



## The Occurrence of Another Highly Pathogenic Avian Influenza (HPAI) Spillover from Wild Birds into Dairy Cattle

### Background

In March 2024, the USDA confirmed the first case of highly pathogenic avian influenza (HPAI) spreading between dairy cattle herds in the United States. This followed reports from dairy producers of an unusual illness in their lactating cows over the preceding 2-3 months. Virus whole genome sequencing and modeling performed by USDA suggested a single spillover of HPAI H5N1, clade 2.3.4.4b, genotype B3.13 from wild birds into dairy cattle likely occurred between October 2023 and January 2024 (1). Since then, federal, state, and industry partners have collaborated to address the HPAI threat in dairy cattle, resulting in two federal orders and the implementation of the [National Milk Testing Strategy \(NMTS\)](#).

States began enrolling in the NMTS in December 2024, in which they are continuing to conduct or now implementing state-wide bulk tank surveillance and/or milk processing plant silo monitoring. Nevada was among the first to participate in the National Silo Monitoring Program, which includes testing milk samples from processing plant silos for HPAI. This sampling scheme coincides with the FDA's existing regulatory program, which requires all raw milk Grade A silos to be sampled four times within 6 months.

### The Detection

In Nevada, 3 of 11 silo samples collected on January 6 and 7, 2025 tested positive for HPAI via polymerase chain reaction (PCR) at the National Veterinary Services Laboratories (NVSL) on January 10. The state was notified, triggering an investigation to trace the source, as up to 12 dairies (in the same geographic region) could have contributed milk to the affected silos. On January 17, regulatory officials collected on-farm bulk milk samples from suspected dairy farms and submitted them to the Washington Animal Disease Diagnostic Laboratory (WADDL), a member of the National Animal Health Laboratory Network (NAHLN). HPAI was confirmed via PCR at NVSL on Friday, January 24, in samples from two of those dairies. NVSL completed whole genome sequencing on January 31 and identified HPAI H5N1, clade 2.3.4.4b, genotype D1.1 in samples from four different bulk tanks from one herd. A second herd also showed a partial sequence consistent with D1.1. Clinical signs were not observed in the cattle prior to the detection, but have been reported since, and the affected dairy producers reported large wild bird die-offs near the dairies.

While genotype D1.1 has been the dominant strain circulating in migratory wild birds across all four North American flyways during the winter of 2024-2025, these Nevada cases represent the

first detection of a genotype other than B3.13 in U.S. dairy cattle and the second known spillover from wild birds into lactating dairy cattle.

### **Virus Epidemiology and Origin**

Since late 2021, six separate introductions of Eurasian HPAI H5N1 clade 2.3.4.4b have been documented into the migratory wild birds in the North American flyways (genotypes A1 through A6). Genotype D1.1 is a reassortant of A3. Genotype A3 first appeared in the Pacific flyway in April of 2022 with detections only in the Pacific flyway until the fall of 2024. Since this fall, genotype A3 has been sporadically reported in migratory wild birds across all four flyways through wild bird surveillance, making up 3.3% of the overall detections to date. Genotype D1.1 retains four genes from the original A3 genotype; hemagglutinin (HA), polymerase basic 1 (PB1), matrix (M) and nonstructural (NS), with other genes originating from other North American lineage viruses found in migratory wild birds. This genotype was first detected in September 2024 and has quickly expanded to all North American flyways. D1.1 is the current predominant genotype in migratory wild birds, making up 6.07% of the total detections since 2022 despite first occurring late 2024.

The D1.1 viruses identified in dairy cattle in Nevada were found to be closely related to other D1.1 viruses recently detected in migratory wild birds across multiple North American Flyways. Analysis of the hemagglutinin gene of the Nevada dairy cattle viruses did not identify changes predicted to impact infectivity or adaptation to mammalian hosts. However, a change of PB2 D701N commonly associated with mammalian adaptation of HPAI virus was identified in viruses sequenced from four separate dairy cattle. To date, this change has not been observed in D1.1 viruses found in wild birds or poultry and is not found in B3.13 genotype viruses detected in dairy cattle. PB2 D701N has previously been associated with mammalian adaptation because it improves RNA polymerase activity and replication efficiency in mammalian cells and has the potential to impact pathogenesis in infected mammals (2,3,4,5,6). The change has previously been identified in human cases of HPAI H5 but with no evidence of onward transmission among humans (7,8). No other changes associated with mammalian adaptation were identified in the sequences. Of note, these D1.1 viruses sequenced from dairy cattle do not contain the PB2 - 631L marker that appeared to be fixed in dairy cattle B3.13 sequences. Following the existing public sharing process, NVSL immediately provided the D1.1 sequence information the Centers for Disease Control and Prevention and will post sequence files to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) within 7 days of analysis, adding metadata as sequences are interpreted and quality checked in light of epidemiological information.

## Summary

This detection indicates that this HPAI virus, genotype D1.1, is the second spillover event from migratory wild birds to dairy cattle following the B3.13 event in late 2023/early 2024.

Investigations are ongoing to fully characterize this event. The Nevada Department of Agriculture acted quickly, by first rapidly enrolling in the NMITS to initiate active surveillance, and then to identify and quarantine the affected dairies before cattle movements could further transmit this virus beyond the local area. This is the first instance where sampling of milk at processing plants rather than individually or directly on farm has detected a high consequence disease, demonstrating silo monitoring as an efficient method to monitor HPAI in the National dairy herd.

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