DHS SCIENCE AND TECHNOLOGY

Master Question List for Highly Pathogenic Avian Influenza (HPAI)

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Foreword

This Master Question List (MQL) was developed by the Department of Homeland Security Science and Technology Directorate (DHS S&T) to present the current state of available information to government decision makers. This MQL quickly summarizes what is known and what additional information is needed to address fundamental questions such as, "What is the infectious dose?" and "How long does the virus persist in the environment?" The information provided is a succinct summary to allow structured and scientifically guided discussions across the Federal Government without burdening them with the need to review scientific reports, and to prevent duplication of efforts by highlighting and coordinating research.

Introduction

Of the four types of influenza viruses (A, B, C, and D), influenza A virus is the only virus known to cause pandemics. Influenza A virus is further classified into subtypes according to two proteins on the surface of each virus that help it invade host cells: haemagglutinin (i.e., H or HA) and neuraminidase (i.e., N or NA). Subtypes (e.g., H5N1) can be classified into clades and further into genotypes based on genetic similarity. Influenza A viruses are found in mammalian species, including humans, swine, canines, and avian species across the globe. Avian influenza viruses (AIVs) naturally circulate among waterfowl and other migratory wild aquatic birds including ducks, geese, shorebirds, and gulls.[1] These bird-specific strains of influenza are typically categorized as having low pathogenicity (low pathogenicity avian influenza [LPAI]), meaning that infected birds show no signs of disease or the symptoms expressed are mild. When LPAI is introduced from waterfowl and other wild aquatic birds into domestic poultry such as chickens or turkeys, LPAI can mutate into high pathogenicity (i.e., HPAI), meaning infection causes severe disease in birds and is often fatal. The distinction between LPAI and HPAI is made based on the lethality of AIV strains to domestic chickens, as the mutation from LPAI to HPAI typically occurs upon replication in domestic poultry species.[2] One exception is known, which are the H5 viruses of the A/Goose/Guangdong/1/1996 lineage (GsGd) that circulate in wild migratory birds as HPAI and can be directly transmitted to domestic poultry as HPAI. Since 2014, GsGd clade 2.3.4.4 viruses have been predominant in global HPAI outbreaks, and responsible for the ongoing outbreak in the United States that began in early 2022.[3-4] The U.S. agricultural concerns and resources have been concentrated on responding to outbreaks and reducing the risk to domestic commercial poultry;[5] however, detections of clade 2.3.4.4b genotype B3.13 viruses in U.S. livestock herds were first reported in March 2024 and are an ongoing concern.[6] As of 31 July 2024, HPAI has been detected in 175 livestock herds in 13 states.[7] The spread to livestock has also been associated with new cases of HPAI in humans with four reported cases since April 2024.[8]

Key Updates

- GsGd lineage HPAI H5N1 clade 2.3.4.4b viruses first emerged Europe in 2020, spread to North America by late 2021,[3] and are responsible for the ongoing global outbreaks.[9-10]
- The ongoing U.S. HPAI outbreak began 8 February 2022 and has affected more than 100.71 million birds from 1,172 flocks in 48 states (as of 31 July 2024).[11]
- The ongoing global HPAI outbreak has impacted wildlife at an unprecedented level with expansion of both the number and diversity of mammalian and avian species infected. Additionally, disease severity has been greater compared to other influenza viruses affecting wildlife.[12-13]
- In early March 2024, HPAI was detected in a juvenile goat in Minnesota. Previously, natural HPAI infection had not been reported in domestic ruminants (i.e., goats, cattle, sheep).[14]
- Samples obtained from Texas and Kansas dairy cattle herds confirmed HPAI on 25 March 2024.[6]
- As of 31 July 2024, HPAI has been detected in 175 livestock herds in 13 states with Texas, Idaho, Colorado, Michigan, and Ohio reporting the highest number of affected herds.[7]
- The spread to livestock has also been associated with new human cases of HPAI in the U.S. with four reported cases in patients exposed to infected livestock and nine reported cases in patients exposed to infected poultry since April 2024.[8]

The cutoff date for information gathering related to this document was 07/31/2024.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
INFECTIOUS DOSE	• The median infectious dose (ID_{50}) of HPAI in birds depends on virus strain and host species, and can range from $<10^{1}$ to $10^{4.6}$ egg ID_{50} • The ID_{50} in humans of HPAI is currently unknown
TRANSMISSIBILITY	• The main exposure and shedding routes for HPAI virus in birds are via oral and cloacal (i.e., urinary, gastrointestinal, genital tract) routes • Migratory waterfowl, primarily of the Anatidae family (ducks, geese, and swans), are major carriers of novel virus strains. The identification of new HPAI strains in poultry is more prevalent within migratory pathways • Farms located near bodies of water, with large flock sizes, or located in an area of high farm density are at increased risk • Dairy cattle may be infected by HPAI via respiratory or mammary exposed routes; however, cattle appear to be more susceptible to mammary exposure and symptoms are similar to what is observed in infected cattle on dairy farms • 2021-2024 reports of confirmed human cases all involve close contact with poultry, infected livestock, or environmental exposure
HOST RANGE	• Migratory aquatic birds are the primary natural reservoir for most subtypes of AIVs, but domesticated poultry and other birds can also be infected • The ongoing global HPAI outbreak has impacted wildlife at an unprecedented level with expansion of both the number and diversity of mammalian and avian species infected. Additionally, disease severity has been greater compared to other influenza viruses affecting wildlife • Since March 2024, HPAI has been detected in over 135 dairy herds in 12 states with Texas, Idaho, Colorado, Michigan, and Ohio reporting the highest number of affected herds. HPAI has also been detected in U.S. goats and alpacas in isolated cases • Since 2020 when H5N1 clade 2.3.4.4b emerged, there have been case(s) reported in Laos, Ecuador, Australia, Chile, India, Spain, Vietnam, China, the U.S., the United Kingdom, and Cambodia
INCUBATION PERIOD	• Incubation periods for HPAI in poultry vary and are dependent on infectious dose, transmission acquisition, and environmental factors. Determination of infection duration and incubation time is confounded when no clinical symptoms are present. Naturally infected chickens have an incubation period from 3-14 days • Humans infected with H5N1 AIV generally show clinical symptoms 2-5 days after exposure, though longer incubation periods (≤17 days) are possible

CLINICAL PRESENTATION	• HPAI and LPAI refer to high or low pathogenicity in domestic chickens (respectively), not humans or other animals • HPAI can cause up to 100% mortality in infected chickens. Clinical presentation in birds can include mild to severe respiratory disease signs as well as neurological issues, problems with egg production and formation, and sudden death • Infected mammals can present with clinical signs often found in other diseases, including fever, coughing, lethargy, diarrhea, and weight loss, with neurological signs such as seizures, ataxia, or tremors also possibly occurring • Dairy cattle may experience reduced appetite, drop in milk production and/or thickened milk, and nasal discharge. Initial reports of illness in dairy cattle indicated that clinical presentation peaks in about three to four days and lasts 10 to 14 days • Fever is common in human infection, but not always present. The less common symptoms to be aware of include nausea or vomiting, diarrhea, or seizures • Recent human cases involving exposure to infected cattle have also presented with fever, chills, cough, and conjunctivitis
BIOSURVEILLANCE AND CLINICAL DIAGNOSIS	• The primary method of detecting AIV in poultry flocks and cattle herds is real-time reverse transcription polymerase chain reaction (rRT-PCR) from cloacal and oropharyngeal/tracheal swabs, sampling from sick and dead birds, manure, and sampling from milk/udder secretions of cattle • Migratory birds that travel long distances have a major role in the global spread of AIVs • Monitoring for HPAI in wildlife is conducted by the U.S. Department of Agriculture (USDA) in the U.S., as well as by international partners in their respective regions • Pre-movement testing is required for all lactating cattle; however, biosurveillance guidelines for cattle are still being established • Due to the multi-faceted nature of the spread of HPAI to cattle, response and monitoring is shared by the USDA, Food and Drug Administration (FDA), and Centers for Disease Control and Prevention (CDC) in the U.S.
VETERINARY MEDICAL COUNTERMEASURES	• There are several medications available that reduce clinical signs and potential for transmission in infected poultry, though they are not used in the U.S. • Palliative care is recommended for infected cattle
HUMAN MEDICAL COUNTERMEASURES	• For humans with confirmed or suspected Influenza A infection, antiviral drugs may be used for treatment and prophylaxis if given early in symptom progression or before symptoms begin • Humans with confirmed or suspected novel influenza should be given neuraminidase inhibitor drugs (e.g., oseltamivir, peramivir, and zanamivir) for treatment

