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SCIENTIFIC REPORT





Risk posed by the HPAI virus H5N1, Eurasian lineage goose/ Guangdong clade 2.3.4.4b. genotype B3.13, currently circulating in the US

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

The emergence of highly pathogenic avian influenza (HPAI) A(H5N1), clade 2.3.4.4b, genotype B3.13 in U.S. dairy cattle marks a significant shift in the virus' host range and epidemiological profile. Infected cattle typically exhibit mild clinical signs, such as reduced milk production, mastitis and fever, with morbidity generally below 20% and mortality averaging 2%. Transmission within farms is primarily driven by contaminated milk and milking procedures, while farm-to-farm spread is mainly linked to cattle movement and shared equipment. The virus demonstrates high replication in mammary glands, with infected cows shedding large quantities of virus in milk for up to 3 weeks, even in the absence of clinical signs. Shedding through other routes appears limited. Infected cattle develop virus-specific antibodies within 7–10 days, offering short-term protection, though the duration and robustness of immunity remain unclear. Between March 2024 and May 2025, the virus was confirmed in 981 dairy herds across 16 U.S. states, with California particularly affected. Risk factors identified for between-farm spread include cattle movement, shared equipment and contact with external personnel, while biosecurity measures, including waste management and wildlife deterrence, may reduce the risk of virus introduction. In response to the outbreaks, U.S. authorities implemented strict movement controls, mandatory testing and enhanced biosecurity protocols. Potential pathways of introduction of HPAI B3.13 virus into EU via trade from US could be the import of lactating cows and bovine meat, although strict trade regulations, absence of animal import and limited virus detection in meat, especially in muscle tissue, do not support this occurrence. Import of products containing raw milk could also be potential pathways for virus introduction. Migratory birds – particularly waterfowl – pose potential pathways for introduction during seasonal migrations. The detection of mammalian-adaptive mutations and zoonotic cases underscores the virus' public health relevance and the need for research, surveillance and cross-sectoral preparedness.

KEYWORDS

H5N1 genotype B3.13, highly pathogenic avian influenza virus, introduction, outbreak, US dairy cows

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1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

As from late March 2024, the United States of America (US) are facing unprecedented events of detection of highly pathogenic avian influenza (HPAI) virus in bovine animals. US Department of Agriculture (USDA), Food and Drug Administration (FDA), Centres for Disease Control and Prevention (CDC), and state veterinary and public health officials have been investigating the occurrence of infection of dairy cows with HPAI H5N1 virus considering animal health, food safety and public health angles. The virus has spread between herds by animal movements specially for lactating cows, but the most reported spreading is within herds. Milk has been demonstrated to be contaminated with the virus, and the main intra-herd transmission pathway is considered to be the aerosols with milk droplets in milking parlours. Although the cows do not show clinical signs of respiratory disease, they suffer with mastitis and there is a change in the quality of milk. In addition, this virus, which is also occurring in wild birds and poultry (as well as two cases recently detected in pigs), has demonstrated certain zoonotic ability: as of 6 November 2024, 25 mild human cases have been reported in individuals exposed to dairy cows that were infected or presumed to be infected, and one unexplained event without evident contact with animals. However, there has been to date no evidence of human-to-human transmission.

The US veterinary authorities, chiefly USDA-APHIS services, have taken several measures (some regulatory, but mainly advisory and financial) to limit the spread of the virus, still the virus has spread to 15 States so far and is still spreading within few of them. The US federal authorities provided for numerous recommendations on the animal health and public health sides. They are also requiring compulsory pasteurisation of milk of affected or at-risk herds as a risk mitigating measure to prevent the spread of the virus from animals to humans.

The virus type affecting dairy cows in the US (H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b genotype B3.13) has not been reported so far in the EU or elsewhere than USA. The EURL, EFSA and ECDC are closely monitoring this situation. Even if the EU is not currently affected by this virus genotype, it is prudent to consider various potential options for risk assessment of the situation with a view of exploring possible action in animals, notably in dairy cows, ahead of a possible future introduction.

It is relevant to ask support from EFSA and EURL, to analyse the situation in the US and get scientific advice assessing animal health and food safety risks linked with this HPAI strain. The scientific advice should address in particular its likelihood of entry into Europe through trade and migratory wild birds, and if likely, the estimated expected timespan for entry via migratory wild birds, its potential impact on the EU and possible risk mitigation measures.

1.2 | Terms of Reference

In accordance with Article 31 of Regulation (EC) No 178/2002, the Commission requests EFSA to provide scientific and technical assistance on the risk posed by the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13, currently circulating in the US; the following aspects are of particular relevance for the scientific report:

ToR 1a. provide a summary of the virological information currently available on the virus, of the outbreaks in dairy cattle in the US, and the measures that have been recommended or applied by the US authorities;

ToR 1b. describe the potential pathways for entry of the virus into the European continent via trade and via migratory birds, and timelines associated with the potential entry via migratory birds.

1.3 | Interpretation of the Terms of Reference

The scope of the assessment is limited to the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13 that has caused an outbreak in US dairy cattle.

The mandate ToRs were translated into the following assessment questions (AQs) and subquestions (SQs):

ToR 1a:

Assessment question 1:

What virological information is currently available on the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13?

- Genomic characterisation.
- Transmission, incubation period, clinical signs, viremia onset and duration, shedding (how, onset, viral loads, duration), immunity.
- Infectivity and pathogenicity for other hosts.

Describe the outbreak in US dairy cattle caused by the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13.

- How many individuals and herds have been infected by location over time (absolute numbers and proportion of all affected and of all susceptible)?
- What are the age, sex, breed and production system of the infected animals?
- Which risk factors for introduction and spread have been identified?

Assessment question 3:

Which measures have been recommended or applied by the US authorities to control the outbreak in US dairy cattle caused by the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13?

- Which measures to prevent spread have been recommended, where and when?
- Which measures to prevent spread have been applied, where and when?

ToR 1b:

Assessment question 1:

What are the potential pathways for entry of the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13 into the European continent via **trade**?

- a. Which commodities can be contaminated with the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13?
- b. Which of these commodities are traded from the US to the EU?

Assessment question 2:

What are the potential pathways for entry of the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13 into the European continent via **migratory birds** and what are the associated timelines?

- a. What are the migratory bird species in the US that could carry the HPAI virus H5N1, Eurasian lineage goose/ Guangdong clade 2.3.4.4b. genotype B3.13?
- b. What are the direct flyways of these migratory birds from the US to the European continent and when does this migration occur?
- c. Through which indirect pathways (overlaps with other flyways) could the virus be carried by migratory birds to the European continent from the US?
- d. Where could the migratory bird species from the US potentially enter the European continent?

2 | DATA AND METHODOLOGIES

2.1 | ToR 1a Assessment question 1: Virological information currently available on the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13

The collection of evidence to address this question involved an extensive review of online resources, including official government websites and structured media monitoring, and of scientific literature. All studies and reports relating to the H5N1 B3.13 virus have been included.

2.1.1 | Sources

Official web sources that were screened include government press releases, legal documents and publications from the U.S. Department of Agriculture–Animal and Plant Health Inspection Service (USDA-APHIS), United States Department of Agriculture-Center for Veterinary Biologics (USDA-CVB), U.S. Department of Agriculture–National Agricultural Statistics Service (USDA-NASS), Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC), US state authorities the World Health Organization (WHO), and the World Organization for Animal Health (WOAH). Additional insights were obtained through daily monitoring of media retrieved from five key sources: ProMed (Program for Monitoring Emerging Diseases), TIM dashboard, EIOS digest (provided by WOAH), Pulsar (based on social media platform X) and EFSA's Daily Media Review (DMR). This process aimed at capturing events that may not have been reported yet in the official sources, and collect supplementary

data not included in official sources. In addition, a search for peer-reviewed scientific literature was conducted in PubMed and the WG experts were requested to supplement the identified information with additional data they were aware of.

2.1.2 | Search strings used

The terms used for identifying relevant information from official web sources included HPAI and animals. The search string used for the PubMed search is available in Appendix 1. The search considered terms for the key elements of the question: Highly pathogenic avian influenza (HPAI) as 'agent' and animals for 'receptor'. A wide range of term variations and synonyms were included for each element. The daily monitoring of media involved screening five key sources that provide summarised data, which had been previously selected and reviewed by expert panels. These sources filter the information using search strings specifically tailored for avian influenza, incorporating terms such as "avian influenza", "HPAI", "bird flu" and other related keywords. The search strings were designed to capture relevant reports on outbreaks, clinical signs, mortality and control measures. Additionally, the search was conducted across multiple languages to ensure comprehensive coverage of global reports, ensuring that no significant information was overlooked. The key sources included:

- ProMED, which gathers and disseminates infectious disease outbreak reports from official health agencies, media, scientific publications and expert contributions, maintained by the International Society for Infectious Diseases (ISID).
- TIM Dashboard, generated from ProMED reports, which aggregates data on emerging threats.
- EIOS Digest, provided by WOAH, which compiles information related to animal health and emerging diseases.
- Pulsar, based on social media platform X, capturing public and expert discussions.
- EFSA's DMR, which monitors food safety and public health news from media outlets, scientific publications, government agencies and stakeholder reports.

2.1.3 | Study selection process

The screening process of the records identified in official websites and the media considered whether the record contained information about the H5N1 B3.13 virus.

The screening process of records identified in the PubMed database was undertaken in two steps: screening of (1) title and abstract and (2) full-text documents to identify records to be excluded considering whether the record contains information about the H5N1 B3.13 virus. Only English language papers were screened for relevant information.

For the media monitoring, a decision tree was used to validate and ensure the relevance of recorded information for this mandate (see Annex E). The focus of the activity were new or unusual events that were not otherwise captured through usual sources such as the Animal Disease Information System (ADIS) for wild and domestic birds in Europe, and WOAH-WAHIS for all animal species. Data regarding outbreaks in different bird species in Europe are reported to the ADIS, with additional details from online sources recorded in the database when relevant. For mammalian species, all events are documented. Outside Europe, all identified events are recorded except those already reported by the World Animal Health Information System (WAHIS). Relevant preprints of new research on H5N1 B3.13 epidemiology were also included. The media monitoring team met weekly to identify important developments, and to review the reliability of sources and recorded data, keeping the database up to date.

2.1.4 | Data extraction

Data on (1) the genomic characterisation of the H5N1 B3.13 virus, (2) information about transmission, incubation period, clinical signs, shedding and immunity, and (3) infectivity and pathogenicity for other hosts were extracted from the identified sources. In addition, information about the virus collated in previous avian influenza monitoring reports done by EFSA was added. The data identified during the media monitoring were recorded in a database, including outbreak timelines, locations, host species, virus subtypes and production systems. When available, details on affected populations, clinical signs, mortality and control measures were also documented.

2.1.5 | Evidence appraisal

The evidence identified was appraised through collegial discussion within the working group.

2.1.6 | Evidence synthesis

The evidence was synthesised and integrated using a qualitative approach. The working group experts narratively summarised the data, weighing the evidence and discussing the uncertainties and their potential impact. The draft summary

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was shared with the US competent authorities United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for their review and completion, together with a list of questions. Feedback and further information provided by USDA and APHIS was used to finalise the relevant scientific report sections.

2.1.7 | Overall uncertainty analysis

The uncertainty analysis was limited to the identification of the main sources of uncertainty that could affect the scientific assessment process.

2.2 | ToR 1a Assessment question 2: Description of the outbreak in US dairy cattle caused by the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13

The collection of evidence to address this question involved an extensive review of online resources, including official government websites and structured media monitoring, and of scientific literature. Evidence collected included the number of individual animals and herds that have been infected by location over time (absolute numbers and proportion of all affected and of all susceptible), the age, sex, breed, production system of the infected animals, and the risk factors for introduction and spread that have been identified by the US competent authorities. The sources and approaches for data extraction, evidence appraisal and synthesis used were those described in section 2.1.

2.3 | ToR 1a Assessment question 3: Measures recommended or applied by the US authorities to control the outbreak in US dairy cattle caused by the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13

The collection of evidence to address this question involved an extensive review of online resources, including official US government websites and information directly received from US competent authorities, structured media monitoring, and of scientific literature. Evidence collected included the control measures that have been recommended or applied by the US authorities, including the location and the period of time. The sources and approaches for data extraction, evidence appraisal and synthesis used were those described in section 2.1.

2.4 | ToR 1b Assessment question 1: Potential pathways for entry of the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13 into the European continent via trade

To answer this ToR, the WG experts assessed the information collected for ToR 1a and identified which commodities could be contaminated with the HPAI B3.13 virus. To identify which of these commodities could be legally traded from the US to the EU, relevant legislation, including Commission Implementing Regulation 2021/404¹ and Annex XIV to Commission Regulation (EU) No 142/2011,² was reviewed.

2.4.1 | Data collection and sources of data

For the commodities that could be contaminated with the HPAI B3.13 virus and could be legally traded from the US to the EU, data were obtained for the period 2023–2025 from TRACES³ and from EUROSTAT.⁴ The data extracted includes the type of the commodity, the amount traded and the destination in the EU.

2.4.2 | Data check and validation

Data were checked to identify evident mistakes or inconsistencies across related variables. Evident mistakes were corrected after consultation with the EC database managers.

¹https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32021R0404.

²EUR-Lex - 02011R0142-20240711 - EN - EUR-Lex.

³https://food.ec.europa.eu/horizontal-topics/traces_en.

⁴https://ec.europa.eu/eurostat/en/.

2.4.3 | Overall uncertainty analysis

An overall uncertainty analysis was performed based on the identification of the main sources of uncertainty that could affect the scientific assessment process.

2.5 | ToR 1b Assessment question 2: Potential pathways for entry of the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13 into the European continent via migratory birds and the associated timelines

A group of contractors with expertise on migratory birds addressed the questions regarding which migratory bird species in the US could carry the H5N1 B3.13 virus, which direct flyways are used by these migratory birds from the US to the European continent and when this migration occur. They also described through which indirect pathways (overlaps with other flyways) the virus could be carried by migratory birds to the European continent, and where these birds could potentially enter the European continent.

2.5.1 | Data sources

The contractors consulted existing databases and atlases, including the information collected by Cornell University in the online resource 'Birds of the World'⁵ and references therein, as well as family-specific atlases. Species-specific articles in scientific journals were also used for detailed (tracking) studies, but such information was not searched for systematically. The taxonomy and species names of birds by the International Ornithological Committee (Gill et al., 2025) was followed.

2.5.2 | Data needs

For long-distance migratory waterbirds, the general breeding range, non-breeding range and general migration pathways of all long-distance migratory waterbirds known or likely to be carriers of avian influenza viruses was reviewed. Particular attention was paid to areas where contact between waterbirds migrating from North America and waterbirds routinely migrating to and from Europe may most likely occur.

For each species, the contact regions ('North American Arctic', 'Siberia' or 'Africa') were identified, when and how long each species generally stays in these contact regions, via which general route they would reach Europe, and in which part of Europe they would be most likely to arrive, making a general distinction between 'Iceland', 'Scandinavia', 'Eastern Europe' and 'Mediterranean'. Periods of occurrence in either contact zones or Europe were expressed in months.

2.5.3 | Evidence synthesis

The evidence was synthesised and integrated using a qualitative approach. The contractors narratively summarised the data.

2.5.4 | Overall uncertainty analysis

An overall uncertainty analysis was performed based on the identification of the main sources of uncertainty that could affect the scientific assessment process.

3 | ASSESSMENT

3.1 | Virological information currently available on the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13

3.1.1 | Genomic characterisation of HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13

Since the first detection on 25 March 2024 and as of 18 May 2025, 981 outbreaks of HPAI A(H5N1) Eurasian lineage goose/ Guangdong clade 2.3.4.4b genotype B3.13 have been reported in the dairy cattle population in the US. Genomic analyses suggest that a single spillover event from wild birds introduced the virus into the dairy cattle population between October 2023 and January 2024, followed by farm-to-farm spread between dairy premises (Nguyen et al., 2024).

The HPAI A(H5N1) B3.13 genotype is a reassortant virus that was first detected in Colorado on 26 November 2023 in a Canada goose (Caserta et al., 2024; Hu et al., 2024). It contains four genes (PA, HA, NA and MP) from the Eurasian H5N1 genotype and four genes (PB2, PB1, NP and NS) from LPAI viruses of the American bird lineages (Caserta et al., 2024; Hu et al., 2024; Nguyen et al., 2024; Worobey et al., 2024). Assessments of the B3.13 virus sequences in cattle identified multiple mutations (in PB2, HA, NP, NA and NS) associated with increased virulence and adaptation to mammals (Dholakia et al., 2025; Good et al., 2024; Nguyen et al., 2024). Immediately prior to the introduction of the B3.13 virus in cattle, a reassortment event in the NP and PB2 gene segments took place (Guan et al., 2024; Nguyen et al., 2024). Notably, the PB2 M631L amino acid change, a mutation associated with increased adaptation to mammalian hosts, was present in 99% of the sequences from 214 cattle but has only been detected sporadically in birds (Nguyen et al., 2024; Oguzie et al., 2024).

