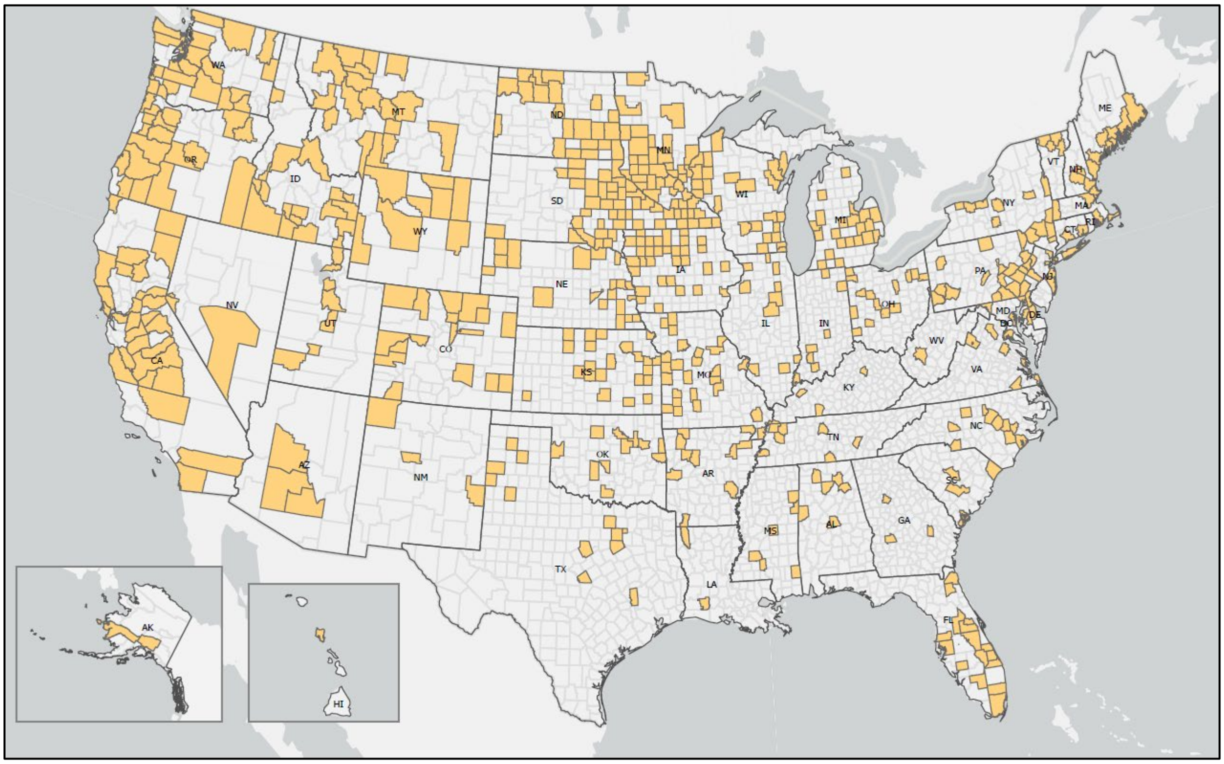
Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Status and Epidemiologic Analyses of HPAI-affected Poultry Flocks December 31, 2024 Interim Report



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For more information, contact:

Sarah Blasko
Acting Director, Center for Epidemiology and Animal Health
USDA–APHIS–Veterinary Services
Email: vs.ceah@usda.gov
Web: https://www.aphis.usda.gov/livestock-poultry-disease/epidemiology

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Email: vs.ceah@usda.gov
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EXECUTIVE SUMMARY

On January 13, 2022, the USDA's Animal and Plant Health Inspection Service (APHIS) identified highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b in a wild bird sample from Colleton County, South Carolina. This detection initiated the largest avian influenza event in U.S. history, involving many wild bird species and virus reassortments, with spillover into poultry, dairy cattle, wild and captive mammals, and outdoor domestic cats. As of December 31, 2024, the National Veterinary Services Laboratories (NVSL; National Centers for Animal Health, Ames IA) confirmed HPAI detections in poultry and other domestic birds in all 50 States, including 623 WOAH (World Organisation for Animal Health) poultry[1] [commercial],[2] 164 WOAH poultry [backyard],[3] 569 WOAH non-poultry,[4] and 20 WOAH poultry [live bird market] premises. This report includes data collected from January 13, 2022, to December 31, 2024.

On March 14, 2024, NVSL confirmed HPAI H5N1 clade 2.3.4.4b genotype B3.13 for the first time in a Texas dairy herd. This ongoing dairy incident is linked to an initial spillover event from migratory birds to dairy milking cows. Once in dairy cattle, analysts determined that the virus spread from farm to farm with subsequent disease spread likely caused by animal movements, shared vehicles and equipment, lack of strict biosecurity practices, and other unknown factors. As of December 31, 2024, the USDA has confirmed genotype B3.13 on 914 dairy cattle premises in 16 States.

Phylogenetic analysis demonstrates that HPAI viruses continue to evolve, and NVSL has identified many different genotypes found in wild bird, mammalian, and poultry detections. Additionally, HPAI has been identified in all four administrative migratory flyways across the United States, indicating that wild birds continue to play a distinct role in disease spread except for genotype B3.13. Genotype B3.13 was first detected in wild birds in October 2023. This genotype was then transmitted onward among dairy cattle, with subsequent spillback into poultry.

Whole genome sequencing and epidemiological data indicate genotype B3.13 spread from dairy premises to poultry premises. A case summary report focusing on the period between October 11, 2024 and December 17, 2024 found that the high density of dairy premises, coupled with high poultry density in the same parts of California, attributed to rapid spread of genotype B3.13 in both dairy and poultry premises. A spatial kernel analytical report of dairy-to-poultry spread of genotype B3.13 in California indicated increased risk of spread from dairy cattle to WOAH poultry [commercial] premises when compared to spread from other WOAH poultry [commercial] premises.

The U.S. National Surveillance Plan for HPAI in Wild Birds was developed to detect avian influenza virus in wild waterfowl. This data informs epidemiologic investigations across the United States. Between December 30, 2021 and December 31, 2024, USDA–APHIS–Wildlife Services staff sampled

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[1] WOAH poultry are defined as "all birds reared or kept in captivity for the production of any commercial animal products or for breeding for this purpose, fighting cocks used for any purpose, and all birds used for restocking supplies of game or for breeding for this purpose, until they are released from captivity."

[2] Commercial premises are defined in 9 C.F.R. 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.

and tested 116,974 apparently healthy wild birds for HPAI, resulting in 5,477 detections of HPAI H5Nx across the four U.S. migratory flyways. The proportion of individuals testing positive for HPAI H5Nx among apparently healthy wild birds appears to be highest during the fall months and lowest during the spring and early summer. Additionally, in this same period, morbidity/mortality investigations resulted in 5,856 detections of HPAI H5Nx in sick or dead birds across all four migratory flyways.

As of December 31, 2024, there have been 412 HPAI H5N1 detections in at least 35 wild or captive wild mammal species in 32 States since the start of the HPAI event on January 13, 2022. The majority of HPAI detections in mammals have been from sick or dead animals, although recent surveillance of peri-domestic wildlife found in and around HPAI-positive farms has resulted in detections of HPAI in apparently healthy wild mammals.

INTRODUCTION

A. Description of HPAI Event in Poultry

USDA–APHIS identified the Eurasian clade 2.3.4.4b H5N1 highly pathogenic avian influenza (HPAI) on January 13, 2022, in a wild bird in Colleton County, South Carolina (APHIS, 2022). This detection was the first Eurasian HPAI H5Nx detection in the United States since December 2016. On February 7, 2022, the National Veterinary Services Laboratories (NVSL; National Centers for Animal Health, Ames, IA) confirmed the first detection of HPAI in a poultry premises on a commercial meat turkey operation in Dubois County, Indiana. As of December 31, 2024, NVSL has confirmed HPAI in all 50 States and in all four U.S. migratory flyways (**Figure 1**). Detections included 229 premises in the Atlantic flyway, 452 in the Mississippi flyway, 327 in the Central flyway, 366 in the Pacific flyway, and 2 in the West Pacific flyway (Hawaii).

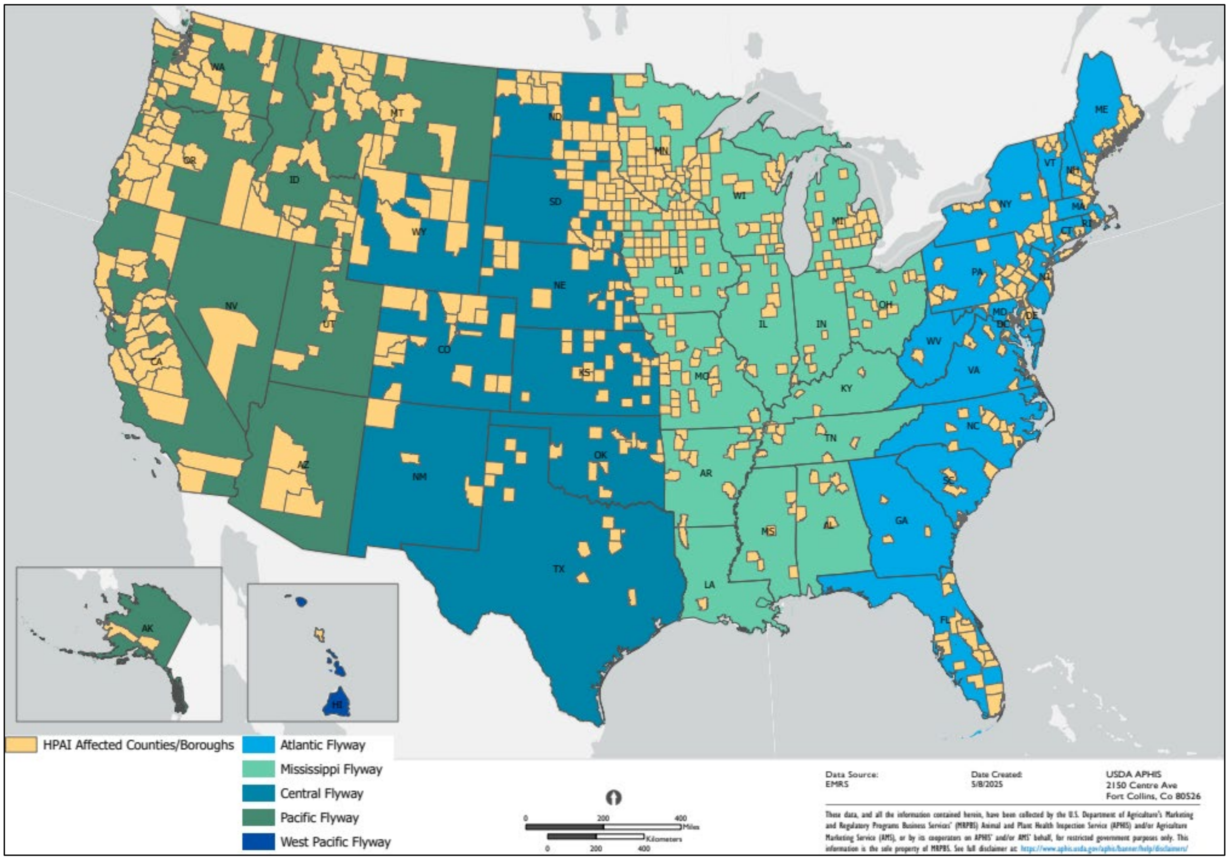

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Figure 1. Counties, boroughs, and parishes with HPAI detections in poultry by migratory flyways, as of December 31, 2024.

Seasonal detections are likely caused by independent wild bird introductions and increase due to the volume of birds transiting an area during migration season. Phylogenetic analyses were correlated with wild bird migrations, as noted in the Analysis of eBird and BirdCast Migration Data: Implications for Disease Introduction, Spread, and Prevention sections of the *Epidemiologic and Other Analyses of HPAI-affected Poultry Flocks* July 2022 and June 2023 interim reports.

HPAI detections in commercial poultry have seasonally fluctuated in the United States since February 8, 2022, through the drafting of this report (**Figure 2**). The epidemiological curves show that the most prominent peaks occur with the fall wild bird migration pattern between September and December. The peak in 2024 lagged in comparison to 2023, due to the late start of the wild bird migration in North America that year. This figure also illustrates that 58,049,481 birds were affected in 2022; 21,976,182 in 2023; and 50,728,638 in 2024; for a total of 130,754,301 birds between 2022 and 2024.

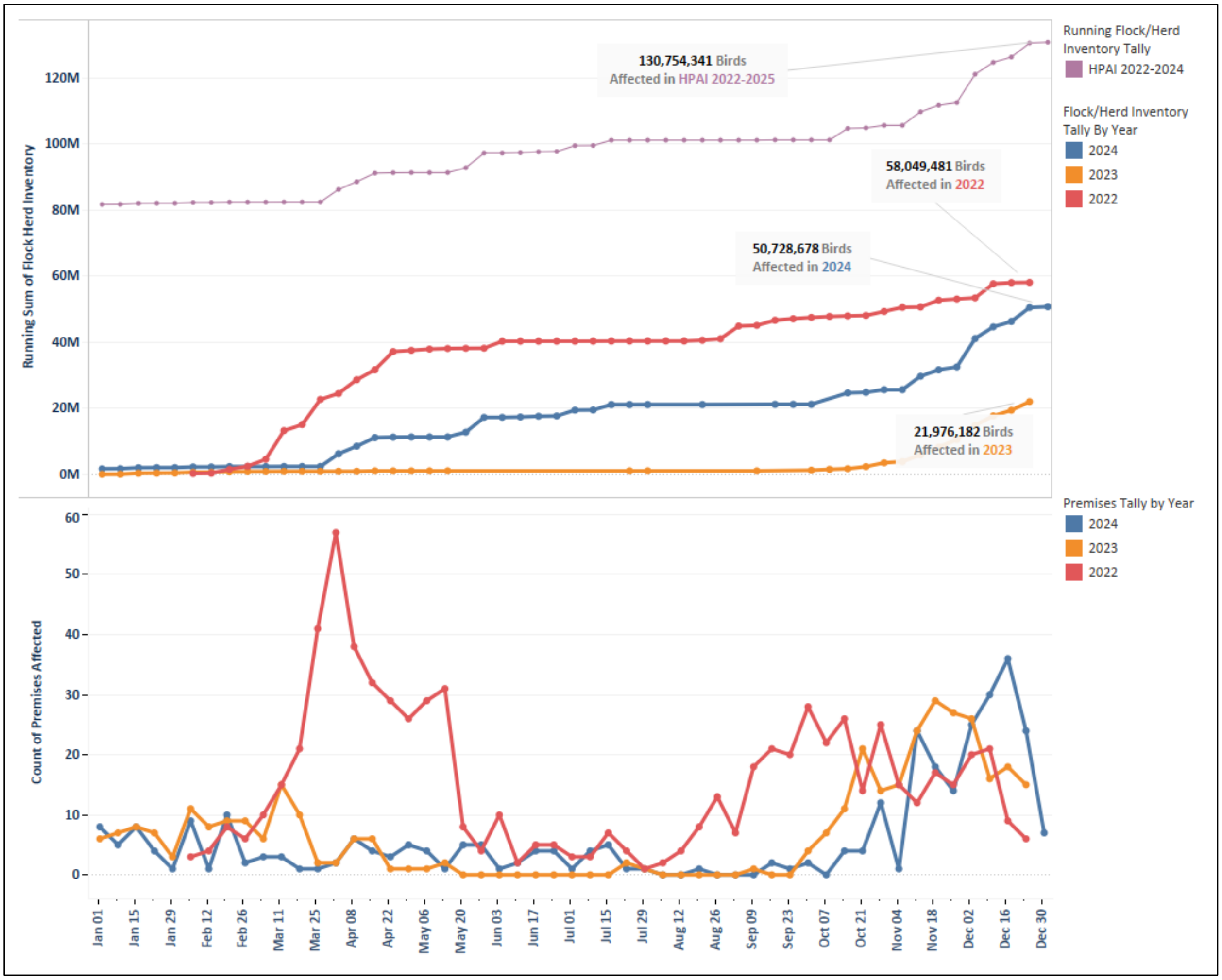
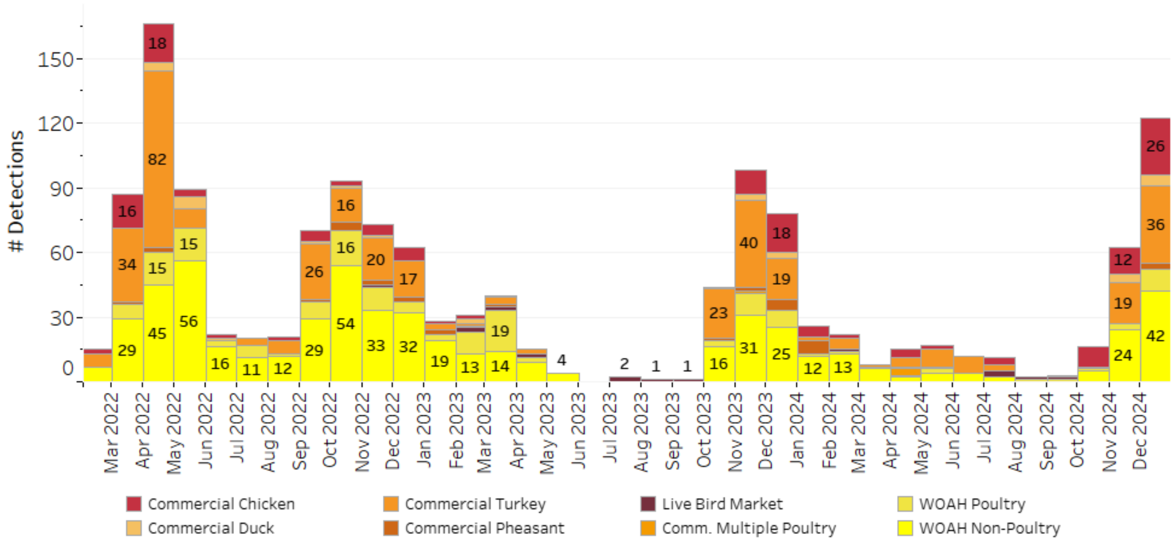


Figure 2. Epidemiological curves of poultry flock/herd inventory, as of December 31, 2024. Cumulative tally by incident year (top) and count of premises affected by week of confirmed diagnosis date (bottom).

