infrastructure and structures, even temporary ones, aid in reducing the chances for introduction and farm risk.

For permanent investments, older barns that invested in permanent improvements, including Danish entry, barn ventilations systems, and feed bins, reduced their likelihood of contracting HPAI. Specifically, farms investing in feed bin improvements decreased their likelihood of contracting HPAI by 41.2 percent ($p < 0.1$). Similarly, farms investing in permanent improvements in barn ventilation systems decreased the likelihood of being a case farm by 93.6 percent ($p < 0.01$), however the interaction of barn age was positive (10.3 percent; $p < 0.1$), suggesting as a barn ages, the effectiveness of these improvements diminish. These results may speak to other structural entry points for wild birds or other vectors that cannot be mitigated with new ventilation systems. Conversely, older barns investing in permanent employee wash stations had more positive outcomes when compared to younger barns. Overall, these results indicate the value of permanent investments in mitigating disease risk.

There is also heterogeneity in the farm type making different investments. Hen farms were found to be 18.4 percent ($p < 0.05$) more likely to invest in permanent biosecurity in the previous two years than tom farms. This may reflect a more modernized infrastructure or an inclination for permanent investments over temporary ones for this production type. In contrast, farms with livestock access were 24.1 percent ($p < 0.1$) less likely to invest in permanent biosecurity and 27.6 percent ($p < 0.1$) less likely to have ongoing monthly biosecurity expenses. Farm diversification may signal cyclical trends in the livestock market that may influence investment decisions. This could be an area for future research.

Estimating the Time of H5N1 HPAI Introduction Into WOAH Poultry [Commercial] Flocks Using Diagnostic Test Results and Production Data 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 disease spread pattern. In collaboration with researchers in the Secure Food Systems team at the University of Minnesota,[7] time of introduction analyses were conducted on a subset of premises. These analyses were dependent on the willingness of producers to provide the necessary data. Additionally, those premises initially thought to be involved with lateral spread clusters were prioritized. Although most premises have more than one house of birds, time of introduction analysis was typically performed only 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 69 WOAH poultry [commercial] houses were analyzed, including 40 meat turkey, 8 broiler chicken, 15 table egg layer, 1 table egg layer pullet, 3 duck breeder, and 2 duck meat bird flocks. Detailed modeling methodology can be found in Appendix A: Time of Introduction Modeling Methods.

A. 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 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 (IND) 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 (INR), 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 18 of the analyzed premises were IND, 7 premises were INR, and 43 were CS LT. One premises included in this analysis was unable to be sequenced. For visual purposes, IND and INR introductions were grouped together and reported as independent introductions.

[7] https://securefoodsystems.umn.edu/

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 INDs. One of the IND 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.

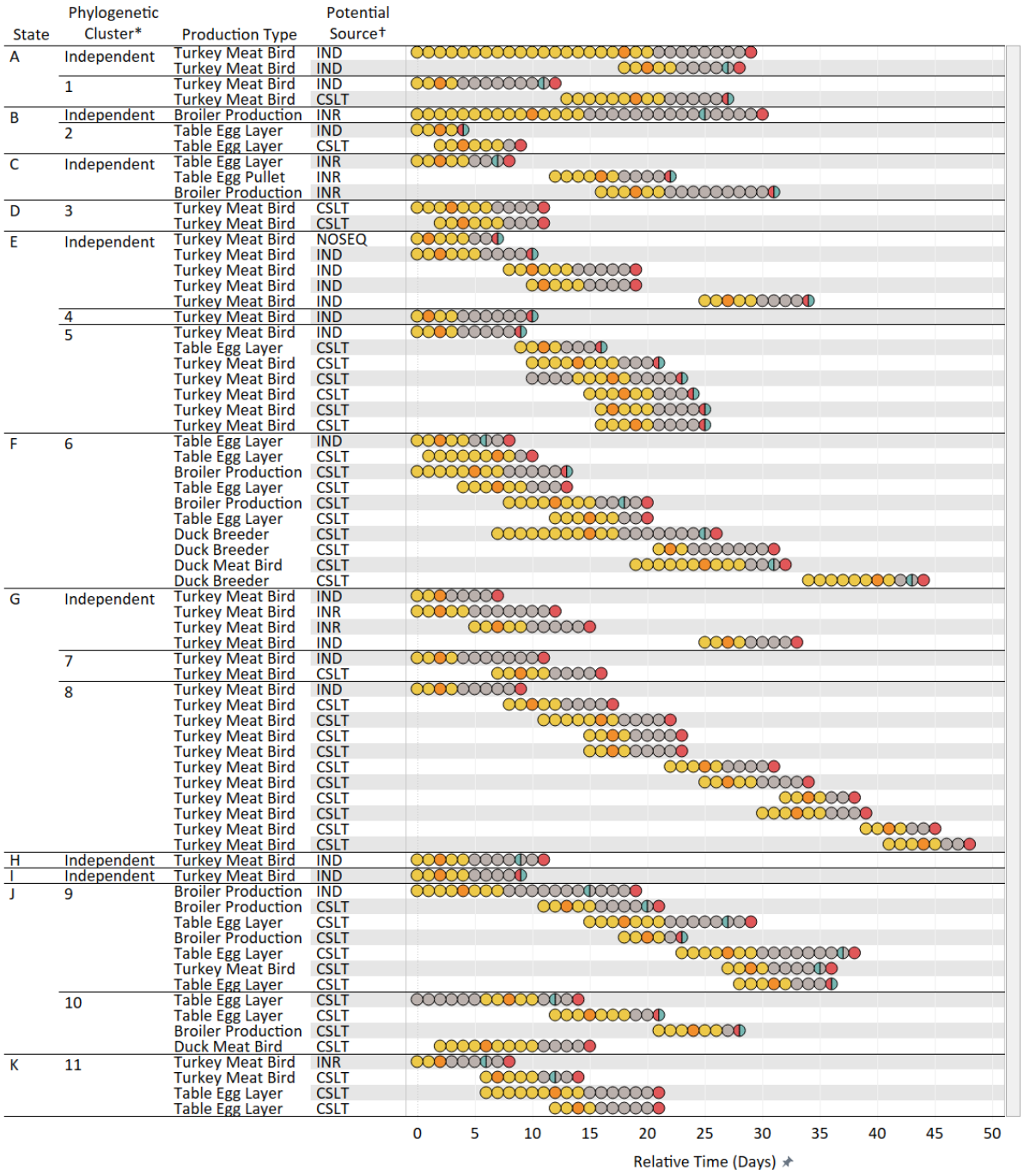
Results are presented for 10 out of 16 premises in a phylogenetic cluster in State F. Time of introduction results for the WOAH poultry [commercial] duck premises should be cautiously interpreted due to limited information to inform modeling priors and the unique structure of WOAH poultry [commercial] 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 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, investigators speculated that rendering was 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, three premises were infected via the movement of live birds. 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.