VACCINES	• The human seasonal flu vaccines do not protect against AIV H5N1 • Globally, there are several existing vaccines against AIV in poultry, though their use is not consistent across impacted countries. USDA maintains emergency poultry vaccination guidelines, procedures, and vaccine recommendations • One complication in vaccination campaigns is vaccinated birds become difficult to differentiate from infected birds, which may impact trade • HPAI vaccination of ducks and poultry may reduce virus shedding following challenge with GsGd lineage HPAI virus
DEPOPULATION/ CARCASS DISPOSAL	• Within 24-48 hours of HPAI notification on farms, the USDA defined standard practice is depopulation with water-based foam systems for floor-raised birds or gassing for caged birds • USDA disposal methods for poultry include composting, burial, incineration, rendering, and landfilling • Early detection and reporting and time to depopulation directly impacts the spread of HPAI and successful containment. On average, 12 days are needed for on-site staff to recognize illness and initiate reporting • USDA APHIS does not currently recommend depopulation of cattle. Infected livestock should be monitored for disease progression and supported with palliative care. Return to the herd should be determined with the assistance of veterinarians
VIRAL PERSISTENCE AND ENVIRONMENTAL STABILITY	• Influenza viruses may remain viable on surfaces for up to two weeks • AIV persistence varies based on the environmental matrix and exposure to natural environmental factors (heat, ultraviolet [UV] exposure, salinity, and pH) • AIVs are extremely stable in water, showing infectivity after several months in cold weather natural wetlands • Initial studies indicate that H5N1 genotype B3.13 persists in milk on stainless steel milking equipment for over an hour and on rubber components for over 3 hours
DECONTAMINATION	• The U.S. Environmental Protection Agency (EPA) maintains a list of registered chemical compounds for use in disinfection against avian influenza on farm settings, including bleach, alcohol, and quaternary ammonium-based compounds such as Lysol® and Formula 409® all-purpose cleaners • The Animal and Plant Health Inspection Service (APHIS) of the USDA maintains protocols for cleaning and disinfection of facilities affected by HPAI • Various decontamination methods have been evaluated for poultry and cattle products to control the spread of AIV • The FDA and USDA recommend that any discarded milk should be heat-treated or pasteurized before disposal

	• The situation in livestock is rapidly evolving and specific guidance for decontamination of milking equipment, dairy products, and meat processing is likely to forthcoming
PERSONAL PROTECTIVE EQUIPMENT (PPE)	• There is effective PPE for those with potential exposures to HPAI, with the recommended type of PPE dependent on the type of exposure (e.g., poultry workers, laboratory staff, depopulation workers) • Recommended PPE for poultry workers includes safety goggles, disposable gloves, boots, a respirator (National Institute for Occupational Safety and Health [NIOSH]-certified at N95 or higher), apron, disposable head/ hair cover, and disposable fluid-resistant coveralls
GENOMICS	• AIVs are defined by the presence (HPAI) or absence (LPAI) of a polybasic cleavage site in the HA gene • Since at least 2014, most of the continuously evolving and circulating GsGd lineage HPAI H5 variants have belonged to clade 2.3.4.4 • As of 20 June 2024, GsGd H5N1 clade 2.3.4.4b viruses have been detected on all continents (i.e., Europe, Asia, Africa, North America, South America, and Antarctica), except Oceania (i.e., Australia and other islands between mainland Asia and the Americas) • Circulating viruses are often screened for mutations known to reduce efficacy of antivirals. Clade 2.2 viruses appear to retain susceptibility to neuraminidase inhibitor drugs and baloxavir. However, novel mutations have been identified in these viruses that reduce susceptibility to adamantane, oseltamivir, baloxavir, zanamivir, or peramivir • Viruses isolated from infected dairy cattle are from the 2.3.4.4b clade but belong to a new genotype, B3.13
VIRUS IMPORTATION	• Importation predominately occurs via close interactions between wild migratory birds and domestic poultry, though other sources may also play a role • Modeling suggests that wild bird migration and illegal poultry trade are primary forms of HPAI introduction, and that the legal poultry trade is not a major importation risk • Movement and trade of livestock within the U.S. is encouraged to be minimized at this time and should not occur if any cattle or other animals on the premises display disease symptoms. Pre-movement testing is required for all lactating cattle and a 30 day quarantine is recommended after arrival of dairy cattle

Infectious Dose – How much agent will cause illness?

What do we know?

Typically, the median infectious dose (ID_{50}) of AIV in birds is measured in median egg infectious dose units (EID_{50}), representing the amount of virus needed to infect 50% of inoculated eggs. For each measure, lower values indicate greater infectivity, as less virus is needed for infecting a host.

- The ID_{50} of HPAI in birds depends on virus strain and host species, and can range from $<10^{1}$ to $10^{4.6}$ EID_{50} (estimated from reported values among ducks, chickens, and turkeys infected by intraocular, intratracheal, intrachoanal, and intranasal inoculation).[15-17]

The infectious dose of HPAI is dependent on HPAI strain and species infected.

- Experimental intrachoanal inoculation of chickens and turkeys with different GsGd lineage HPAI H5N2 strains resulted in similar ID_{50} values ranging from 10^{3} to $10^{5.1}$ EID_{50} for A/turkey/MN/12582/2015, A/turkey/SD/12511/2015, A/chicken/IA/13388/2015, A/northern pintail/WA/40964/2014, and A/turkey/Arkansas/7791/2015.[18-19]
- Experimental intranasal inoculation of ducks, with three GsGd lineage HPAI H5N2 strains (A/Tk/MN/15, A/Ck/IA/15, and A/Np/WA/14) determined a low infectious dose of $<10^{2}$ EID_{50} with no mortality observed at low (10^{2} EID_{50}) or high (10^{6} EID_{50}) doses. In contrast, a GsGd lineage HPAI H5N1 strain (A/Ws/Mongolia/05) had a mortality of 100% at the lowest dose (10^{2} EID_{50}) despite having a similar ID_{50} of $<10^{2}$ EID_{50} dose.[18]
- Six-week-old chickens intranasally inoculated with 10^{6} EID_{50} of GsGd lineage HPAI H5N8 (A/Wildbird/Cixi/Cixi02/2020) began to die 3 days post-infection (d.p.i.) with all infected chickens dying by 5 d.p.i.[20] Comparing GsGd lineage HPAI H5N8 strains isolated in Japan, the ID_{50} for chickens varied between strains from $10^{2.75}$ to $10^{3.50}$ EID_{50} and time to death ranged from 4 to 9 d.p.i depending on dose and strain.[21]
- The ID_{50} of GsGd lineage HPAI H5N1 A/chicken/England/053052/2021 strain was determined through oculo-nasal infection of chickens and ducks. The ID_{50} was determined to be $<10^{3}$ EID_{50} for ducks and $10^{4.67}$ EID_{50} for chickens.[22]
- The ID_{50} of a non-GsGd lineage HPAI H7N8 strain A/turkey/IN/16-001403-1/2016 was determined to be $10^{3.2}$ EID_{50} for chickens and $10^{2.5}$ EID_{50} for ducks; however, it was lowest for turkeys $<10^{2}$ EID_{50}.[23]

Wild fowl ~~also~~ show lower mortality than domestic poultry when experimentally infected with the same HPAI strain. Wild fowl may appear clinically normal while harboring systemic infections and shedding infectious virus for several days,[24-26] but this will vary depending on the HPAI strain.[27]

- Intranasal inoculation of rooks (*Corvus frugilegus)* with GsGd lineage HPAI H5N1 (A/mandarin duck/Miyazaki/22M807-1/2011) resulted in subclinical infection, but viral shedding from the oral cavity was $<10^{3}$ EID_{50} 1-5 days post-infection (d.p.i).[28]
- Intranasal inoculation of ducks with $\geq 10^{4}$ EID_{50} GsGd lineage HPAI H5N6 (Clade 2.3.4.4e HPAI Tottori/1) caused subclinical infection with low oral viral shedding, but systemic infection from higher dosing led to higher viral shedding ranging from $10^{4.5}$-$10^{5.7}$ EID_{50}.[29]

The median human infectious dose (HID_{50}) of HPAI is currently unknown; however, controlled studies have determined the ID_{50} for mammalian model organisms.

- Infection of ferrets with three strains of GsGd lineage HPAI H5N1 viruses (A/Vietnam/1203/2005, A/Muscovy duck/Vietnam/209/05, A/Whooper swan/Mongolia/244/05) was achieved with 10^6 EID_{50} intranasally and ~$10^{9.5}$ EID_{50} through consumption of infected meat. Infection occurred with $10^{8.3}$ EID_{50} after direct gastric exposure to meat infected with A/Vietnam/1203/2005.[30]

- Non-human primates (NHPs) exposed to 4 x 10^7 plaque forming units (PFU) of aerosolized GsGd lineage HPAI H5N1 (A/Vietnam/UT3040/2004) presented viral titers of $10^{3.60}$, $10^{2.90}$, and $10^{2.34}$ PFU/mL 1 d.p.i. from nasal swabs. Conventional inoculation of NHPs presented higher or equivalent viral titers of $10^{2.40}$, $10^{2.30}$, $10^{4.53}$, and $10^{4.28}$ PFU/mL 1 d.p.i.[31]
- Experimental inoculation of guinea pigs with a non-GsGd lineage HPAI H7N9 virus (A/Anhui/1/2013) determined an ID_{50} of 3 PFU.[32]

What do we need to know?

- How infectious are AIVs in humans compared to seasonal influenzas?
- Is the infectious dose of AIV route-dependent? How does the aerosol route of exposure compare?
- What is the infectious dose in dairy cattle for respiratory and mammary routes of infection?

Transmissibility – How does it spread from one host to another? How easily is it spread?

What do we know?

The main exposure and shedding routes for HPAI virus in birds are via oral and cloacal (i.e., urinary, gastrointestinal, genital tract) routes, although respiratory exposure may also lead to HPAI infection.[33]

- HPAI H5N1 viruses replicate to high titers in the respiratory tract and intestinal tract, and virus is excreted in high titers in both feces and oral secretions.[34-35]
- Environmental transmission of HPAI H5N8 virus occurs via fecal contaminated water.[36] The estimated average number of secondary infections from a contaminated environment was three.[36]

Waterfowl, including ducks, appear to be driving the transmission of LPAI and HPAI to domestic poultry.