Production types vary in health and management practices used to monitor HPAI on-farm, such as changes in water consumption and egg production, mortalities, and movements on and off a premises. Therefore, it is important to understand the trends within each production setting and if certain production types have increased susceptibility. **Figure 3** illustrates an epidemiological curve broken down by commercial and WOAH premises types: commercial chicken, commercial turkey, commercial duck, commercial pheasant, multiple commercial poultry types, WOAH poultry, WOAH non-poultry, and live bird markets.



All commercial, live bird market, and backyard premises are defined as WOAH Poultry premises for reporting purposes.

Figure 3. Monthly HPAI detections by premises type, as of December 31, 2024.

As of December 31, 2024, the NVSL confirmed HPAI detections in all 50 States (**Table 1**). These NVSL-confirmed detections included 623 WOAH poultry [commercial], 166 WOAH poultry [backyard], 569 WOAH non-poultry [backyard], and 20 WOAH poultry [live bird market] premises. WOAH poultry [commercial] detections included 394 turkey, 88 table egg, 70 broiler, 33 duck, 31 pheasant, 1 goose, and 5 multiple bird species premises.

The contribution of WOAH poultry [commercial] premises to the total number of detections was higher for the inland flyways than coastal flyways (**Figure 4**), when compared over the course of the entire event. Along the Mississippi and Central flyways, WOAH poultry [commercial] premises accounted for 59.7 percent (270 out of 452) and 51.1 percent (167 out of 327) of detections, respectively. In contrast, WOAH poultry [commercial] premises only accounted for 24.9 percent (57 out of 229) of detections in the Atlantic flyway and 35.2 percent (129 out of 366) of detections in the Pacific flyway.

Table 1. Confirmed detections of HPAI by production type and WOAH reportable species, as of December 31, 2024.

Production Type	Chicken	Turkey	Duck	Pheasant	Goose	Other*
WOAH Poultry						
Commercial Breeder Operation	3	5			1	1
Commercial Broiler Production	47					1
Commercial Broiler Breeder	16					
Commercial Broiler Breeder Pullets	4					
Commercial Table Egg Layer	74					1
Commercial Table Egg Pullets	11					
Commercial Table Egg Breeder	3					
Commercial Turkey Meat Bird		354				2
Commercial Turkey Breeder Hens		26				
Commercial Turkey Breeder Replacement Hens		4				
Commercial Turkey Breeder Toms		4				
Commercial Turkey Poult Supplier		1				
Commercial Duck Breeder			18			
Commercial Duck Meat Bird			14			
Commercial Raised for Release			1	5		
Commercial Upland Gamebird Producer				26		1
Live Bird Market	11					9
Backyard	74	3	18	8	2	61
WOAH Non-Poultry [Backyard]	373	3	41	3	17	132
Total	616	400	92	42	20	208

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

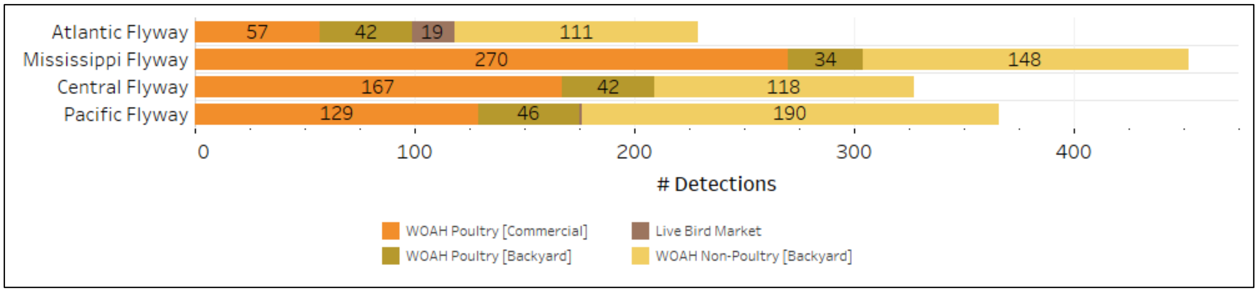


Figure 4. HPAI detections by premises type in each migratory flyway, as of December 31, 2024.

Phylogenetic analysis indicates most HPAI detections are the result of independent wild bird introductions (except for genotype B3.13); however, spillover from dairies (genotype B3.13) and lateral spread between poultry premises have also occurred. 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 (**Figure 5**).

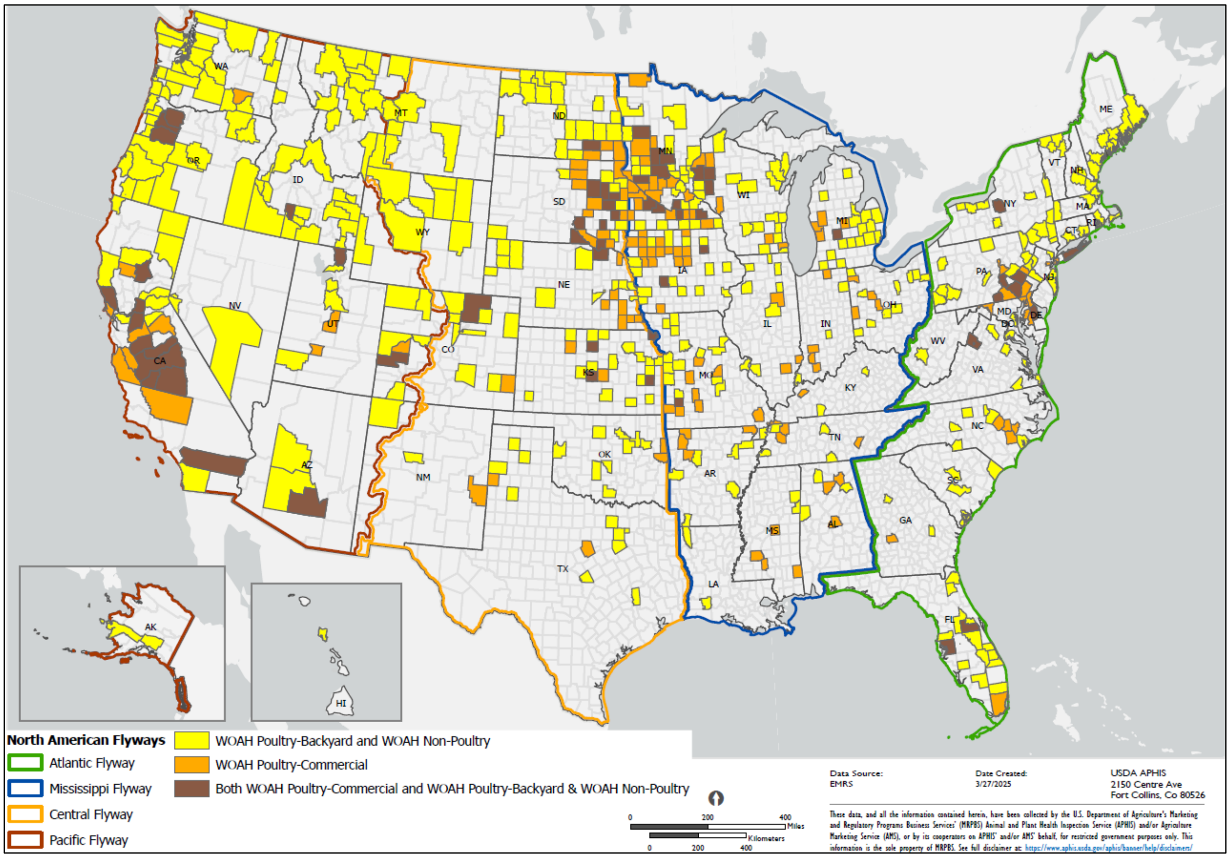


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Figure 5. Counties, boroughs, and parishes with HPAI detections in poultry by migratory flyway and premises type, as of December 31, 2024.

B. Description of HPAI Event in Dairy Cattle

USDA–APHIS announced the first detection of HPAI in domestic livestock on March 25, 2024. Oropharyngeal swabs collected from sick dairy cattle and samples of unpasteurized milk from a farm in Texas tested positive for the virus, followed by detections in Kansas on March 26, 2024. The NVSL identified HPAI H5N1 clade 2.3.4.4b genotype B3.13 in the samples. Whole genome sequencing and epidemiological data indicated the first detection in livestock was linked to an initial spillover event from migratory birds to dairy cattle. The disease then moved between dairies, and in some instances, from dairies to poultry premises. As of December 31, 2024, genotype B3.13 affected a total of 914 dairies in 16 States, with no evidence of additional spillover events from migratory birds into cattle (**Figure 6**).

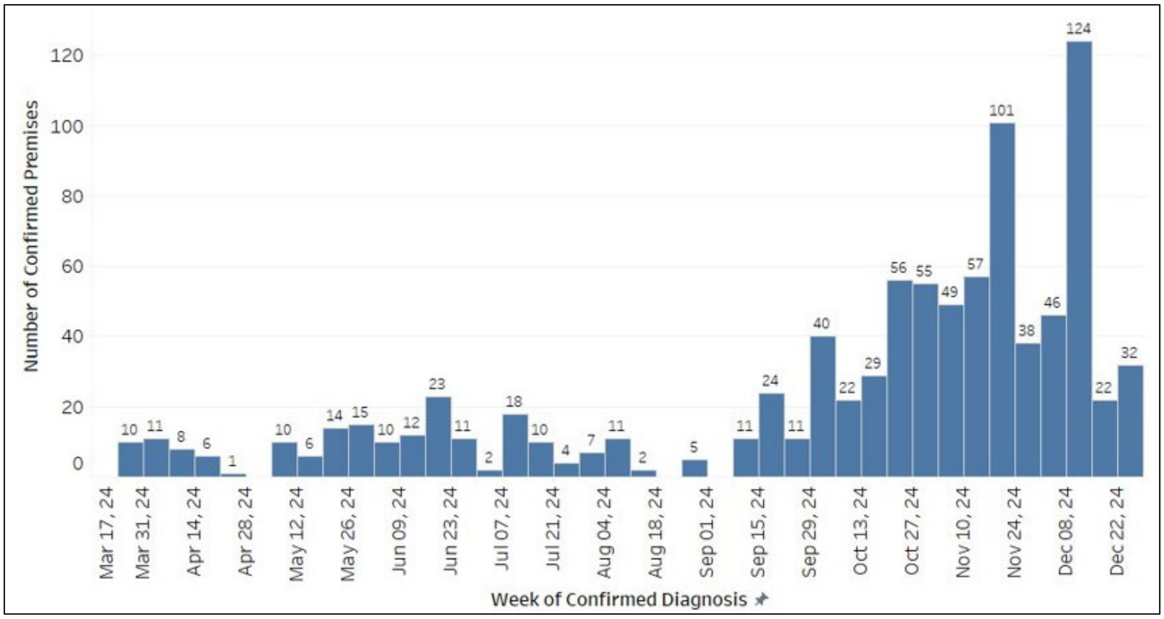


Figure 6. Epidemiological curve of confirmed positive dairies with genotype B3.13 from March 25, 2024, to December 31, 2024.

Between March 25, 2024, and December 31, 2024, genotype B3.13 presumptive positive cases were found in 17 States (**Figure 7**). In nine States, both dairies and poultry premises were impacted (counties shown in blue); six States had counties with only dairies impacted by genotype B3.13, and not poultry premises (counties shown in orange); and six States had counties with only domestic poultry premises impacted (counties shown in yellow). The USDA–APHIS Detections of HPAI in Livestock webpage provides up-to-date information for this event.

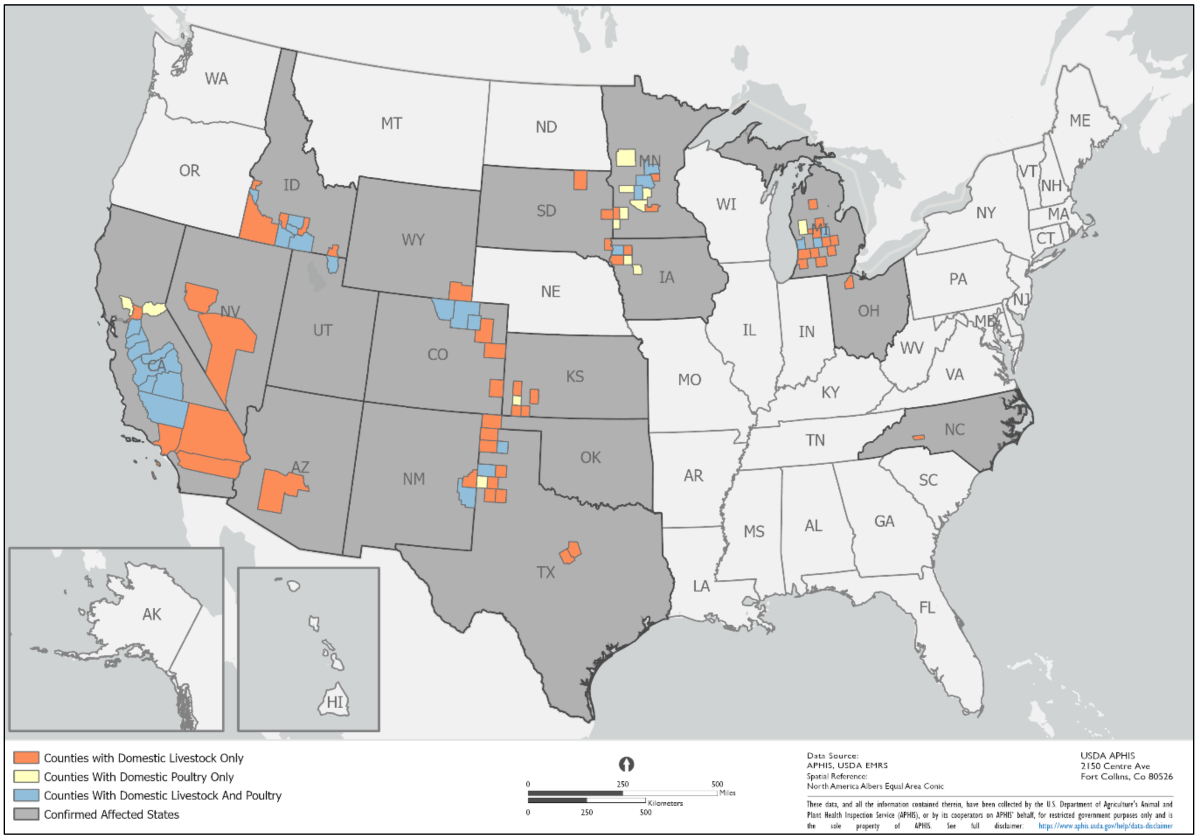


Figure 7. Genotype B3.13-positive detections in States (dark grey) with associated counties highlighted by premises type (domestic livestock only, orange; poultry only, yellow; domestic livestock and poultry, blue).

C. Scope of December 31, 2024 Poultry Status Report

In response to the HPAI H5 clade 2.3.4.4b detections in WOAH poultry [commercial] and WOAH poultry [backyard] across the United States, USDA–APHIS–Veterinary Services, Wildlife Services, and the affected States have initiated epidemiologic, genetic, and wildlife investigations. These investigations help provide a better understanding of factors associated with HPAI transmission and introduction into poultry flocks.