State J had 11 premises analyzed, and these premises were a part of two regional phylogenetic clusters that contained a total of 17 premises. An in-depth investigation of these premises was conducted in attempt to identify the routes of spread between premises. Within production types, the potential routes of spread identified included shared ownership, shared personnel and equipment, cohabitation of employees, sample collection practices, and movement of nest run eggs. However, within clusters with multiple production types (e.g., table egg layers, broilers, turkey) epidemiological links between the different production types were not identified.

State K had a total of four houses from three premises analyzed that were part of a temporal cluster of six premises; two houses were analyzed for the table egg layer premises presented in Figure 9. The first premises infected in this cluster had phylogenetic similarities with a premises in a separate geographical region in the State (premises not analyzed), though no epidemiological links were identified, and these premises were assumed to be infected via separate wild bird introductions. For the remaining premises analyzed in this cluster, no definitive evidence of epidemiological lateral spread was identified—the only potential epidemiological link identified was shared corporate affiliations.



Time of Introduction‡
- 95% Confidence Interval
- Day of Introduction
- Silent Spread
- Onset of Clinical Signs
- Presumptive Diagnosis

*† Phylogenetic Cluster and Potential Source data are based on supporting genetic evidence which should be interpreted in the context of all available virus information. Relative Time (Days) is adjusted to reflect Day 0 as the suspected outbreak start date for each Phylogenetic Cluster.

‡ Time of Introduction data are kindly provided by the University of Minnesota Secure Poultry Supply modeling team.

Abbreviations:

IND = Independent Wild Bird Introduction
INR = Independent Wild Bird Introduction with Genotypic Similarities
CSLT = Common Source/ Lateral Transmission
NOSEQ = Sequencing Unavailable

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 within-house virus-spread dynamics and how rapidly cases were detected following virus introduction. To assess the timeline of case detection, investigators 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 2 to 11 days. Broiler production premises had the longest TFPS, with a range of 3 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 13 out of 15 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 6 days for premises under ongoing surveillance testing (e.g., testing within a Control Area or Surveillance Zone or for movement permits), and 9 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 6 days for common source or lateral spread introductions and 8 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 and contact tracing activities that occur after a detection.

