

**California HPAI Epidemiology Investigation: Final Summary Report**

The 2022-2023 Highly Pathogenic Avian Influenza H5N1 Clade 2.3.4.4b (HPAI) outbreak has been the most widespread Foreign Animal Disease (FAD) incursion event in birds in late US history. Recent National Veterinary Services Laboratory (NVSL) sequencing identified two clusters of California (CA) infected premises (IPs) in Sonoma, and Stanislaus & Merced Counties, in which either common introduction or lateral transmission was the source of HPAI infection.

Epidemiologic interview (EI) questionnaires were reviewed for the IPs of interest. Communications with producers and state and federal responders were also taken into consideration. Wild bird genomic sequencing data was supplied by NVSL. Time of Introduction (TOI) modeling was performed for several IPs to estimate the most likely dates the flocks became infected. Additionally, wind data was collected and analyzed for several IPs, in terms of association with flock clinical onset dates. A genomic epi diagram to aid visualization of lateral transmission suggested by NVSL sequencing was also prepared.

Bird migration data estimates that approximately 792 thousand birds migrated in these three counties per night in early November, about 14 days prior to the initial detections. The estimate for Sonoma County alone was 520 thousand birds per night, a significant number, more than half of the total. NVSL genomic sequencing analyses of wild bird samples found several samples similar to those of the 2 cluster IPs.

Notably, all the IPs that were part of this investigation are in close geographic proximity to others, each premises being in at least 1 Infected Zone of another IP, which has been shown to be a significant risk factor for subsequent infections in previous HPAI studies.

**Security Breaches**

Animal Sanctuary activists were videoed trespassing inside poultry barns and removing ducks on the nights of 10/24/23 and 11/14/23; decreased egg production was noted 8 days after the 2<sup>nd</sup> illegal entry. Given the high number of wild birds migrating through the area at that time shedding virus into the environment, as well as the egregious nature of these biosecurity breaches, it is plausible that virus was introduced into the index Sonoma premises during those incidents. Sequencing from the initial Sonoma detection indicated likely wild bird introduction. Activists posted on Social Media that they also had “rescued” chickens from another poultry premises in the area, which was the second premises where HPAI was detected; no video evidence exists. Whole Genome Sequences of collected samples from both IPs are similar.

**Biosecurity Concerns**

Responders noted biosecurity concerns at all Sonoma IPs and some additional biosecurity risks were documented on EI questioners. Examples: Two Sonoma IPs owned by the same producer, indicated shared personnel and equipment on their EI questioners. On others with shared ownership, sample collection practices were noted as potential biosecurity risks. For example, an individual collected samples at two different IPs on the same day, with one testing non-negative on that day (early detection so signs of disease not evident), and the other, several days later. It’s unknown which premises was sampled first; however, genome sequencing results suggested directionality in agreement with the

detection order. A similar situation occurred in the Central Valley cluster also, and TOI modeling estimates the most likely viral introduction date in the second IP of the pair to be the date of sampling.

In the Sonoma cluster, two premises share Ranch Managers, who were supposed to shower, change clothing, and wait 24 hours prior to entering the next premises; it's not known if this company policy was always adhered to. In another situation, two employees were discovered to be cohabitating which was against the companies' policies. There were also reports that some employees of a subsequently infected premises participated in depopulation and disposal activities on a previously detected IP. The genome sequences from both ranches are closely related.

Other biosecurity concerns such as routine daily activities for layer premises also could have potentially played a role in lateral transmission. Three Sonoma IP's regularly sent nest run eggs to be processed at a fourth IP. These four premises shared a closely related virus with 3 other premises in the area. Similarly, in the Merced cluster, three IPs all had their eggs picked up by the same egg trucks that were reported to go to multiple premises in succession to collect sufficient eggs to make up the total needed for orders. The clinical onset date for one was the day after another IP's on the route. The gene sequences for all three are closely related. Other trucks such as feed delivery, rendering, and trash collection were common to multiple premises. It's not known if these trucks were appropriately cleaned and disinfected between premises. Therefore, they could be one of the contributing factors to the spreading of the virus in the region.

The biosecurity concerns described could have contributed to lateral HPAI transmission. *There is no absolute evidence that any of these risks caused such transmission;* the sequence subcluster IPs could have had common source introductions of virus, given the proximity of the premises. The presence of wild birds was clear with Egrets, Ducks, Geese, and wading birds, reported on the Central Valley farms before the onset of clinical signs. However, as biosecurity risks may lead to disease transmission, it is crucial for improved biosecurity to be implemented in the cluster IPs, particularly the Sonoma premises. All poultry premises should have had enhanced biosecurity protocols in place, but it is not clear that was the case for some of the IPs at the time of detection. Most importantly, producers and employees should understand and implement appropriate biosecurity measures during their normal operation to protect the health of the birds - not just during times of foreign animal disease incursion response.