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

- Updated virus phylogenetic analyses
- Case summaries of poultry infected with genotype B3.13 in California
- Spatial kernel analysis of genotype B3.13 spread from dairy cattle to poultry farms in California
- Updated descriptions of wild bird and mammal surveillance

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

PHYLOGENETIC OVERVIEW

A. Background

Whole genome sequencing of viruses provides information on the relationship between the viruses in different animals and on different farms, providing context and support to guide epidemiologists in their investigations. The NVSL sequences IAVs from a variety of sources, including wild birds and mammals, poultry, and dairy cattle. Analysis of viral sequences allow staff to identify viruses that are more closely related and may have been caused by movement within domestic animal populations, versus those that are most likely spillover events stemming from wildlife sources. The data pipeline for influenza A includes bioinformatics tools, such as reference guided assembly of genome sequences using IRMA v1.1.4, GenoFLU, vSNP, and evaluation for potential mutations of interest. All high quality raw genetic sequences are uploaded to the National Center for Biotechnology Information (NCBI) Sequence Read Archive, with consensus genomes assembled and submitted to NCBI GenBank, as appropriate.

B. Distribution of GenoFLU Genotypes

GenoFLU, developed by the NVSL for North America and using references detected primarily within the United States, identifies and tracks introductions of HPAI H5Nx goose/Guangdong clade 2.3.4.4b viruses into North America and their further re-assortments with North American wild bird IAVs. There have been six distinct introductions of Eurasian virus in North America during the current event.

Using GenoFLU, fully Eurasian and distinct introductions of H5Nx clade 2.3.4.4b virus are denoted by serially numbered genotypes starting with “A.” Reassortments of genotype A1 viruses with North American low pathogenic viruses by their initial introduction are denoted by genotypes starting with “B,” with serial numbering of independent reassortment events. Reassortments of A2 viruses with North American low pathogenic viruses are similarly denoted by genotypes starting with “C,” and reassortments of A3 viruses with North American low pathogenic viruses are denoted by genotypes starting with “D.”

Prior to March 2024, the genotype distribution of HPAI detections in poultry closely followed that of wild bird detections (**Figure 8**). Following an initial spillover event of genotype B3.13 from wildlife to dairy cattle in late fall 2023 with spread and detection in March 2024, dairy-to-poultry spillover was established as a new route of virus introduction with poultry detections of genotype B3.13 closely mirroring the level of virus present in dairy cattle. In addition, rare wild bird detections of genotype B3.13 following this initial spillover have been associated with peri-domestic species and predominantly targeted peri-domestic sampling of affected premises by USDA—APHIS—Wildlife Services.

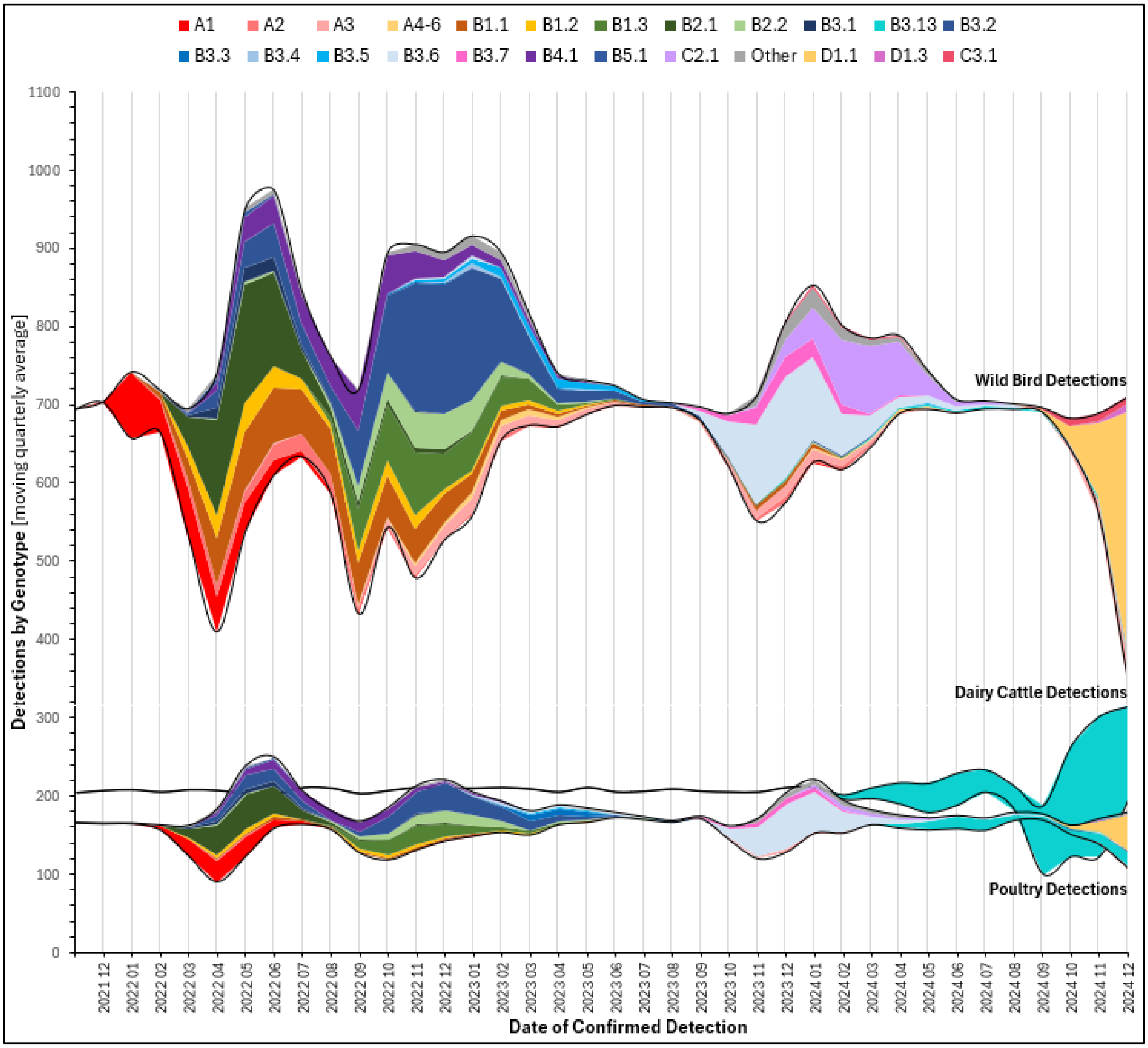


Figure 8. Genotype distribution of wild bird (top), dairy cattle (middle), and poultry detections (bottom), represented as moving quarterly averages normalized to detections in each animal category, as of December 31, 2024.

In contrast to genotypes A1 and A2, which were most likely introduced via the Atlantic flyway, genotype A3 was most likely introduced via the Pacific flyway and was first detected in Alaska early in 2022. Circulation of genotype A3 remained limited to the Pacific flyway until late fall and winter of 2024, when A3 and its associated North American low pathogenic virus reassortants ("D" genotypes) started to move eastward across all four migratory flyways (**Figure 9**). In particular, the D1.1 genotype, which was first detected in September 2024, has affected wild birds, poultry, and wild mammals across the four migratory flyways (**Table 2**).

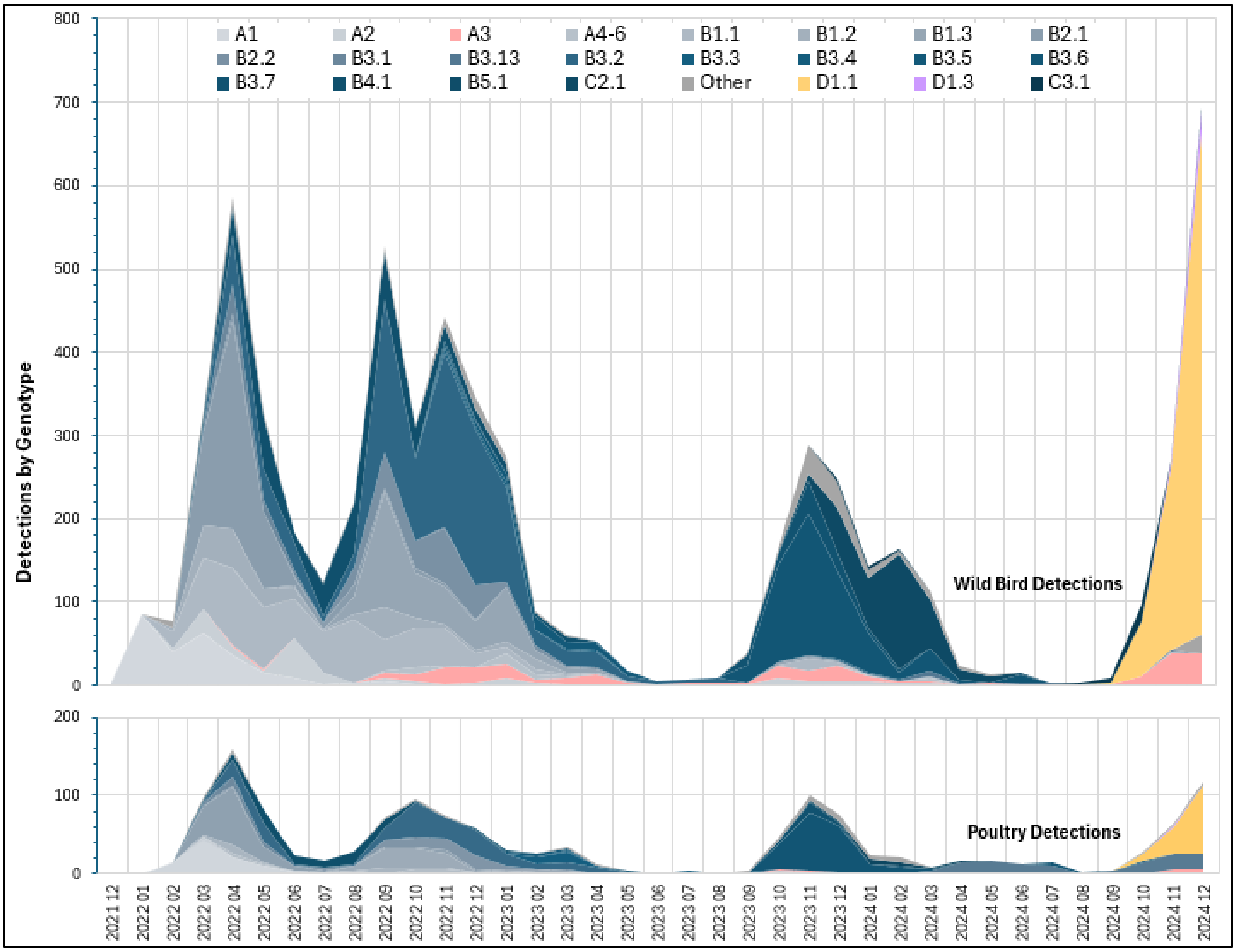
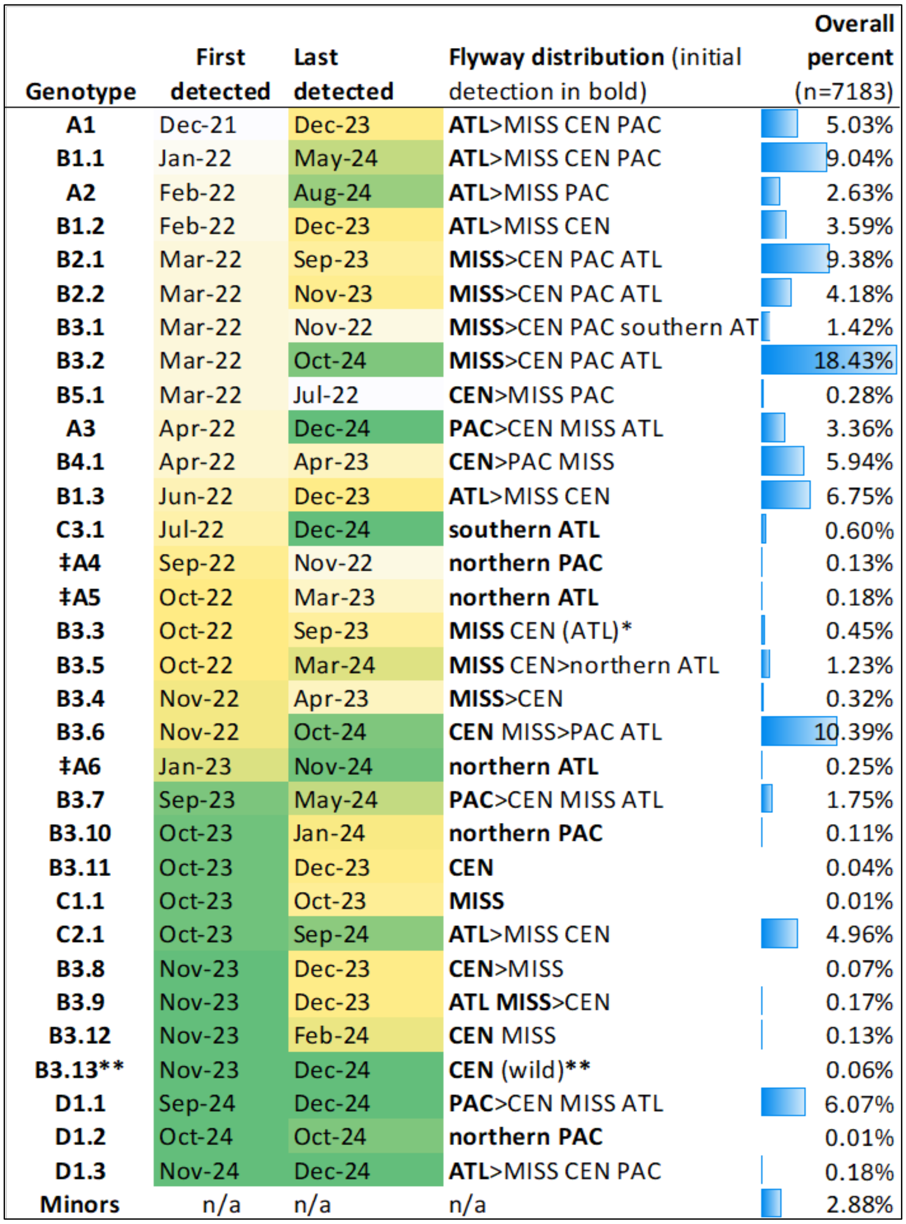


Figure 9. Genotype distribution of wild bird (top) and poultry (bottom) detections of A3 and associated North American low pathogenic virus reassortants, or "D" genotypes.

Table 2. GenoFlu genotype detections by dates of first and last detections, migratory flyway distribution, and overall percent, as of December 31, 2024. The white-yellow-green fill gradient in each date column represents oldest (white) and newest (green) dates detected. Blue fill bars represent the overall percentage of detections of each genotype.



Genotype	**First detected**	**Last detected**	**Flyway distribution** (initial detection in bold)	**Overall percent** (n=7183)
A1	Dec-21	Dec-23	**ATL**>MISS CEN PAC	5.03%
B1.1	Jan-22	May-24	**ATL**>MISS CEN PAC	9.04%
A2	Feb-22	Aug-24	**ATL**>MISS PAC	2.63%
B1.2	Feb-22	Dec-23	**ATL**>MISS CEN	3.59%
B2.1	Mar-22	Sep-23	**MISS**>CEN PAC ATL	9.38%
B2.2	Mar-22	Nov-23	**MISS**>CEN PAC ATL	4.18%
B3.1	Mar-22	Nov-22	**MISS**>CEN PAC southern AT	1.42%
B3.2	Mar-22	Oct-24	**MISS**>CEN PAC ATL	18.43%
B5.1	Mar-22	Jul-22	**CEN**>MISS PAC	0.28%
A3	Apr-22	Dec-24	**PAC**>CEN MISS ATL	3.36%
B4.1	Apr-22	Apr-23	**CEN**>PAC MISS	5.94%
B1.3	Jun-22	Dec-23	**ATL**>MISS CEN	6.75%
C3.1	Jul-22	Dec-24	**southern ATL**	0.60%
‡A4	Sep-22	Nov-22	**northern PAC**	0.13%
‡A5	Oct-22	Mar-23	**northern ATL**	0.18%
B3.3	Oct-22	Sep-23	**MISS** CEN (ATL)*	0.45%
B3.5	Oct-22	Mar-24	**MISS** CEN>northern ATL	1.23%
B3.4	Nov-22	Apr-23	**MISS**>CEN	0.32%
B3.6	Nov-22	Oct-24	**CEN** MISS>PAC ATL	10.39%
‡A6	Jan-23	Nov-24	**northern ATL**	0.25%
B3.7	Sep-23	May-24	**PAC**>CEN MISS ATL	1.75%
B3.10	Oct-23	Jan-24	**northern PAC**	0.11%
B3.11	Oct-23	Dec-23	**CEN**	0.04%
C1.1	Oct-23	Oct-23	**MISS**	0.01%
C2.1	Oct-23	Sep-24	**ATL**>MISS CEN	4.96%
B3.8	Nov-23	Dec-23	**CEN**>MISS	0.07%
B3.9	Nov-23	Dec-23	**ATL MISS**>CEN	0.17%
B3.12	Nov-23	Feb-24	**CEN** MISS	0.13%
B3.13**	Nov-23	Dec-24	**CEN** (wild)**	0.06%
D1.1	Sep-24	Dec-24	**PAC**>CEN MISS ATL	6.07%
D1.2	Oct-24	Oct-24	**northern PAC**	0.01%
D1.3	Nov-24	Dec-24	**ATL**>MISS CEN PAC	0.18%
Minors	n/a	n/a	n/a	2.88%

Figure provided by NVSL. Previously published on the NVSL GitHub repository for GenoFLU.