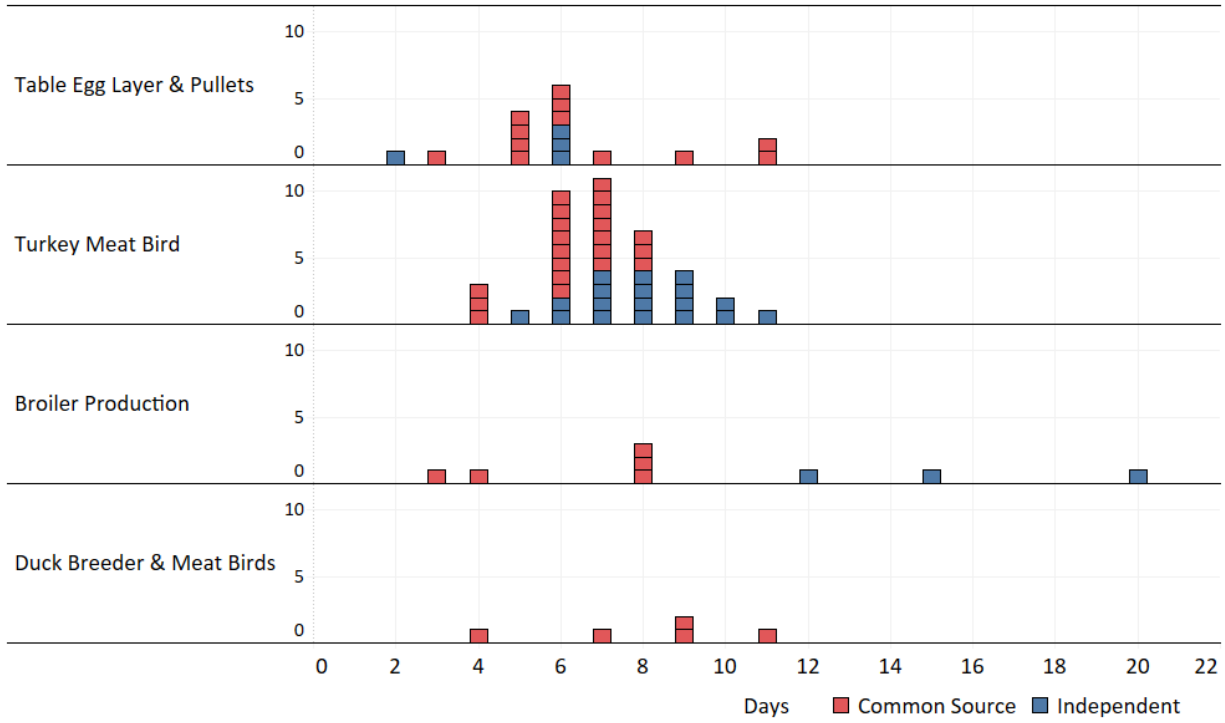
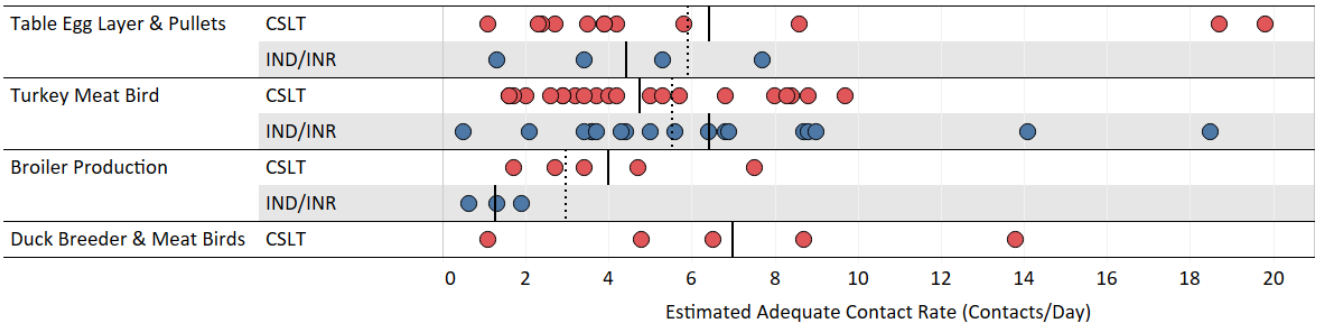


Figure 10. Time from estimated HPAI introduction to detection by production type and likely source of introduction.

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 5.3 contacts per day (range 0.5–19.8; Figure 11). Contact rates were similar across all production types, except for broiler production premises. The mean contact rate was 5.5 contacts per day (range 0.5–18.5) for turkey premises, 5.9 contacts per day (range 1.1–19.8) for table egg layer and pullet premises, 5.3 contacts per day (range 1.1–8.7) for duck premises, and 2.3 contacts per day (range 0.6–7.5) 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; conversely, 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 et al., 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 when comparing them to broilers. When grouped by the source of introduction, the estimated mean contact rate was 5.2 for CS LT and 5.5 for IND/INR.

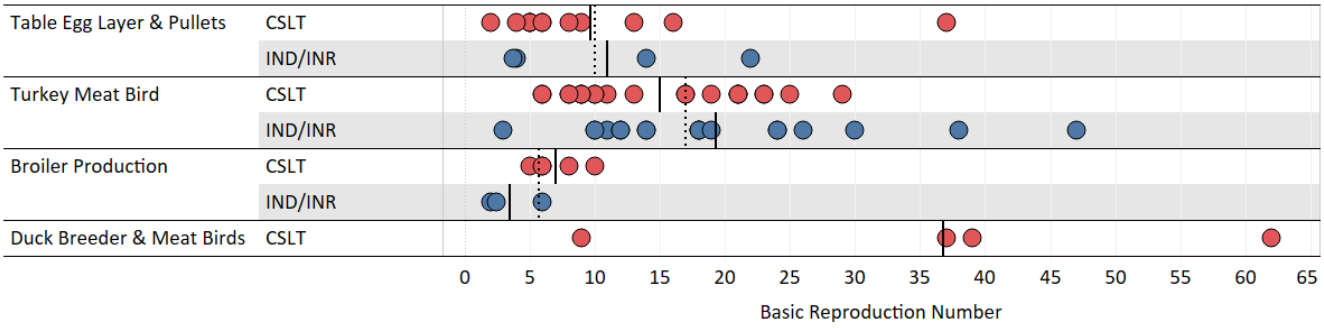


Note:
Solid lines reflect the average value for the production type and source of introduction.
Dashed lines reflect the overall average value for the production type.
Abbreviations:
IND = Independent Wild Bird Introduction
INR = Independent Wild Bird Introduction with Genotypic Similarities
CSLT = Common Source/ Lateral Transmission