Compared to the sequence of the first HPAI H5N1 clade 2.3.4.4b sequence A/chicken/NL/FAV-0033/2021 detected in Canada, 15 mutations emerged in viruses of genotypes circulating in late 2023 (for example, A2 and B3.6) and were maintained in genotype B3.13 viruses in 2024, including mutations in PB2 (V109I, V139I, V495I and V649I), PB1 (E75D, M171V, R430K and A587P), PA (K113R), HA (T211I), NA (V67I, L269M, V321I and S339P), NP (S482N) and NS1 (C116S) genes (Caserta et al., 2024). Seven additional mutations were detected exclusively in genotype B3.13 viruses, including five substitutions in PB2 (T58A, E362G, D441N, M631L and T676A), one in PA (L219I) and one in NS1 (S7L). When compared with the first reported B3.13 sequences, the cow HPAI H5N1 viral sequences presented five amino acid substitutions, including three in PB2 (E362G, D441N and M631L), one in PA (L219I) and one in NS (S7L), suggesting that these could have emerged following spillover in cattle.

Studies of the distribution of avian and human influenza A virus receptors (sialic acid α2,3 and α2,6) indicate that both receptors are widely expressed in the mammary glands of cows (Kristensen et al., 2024; Nelli et al., 2024; Ríos Carrasco et al., 2024). The receptors were displayed to a smaller extent in the respiratory tract and cerebrum of cows. While two studies found that the avian and human influenza A virus receptors were only significantly expressed in the lower respiratory tract of non-infected cattle (Kristensen et al., 2024; Ríos Carrasco et al., 2024), one study showed that the receptors were displayed in both the upper and lower respiratory tract of cattle (Nelli et al., 2024).

3.1.2 | Transmission, incubation period, clinical signs, viremia, shedding and immunity

3.1.2.1 | Clinical signs

According to a national epidemiologic brief by USDA, the morbidity in the affected dairy cattle herds was less than 20% on average and the mortality and culling was up to 2% on average (USDA-APHIS, online-a). However, the industry reported clinical cases with up to 15% mortality (California Dairy Quality Assurance Program, online; Los Angeles Times, online-a). Clinical signs included reduced milk production and mastitis (with milk of infected dairy cows appearing thickened and discoloured), decreased feed consumption and rumination, lethargy, fever, dehydration, abnormal faeces (tacky or loose) and/or mild respiratory signs (USDA-APHIS, online-a; Baker et al., 2024; Burrough et al., 2024).

In experimental studies, clinical signs in lactating cattle infected with HPAI A(H5N1) virus via intramammary route were consistent with naturally infected dairy cattle (mastitis, fever), while mild respiratory signs such as nasal secretions and coughing were observed in Holstein calves experimentally infected by aerosol inoculation (Baker et al., 2024; Halwe et al., 2024).

Affected dairy cattle from the US outbreaks had clinical signs for up to 21 days (range: 0–21 days, average: 6 days), except for changes in milk (USDA-APHIS, online-a). The milk production was reportedly reduced for up to 45 days (range: 0–45 days, average: 12 days). Experimentally infected lactating cattle displayed clinical signs for 7–14 days and appeared to be recovering from clinical signs 14–24 days after inoculation, while the milk production steadily declined from 1 to 4 days post-infection and remained low until 10–12 days after inoculation and was 71%–77% of the baseline production at 23 days after inoculation (Baker et al., 2024). Similarly, the milk production of infected cattle remained low in another experimental study, where the recovery of baseline milk production ranged from less than 3% to up to 25% at 21 days after inoculation (Halwe et al., 2024). In a recent preprint examining the impact of an HPAI H5N1 genotype B3.13 outbreak in a dairy herd, a cumulative milk loss of 900 kg per lactating dairy cow showing clinical signs was reported over a period of 60 days after the outbreak (Peña-Mosca et al., 2025). The authors found a seroprevalence of 89% in the herd, where 76% of the seropositive cows had been subclinically infected. Moreover, cows with clinical signs had an increased risk of death compared to cows without.

3.1.2.2 | Transmission

According to the USDA, the spread of HPAI A(H5N1) virus between farms within and between States is likely linked to movement of lactating dairy cattle and shared equipment or workers between farms, while within farm spread is considered to be driven by mechanical cow-to-cow transmission, for example through contaminated milking equipment (USDA, onlineb; Le Sage et al., 2024a). A national epidemiologic brief by USDA on affected dairy herds up until August 2024 showed that more than 45% of affected farms continued to move cattle from the farm after clinical signs had been observed, while more than 50% of the affected farms had received new cattle in the herd 30 days prior to onset of clinical signs (USDA-APHIS, online-a). In addition, 34% of affected farms shared vehicles with other farms (of which 50% did not clean vehicles before use), 21% shared workers with other farms and 64% disposed waste milk in the lagoon (of which most was not treated before disposal), all of which could be other potential routes of transmission.

Experimental studies supported that the primary transmission route of HPAI A(H5N1) virus between dairy cattle is milk and milking procedures rather than the respiratory route (Baker et al., 2024; Halwe et al., 2024). This includes the use of shared milking machines, which can act as fomites: raw milk spiked with HPAI H5N1 has been shown to remain infectious on milking machines for several hours (Le Sage et al., 2024b), and can be detected in environmental samples collected from milking parlours (Singh et al., 2024), highlighting the risk of indirect transmission during routine milking. Experimental infection of heifers through the respiratory route was observed, but transmission to sentinel calves did not occur. However, transmission via the respiratory route may be relevant in farms with a high density of animals (Halwe et al., 2024).

Mice studies suggest that the invasion of the mammary gland can occur following intranasal inoculation (Eisfeld et al., 2024). In cows, however, this has not yet been demonstrated. Given the limited viral replication observed in extramammary tissues of infected cows, systemic spread following replication in the respiratory tract, while possible, appears unlikely to be the primary mechanism of mammary gland infection (Halwe et al., 2024).

3.1.2.3 | Incubation period

According to the USDA, the incubation period of HPAI A(H5N1) genotype B3.13 in dairy cattle is 12–21 days at herd level (USDA-APHIS, online-c). A recent preprint study describing an outbreak in a dairy herd reported that the first clinical signs appeared 13 days after introducing seemingly healthy lactating cattle from an affected farm (Peña-Mosca et al., 2025). In an experimental study, intramammary inoculation of HPAI A(H5N1) genotype B3.13 in lactating cows resulted in signs of mastitis (positive California Mastitis Test and changes in milk colour and consistency) 2 days after inoculation and until 14 days after inoculation (Baker et al., 2024). The signs were only present in inoculated quarters. Rumen motility decreased 1 day after inoculation, and milk production declined 1–4 days after inoculation. Another experimental study reported that the first clinical signs (impaired general condition, abnormal posture, lethargy) were observed as early as 1 day after intramammary inoculation of lactating cattle, while the earliest clinical signs (nasal secretion and coughing) were observed 2 days after oronasal inoculation of Holstein calves (Halwe et al., 2024).

3.1.2.4 | Viremia

Viral RNA was sporadically detected by RT-PCR in samples of whole blood (3 of 25 samples) and serum (1 of 15 samples) collected from nine infected farms (Caserta et al., 2024). In comparison, 10 of 47 nasal swabs and 129 of 192 milk samples from the same farms contained viral RNA. Blood samples from heifers and dairy cattle experimentally infected with B3.13 virus were RT-qPCR negative at all time points in the experiment (Baker et al., 2024), and another experimental study reported no evidence of systemic infection (Halwe et al., 2024). The wide expression of both avian and human influenza A virus receptors in the mammary glands of cows combined a high viral load in milk (ranging from 10^{4.0} to 10^{8.8} TCID50 per millilitre, Nooruzzaman, Covaleda, et al., 2025) suggest local replication of the B3.13 virus (Imai et al., 2025; Kristensen et al., 2024).

3.1.2.5 Shedding

Shedding of B3.13 viruses can occur in clinically and non-clinically affected dairy cattle, as well as during the incubation period (Nguyen et al., 2024). Viral RNA has been detected in milk, nasal swabs and urine of infected cattle (Baker et al., 2024; Halwe et al., 2024). While the virus load was lower in nasal swabs and urine samples, the highest concentrations of infectious virus have consistently been found in milk and mammary tissue (Baker et al., 2024; Burrough et al., 2024; Caserta et al., 2024). Spackman, Jones, et al. (2024); Spackman, Anderson, et al. (2024) detected a mean virus titre of 3.5 log₁₀ 50% egg infectious doses per mL, or about 3000 virus particles per mL in bulk tank milk from affected farms. Moreover, the highest viral antigen expression was documented in the mammary gland (Kristensen et al., 2024). Naturally infected cattle may shed viruses for 14–21 days (Nguyen et al., 2024), with onset of shedding 1 day after infection in experimentally inoculated cattle (Halwe et al., 2024). Two experimental studies investigated viral shedding in lactating dairy cattle and yearling heifers infected with B3.13 virus through intramammary and aerosol inoculation, respectively. Baker et al. (2024) detected viral RNA in milk samples from inoculated quarters 1 day after inoculation of dairy cows, and replicating virus was still present in the inoculated mammary gland quarters at necropsy 24 days after inoculation. In addition, samples from inguinal and intramammary lymph nodes were RT-qPCR positive at necropsy. Lung tissue from the heifers was RT-qPCR positive at necropsy 7 days after inoculation. Samples from faeces, blood and other organs from heifers and dairy cattle were RT-qPCR negative at all time points in the experiment (Baker et al., 2024). Virus was isolated from bulk tank samples up to 10 days after inoculation, but no viable virus was found in individual milk samples and pooled bulk tank samples 12 days after inoculation (Baker et al., 2024). Similarly, Halwe et al. (2024) detected high-titre infectious virus in milk from infected cattle for at least 8 days, with a peak of 10⁹ 50% tissue culture infectious dose (TCID₅₀) per ml recorded 2 days after infection. Meanwhile, viral genome was detected in milk up to 20 days after inoculation. For the experimentally infected calves, nasal shedding of infectious virus was observed for a maximum of 8 days (Halwe et al., 2024), and viral RNA was sporadically detected by RT-qPCR in nasal, oropharyngeal, saliva and ocular samples from 1 to 7 days after inoculation (Baker et al., 2024).

Although viral antigen had been detected in lung tissue 7 days after inoculation (Baker et al., 2024), no transmission from infected calves to sentinel calves was observed (Halwe et al., 2024).

3.1.2.6 | Immunity

Two studies examined the development of influenza A virus (IAV)-specific antibodies in experimentally infected lactating cattle and calves. One study found that experimentally infected dairy cows developed NP antibodies 7 days after inoculation (Baker et al., 2024), while another study detected H5 specific antibodies in the milk of dairy cattle 9 days after inoculation, with the maximum level reached 11 days after inoculation and remaining stable for the rest of the study period (Halwe et al., 2024). In addition, IAV-specific antibodies were detected in serum samples with the first signs of seroconversion in dairy cattle 7 days after inoculation and 10 days after inoculation in calves. Neutralising antibodies were recorded in calves at 10–14 days after inoculation, with titres ranging from 1:5 to 1:80, persisting until the end of the study period. In lactating cattle, neutralising antibodies could be detected in milk samples at 9 days after inoculation, with titres ranging from 1:25 to 1:813. Baker et al. (2024) recorded virus-neutralising antibodies in the inoculated mammary gland quarters at the same time as negative virus isolation (12 days after inoculation). There is initial evidence that natural immunity following resolution of the infection is protective against reinfection. Facciuolo et al. (2025) observed that cows reinfected 31 days after first infection did not develop clinical signs.

3.1.3 | Infectivity and pathogenicity for other hosts

The HPAI A(H5N1) B3.13 genotype has been identified in dairy cattle, wild and domestic birds, and mammals, indicating its ability to infect multiple species. Transmission events from infected cattle to wild birds (great-tailed grackles), peridomestic birds (pigeons), domestic birds (chicken), wild mammals (raccoons), peri-domestic mammals (mice), domestic mammals (cats) and humans have been reported on affected cattle premises (Caserta et al., 2024; Nguyen et al., 2024; Uyeki et al., 2024). This has been supported by phylogenetic analyses, however, the limited and incomplete surveillance data currently available constrain the ability to confidently infer the direction and extent of transmission in all cases. High mortalities were recorded in domestic cats, with more than 50% of the cats becoming sick and dying after consuming raw milk from infected cattle (Burrough et al., 2024). Moreover, fatal cases have been described in indoor cats with owners who worked on dairy farms in affected states (CDC, online-a). Affected cats showed clinical signs including depression, stiff body movements, ataxia, blindness, circling and oculo-nasal discharge. IAV antigen was detected in the brain, lung, heart and retina of the diseased cats. The national epidemiologic brief by USDA on affected dairy herds up until August 2024 showed that 80% (12/15) of the affected dairy farms had cats. Among these farms, 58% (7/12) reported observing sick or dead cats (USDA-APHIS, online-a). Additionally, 40% of farms (6/15) had chickens or poultry, and 13% (2/6) of those reported sick or dead poultry. All affected farms had some type of wild peri-domestic birds present near cattle (within 30.84 meters), and 25% of the farms reported sick or dead wild birds within 30 days before observing the first clinical signs in cattle. Small perching birds, pigeons and doves, and blackbirds, crows, cowbirds and grackles were most frequently observed around dairy farms. A review of spillover events of HPAI virus in wild mammals, based on peer-reviewed publications and ENETwild reports, indicated that carnivores are important epidemiological drivers through their predatory and scavenging behaviour, including contact with wild birds and potentially contaminated environments (ENETwild Consortium, 2024).

Mice orally inoculated with a milk sample from an infected cattle herd developed lethargy and ruffled fur on day 1 (Guan et al., 2024). After euthanasia on day 4, high virus titres were detected in respiratory organs (lungs, trachea and nasal turbinate), while moderate virus titres were detected in several other organs (spleen, heart, mammary gland, brain, kidney and liver), indicative of systemic infection in mice.

Pigs are natural hosts to influenza A viruses and considered mixing vessels, i.e. they can be infected by both human and avian influenza viruses (Zhang et al., 2020). A recent preprint demonstrated the experimental oro-respiratory infection of pigs with bovine-derived B3.13 viruses (Kwon et al., 2025). The pigs had no apparent clinical signs during the study period (3–14 days). Infectious virus was isolated from nasal swabs at the earliest 2 days after infection, and oropharyngeal swabs tested positive to the virus at the earliest 1 day after infection (at the limit of detection). From 1 to 5 days after infection, viral RNA was detected at least once in the nasal or oropharyngeal swabs from seven out of nine pigs. Infectious virus was detected in the lower respiratory tract in low concentrations on days 3 and 5 after infection. Based on viral genome sequencing, the mutation HA-S136N which has been associated with increased binding ability of H5 HA to human influenza A virus receptors (α2,6-linked sialic acids) was identified in one pig. Transmission to sentinel pigs was not observed.

Since 2024 and up to 18 May 2025, the U.S. CDC has confirmed 70 human cases of avian influenza. Based on exposure histories collected via epidemiological investigations, the likely source of infection for 41 of these 70 human cases was exposure to affected dairy cattle herds (CDC, online-b). According to CDC, dairy workers in milking parlours and areas with contaminated surfaces may have a particularly high risk of exposure to B3.13 viruses. In this group, the use of personal protective equipment is reported to be low (CDC, online-c). Clinical signs included conjunctivitis and mild respiratory signs such as coughing (CDC, online-c; Garg et al., 2024). The B3.13 virus has been isolated in both conjunctival and nasopharyngeal swabs (Uyeki et al., 2024). In addition, a change in PB2 E627K (a mutation associated with viral adaptation to mammalian hosts) was identified in the virus in human specimens from a case in a dairy worker from Texas in March 2024. At that time, the E627K mutation had not yet been identified in cattle samples, but it was subsequently detected in four dairy cow

11 of 55

herds on 11 March 2025 (Los Angeles Times, online-b). Virus isolated from a human case in Texas was used to infect ferrets in an experimental study (CDC, online-d). The study demonstrated efficient spread of the virus to three out of three ferrets through direct contact with infected ferrets in the same enclosure, and to one of three ferrets via respiratory droplets. The infection caused severe illness in all ferrets with 100% mortality.

3.2 | Description of the outbreak in US dairy cattle caused by the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13

Since the first detection on 25 March 2024 and as of 18 May 2025, the USDA has confirmed a total of 981 cases⁶ of HPAI virus H5N1, clade 2.3.4.4b. genotype B3.13 in dairy cattle in 16 states: 762 herds in California, 64 in Colorado, 36 in Idaho, 31 in Michigan, 27 in Texas, 13 each in Iowa and Utah, 9 each in Minnesota and New Mexico, 7 in South Dakota, 4 in Kansas, 2 in Oklahoma and 1 each in Nevada, North Carolina, Ohio, Wyoming (Figures 1–6).

Despite a comprehensive review of official sources and news reports, detailed information on the reported cases – including age, sex, breed and production system of the infected animals – remains limited. The following sections provide a chronological overview of case occurrences, summarising all available data.

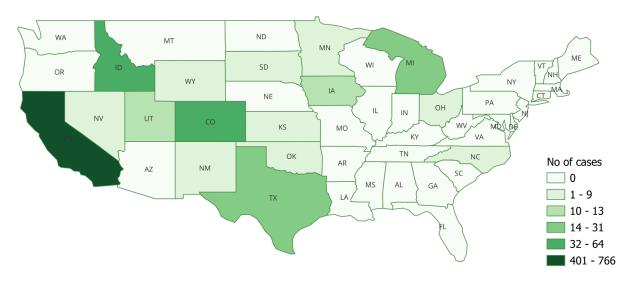


FIGURE 1 Cumulative monthly number of HPAI H5N1 B3.13 reported cases in dairy farms per state in US (25 March 2024 to 30 April 2025).