‡ All detections have been limited to wild birds only

* All detections have been limited to WOAH poultry [live bird market] flocks in 2023 only

** Data shown are limited to wild mammal detections only (pre-dairy cattle event)

CASE SUMMARY REPORT: GENOTYPE B3.13 IN CALIFORNIA POULTRY PREMISES BETWEEN OCTOBER 11, 2024, AND DECEMBER 17, 2024

A. Summary

Of all confirmed genotype B3.13 detections in dairy cattle premises nationwide, 75.5 percent (567 out of 751) were in California, most of which occurred between October 11, 2024, and December 17, 2024 (**Figure 6**). This case summary report focuses on this period to assess genotype B3.13 dairy cattle and poultry cases for possible connections and risk factors between commodities. The high density of dairy premises, coupled with high poultry premises density in the impacted parts of California, contributed to the rapid spread of genotype B3.13 in both dairy and poultry premises.

Genotype B3.13 poultry cases steadily increased between October 11, 2024, and December 17, 2024 (44 out of 143 confirmed HPAI detections). Of the 44 genotype B3.13-confirmed poultry premises within the United States, 43 cases (40 WOAH poultry [commercial], 1 WOAH poultry [backyard]and 2 WOAH non-poultry) were in California and 1 WOAH poultry [commercial] case was in Utah. Furthermore, of the 40 WOAH poultry [commercial] flocks impacted in California, 15 broiler production, 3 duck breeder, 1 duck meat, 7 table egg layer, 2 table egg pullet, and 12 turkey meat flocks were impacted.

On August 30, 2024, the NVSL confirmed the first case of genotype B3.13 in dairy cattle in California (Tulare County). The first California genotype B3.13 detection in WOAH poultry [commercial] premises was confirmed on September 18, 2024, in Merced County, notably separated by over 100 miles as the crow flies and multiple non-adjacent counties from the initial dairy detection in Tulare County (**Figure 10**).

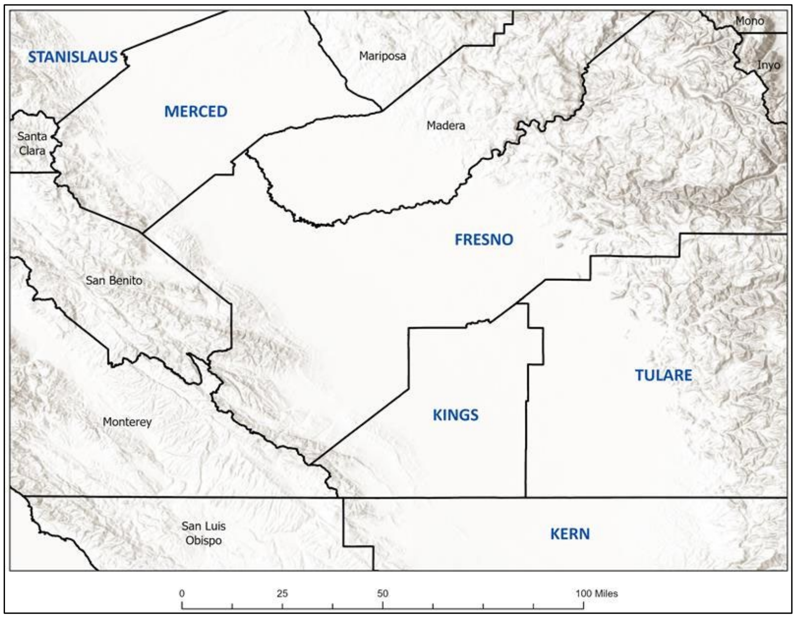


Figure 10. Map of California counties with confirmed positive poultry detections of genotype B3.13 (county names shown in bold blue and capitalized).

Interestingly, although there were no confirmed dairy premises in Merced County until October 3, 2024, the virus from the first detection of B3.13 in poultry was genomically linked to other viruses from dairies. The next confirmed positive WOAH poultry [commercial] premises in California did not occur until a Tulare County detection on October 16, 2024.

Some genotype B3.13-positive flocks had poultry company connections that may have increased the risk of in-network HPAI spread through shared services, crews, or delivery providers, though this was not consistently identified among all impacted flocks. Proximity of dairy premises to poultry premises did not appear to be the only, or even primary, risk factor of genotype B3.13 viral spillback into poultry premises. In fact, nationwide only 19 out of 44 poultry premises impacted by genotype B3.13 infection during this period were phylogenetically linked to the nearest dairy premises. These included nine commercial broiler production, three commercial table egg layer, three commercial turkey meat bird, and one WOAH non-poultry case.

At the time of writing, B3.13 was the only genotype found in dairy cattle. This case report examined key connections and risk factors between poultry flocks with presumptive positive detections of genotype B3.13 and focused on additional information-gathering requirements needed for continued analysis.

B. Genetic Clusters

Phylogenetic sequencing was performed and considered alongside epidemiological links to identify disease clusters. Some clusters of genotype B3.13-positive cases showed a singular poultry premises in a cluster of dairies, whereas other clusters showed multiple poultry premises in a cluster of dairy premises. The phylogenetic and epidemiologic links and risk factors discussed in the remainder of this section are for California poultry and dairy premises in the timeline shown in **Figure 11**.

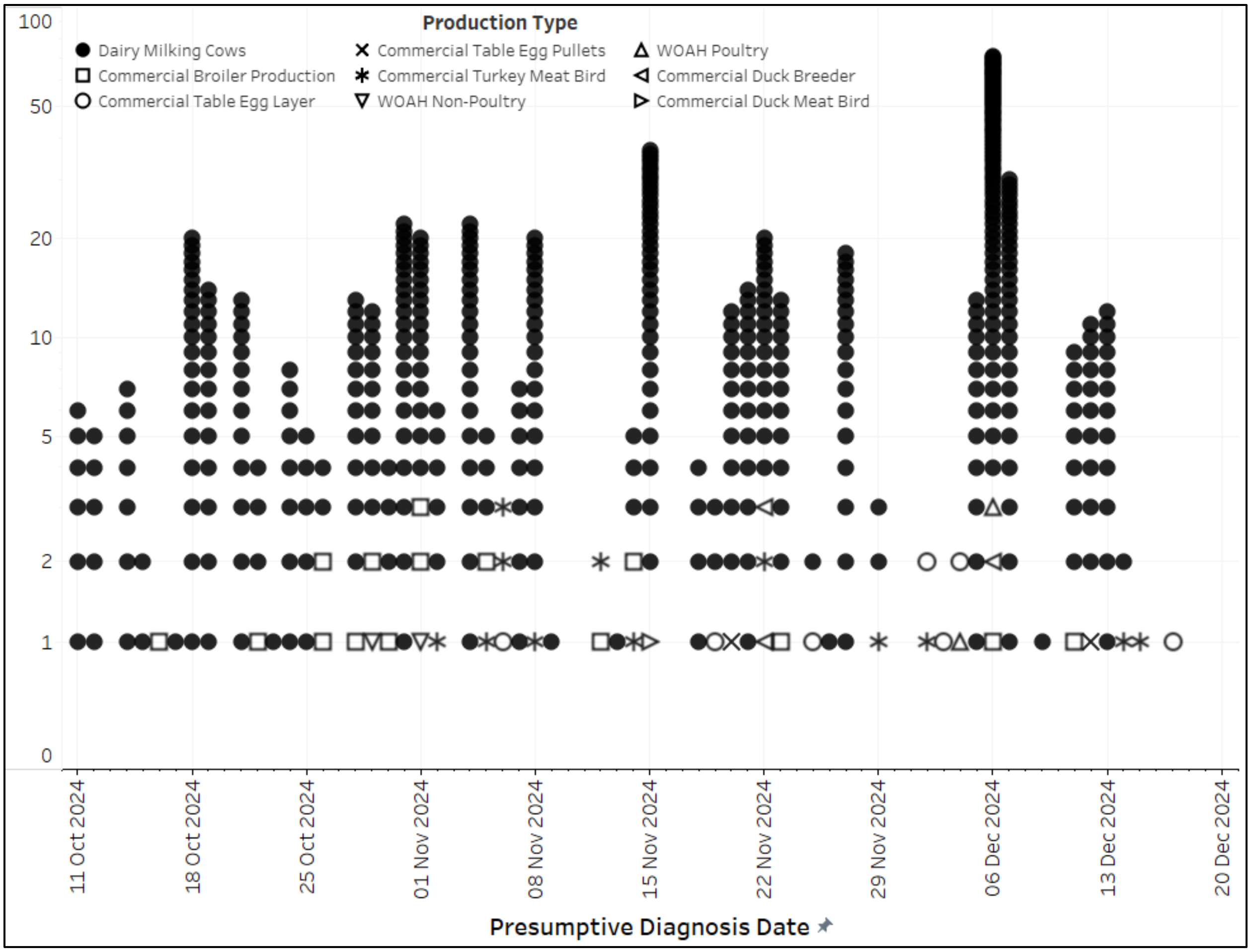


Figure 11. Timeline of California poultry and dairy premises impacted by genotype B3.13 in the period between October 11, 2024, and December 17, 2024, by week of presumptive diagnosis date.

The genetic sequence association and analyses of producer responses to the HPAI Response Initial Epidemiological (Epi) Interview indicate that both Tulare County poultry premises were likely infected by dairy-to-poultry spillover events, although not from the same originating dairy premises. Two Merced County presumptive positive poultry premises detected two weeks apart were genetically closely related and spatially close to each other, but not spatially close to any linked dairy premises. This suggests long-distance transmission of virus between an affected dairy and a poultry premises, even though these Merced County poultry premises were the same production type and owned by the same company.

Three Kings County premises infected with genotype B3.13 after the first Tulare County premises had more than one sequence submission fall on more than one dairy cluster, making the connections more challenging to ascertain from the genetic data. Epidemiological assessment does not further clarify these dairy connections, and either dairy-to-poultry or poultry-to-poultry spread could have occurred.

In late October 2024, the first two premises in Fresno County with genotype B3.13 were presumptive positive one day apart; however, neither premises were in the same cluster. The first Fresno County premises was genomically linked to a dairy in close proximity and was more likely dairy-to-poultry spread. The second Fresno County poultry premises was more closely related to a Kings County premises not owned by the same company. Based on epidemiological assessments of dairy premises, most Fresno County cases fell into separate sub-clusters from one another. These instances illustrate the complexity of virus transmission beyond straightforward in-network spread and proximity of premises, as seen by the transmission of genotype B3.13 in Fresno County. Three premises were presumptive positive within a one-day window and contracted with the same company but represent different production types, and despite their genomic links were not geographically close to one another. One premises shares an identical sequence to a dairy premises, yet that linked dairy was not the closest geographically.

The first Kern County poultry premises in early November 2024 was presumptive positive on the same day as the sixth Fresno County premises; however, it was on an entirely separate cluster closely related to a nearby dairy premises. The second Kern County poultry case, approximately two weeks later, was linked to the first Kern County poultry case; both are commercial table egg layer premises, though not owned by the same company. However, due to the time elapsed between presumptive positive detections of these Kern County premises, separate dairy-to-poultry transmissions were very probable. Prior to this point, the predominant production types involved were meat birds (chicken or turkey).

In early November, Stanislaus County, the northernmost county in this timeframe, was included in a dairy cluster with no other poultry premises or genomic linkages to concurrent genotype B3.13 poultry cases. The second Stanislaus County poultry premises was of the same production type and owned by the same company as the first, but was not presumptive positive until three weeks later, at the end of November 2024. This poultry premises shared identical sequences with multiple dairy premises, suggesting dairy-to-poultry spread. It is important to note that these dairies were not close geographically. Within this dairy cluster were three commercial table egg layer premises in Merced and Stanislaus Counties. Genomic analysis of the cluster suggested directional transmission from the fourth and/or sixth Merced commercial egg layer premises to the eighth premises. The combination of genomic analysis, epidemiological assessment analysis, and geographic relationships to related dairies suggest poultry-to-poultry premises spread is more likely than dairy-to-poultry spread in this case.

By early December 2024, there were over 500 confirmed positive dairy premises in California, and the genetic analysis and determination of clusters and branches became exponentially complex. The remaining poultry genotype B3.13 cases in this period—three Merced County, two Tulare County, and three Stanislaus County premises—were on various dairy clusters, where only the Tulare premises were infected via lateral poultry-to-poultry transmission.

C. Risk Factors

Many risk factors are considered in the movement of HPAI from premises to premises (dairy-to-poultry or poultry-to-poultry). Wild bird introduction is unlikely because the B3.13 genotype was not circulating in wild bird populations. Therefore, the risk factors are concentrated on proximity and its associated factors, as well as a variety of shared connections, services, and personnel. Proximity must be assessed carefully, as many poultry premises with nearby dairies were not genomically linked to those closer dairy premises. The discussion of the risk factors is not in order of likelihood of significance in the genotype B3.13 virus spread, as priority was not able to be determined at this time, based on the information available.

Proximity of a poultry premises to an infected dairy is a significant potential risk factor of genotype B3.13 spread. The average distance of a poultry premises to a dairy premises was 4,883 meters (3.0 miles), median was 1,575 meters (1.0 miles), and adjusted average (minimum and maximum values removed) was 2,101 meters (1.3 miles). The exact modality of the transmission route between premises with different species is more complicated. From October 11, 2024 to November 14, 2024, 13 of the 20 presumptive positive premises were genomically linked to their closest dairy farm; however, for the rest of this reporting period, from November 15, 2024 through December 17, 2024, only two additional genotype B3.13-affected poultry premises were in close proximity to a genomically linked dairy. Furthermore, some genomically linked dairies were several miles away, and often with other infected, but not genomically linked, dairy premises in between. In total, only 16 out of 44 (37 percent) B3.13 poultry cases were genomically linked to their closest dairy and none of the presumptive positive poultry cases detected between November 14, 2024, and December 14, 2024, were genomically linked to nearby dairies. A spatial transmission kernel analysis that further describes the geospatial features of dairy-to-poultry and poultry-to-poultry transmission is provided in the next section of this report.

Virus transmission routes for the poultry premises that were close to their genomically linked dairy premises include people movement as a risk factor, though it is uncommon to have the same personnel working on both a dairy and a commercial poultry premises. There was only one known instance of a poultry farm worker going to a dairy premises within 28 days prior to onset of clinical signs. In this instance, the exact dairy premises visited was not provided and the genomically linked dairy premises to this infected poultry premises was not the closest dairy farm. Poultry producers and managers did not report community living of dairy farm workers with poultry farm workers on most of the epidemiological questionnaires. The one poultry premises that did note community living of poultry farm workers with dairy farm workers did not have any closely related dairy genetic sequences. Lack of community living for most premises does not rule out commingling at common, shared destinations visited by workers from either type of premises, such as local restaurants or supply stores.

Another risk factor related to proximity of poultry premises to dairy premises is vehicle movement. This is more of a risk if there are movements occurring between premises in a local area, such as shared services like utilities, repairs, or routine trash pickup, and contaminated common road surfaces could also play a role if virus-laden milk has been spilled and carried on to vehicle tires. More information is needed to determine the ability of the HPAI virus to remain infectious and stable in such environments, especially considering weather conditions, such as temperature and humidity. The biosecurity plan of a commercial poultry premises should account for such variables. Workers should be conducting truck or tire washes for vehicles coming onto a premises, especially if vehicles are traveling inside the perimeter buffer area of a poultry premises.

A broader category of risk factors related to proximity is the movement of pest and peri-domestic animals, including flies, mice, rats, cats, and perhaps even feral swine. The potential that these insects or animals can become mechanical or even biological vectors is important and detailed analysis must be performed to ascertain this risk. For example, the median proximity of a poultry premises to a dairy premises was about 1 mile. A housefly typically travels 1 to 2 miles, a rat will usually travel only up to 300 feet from their nests but can travel up to a mile or more, and an average farm cat is likely to roam up to 2 miles away but can travel much further. This suggests that there is a likelihood that these vectors might have a role to play in virus transmission between dairy and poultry premises. It is important to note that this median value of closest dairy premises is not always the genomically linked dairy, as only 35 percent of the poultry premises were closest to their genomically linked dairy premises. Therefore, the typical distance traveled by these vectors may be shorter than the distance of a dairy premises linked to that poultry premises. Further analysis is needed to determine the likelihood of risk with this transmission route.