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 15 (range 2–62). The mean R0 for turkeys was 17 (range 3–47); for the table egg layer and pullet premises, 10 (range 2–37); for the broilers, 5.7 (range 2.4–10); and for ducks, 36.8 (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 R0 was 14.6 for CS LT and 16.1 for INDI/INR.



Note:
Solid lines reflect the average value for the production type and source of introduction.
Dashed lines reflect the overall average value for the production type.
Abbreviations:
IND = Independent Wild Bird Introduction
INR = Independent Wild Bird Introduction with Genotypic Similarities
CSLT = Common Source/ Lateral Transmission

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

B. 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 69 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-credibility 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 HPAI. Influenza A viruses in wild birds tend to circulate seasonally within migratory flyways, and subtype prevalence can wax/wane in multi-year cycles. Areas where birds from different flyways congregate provide opportunities for viruses to mix across flyways.

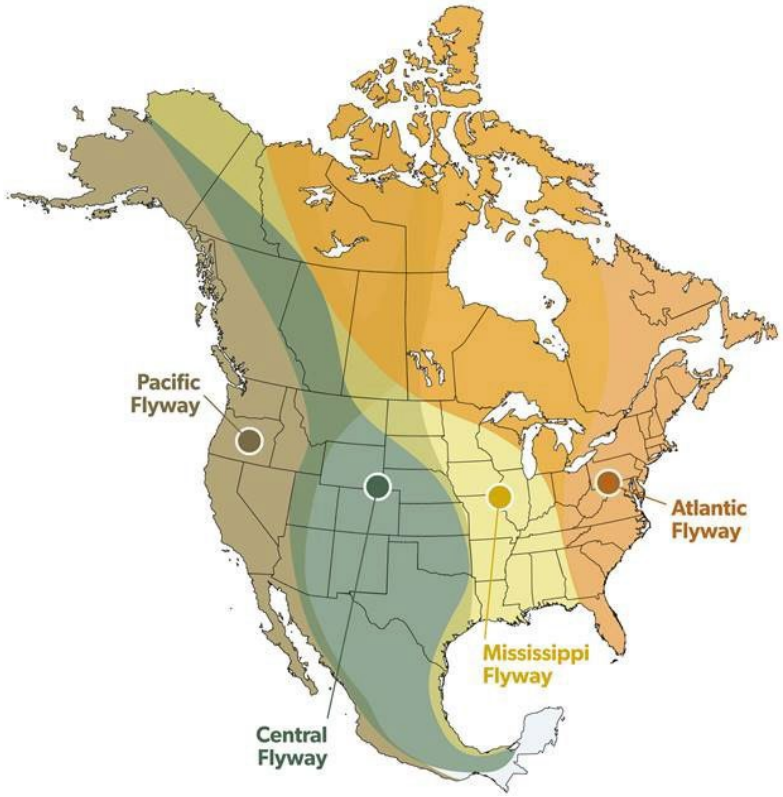


Figure 13. Map depicting the four primary North American waterfowl flyways.[8]

Waterfowl migration in North America generally consists of north-south seasonal movements between breeding grounds and wintering areas, typically following regular routes known as migratory flyways. There are four major flyways in North America: the Pacific, Central, Mississippi, and Atlantic flyways (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. Many migratory bird species use specific flyways during spring and fall, while other species migrate

[8] 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

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 highly pathogenic avian influenza virus in North America occurred in a wild 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 United States was reported in January 2022 in a wild dabbling duck from South Carolina. The bird was exhibiting no neurologic signs and was an apparently healthy bird collected during hunter harvest. The initial spread of EA H5N1 along the Atlantic flyway, as well as the subsequent spread to the other three North American flyways, has been a direct result of wild bird movement.

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 migratory birds. Surveillance helps to

a. understand how HPAI is distributed in the United States,

b. detect the spread of HPAI to new areas of concern,

c. provide a flexible surveillance framework that accounts for changing disease risks through time, and

d. obtain sequence data to better understand HPAI transmission dynamics and spillover risk to domestic poultry.

The surveillance plan focuses on sampling apparently healthy dabbling ducks, which have been identified as the primary reservoir for HPAI and other IAV of concern. While HPAI has been detected in many non-target birds, such as raptors, gulls, and passerines (Appendix B. H5Nx HPAI Wild Bird Detections in the United States), HPAI infections in apparently healthy birds of these avian groups are less common than in dabbling ducks and would require considerably larger sample sizes to obtain robust detection data. Targeting apparently healthy individuals of reservoir host populations maximizes the plan's potential to detect and monitor HPAI, while also providing relevant information on which IAVs are moving throughout the landscape concurrent with wild bird movement.

