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An Assessment of the Risk Associated with the Movement of Raised-for-Release Mature Upland Gamebirds from a State within the United States with a Highly Pathogenic Avian Influenza Detection to a Hunting Preserve Located Within or Outside of the Infected State

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1 Abbreviations and Definitions

AC	Antigen capture (as in "AC testing")
AI	Avian influenza
APHIS	Animal and Plant Health Inspection Service (USDA:APHIS)
CEAH	Center for Epidemiology and Animal Health (USDA:APHIS:VS:CEAH)
CFR	U.S. Code of Federal Regulations
C&D	Cleaning and disinfection, or cleaned and disinfected
dpi	Days post-inoculation (or days post-infection)
EA/AM	Eurasian/American
EPA	U.S. Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
GIS	Geographic Information System
GLEWS	Global Early Warning System for Major Animal Diseases Including Zoonoses
HA	Hemagglutinin
HI	Hemagglutination inhibition
HPAI	Highly pathogenic avian influenza
ILT	Infectious laryngotracheitis
IP	Infected premises
LPAI	Low pathogenicity avian influenza
NA	Neuraminidase
NAHLN	National Animal Health Laboratory Network
NAHMS	National Animal Health Monitoring System (USDA)
NAGA	North American Gamebird Association
NPIP	National Poultry Improvement Plan
NVSL	National Veterinary Services Laboratory (USDA)
PBA	Perimeter buffer area
PMIP	Pre-Movement Isolation Period
PPE	Personal protective equipment
PRRSV	Porcine reproductive and respiratory syndrome virus

rRT-PCR	Real-time reverse transcription polymerase chain reaction
SAHO	State animal health official
SPF	Specific pathogen free
U.S.	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet light
VS	Veterinary Services (USDA:APHIS:VS)
WOAH	World Organisation for Animal Health (WOAH; formerly known as Office International des Epizooties [OIE])
WHO	World Health Organization

AERMOD

Aerosol dispersion model developed by the EPA and recommended to be used for regulatory decisions associated with air quality.

BID_{50}

50 percent bird infectious dose. One BID_{50} unit is the amount of virus that will infect 50 percent of inoculated birds.

Biosecurity

A comprehensive approach of measures undertaken to prevent the introduction of disease agents into a specific area.

Buffer zone

The zone immediately surrounding the infected zone. The buffer zone and the infected zone comprise the Control Area.

Control Area

Consists of an infected zone and a buffer zone and established to ensure the rapid and effective containment of the disease. Initially, the entire State, commonwealth, Tribal Nation or territory may be declared a Control Area and subject to movement restrictions until appropriate surveillance and epidemiological evidence has been evaluated and the extent of the outbreak is known. All susceptible bird and other livestock movement will be stopped for a period long enough to determine the scope of the disease outbreak. The potential modes of transmission of HPAI will be considered when determining the minimum size and shape of a Control Area. Movement control using permits should be maintained until the disease is eradicated.

CID_{50}

50 percent chicken infectious dose. One CID_{50} unit is the amount of virus that will infect 50 percent of inoculated chickens.

Conventional poultry

Poultry produced by the most prominent commercial sectors of the poultry industry, including the egg laying industry, broiler industry, and turkey industry.

Downtime for visitors and personnel

For purposes of this assessment, downtime when associated with visitors or personnel refers to the time interval between when a visitor enters the hatchery and the time of last contact with other domestic poultry, other avian species, and/or related organic material from the Control Area.

Downtime for a farm

For purposes of this assessment, downtime when associated with a farm refers to the time interval when no birds are being produced for the market (e.g., for release on a hunting preserve)

Egg

The hatching egg of upland gamebirds. While mentioned, the movement of eggs is not assessed in this risk assessment.

EID_{50}

50 percent chicken embryo infectious dose. One EID_{50} unit is the amount of virus that will infect 50 percent of inoculated embryos.

ELD_{50}

50 percent chicken embryo lethal dose. One ELD_{50} unit is the amount of virus that will be lethal to 50 percent of inoculated embryos. Since most HPAI viruses are embryo lethal, the ELD_{50} estimates would be similar to EID_{50}.

Flight-ready upland gamebirds

Upland gamebirds that have reached the proper age and are in the proper feather- and physical-condition to be sold to hunting preserves and perform well when flushed (i.e., spooked into the air to be hunted).