There are similar risk factors for transmission of genotype B3.13 from dairy-to-poultry and poultry-to-poultry. These include geographical proximity, feed or bedding deliveries, shared services (fuel, repair/maintenance, trash pickup), and people movement. For commodities like feed and bedding, most company-owned poultry premises function within a specific network of shared services and personnel. This includes company-specific feed mills, company field supervisors, catch crews, and transport vehicles, which can increase the potential risk of within-network spread of disease depending on the biosecurity practices for vehicles and personnel moving on and off premises. However, this does not explain the increased risk for other out-of-network poultry premises, independent producers, or introduction risk from dairy premises. In California, of the 43 total genotype B3.13 poultry cases, only 4 premises did not have a company connection (no corporate account listed), and 2 of those premises were WOAH non-poultry [backyard] premises and would not be expected to have a company contract. Even though the other 39 premises were linked to a company contract, there were not always in-network connections; there were a few occurrences of genomically related sequences from premises that were not in the same company network. Only 8 of these 39 cases had genomic or epidemiological links to other poultry premises within the same company network.

General agricultural and non-agricultural services outside of the poultry company network may also play a role in virus transmission. These potentials include, but are not limited to, contracted workers, rendering services, trash pickup, propane or other fuel delivery, electricians, water services, and portable restroom services. Producer responses to the questionnaires indicate 16 of the 43 California premises employed contract workers from an agency, and in total, 5 different agencies were identified. Usually, poultry premises within the same company network used the same agency, but not always. This did not appear to increase the risk as there were not always in-network genetic links between poultry premises, and more information is needed on the role of contracted employees and the likelihood of virus transmission risk with their employment.

Another possible risk for genotype B3.13 transmission is using rendering to dispose of mortalities. Many dairy premises use rendering services for removal of their deadstock, and this was a common service used by many poultry premises, including 20 of the 44 total genotype B3.13 cases in this reporting timeframe. Most contracted with one company, accounting for 18 of the 20 cases that used rendering. For 14 of the 20 poultry operations that used rendering services, carcasses were transported to the rendering facility by a poultry company transport. The other four cases used either farm-owned transport or a renderer company transport. While this service could play a role in virus transmission, there is more information needed to ascertain the true likelihood of this risk, especially as many poultry premises have the carcass bin pick up outside of the perimeter buffer area. Similarly, more information is needed on other services and how they connect between poultry and dairy premises, such as trash pickup and fuel deliveries, to determine what role these services play—not enough information is provided on the existing poultry or dairy epidemiological questionnaires to determine this.

D. Additional Information Needed and Questions

While this report clarifies some key potentials for virus transmission and spread between and among dairy and poultry premises, there remains uncertainty with the likelihood of multiple risk factors. This uncertainty is simply due to information not otherwise provided or asked for on a typical poultry or dairy epidemiological questionnaire. Some of that information is not even necessarily premises-specific, but more so related to how certain services operate and products or people usually move. The topic areas and related questions to gather more information for analysis are detailed below.

Feed Mills and Delivery	Some feed mills produce grain for multiple species, including poultry and dairy cattle. • What is the crossover risk? ○ Do trucks deliver grain to different species premises on the same day? ○ Do trucks deliver grain to different species premises on the same load? ○ If so, does the delivery driver exit the cab of the vehicle on both dairy and poultry premises? ▪ If they do exit the cab of the vehicle, do they put on protective attire, such as disposable boot covers and gloves? • What are the sources of corn for producing grain supplied to poultry and dairy premises?
Rendering	Some rendering plants process both poultry and dairy cattle. • What is the crossover risk? ○ What are the usual routes for rendering trucks? ○ Does a truck only service poultry premises in a day or can it service both poultry and dairy premises on the same pickup route? ○ Is the risk of virus transmission reduced when producer or poultry company transports carcasses themselves to the rendering facility?

Contract Workers	• Is hiring contracted workers a risk and how do contracts work? ○ How long are employees contracted with a poultry premises? ▪ Does this depend on certain factors? If so, what are those factors? • Are contracted employees forbidden from being assigned to or taking employment on other agricultural premises? • Are there limitations to what the poultry producer/grower can require a contracted worker do or not do, such as working on other poultry or livestock premises?
Proximity and Virus Spread	More information is needed to determine risk factors for poultry premises that are closest to their genomically linked dairy premises. • Positive avian influenza polymerase chain reaction (PCR) peri-domestic sampling: What is the distance between the poultry premises and its closest dairy premises? Do peri-domestic samples genomically link the premises? • “Spilled milk” theory: Is there gross contamination of virus-laden milk on roadways used by more than one operation? If so, what is the duration of viable virus? • What is the risk of airborne spread between premises? ○ It would take nearly “perfect” conditions to allow for aerosolized milk from a dairy operation to go to a nearby poultry operation. It would also have to travel at least a mile in many cases. This is not likely considering low humidity and lack of air movement in the Central Valley of California; however, it should be assessed.

MODELING GENOTYPE B3.13 TRANSMISSION FROM DAIRY FARMS AND POULTRY PREMISES INTO POULTRY PREMISES IN CALIFORNIA USING A SPATIAL TRANSMISSION KERNEL

A. Overview

Between August 15, 2024, and December 31, 2024, hundreds of dairies and dozens of poultry premises in California became infected with genotype B3.13. A combination of epidemiological and genomic data suggests that genotype B3.13 first spread from dairies to poultry premises, with subsequent dairy-to-poultry and poultry-to-poultry spread in the State.

Center for Epidemiology and Animal Health (CEAH) analysts quantified the geospatial dependence of genotype B3.13 transmission from infected dairy farms and poultry premises to susceptible poultry premises using a spatial transmission kernel. A spatial transmission kernel refers to a continuous mathematical function that captures how the daily transmission rate declines with distance from each infectious dairy farm or poultry premises to a susceptible poultry premises. With this method, the total rate at which a given susceptible poultry premises becomes infected is a function of the number of infectious dairy farms and poultry premises, the distance to these farms and premises, and the fitted dairy-to-poultry and poultry-to-poultry transmission kernels that describe how the transmission rate from each infectious farm and premises decreases with distance.

This report describes the geospatial features of dairy-to-poultry and poultry-to-poultry transmission as estimated by the spatial kernel fit using 50 poultry premises (42 WOAH poultry [commercial] premises and 8 backyard flocks) and 692 dairy herds that tested positive for genotype B3.13 in California between August 15, 2024, and December 31, 2024. The report also describes the probability that susceptible poultry premises would become infected over time (i.e., the assumed infectious period of a dairy farm or poultry premises) for a range of scenarios for the number of, and distance to, infectious dairy farms and/or poultry premises.

The results are most directly applicable to the assessment of risk associated with the restocking of poultry premises after a recently cleared infection while dairy farms and/or poultry premises in the vicinity remain infected. Spatial transmission kernel model fits indicate that re-infection of poultry premises following restocking is unlikely when few nearby dairy farms or poultry premises are infected. However, when infection in dairy farms or poultry premises is common, restocking could lead to a high probability of re-infection. Modeled results indicate that over 10 percent of poultry premises experienced more than a 5 percent probability of infection over a 14-day period in late November and early December 2024.

B. Fitted Kernel Model

The fitted kernels show a rapid decline in transmission rate as distance between farms and premises increased (**Figure 12**). The daily transmission rate per dairy farm to a susceptible poultry premises (dairy-to-poultry spread) was estimated to be similar to the transmission rate per poultry premises (poultry-to-poultry spread). Though median transmission estimates indicate that the rates of dairy-to-poultry and poultry-to-poultry spread are different, the wide credible interval (CI) for poultry-to-poultry spread and overlap with the dairy-to-poultry spread CI do not indicate that that one rate is significantly higher than the other. The Bayesian 95 percent CI on daily poultry-to-poultry transmission rate is much wider than for daily dairy-to-poultry transmission rate because California had far fewer poultry premises infections than dairy infections in the period between August 15, 2024, and December 31, 2024.

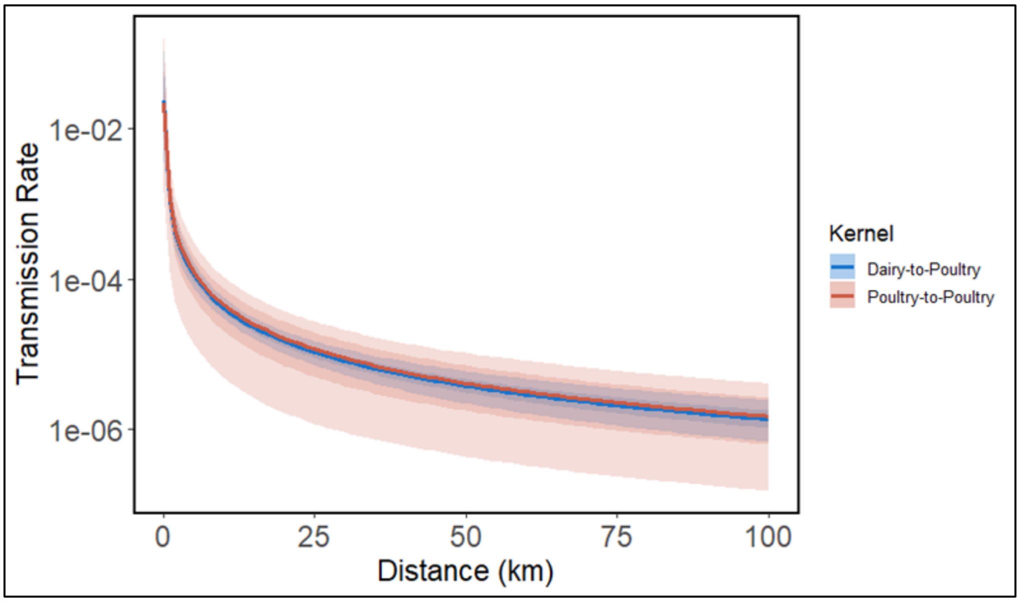


Figure 12. Estimated spatial transmission kernel. Solid lines show medians; darker envelopes indicate 60% and lighter envelopes indicate 95% credible intervals (CIs) for dairy-to-poultry (blue) and poultry-to-poultry (red) spatial transmission kernels, respectively.

Given that the number of infectious dairy farms in California greatly outnumbered the number of infectious poultry premises at all timepoints in this period, dairy-to-poultry transmission contributes to total force of infection (FOI; the rate at which a susceptible premises becomes infected) by 5.5 times more, on average, than poultry-to-poultry transmission (**Figure 13**).

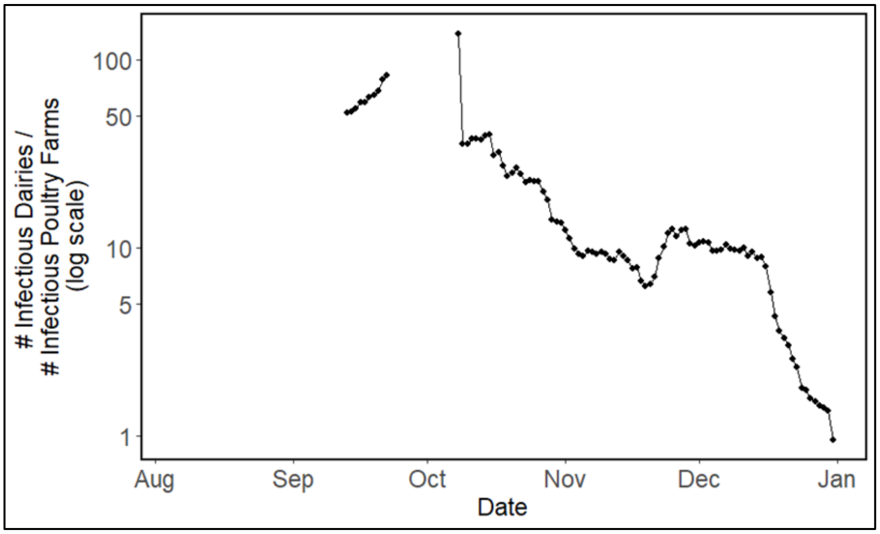


Figure 13. Ratio of the number of infectious dairies to infectious poultry premises between August 15, 2024, and December 31, 2024, assuming a 25-day infectious period for dairies and poultry premises infectious until initial virus elimination (IVE). Timepoints without points have no actively infectious poultry premises.

Estimated Poultry Premises Infection Probabilities

Figure 14 shows the predicted probability that a susceptible poultry premises would become infected by a single nearby infectious dairy or poultry premises across the full infectious period (25 days for infectious dairies and 32 days for infectious poultry premises; see **Model Specifications and Assumptions**). Over the full infectious period of a dairy, the predicted transmission probabilities to a susceptible poultry premises at 1 km and 2 km were 2.8 percent (95 percent CI: 1.5 to 5.2 percent) and 1.1 percent (95 percent CI: 0.6 to 1.8 percent), respectively. Over the full infectious period of a poultry premises, the predicted transmission probabilities to a susceptible poultry premises at 1 km and 2 km were 3.6 percent (95 percent CI: 0.4 to 10.2 percent) and 1.4 percent (95 percent CI: 0.2 to 3.8 percent), respectively.

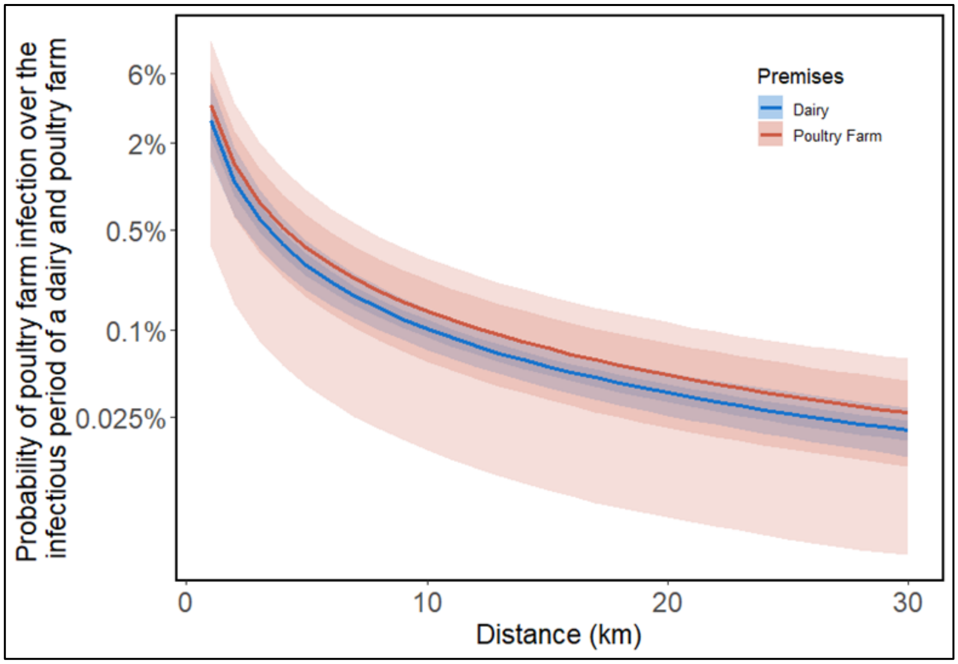


Figure 14. Predicted probability of a susceptible poultry premises becoming infected by a single nearby infected premises over the infectious period of that premises, as a function of the distance between them. Solid line shows median; darker envelopes indicate 60% and lighter envelopes indicate 95% CIs, respectively.

Throughout the HPAI event in California dairies, few poultry premises were located near only one infectious dairy, while being near only one infectious poultry premises was common. The predicted infection probabilities experienced by individual poultry premises throughout the event in California were often much larger (**Figure 15**), with greater than 10 percent of poultry premises experiencing more than a 5 percent probability of infection over a 14-day period in late November and early December 2024 (**Figure 16**).

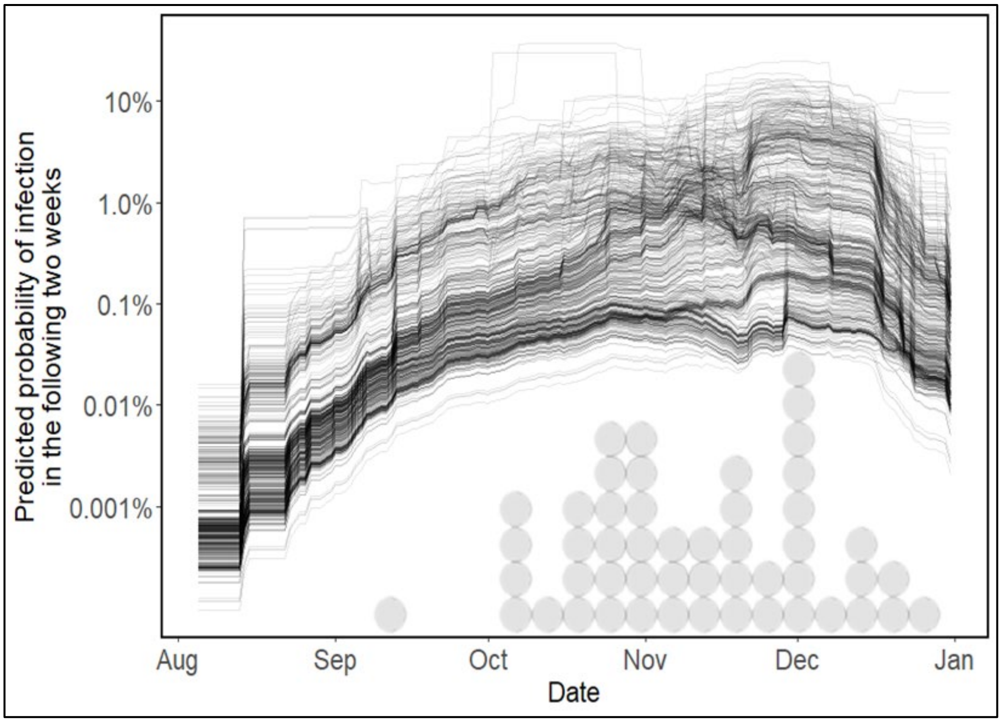


Figure 15. Lines show how the predicted infection probability over a 14-day window to all poultry premises changed over time. Each of the 663 lines plotted here represents a single poultry premises. Dots show when each poultry premises became infected (binned to a 5-day window).

