Epidemiological Bulletin: 2024 Highly Pathogenic Avian Influenza (H5N1) Livestock Event – Nevada and Arizona H5N1 D1.1 HPAI Dairy Investigation

Please note: This report is provisional and may be revised at a later date pending further investigation. Data cut-off is April 22, 2025.

The Center for Epidemiology and Animal Health developed this technical brief to give an interim update on highly pathogenic avian influenza spillover events detected in Nevada and Arizona via the National Milk Testing Strategy.

Introduction

APHIS first confirmed highly pathogenic avian influenza (HPAI) H5N1 viral infection in dairy cattle in the United States on March 25, 2024. The introduction of H5N1 in dairy cattle was caused by an initial spillover event from migratory waterfowl, with further virus spread linked to both direct and indirect transmission.

Building on activities taken since the initial detection to minimize disease spread, USDA announced the implementation of a National Milk Testing Strategy (NMTS) in December 2024. The NMTS includes a combination of dairy processing plant silo monitoring and State-specific surveillance, ongoing testing for case investigation and response, and the mandatory testing for interstate movement of lactating dairy cattle (per the <u>Federal Orders</u> issued April 24 and December 6, 2024), to identify states and herds affected with H5N1 and respond appropriately.

In early 2025, through the NMTS, the USDA, the Nevada Department of Agriculture, and the Arizona Department of Agriculture detected HPAI in a silo milk sample On January 31, 2025, the USDA's National Veterinary Services Laboratories (NVSL) confirmed the detection of H5N1 virus as Eurasian lineage goose/Guangdong clade 2.3.4.4b genotype D1.1, in bulk tank milk samples submitted from two Nevada dairies. This represented the first detection of genotype D1.1 in dairy cattle in the United States; all previous dairy detections were the B3.13 genotype.

On February 13, 2025, NVSL confirmed a second detection of genotype D1.1 in dairy cattle in Arizona. NVSL completed whole genome sequencing demonstrating that the Arizona detection was a distinct introduction unrelated to the Nevada detection. APHIS epidemiological teams deployed to Nevada and Arizona to investigate the new findings at the request of the State Animal Health Officials.

The rapid identification of HPAI-positive dairy herds and the subsequent response measures by the Arizona and Nevada Departments of Agriculture highlight the utility of the NMTS as an effective surveillance tool for the national HPAI event in dairy cattle.

Key Findings of Epidemiological Investigations

• Based on whole genome sequencing, the Nevada and Arizona viruses represent separate introductions or spillover events that are genetically distinct from each other *and* previous detections of H5N1 genotype B3.13 in the United States.

- The virus isolated in the Nevada detection contained a mutation at D701N in the PB2 protein, which is known to promote adaptation of influenza A in mammalian hosts. The virus found in Arizona did not contain this mutation.
- APHIS sampled wildlife in both States.
 - In Nevada, APHIS found no direct link between wild birds or other wildlife and the introduction of the virus into cattle; suspecting an unsampled intermediate host. Wildlife surveillance did not provide sufficient evidence to implicate wildlife as a source of lateral viral transmission between dairies. Of 775 combined wildlife samples collected across 6 positive Nevada dairy premises, only 4 were positive. Three of those samples had enough virus to sequence and genetically matched the dairy where they were collected.
 - In Arizona, a wild duck sampled on the affected farm showed infection with a highly similar ancestral virus. An additional sample of pigeon lung was positive; however it did not contain enough virus for genetic sequencing. The remaining 230 surveillance samples from wildlife were negative.
- NVSL whole genome sequencing shows genomically similar virus on multiple farms in both Nevada and Arizona. APHIS identified multiple transmission risk factors during epidemiologic investigations but determined no definitive mode of transmission in Nevada.
 - In Arizona, the virus spread between two farms that function as a single unit, with cattle moving between the farms weekly. An additional neighboring farm that shares equipment but not cattle was also positive for H5N1; with genomics from this premises consistent with common source or lateral transmission.
- In the Arizona epidemiological investigation, APHIS testing of individual cows, pools of cows, and milking string or pen samples indicated minimal disease spread over 21 days after positive bulk milk samples, even in positive pens. Testing of individual cattle in positive pens showed ~2 percent positivity among 327 cattle, despite shared housing and milking equipment.
- H5N1 D1.1 clinical presentation is mild or subclinical when compared to H5N1 B3.13-positive herds.
 - This observed difference in clinical course and clinical signs is based on a small number of D1.1 affected herds and may not be the case in a broader population.
 - The majority of D1.1 impacted dairies reported no clinical signs at the time of the initial detection.
 - In Arizona, the only clinical sign, and only in some individual cows, was decreased milk production.
 However, due to lack of intra-herd spread, HPAI did not result in a decline in milk production for the herd overall.
- The use of bulk tank milk and silo milk sampling as part of the NMTS allowed identification of new spillover events that otherwise might have gone undetected due to lack of or mild clinical signs. This demonstrates the success of the NMTS to bolster passive surveillance and identify additional spillover events into cattle.