Flock

The unit of upland gamebirds describing all the birds within a single pen or barn on an upland gamebird farm.

Fomite

An inanimate object, such as boots, clothing, etc., that when contaminated with a viable disease agent, can serve as a source of infection for a susceptible host.

Free Area

Any area outside of the Control Area. The Surveillance Zone is a part of the Free Area.

Hunting Preserve

A public or private commercial enterprise that owns and maintains land where hunting is controlled, usually providing guided or staged hunts for patrons. Can

often include commercial accommodations and other activities such as clay shooting for patrons.

Incident Command System (ICS)

A management system designed to enable effective and efficient domestic incident management by integrating a combination of facilities, equipment, personnel, procedures, and communication within a common organizational structure.

Infected Zone

In an outbreak of HPAI, the Infected Zone will encompass the perimeter of all presumptive or confirmed positive premises ("Infected Premises") and include as many "Contact Premises" as the situation requires logistically or epidemiologically. Activities in an infected zone include:

Preventing products from birds and other susceptible animals from leaving the zone unless a risk assessment determines that such movement can be permitted.

Preventing movement of vehicles, equipment, and non-susceptible animals out of the zone unless appropriate biosecurity procedures (as determined by a risk assessment) are followed.

Infectious period

The period that an individual bird is infectious (i.e., shedding HPAI virus at sufficient levels that transmission could result if there is adequate contact with a susceptible host).

Latent period

The period of time between infection of a bird and when it becomes infectious. Also known as the *eclipse period*.

Line of Separation (LOS)

The LOS is a clearly identified boundary around or within a poultry premises to separate off-farm traffic from on-farm movements of vehicles, people, and animals. The purpose of the LOS is to prevent movement of HPAI onto or from a premises. Crossing the LOS through a controlled access point requires following appropriate biosecurity measures.

Local area spread

Refers to risk pathways that have an increased likelihood for disease transmission with proximity to infected flocks.

Mature upland gamebirds

Upland gamebirds that have reached peak age to be released and are flight ready. Peak age ranges from roughly 16 to 28 weeks, depending on the species.

Movement permit

A VS Form 1-27, a State-issued permit or letter customized to the applicant's situation, generated by the Permit Team and issued at the discretion of Incident Command to allow the movement of poultry (including upland gamebirds) industry products from a premises or a geographic area described in a quarantine order.

National Poultry Improvement Plan (NPIP)

A cooperative State-industry-Federal program that establishes guidelines for evaluation of poultry products and poultry production relative to disease and eligibility for interstate/international trade.

Personal Protective Equipment (PPE)

Special clothing and equipment designed to act as a barrier between an individual and a hazard; in this case, the hazard is a highly contagious pathogen (HPAI). In the event of an HPAI outbreak, PPE serves to prevent the spread of the disease agent between animals and locations. For purposes of this report, appropriate PPE is considered protective boot covers, clothing, and gloves.

Poultry

Domesticated gallinaceous birds grown for commercial purposes (i.e., direct production and breeding stock), specifically chickens for egg laying and meat (i.e., broilers) and turkeys.

Premises

A geographically and epidemiologically defined location, such as a ranch, farm, plant, or other establishment.

Raised-for-release upland gamebirds

Upland gamebirds that are commercially raised in manner that allows for proper flight conditioning, with the specific purpose of being released on hunting preserves.

Secure Broiler Supply Plan (SBS Plan)

A science-based plan composed of outbreak measures and protocols proposed by the Broiler Sector Working Group to mitigate the risk of HPAI spread associated with the movement of hatching eggs and day-old chicks into, within, and outside of a Control Area. The SBS Plan includes various categories of measures, such as active surveillance, holding time, biosecurity, cleaning, and disinfection.

Secure Poultry Supply (SPS) Plan

A harmonized plan to facilitate poultry industry and State regulatory agency preparedness for product movement in an HPAI outbreak.

Secure Turkey Supply (STS) Plan

A set of science-based outbreak measures developed by the Turkey Sector Working Group to mitigate the risk of HPAI spread associated with the movement of turkeys, turkey eggs, and turkey semen in a Control Area.

Secure Upland Gamebird Supply (SUGS) Plan

A set of science-based outbreak measures developed by the SUGS Sector Working Group to mitigate the risk of HPAI spread associated with the movement of raised-for-released mature upland gamebirds to a hunting preserve from a premises that is in a State with an active HPAI outbreak, but not located within a Control Area.