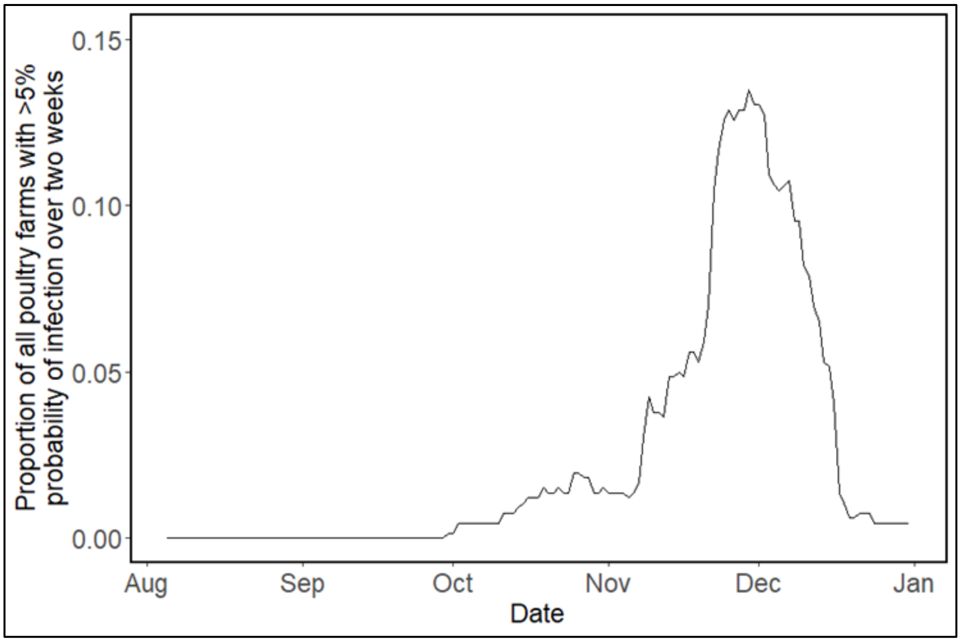


Figure 16. The proportion of all poultry premises experiencing greater than a 5% infection probability over a two-week period.

Given the spatial dependence of transmission (**Figure 12**, **Figure 14**), the estimated total cumulative probability that a given poultry premises would have become infected by December 31, 2024 is naturally an increasing function of the total number of nearby infectious premises, how close they were located, and how long they were infectious. Given the smaller number of infectious poultry premises in this period and the smaller contribution of poultry premises to overall FOI, the analysis initially focused on dairy-to-poultry transmission. **Figure 17** shows these dependencies for the 335 poultry premises (51 percent of the poultry premises in the dataset) that experienced at least one day when greater than 50 percent of dairies within 20 km were infectious. The predicted cumulative probabilities result in an estimate of 37 infections (60 percent CI: 32 to 41 infections; 95 percent CI: 26 to 48 infections). The data show that 44 actually became infected.

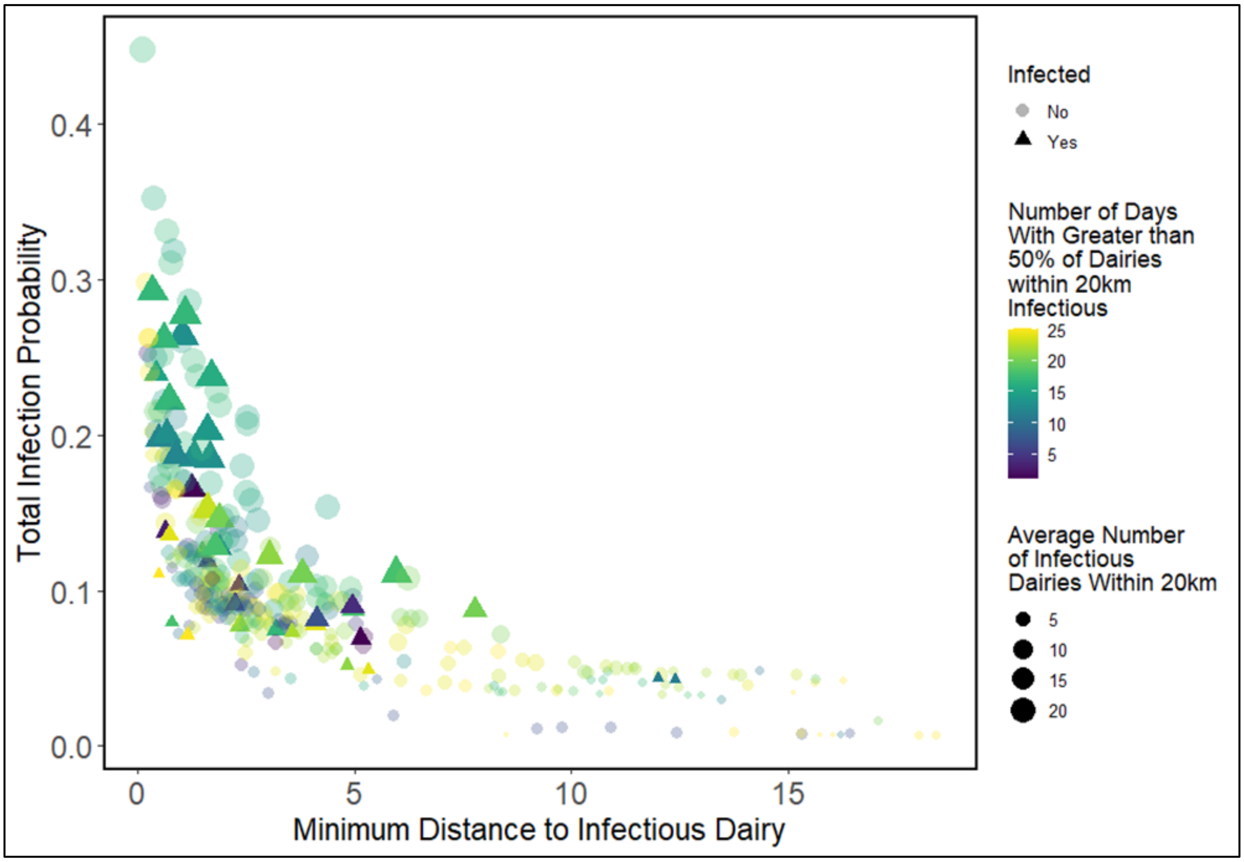


Figure 17. Total infection probability against minimum distance to infectious dairies, for the 335 poultry premises (51% of the poultry premises in the dataset) that experienced at least one day when greater than 50% of dairies within 20 km were infectious. Each point shows one poultry premises; infected poultry premises are shown as triangles and point size shows the average number of infectious dairies within 20 km. Colors show the total number of days when greater than 50% of all dairies within 20 km of a given poultry premises were infectious. Premises that became infected are more concentrated at higher estimated infection probabilities.

Although the FOI of infection was higher for dairy-to-poultry transmission, there were several instances where poultry premises had increased risk due to their proximity to other infected poultry premises. Three poultry premises had greater than 50 percent of poultry premises within

20 km infectious. The kernel model predicted the following: these three premises experienced 5, 21, and 26 days in which greater than 50 percent of poultry premises within 20 km were infectious; a maximum number of 5, 9, and 5 infectious poultry premises; and a minimum distance to the closest infectious poultry premises of 9.5, 0.9, and 6.8 km, respectively. The kernel model also predicted an infection probability of 0.6, 8, and 1 percent for these three premises, the second of which became infected.

Comparison of Model Predictions to Empirical Data

In total, 50 out of 663 poultry premises (7.5 percent of those in the dataset) became infected with genotype B3.13 between August 15, 2024, and December 31, 2024. The expected number of infected poultry premises in the spatial kernel model fit, based on the known infectious dairy and poultry premises, is 43 (95 percent CI: 28 to 62 premises). The model prediction provides one indication of realistic infection probabilities—the real value of 50 falls well within this 95 percent CI. Further, using the fitted kernel and the observed dairy infections, analysts simulated 1,000 new sets of poultry premises infections. Across these simulations, the median infected poultry premises from both dairies and other poultry premises was 51 (60 percent CI: 40 to 62 premises; 95 percent CI: 30 to 76 premises). Thus, the predicted number of premises is close to the observed 50 infected premises.

Simulating infection from only dairies (i.e., setting poultry-to-poultry transmission to zero), the median number of poultry premises infected was 42 (60 percent CI: 36 to 51 premises; 95 percent CI: 27 to 62 premises). Epidemiological and genomic data suggests at least six poultry premises were likely the result of lateral spread between poultry premises (see **Genomic and Epidemiological Evaluation**). Furthermore, these simulations predict that most infected poultry premises were a median distance of 2.60 km from the closest infected dairy; this value is 2.74 km in the empirical data.

E. Potential Applications

Risk Evaluation

These fitted kernels can be used to determine the conditions (e.g., percent of surrounding dairy farms and poultry premises at what distances) under which restocking of poultry premises could result in a probability of repeat introduction of genotype B3.13 above a given risk threshold. In California, no genotype B3.13-infected poultry premises had repeated introductions of the same genotype; however, the model predicted that if a high density of infectious dairy farms remained in close proximity, restocking of poultry premises soon after the virus was cleared would result in greater than 5 percent probability of reintroduction over two weeks following restocking in up to 13 percent of poultry premises.

Supporting the Development of Interventions on At-risk Farms

These fits can be used to predict the impact that interventions initiated in response to a nearby infection on susceptible dairy farms or poultry premises would have on curtailing spread to new premises.

Inform Surveillance and Regions of Critical Need for Increased Biosecurity

A kernel model can help in identifying regions of elevated risk in the early to middle phases of an event when genomic data is unavailable or phylogenetic analysis to construct transmission pathways is incomplete. For example, the impact of therapeutic intervention (e.g., vaccination) could be estimated by forecasting an event forward in time while treating a subset of susceptible premises either completely or partially resistant to infection because of vaccination.

F. Strengths and Limitations

One strength of a spatial kernel model is that it can be fit with relatively little data. A spatial kernel is particularly useful in the early to middle phases of an event to estimate which single premises or clusters of premises have the highest predicted risk for infection. Despite its simplicity, a kernel can be quite accurate in describing transmission patterns and used to forecast cases forward over short time windows when it is more realistic to assume that little about the event will change (e.g., no implementation of new interventions).

A limitation of a spatial kernel is that it only provides a description of the observed spatial dependence of transmission. Therefore, it is not helpful in evaluating mechanisms of disease spread. Because of the simplicity of a spatial kernel, it can become superseded by more complex mechanistic models if better data becomes available. This report provides precise quantitative estimates unique to California in 2024. It may have little direct application to HPAI transmission in other States, though the methodology may be applied to other geographic regions to assess the local spatial transmission risk based on the relative density of both dairy farms and poultry premises.

G. Results and Discussion

Summary of Empirical California HPAI Event Data

Averaged across all poultry premises in California between August 15, 2024, and December 31, 2024, unaffected poultry premises were located within 20 km of 4.5 infectious dairies (12 percent of all dairies within 20 km); however, 335 poultry premises (51 percent of the poultry farms in the dataset) experienced at least one day when greater than 50 percent of dairies within 20 km were infectious. On average, these 335 poultry premises experienced 16.5 such days; 44 of the 50 poultry premises that became infected were part of this group of 335 premises.

Identical calculations for infectious poultry farms revealed that, on average, unaffected poultry premises were located within 20 km of 0.47 infectious poultry premises (1.5 percent of all poultry premises within 20 km); only three unaffected poultry premises had at least one day when greater than 50 percent of poultry premises within 20 km were infectious; and though a rare occurrence for poultry premises in general, these three poultry premises experienced 17 such days, on average.

Genomic data revealed that isolates from 23 poultry premises share high levels of whole genome sequence similarity with one or more isolates from other poultry premises. Phylogenetic reconstruction provides strong evidence for at least six poultry premises being infected by another poultry premises.

Infected dairies implicated as potential infectors of poultry premises, based on similarly of whole genome sequences, were often physically closer based on straight-line distance to infected poultry premises than all non-implicated dairies infectious at the time of new poultry premises infections. Specifically, across all infected poultry premises for which there were complete phylogenetic relationships, the median distance to genomically-implicated dairies was 14 km, compared to 49 km for all dairies infectious at the time of poultry infection.

Summary of Results from the Spatial Transmission Kernel Model

Analysts estimated that between August 15, 2024, and December 31, 2024, susceptible poultry premises in California experienced a similar infection pressure from each individual infected poultry premises and infected dairy. For example, there is an estimated 1.1 percent (95 percent CI: 0.6 to 1.8 percent) and 1.4 percent (95 percent CI: 0.15 to 3.8 percent) probability that a *single* infected dairy or poultry premises, respectively, would infect a susceptible poultry premises at a distance of 2 km across their full infectious period. However, because far more dairies than poultry premises were infected in California during this period, it is estimated that susceptible poultry premises, on average, experienced approximately a 5.5 times higher transmission rate from infectious dairies, *collectively*, than from infectious poultry premises.

Considering the precise distances of the infectious dairies within 20 km of the 335 poultry premises that experienced at least one day when greater than 50 percent of dairies within 20 km were infectious, the kernel model predicted an 11 percent probability that these poultry premises would, on average, become infected. In reality, 44 (13 percent) of these poultry premises became infected. The three poultry premises with greater than 50 percent of poultry premises within 20 km infectious had a maximum of 4, 5, and 9 poultry premises infectious. These three premises experienced an average of 3 percent probability of becoming infected in total across the 17 days. One of the three premises became infected.

Kernel-based infection patterns are strongly supported by the available genomic data. In brief, simulated epidemics using the fitted kernel predicted a median of 18 percent of poultry infections were due to transmission from another infected poultry premises. Based upon the available epidemiologic data (e.g., temporal nature of the detections in poultry premises and shared ownership of farms and/or species) and implicated directionality in infector-infected pairs based upon genomic data, analysts estimate that 6 out of 50 (12 percent) of the poultry transmission events may have been the result of lateral transfer from another poultry premises (see **Genomic and Epidemiological Evaluation**).

HPAI SURVEILLANCE IN WILD BIRDS AND MAMMALS

A. Background

Waterfowl are the primary reservoir hosts for the greatest diversity of influenza A viruses (IAVs; subtypes H1–H16), and detections of HPAI in wild birds have been increasing globally since the emergence of clade 2.3.4.4b goose/Guangdong lineage viruses at the turn of the century. IAVs 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 migratory flyways congregate provide opportunities for viruses to mix across migratory flyways.

Waterfowl migration in North America generally consists of north-south seasonal movements between breeding grounds and wintering areas, typically following the migratory flyways. Migratory 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 migratory flyways have areas of overlap and convergence, particularly at the north and south ends. Migratory 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 across flyways. During migratory movement, wild birds have the potential of dispersing pathogens, such as IAV, across wide geographic distances.

The first detection of HPAI H5N1 Eurasian (EA) clade 2.3.4.4b virus in North America occurred in a wild Great black-backed gull in December 2021 in Newfoundland and Labrador, Canada. The bird showed neurologic signs and was part of a large mortality event. The first subsequent detection of HPAI H5N1 in the United States was reported in January 2022 in a wild dabbling duck from South Carolina. This bird exhibited no neurologic signs and was an apparently healthy bird collected during hunter harvest. The initial spread of HPAI along the Atlantic flyway, as well as the subsequent spread to the other three migratory flyways, has been primarily attributed to wild bird movement.

B. Wild Bird Surveillance Program

The U.S. National Surveillance Plan for Highly Pathogenic Avian Influenza in Wild Birds was developed in 2006 to maximize the ability to detect IAV in wild migratory birds. Surveillance helps to understand how HPAI is distributed in the United States, detect the spread of HPAI to new areas of concern, provide a flexible surveillance framework that accounts for changing disease risks through time, and obtain sequence data to better understand HPAI transmission dynamics and spillover risk to poultry.