Between 30 December 2021 and 29 February 2024, over 82,000 apparently healthy wild birds were sampled and tested by rRT-PCR for IAV (Table 2). Wild bird surveillance testing follows the National Animal Health Laboratory Network (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 the NVSL for confirmatory testing. The number of H5Nx HPAI lineage virus detections is based on viruses confirmed at the NVSL from H5 HPAI presumptive samples forwarded by NAHLN laboratories. Overall, targeted wild bird surveillance conducted by USDA–APHIS–WS has resulted in 4,290 detections of H5Nx HPAI lineage virus across the four administrative flyways. H5Nx detections are reported as opposed to H5N1 because there are occasionally reassortants in wild birds that result in other N detections, and because there are a number of HPAI positives where the N-specific assay is not successful.

Table 2. Number of H5Nx HPAI detections from apparently healthy wild birds sampled by USDA–APHIS–WS between 30 December 2021 and 29 February 2024.

Flyway	# Birds Sampled	# H5Nx HPAI Detections
Atlantic	24,148	1,191
Mississippi	24,504	1,175
Central	10,549	741
Pacific	23,094	1,183
Total	82,295	4,290

Prevalence of H5Nx HPAI (i.e., the proportion of individuals testing positive for H5Nx HPAI) among apparently healthy wild birds appears to vary based on the time of year, with prevalence being the highest during the fall months and lowest during the spring and early summer (Figure 14). This pattern has been observed in numerous other studies globally and is likely driven by the large influx of juvenile birds during fall migration that have not yet been exposed to IAV. As juveniles are exposed to IAV over the course of their first year and immunity builds among wild birds, the overall number of infected individuals decreases. Continued monitoring of wild birds will be critical for understanding the spatiotemporal differences in HPAI prevalence and the associated risks to domestic animal populations.

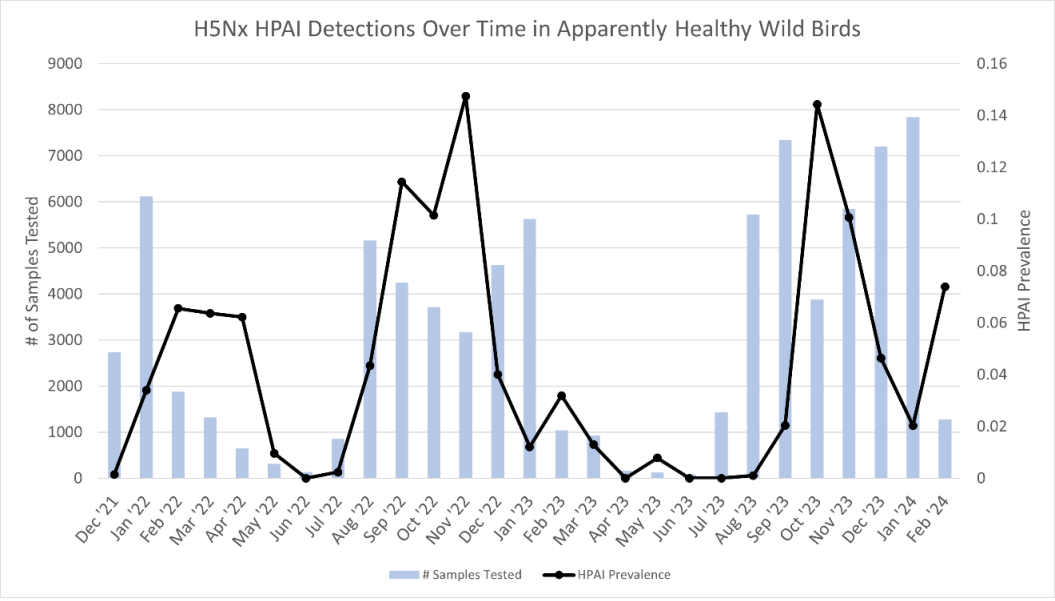


Figure 14. Sampling effort and H5Nx HPAI prevalence in apparently healthy wild birds sampled from December 2021–February 2024 as part of the U.S. National Surveillance Plan for Highly Pathogenic Avian Influenza in Wild Birds. Blue bars represent the total number of wild bird samples collected in a given month, and black dots represent the point prevalence of birds with H5Nx HPAI in a given month.

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 HPAI 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, U.S. Geological Survey's (USGS) National Wildlife Health Center, and universities.

Between 30 December 2021 and 29 February 2024, morbidity/mortality investigations resulted in 5,446 detections of H5Nx HPAI lineage virus in sick or dead birds across all four flyways: Atlantic: 1,551; Mississippi: 1,550; Central: 952; and Pacific: 1,393. Altogether, targeted surveillance samples collected by USDA–APHIS–WS and other agencies, plus morbidity/mortality investigations of sick or dead birds during this time period, have resulted in 10,142 detections of H5Nx HPAI lineage virus in at least 166 wild bird species across 49 States and Washington, D.C. (Figure 15). The true number of wild birds that have been infected with H5Nx HPAI in the United States is assumed to be much higher than the number of confirmed detections, due to the fact that when a large mortality event is observed, only a subset of animals is screened for HPAI. Combined data from the USDA–APHIS–WS database[9] and USGS Wildlife Health Information Sharing Partnership database[10] estimates that at least 33,500 wild birds across the United States have been infected with H5Nx HPAI since the outbreak began in late 2021.