- While GsGd lineage viruses are the only HPAI viruses known to circulate in wild birds, HPAI viruses that have emerged from LPAI have been isolated from wild birds during outbreaks in poultry.[37]
- Migratory waterfowl, primarily of the Anatidae family (ducks, geese, and swans), are major carriers of novel virus strains. The identification of new HPAI strains in poultry is more prevalent within migratory pathways.[38]
- Higher cloacal virus shedding of both wild and domestic ducks appears to be a factor in transmission between wild birds and poultry.[22, 36, 39-40] Ducks appear to have age-dependent symptoms and shedding, with younger ducks experiencing higher levels of shedding and mortality.[41]
- The infected goats were located on a farm that had a known HPAI detection and it is speculated that the goats had been exposed via access to a shared water source.[14]
- Similarly, known dairy herd infections are believed to have originated from wild birds.[6]

The R_0 is the calculated value for communicable diseases that represents the number of additional birds that one infected bird can infect or the number of additional farms that an outbreak spreads to.

- Analysis of outbreaks between 2003-2018 in South Korea provided an R_0 estimate of 1.69, 1.60, and 1.49 for H5N1, H5N8, and H5N6, respectively.[42]
- Analysis of four commercial poultry HPAI H7 outbreaks estimated the farm-to-farm $R_{0,}$ pre-intervention mean range 1.1 to 2.4.[43] Analysis of the 2016/17 HPAI H5N8 poultry farm outbreaks in in Hungary, Germany, Poland, and the Czech Republic estimated R_0 range 0.2-1.3 for farm-to-farm within countries.[44]
- The generation time between reported infection in one farm and confirmed infected in the next farm varied between 1.9-8.4 days, suggesting substantial variation in farm-to-farm spread.[43]
- Laboratory experiments of airborne transmission rates of HPAI H5N1 strain A/turkey/Turkey/1/2005 between chickens was low: 0.13 and 0.10 new infections per infectious bird at 0.2 meters and 1.1 meters distance, respectively,[45] which suggests bird-to-bird airborne transmission contributes less than other routes.
- Culling birds on infected farms, culling birds on contiguous premises, banning the restocking of emptied farms, and enforcing biosecurity measures including restrictions to reduce the number of vehicles and staff on and among farms can reduce the R_0.[43-44, 46-47]
- Farms located near bodies of water, have large flock sizes, or are in an area of high farm density are at increased risk for HPAI.[48-49] Another important aspect of disease transmission is negligence, or the loose implementation of biosecurity and preventive measures combined with low levels of surveillance.[50-51]

Risk factors for human HPAI infection are direct contact with or close exposure to sick or dead poultry, or visiting a live poultry market.[52-5334, 51] 2021-2024 reports of confirmed human cases all involve close contact with poultry, infected dairy cattle during milking operations, or environmental exposure.[9]

- Generally, there is low risk of human infection.[54-55] HPAI viruses can be transmitted by direct contact and aerosol in mammals,[56-57] including aerosols generated during poultry slaughter.[58]
- H5N8 was detected in human poultry workers during an outbreak on poultry farms in Russia, although the humans did not have symptomatic disease.[59]
- There is no direct evidence that HPAI viruses are transmitted to humans via consumption of contaminated poultry products,[60] but there is evidence that other mammals have become infected after eating contaminated poultry meat and blood[61-63], or raw milk from infected dairy cattle.[64]
- The USDA and the FDA have assessed the risk of humans becoming infected with HPAI virus through contaminated cooked poultry meat, shell eggs, egg products, or pasteurized dairy products to be low.[65-66]
- Studies in both the U.S. and Canada testing retail dairy products have not detected infectious virus; however, HPAI RNA was detected in retail pasteurized milk in the U.S.[67-70]

Sustained transmission in mammal populations is uncommon.

- Although there are cases of mammalian host-to-host transmission of HPAI viruses,[71-76] sustained transmission is uncommon in mammals.[24]

- In October 2022, farmed mink were found to be highly susceptible to GsGd lineage HPAI H5N1 and a mutation in the PB2 gene that enhance the polymerase activity of influenza in mammalian cells. Mink-to-mink transmission was indicated to have occurred.[71]
- Human cases of H5N6 have occurred with severe disease and 55% fatality rates, but there has been no evidence of human-human transmission.[77]
- The USDA has noted spread of HPAI between cows, cows to poultry, and farm to farm spread associated with cattle movement.[78]
- Dairy cattle may be infected by HPAI genotype B3.13 via respiratory or mammary exposed routes; however, cattle appear to be more susceptible to mammary exposure and symptoms are similar to what is observed in infected cattle on dairy farms.[79-80]
- The FDA recommends that any discarded milk or diary product intended for animal consumption be heat-treated or pasteurized to prevent transmission.[81]

What do we need to know?

- What is the relative contribution of factors that influence transmissibility between farms (e.g., wild birds, shared farm equipment, human movement, livestock movement)?
- What is the typical generation time or serial interval for infections in poultry? For wild birds?
- Is transmission heterogeneous, in the sense that only a few animals contribute the most to new cases?
- What is the potential for HPAI viruses to transmit to and among humans and what is the likely route of transmission?

Host Range – How many species does it infect? Can it transfer from species to species?

What do we know?

Migratory aquatic birds are the primary natural reservoir for most subtypes of AIVs,[38] but domesticated poultry and other birds can also be infected.[75]

- HPAI virus has been found in gallinaceous poultry (pheasants, quail, guinea fowl), game birds, ducks, geese, ratites, pigeons, vultures, raptors, and passerines.[24, 75, 82-83]
- GsGd lineage HPAI H5N1 was initially detected in geese and emerged among poultry in China.[84-85]

Many animal species, including humans, are susceptible to AIVs, despite not being the primary reservoir hosts. The ongoing global HPAI outbreak has impacted wildlife at an unprecedented level with expansion of both the number and diversity of mammalian and avian species infected. Additionally, disease severity has been greater compared to other influenza viruses affecting wildlife.[12-13]

- Assessment of the ongoing HPAI outbreak revealed that by the end of 2023, there had been a minimum 2.8-fold expansion in mammalian species impacted and 2.2-fold expansion in wild bird species impacted compared to the onset of the outbreak in 2021.[12]
- The USDA tracks reports of HPAI in mammals during the ongoing U.S. HPAI outbreak, and as of 31 July 2024, HPAI H5N1 has been detected in over 350 samples. Species impacted include rodents, felids, canids, bears, seals, and dolphins. HPAI has most commonly been reported in red fox (96 detections), house mouse (82 detections), striped skunk (36 detections), and domestic cat (36 detections).[86]
- As of 31 July 2024, HPAI has been detected in 175 dairy herds in 13 states with Texas, Idaho, Colorado, Michigan, and Ohio reporting the highest number of affected herds.[7]

- Since March 2024, HPAI has also been detected in U.S. goats and alpacas in isolated cases.[87]
- Foxes have also tested positive for GsGd lineage HPAI H5 in Canada,[83, 88-89] East Asia,[90] and Europe.[91-94]
- There is evidence domestic cats and dogs have been infected with H5N1 in multiple locations, including Europe, Thailand, and North America.[62, 95-97]
- GsGd lineage HPAI H5N2 was recovered from a dog and was transmissible to other dogs, chickens, and cats,[75] and has been associated with multiple ostrich outbreaks.[98]
- GsGd lineage HPAI H5N8 has appeared in poultry, wigeons, mute swans, gyrfalcon, ostrich, penguins, wild waterfowl, domestic ducks, and pigs.[27, 98-103]
- GsGd lineage HPAI H5N6 has been isolated from pigs[75] and detected in wild birds.[104]

WHO has reported several human cases of HPAI H5N1 since 2003.[105]

- Since 2020 when H5N1 clade 2.3.4.4b emerged, there have been human case(s) reported in Laos, Ecuador, Australia, Chile, India, Spain, Vietnam, China, the U.S., the United Kingdom, and Cambodia.[105]

What do we need to know?

To better understand the risk of transmission to species other than birds, we need more information on the role of viral diversity on host susceptibility:

- What is the risk of human and animal infection and subsequent transmission due to natural diversity of H5N1 subtypes?
- What is the role of domestic animals in transmitting and maintaining H5N1?
- Will genotype B3.13 currently impacting dairy cattle lead to larger outbreaks in other mammalian species?

Incubation Period – How long after infection do symptoms appear? Are animals infectious during this time?

What do we know?

Birds generally exhibit clinical symptoms of infection such as coughing, sneezing, and nasal discharge hours to days after becoming infected with an HPAI virus, however they can shed HPAI virus during the incubation period prior to clinical signs.

- Incubation periods for HPAI vary and are dependent on infectious dose, transmission acquisition, and environmental factors. Determination of infection duration and incubation time is confounded when no clinical symptoms are present. Naturally infected chickens have an incubation period from 3-14 days.[106]
- In poultry, the incubation period can range from hours to days. For disease control considerations, a 28-day incubation period is used for avian populations.[107] Mammals are thought to have short incubation periods of 1-2 days.[75]
- The duration of infection depends on the host species, virus strain, and severity of infection.[75] Waterfowl can shed virus before clinical signs appear.[24, 75] Studies have found virus shedding in chickens and wild birds within 1-2 days following exposure.[108-109]

Humans infected with H5N1 AI generally show clinical symptoms 2-5 days after exposure, though longer incubation periods (≤17 days) are possible.