On 25 March 2024, the USDA, Food and Drug Administration (FDA) and CDC announced the ongoing investigation into an emerging illness primarily affecting older dairy cows in Texas, Kansas and New Mexico. The condition was characterised by unspecific clinical signs such as reduced milk yield, decreased appetite and various other clinical signs. Concurrently, reports from farmers indicated the discovery of dead wild birds on affected cattle farms (USDA-APHIS, online-c). The H5N1 B3.13 genotype of the avian influenza virus was subsequently confirmed in clinical samples from the sick cows, including milk and oropharyngeal swabs. Investigations indicated that wild birds were likely the source of the virus, with the *Eurasian lineage goose/Guangdong clade 2.3.4.4b strain of H5N1* being identified in affected states. On 29 March, Michigan reported its first case of H5N1 B3.13 in a dairy herd that had recently received cows from Texas. The virus strain in Michigan closely matched with that found in Texas and Kansas. By the end of March 2024, the USDA had confirmed 10 cases across farms in Texas, Kansas and Michigan (USDA-APHIS, online-c).

By April 2024, the number of confirmed cases had risen to 26, spanning nine states. The USDA identified several transmission pathways, including spread within herds, transmission from cows to poultry and between dairies associated with cattle movements. Additionally, cases involving cows without clinical signs that tested positive for the virus were reported. The situation raised further concerns when USDA-APHIS microbiologists detected a genetic shift in an H5N1 sample from a cow in Kansas, which could indicate potential adaptation of the virus to mammals (USDA, online-d).

The outbreak expanded to 45 new cases in five states in May 2024 and to 56 new cases in seven states in June, with Wyoming reporting its first case in June. As the number of cases increased, a broader range of clinical signs were observed, including reduced rumen motility, constipation, increased respiratory rate, laboured breathing, nasal discharge, epistaxis, pneumonia, neurological signs, blisters or ulcers, abortions, lameness, fever, dehydration, abnormal manure consistency, thickened or colostrum-like milk, milk discoloration (ranging from grey to yellow) and low-grade fever (Wyoming

⁶According to the USDA definition, a *confirmed case* of HPAI H5N1 clade 2.3.4.4b B3.13 genotype in non-avian species is determined at the NVSL through molecular assay or genome sequencing. The confirmation of an affected dairy herd in a new state requires PCR detection of the virus targeting at least two distinct gene regions, along with genotype determination (USDA-APHIS, online-b). Official reports and management strategies focus on individual farms or herds as the epidemiological units for surveillance, testing and control measures: a 'case' of HPAI H5N1 in dairy cattle is effectively defined as the identification of the virus within a single farm (epidemiological unit). In the context of this report, 'case' and 'outbreak' are used interchangeably.

Department of Agriculture, online; USDA-APHIS, online-a). The American Veterinary Medical Association (AVMA) reported that while avian influenza virus type A (H5N1) caused high morbidity and mortality in birds, it had a lower impact on dairy cattle, with most animals recovering through supportive treatment and a low mortality/culling rate of 2% or less (AVMA, online). The virus appeared to spread between cattle, with animal movement being a recognised risk for transmission between farms. This was also confirmed by the USDA's epidemiological investigation of Michigan dairy herds and poultry flocks, published on 9 June 2024 (USDA, online-j). Based on contact tracing histories, the report indicated that the initial introduction of the disease in the state resulted from interstate animal movement, while in-state transmission between dairy farms likely occurred through indirect epidemiological links associated with routine business operations, including frequent movement of people, vehicles and other conveyances on and off affected premises. The majority of connections between affected dairy farms, as well as between dairy and poultry farms, were indirect, primarily due to shared personnel, vehicles and equipment. According to the USDA, at the time of the epidemiological investigation, there was no genomic evidence indicating that the outbreak in Michigan resulted from a spillover from wild birds. On the contrary, transmission of H5N1 genotype B3.13 from dairy cattle to wild birds has only occurred to resident wild birds on or directly surrounding an affected dairy cattle farm. The H5N1 genotype B3.13 has not been found in migratory wild bird surveillance in the U.S. since the first detection in dairy cattle in March 2024 (USDA, confirmed by e-mail on 19 May 2025). However, the potential spread of the disease through the movement of resident wild birds or other peri-domestic mammal species could not be ruled out.

In July 2024, 40 additional cases were confirmed in eight states, and in August, 19 new cases were reported, including California's first case of the year. The situation worsened in September and October 2024, with 47 and 192 new cases confirmed, respectively, primarily in California, where the number of detections surged, reaching 182 new cases in October. November 2024 saw 254 new cases, with California and Utah continuing to bear the brunt of the outbreak. Although the situation seemed to stabilise somewhat across the U.S. by December 2024, 228 new cases were confirmed, especially in California, Michigan, Nevada and Texas (USDA, online-i).

In January and February 2025, the overall number of new detected cases dropped significantly. According to the USDA, in January a total of 39 new cases of HPAI H5N1 B3.13 were reported from two states, with California continuing to report the highest number of these (38 out of 39 cases). In February, the total number of new detected H5N1 B3.13 cases dropped to 10 (California), while the overall number of affected states remained 16.

In March and April, new cases were detected in California, respectively 11 and 7 cases, while in May no B3.13 genotype cases were detected. Up to 18 May 2025, California has reported the highest number of cases: since the first detection of H5N1 B3.13 on 30 August 2024, a total of 766 farms have been affected. Of these, 613 farms have fully recovered and been released from quarantine (see Section 3.4). According to the CDFA, premises are typically quarantined if they are within a designated Control Area or have business connections to other quarantined premises (CDFA, online-d; CDFA, online-e).

Since 31 January 2025 up to 18 May 2025, the USDA-APHIS National Veterinary Services Laboratories (NVSL) confirmed further detections of highly pathogenic avian influenza H5N1 in 81 farms: 10 in Nevada, 4 in Arizona, 65 in Idaho and two in Texas. However, it is not indicated if these outbreaks were caused by the B3.13 genotype.

The maps in Figure 2 illustrate the cumulative monthly number of HPAI H5N1 B3.13 reported cases over time, while the maps in Figure 3 illustrate the number of the HPAI H5N1 B3.13 reported cases by month.

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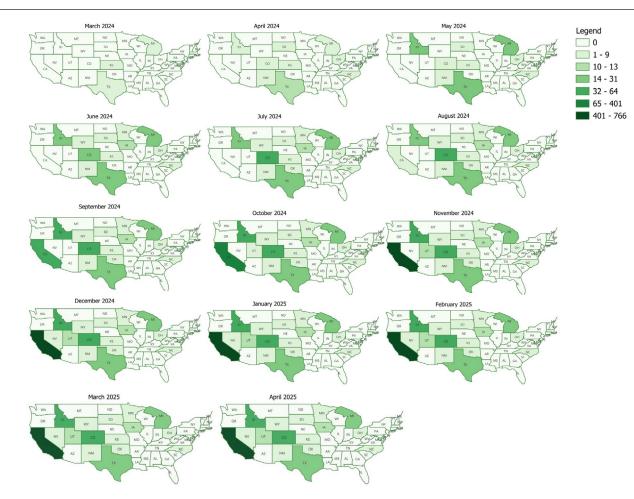


FIGURE 2 Cumulative monthly number of HPAI H5N1 B3.13 reported cases in dairy farms per state in US (25 March 2024 to 30 April 2025).

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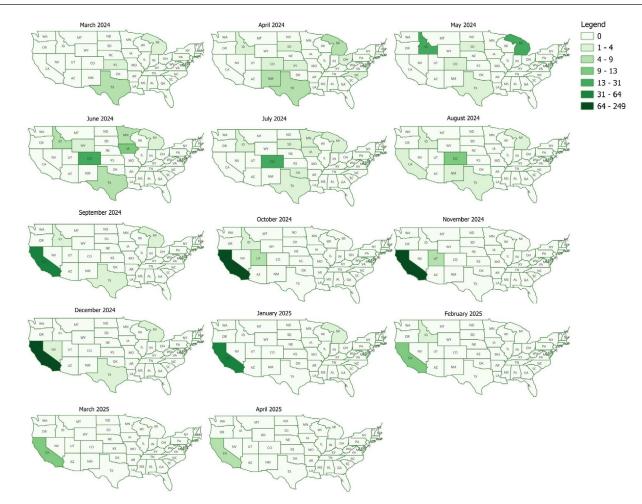


FIGURE 3 Monthly number of HPAI H5N1 B3.13 reported cases in dairy farms per state in US (25 March 2024 to 30 April 2025).

According to the USDA's National Agricultural Statistics Service 2022 Census of Agriculture, the 16 affected states account for approximately 58% of the U.S. dairy cattle population. These states also have the largest average farm sizes in the country. California ranks first in dairy cattle population, with 1,688,202 cattle housed in 1117 dairy farms. The state's average farm size is 1511 animals, making it the second largest in the U.S., after Arizona where the average farm size is 1633 animals. Approximately 69% of California's dairy farms have been affected by the outbreak (Figure 4), which can be explained by the density of dairy farms in the state, and the interconnected relationships between those farms, leading to increased risk for between farm transmission (USDA, confirmed by e-mail on 19 May 2025). According to the CDFA, the cases are concentrated in Central California. According to the USDA's National Agricultural Statistics Service 2022 Census of Agriculture California (USDA-NASS, online), only 3% of the total number of dairy farms in the nation (Figure 5) account for 77% of the reported HPAI H5N1 B3.13 cases in US dairy cattle farms (Figure 6).

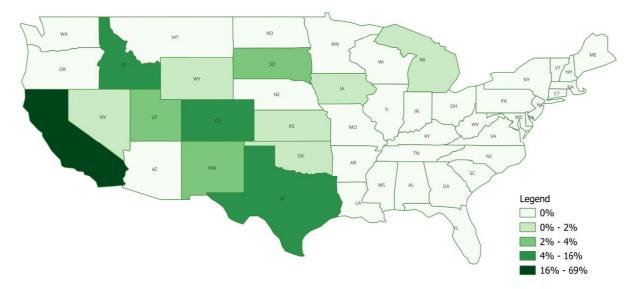


FIGURE 4 Percentage of Affected Dairy Farms by State (Calculated as the number of HPAI H5N1 B3.13 reported cases divided by the total number of dairy farms in the state; cases reported from 25 March 2024 to 30 April 2025).

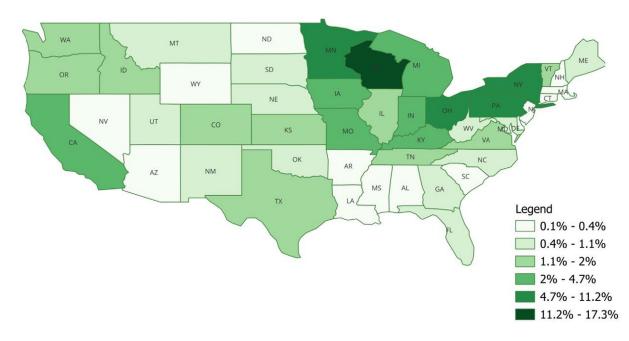


FIGURE 5 Percentage of dairy cattle farms by state relative to the national total (Calculated as the number of dairy farms in a state divided by the total number of dairy farms in the U.S., cases reported from 25 March 2024 to 30 April 2025).

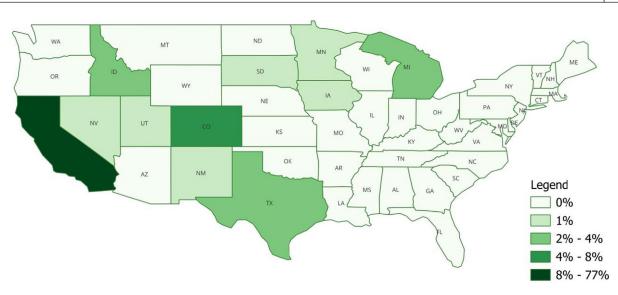


FIGURE 6 Percentage of Total Cases by State in U.S. Dairy Cattle Farms (Calculated as the number of HPAI H5N1 B3.13 reported cases in the state divided by the total number of HPAI H5N1 B3.13 reported cases from 25 March 2024 to 30 April 2025, across all states.)

3.3 | Risk factors for introduction and spread of HPAI virus H5N1, Eurasian lineage goose/ Guangdong clade 2.3.4.4b. genotype B3.13 identified by the US authorities

According to national epidemiologic briefs by USDA on the investigation of the outbreaks in dairy herds up until August 2024 (USDA-APHIS, online-a; USDA, online-a; USDA, confirmed by e-mail on 19 May 2025), risk factors for disease introduction included presence of wild birds in dairy cattle farms (whole genome sequencing and modelling indicate single spillover event of HPAI H5N1, clade 2.3.4.4b, genotype B3.13 from wild bird-origin into dairy cattle likely occurred between October 2023 and January 2024, while in January 2025, two additional spillover events from wild bird into dairy cattle of HPAI H5N1, clade 2.3.4.4b, genotype D1.1 were detected). Risk factors for disease for between farm transmission included, not in order of relevance, the movement of cattle, presence of other animal species on dairy farms (including cats and poultry), presence of wild birds around the farm, vehicles shared with other farms and lack of cleaning prior to use, using the same equipment to handle manure and feed without cleaning prior to use, shared workers between farms or contact with other cattle herds or poultry flocks, and regular visitors with frequent contact with cattle (such as veterinarians, consultants, technicians, hoof trimmers and deadstock, manure or milk haulers). In addition, the USDA indicated that certain management practices may have a protective effect against the introduction and spread of the virus. These management practices included storing feed and bedding in a way that prevents attraction of wildlife, preventing other potential fomites, following best waste management of dairy wastes (including unpasteurised waste milk, process wastewater (from equipment, floors and alleys), flushing water and any waste materials containing raw milk such as manure, slurry, bedding, urine or feed). However, the potential risk factors and potentially protective factors have not been statistically tested.

In June 2024, the Michigan Department of Agriculture invited the USDA to investigate the potential epidemiological link between infected dairy herds and poultry flocks (USDA, online-j). A summary of the epidemiological investigations in 15 affected dairy herds and 8 poultry flocks indicated that the virus was initially introduced into Michigan via movement of cattle (without clinical signs at the time of the movement) from a farm in Texas which later tested positive for the B3.13 genotype. The subsequent spread within Michigan (i.e. between dairy farms with spillover into poultry farms) was considered multifactorial, based on the epidemiological investigations reported in the national epidemiologic brief by USDA. Introduction of the virus into dairy herds and poultry flocks from wild waterfowl was considered unlikely based on genomic and epidemiological analyses. Moreover, shared workers between farms, sharing vehicles between farms without cleaning prior to use and regular visitors with frequent contact with cattle (such as veterinarians, consultants, technicians, hoof trimmers and deadstock, manure or milk haulers) were identified as potential risk factors for within-state transmission in Michigan.

3.4 | Measures recommended or applied by the US authorities to control the outbreak in US dairy cattle caused by the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13

In the following section, the control measures taken by US authorities are presented in chronological order, while an overview of the control measures is given in Table 1.

In response to the reports of an emerging disease in cattle in March 2024, the USDA and individual states implemented measures to prevent the spread of disease between dairy establishments, including movement restrictions for importations of cattle exhibiting clinical signs or originating from affected premises/states, either through state quarantine authorities or herd plan agreements, requiring health certificates of veterinary inspection prior to transportation (including the individual official identification and a statement that the cattle are free from and have not been exposed to a known contagious and infectious disease), testing of dairy cattle prior to interstate movement and testing of dairy cattle intended for import from the affected states, biosecurity measures, exhibition restrictions and quarantine (Table 1). Alongside the legislative measures, the authorities recommended enhancing biosecurity practices on cattle farm level such as limiting visitors, implementing cleaning and disinfection procedures of workers, vehicles and equipment, avoid mixing species, increase awareness of clinical signs of disease and quarantining new animals in the herd (USDA, online-b; USDA-APHIS, online-e). According to the USDA, cattle showing clinical signs compatible with those observed in dairy cows infected with avian influenza should be placed in isolation pens with other affected cattle until the clinical signs are resolved and should not be moved off the premises, although certain movements may be allowed to prevent animal welfare issues and support continuity of business. All animals that need to be moved on/off premises should be isolated for 30 days to prevent the spread of the disease (USDA-APHIS, online-e). In addition, the US authorities implemented measures and recommendations with the aim to protect human health, but those were outside the scope of this report.

On 19 March 2024, before the first outbreak of HPAI A(H5N1) was confirmed in cattle, the states of Idaho and Delaware implemented movement restrictions for cattle from Texas, Kansas and New Mexico due to the detection of health issues in dairy herds linked to reduced feed intake, drop in milk production and changes in milk appearance in these states (Farm Progress, online; no official record). Following the announcement of confirmed cases of HPAI virus in cattle farms, on 26 March 2024, Utah required certificates of veterinary inspection issued 7 days before the importation of lactating dairy cattle from states affected by the emerging disease (Utah Department of Agriculture and Food, online).