[9] https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/wild-birds
[10] https://whispers.usgs.gov/home

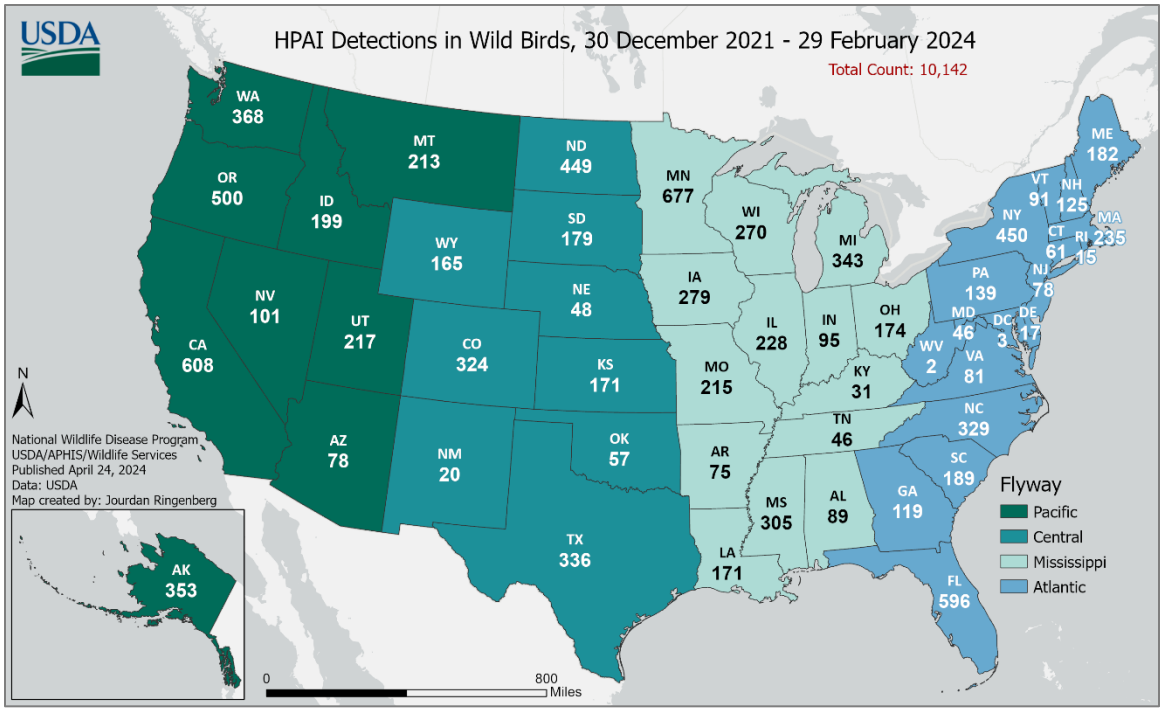


Figure 15. Number of H5Nx HPAI detections in wild bird species tested between 30 December 2021 and 29 February 2024. State totals include detections from both apparently healthy birds from targeted surveillance efforts and sick/dead birds from morbidity/mortality investigations. State totals also include captive wild birds, which are often birds found sick in the wild and submitted to animal rehabilitation centers.

D. HPAI Detections in Mammals

Although IAV primarily affects wild birds and poultry, 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 United States (Figure 16). In some cases, infection may cause illness, including severe disease and death. As of 29 February 2024, there have been 212 H5N1 HPAI detections in at least 20 wild mammal species in 28 States since the start of the H5N1 HPAI outbreak. All the H5N1 HPAI detections in mammals have been from sick or dead animals, and there is currently no active nationwide HPAI surveillance effort in apparently healthy wild mammals.