- For AIV (H5N1) infections in humans, incubation periods average 2-5 days after virus contact or contact with exposed live poultry, often being described as "within the week prior" [110-112,113] and while rare, on the high end can range from 8-17 days.[59, 75, 114] For human infections with the HPAI (H7N9) virus, incubation period ranges from 1-13 days,[75] with an average of 3-5 days.[111]
- There are limited examples of possible human-to-human transmission of HPAI[115] however in the few documented cases of likely spread, the incubation period was measured at 3-5 days, with one instance of an incubation period of 8-9 days.[116]

Initial reports of illness in dairy cattle indicated that the disease symptoms peak in about three to four days and lasts 10 to 14 days.[117]

- Herd level incubation is estimated to be variable and between 12 to 21 days.[118]
- Cows without signs of infection have been linked to spread of HPAI.[78]
- Cows exposed to genotype B3.13 via the mammary route showed clinical signs within 48 hours of infection similar to those observed on dairy farms, while cows exposed via respiratory route showed limited clinical signs of disease including increased nasal discharge 1 to 3 days d.p.i.[79-80]

What do we need to know?

- How infectious are individuals during the incubation stage relative to the symptomatic stage?
- Does the route of transmission for human AIV infections influence the incubation time?
- To inform sensitivity of diagnostic tests and improve modeling, to what extent is HPAI H5N1 shed during the incubation period?

Clinical Presentation – What are the signs of infected individuals?

What do we know?

HPAI and LPAI refer to high or low pathogenicity in chickens (respectively), not humans or other animals.[119]

- LPAI viruses can cause serious illness in humans, but generally not in chickens.[119] Clinical symptoms of LPAI in humans can include conjunctivitis, fever, runny nose, sore throat, cough, and severe respiratory symptoms such as pneumonia and respiratory failure, even death. Few cases are asymptomatic.[120]
- HPAI infected wild birds such as waterfowl or migratory birds are often asymptomatic, however recent surveillance shows the possibility that a clinical sign of HPAI infection in wild birds could be a reduction in the number of movements over a time period or decreased flight distance.[3]

HPAI can cause up to 100% mortality in infected chickens.[121] Clinical presentation in birds can include mild to severe respiratory disease signs as well as neurological issues, problems with egg production and formation, and even sudden death.

- Clinical signs of HPAI in birds can include common respiratory illness signs such as nasal discharge, coughing, sneezing, and general fatigue. More severe symptoms are facial swelling, comb and wattles turning blue,[122] green feces/diarrhea, loss of muscle control, involuntary muscle movements and spasms, immobility, death,[123] and egg abnormalities such as soft or misshapen eggs, reduced egg production,[124,125,75] or eggs without shells.[126] Mortality can occur without external clinical signs of infection,[35] and an increase in mortality within flocks is sometimes the only sign of this virus.[125]

- Raptors (e.g., eagles, hawks, and owls) can present severe neurologic symptoms from HPAI infections.[127]

Mammals infected with AIV often present with symptoms that are common for other diseases and infection can be fatal.[62, 95]

- Infected mammals can present with clinical signs often found in other diseases, including fever, coughing, lethargy, diarrhea, and weight loss,[95, 128-129] with neurological signs such as seizures, ataxia, or tremors also possibly occurring.[12, 130] Laboratory animal models show these clinical signs can range from mild to fatal.[128]
- HPAI infection in mammals has presented as neurological issues in several species including porpoises, grizzly bears, bush dogs, domestic cats, dolphins, and seals.[12, 89, 131-135]
- HPAI infection of marine mammals has been linked to mass mortality events.[12, 136]
- Symptoms of HPAI in cattle include decreased feed intake, altered fecal consistency, respiratory distress, and decreased milk production with abnormal milk (e.g., thicker, concentrated, colostrum-like milk), lethargy, dehydration, and fever.[137] Older dairy cattle appear to be more clinically affected with more severely affected lactation.[66]
- The American Association of Bovine Practitioners (AABP) has indicated that impacted herds can experience a loss of about 20% of milk production for 14 to 21 days.[117]

Humans infected with HPAI H5 virus generally exhibit acute illness including fever, upper respiratory tract symptoms, myalgia, and lower respiratory tract illness.[124] However, humans can present with more severe symptoms including pneumonia, gastrointestinal issues, encephalitis, septic shock, multi-organ failure, and even death.[59, 124]

- Fever is common, but not always present. The less common symptoms to be aware of include nausea or vomiting, diarrhea, or seizures.[138]
- A strong index of suspicion of human H5N1 virus infection is warranted with cases of rapid onset fever and respiratory illness after having exposures to potentially infected poultry.[52]
- Personnel involved in culling operations or others with close contact of known infected birds should monitor closely for neurological or respiratory symptoms, as well as conjunctivitis, for at least 10 days following the exposure.[139]

Prior to 2022, there were no confirmed HPAI H5 infections in humans in the U.S.,[124] though sporadic human infections have been reported in other countries (e.g., H5N8 in Russia,[140] H5N6 in China,[141] H5N1 in China,[142] Cambodia,[143-144] Chile,[145] Europe,[146] Ecuador, [147] Vietnam, Laos, Egypt, and Indonesia).[148] However, since April 2022, fourteen cases of H5N1 have been identified in the U.S.[149-150]

- The first U.S. patient was involved with culling infected poultry at a Colorado farm with confirmed H5N1 cases in the poultry. He presented with fatigue and was treated with antivirals and recovered without hospitalization, with no further spread to close contacts.[149]
- Four U.S. cases have been reported since April 2024 in patients in contact with infected dairy cattle.[150] Cases have been mild with two patients only presenting with conjunctivitis.[151]
- Nine U.S. cases have been reported since April 2024 in patients in contact with infected poultry.[150]
- The WHO tracks all human cases of H5N1, which as of 31 July 2024 includes data from 2003 through 19 July 2024.[105]

- Australia reported its first human case of AIV on 22 May 2024. H5N1 clade 2.3.2.1a was detected in a 2.5 year old child and is suspected to have been acquired while the child was in India.[152]

What do we need to know?

- To what extent have subclinical or asymptomatic HPAI infections been underreported in humans?
- Is there any discernable pattern based on timing or progression of symptoms that would allow farmers to recognize HPAI infections more quickly in their flocks?

Biosurveillance and Clinical Diagnosis – Are there tools to diagnose infected individuals? When during infection are they effective? Are there ongoing surveillance efforts to detect HPAIs?

What do we know?

The primary method of detecting AIV in poultry flocks and cattle herds is real-time reverse transcription polymerase chain reaction (rRT-PCR) from cloacal and oropharyngeal/tracheal swabs,[153] sampling from sick and dead birds,[154] manure,[155] sick cattle, and milk/udder secretions.[156]

- RT-PCR is used for evaluating the presence of HPAI in manure from commercial flocks,[155] and along with sequencing provide confirmatory testing for HPAI presence.[38] Validated RT-PCR assays allow for detection of HPAI H5 viruses and co- circulating LPAI viruses, and considerably reduce diagnosis times.[157]
- RT-PCR viral detection is typically possible within a few days of disease onset.[158]
- Both RNA and infectious virus are detected in raw milk collected from affected cows.[159]
- Pre-movement testing is required for all lactating cattle.[81, 160]
- Due to the multi-faceted nature of the spread of HPAI to cattle, response and monitoring is shared by the USDA, FDA, and CDC in the U.S.

Laboratory diagnoses also include immunodetection of virus antigen/antibody.

- According to the CDC and World Health Organization (WHO) guidelines, rapid antigen detection tests, such as immunofluorescence or enzyme immunoassay, should not be the diagnostic method of choice in the event of a suspected outbreak of AIV.[59, 161] Rapid antigen testing for HPAI is often falsely negative in confirmed cases.[158]
- Antigen detection is widely used globally for AIV identification in poultry flocks for early detection and containment initiation.[2, 162-163]

Migratory birds that travel long distances have a major role in the global spread of AIVs.[164]

- An important component of biosurveillance is wild bird carcass surveillance from target species.[165] In wild birds, passive surveillance (from dead birds) is an appropriate method for HPAI surveillance when HPAI infections are associated with bird mortality, whereas active surveillance (from live birds) has an extremely low efficiency for detecting HPAI virus.[166] The presence of important long-range HPAI vectors is generally seasonal, which should influence active sampling schemes to supplement passive sampling.[25]
- When positive cases of HPAI are detected in a country or region, surveillance protocols for wild birds should be initiated, as the movement of migratory waterfowl is considered a potential risk for virus transmission into non-infected areas.[163]

- Characterizing local bird communities and their compositions can help determine which species are likely to come into contact with local poultry farms. This data can help streamline surveillance and management techniques during HPAI outbreaks.[167]

Monitoring for HPAI is conducted by the USDA in the U.S., as well as by international partners in their respective regions.