The surveillance plan focuses on sampling apparently healthy dabbling ducks, which have been identified as the primary reservoir for HPAI and other IAVs of concern. While HPAI has been detected in many non-target birds, such as raptors, gulls, and passerines (see the Detections of Highly Pathogenic Avian Influenza in Wild Birds webpage for the current list), 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 December 31, 2024, over 116,974 apparently healthy wild birds were sampled and tested by reverse transcription-quantitative polymerase chain reaction (rRT-PCR) for IAV (**Table 3**). Wild bird surveillance testing follows the National Animal Health Laboratory Network (NAHLN) testing algorithm: samples are first tested by a Type A-specific matrix assay (IAV-M), then by H5/H7 subtyping assays in samples where viral RNA is detected. H5- and H7-positive samples are forwarded to the NVSL for confirmatory testing. The number of HPAI virus detections is based on viruses confirmed at the NVSL from HPAI presumptive positive samples forwarded by NAHLN laboratories. Overall, targeted wild bird surveillance conducted by USDA–APHIS–Wildlife Services has resulted in 5,477 detections of H5Nx HPAI virus across the four migratory flyways. HPAI detections are reported by Wildlife Services staff as H5Nx detections, as opposed to H5N1, because there are occasionally reassortants in wild birds that result in other N detections and some HPAI positives where the N-specific assay is not successful.

Table 3. Number of H5Nx HPAI detections from apparently healthy wild birds sampled by USDA–APHIS–Wildlife Services, as of December 31, 2024.

Migratory Flyway	# Birds Sampled	# HPAI H5Nx Detections
Atlantic	33,051	1,436
Mississippi	35,811	1,571
Central	15,469	941
Pacific	32,643	1,529
Total	116,974	5,477

Prevalence of HPAI H5Nx (i.e., the proportion of sampled individual birds testing positive for HPAI H5Nx) 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 18**). 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.

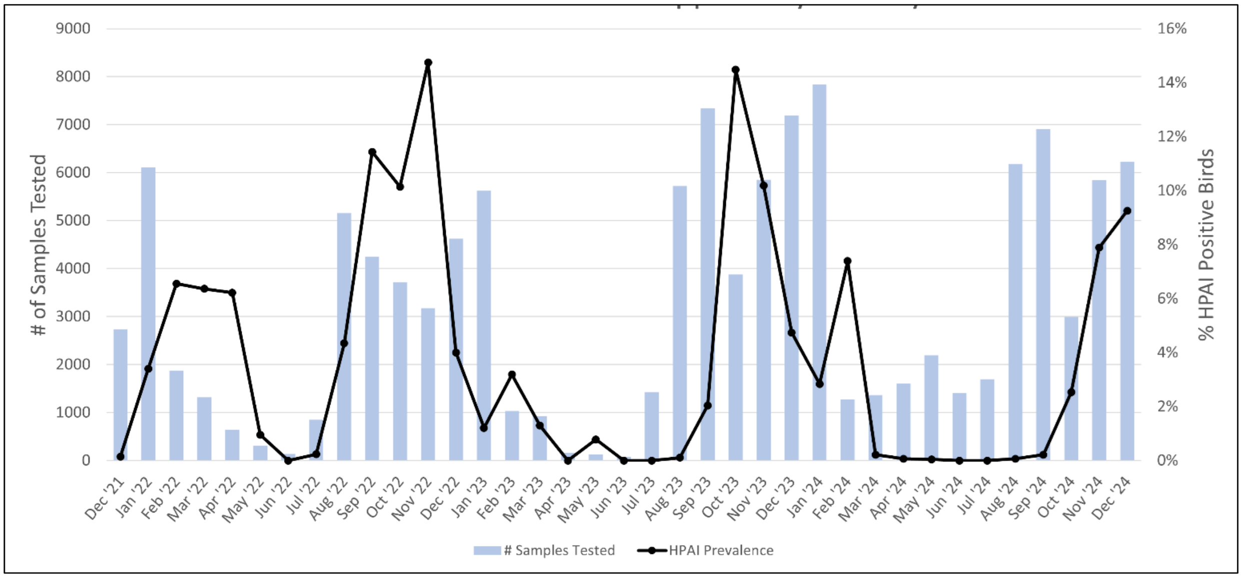


Figure 18. Monthly sampling effort (number collected; blue bars) and H5Nx HPAI prevalence (percentage of samples found to be HPAI H5Nx-positive; black line) in apparently healthy wild birds as part of the U.S. National Surveillance Plan for Highly Pathogenic Avian Influenza in Wild Birds, as of December 31, 2024.

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, as previously described. 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–Wildlife Services, State wildlife agencies, the U.S. Fish and Wildlife Service, the U.S. Geological Survey's (USGS) National Wildlife Health Center, and universities.

Between December 30, 2021 and December 31, 2024, morbidity/mortality investigations resulted in 5,856 detections of HPAI in sick or dead birds across all four migratory flyways: 1,784 in the Atlantic flyway; 1,653 in the Mississippi flyway; 979 in the Central flyway; and 1,440 in the Pacific flyway. Altogether, targeted surveillance samples collected by USDA–APHIS–Wildlife Services, samples collected as part of other Wildlife Services programs and external research groups, and morbidity/mortality investigations of sick or dead birds during this time period have resulted in 12,055 detections of HPAI in at least 187 wild bird species across all 50 States and Washington, D.C. (**Figure 19**). The true number of wild birds that have been infected with HPAI in the United States is assumed to be much higher than the number of confirmed detections, because only a subset of animals are screened for HPAI when a large mortality event is observed.

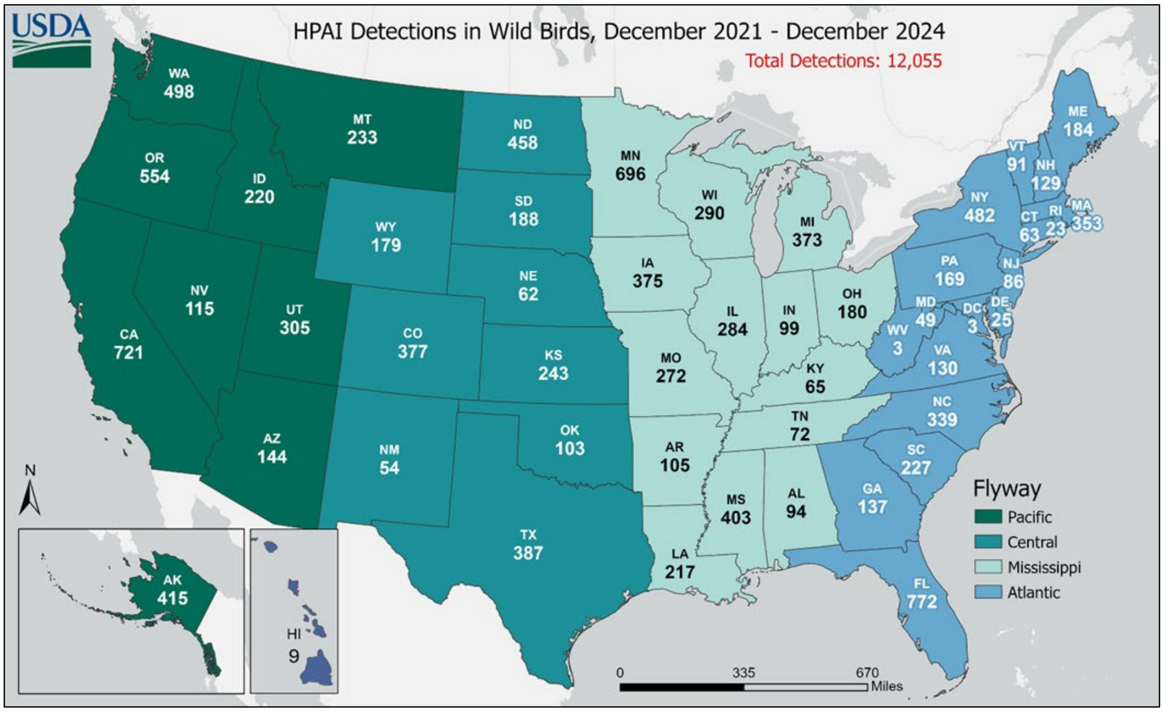


Figure 19. Number of HPAI detections in wild bird species tested between 30 December 2021 and December 31, 2024. State totals include detections from both apparently healthy birds from active surveillance efforts, sick/dead birds from morbidity/mortality investigations, and captive wild birds, which are often birds found sick in the wild and submitted to animal rehabilitation centers.

D. HPAI Detections in Mammals

Although HPAI primarily affects wild birds and poultry, these viruses can occasionally be transmitted to mammals. A rising number of HPAI cases have been reported in several terrestrial and aquatic mammalian animals across the United States (**Figure 20**). In some cases, infection may cause illness, including severe disease and death. As of December 31, 2024, there have been 412 HPAI detections in at least 35 wild or captive wild mammal species in 32 States since the start of the HPAI event. The majority of HPAI detections in mammals have been from sick or dead animals, although recent surveillance of peri-domestic wildlife found in and around HPAI-positive farms has resulted in detections of HPAI in apparently healthy wild mammals.

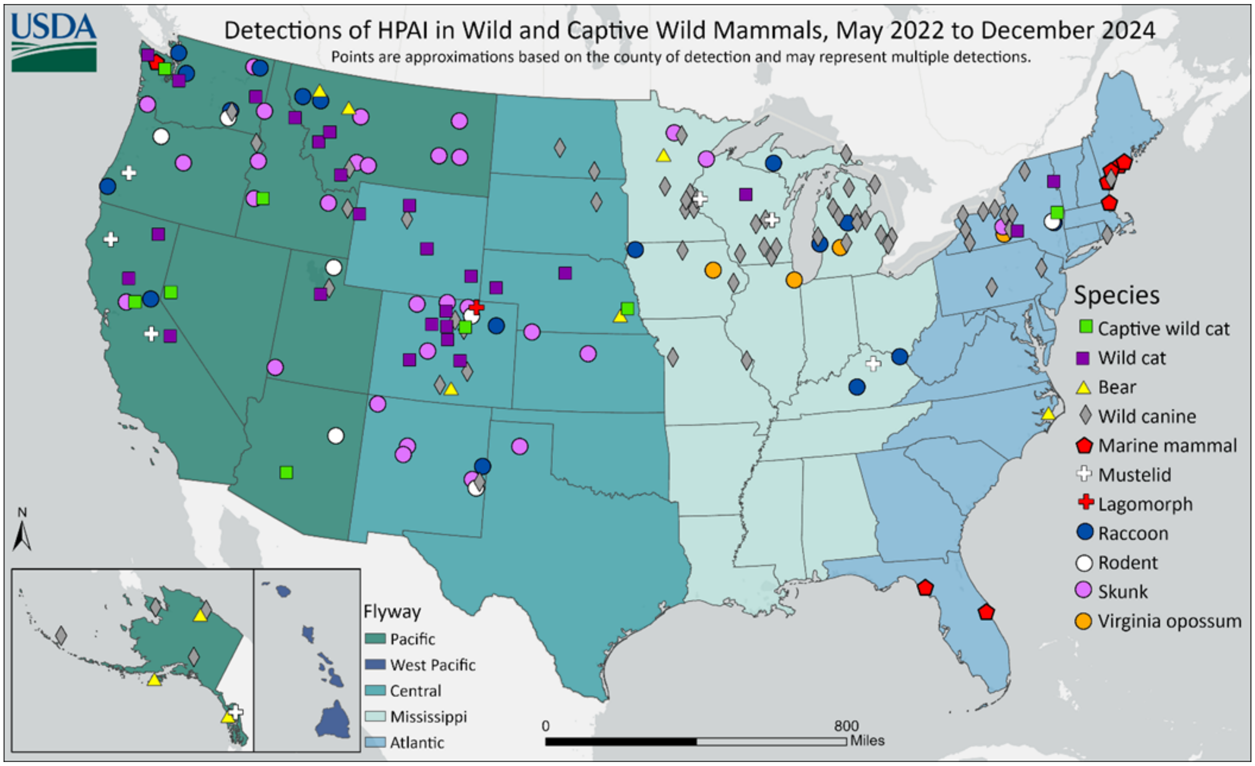


Figure 20. Detections of HPAI virus in wild mammals and captive wild mammals in the United States as of December 31, 2024.

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- Carolann Knutson, Jared Luxton, Philip Riggs (CFI)
- Valerie Koenig (FiOps)

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APPENDIX A. CALIFORNIA KERNEL MODEL METHODOLOGY

A. Data Requirements

CEAH analysts used data from California dairy and poultry premises. Specifically, the model fits presented here used: a) information on where 999 California dairy and 663 poultry premises are located as given by the latitude and longitude of their front gate; and b) when 692 dairy and 50 poultry premises became infected with genotype B3.13 (defined here as the date of onset of clinical signs minus 10 or 5 days, respectively) between 10 August 2024 and 25 December 2024. All data was retrieved from the Emergency Management Response System (EMRS).

Key data processing steps required to build the analysis dataset included: 1) filling in missing values for the date of onset of clinical signs in dairies (33 percent of all dairy infections were missing a date of onset of clinical signs; no infected poultry premises were missing this information) using the mean difference between the date of onset of clinical signs and the date of presumptive diagnosis calculated from all premises with both values; 2) dropping one dairy premises that appeared to have been infected from out of the State based on low genetic similarity to any California dairy infection; 3) dropping one dairy premises with missing latitude and longitude data; and 4) generating two pairwise distance matrices—one between all dairy premises and all poultry premises and a second between all pairs of poultry premises.

B. Model Specifications and Assumptions

Kernel Shape and Force of Infection

Analysts modeled both dairy-to-poultry and poultry-to-poultry transmission kernels (k_{dp} and k_{pp}, respectively) with a three-parameter function commonly used to model disease transmission in livestock (see Boender et al., 2010; Bonney et al., 2018; Chanchaidechachai et al., 2021). Given few poultry premises infections relative to dairy infections and a short poultry premises infectious period (see **Additional Model Assumptions**), analysts fit a constrained model using four parameters instead of six, allowing all three kernel parameters to differ between k_{dp} and k_{pp}. Specifically, these two kernels were parameterized as:

$$k_{dp}(r_{ij(d)}) = \frac{k_0}{1 + \left(\frac{r_{ij(d)}}{r_0}\right)^a} \qquad k_{pp}(r_{ij(p)}) = \frac{k_0 \delta}{1 + \left(\frac{r_{ij(p)}}{r_0}\right)^a}$$

where k_0 is the dairy-to-poultry transmission rate per day at zero distance, δ is a scaling factor for poultry-to-poultry transmission at zero distance, r_0 gives the distance at which transmission rate is half k_0, a gives the slope by which transmission decreases over distance, and $r_{ij(d/p)}$ gives the distance between susceptible poultry premises *i* and infectious dairy/poultry premises *j*. The constraint resulted in single values for r_0 and a, which together control how transmission rate decays with distance.

The total FOI, the rate at which a susceptible premises becomes infected, experienced by a given susceptible poultry premises *i* on a given day *t,* is calculated as the sum of the kernel-based transmission rates from all infectious premises *j* on day *t*:

$$\lambda_i(t) = \sum_{j(d)} k_{dp}(r_{ij(d)}) + \sum_{j(p)} k_{pp}(r_{ij(p)})$$

Maximum Likelihood Function

The likelihood function that is maximized to estimate these three kernel parameters is composed of the product of three terms: 1) for premises that became infected, the probabilities of these premises escaping infection until the day before they became infected; 2) for premises that became infected, the probabilities of these premises becoming infected on the day they became infected; and 3) for premises that did not get infected, the probabilities of premises escaping infection over the whole study period. A mathematical description of a similar likelihood function with one type of infectious premises is available in Chanchaidechachai et al. (2021). A translation of this likelihood function into R code is available upon request.

Additional Model Assumptions

Analysts assumed point values for three parameters. First, a period of 10 days from initial dairy infection to the onset of clinical signs. This assumption was based on within-herd modeling and surveillance of bulk tank milk in California. Second, poultry premises were assumed to be infectious for an average of 6 days prior to presumptive diagnosis, based on the estimated median time to first positive sample from an HPAI time of introduction analysis (Ssematimba et al., 2024). Third, poultry premises were assumed to be infectious until IVE. Thus, unlike dairies, which were each assumed to be infectious for 25 days, poultry premises varied in their infectious period given differences among premises in how long it took to reach IVE. The median infectious period for the 50 poultry premises infections in the dataset was 32 days.

Third, both infected dairies and poultry premises were assumed to become infectious directly following exposure—for dairies, this assumption was partially based on observational evidence of milking cows being sold directly following a reduction in their milk production—and remained infectious at a constant level of infectiousness (i.e., contributes equally each day to the FOI) for *n* days. For poultry premises, *n* was allowed to vary among premises given variability in IVE date as described previously. For dairies, analysts explored what value of *n* was best supported by the data in the first step of model fitting.