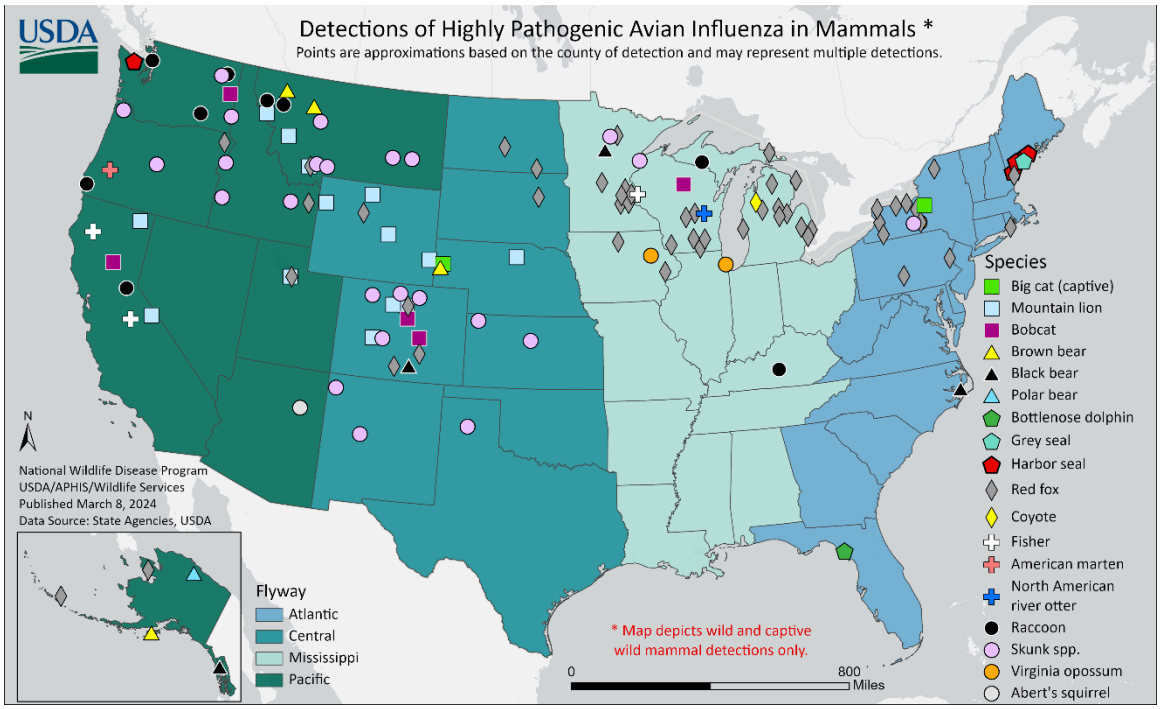


Figure 16. Detections of H5N1 HPAI virus lineage in wild mammals and captive wild mammals in the United States as of 29 February 2024.

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

We also appreciate the diligent efforts of State and Federal responders to collect epidemiologic information while working to control this devastating disease, and their partnership on the resulting analyses.

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

APPENDIX A: TIME OF INTRODUCTION MODELING METHODS

CEAH analysts 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 A1 and Table A2, 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 A1. 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 A2. 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)

APPENDIX B. H5NX HPAI WILD BIRD DETECTIONS IN THE UNITED STATES

Number of H5Nx HPAI detections in apparently healthy and sick/dead wild and captive wild birds in the United States from 30 December 2021 to 29 February 2024.

Species	# HPAI Detections in Apparently Healthy Birds	# HPAI Detections in Sick/Dead Birds
African crowned crane	0	1
American black duck	75	1
American coot	0	4
American crow	0	100
American kestrel	0	5
American robin	0	1
American white pelican	1	112
American wigeon	436*	9
American wood stork	0	2
Arctic tern	0	2
Baer's pochard	0	1
Baikal teal	0	1
Bald eagle	0	559
Barn owl	0	3
Barred owl	0	23
Bird (NOS**)	0	1
Black scoter	0	6
Black skimmer	0	3
Black swan	0	4
Black turnstone	0	2
Black vulture	0	593
Black-bellied plover	0	1
Black-billed magpie	0	3
Blackbird (NOS)	0	2
Black-crowned night heron	0	7
Black-legged kittiwake	0	1
Black-neck swan	0	2
Blue-winged teal	575*	11
Boat-tailed grackle	0	1
Bonaparte's gull	0	2
Brandt's cormorant	0	1
Brant	0	8
Brazilian teal	0	1
Broad-winged hawk	0	2
Brown pelican	0	17
Bufflehead	3	13
Cackling goose	4*	63

California condor	0	22
California gull	0	10
California quail	0	6
Canada goose	17	775
Canvasback	7	1
Caspian tern	0	26
Cattle egret	1	0
Chilean flamingo	0	2
Cinnamon teal	25*	2
Common eider	0	30
Common goldeneye	3	7
Common grackle	0	3
Common loon	0	10
Common merganser	0	3
Common murre	0	18
Common raven	0	50
Common tern	0	11
Cooper's hawk	0	31
Cormorant (NOS)	0	9
Crane (NOS)	0	1
Crested caracara	0	1
Crested screamer	0	2
Crow (NOS)	0	16
Dalmatian pelican	0	1
Dark-eyed junco	0	1
Double-crested cormorant	1*	31
Duck (NOS)	0	15
Dunlin	0	3
Eagle (NOS)	0	4
Eared grebe	0	14
Eastern screech owl	0	4
Egyptian goose	0	1
Eider (NOS)	0	1
Emu	0	1
Ferruginous hawk	1	1
Finch (NOS)	0	1
Fish crow	0	6
Forster's tern	0	1
Fulvous whistling duck	1	1
Gadwall	328*	14
Gannet	0	2
Glaucous gull	0	17
Glaucous-winged gull	0	3