Secure Upland Gamebird Supply Working Group

A working group made up of representatives from the upland gamebird industry, academia, SAHOs, and USDA:APHIS to support evaluation of the movement of upland gamebird live birds and products during an HPAI outbreak.

Standard Operating Procedure (SOP)

Established or prescribed methods to be followed routinely for the performance of designated operations in a designated situation.

Started upland gamebirds

Upland gamebirds that are roughly 5 weeks of age (depending on the species) that can live in outdoor pens.

NPIP Subpart J

Subpart of the National Poultry Improvement Plan (NPIP) specifically addressing the commercial raised-for-release upland gamebird industry.

$TCID_{50}$

50 percent tissue culture infectious dose. One $TCID_{50}$ unit is the amount of virus that will cause cytopathic effects in 50 percent of exposed host cells. The Madin-Darby Canine Kidney cell line is often used to estimate $TCID_{50}$ for HPAI viruses.

Upland gamebirds

Defined as the most common commercially raised types of upland gamebirds for the purposes of release in game preserves, including pheasant, bobwhite quail, and chukar. By species, these include *Phasianus colchicus* (Mongolian or Chinese pheasant), quail of the genus *Colinus* (bobwhite quail), and *Alectoris chukar* and *Alectoris rufa*, (chukar or red-legged partridge). Gamebird species that are sold for slaughter or live bird market sale are not within the scope of this risk assessment.

Upland gamebird farm

A commercial farm that produces only pheasants, quail, and/or partridge that are raised under confinement in outdoor netted pens or barns for release into hunting preserves.

Zoonosis

A disease caused by an infectious agent that can be transmitted between (or shared by) animals and humans.

2 Executive Summary

Industry, local, State, and Federal authorities implement a foreign animal disease emergency response during outbreaks of highly pathogenic avian influenza (HPAI). In these events, permit requests will or may (given the circumstances) be required to move poultry, including upland gamebirds, and poultry products must be supported by risk assessments that demonstrate the risk of HPAI spread associated with the movement is acceptable. Risk assessments can enhance emergency response and facilitate timely movement permitting decisions during an outbreak. This document assesses the risk that the movement of mature, flight-ready upland gamebirds to hunting preserves (i.e., upland gamebird to release), during an HPAI outbreak from a premises located outside of Control Area but in an HPAI-infected State will result in HPAI virus spread to a virus-free poultry premises.

This assessment is **based on information, data, and literature published or obtained as of November 2020[1],** but is an evolving product-specific risk assessment that will be reviewed and updated as necessary before and during an outbreak to incorporate the latest scientific information and preventive measures. This risk assessment is a joint effort of the Secure Upland Gamebird Supply (SUGS) Working Group, which is made up of representatives from the upland gamebird industry, academia, State Animal Health Officials (SAHOs), and the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA:APHIS). This working group and the assessment herein support permits for the terminal movement of upland gamebirds to release during an HPAI outbreak. This assessment is applicable to commercial raise-for-release upland gamebird premises that do not have other poultry or any waterfowl on the premises and do not participate in any live bird market activities. These upland gamebird facilities must participate in the SUGS Plan in the event of an HPAI outbreak. The SUGS Plan contains science-based outbreak measures developed by the SUGS working group to mitigate the risk of HPAI spread associated with the terminal movement of upland gamebirds to release.

This risk assessment considers applicable current industry practices and biosecurity measures (e.g., the NPIP) as well as outbreak-specific measures stipulated within the SUGS Plan. The main categories of outbreak measures outlined in the SUGS Plan for upland gamebird premises wishing to move birds to release include:

- Establishing criteria that are equivalent to those of a Monitored Premises designation to demonstrate that it is not an infected, suspect, or a contact premises
- Active surveillance (e.g., rRT-PCR [real-time reverse transcription polymerase chain reaction] and antigen capture testing, detection of abnormally high mortality)
- Observing the enhanced biosecurity measures of the Pre-Movement Isolation Period (PMIP)

The Pre-Movement Isolation Period (PMIP) is a critical biosecurity component that involves a period of greatly intensified biosecurity for an entire premises located outside a Control Area but in a State with an active HPAI outbreak prior to movement of upland gamebirds to release. In the case of upland gamebird premises, due to the frequency of movements, the PMIP lasts the