In April 2024, the authorities of North Carolina, Delaware, Mississippi, Alabama, Arkansas, Hawaii, Pennsylvania, Maryland, Florida, Oregon and New York ordered that no dairy cattle from affected herds were allowed to enter the states (Alabama Department of Agriculture and Industries, online; Arkansas Department of Agriculture and Arkansas Department of Health, online; Mississippi Board of Animal Health, online; North Carolina Department of Agriculture and Consumer Services, online; Delaware Department of Agriculture, online-a; Hawaii Department of Agriculture, online; Oregon Department of Agriculture, online-a; New York State Department of Agriculture and Markets, online-a; Florida Department of Agriculture and Consumer Services, online-a; Pennsylvania Department of Agriculture, online-a; Maryland Department of Agriculture, online-a). Pennsylvania was the first state to order testing of milk and nasal swabs for influenza A virus (within 7 days of movement, it may vary among States) before the import of dairy cattle from any affected state. According to the 'Interstate and International Quarantine Order' of the Pennsylvania Department of Agriculture, a representative sample of 30 animals per shipment must be tested for Influenza A within 5 days before movement; if fewer than 30 animals are present, all must be tested. Animals must have been kept together for at least 30 days before testing, without new additions; otherwise, all animals must be tested regardless of group size. Milk specimens (for lactating animals) and nasal swabs (for non-lactating animals) must be collected by an Accredited Veterinarian following NVSL guidance. All samples must be tested individually (not pooled) using PCR at a NAHLN laboratory (Pennsylvania Department of Agriculture, online-a). Moreover, Delaware required health certificates signed within 48 hours of entry and quarantine of all imported dairy cattle for 30 days (Delaware Department of Agriculture, online-a), Hawaii and New York State required a statement about examination for HPAI by an accredited veterinarian (it may vary among States) on the health certificates of imported dairy cattle (Hawaii Department of Agriculture, online; New York State Department of Agriculture and Markets, online-a), and all importations of dairy cattle into Arkansas from affected states needed a permission of entry and pre-movement testing by the competent authority (Arkansas Department of Agriculture and Arkansas Department of Health, online).

During April 2024, more states implemented measures to reduce the spread of influenza A virus. The authorities of Nebraska, California, Louisiana, Oregon and Kansas only allowed importations of cattle with health certificates of veterinary inspection and/or a permission from the competent authority (CDFA, online-a; Nebraska Department of Agriculture, online; CDFA, online-b; Kansas Department of Agriculture, online; Louisiana Department of Agriculture and Forestry, online-a), while Oregon also required negative individual or laboratory-pooled PCR test for influenza A virus on milk samples before importation (Oregon Department of Agriculture, online-a). Similarly, Tennessee prohibited the import of any dairy cattle testing positive for influenza A virus, and all adult cattle from affected states needed a permission of entry by the competent authority (Tennessee Department of Agriculture, online-a). West Virginia was reported to require a certificate of veterinary inspection for cattle intended for import from affected states (Farm and Dairy, online); however, no official record was found. According to the American Veterinary Medical Association, the states of Arizona, Idaho and Kentucky increased the requirements for cattle importations as well (American Veterinary Medical Association, online).

Besides the state-specific legislative measures, some dairy farmers have also implemented additional voluntary measures such as limiting visitors, removing trees to discourage wild birds from landing on the property and disinfecting visiting vehicles (Fast Company & Inc., online).

To curb the spread of the disease, the USDA issued a Federal Order effective on 29 April 2024 requiring all dairy cattle to undergo mandatory testing for influenza A virus at an approved National Animal Health Laboratory Network (NAHLN) laboratory prior to any interstate movement and mandatory reporting of any positive Influenza A diagnostic results in livestock (USDA, online-c). The negative test result should be valid for 7 days from the date of sample collection; however, individual states were able set their own validity periods. For cattle moving interstate to exhibitions, shows or sales, with a return to their home herd, the test result could remain valid for up to 10 days (USDA-APHIS, online-f). Additionally, cattle owners had to provide comprehensive epidemiological information, including detailed animal movement tracing. Laboratories and state veterinarians are required to report any positive Influenza A test results to USDA-APHIS. The USDA

further emphasised that only milk from healthy, uninfected animals is permitted for human consumption. Milk from infected cows (identified through testing and/or clinical examination) is diverted or destroyed to prevent any potential contamination of the food supply (USDA, online-d).

Following the USDA Federal Order and guidelines, the states of Idaho and Florida required health certificates of veterinary inspection for imported dairy cattle (Idaho State Department of Agriculture, online; Florida Department of Agriculture and Consumer Services, online-b), and the state of Delaware required testing of dairy cattle intended for importation in addition to veterinary inspection (Delaware Department of Agriculture, online-b). Michigan issued an Order effective on 8 May 2024, stating that all commercial dairy and poultry farms had to implement biosecurity practices, including designating a biosecurity manager, limiting access points, establishing cleaning and disinfection procedures for vehicles and visitors, and keeping visitor logbooks. Additionally, Michigan prohibited exhibitions of dairy cattle of any age from affected herds (even if negative at pre-movement test) and of all lactating dairy cattle until the state has no new cases of influenza A virus in dairy cattle for a minimum of 60 days (Michigan Department of Agriculture and Rural Development, online). Pennsylvania implemented additional restrictions on 9 May 2024, including prohibiting the importation of dairy cattle from farms located within 3 km of domestic birds infected with HPAI, and requiring dairy cattle to be tested for influenza A virus 7 days prior to importation (Pennsylvania Department of Agriculture, online-b).

On 30 May 2024, the USDA announced the start of the 'Voluntary H5N1 Dairy Herd Status Pilot Program' with the final goal to increase monitoring capabilities, increase surveillance and expand knowledge of the disease (USDA, online-e). Enrolment in the Dairy Herd Status Program includes weekly testing of bulk milk samples from the herd and offers dairy producers the option to monitor their herds via bulk milk test rather than individual animal tests as required by the Federal Order for interstate movement. After three consecutive weeks of negative test results, herds with no history of HPAI detection are designated the 'monitored unaffected' herd status, which allows interstate movement of cattle without additional individual testing, beside weekly bulk milk testing, as required under the Federal Order of April 2024. As of 4 February 2025, 80 herds distributed in 16 States are enrolled in the program (USDA, online-f). Once the herd status is established, weekly testing of bulk milk samples (representative of all lactating animals) continues according to herd monitoring plan.⁷

In June and July 2024, the states of Wisconsin, Delaware, New York, Minnesota, Iowa, Tennessee, Oklahoma and Texas implemented exhibition restrictions stating that dairy cattle to be exhibited should have a negative test for influenza A virus 7 days prior to the event (New York State Department of Agriculture and Markets, online-b; Minnesota Board of Animal Health, online-a; Wisconsin Department of Agriculture, Trade and Consumer Protection, online; Delaware Department of Agriculture, online-c; Oklahoma Department of Agriculture, Food and Forestry, online; Texas Animal Health Commission, online; Iowa Department of Agriculture and Land Stewardship, online; Tennessee Department of Agriculture, online-b). Additionally, Delaware and Minnesota required health certificates of veterinary inspection before exhibition, while Iowa and Oklahoma required both health certificates of veterinary inspection before exhibition. For Iowa, the measures to be taken at the exhibition included cleaning and disinfection of milking equipment between milking of cattle from different farms, and having a written protocol describing the measures to take in case a cow develops clinical signs during the exhibition, including the place where cattle showing clinical signs should be isolated. For Oklahoma, the measures to be taken at the exhibition included prohibition of sharing portable milking machines, feed, water and grooming equipment. On 22 July 2024, Colorado ordered weekly bulk tank testing for influenza A virus in dairy herds (Colorado Department of Agriculture, online).

In August 2024, the state of Louisiana followed suit by ordering pre-exhibition testing of milk and health certificates of veterinary inspection from lactating dairy cattle (Louisiana Department of Agriculture and Forestry, online-b). Meanwhile, the state of New York implemented further restrictions for imported non-lactating cattle to have negative test results for influenza A viruses and signed health certificates 3 days before entry into the state (New York State Department of Agriculture and Markets, online-c). In the end of August 2024, the USDA announced the authorisation of a field trial of an A(H5N1) vaccine in cattle. The USDA Center for Veterinary Biologics (CVB) published a notice regarding veterinary biologics product licence applications for veterinary biological products used to vaccinate livestock for HPAI H5N1, clade 2.3.4.4b (USDA CVB, online).

On 16 September 2024, Massachusetts was the first state to complete its testing of all licensed dairy farms, which all tested negative for HPAI H5N1 (Massachusetts Department of Agricultural Resources, online).

Further control measures were implemented in Florida on 4 October 2024, where dairy cattle from both affected and unaffected states were required to have health certificates of veterinary inspection, including negative test results for influenza A (H5N1) virus of lactating dairy cattle within 7 days before entry into Florida (Florida Department of Agriculture and Consumer Services, online-c). Following the measures previously taken in other states, Florida also implemented exhibition restrictions. On 17 October 2024, Arizona ordered, in addition to all lactating cows, also all non-lactating dairy cattle to be tested for influenza A viruses and have a health certificate of veterinary inspection prior to importation. Moreover, all imported dairy cattle should be quarantined for at least 21 days before being included in the herd (Arizona Department of Agriculture, online). In South Dakota, importations of dairy cattle were required to have a permit and a health certification of veterinary inspection (South Dakota Animal Industry Board, online).

⁷https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/livestock/dairy-herd-status-program.

On 20 November 2024, Pennsylvania issued an order for mandatory testing of bulk milk from dairy farms at least once every 2 weeks. Farms testing positive for influenza A virus would be quarantined and the surveillance would continue until the farm is declared safe (Pennsylvania Department of Agriculture, online-c).

On 6 December 2024, USDA-APHIS announced a second Federal Order with additional measures related to raw milk testing and reporting. The order stipulated that any individual responsible for a dairy farm, bulk milk transporter, bulk milk transfer station or dairy processing facility that ships or stores raw (unpasteurised) milk intended for pasteurisation had to comply with a request of APHIS or its designated representatives to obtain a sufficient quantity of raw milk for the purpose of testing for the HPAI virus. Further, laboratories and state veterinarians had to report positive influenza A test results (e.g. PCR and genetic sequencing) in livestock samples, including raw milk, to USDA-APHIS. Additionally, herd owners whose milk tests positive for HPAI had to provide basic epidemiological details, including animal movement information (USDA, online-i). This is also specifically mandated for the HPAI livestock response through the April 2024 Federal Order.⁸

From December 2024 to February 2025, further requirements of testing of bulk milk from commercial dairy farms in the states were implemented in Oregon, Maryland, New York and Minnesota (Maryland Department of Agriculture, online-b; Oregon Department of Agriculture, online-b; Minnesota Board of Animal Health, online-b; New York State Department of Agriculture and Markets, online-d). Following the continuous spread of disease, statewide bans of all dairy cattle and poultry exhibitions were issued in California effective from 8 January 2025 and in Nevada effective from 14 January 2025 until further notice (CDFA, online-c; Nevada Department of Agriculture, online-a, online-b).

As of 6 March 2025, New Mexico, Ohio and Wyoming were the only affected states where no additional measures than the Federal Orders were implemented.

USDA recommends dairy waste should not be land-applied within 5 kilometres of other livestock or poultry premises. Additionally, vehicles transporting untreated dairy waste should avoid travelling near livestock and poultry facilities. Producers are also advised to pasteurise, heat-treat, chemically treat or store dairy waste onsite instead of applying it directly to land. Additionally, raw milk should always be pasteurised before disposal or feeding to calves or other animals (USDA-APHIS, online-d).

TABLE 1 Legislative measures applied by the US authorities to control the outbreak in US dairy cattle caused by the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13. The measures are categorised into biosecurity measures (at the farm or at exhibitions), exhibition ban (of all dairy cattle or of dairy cattle from affected farms), health certification (veterinary inspection of dairy cattle intended to be moved), movement restriction (e.g. ban of cattle movements from affected farms or states), quarantine (quarantine of imported cattle or cattle with clinical signs), testing in the state (testing of bulk milk) and testing prior to movement (testing of milk and/or nasal swabs). Please refer to the details in the text. Measures listed under 'All States' refer to USDA-issued Federal Orders that apply nationwide. Numbers indicate the legislative orders emitted from each state. The states marked in bold have detected B3.13 viruses in cattle.

State	Biosecurity measures	Exhibition ban	Health certification	Movement restriction	Quarantine	Testing in the state	Testing prior to movement	Total
All states						2	1	3
Alabama				1				1
Alaska								0
Arizona			1	1	1		1	4
Arkansas			1	1	1			3
California		1	3					4
Colorado					1	1		2
Connecticut								0
Delaware			3	3	1		2	9
Florida			3	2			2	7
Georgia								0
Hawaii			1	1				2
Idaho			1	2			1	4
Illinois								0
Indiana								0
lowa	1	1	1				1	4
Kansas			1					1
Kentucky				1				1
Louisiana			2	1			1	4
Maine								0

TABLE 1 (Continued)

State	Biosecurity measures	Exhibition ban	Health certification	Movement restriction	Quarantine	Testing in the state	Testing prior to movement	Total
Maryland			1	1	1	1		4
Massachusetts						1		1
Michigan	1	1					1	3
Minnesota			1			1	1	3
Mississippi				1				1
Missouri								0
Montana								0
Nebraska			1					1
Nevada		1						1
New Hampshire								0
New Jersey								0
New Mexico								0
New York			2	1		1	2	6
North Carolina				1				1
North Dakota								0
Ohio								0
Oklahoma	1		1				1	3
Oregon			1	1		1	1	4
Pennsylvania				3	4	1	3	11
Rhode Island								0
South Carolina								0
South Dakota			2					2
Tennessee			1	1	1		2	5
Texas							1	1
Utah			1					1
Vermont								0
Virginia								0
Washington								0
West Virginia			1					1
Wisconsin							1	1
Wyoming								0
Total	3	4	29	22	10	9	22	99

Note: These are the number of legislative measures related to biosecurity implemented in individual states. In general, the USDA and individual states recommend enhanced biosecurity. For lowa, the measures were concerning cattle exhibitions and included cleaning and disinfection of milking equipment between milking of cattle from different farms, and preparing a written protocol describing response in case a cow develops clinical signs during the exhibition, including planned place to isolate cattle showing clinical signs. For Michigan, all commercial dairy and poultry farms had to designate a biosecurity manager, limit access points, establish cleaning and disinfection procedures for vehicles and visitors, and keep visitor logbooks. For Oklahoma, the measures were related to cattle exhibitions and included prohibition of sharing portable milking machines, feed, water and grooming equipment. The graphs below (Figures 7–12) provide a visual representation of the distribution of control measures in relation to the outbreak timeline. Individual affected state graphs depict the progression of outbreaks and the timing of USDA or stateissued orders. The rise in detections across most states appears to be closely linked to the implementation of bulk milk testing or nasal swab testing requirements before cattle movement, whether voluntary or mandatory.

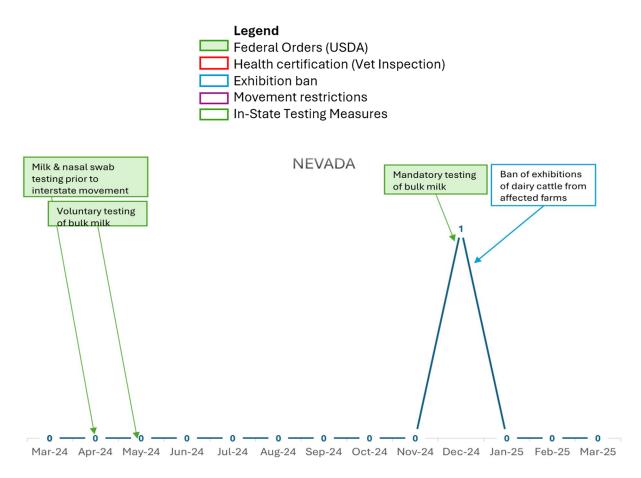


FIGURE 7 Monthly outbreak distribution by state and the timeline of control measures implement.

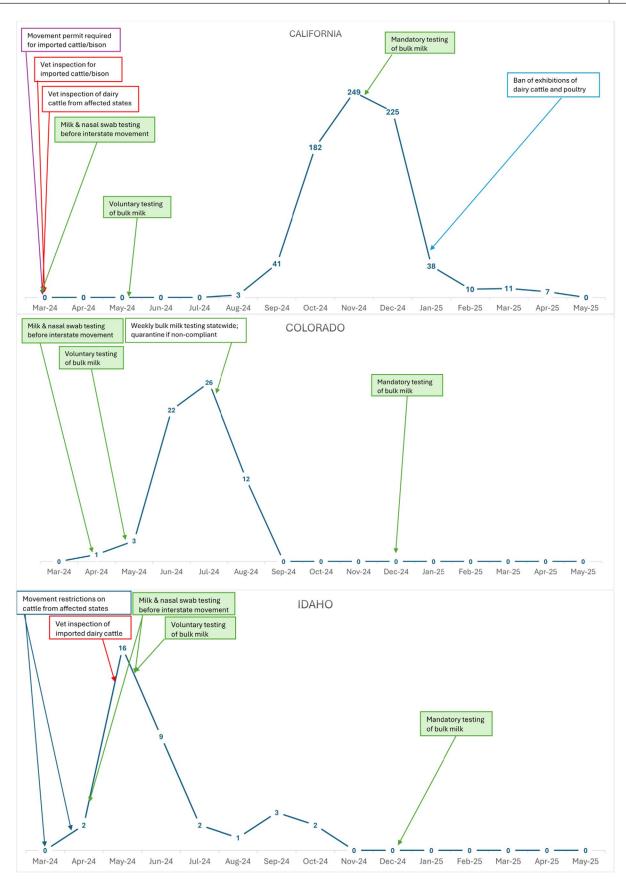


FIGURE 8 Monthly outbreak distribution by state and the timeline of control measures implemented.