- The U.S. Geological Survey National Wildlife Center in Wisconsin conducts enhanced HPAI surveillance and has identified lethal HPAI infections in diverse raptor populations.[168] USDA APHIS performs AI surveillance in migratory birds.[82, 169] WHO continuously monitors AIV and other zoonotic influenza viruses through its Global Influenza Surveillance and Response System, and in collaboration with the World Organisation for Animal Health (WOAH) and the Food and Agriculture Organization (FAO), conducts human-animal surveillance.[59]
    - USDA, together with the Center for Food Security and Public Health, implemented a biosecurity resource based off of the Checklist for Self-Assessment of Implementing Poultry Biosecurity as part of initial response.[170]
    - Appraisal and Indemnity resources are available for backyard flocks affected by HPAI.[171]
- Wild bird LPAI/HPAI viral sampling is primarily from several genera from the family Anatidae.[172] USDA determines which watersheds to conduct virus surveillance.[172]
- The National Wildlife Disease Program (USDA/APHIS/Wildlife Services) monitor HPAI in mammals across the country.[86]
- The 25 member nations in the Global Consortium for H5N8 and Related Influenza Viruses monitor global circulating AIVs.[164] Similarly, 31 European countries routinely sample commercial and backyard poultry flocks, as well as wild birds for circulating LPAI and HPAIs.[173] This type of surveillance can help establish, for instance, whether new genomic variants likely arose from locally circulating strains, or whether they were imported from other sources.[174]
- Coinciding with HPAI infections of dairy cattle, H5 markers have been detected in nearby wastewater plants that permit discharge of animal waste, including milk byproducts.[175]
- Researchers are developing open platforms that helps to eliminate barriers to access of HPAI outbreak information and enable policymakers to rapidly respond to outbreaks, conduct investigations, and communicate risk information to the public.[176]

What do we need to know?

- How effective are methods for identifying LPAI with the potential to develop into HPAI?
- What is the most effective approach to conduct surveillance in dairy herds and in dairy workers?

Veterinary Medical Countermeasures – Are there effective treatments?

What do we know?

In the U.S., the primary method of HPAI virus control and eradication in poultry is depopulation, rather than use of veterinary countermeasures to treat infected animals.[106]

- USDA instructs responsiveness to HPAI virus by isolating and depopulating an infected population. In extreme cases, emergency vaccinations can be administered to the animals.[106]

There are several medications available that reduce clinical signs and potential for transmission in infected poultry,[177] though they are not used in the U.S..

- Experimental intranasal infections in chickens with HPAI H5N6 were treated with oral baloxavir or peramivir either immediately or 24 hours post-challenge. Only those chickens treated immediately post-challenge with baloxavir showed significant reduction in viral titers and protection from death.[178]
- Oseltamivir reduced mortality and transmission when administered to chickens infected with HPAI H5N2, but transmission resumed once antiviral treatment ended.[179] Zanamivir was ineffective at reducing HPAI mortality or transmission between chickens.[179]
- Resistance to amantadine has been resolved to a single mutational polymorphism that is present in AI H5N1 and H7N9 subtypes.[180-181] Experimental studies using site-directed drug development have shown that M2-inactivation drugs can still be used against resistant strains and remain viable future treatment options.[180-181]

Vaccination efficacy is typically limited to the same subtype and clade; however, it is a strategy that can be used alongside other methods to control and prevent the spread of AIV.[182]

Palliative care is recommended for infected cattle.[81]

What do we need to know?

- Are there effective measures for reducing viral load in infected poultry aside from vaccines?
- Are there effective measures for reducing or preventing disease in infected livestock?
- Can currently resistant strains of influenza develop additional resistance to existing treatments for animals?

Human Medical Countermeasures – Are there effective treatments?

What do we know?

For humans with confirmed or suspected novel influenza, antiviral drugs may be used for treatment and prophylaxis if given early in symptom progression or before symptoms begin.

- There is no clinical trial data for use of antivirals in human cases of novel influenza.[183]
- Humans with confirmed or suspected novel influenza should be given neuraminidase inhibitor drugs (e.g., oseltamivir, peramivir, and zanamivir) for treatment.[183]
- In a small sample size, baloxavir as an alternative to oseltamivir was efficacious as an antiviral treatment for people with H5N6 infections.[184]
- Phenotypic testing of 22 clade 2.3.2.1a and 2.3.4.4b viruses revealed broad susceptibility to neuraminidase inhibitor drugs and baloxavir concluding that most contemporary HPAI A(H5N1) viruses retain susceptibility to antiviral drugs.[185]
- Household or close family members with highest risk of exposure to individuals having confirmed influenza caused by H7N9 or H5N1 viruses should be given oral oseltamivir or inhaled zanamivir as chemoprophylaxis within 48 hours of exposure to reduce likelihood of additional transmission.[186]
- Some HPAI strains (H7N9, H5N6, and H5N1) are resistant to antiviral medications amantadine and rimantadine, which should not be used.[183]
- The anti-influenza drug, favipiravir, a chain terminator of viral RNA only approved for use in Japan.[187]
- Purified antibodies against H5N1 have been tested in animal and small human trials and appear safe, with some efficacy in *in vitro* studies.[188-189]

What do we need to know?

- What alternatives can be developed if antiviral resistance becomes widespread?
- Can current therapeutic options be modified to counter resistance to amantadine class drugs?

Vaccines – Are there effective vaccines?

What do we know?

Globally, there are several existing vaccines against AIV in poultry, though their use is not consistent across impacted countries.[190] USDA maintains emergency poultry vaccination guidelines, procedures, and vaccine recommendations.[106]

- One complication in vaccination campaigns is vaccinated birds become difficult to differentiate from infected birds.[191-192]
- The role of vaccines in the prevention and control of HPAI is a topic being actively explored,[193] with the European Council releasing a press release in May 2022 regarding the decision by the agriculture ministers on a strategic vaccination approach.[194] Vaccination has often been thought of as a last resort, but the 2021-2024 epidemic is causing countries to debate revising their vaccination strategies.[195-196]
- An International Alliance for Biological Standardization international meeting was held in October 2022 to discuss challenges and barriers to AIV vaccine use, such as trade concerns and availability of suitable vaccines, and recommendations have been made to support greater use of vaccination to help control the spread of HPAI.[197]
- Due to the 2023 spread of HPAI within the U.S., in April 2023 the USDA began HPAI vaccine trials of four candidates to test their efficacy in poultry against GsGd lineage H5N1 clade 2.3.4.4b, the strain causing the current outbreak.[198-199]
- Vaccines used in birds are either “homologous,” (the HA and subtypes both match the virus to be protected against), or “heterologous” (where the NA subtype differs). For all inactivated AIVs, the HA vaccine strain subtype needs to match the wild virus strain HA subtype. Heterologous vaccines are most often used for HPAI, and permit differentiation of birds infected with vaccine or field strains.[190]
- A 2024 meta-analysis focusing on vaccine studies from 2010-2023 concluded the following efficacies:[200]
  - Inactivated vaccines: efficacy of 95% against homologous strains and an efficacy of 78% against heterologous strains.
  - Live recombinant vaccines: overall efficacy of 97%.
  - Inactivated recombinant vaccines: overall efficacy of 90%.
  - Commercial vaccines: overall efficacy of 91%, with 96% efficacy against homologous strains and 90% efficacy against heterologous strains.
- Significant antigenic differences between commercially available poultry vaccines and currently circulating HPAI viruses suggests that vaccines may be suboptimal in controlling current poultry outbreaks.[201]
- Vaccines have historically been used to help control outbreaks of HPAI in poultry flocks in Mexico, Pakistan, Egypt, Indonesia, Vietnam, and China.[190, 202-203]
- Eradication of HPAI through vaccination campaigns, in coordination with other measures, has occurred in a few countries, and typically where either a high level of competence in

veterinarian services exists, or where the geography and density of bird populations have helped lead to the success.[192]

- After 19 critically endangered California condors were found dead due to HPAI infection, APHIS approved the emergency use of an HPAI vaccine. Vaccination will be limited to the condors, which are wild birds and not poultry, so it will not impact trade.[204-206]

There are multiple vaccines for use in humans for protection against H5N1, however they have typically been developed for the Strategic National Stockpile or for pandemic preparedness and are not produced in large quantities nor available for general population use.[207]

- The human seasonal flu vaccines do not protect against AIV H5N1.[208]
- The CDC, in coordination with the WHO, developed a curated bank of Candidate Vaccine Viruses (CVVs) that are a library of influenza viruses, including both seasonal and HPAI influenza viruses, which can be used for expedited development of human vaccines if needed. The CVV library contains virus nearly identical to H5N1 clade 2.3.4.4b.[208-212]
- The U.S. Government, Biomedical Advanced Research and Development Authority (BARDA), is working with multiple vaccine manufacturers, to test the safety of H5 vaccine candidates similar to the current outbreak strains.[213]

AIV vaccines for birds do not prevent infection but reduce clinical signs and mortality. Vaccinated birds can still transmit infection to other birds, albeit at a lower rate than unvaccinated birds.