[1] During the charter review process of the risk assessment herein, Reyna et al (2021)[434] and personal communications were referenced to inform content. A systematic updating of the information, data, and literature in the risk assessment herein with studies and reports published after 2020 is still needed to accurately reflect the most up to date understanding of HPAIVs.

duration of an active HPAI outbreak in the State in which the premises is located. The PMIP to move upland gamebirds to release includes the following stipulations:

- No live or dead poultry or upland gamebirds will be moved onto the premises
- Only critical operational visits to the premises will continue
- Manure and litter will be managed on-premises; the producer is responsible for managing the risks associated with any on-site movement or handling of manure, litter, and garbage that must occur
- Garbage pick-up vehicles and personnel should not cross the PBA at any time
- Enhanced biosecurity will be implemented for people, vehicles, and equipment entering the premises; garbage pick-up sites on the farm must be located outside of the Perimeter Buffer Area (PBA)

The SUGS working group selected a PMIP that would last the duration of the outbreak and generally provides a high probability of detection. A **perpetual, repeating 8-day** PMIP is sufficiently robust to allow high probabilities of detection (i.e., > 95 percent) for all potential HPAI virus strains and contact rates.

The emphasis in this assessment is on the risk of HPAI virus spread to a susceptible poultry premises associated with the movement of upland gamebirds from outside of a Control Area, but within an HPAI-infected U.S. State. We assume that movement of infected and undetected upland gamebirds to release may pose some likelihood of HPAI spread to susceptible poultry with associated adverse consequences, and therefore, we rated the overall risk according to the likelihood of moving infected and undetected birds. The probability of detection before movement improves as the number of days after exposure increases. As HPAI moves through the flock, there is an exponential increase in mortality, which consequently increases the likelihood of including at least one infected bird in the pooled mortality sample taken for diagnostic testing or of observing total mortality above the threshold amount. Thus, the PMIP serves a dual purpose of (1) reducing the chances of exposure to HPAI close to the time of movement, and (2) allowing sufficient time for the infection to manifest itself within the flock and be detected.

To assess the overall risk of moving upland gamebirds to release, this risk assessment evaluated the possible pathways for virus transmission to upland gamebird premises. Each pathway may consist of combinations of several activities. We have grouped these pathways into several categories: 1) components of local area spread; 2) people and vehicles; and 3) load-out processes and equipment. Local area spread refers to risk pathways that cause an increased likelihood of disease transmission with proximity to infected poultry and gamebird flocks. If, due to a lapse in PMIP biosecurity practices or other unforeseen events, upland gamebirds are moved from the pen within a short time after being exposed to the HPAI virus, it is unlikely that HPAI would be detected by the time of movement. Therefore, pathways for HPAI infection of mature, flight-ready upland gamebirds close to scheduled movement, combined with the likelihood of detecting the infection prior to movement and the likelihood of infection during the load-out process, were considered when evaluating the overall risk of spread associated with movement of upland gamebirds to release. These pathways and the corresponding likelihood and risk ratings are described below. The overall finding and conclusion qualitatively integrate the results from the pathway assessments.

2.1 Likelihood of an Upland Gamebird Flock Becoming Infected with HPAI via Components of Local Area Spread Resulting in Infected but Undetected Movement to Release

- **Insects.** The likelihood of an upland gamebird premises becoming infected with HPAI virus via insect transmission varies with distance and with source premises' infection status, because proximity to a known infected premises directly influences likelihood. Of note, there are too many variables to accurately assess the risk of becoming infected with HPAI via insect transmission for premises located closer than 1.5 km to an infected flock. The following is a breakdown for the likelihood of HPAI spread to an upland gamebird flock via insect transmission:

Source premises type	Composite likelihood rating Distance from source (km)			
	1 km	5 km	10 km	15+ km[a]
Infected but undetected premises	*Low*	*Negligible to low*	*Negligible*	*Negligible*
Known-to-be-infected premises	*Not applicable*	*Not applicable*	*Negligible*	*Negligible*

[a] 15.42 km is the average distance an upland gamebird farm is located in relation to a poultry farm or other upland gamebird farm in the State of MN.[14]