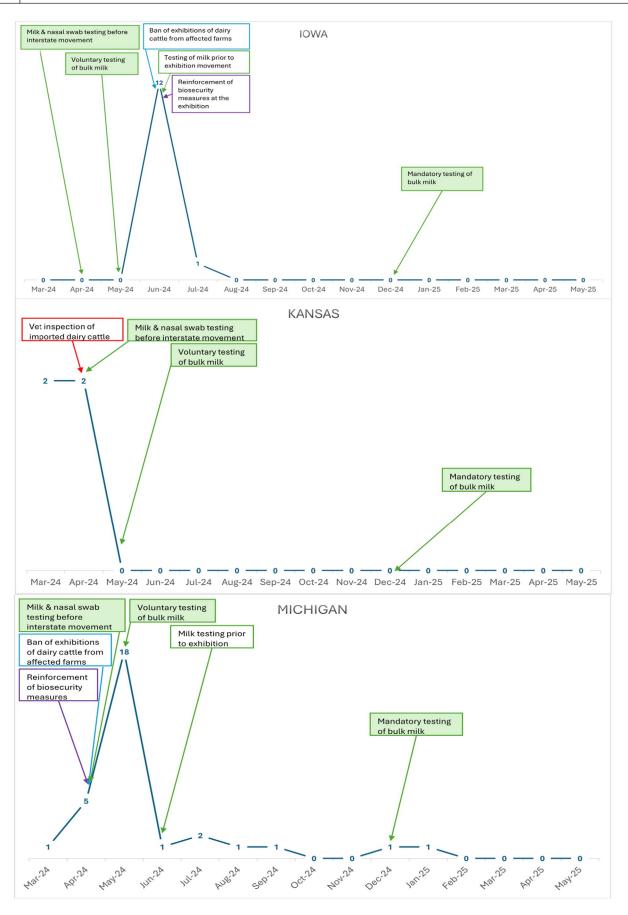


FIGURE 9 Monthly outbreak distribution by state and the timeline of control measures implemented.

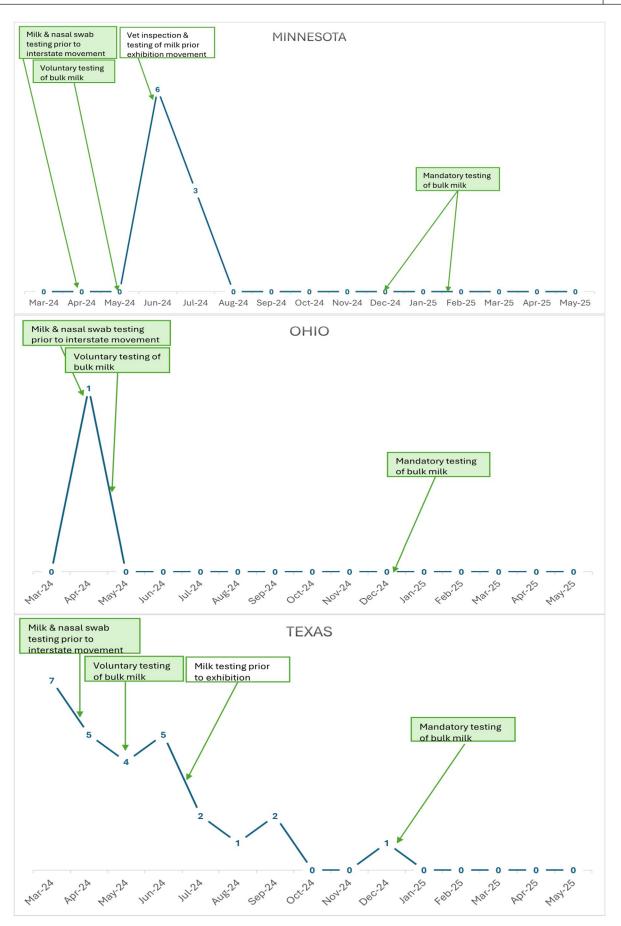


FIGURE 10 Monthly outbreak distribution by state and the timeline of control measures implemented.



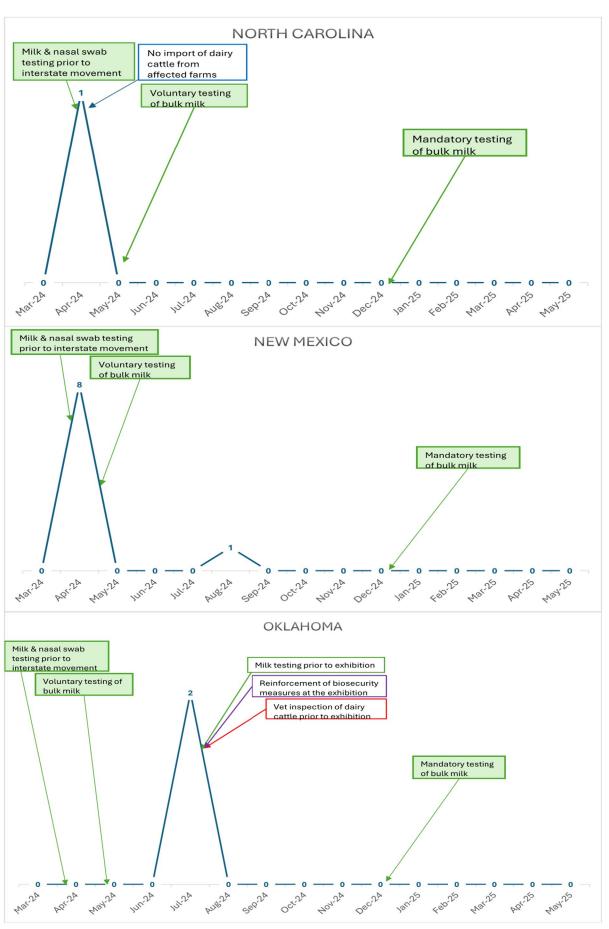


FIGURE 11 Monthly outbreak distribution by state and the timeline of control measures implemented.

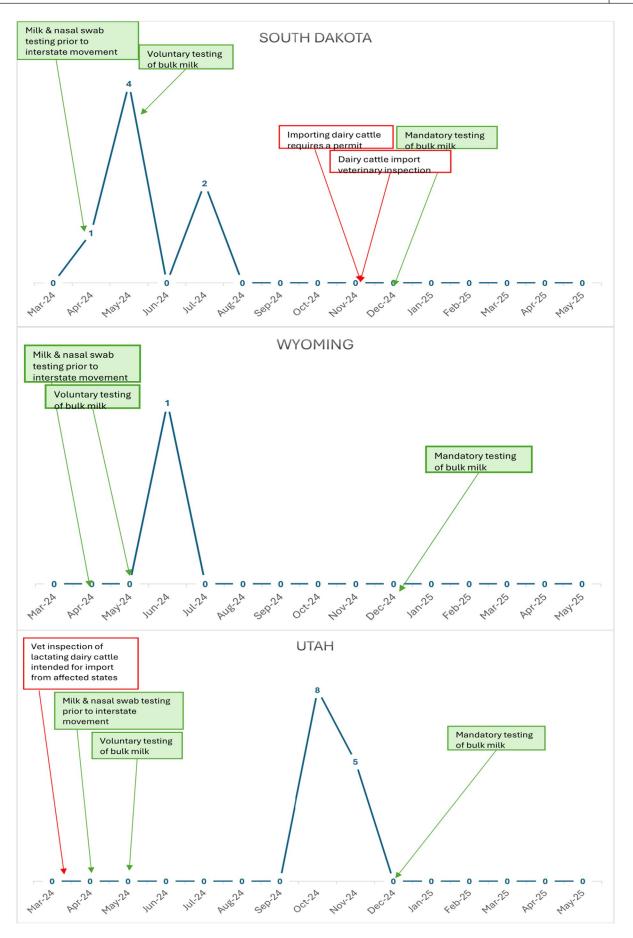


FIGURE 12 Monthly outbreak distribution by state and the timeline of control measures implemented.

3.5 | HPAI outbreaks in poultry in the US

Since the start of the epidemic on 8 February 2022 up to 19 May 2025, 171.55 million birds have been affected. HPAI (subtype not specified) has been detected in 1702 flocks in 51 States (784 commercial flocks, 918 backyard flocks, Figures 13 and 14).

There are more than 378.5 million egg-laying chickens in the United States. In 2023, more than 9400 million heads of broiler chickens and 218 million turkeys were processed in the United States (USDA⁹).

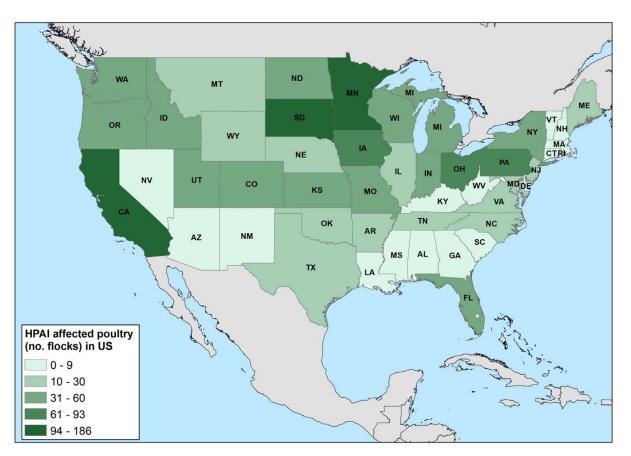
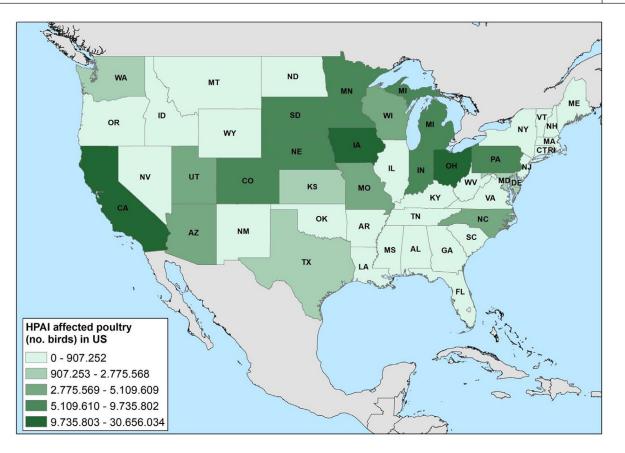


FIGURE 13 Spatial distribution of reported HPAI outbreaks in poultry flocks (commercial and backyard flocks) in US since the beginning of the epidemics, since 8 Feb 2022 up to 19 May 2025.





The sequences of HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13 submitted to GISAID¹⁰ from the US have been reviewed. Since 1st January 2023 until 30 April 2025, 2774 viruses belonging to all H5N1 clades/genotypes identified in poultry species have been submitted from the US to GISAID. Of these, 469 viruses from poultry species (chicken, duck goose, turkey) belong to clade 2.3.4.4b and genotype B3.13 (16.9%) (see Table 2); 339 sequences were from chicken, 112 from turkeys, 17 from ducks, 1 from geese. For several submissions the State of collection as well as the collection date were not available.

TABLE 2	Number of HPAI H5N1 clade 2.3.4.4b genotype B3.13 genetic sequences available on GISAID per infected poultry host species per state				
and year in US (5 May 2025).					

Location	Host	Collection year	Collection month	Total
US (State not specified)	Chicken	2024		74
		2025		9
	Duck	2024		3
		2025		12
	Turkey	2024		20
		2025		1
California	Chicken	2024	10	81
			11	15
			12	76
	Duck	2024	12	1
	Turkey	2024	10	25
			11	2
			12	3
		2025	01	4
Colorado	Chicken	2024	06	4
			07	32
	Goose	2023	11	1
				(Continued)

(continued)				
Location	Host	Collection year	Collection month	Total
Idaho	Chicken	2024	04	1
			05	2
			06	1
			07	1
			09	2
	Duck	2024	09	1
	Turkey	2024	09	3
lowa	Chicken	2024	05	2
	Turkey	2024	06	4
Kansas	Chicken	2024	04	1
Michigan	Chicken	2024	04	9
			05	2
	Turkey	2024	04	5
			05	2
Minnesota	Chicken	2024	05	7
			06	2
	Turkey	2024	04	5
			05	15
			06	18
			07	5
New Mexico	Chicken	2024	04	6
Texas	Chicken	2024	03	8
			04	2
Utah	Chicken	2024	10	2

3.6 Potential pathways for entry of the HPAI virus H5N1, Eurasian lineage goose/ Guangdong clade 2.3.4.4b. genotype B3.13 into the European continent via trade

Commission Implementing Regulation 2021/404¹¹ lays down the lists of third countries, territories or zones thereof from which the entry into the Union of animals, germinal products and products of animal origin is permitted in accordance with Regulation (EU) 2016/429 of the European Parliament and the Council. The commodities listed in the relevant annexes to that Implementing Regulation for bovine animals and for poultry are shown in Table 3.

TABLE 3 Animals, germinal products and products of bovine and poultry origin that can be imported to the EU from the US based on Commission Implementing Regulation 2021/404.

Animals of Bovidae family from confined establishments to confined establishments
Semen
Oocytes and embryos
Semen
Fresh meat
Meat products which have undergone the non-specific risk-mitigating treatment A
Casings
Milk, colostrum, colostrum- based products, dairy products derived from raw milk and dairy products that are not required to undergo a specific risk-mitigating treatment against foot and mouth disease

18314732, 2025, 7, Downloaded from https://efsa.onlinelibrary.wiley.com/doi/10.2903j.efsa.2025.9508 by Cochranettalia, Wiley Online Library on [03/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons

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TABLE 3	(Continued)
Poultry	Specified pathogen-free eggs
	Breeding poultry and productive poultry
	Day-old chicks of poultry
	Poultry intended for slaughter
	Less than 20 heads of poultry
	Hatching eggs of poultry
	Less than 20 hatching eggs of poultry
	Captive birds and hatching eggs of captive birds
	Fresh meat of poultry, ratites and game birds
	Processed meat products of poultry that have undergone the non-specific risk-mitigation treatment A or treatment D for meat products
	Casings
	Eggs and egg products

In addition, a range of animal by-products and derived products from bovines and poultry or other avian species can be imported into the EU from the US, based on Annex XIV to Commission Regulation (EU) No 142/2011¹² (Table 4).

TABLE 4 Animal by-products and derived products from US bovine animals or poultry and other avian species that can be imported into the EU.

Animal by-products and derived products	Countries from which entry is allowed
Blood products for feed material	Third countries or parts of third countries listed in Part 1 of Annex XIII to Implementing Regulation (EU) 2021/404 or Annex I to Implementing Regulation (EU) 2021/405, from which imports of all categories of fresh meat of the respective species are authorised.
Untreated Blood products from ungulates for the manufacture of derived products for uses outside the feed chain for farmed animals	Third countries or parts of third countries listed in Part 1 of Annex XIII to Implementing Regulation (EU) 2021/404 from which imports of fresh meat of any domestic ungulate species is authorised and only for the period indicated in columns 7 and 8 of that Part.
Untreated blood products from poultry and other avian species for the manufacture of derived products for uses outside the feed chain for farmed animals	Third countries or parts of third countries listed in Part 1 of Annex XIV to Implementing Regulation (EU) 2021/404.
Raw petfood	Third countries listed in Part 1 of Annex XIII, Part 1 of Annex XIV to Implementing Regulation (EU) 2021/404 or Annex I to Implementing Regulation (EU) 2021/405, from which Member States authorise imports of fresh meat from the same species and where bone in meat is authorised.
Dog chews	Third countries listed in Part 1 of Annex XIII, Part 1 of Annex XIV or Part 1, Section A, of Annex XV to Implementing Regulation (EU) 2021/404
Animal by-products for use as raw petfood	Third countries listed in Part 1 of Annex XIII or Part 1 of Annex XIV to Implementing Regulation (EU) 2021/404, or in Annex I to Implementing Regulation (EU) 2021/405, from which Member States authorise imports of fresh meat from the same species and where bone in meat is authorised.

For products originating from bovine animals that are imported from the US to the EU, TRACES¹³ and EUROSTAT data has been collected for the period of 1/1/2023–12/3/2025 (TRACES, Annex B) and Jan 2023 – Jan 2025 (EUROSTAT, Annex C).

For each possible pathway, the evidence of potential contamination of the commodity with the virus, the amount traded, and a conclusion regarding the possibility of introducing the virus through the pathway are provided below.

3.6.1 | Pathway 1: Live bovine animals traded from US to EU

3.6.1.1 Evidence of potential virus contamination in the commodity

The trade of live bovine animals from the USA to the EU is regulated by strict animal health requirements to ensure the safety and health of the animals and prevent the transmission of diseases.^{14,15} For entry into the EU, live animals must be

¹²EUR-Lex - 02011R0142-20240711 - EN - EUR-Lex.