- HPAI vaccination of ducks and poultry may reduce virus shedding following challenge with GsGd lineage HPAI virus.[214-215]
- Benefits of vaccination are extremely limited for short-lived poultry such as broiler chickens.[216]

Vaccines exert selective pressures on AIVs,[217] hastening evolution and vaccine resistance.[218]

- Antigenic drift in Egypt has reduced the efficacy of an existing H5N2 vaccine against circulating HPAI H5N1 strains.[219]
- Similarly, researchers have found novel HPAI H7N9 strains in China have the ability to partially escape neutralization by vaccines, with those vaccines introduced only 6 months prior,[220] which is suggestive of rapid evolution due to vaccine-induced selective pressures. The control of H5N1 in China has resulted in seven different vaccines being introduced over a 10-year period.[192]
- The WOAH's General Assembly debated the use of vaccination as a complementary tool and extensively discussed its associated implementation challenges.[221] It was recognized that a successful vaccination strategy must rely on authorized vaccines that closely match the virus strains in circulation. Furthermore, it must be accompanied by robust disease surveillance, which is able to demonstrate freedom from infection in the domestic animal population as recommended by WOAH Terrestrial Animal Health Code.[221]

What do we need to know?

- For increased resistance to infection as the influenza strains change over time, and reduced time for production of new strain specific vaccines, could a “universal” AIV vaccine be developed?

- How effective is prophylactic vaccination at reducing depopulation needs? (To understand the cost and risk benefit of vaccination versus mandatory culling if a farm's flock becomes infected).
- Should vaccination of high-risk poultry and livestock workers be implemented?
- Is vaccination an effective approach to protecting dairy cattle from AIV infection?

Depopulation / Carcass Disposal – What are safe and effective ways to minimize the spread of HPAI in agricultural settings?

What do we know?

Within 24-48 hours of HPAI notification on farms, the USDA defined standard practice for commercial poultry is depopulation with water-based foam systems (e.g., National Veterinary Stockpile Kifco Avi-Guard, or Spumifer handheld nozzles) for floor-raised birds or gassing (e.g., carbon dioxide, carbon monoxide, argon, or nitrogen) for caged birds. These processes are generally safe and effective, and gassing is identified as an accepted practice for euthanasia by the American Veterinary Medical Association (AVMA),[222] though efficacy depends on poultry species.[223-224]

- Efficacy of foam versus gassing for depopulation is species-dependent. While water-based foam (Spumifer with 1% Phos-Chek and water foam) resulted in more rapid brain death in turkeys,[225-226] 100% CO_2 gas outperformed water-based foam in four physiological categories (time to unconsciousness, motion cessation, brain death, and altered terminal cardiac activity) in ducks.[226-227]
- Gas concentration in depopulation is also species-dependent; 40% CO_2 concentrations are effective euthanasia for chickens within 2-4 minutes, although >70% concentration is required for ducks and geese.[222-223]

Alternative methods such as Ventilation Shut Down (VSD) are conditionally approved as adjunct methods by USDA, but must meet additional policy requirements before use.[224]

- VSD is considered a controversial practice by some veterinarians.[228]
- Continuing research shows improvement of VSD efficacy with addition of supplemental heat.[229-231]

USDA disposal methods include composting, burial, incineration, rendering, and landfilling.

- The disposal method to be used is selected by the disposal group comprised of federal partners and incident command staff based on several considerations.[232]
- Research indicates increasing temperature from 35°C to 55°C during carcass composting reduces the time required to achieve greater than 99.999% reduction in viral activity from 6.4 hours to 29 minutes.[233] USDA suggests maintaining a temperature of 135-140°F for 3-12 weeks to ensure full decomposition.[232]

Early influenza virus detection and reporting and time to depopulation directly impacts the spread of HPAI and successful containment. On average, 12 days are needed for on-site staff to recognize illness and initiate reporting.[234-237]

- Expedited bird depopulation can greatly reduce HPAI spread.[223, 236, 238-239]
- Reporting delays can result in increased culling.[237]
- Geographic containment zones are established immediately upon HPAI notification per USDA guidance control strategies.[232]

- Depopulation or carcass disposal strategies have not been determined for migratory and wild birds, but USDA recommends separating and securing water, food and other materials in locations difficult for wild birds to access.[240]
- Machine learning is being considered to pre-emptively identify quarantine and culling zones.[241]

USDA APHIS does not currently recommend depopulation of cattle. Infected livestock should be monitored for disease progression and supported with palliative care. Return to the herd should be determined with the assistance of veterinarians.[81]

What do we need to know?

- What are the current barriers to on-site recognition of illness and initiation of reporting?
- What is the risk of on-site handling procedures during culling and disposal for accidental contamination?
- Would further evaluation of alternative depopulation methods provide time-savings, efficacy, or cost burden benefits?
- What is the most effective approach to contaminated milk disposal?

Viral Persistence and Environmental Stability – How long does the virus live in the environment?

What do we know?

Avian influenza virus persistence varies based on the environmental matrix and exposure to natural environmental factors (heat, ultraviolet [UV] exposure, salinity, and pH).

- Avian influenza virus can persist in aerosols for 24-36 hours, which is longer than human influenza viruses (6-15 hours).[242] [243-244]
- The duration of AIV virus persistence decreases with increasing temperature.[245-250] Survival rates at 4ºC, 20ºC, and 30ºC in saline solution were measured to be 3213 at 4°C, 293 at 20°C, and 58 days at 30°C.[246]
- UV light exposure for 30 minutes and pH of less than 2 for 30 minutes have been shown to inactivate H7N9.[249]

AIVs are extremely stable in water, showing infectivity after several months in cold weather natural wetlands.

- Using a combination of field-and laboratory-based approaches, five subtypes of AIV were found to be infectious after at least 7 months in Alaskan and Minnesotan wetlands,[251] suggesting a key source of natural infection in waterfowl.
- Eurasian HPAI H5N1 showed persistence in water similar to several LPAI strains,[252] though HPAI persistence depends on the specific strain.[253] All avian influenza viruses appear more stable in cooler, less acidic water with low salinity.[252, 254-255]
- HPAI H5N1 viruses were used to experimentally inoculate artificial aquatic biomes. Infectious virus was only recovered from rainwater 4 days post-contamination at 25°C. Infectious virus and viral RNA was detected in few cases in the aquatic fauna and flora, especially in bivalves and labyrinth fish.[256]
- The infectivity of 12 Influenza A viruses that were isolated from naturally infected ducks were monitored for approximately one year. A single replicate from two viruses tested remained viable for 361-377 days post-sample collection when maintained in surface waters under ambient temperatures.[257]

- HPAI viruses are relatively stable in duck feathers,[245, 258] maintaining infectivity for up to 160 days in experimental trials.[245]
- AIV viruses may remain stable in duck or poultry feces for days to weeks.[245, 247-248]

Influenza A virus surface persistence is dependent on the surface, strain, and environmental conditions.

- Influenza viruses may remain viable on non-porous surfaces (stainless steel) for up to two weeks.[259]
- AIV persisted for 24-72 hours on non-porous surfaces such as stainless steel and plastic, and for <8-12 hours on cloth, paper, or tissues.[260-262]
- AIV H13N7 infectivity persisted for ≥6 days on latex and feathers; ≥3 days on steel, tile, rubber gumboots, rubber tires, egg shells, and plastic; ≥2 days on wood; ≥1 day on cotton fabric; and ≤1 day on egg trays and polyester fabric.[263]
- In poultry litter, HPAI can persist for up to 60 hours compared to 24 hours for LPAI.[264]

HPAI maintains infectivity in fresh and frozen poultry products, creating a potential importation hazard.

- H7N9 on raw chicken remained viable at -20°C for 9 days, 4°C for 7 days, and 25°C for 4 days; therefore, H7N9 on raw chicken could be a potential source of transmission domestically and internationally.[265]

The stability of HPAI in unpasteurized dairy products and on milking equipment is not well understood.

- Initial studies indicate that H5N1 genotype B3.13 persists in milk on stainless steel milking equipment for over an hour and on rubber components for over 3 hours.[266]

What do we need to know?

- How long does infectious virus persist on dairy milking equipment?
- How long does infectious HPAI persist in unpasteurized dairy products?
- How long do HPAI strains maintain infectivity in frozen poultry carcasses?

Decontamination – What are effective methods to kill the agent in the environment?

What do we know?

The U.S. Environmental Protection Agency (EPA) maintains a list of registered chemical compounds for use in disinfection against avian influenza on farm settings, including bleach, alcohol, and quaternary ammonium-based compounds.[267]

- The EPA’s List M for registered antimicrobial products with label claims against AI: List M: Registered Antimicrobial Products with Label Claims for Avian Influenza | U.S. EPA.[268]

USDA APHIS maintains protocols for cleaning and disinfection of facilities affected by HPAI, and decontamination is a crucial component of HPAI response. HPAI-affected farms must undergo cleaning and removal of bulk debris, followed by disinfection by drying and heating (100-120ºF for 7 days) or wet disinfection with an approved product, and fumigation if needed.[269]

- During 2014-2015 outbreak, APHIS found that dry cleaning and heat disinfection of barns was most cost- and time- effective.[270]

- Sodium bisulfate is used to acidify poultry litter, and is largely effective at inactivating LPAI within 36 hours.[264] Acidification (exposure to pH 1 or pH 3 for 6 hours) is known to inactivate low levels of HPAI (H5N1) when suspended in peptone water.[250]
- Soap, detergent, and alkali (Surf Excel®, Life bouy®, and caustic soda) at 0.05% concentration at 28°C was not sufficient in destroying H5N1 virus, but increasing concentrations above 0.1% inactivated the virus after 5 minutes contact time at 28°C.[250]
- Chemical disinfectants (formalin, iodine crystals, phenol crystals, CID 20, Virkon-S, Zeptin, KEPCIDE 300, and KEPCIDE 400) can inactivate H5N1 at the recommended concentrations at 28°C.[250]
- Burning of contaminated poultry carcasses, litter, and feed in pyres or incinerators is another option for the decontamination and disposal of large amounts of contaminated waste resulting from HPAI outbreaks, if other methods are not feasible.[106, 271]

For facilities that cannot be adequately cleaned and disinfected, a fallowing period (allowing to lie dormant and unoccupied) is required.[269]

- The fallowing period is typically 120 days, but is dependent upon temperature and season.[269] Rapid depopulation to allow for a fallow period can prevent millions of U.S. dollars in lost profits.[272]

Various decontamination methods have been evaluated for poultry and cattle products to control the spread of AIV.