- **Aerosols.** The likelihood of an upland gamebird premises becoming infected with HPAI virus via bio-aerosols varies with distance and viral load at the source premises. Literature reviews and outbreak reports as of 2020 indicated that aerosol transmission was not an important factor at distances more than 1.5 km from an infected flock. However, there is some evidence of aerosol transmission over shorter distances. The following is a breakdown for the likelihood of HPAI spread to an upland gamebird flock via bio-aerosol transmission:

Source premises type	Composite likelihood rating Distance from source (km)			
	1 km	5 km	10 km	15+ km
Infected but undetected premises	*Low*	*Negligible*	*Negligible*	*Negligible*
Known-to-be-infected premises	*Not applicable*	*Not applicable*	*Negligible*	*Negligible*

- **Wild birds**. The likelihood of HPAI virus spread to an upland gamebird premises via wild birds depends upon the type of wild birds, the propensity for specific types of wild birds to be carrying different virus strains, and the degree and frequency of upland gamebird exposure to the wild birds. Aquatic species and larger non-aquatic species

typically do not come onsite unless attracted by ducks raised onsite or bodies of water are present. However, passerine birds may access the inside of upland gamebird pens and sit on top of netting, and predatory species may attempt to gain access into pens or prey upon upland gamebirds through the netting. With an effective PMIP, the increased pen-to-pen biosecurity, specifically the use of pen-specific footwear, may decrease HPAI infection via wild aquatic birds, as their waste is unlikely to access or be tracked into a pen and direct fly overs are variable. Additionally, the birds that are larger than the size of a passerine have very limited contact with potentially infected conventional commercial poultry raised in barns (e.g., turkeys, broilers, egg laying chickens). Given that passerine birds and predatory species may access pens or contact upland gamebirds (even during a PMIP) and have been shown capable of shedding the virus, the likelihood of HPAI spread to an upland gamebird premises via each of these bird categories is described below:

Wild bird category	Composite likelihood rating (Wild birds)
Aquatic wild birds	*Low*
Non-aquatic wild birds (Primarily attracted to feed [Passeriformes and Columbiformes])	*Low to moderate*
Non-aquatic wild birds (predatory and scavenger [Accipitriformes, Strigiformes, scavenging Passeriformes under the family of Corvidae, and Falconiformes])	*Low*

- **Wild Mammals.** The likelihood of HPAI virus spread to an upland gamebird premises via wild mammals depends upon the type of exposure to and the species of the wild mammals. While large mammals do not typically scavenge on upland gamebird farms, they may access pens and prey upon birds; however, proper fencing and mitigations can help prevent predator access. These types of mammals would have no contact with potentially infected conventional commercial poultry raised in barns (e.g., turkeys, broilers, egg laying chickens), though access to mortality storage is possible. Home ranges of predatory mammalian species are typically smaller than the minimum distance between a known-to-be-infected farm and an upland gamebird premises participating in the SUGS Plan. Similarly, rodents can access pens, but the likelihood of rodents travelling between poultry premises is small. With an effective PMIP, the increased pen-to-pen biosecurity, specifically the use of pen-specific footwear and handwashing, may decrease HPAI infection via wild predators handled when trapped or dispatched onsite. Thus, the likelihood of HPAI spread to an upland gamebird premises via each of these bird categories is described below:

Wild mammal category	Composite likelihood rating (Wild birds)
Rodents	*Very Low*
Predatory mammals	*Low*

- **Live-haul routes.** The risk of HPAI virus spread to upland gamebird premises near poultry live-haul routes via feathers, feces, and other fomites is both distance- and source flock-dependent. Given that poultry and live-haul vehicles passing a susceptible upland gamebird premises would originate from within or outside a Control Area, the following risk ratings are provided:

Characteristics of live-haul vehicle	Risk rating at given distance (between live-haul road and poultry premises)		
	<100 meters	100-1000 meters	>1000 meters
Truck hauling poultry that had no PMIP and no tests	*High*	*Moderate*	*Low*
Truck hauling poultry that had less than optimum PMIP and tests (80% effective PMIP, delayed testing, or load-out >24 hours)	*Low*	*Very Low*	*Negligible*
Truck hauling poultry that had a PMIP & rRT-PCR- / AC-negative birds (100% effective PMIP; rRT-PCR testing consist of 11 swabs at the start of an 8-day PMIP and samples for AC testing consist of pools with five swabs taken at the same time, immediately prior to the start of load-out.)	*Very Low*	*Negligible*	*Negligible*