¹³https://food.ec.europa.eu/horizontal-topics/traces_en.

¹⁴https://food.ec.europa.eu/animals/live-animal-movements_en.

¹⁵ https://www.aphis.usda.gov/live-animal-export.

accompanied by health certificates signed by an official veterinarian from the country of origin, and they are subject to checks at designated Border Control Posts upon arrival.

3.6.1.2 | Amount traded

In TRACES and EUROSTAT database, there has been no report of exported live bovine animals from USA to the EU in 2023 and 2024. No indirect exports via other countries such as Canada or Mexico have been recorded in Eurostat, while TRACES shows that eight live bovine animals have been imported from Canada in 2023.

3.6.1.3 | Conclusion

As the B3.13 genotype virus can be transmitted from imported lactating cows (see Section 3.1), this is a pathway through which the virus could be introduced, though the trade data does not indicate any introduction during the outbreak period.

3.6.2 | Pathway 2: Fresh bovine meat traded from US to EU

3.6.2.1 | Evidence of potential virus contamination in the commodity

There is limited scientific literature on AIV infection of bovine muscle.

Until October 2024, 192 dairy farms had tested positive for HPAI A(H5N1) (Rodriguez et al., 2024) with further transmission between herds associated mainly with cattle movement. High viral loads have been found in milk, while moderate to low titres have been found in samples from the respiratory and gastrointestinal (GI) tracts. Although most beef in the USA comes from beef herds, spent dairy cattle may also be used, particularly for ground beef, but it is unlikely that this product of second choice is traded abroad.

On May 30, 2024, FSIS announced the final results of its beef muscle sampling of cull dairy cows condemned at select FSIS-inspected slaughter facilities. No meat from these dairy cattle entered the food supply. According to APHIS,¹⁶ a total of 109 muscle samples were collected and tested in May 2024. The samples were analysed by APHIS using PCR to determine the presence of HPAI virus particles. HPAI virus particles were detected in 1 out of 109 muscle samples.

In September 2024, FSIS began testing for H5N1 influenza A virus in muscle samples from culled dairy cows as part of the national surveillance programs for pathogens and chemical contaminants. By 1 January, 2025, FSIS had results from 208 diaphragm muscle samples. Among these, samples from one dairy cow, including its kidney, tested positive for H5N1 influenza A at very low levels (APHIS, 2025). Currently, there is no evidence of clinical signs in non-lactating cattle, and there is a lack of studies of infected beef cattle or surveillance of beef herds for H5N1. Some beef cattle that had been directly exposed to infected dairy cattle, have been found to be antibody positive with no reports of clinical illness (USDA, confirmed by e-mail on 19 May 2025).

An unspecified number of ground beef samples obtained at retail outlets in the States in which dairy cattle herds have tested positive for H5N1 influenza virus were analysed by APHIS using PCR to identify whether any H5N1 virus particles were present. No virus particles were found to be present.¹⁷

Inoculation studies by the US Agricultural Research Service (ARS) on ground beef using a reverse genetics LPAI virus that contained the modified HA gene from a clade 2.3.4.4c HPAIV strain showed that cooking temperatures of 63°C and 71°C completely inactivated the virus, while it was reported that cooking temperatures of 49°C achieved a 3.5 log reduction (Luchansky et al., 2024).

As shown in Section 3.1, udder tissue of infected animals is likely containing a high amount of virus.

3.6.2.2 | Amount traded

According to TRACES and EUROSTAT data, different types of fresh bovine meat are imported to the EU from the US (carcasses, fresh boneless meat, minced meat, salted or dried meat), amounting to around 20,000 tons per year.

3.6.2.3 | Conclusion

Based on the available evidence, HPAIV is rarely found in muscle tissue, but there is uncertainty regarding the representativeness of the populations studied in the experiments regarding the infected US cattle population. Therefore, this is still a potential pathway through which the virus could be introduced into the EU.

Udder tissue of infected animals is likely containing a high amount of virus, therefore, the import of udder tissue, especially if chilled or frozen, is a potential pathway for introducing the virus to the EU.

¹⁶https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/livestock/testing-and-science/meat-safety.

¹⁷https://www.usda.gov/about-usda/news/press-releases/2024/05/24/updates-h5n1-beef-safety-studies.

3.6.3 | Pathway 3: Milk and milk products traded from US to EU

3.6.3.1 | Evidence of potential virus contamination in the commodity

Viable/infectious virus of HPAI H5N1 clade 2.3.4.4b genotype B3.13 has been detected in raw milk samples from bulk tanks from four US states and in a retail sample of raw milk¹⁸ but has, to date, not been detected in retail samples of pasteurised milk or milk products. In their sampling of raw milk from bulk tanks, U.S. Food and Drug Administration (FDA) found 57.5% of 275 samples positive for viral RNA, with 24.8% of those containing infectious virus. Titres ranged from 1.3 to 6.3 log₁₀ 50% egg infectious doses (EID₅₀) per mL with a median titre of 3.5 log₁₀ EID₅₀/mL (Spackman, Anderson, et al., 2024). Viral RNA was also found in 20% of pasteurised retail milk products (N= 297) sampled in April 2024 by the U.S. FDA (Spackman, Jones, et al., 2024) and in 17.4% of the tested retail dairy products, including a variety of cheeses, butter, ice cream and fluid milk (i.e. whole, 2%, 1%, skim and heavy cream) (N= 167) in June–July 2024 (Suarez et al., 2024). Although detectable viral RNA was found in these retail samples, all samples were negative for viable/infectious virus as shown by failure of isolation attempts by inoculation of embryonated chicken eggs.

According to Martin et al. (2024), it is likely that H5N1 would retain infectivity in raw milk until pasteurisation, as in the dairy industry this is usually performed soon after collection to preserve the quality and the virus remains stable for long periods of time at low temperatures. For example, the study by Guan et al. (2024) suggests that infectious virus can persist for more than 5 weeks (observing only a 2 log decline) in contaminated milk when stored at 4°C. This was confirmed by Lenz-Ajuh et al. (2025) keeping milk at 4°C for up to 4 weeks.

Laboratory scale studies did find detectable levels of infectious H5N1 virus after pasteurisation treatments of at least 72°C for 15 s, but these studies did not use continuous flow systems (for example Guan et al. (2024) and therefore do not fully replicate commercial systems. Browne et al. (2025) assume that the amount of virus will be significantly reduced by between 4.4 and > 6 log using standard pasteurisation methods (Low Temperature Long Time and High Temperature Short Time), based on the study by Alkie et al. (2025). The FDA study used a continuous flow system and found no infectious virus after treatment at 72°C for 15 s and estimated that reductions of > 12 $\log_{10} EID_{50}$ could be expected from industry-standard pasteurisation practices (Spackman, Anderson, et al., 2024).

To date there are few studies evaluating the persistence of H5N1 in raw dairy products like cheese. Lenz-Ajuh et al. (2025) evaluated the impact of two important parameters in cheese production, pH and temperature, on infectivity of H5N1 avian influenza viruses. They found that H5N1 HPAIV (B3.13 genotype strain) remained infectious in previously heat-treated milk at 4°C for up to 4 weeks, with a gradual decrease at 21°C and complete inactivation at 37°C after 4 weeks. The virus was stable at pH levels between 6 and 10, while a gradual reduction of the infectious titre was observed when the pH was lowered stepwise from pH 6.0 to 4.0, with no residual infectivity left at pH 4.0. During yogurt production, H5N1 LPAIV was completely inactivated at pH 4.3. In cheese production, the lowest pH reached was between 5.0 and 5.3. Overall, this study suggests that H5N1 avian influenza viruses are largely inactivated during the lactic acid fermentation of raw milk. Nooruzzaman, de Oliveira, et al. (2025) also showed a pH-dependent survival of the virus, with infectious virus persisting throughout the cheese making process and for up to 60 days of aging using a mini cheese model prepared with HPAI-spiked raw milk under pH 6.6 and 5.8; whereas at pH 5.0, the virus did not survive the cheese making process. These findings were validated using commercial raw milk cheese samples, naturally contaminated with H5N1 genotype B3.13 virus, in which infectious virus was detected for up to 60 days of aging.

3.6.3.2 | Amount traded

A number of commodities related to milk and dairy products that may not have undergone sufficient thermal treatment, such as pasteurisation have been imported from the US to the EU during 2023–2025 (TRACES, see Annex B). Eight consignments (899 tons) of 'Milk and cream, not concentrated nor containing added sugar or other sweetening matter, for human consumption' and four consignments of fresh (unripened or uncured) cheese (10 tons), including whey cheese and curd, preserved at frozen/chilled temperature, have been imported over the last 3 years. It is not indicated if any thermal treatment (such as pasteurisation or UHT) has been applied to these commodities.

3.6.3.3 | Conclusion

Milk and milk products that may not have undergone sufficient thermal treatment, such as pasteurisation, that are derived from raw milk from H5N1 infected asymptomatic cows that accidently entered the milk processing plants are a potential pathway for introduction of the virus to the EU. There is uncertainty regarding the efficacy of non-thermal treatments to inactivate the virus due to the limited number of studies performed, and regarding the extent to which the imported milk products contain unpasteurised milk.

3.6.4 | Pathway 4: Bovine semen traded from US to EU

3.6.4.1 | Evidence of potential virus contamination in the commodity

While HPAI virus has been detected in cattle, particularly in dairy cows, there is no evidence suggesting that bovine semen can contain avian influenza virus. The potential role of semen in cattle-to-cattle transmission is currently being investigated (EFSA and ECDC, 2024). Generic biosecurity measures cannot guarantee the absence of H5N1 virus in semen.

3.6.4.2 | Amount traded

According to TRACES data, 12 consignments of bovine semen consisting of 242,488 packages with a total gross weight of approximately 84,740 kg have been imported from the US to the EU during 2023–2025.

3.6.4.3 | Conclusion

No evidence for infection of bulls with virus exists so far, but it is unknown how many studies have been carried out. Infection of bulls would occur through the respiratory tract, which is less efficient than mammary infection. Further, it is not known if this would lead to viremia and secretion through semen. To date, there is no indication that import of semen is a pathway for introduction of the virus to the EU, but there is uncertainty due to the lack of studies.

3.6.5 | Pathway 5: Bovine oocytes and bovine embryos

3.6.5.1 | Evidence of potential virus contamination in the commodity

No evidence that bovine oocytes and bovine embryos of infected cows become infected with the HPAI virus and contain viable virus has been identified.

3.6.5.2 | Amount traded

No imports of bovine oocytes and bovine embryos have been recorded in TRACES for the period of 2023–2025.

3.6.5.3 Conclusion

While no evidence that bovine oocytes and bovine embryos of infected cows become infected with the HPAI virus has been found, due to the lack of studies investigating this pathway, it cannot be excluded that trade with these commodities is a potential pathway for introducing the virus to the EU.

3.6.6 | Pathway 6: Foetal calf serum

3.6.6.1 | Evidence of potential virus contamination in the commodity

Foetal calf serum is usually obtained from the blood of bovine foetuses removed during the slaughter of pregnant cows. Systemic infection (viremia) in the cow may permit transplacental transmission of HPAI. However, no confirmed cases of HPAI transmission to bovine foetuses have been documented to date.

3.6.6.2 | Amount traded

No imports of foetal calf serum have been recorded in TRACES for the period of 2023–2025.

3.6.6.3 | Conclusion

While no evidence currently confirms that there is transplacental transmission and foetuses of HPAI-infected cows acquire the virus intrauterine or become viraemic, due to the lack of studies investigating this pathway, it cannot be excluded that trade with foetal calf serum is a potential pathway for introducing the virus to the EU.

3.6.7 | Pathway 7: Live poultry and poultry meat traded from US to EU

3.6.7.1 Evidence of potential virus contamination in the commodity

Poultry flocks of gallinaceous species exhibit severe clinical signs and high mortality. Infection of Anseriformes flocks may lead to less prominent clinical signs, yet some clinically affected birds will be present in any affected flock. Consequently, poultry infected with (H5N1 B3.13 and other) HPAI viruses can effectively be detected before being exported. For the same reason, infected poultry will most probably be detected and removed before entering a slaughtering line. Still, meat from infected poultry is not likely to enter the food/feed chain in the EU, as evidenced by the lack of transmission events from the US to the EU occurring since 2014.

3.6.7.2 | Amount traded

According to EUROSTAT data, 2 and 3 tons of live poultry consignments have been imported from the USA to the EU in 2023 and 2024, respectively. In relation to poultry meat, 38 and 1421 Kg poultry meat are reported in Eurostat to be imported from US to EU in 2023 and 2024, respectively.

3.6.7.3 Conclusion

Due to the clear and rapid onset of clinical manifestations in infected poultry flocks and the absence of evidence indicating that the H5N1 B3.13 virus behaves differently than other HPAI viruses, trade of live poultry from US is not considered a potential pathway for introduction of the virus into the EU. The certainty regarding poultry meat not being a potential pathway is lower than for live birds due to the possibility that poultry in the initial stages of infection may not always be detected at slaughter.

3.7 | Waterbird flyways as potential pathways for entry of the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13 into the European continent and the associated timelines

In late 2021, H5N1 clade 2.3.4.4b virus crossed the Atlantic Ocean from Europe to North America via migratory birds (Caliendo et al., 2022). The first detections in wild birds in North America were in American Wigeon and Blue-winged Teal in December 2021 (Bevins et al., 2022). Subsequently, it spread through wild birds and commercial poultry over North and South America. Up to now, it has been reported in more than 9000 wild birds belonging to at least 163 species throughout the USA (Nguyen et al., 2024). While genotype B3.13 has been predominantly associated with dairy cattle, it has also been detected in wild birds and poultry. The few known wild bird transmission events were mostly restricted to passerines and pigeons that were collected from premises with cattle where genotype B3.13 was identified (Nguyen et al., 2024). Against this background a study was performed to evaluate the likelihood of HPAI viruses entering Europe via migratory wild birds. The focus was on ornithological data.

The general breeding range, non-breeding range and migration pathways of all long-distance migratory waterbirds known or likely to be carriers of avian influenza were extracted from existing databases and atlases. The primary source was the information collected by Cornell University in the online resource Birds of the World (https://birdsoftheworld. org/) and references therein, as well as family-specific atlases (Delany et al., 2009; Lappo et al., 2012; Madsen et al., 1999). Although many wild bird species may carry influenza viruses, birds of wetlands and aquatic environments such as the Anseriformes (ducks, geese and swans) and Charadriiformes (particularly gulls, terns and waders) have repeatedly been shown to constitute the major natural virus reservoir of avian influenza (Olsen et al., 2006; Webster et al., 1992). In addition, a few seabird species (e.g. Northern Gannet and Leach's Storm Petrel) were also considered, as they are long-distance migrants exhibiting intercontinental movements and/or occur in regions where European-breeding birds may meet conspecifics that breed in North America. Further, at least some species breed in dense colonies where transmission of viruses is likely enhanced and some of these species have been shown to be infected with H5N1 viruses, e.g. gulls which can harbour a greater diversity of AIVs than waterfowl and may have the role of mixing vessels for different lineages of AIVs (Jahid & Nolting, 2025).

The focus of this study is on genotype B3.13, that currently only occurs in North America. Therefore, particular attention was paid on areas where contact between waterbirds migrating from North America and waterbirds routinely migrating to and from Europe may most likely occur. Such areas primarily exist in the Arctic, where many migratory waterbirds breed (Ganter et al., 2013; Smith et al., 2020). Waterbird populations breeding in specific geographic regions often follow similar migratory flyways (Figure 1). Three flyways are distinguished across North and South America (Figure 1, referred to as 'American flyways' in this study). Two of these flyways overlap with the East Atlantic flyway, used by birds migrating to or through Europe, in northeast Canada and northwest Greenland (referred to as the 'North American Arctic' in this study; Figure 2) and one American flyway overlaps with the East Asian/Australasian flyway in west Alaska and east Siberia (referred to as 'Siberia' in this study; Figures 1 and 2). The East Asian/Australasian flyway connects with the East Atlantic flyway (Figure 1) but this route from North America to Europe is less direct. It is however important to bear in mind that the routes

of all flyways in Figure 1 are coarse indications and the borders are thus not rigid, and it is therefore possible that (for some species) there is overlap in Siberia between e.g. the East Atlantic flyway and the American flyways. All migratory waterbirds occurring (i.e. almost always for reproduction) in either the North American Arctic or Siberia (red areas in Figure 2) are of interest because of the possibility of intercontinental transmission of viruses between North America and Europe.

HPAI can potentially also be transmitted across flyways via mixing of birds that use different flyways in African nonbreeding areas after intercontinental transmission from the American flyway in Siberia. If birds using the West Asian/African flyway (Figure 1) get infected, they can carry a virus to the non-breeding areas in Africa, where they in the non-breeding season (September–April) mix with birds using the East Atlantic flyway that can, after secondary infection, carry the virus to Europe during their northward migration in April–May (Figure 2). In this scenario, the route to Europe runs via western or southern Africa (referred to as 'Africa' in this study). Compared with the route via the North American Arctic, where there is direct contact possible between birds coming from the USA and birds wintering or migrating via Europe, the route via Siberia is less direct. The route via Siberia would take secondary transmission in Africa and therefore at least an extra half year because this transmission in Africa would take place in the non-breeding season (September–April) following a first transmission in Siberia in the summer (June–August).