- According to USDA, AIVs can be inactivated in egg products and poultry meat by heating processes (e.g., 60°C for 188 seconds for whole eggs, 65°C for 42 seconds for poultry meat).[106]
- The guidelines provided by USDA Food Safety and Inspection Service time and temperature for cooking chicken meat to achieve a 7-log reduction of *Salmonella* is also applicable to AIV strains. AIV strains including HPAI were effectively inactivated in chicken meat held at 70 or 73.9°C for less than 1 second.[273]
- Pasteurization temperatures of both 63°C and 72°C rapidly and effectively inactivated influenza viruses in milk.[274] The FDA "does not currently have concerns about the safety and availability of pasteurized milk products".[66]
- USDA has concluded that there is no risk from beef cooked to 145 – 160°F.[275]
- The FDA and USDA recommend that any discarded milk should be heat-treated or pasteurized before disposal and that milk producers consult their respective State regulatory officials for any state-specific requirements.[81]

What do we need to know?

- What are additional cost-effective means of HPAI poultry virus decontamination?
- What are the risks of reinfection given different means of decontamination?
- What are the most effective means of decontamination for milking equipment and meat processing equipment?

Personal Protective Equipment (PPE) – What PPE is effective and who should be using it?

What do we know?

There is effective PPE for those with potential exposures to HPAI, with the recommended type of PPE dependent on the type of exposure (e.g., poultry workers, laboratory staff, depopulation workers).

- The greatest risk for AIV infection are those who have direct physical contact or close proximity (2 meters) to infected birds, contact with contaminated surfaces, or at a live poultry market.[276] In addition to frequent handwashing, PPE must be used when in direct contact with possible infected birds as wells poultry carcasses, poultry feces or litter, or when entering any premises with diseased or dead poultry.[276]
- Current CDC HPAI H5 or H7 virus human exposure monitoring guidance for avian outbreak responders: self-reporting illness (passive monitoring) for those wearing adequate PPE. For those with inadequate or lacking PPE, active disease monitoring is advised. For those responding to an AIV of unknown origin, active monitoring during exposure and continuing for 10 days post-exposure are recommended, regardless of PPE use.[277]

Recommended PPE for poultry workers includes safety goggles, disposable gloves, boots, a respirator (NIOSH-certified at N95 or higher), apron, disposable head/ hair cover, and disposable fluid-resistant coveralls.[276]

- Respirators should be used in a comprehensive respiratory protection program in accordance with the Occupational Safety and Health Administration (OSHA) Respiratory Protection standard (29 CFR 1910.134) and other requirements. Staff required to wear N95 (or higher) respirators require medical clearance, training, and fit-testing for respirator use.
- Reusable PPE (e.g. rubber boots, rubber apron) should be cleaned until visible dirt is removed, and then disinfected with an EPA approved disinfectant.[276]
- Poultry workers involved in depopulation should wear full PPE consisting of lightweight, disposable or heavy-duty rubber work gloves that can be disinfected, disposable outer garments, coveralls or surgical gowns with long, cuffed sleeves and a sealed apron, disposable shoe covers or boots that can be cleaned and disinfected, safety goggles and disposable head/hair cover, and an N95 or higher respirator.[278]
- To reduce risk of HPAI virus infection, landfill workers having contact with AIV-infected carcasses or potentially infected materials should use appropriate PPE when disposing of poultry carcasses during HPAI outbreaks,[276] including disposable gloves, boots, protective disposable fluid-resistant coveralls, goggles, and a NIOSH-certified respirator (e.g., N95 or higher) when in direct contact with infected birds, poultry carcasses, and/or poultry feces or litter.[276]

Recommended PPE for laboratory workers depends on the purpose of the work, the biosafety level of the laboratory, and the country of operation.

- Laboratory research with HPAI requires development and implementation of a written biosafety plan is proportionate to the risk of the select agent (9 CFR §121.12(a)).[279-280]
- Biosafety Level-2 (BSL-2) Laboratories: Laboratories such as veterinary diagnostic laboratories conducting routine screening surveillance on samples collected from wild birds and domestic poultry. In the U.S. and regions known to be HPAI-free, these are considered low-risk materials, and this work can be conducted in a BSL-2 laboratory.[281] Personnel are required to use , disposable gloves, laboratory coat, eye protection.[280]
- Biosafety Level-3 (BSL-3) Laboratories: In addition to standard BSL-2 practices, the following additional PPE and laboratory practices are used: powered air-purifying respirators, protective suit (e.g., wrap-back disposable gown, protective suit, disposable Tyvek gown), and double disposable gloves. For research with mammalian-transmissible HPAI viruses, disposable sleeves are worn over the gown while working in a biosafety cabinet, as well as shoe coverings (e.g., double disposable shoe coverings; single

disposable shoe coverings if worn with footwear dedicated to BSL-3 enhanced laboratory use, or impervious boots or shoes of rubber or other suitable material that can be decontaminated), and protective eyewear.[279-280]

- Biosafety Level-4 (BSL-4) Laboratories: Additional measures beyond the facility requirements for BSL-3 are not needed. The BSL-3 criteria are sufficient for appropriate HPAI biocontainment.[279]
- A cross-contamination event occurred in 2014 at the CDC between LPAI H9N2 (non-select agent) and HPAI H5N1 (select agent) viruses. Because proper biocontainment and PPE procedures were followed, the virus remained contained and no illness or injury occurred.[282]
- Those visiting a HPAI infected herd should refer to the USDA APHIS recommendations and follow any additional PPE recommendations of the local Incident Commander and Safety Officer.[283]

What do we need to know?

- Are PPE stocks sufficient in the event of a large-scale outbreak or multi-species outbreak?

Genomics – How does the disease agent compare to previous strains?

What do we know?

AI viruses are defined by the presence (HPAI) or absence (LPAI) of a polybasic cleavage site in the HA gene.

- LPAI viruses contain HA proteins that can only be cleaved (required for cell entry) by a limited number of enzymes; however, HPAI viruses contain HA that can be cleaved by a broader set of enzymes.[284] The transition from LPAI to HPAI can occur from mutations at the cleavage site during circulation of the virus in natural hosts, and these transitions can be documented by identifying the LPAI ancestors of HPAI strains in phylogenetic studies.[285]
- Between 1959 and 2019, there have been 42 observed transition events from LPAI to HPAI in H5 and H7 AIs.[284] While most led to restricted outbreaks, several, including H5 HPAI, continue to cause outbreaks in poultry.[284]
- Interspecies transmission of HPAI was enhanced through genetic reassortment of H5N8 with a North American avian origin LPAI virus resulting in the generation of H5N2 HPAI virus responsible for the outbreaks in Canada and the U.S. in 2015.[286]
- The high mortality of GsGd lineage HPAI H5N8 clade 2.3.4.4b virus in ducks is associated with a number of genome segments, not just HA,[27] and amino acid substitutions in polymerase genes have also been linked to elevated mortality.[26]

As with all influenza viruses, evolution of HPAI viruses is rapid, which contributes to the diversity of these viruses.

- The nomenclature for variants is complex and requires continuous revision. The H5N1 Evolution Working Group was established in 2007 to develop a unified nomenclature.[287] An influenza clade or group is an additional classification beyond subtypes or lineages. For GsGd lineage HPAI H5, several viral clades have been identified (0 to 9), with respective hierarchical orders denoted using decimals. For example, subclades 2.2 and 2.3 are genetically similar and part of clade 2, and 3rd and 4th order subclades (e.g., 2.3.2 and 2.3.2.1, respectively) denote further genetic variation within the subclade 2.3.[288]
- Since at least 2014, most of the continuously evolving and circulating GsGd lineage HPAI H5 variants have belonged to clade 2.[288-291]

- As of 20 June 2024, GsGd H5N1 clade 2.3.4.4b viruses have been detected on all continents except Oceania[292-293] (i.e., detected in Europe,[126] Asia,[294] Africa,[295] North America,[3] South America,[296-298] and Antarctica[299-300]). Other HPAI viruses have been identified in Oceania and as of 22 May 2024 this is an active outbreak of HPAI H7 strains in Australia.[301]
- The geographic spread of AIV has been reflected in reported human cases across the globe.[302]

Exchange of genetic material among co-circulating AI strains is a primary driver of evolutionary change.