Glossy ibis	0	1
Golden eagle	0	12
Goose (NOS)	0	87
Grackle (NOS)	0	2
Great black-backed gull	0	60
Great blue heron	0	11
Great egret	0	4
Great horned owl	0	360
Greater rhea	0	1
Greater sage grouse	0	1
Greater scaup	3	1
Greater white-fronted goose	2	24
Great-tailed grackle	0	3
Green heron	0	3
Green-winged teal	775*	13
Gull (NOS)	0	29
Harris hawk	0	2
Hawk (NOS)	0	27
Heron (NOS)	0	1
Herring gull	0	53
Hooded merganser	3	32
Horned grebe	0	3
House sparrow	6*	2
King vulture	0	1
Laughing gull	0	1
Lesser flamingo	0	1
Lesser scaup	26*	39
Long-eared owl	0	1
Long-tailed duck	3	0
Lorikeet (NOS)	0	1
Magpie (NOS)	0	14
Mallard	1,511*	95
Mallard/Black duck hybrid	4	0
Merganser (NOS)	0	9
Merlin	0	3
Mottled duck	9	1
Muscovy duck	0	38
Mute swan	1	26
Neotropic cormorant	0	3
Northen pintail	0	1
Northern fulmar	0	2
Northern gannet	0	3
Northern harrier	0	3

Northern pintail	196*	7
Northern shoveler	200*	0
Osprey	0	7
Owl (NOS)	0	14
Pacific loon	0	1
Parasitic jaeger	0	2
Peafowl (NOS)	0	4
Pelican (NOS)	0	31
Peregrine Falcon	0	81
Pheasant (NOS)	0	3
Pied-billed grebe	0	2
Pigeon (NOS)	0	1
Plush-crested jay	0	1
Prairie falcon	0	1
Puna teal	0	1
Razorbill	0	2
Red-breasted goose	0	4
Redhead duck	20	10
Red-necked grebe	0	2
Red-necked phalarope	0	1
Red-shouldered hawk	0	21
Red-tailed hawk	0	379
Red-winged blackbird	0	1
Rhea (NOS)	0	4
Ring necked duck	1	0
Ring-billed gull	0	15
Ring-necked duck	71	9
Ring-necked pheasant	0	4
Roseate spoonbill	0	1
Ross's goose	28	95
Rough-legged hawk	0	8
Royal tern	0	15
Ruddy duck	5	4
Ruddy turnstone	0	2
Ruffed grouse	0	1
Sabine's gull	0	3
Sanderling	0	36
Sandhill crane	0	13
Sandwich tern	0	1
Scaly-sided merganser	0	1
Scarlet ibis	0	1
Sharp-shinned hawk	0	6
Short-billed gull	0	1

Short-eared owl	0	1
Short-tailed shearwater	0	3
Smew	0	1
Snow goose	131*	535
Snowy egret	0	7
Snowy owl	0	20
Snowy plover	0	5
Swainson's hawk	0	8
Swan (NOS)	0	22
Swan goose	0	1
Teal (NOS)	0	1
Thayer's gull	0	1
Tree swallow	0	1
Trumpeter swan	3	40
Tundra swan	1	16
Turkey vulture	0	132
Vulture (NOS)	0	7
Warbler (NOS)	0	1
Western grebe	0	1
Western gull	0	19
Western sandpiper	0	2
Western screech owl	0	2
White-faced ibis	0	5
White-winged scoter	0	1
Wild turkey	0	21
Willet	0	3
Wood duck	216	97
Grand Total	4,696*	5,446

*Includes apparently healthy wild bird samples collected by other agencies that are not participating in the USDA–APHIS–WS targeted surveillance program.

**NOS = not otherwise specified

Epidemiologic and Other Analyses of HPAI-affected Poultry Flocks
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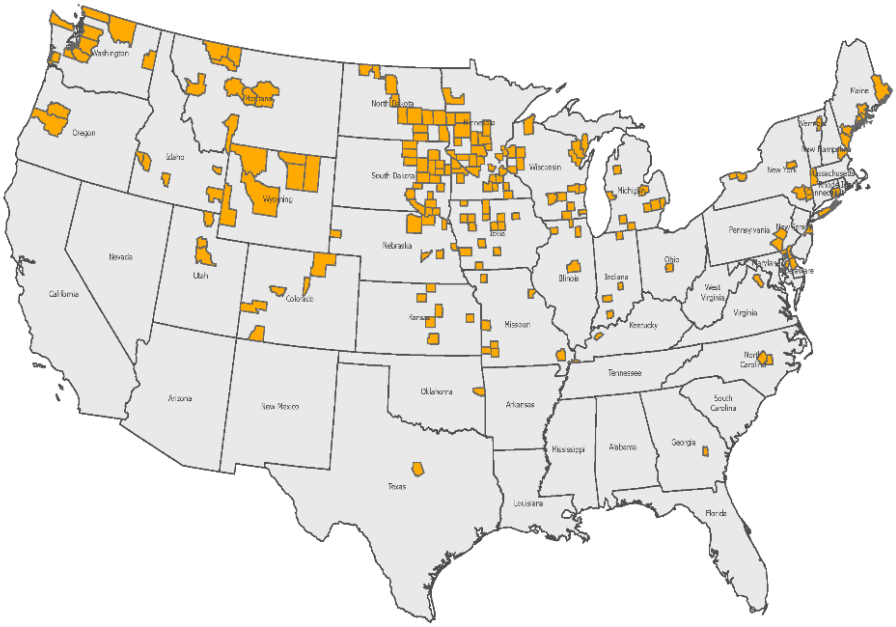

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