Each waterbird population has its own breeding and non-breeding range between which birds seasonally migrate. While the actual regions where mixing of birds using different flyways occurs is likely relatively small (Figure), the ranges of birds in the literature may not always be up to date or very accurate. Therefore, large areas were considered as potential areas for intercontinental transmission (the red areas in Figure) to avoid missing potential species of relevance.

For each species, the contact regions ('North American Arctic', 'Siberia' or 'Africa') were identified, when and how long each species generally stays in these contact regions, via which general route they would reach Europe and in which part of Europe they would be most likely to arrive (general distinction between 'Iceland', 'Scandinavia', 'Eastern Europe' and 'Mediterranean' (see Annex D. Sites or regions suitable for surveillance of the virus, given these general areas of entry into Europe, are mentioned. Periods of occurrence in either contact zones or Europe are expressed in months. Inevitably, this information is coarse and generalised because of substantial within-species variation in migration routes and timing of migration. Finally, for each species considered it is mentioned whether infections with HPAI (not specifically genotype B3.13) have been reported in the USA by the Animal and Plant Health Inspection Service (U.S. Department of Agriculture) per 11 February 2025 (Figures 15 and 16).

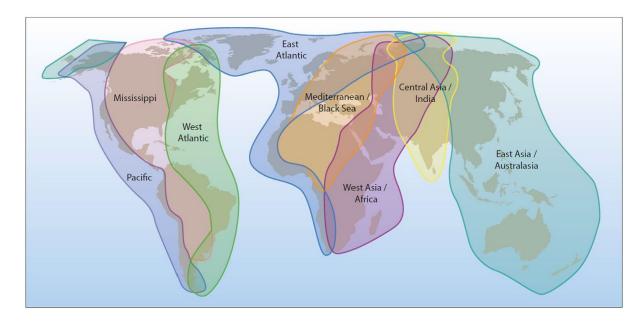


FIGURE 15 The main general global migratory flyways of wild waterbird populations (Source: Ganter et al. 2006, based on information collected and analysed by Wetlands International). Bird migration links Arctic breeding areas to all other parts of the globe. These global migration flyways are generalisations and there are situations where populations behave differently from these common patterns and overlap between flyways may be larger.

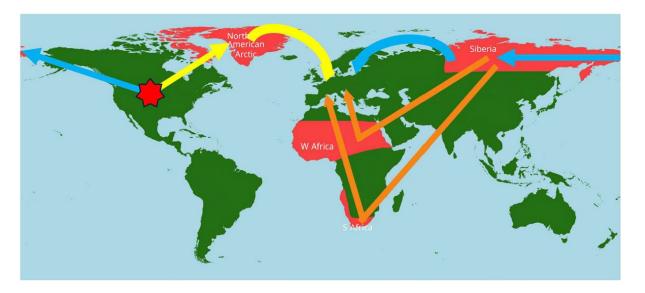


FIGURE 16 A schematic illustration of routes and corresponding seasons via which HPAI viruses can spread from North America (red star) to Europe via wild birds. The main routes will be via the Arctic region, where waterbirds that spend the non-breeding season in the USA migrate to the 'North American Arctic' or to 'Siberia'. The possible routes of the virus via wild migratory waterbirds from the USA to Europe are depicted by yellow arrows (intercontinental transmission via the North American Arctic), and blue arrows (intercontinental transmission via Siberia). A third general route is via secondary transmission in West or South Africa (orange arrows).

In total, 57 waterbird species were considered to possibly be in contact with North American conspecifics (birds of the same (or closely related) species but from a different flyway). These included four colony-breeding seabirds (Leach's Storm Petrel, Northern Fulmar, Northern Gannet and Arctic Tern), three gull species, three skua species, nine goose species, 15 ducks (both freshwater ducks and sea ducks) and 23 shorebirds (Annex D). A few bird species were identified that are not regularly reported in Europe (except as vagrants) but migrate to the North American Arctic where they can be in close contact with species of the same families that are reported in Europe (two ducks: Lesser Scaup, American Wigeon, two geese: Snow Goose, Ross's Goose). Several other species may occur as vagrants, but their occurrence was considered too infrequent for inclusion in Annex D.

Of these 57 species, 36 (57%) occur in the contact zone in the North American Arctic (Figure 14) and could, after infection there in the Arctic summer (typically June–August) directly transport the virus towards Europe during southward migration (August–October in most species). Even more species, namely 40 (70%), (also) occur in the Siberian contact zone (Annex D) and could transport the genotype B3.13 virus towards Europe from the east. Sixteen of the forty species using the Siberian route (40%) could also transport the virus towards Europe via a secondary transmission in Africa (Figure 14). Several seabird species migrate between the Atlantic Ocean and Europe, and thus use different flyways than presented in Figure 1. Purple Sandpipers also use a migration route deviating from the general ones presented in Figure 1 and migrate between Newfoundland (east Canada) and Ireland and Britain (Summers et al., 2024), which is a route between North America and Europe that is considerably further south compared with most shorebird species that cross the icecap of Greenland to the North American Arctic. Typically, the route across the Atlantic will take place via Greenland and Iceland or via seabirds using pelagic routes (Annex D).

HPAI viruses have been recorded in 27 species in the USA (Annex D). This especially occurred in the larger species (nearly all geese and ducks considered), indicating that detectability, particularly the probability of encountering a dead bird, may play a role.

The contact zone for transmission of HPAI viruses from birds using the American flyway to birds that routinely migrate to Europe, lies primarily in the North American Arctic. In the northeastern Canadian Arctic and a small northwestern part of Greenland, birds that have wintered in North America may encounter birds that migrate to Europe after the breeding season, which typically lasts from June to August. The North Atlantic has been shown to be an important corridor for the movement of AI viruses between Europe and North America, where viruses were detected in shorebirds, gulls and waterfowl that belonged to American, Eurasian and mixed American-Eurasian virus lineages (Dusek et al., 2014; Hall et al., 2014). A transatlantic spread of HPAI from western Europe to Newfoundland (Canada) by wild migratory birds has been identified (Caliendo et al., 2022) and, given the annual migrations in both directions between the continents, this could eventually also be the (inverse) route followed by genotype B3.13 from the USA to Europe.

While there is considerable movement of birds between Greenland and Europe (Lyngs, 2003), it is important to keep in mind that birds using the American flyways generally breed on the western side of Greenland and are separated by a large distance and a large icesheet from birds breeding on the eastern side which are part of the East Atlantic flyway and which routinely migrate to Europe. In the northeastern Canadian Arctic (e.g. Ellesmere Island) there is a contact zone of shorebirds and waterfowl that use both the American and East Atlantic flyways, and this high arctic area is the most likely region where HPAI viruses could spread across flyways. Many of the birds migrating from here to Europe routinely use Iceland, the Faroe Islands, Scottish islands and wetlands along the Norwegian west coast to stop over during both northward and southward migration.

North American migratory birds breeding in Alaska or crossing the Bering Strait and reproducing in the East Siberian tundra may encounter bird species in these areas that also use the East Atlantic flyway and migrate to Europe and/or Africa after the breeding season, generally in August–October and would arrive via Scandinavia or eastern Europe. Although the route via Siberia contains more species with a connection to Europe than the North American route (Table 5), these Siberian-breeding birds usually have a wide breeding range from west to east Siberia and birds breeding in the eastern parts of this range are more likely to migrate via the East Asia - Australasian flyway to Asian and Australian non-breeding areas, the Central Asian flyway and the East African/West Asian flyways (Figure 14). Birds using the latter flyway will, in the non-breeding season (typically lasting from September to April) in West and South Africa, occur in potential contact with birds routinely migrating between Africa and Europe via the East Atlantic flyway and Black Sea/Mediterranean flyways. Consequently, a spread of HPAI from North America to Europe via the Siberian route will likely take considerably longer (i.e. a few years) than the American route because it is more likely to occur via repeated infection events and across multiple breeding seasons, slowly spreading westwards in the Siberian arctic until it arrives in the Siberian-breeding areas of birds routinely migrating to Europe, or via African non-breeding grounds. Birds using the route via Africa would arrive in Europe from the south (Mediterranean) in spring (March–May), but many species make direct non-stop flights and arrive in any European wetlands after northward migration from Africa.

Given that transmission of HPAI viruses from North American birds to Europe is more likely and faster via the American route than via the Siberian and indirect routes, surveillance enabling an early detection in Europe should preferably be focused on areas with high concentrations of waterbirds in Iceland, Britain and Ireland, and western Scandinavia (Norway). It should be noted though that especially shorebirds, that annually migrate between Europe and the North American Arctic can make direct long-distance migratory flights between the European mainland and Greenland or northeast Canada (e.g. Kok et al., 2020; Reneerkens et al., 2020) and can arrive in any European wetland. Large wetlands, such as the international Wadden Sea, where large congregations of birds of multiple species flock together on high tide roosts, would then be a suitable location for surveillance of HPAI viruses too.

Transmission is most likely in areas where birds congregate in high densities. In seabirds, this is most likely occurring in breeding colonies, whereas coastal shorebirds are more likely to be close to conspecifics at high tide roosts and ducks and geese at communal feeding and roosting areas and during post-breeding moult. The considered seabirds occur in pelagic areas on the Atlantic where they may encounter (infected) conspecifics from North America, but in these areas on the open ocean, they usually occur in relatively low densities and less often get close to other birds, except for skuas that chase other seabirds to steal regurgitated food from them.

In the most direct, and perhaps the most likely, North American route, but also via the Siberian route, birds infected in the Arctic would appear in Europe after 'southward' migration when the Arctic breeding season has ended. They would typically appear in Europe in large numbers in August–September (shorebirds) or September–November (ducks and geese). Species that deserve special attention as they can be relevant for early detection of an HPAI virus introduction from the US to the EU, based on their migratory movements, are shown in Table 5.

Species' vernacular names	Route	Period in Europe	European entry location	Remarks
Brant Goose	Siberia, America	Year-round	Iceland, Scandinavia	Populations migrating from Greenland to Iceland in autumn most relevant; in winter in wetlands and agricultural areas mainly near coasts
Barnacle Goose	America	Year-round	Iceland	Populations migrating from Greenland to Iceland in autumn most relevant; in winter in wetlands and agricultural areas
Pink-footed Goose	America	Oct-May	Iceland	Populations migrating from Greenland to Iceland in autumn most relevant; in winter in wetlands and agricultural areas
Greater White- fronted Goose	Siberia, America	Sep-Apr	Siberia, Iceland	Subspecies <i>flavirostris</i> migrating from Greenland to Iceland and Britain and Ireland in autumn most relevant; in winter in wetlands and agricultural areas
Ruddy Turnstone	America, Siberia, Africa	Year-round	Iceland, Scandinavia, Mediterranean	Mostly a coastal shorebird
Red Knot	America, Siberia, Africa	Aug-May	Iceland, Scandinavia, Mediterranean	Mostly a coastal shorebird
Arctic Tern	Siberia, America, Africa	Apr-Aug	Atlantic	Mostly pelagic in the non-breeding season
Black-legged Kittiwake	America	Year-round	Atlantic	Mostly pelagic in the non-breeding season
Northern Fulmar	America	Year-round	Atlantic	Mostly pelagic in the non-breeding season

TABLE 5 Relevant species for early detection of HPAI virus introduction into the EU.

3.8 | Uncertainty analysis

Table 6 lists the sources of uncertainty that could impact the outcome of the scientific assessment related with the assessment inputs and methodology that were identified.

ToR	Source of uncertainty	Impact on the assessment
1a	The search of relevant peer-reviewed scientific literature was limited to articles in English	Relevant information could be missed
1a	The decision tree used to evaluate the relevance of media news could misidentify relevant information	Relevant information could be missed, or non- relevant data wrongly included
1a	Lack of data and underreporting of infection in cattle in the US could lead to data gaps related with transmission, incubation, clinical signs, shedding and immunity in cattle	Estimates for transmission, incubation, etc. could be over/underestimated
1b	Evidence available was assessed by a limited number of experts	The potential of certain pathways for introduction of genotype B3.13 could be over/underestimated
1b	Only data from commodities legally traded from the US to the EU were considered	The potential of certain commodities for introduction of genotype B3.13 could be underestimated
1b	The possibility of infected poultry entering the food/feed chain cannot be ruled out completely	The potential of poultry meat for introduction of genotype B3.13 could be underestimated
1b	Only trade data from the 2023 to 2025 period was considered in the assessment	If trading patterns change the potential of certain commodities for introduction of genotype B3.13 could be over/underestimated
1b	Little evidence on the distribution of genotype B3.13 in cattle tissue and germinal products and on the efficacy of several processing steps for milk and milk products exist	The potential of certain commodities for introduction of genotype B3.13 could be over/ underestimated
1b	Analysis of the overlap of migratory routes was based on certain limits of the main flyways even though for certain species these limits may not be fully applicable and thus additional overlap between routes may exist	Additional indirect routes for introduction of genotype B3.13 through migratory birds could be possible

4 | KEY MESSAGES

Virological information

- Clinical impact: Infected dairy cattle typically show a range of mild clinical signs such as reduced milk production, mastitis, fever, lethargy and digestive issues. Clinical signs can last up to 21 days, with reduced milk production for up to 45 days. Morbidity in dairy cattle herds is less than 20%, while mortality is usually low, on average 2%, with few outbreaks reporting up to 15%.
- Transmission pathways: The primary route of HPAI A(H5N1) within-farm transmission among dairy cattle is through milk
 and milking procedures. Farm-to-farm spread is linked to cattle movement, shared equipment and personnel.
- Incubation period: The incubation period ranges from 12 to 21 days at the herd level observed in the field. In individual cows, clinical signs can appear as early as 1–2 days after infection following intramammary inoculation, according to experimental evidence.
- Viremia and viral replication: Evidence of systemic infection (viremia) in cattle is limited. High viral loads in milk and receptor expression in mammary glands suggest local replication of the virus rather than widespread systemic infection. More studies in cattle are warranted to elucidate whether the B3.13 virus is introduced into the mammary gland via an ascending infection or by systemic infection.
- Virus shedding in milk: Infected dairy cattle, including those without clinical signs, can shed high levels of infectious HPAI A(H5N1) virus in milk, starting as early as 1 day after infection and lasting up to 14–21 days. Milk consistently shows the highest viral loads compared to other sample types (nasal swabs, urine).
- No transmission was observed from infected calves to co-housed sentinel animals, suggesting limited transmissibility by the respiratory and oro-faecal routes.
- Immune response and protection: Infected cattle develop virus-specific antibodies in blood and milk within 7–10 days
 post-infection. These antibodies can offer short-term protection against clinical signs in case of reinfection, but further
 research is needed to understand the onset, duration and strength of immunity.
- Cross-species transmission and risk to humans: The HPAI A(H5N1) B3.13 virus has infected a wide range of hosts including birds, mammals and humans, highlighting a significant host range and zoonotic potential.
- Viral adaptation and public health concern: Mutations of HPAI A(H5N1) B3.13 virus associated with mammalian adaptation (e.g. PB2 E627K) have been detected in both human and cattle samples, underscoring the need for strict biosecurity and protective measures for farm workers.

Outbreaks in dairy cattle in US

- Widespread outbreak across major dairy US states: between March 2024 and 18 May 2025, the HPAI H5N1 B3.13 virus was confirmed in 981 dairy herds across 16 U.S. states, California alone accounted for 762 cases. These 16 states represent about 58% of the national dairy cattle population. Up to May 2025, approximately 69% of California's dairy farms were affected.
- Rapid spread and diverse clinical signs: initially presenting as mild illness in cows, the outbreak expanded rapidly, with increasing reports of severe clinical manifestations, including respiratory, neurological and reproductive signs. Between-farm transmission was linked to cattle movement and shared farm resources.
- Ongoing recovery: As of the beginning of 2025, the number of reported new cases declined.

Risk factors and protective factors

- Key risk factors for virus spread: USDA identified several potential risk factors for the introduction and spread of HPAI H5N1 B3.13 in dairy herds, including cattle movement, shared equipment and vehicles, presence of wild and domestic animals, and frequent contact with external personnel such as veterinarians and haulers.
- Protective factors: While certain farm management practices like proper waste handling and wildlife deterrence may reduce risk, these measures lack robust validation.

Control measures

- Comprehensive movement and testing controls: In response to the HPAI H5N1 B3.13 outbreak, U.S. authorities implemented strict interstate movement restrictions, mandatory reporting of dairy cattle for Influenza A virus, testing of bulk milk and quarantine protocols to limit disease spread between farms and states.
- Enhanced farm-level biosecurity: Authorities recommended that farmers adopt biosecurity measures such as isolating diseased cattle, disinfecting equipment and vehicles, limiting visitors and improving waste management to reduce the risk of virus introduction and transmission.
- State-specific import regulations: Multiple states enacted additional entry requirements, including health certificates, pre-movement testing and quarantine mandates for dairy cattle from affected areas.