- H5 AIVs (H5N1, H5N2, H5N6, and H5N8) infecting wild birds in China acquired different NA types through reassortment with other strains (H3N2, H6N6, H3N8).[303] HPAI H5 clade 2.3.4.4b reassortant viruses were detected in wild birds in The Republic of Korea in 2022.[304] Similarly, sequencing of strains from the Czech Republic showed a high propensity of HPAI H5 to reassort with LPAI strains.[305]
- AI viruses circulating in wild birds have extensive reassortment, rather than more stable, isolated evolutionary lineages,[306] suggesting that outbreaks of novel avian influenzas in wild birds may be related to the timing of reassortment events in natural populations.[307]
- The global diversity of HPAI viruses is not fully characterized, but the GsGd lineage HPAI H5Nx lineage is known to frequently reassort and have a relatively high evolutionary rate compared to LPAI resulting in high virus diversification.[119]
- Evidence suggests that control measures, primarily in China, aimed at reducing the global spread of GsGd lineage HPAI H5N1 may have facilitated the emergence of novel H5Nx lineages.[308]

While rare, human cases of HPAI may increase due to new mutations in circulating viruses. There is concern that GsGd lineage HPAI H5 viruses will gain human-to-human transmissibility.[309]

- The receptor binding preference could influence the probability of spillover events from avian species to humans. Avian-lineage influenza viruses differ from human-lineage influenza viruses in that they generally prefer to bind different sialic acids on cell surfaces, but adaptations and mutations have been documented in H5N1, H7N2, and H9N2 avian-lineage isolates recovered from humans.[310]
- Mutations to gene segments (HA) have been associated with increased affinity for human type receptors as opposed to avian type receptors. Mutations in other gene segments (PB2, M1) have been shown to enhance replication in mammalian cells.[92, 305, 311-312] PB2 mutations continue to be detected in viruses isolated from infected mammals.[93-94, 97, 313]
- Circulating viruses are often screened for mutations known to reduce efficacy of antivirals. Clade 2.2 viruses appear to retain susceptibility to neuraminidase inhibitor drugs and baloxavir.[185] However, novel mutations have been identified in these viruses that reduce susceptibility to adamantane, oseltamivir, baloxavir, zanamivir, or peramivir.[185, 314-316]

HPAI H5N1 Clade 2.3.4.4b, genotype B3.13 is associated with outbreaks in U.S. livestock.

- Viruses isolated from infected dairy cattle are from the 2.3.4.4b clade but belong to a new genotype, B3.13. B3.13 viruses have mutations in HA, M1, and NS genes but do not have mutations in PB2 or PB1.[317]

- The respiratory tract and mammary tissues of dairy cattle express the AIV-specific receptor sialic acid α2,3-gal.[318]
- AIV from the HPAI positive patient in Texas was closely related to isolates from Texas dairy cattle as well as wild birds. Overall, it was concluded that the virus lacked changes that would indicate adaptation to human or mammalian hosts. However, the isolate has a mutation in PB2, which has been associated with adaptation to mammalian hosts.[319]

What do we need to know?

- What biological factors influence spillover probability?
- What factors lead to LPAI viruses becoming HPAI viruses?
- What fraction of the global genetic diversity of HPAI poses a threat to human and animal health?
- How can we predict which HPAI viruses pose a pandemic threat?
- What conditions favor genomic reassortment between HPAI H5 viruses?

Virus Importation – What are the main routes of entry into the United States? Are there effective mitigation strategies to limit HPAI importation?

What do we know?

Importation predominately occurs via close interactions between wild migratory birds and domestic poultry,[320,321] though other sources may also play a role.

- Modeling suggests that wild bird migration and illegal poultry trade are primary forms of HPAI introduction, and that the legal poultry trade is not a major importation risk.[164]
- Some outbreaks in domesticated poultry[322] have been linked to imported contaminated carcasses, as imported HPAI can maintain infectivity in fresh[323-324] and frozen poultry products.[322-323]
- HPAI H5N1 outbreaks with novel genetic mutations have recently occurred in farmed American mink, suggesting that both imported and domestic mink farming are potential sources of HPAI importation.[71, 325]

HPAI outbreaks are associated with wildfowl migratory seasons and routes.[326]

- Northern Mexican poultry farms have experienced HPAI H7N3 outbreaks occurs since 2012, which may be a risk to U.S. poultry.[327]
- Korea appears vulnerable to HPAI outbreaks due to the East Asian-Australasian migratory flyway for waterfowl.[328-329]
- Phylogenetic analysis revealed two main pathways into Europe,[330] including spread from central Asia.[331-332]
- In 2014, clade 2.3.4.4 H5N8 HPAI viruses spread across Korea and to China, Japan, Russia, and Europe, and were eventually discovered in wild birds in Canada and the Northwestern U.S. from wild waterfowl in the Pacific Flyway.[333-334]
- GsGd lineage HPAI H5 clade 2.3.4.4b viruses are currently circulating in wild birds in the U.S..[82] Migration studies indicate that this virus has been imported into the U.S. across the Atlantic ocean via Iceland, Greenland/Arctic and/or pelagic routes.[4] Bird banding data showed widespread movement of waterfowl within the Atlantic Flyway and between neighboring flyways and northern breeding grounds.[335]

- There is an extremely low risk that HPAI could be transmitted to domestic poultry from corn or feed contaminated by feces of infected wild migratory birds.[336] Nevertheless, securing poultry food bins and cleaning up wasted or spilled feed is recommended.[337]

After importation, spread can be rapid,[338] and losses to poultry flocks and the economy can be severe.[334]

- The 2014-2015 HPAI H5 outbreak affected 21 Western and Upper Midwestern States and had a $3.3 billion impact on the economy.[334]

Movement and trade of livestock within the U.S. is encouraged to be minimized at this time and should not occur if any cattle or other animals on the premises display disease symptoms. Pre-movement testing is required for all lactating cattle and a 30 day quarantine is recommended after arrival of dairy cattle.[81, 160]

What do we need to know?

- Illegal poultry trade contributes to importation and spread of HPAI. How can illegal poultry trade be addressed to reduce the risk of HPAI importation?
- How will interstate and international trade of livestock impact the spread of genotype B3.13 HPAI?

Definitions of Commonly Used Acronyms and Names

Acronym/Term	Definition	Description
AABP	American Association of Bovine Practitioners	N/A
AIV	Avian Influenza Virus	Virus responsible for causing avian influenza
APHIS	Animal and Plant Health Inspection Service	N/A
AVMA	American Veterinary Medical Association	N/A
BARDA	Biomedical Advanced Research and Development Authority	N/A
BSL	Biosafety Level	N/A
CDC	Centers for Disease Control and Prevention	N/A
Clade	Closely related viruses based on the similarity of their HA genes	Influenza A virus subtypes are based on two proteins on the surface of the virus: hemagglutinin (HA) and neuraminidase (NA). Subtypes are further divided into clades, which are based on the genetic similarity of the HA gene.
CVV	Candidate Vaccine Viruses	N/A
d.p.i.	Days Post-Infection	N/A
DHS S&T	U.S. Department of Homeland Security	N/A
EID_{50}	Median egg infectious dose	The dose at which 50% of the inoculated eggs become infected. Used as a standard measure of infectivity.
EPA	U.S. Environmental Protection Agency	N/A
FAO	Food and Agriculture Organization	N/A
FDA	U.S. Food and Drug Administration	N/A
GsGd lineage HPAI	A/Goose/Guangdong/1/96 (GsGd) lineage of HPAI H5 virus	GsGd lineage HPAI circulates in waterfowl and other migratory wild birds as HPAI. This lineage is unique as other HPAI viruses typically emerge from LPAI after replication in a domestic poultry species.
Hemagglutinin (H or HA)	A glycoprotein found on the surface of cells and viral envelopes	Hemagglutinin on the surface of influenza binds to sialic acid to facilitate importation of the virus.
HID_{50}	Median Human Infectious Dose	The dose at which 50% of humans become infected. Used as a standard measure of infectivity.
HPAI	Highly Pathogenic Avian Influenza	Disease caused by a highly pathogenic avian influenza virus
ID_{50}	Median Infectious Dose	The dose necessary to infect 50% of the target population (e.g., birds). Generally, assumes typical, healthy, adult individuals.
LPAI	Low Pathogenicity Avian Influenza	N/A
MQL	Master Question List	N/A

Acronym/Term	Definition	Description
Neuraminidase (N or NA)	An enzyme that cleaves neuraminic acids	Newly replicated viral particles use neuraminidase to cleave sialic on the surface of the host cell, which allows the viral particle to be released from the host cell.
NHP	Non-Human Primate	N/A
NIH	National Institutes of Health	N/A
NIOSH	National Institute for Occupational Safety and Health	N/A
OSHA	Occupational Safety and Health Administration	N/A
PFU	Plaque Forming Unit	A measure of virus infectivity per unit volume. Infectious virus particles form a plaque in cultured cells.
PPE	Personal Protective Equipment	N/A
R_0	Calculated value for communicable diseases that represents the number of additional animals that one infected animal can further infect	N/A
Reassortant	Strain having genetic material from two or more related strains	Reassortment occurs when individual hosts are infected with multiple related virus strains simultaneously and those strains exchange genetic material; this genetic mixing leads to reassortants, which are the strains that result from such exchange.
RT-PCR	Real-Time Polymerase Chain Reaction	N/A
USDA	U.S. Department of Agriculture	N/A
UV	Ultraviolet	N/A
VSD	Ventilation Shut Down	N/A
WHO	World Health Organization	N/A
WOAH	World Organisation for Animal Health	N/A

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