C. Model Fitting

The model was fit in two stages. Using the base R function *optim,* analysts estimated kernel parameters by maximizing the likelihood function for a range of different assumptions for the duration of the infectious period in dairies—how long an infected dairy contributes to FOI. Specifically, the infectious periods were fit between 10 and 35 days. Each fit was repeated 5 times from different random starting parameter sets to confirm model convergence to the global likelihood maximum. For all considered infectious periods, at least three different random starting conditions converged on the same parameter set; in all cases this parameter set had the maximum likelihood. This fitting step revealed a maximum likelihood at an infectious period of 25 days.

Using an infectious period of 25 days, kernel parameters were estimated using a custom Markov chain Monte Carlo (MCMC) sampler. MCMC was primarily used as a convenient method of obtaining uncertainty on the full kernel and was fit using three chains at different starting conditions: 5,000 burn-in samples, 5,000 saved samples, and a thinning of 10 (saving every tenth sample to reduce autocorrelation). Thus, the saved posterior consisted of 1,500 total samples. All parameters were fit on the log scale.

D. Model Diagnostics

CEAH analysts checked for MCMC model convergence using the Gelman-Rubin (R-hat) statistic, which checks for low variance in sampling among chains; Heidel diagnostic test, which checks for chain stationarity; and a visual inspection of MCMC chains. Ideally, R-hat will be less than 1.01 for all parameters, though the default check for convergence in the widely used auto JAGS uses 1.05, and the Heidel diagnostic test will pass for all parameters with an epsilon of 0.10. The largest R-hat value was 1.02 and three of the four parameters passed the Heidel diagnostic test with an epsilon of 0.1 (δ with an epsilon of 0.2). A visual inspection of MCMC chains shows acceptable, if less than ideal mixing, especially for the δ parameter (**Figure B1**). For comparison, a three-parameter model formulation not shown had a maximum R-hat of 1.014 and all parameters passed Heidel with an epsilon of 0.1. Posterior fit distributions for the four parameters are shown in **Figure B2**.

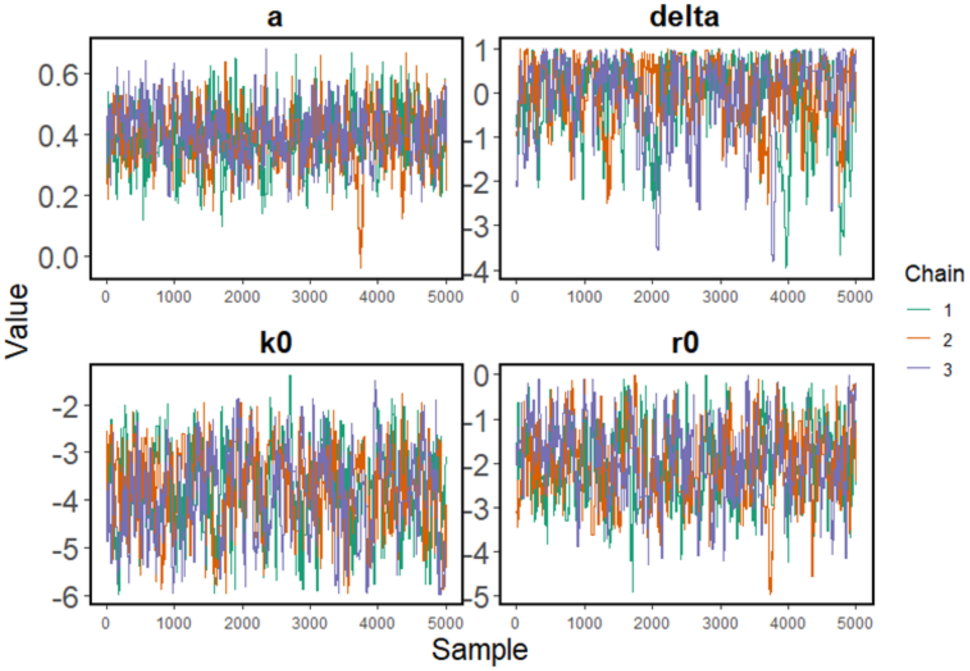


Figure B1. Markov chain Monte Carlo (MCMC) samples. Parameter values shown on the log scale.

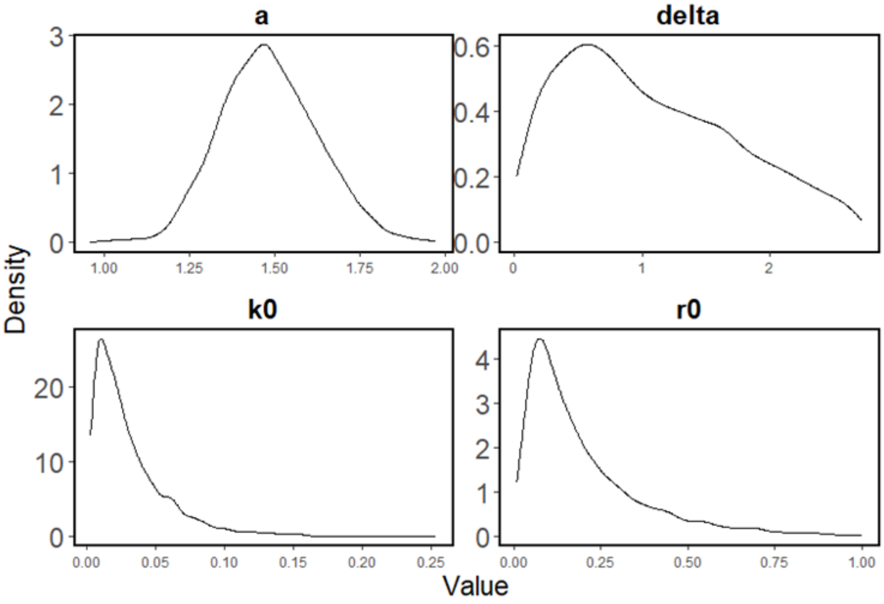


Figure B2. Parameter posteriors. Parameters were fit on the log scale but are presented on the linear scale here.

E. Genomic and Epidemiological Evaluation

It is conceivably possible to reconstruct the full transmission network among dairy and poultry premises using a phylogenetic analysis with complete genomic data paired with a robust epidemiological investigation. However, because phylogenetic analysis had not yet been completed for many dairies and poultry premises at the time of writing and epidemiological data is sparse, a kernel analysis remains a strong candidate model for describing patterns of transmission. The available genomic data and phylogenetic analysis corroborates the predictions of the kernel model in three ways.

Genomic data reveals that isolates from 23 poultry premises share strong full genome sequence similarity with one or more isolates from other poultry premises. Phylogenetic analyses suggest that strong evidence exists for six poultry premises being infected by another poultry premises. Based on realistic timing of infection in these implicated infector-infectee pairs and epidemiological information, such as shared ownership and/or the movement of vehicles, equipment, and personnel among them, it is plausible from an epidemiological perspective that each of these six poultry premises became infected through lateral transmission from another poultry premises, though infection from a dairy remains possible.

The kernel model predicted that susceptible poultry premises, across all premises between August 15, 2024, and December 31, 2024, experienced an average of 5.5 times higher FOI from infectious dairies than from infectious poultry premises. Based on the median across 1,000 simulations using the fitted kernel, simulated epidemics using the kernel predicted 9 lateral poultry-to-poultry premises transmission events and 42 dairy-to-poultry transmission events.

Infected dairies broadly implicated as potential infectors of poultry premises, based on whole genome sequence similarity, were many times physically closer in straight-line distances to infected poultry premises than all non-implicated dairies infectious at the time of new poultry premises infections. Across all infected poultry premises, genomically implicated dairies were 6 times closer than non-implicated dairies based on the median and 49 times closer than non-implicated dairies based on the mean across all possible infector-infectee pairs. After using the non-linear spatial kernel to translate these distances into transmission rates, the probability that infection came from one of the genomically-supported source dairies than other infectious dairies was predicted to between 2.3 and 68 times higher based on the mean and between 1.7 and 7.7 times based on the median.

F. Model Caveats and Future Work

Despite a strong model fit to the data, it may be possible to achieve an improved model fit with a few adjustments and/or extensions.

Analysts assumed that both dairy and poultry premises became infectious the same day that they became infected and remained infectious at a constant level for the duration of their infectious period. However, it is more realistic to consider a lag period as a virus incubates in individual animals and prevalence increases, which would result in a gradual increase, and then decrease as herds/flocks recover in infectiousness over time. These assumptions could be relaxed by fitting across a range of possible values for time from infection to the start of infectiousness and using a variety of "hump-shaped" curves for varying infectiousness over time.

Analysts also assumed a single daily transmission rate from all infectious dairies and poultry premises. This assumption could be relaxed by, for example, allowing premises size to impact transmission rate; however, complete data on premises size would be needed for all dairies and poultry premises, which is not currently available.

This kernel does not allow for long-distance transmission. In California, two poultry premises became infected despite no infected dairies detected and recorded to be infected within 100 km. Though rare in the empirical data, these infections would never be predicted with this kernel model. An additional parameter could be added to this kernel function to allow for some long-distance transmission that does not adhere to the monotonically decreasing kernel.

The current two-step modeling approach does not propagate uncertainty from the fitted dairy infectious period. Fitting dairy infectious period simultaneously with the kernel parameters in a Bayesian MCMC context would allow for a propagation of uncertainty from this parameter. Note, however, that an assumed infectious period for dairies between approximately 23 and 35 days has relatively little impact on maximum likelihood kernel estimates (**Table B1**). This suggests that while this model can give some clarity on dairy infectious period, it is not the most sensible model for estimating this latent parameter with precision.

Table B1. Infectious Period for California Dairies.

Infectious Period (days)	k_0	r_0	a	Negative log likelihood
23	0.0289	0.1416	1.4893	-353.56
27	0.0223	0.1998	1.5877	-349.29
31	0.0256	0.1554	1.5572	-351.73
35	0.0290	0.1223	1.5270	-353.72

This model assumes that infectious period is identical for each dairy. It would be possible to allow infectious period to vary among dairies (e.g., by size). Given high uncertainty in the δ parameter (**Figure 14**, **Figure B3**, **Figure B4**), analysts fit two alternative model specifications to better resolve the δ parameter.

First, fitting a model that allowed each kernel parameter to vary between dairy and poultry premises transmission, resulting in six fitted parameters. Neither maximum likelihood estimates (MLE) nor MCMC fits for this model converged. This fact led to simplifying the model and resulted in the four-parameter model presented here.

Second, fitting an even simpler three-parameter model that excluded δ, thus assuming an equal daily transmission rate from each infectious dairy and poultry premises. Predictions from this model (results not shown) were nearly identical to those presented here, namely due to a similar median estimate between daily transmission rates when δ is included, except with less uncertainty. Analysts feel that assuming an equivalence in daily transmission rate from an infected dairy and an infected poultry premises is a very strong, overly restrictive assumption, and this reduction in uncertainty is more manufactured and underpredicts true uncertainty. For this reason, the model results are presented with δ.

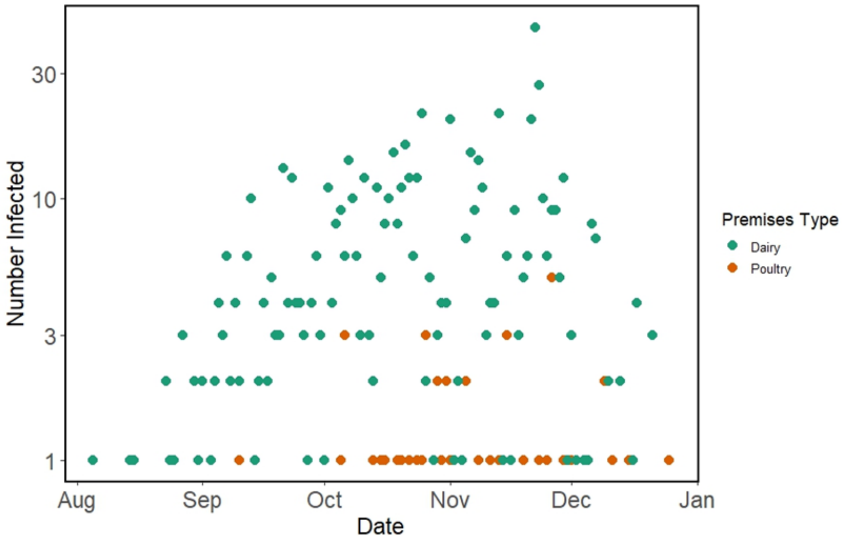


Figure B3. Timing of dairy and poultry infections in California. Timing here is given as date of onset of clinical signs – 10 days.

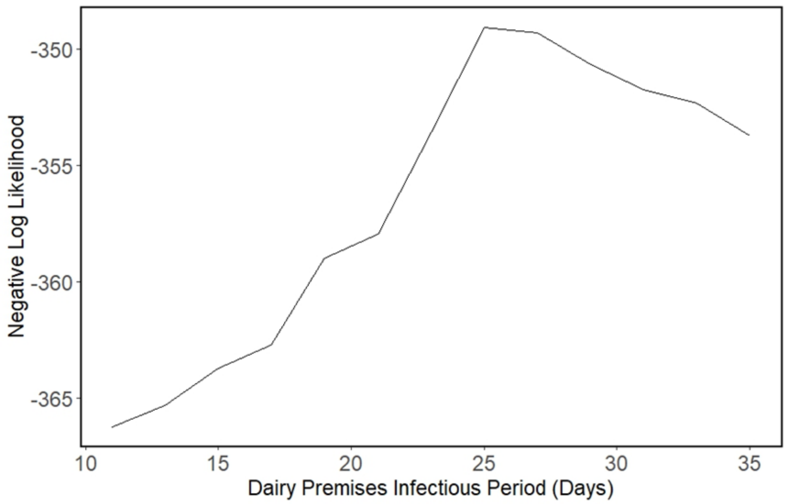
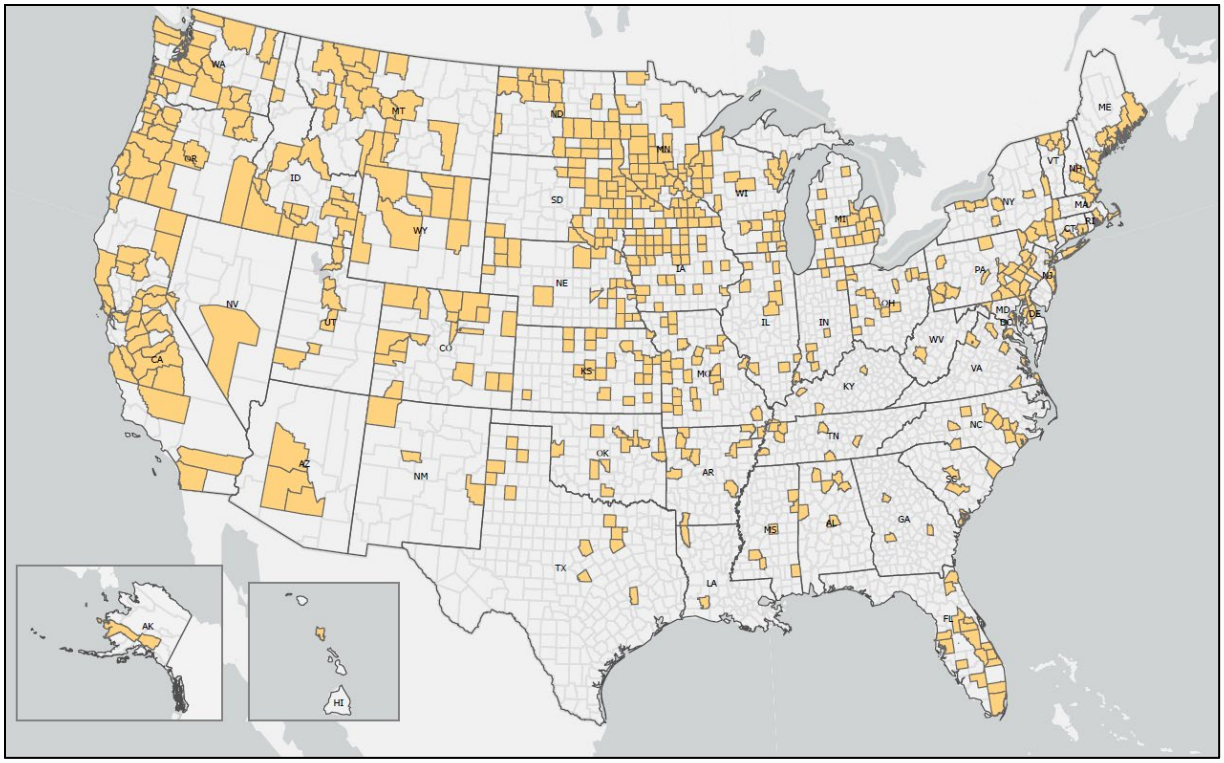


Figure B4. Negative log likelihood from kernel fits using *optim* for different assumptions for the infectious periods of dairies.



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