Pathways for introduction of B3.13 AI virus via trade into EU

- Live bovine and bovine meat: although HPAI H5N1 B3.13 could theoretically be introduced into the EU through live cattle or meat imports from the U.S., strict trade regulations, absence of live cattle exports and limited virus detection in meat – especially muscle tissue – make this pathway unlikely, though uncertainty remains regarding udder tissue and completeness of surveillance data.
- Milk and milk products: while pasteurisation effectively inactivates HPAI H5N1 virus in milk, raw or insufficiently treated milk and dairy products pose a potential risk for virus introduction into the EU, with uncertainty remaining around the efficacy of non-thermal treatments and product labelling.
- Bovine reproductive material and foetal calf serum: no reports have been published investigating whether HPAI H5N1 B3.13 infects bovine semen, embryos, oocytes or foetal calf serum; no such imports have been recorded from the U.S. to the EU during 2023–2025.
- Live poultry and poultry meat: given the rapid onset of clinical signs in infected poultry, especially chicken, adequate surveillance and absence of detection of US HPAIV H5N1 lineages in the EU in the last decade, the trade of live poultry and poultry meat from the U.S. to the EU is considered an unlikely pathway for HPAI H5N1 B3.13 introduction, though the risk associated with early-stage infections for meat contamination results in a minor uncertainty.

Pathways of possible introduction of B3.13 AI virus via migratory birds

- Migratory birds pose a potential entry route for HPAI B3.13 into Europe: Although genotype B3.13 is currently confined to North America and has not yet been identified in wild birds in the US, migratory waterbirds – especially gulls and other long-distance migrating species – could introduce the virus into Europe through overlapping flyways especially in the Arctic, but possibly also in Siberia, or Africa.
- Timing and geography influence risk of introduction: The highest risk periods for virus entry into Europe align with bird migration seasons (April–May and the non-breeding season (September–April), with key entry points likely in Iceland, Scandinavia, Eastern Europe and the Mediterranean, depending on the migratory route.
- North American Arctic is the most likely entry point: among the 57 waterbird species assessed, over half frequent the North American Arctic, where they may come into contact with European migratory birds.
- Surveillance for the early detection of B3.13 introduction in the EU should focus on key European stopovers: given the potential for direct transatlantic spread, early detection efforts should prioritise high-density bird congregation sites such as Iceland, Britain, Ireland, western Scandinavia and large wetlands like the Wadden Sea, where intercontinental migrants are likely to arrive.

ABBREVIATIONS

AQs	assessment questions
CDC	Centers for Disease Control and Prevention
FDA	Food and Drug Administration
HPAI	highly pathogenic avian influenza
NVSL	National Veterinary Services Laboratory
ProMed	Program for Monitoring Emerging Diseases
SQs	subquestions
ToR	Terms of Reference
USDA	U.S. Department of Agriculture
USDA-APHIS	U.S. Department of Agriculture – Animal and Plant Health Inspection Service
USDA-CVB	United States Department of Agriculture-Center for Veterinary Biologics
USDA-NASS	U.S. Department of Agriculture–National Agricultural Statistics Service
WAHIS	World Animal Health Information System
WHO	World Health Organization
WOAH	World Organization for Animal Health

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APPENDIX A

Literature search string

Search string for literature search of phenotypic and genetic characterisation of HPAI viruses circulating in domestic or wild birds or mammals (excluding humans):

P3 Search 11 AND P2 Highly pathogent'table) (DR lighty) pathogen'(Table) AMD ("finiteerazi in Birds'("Medd) GR P2 ((high pathogent'table) (DR lighty) pathogen'(Table) AMD ("finiteerazi in Birds'("Medd) GR Highty pathogenci avian influenza in birds'(Table) OR "hight (Table) O	Search	Query	Concepts
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APPENDIX B

Commodities of bovine origin traded from US to Europe in 2023–2025 as per TRACES (pasteurised and UHT products excluded)

Type of commodities	Net weight (kg)	No. of consignments
Blue-veined cheese and other cheese containing veins produced by Penicillium roqueforti	136	1
Dairy, preserved at frozen/chilled temperature	136	1
Boneless	28,934,090	25
Domestic	7,659,862	8
Fresh meat (not Meat minced, not Meat preparations)	21,119,695	15
Vinced meat	154,534	2
Bovine semen	-	12
Others	-	3
Semen	_	9
Carcases and half-carcases	214	1
Domestic	214	1
Cheese and curd	496,169	14
Colostrum	9	2
Dairy, preserved at frozen/chilled temperature	493,851	11
Drganic	2309	1
Exceeding 1,5% but not exceeding 27%	61,509	3
ABP for feeding of farmed animal	1072	1
Colostrum	562	1
HC, preserved at ambient temperature	59,875	1
Fresh (unripened or uncured) cheese, including whey cheese and curd	10,862	4
Dairy, preserved at frozen/chilled temperature	10,862	4
Nilk and cream, concentrated or containing added sugar or other sweetening matter	27,857	4
Dairy, preserved at frozen/chilled temperature	10,857	3
Organic	17,000	1
Nilk and cream, not concentrated nor containing added sugar or other sweetening matter	899,778	8
Colostrum	13,527	1
Milk for HC	886,251	7
Not containing added sugar or other sweetening matter	2987	2
Dairy, preserved at frozen/chilled temperature	2252	1
HC, preserved at ambient temperature	735	1
Other cheese than 040610; 040620; 040630; 040640	678,789	12

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(Continued)

Type of commodities	Net weight (kg)	No. of consignments
Dairy, preserved at frozen/chilled temperature	678,789	12
Other cuts with bone in	2,458,177	14
Domestic	1,547,206	11
Fresh meat (not Meat minced, not Meat preparations)	910,972	3
Yogurt	6260	2
Dairy, preserved at frozen/chilled temperature	6260	2

APPENDIX C

Commodities of bovine origin (live animals, meat, dairy) and live poultry traded from US to Europe in 2023–2025 (January) as per EUROSTAT

Data extracted on 4/4/2025, quantity (kg).

TIME	2023	2024	2025 - Jan
PRODUCT (Labels)			
Live bovine animals	40	:	:
Live poultry, 'fowls of the species Gallus domesticus, ducks, geese, turkeys and guinea fowls'	2054	3428	:
Meat of bovine animals, fresh or chilled	14,119,406	12,656,111	1,113,330
Meat of bovine animals, frozen	199,884	294,913	20
Edible offal of bovine animals, swine, sheep, goats, horses, asses, mules or hinnies, fresh, chilled or frozen	11,241	5738	:
Meat of bovine animals, salted, in brine, dried or smoked	15	103	7
Milk and cream, not concentrated nor containing added sugar or other sweetening matter	1451	378	14
Milk and cream, concentrated or containing added sugar or other sweetening matter	865,473	1,444,263	230,817
Buttermilk, curdled milk and cream, yogurt, kephir and other fermented or acidified milk and cream, whether or not concentrated or flavoured or containing added sugar or other sweetening matter, fruits, nuts or cocoa	49,978	10,266	15
Whey, whether or not concentrated or containing added sugar or other sweetening matter; products consisting of natural milk constituents, whether or not containing added sugar or other sweetening matter, n.e.s.	1,418,516	934,967	74,793
Butter, incl. dehydrated butter and ghee, and other fats and oils derived from milk; dairy spreads	17,712	243,562	85,450
Cheese and curd	332,572	534,448	37,563

APPENDIX D

Overview of species that may transmit HPAI from the American continent to Europe

The routes are either directly by birds migrating to/from Greenland and the Canadian Arctic where they may encounter conspecifics or other species that spend the non-breeding season in North America (route 'America'), via Siberia (route 'Siberia') where America-wintering birds may mix with European-wintering birds, or indirectly where birds infected in Siberia winter in Africa where they transmit the virus to birds that migrate to Europe (route 'Africa'). For a few European species, closely related American species, often previously considered the same species, with which they may mix in the mentioned contact zone are mentioned between brackets; if the routes and European entry via the American species differ from the European species, then those are also mentioned between brackets. The time and period during which certain species occur in the potential contact zones in America, Siberia or Africa (red areas in Figure 2), when they occur in Europe and where they would enter Europe from the contact zones (coarsely divided in 'Atlantic', 'Iceland', 'Scandinavia', 'East Europe' or 'Mediterranean'). When infections with HPAI (not specifically genotype B3.13) were reported by the Animal and Health Inspection Service (U.S. Department of Agriculture), it was marked Y (yes). In the last column, the relative abundance of the species in Europe is coarsely indicated in four categories ranging from 0 (few individuals (i.e. hundred or less)) to +++ (very abundant (i.e. 100,000s)).

Species Vernacular name	Species scientific name	Route	Time in contact zone (months)	Period in contact zone	Period in Europe	European entry	Known infections with HPAI (Y/N)	Population Size in Europe
Brant Goose	Branta bernicla	Siberia, America	3	Jun-Aug	Year-Round	Iceland, Scandinavia	Y	+++
Red-breasted Goose	Branta ruficollis	Siberia	5	May–Sep	Oct–Apr	East Europe	Y	+
Canada Goose	Branta canadensis	America	5	May–Sep	Year-Round	NA	Υ	++
Barnacle Goose	Branta leucopsis	America	3	Jun–Aug	Year-Round	Iceland	Y	+++
Ross's Goose	Anser rossii	America	6	Apr–Sep	Na	NA	Y	0
Snow Goose	Anser caerulescens	America	6	Apr–Sep	Na	NA	Υ	0
Pink-footed Goose	Anser brachyrhynchus	America	3	Jun–Aug	Oct–May	Iceland	Ν	++
Tundra Bean Goose	Anser serrirostris	Siberia	4	May–Aug	Oct–Mar	Scandinavia, East Europe	Ν	+++
Greater White-fronted Goose	Anser albifrons	Siberia, America	5	May–Sep	Sep–Apr	Siberia, Iceland	Y	+++
Northern Shoveler	Spatula clypeata	Siberia	3	May–Jul	Year-round	Scandinavia, East Europe	Y	+++
Eurasian Wigeon	Mareca penelope	Siberia, (Africa)	3	May–Jul	Year-round	Scandinavia, East Europe, (Mediterranean)	Y	+++
American Wigeon	Mareca americana	America	4	May–Aug	Na	NA	Y	0
Mallard	Anas platyrhynchos	(America)	2	Jun–Jul	Year-round	Iceland	Y	+++
Northern Pintail	Anas acuta	Siberia	2	Jun–Jul	Year-round	Scandinavia, East Europe	Y	++
Common Teal (Green-winged Teal)	Anas crecca	Siberia, (America)	2	Jun–Jul	Year-round	Scandinavia, East Europe, (Iceland)	Y	+++
Greater Scaup	Aythya marila	Siberia	4	May–Aug	Year-round	Scandinavia	Y	++
Lesser Scaup	Aythya affinis	America	4	May–Aug	Na	NA	Y	0
Common Eider	Somateria mollissima	America	4	Jun–Sep	Year-round	Iceland	Y	++
Velvet Scoter (White- winged Scoter)	Melanitta fusca	Siberia	2	Jun–Jul	Aug–Jun	Scandinavia	Y	+

(Continued)

Species Vernacular			Time in contact	Period in	Period in		Known infections	Population Size
name	Species scientific name	Route	zone (months)	contact zone	Europe	European entry	with HPAI (Y/N)	in Europe
Common Scoter (Black Scoter)	Melanitta nigra	Siberia, (America)	2	Jun–Jul	Aug–Jun	Scandinavia, (Iceland)	Y	+++
Long-tailed Duck	Clangula hyemalis	Siberia, (America)	3	Apr–Jul	Nov–Mar	Scandinavia, (Iceland)	Υ	+
Common Goldeneye	Bucephala clangula	Siberia	5	May–Sep	Year-round	Scandinavia	Y	++
Common Merganser	Mergus merganser	Siberia, (America)	3	May–Jul	Year-round	Scandinavia, (Iceland)	Υ	+
Red-breasted Merganser	Mergus serrator	Siberia, America	4	May–Aug	Year-round	Iceland, Scandinavia	Y	+
Grey Plover	Pluvialis squatarola	Siberia, Africa	2–9	Jun–Jul & Aug–May	Aug–May	Scandinavia, Mediterranean	Y	++
Eurasian Golden Plover (American Golden Plover)	Pluvialis apricaria	Siberia, (America)	2	Jun–Jul	Year-round	Scandinavia, (Iceland)	Ν	++
Eurasian Dotterel	Eudromias morinellus	Siberia	3	May–July	Aug-Oct & Apr-May	Scandinavia	Ν	0
Common Ringed Plover (Semipalmated Plover)	Charadrius hiaticula	America, Siberia, Africa	2-9	Jun–Jul	Year-round	Iceland, Scandinavia	Ν	+
Bar-tailed Godwit	Limosa lapponica	Siberia, Africa	2	Jun–Jul	Year-round	Scandinavia, Mediterranean	Ν	++
Jack Snipe	Lymnocryptes minimus	Siberia	4–8	May–Aug & Aug–Apr	Year-round	Scandinavia, East Europe	Ν	+
Common Snipe	Gallinago gallinago	Siberia, Africa	2–9	Jun–Jul & Aug–May	Year-round	Scandinavia, Mediterranean	Ν	++
Red Phalarope	Phalaropus fulicarius	Siberia, America	2–5	Jun–Jul & Nov–Mar	Sep-Feb	Atlantic	Ν	0
Red-necked Phalarope	Phalaropus lobatus	Siberia, America	9	Sep–May	May, Jul–Oct	Scandinavia, Iceland	Y	+
Terek Sandpiper	Xenus cinereus	Siberia, Africa	2	Jun–Jul	Aug–May	Scandinavia, East Europe, Mediterranean	Ν	0
Common Sandpiper	Actitis hypoleucos	Siberia, Africa	2	Jun–Jul	Year-round	Scandinavia, East Europe, Mediterranean	Ν	+
Spotted Redshank	Tringa erythropus	Africa	7–9	Aug–May	Year-round	Mediterranean	Ν	+
Wood Sandpiper	Tringa glareola	Africa	7–9	Aug–May	Apr–Sep	Mediterranean	Ν	+
Ruddy Turnstone	Arenaria interpres	America, Siberia, Africa	2–10	Jun–Jul & Aug–May	Year-round	lceland, Scandinavia, Mediterranean	Ν	++
				Aug–May		Mediterranean		

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(Continued)

Species Vernacular name	Species scientific name	Route	Time in contact zone (months)	Period in contact zone	Period in Europe	European entry	Known infections with HPAI (Y/N)	Population Size in Europe
Red Knot	Calidris canutus	America, Siberia, Africa	2–10	Jun–Jul & Aug–May	Aug–May	lceland, Scandinavia, Mediterranean	Ν	++
Ruff	Calidris pugnax	Siberia, Africa	2	Jun–Jul	Year-round	Scandinavia, East Europe, Mediterranean	Ν	++
Curlew Sandpiper	Calidris ferruginea	Siberia, Africa	2–9	Jun–Jul & Nov–May	Apr–May, Aug–Sep	Scandinavia, Mediterranean	Ν	+
Temminck's Stint	Calidris temminckii	Siberia, Africa	2–7	Jun–Jul & Oct–Apr	Year-round	Scandinavia, East Europe, Mediterranean	Ν	+
Sanderling	Calidris alba	America, Siberia, Africa	2(-10)	Jun–Jul & Aug–May	Aug–May	lceland, Scandinavia, Mediterranean	Ν	++
Dunlin	Calidris alpina	Siberia, America	2	Jun–Jul	Year-round	Iceland, Scandinavia	Ν	+++
Purple Sandpiper	Calidris maritima	America	2–4	Jun–Jul (Aug-Sep?)	Nov–May	Iceland	Ν	+
Little Stint	Calidris minuta	Siberia, Africa	2–9	Jun–Jul & Sep–May	Apr–Sep	Scandinavia, Mediterranean	Ν	+
Pectoral Sandpiper	Calidris melanotus	Siberia, America	2	Jun–Jul	Apr–Oct	Scandinavia, Iceland	Ν	0
Arctic Tern	Sterna paradisaea	Siberia, America, Africa	2–8	Jun–Jul & Oct–May	Apr–Aug	Atlantic	Ν	+
Black-legged Kittiwake	Rissa tridactyla	America	5	Oct-Feb	Year-round	Atlantic	Y	++
Glaucous Gull	Larus hyperboreus	America, Siberia	6	May–Oct	Oct–Apr	Atlantic	Ν	+
Iceland Gull	Larus glaucoides	America	5	May–Sep	Oct–Apr	Atlantic	Ν	0
Long-tailed Skua	Stercorarius longicaudus	Siberia, America	2–5	Jun–Jul & Nov–Mar	Apr–Oct	Atlantic	Ν	+
Parasitic skua	Stercorarius parasiticus	Siberia, America	2	Jun–Jul	Apr–Oct	Atlantic	Ν	+
Pomarine Skua	Stercorarius pomarinus	Siberia	2–5	Jun–Jul & Nov–Mar	Apr–May, Aug–Nov	Atlantic	Ν	+
Leach's Storm Petrel	Hydrobates leucorrhous	America	5	Nov–Mar	Apr–Oct	Atlantic	Ν	+++
Northern Fulmar	Fulmarus glacialis	America	7	Apr–Oct	Year-round	Atlantic	Y	+++
Northern Gannet	Morus bassanus	America	7	Sep–Apr	Year-round	Atlantic	Y	++

APPENDIX E

Decision tree used for the collection of data from the news sources





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