### **Wild Bird Surveillance**

Wild waterfowl spread avian influenza viruses via oropharyngeal and cloacal shedding as they migrate between Canada and Mexico. Migration of wild birds in the Pacific Flyway has led to the introduction of HPAI in domestic poultry and other birds in CA. As the birds travel long distances during migration, they stop along the way to rest, eat, and drink. Bodies of water and fields with grain and other food sources attract migratory birds to stop and spend time replenishing their energy for the remainder of their journey. Weather patterns, such as storms, can influence wild birds to stay in certain locations to take refuge before continuing further. Increased durations of time that wild birds remain in areas lead to greater quantities of avian influenza viruses being shed into the environment. High environmental levels of avian influenza viruses increase the potential for humans, vehicles, equipment, and other fomites to bring these viruses into poultry premises where proper biosecurity measures are not established. Migratory birds can also transmit avian influenza viruses to other non-migratory wild birds that remain in the area; these non-migratory wild birds can bring viruses into poultry houses, if they are not appropriately excluded.

NVSL routinely sequences poultry, and wild bird samples from USDA-APHIS-Wildlife Services (WS) surveillance, as well as morbidity and mortality samples from a variety of sources. From October through December of 2023, NVSL identified fifty-five wild bird HPAI sequences from CA samples. In the recent *California Overview*, CA wild bird sequences in the predominant genotype. There were several wild bird detections from multiple counties that were genetically similar to the 2 IP Clusters of interest, but wild bird sequences also show genetic diversity across the genotype.

### **Time of Introduction Modeling**

To support the epidemiological investigation of two phylogenetic clusters identified in California, the CEAH-TDA team conducted Time of Introduction (TOI) analysis for individual houses for 13 of the 17 infected premises with available data. By narrowing the time window of possible virus introduction, we can better identify the potential transmission routes and enhance our understanding of the pattern of disease spread. The analysis was performed using a within flock HPAI disease transmission model to fit observed mortality and diagnostic test results. .

### **Wind Effects**

Multiple IP producers shared that they thought that wind brought the virus into their premises from other nearby IPs. For this reason, wind analysis was undertaken for several premises. One Sonoma IP provides a notable example, as it's located directly across the road to the west of another IP that was infected first and the poultry were euthanized during a winter storm in which the prevailing winds switched to come from the southeast. The downwind IP clinical onset was 2 days later. However, TOI modeling estimated that the likely window of virus introduction for the downwind premises to be several days earlier. Because these premises were linked by geographic proximity and business practice, there could have been other opportunities for the virus from one to move to the other besides via wind.

Another example of potential wind contribution mentioned was early transmission to the first infected layer facility in the Central Valley. The commercial layer premises is approximately nine miles downwind from a poultry premises with clinical onset of 11/27/23. Sequences from these two IPs were closely related with potential directionality from the upwind premises to the downwind premises. Winds were from the northwest during the time, and the second premises had a clinical onset noted on 12/9/23, raising the possibility of transmission of virus particles carried by the wind. It's important to note that another commercial premises was located in the wind path between these two premises; and, that commercial premises was not detected with HPAI. Further study into the biosecurity practices of the non-infected premises may provide additional information.

Researchers in the Netherlands studied HPAI H7N7 transmission in 227 poultry premises, using hourly meteorological data including wind speed and direction, as well as statistical modeling. They found the spread of the virus to be correlated with the direction of wind at the date of infection, estimating a potential wind-mediated mechanism contribution of 18% to the total amount of transmission. In 2023, British researchers published a study in which they had collected environmental samples from 3 poultry premises (1 each of duck, turkey, and broiler) and tested for the presence of HPAI H5N1 clade 2.3.4.4b. They found particulate matter containing the infectious virus could be moved on the wind short distances of < 10m, though viral RNA could be moved greater distances of ≤ 80m. Other means of HPAI spread, such as direct wild bird interactions and fomites, play much more significant and well-known roles in viral transmission. For this reason, appropriate biosecurity is essential to prevent viral introduction into

premises in geographic locations nearby detected IPs. More extensive research is needed to elucidate the role that wind may play in transmission of HPAI clade 2.3.4.4b between poultry premises.

### **Recommendations to Improve Future Responses**

Besides looking into what could be learned about potential lateral transmission, the CA State Veterinarian requested that recommendations for the future be included with investigation findings. In addition to improved biosecurity, ensuring that mortality is sampled as a priority is important for early detection. Based on laboratory submission paperwork, it appears that 35% of the submissions were from live birds (including sick) and 20% did not include sufficient documentation to know if mortalities were sampled. Recognizing that the increased testing (multiple times per week) in a Disease Control Area is primarily conducted on healthy flocks, so sufficient mortalities may not be available, it should be noted that sampling of healthy gallinaceous birds when mortalities are available can lead to delayed detection, which could in turn have resulted in higher viral loads in the environment after depopulation, raising the risk of introduction to neighboring premises.

Another recommendation would be for USDA-APHIS-WS to start peri-domestic sampling of wild birds during migration periods in the Stanislaus – Merced area for forecasting and sequencing purposes. WS had no previously designated watersheds in the three Counties of interest; thus, there was no hunter-harvested dabbling duck HPAI surveillance to evaluate environmental contamination during the outbreak. Given that this area is the most poultry-dense region in the State, and premises have been detected positive in both the HPAI 2015-16 and 2022-23 outbreaks; an alternate testing stream of wild waterfowl samples seems prudent.