2.2 Likelihood of an Upland Gamebird Flock Becoming Infected with HPAI via Movements of People, Vehicles, or Equipment, Resulting in Infected but Undetected Movement to Release

- **Feed and Critical Operational Visits**. Critical operational visits will be limited during PMIP; however, delivery of feed during this period will continue to occur, and the potential for emergency veterinary visit also exists to ensure bird health. Provided the biosecurity stipulations of the PMIP are in place and strictly followed, the likelihood of an upland gamebird flock becoming infected with HPAI via feed and critical operational visits during PMIP was assessed as follows:

Critical operation component	Composite likelihood rating (Critical Operational Visits)
Contaminated feed	*Negligible*
Feed delivery (i.e., driver and/or vehicle)	*Low*
Other critical visitors (i.e., personnel and/or vehicle)	*Low to moderate*

- **Growers, Employees, and Their Vehicles.** During the PMIP, vehicle and visitor traffic to an upland gamebird premises should only include critical visitors, employees, and growers. Provided the SUGS PMIP measures for people and their vehicles are strictly followed (e.g., use of farm-specific clothing and pen-specific footwear, and proper cleaning and disinfection of the vehicle interior and exterior), we rate the likelihood of an

upland gamebird flock becoming infected with HPAI via people (namely growers or employees) and their vehicles during the PMIP as follows:

Person type	Composite likelihood rating (People)
Persons entering upland gamebird pens	*Low*
Persons not entering upland gamebird pens	*Very low*

- **Dead Bird Disposal.** Onsite mortality disposal, such as composting or burial, and depending on the management of compost or burial sites and/or the volume of mortality, scavengers may be attracted to the site. These species can biologically or mechanically carry HPAI virus from different poultry sites; however, the home ranges of these animals are typically smaller than the minimum distance between a known-to-be-infected farm and an upland gamebird premises participating in the SUGS Plan. As such, access to any on-farm dead bird storage container or disposal method presents a pathway for HPAI spread, but during a PMIP, pen-to-pen biosecurity, including pen specific footwear, minimizes transmission from the environment into the pen. The only offsite mortality disposal method used by the upland gamebird industry is landfills, which carry the same risk of HPAI transmission to the farm as is depicted in the Garbage Management risk evaluation (see below). Provided the SUGS PMIP measures (specifically discontinuing any off-farm mortality disposal and utilizing pen-specific footwear) are strictly followed, we rate the likelihood of an upland gamebird flock becoming infected with HPAI via dead bird disposal as follows:

Mortality disposal practice	Composite likelihood rating (Dead bird disposal)
Likelihood of an upland gamebird flock becoming infected via the mechanical or biological transfer of HPAI virus from on-farm dead bird disposal during PMIP	*Very Low*
Likelihood of an upland gamebird flock becoming infected via the mechanical or biological transfer of HPAI virus from off-site dead bird disposal that takes place prior to the PMIP	*See Garbage Management likelihood rating below*

- **Garbage Management.** Multiple types of potentially contaminated items have been reported to be disposed of in garbage on poultry operations which can share garbage routes with upland gamebirds depending on proximity, and there is potential for HPAI virus associated with garbage management to be tracked into an upland gamebird pen. Provided the SUGS PMIP measures (specifically placement of garbage dumpsters outside of the perimeter buffer area and use of pen-specific footwear) are strictly followed, we rate the likelihood of an upland gamebird flock becoming infected with HPAI via garbage management during the PMIP as *low.*

Pathway	Composite likelihood rating (garbage)
Garbage management	*Low*

2.3 Likelihood of an Upland Gamebird Flock Becoming Infected with HPAI via Load-out Crews, Vehicles, or Equipment Resulting in an Infected but Undetected Movement to Release

Load-out. Previous outbreaks have implicated contaminated load-out crews and equipment in the spread of HPAI. However, the load-out process for upland gamebirds differs from that of conventional poultry sectors (e.g., broiler and turkey) where no outside crews or equipment are used and load-outs are completed within 24 hours. Given that PMIP enhanced biosecurity and testing measures are strictly implemented, the risk of an upland gamebird flock becoming infected with HPAI virus via load-out operations and resulting in an infected but undetected movement to release is estimated to be *very low to low*.

Pathway	Composite risk rating (Load-out)
Load-out and transport to release	*Very low to low*

This assessment aids, but does not replace, the judgment of officials. This document is an evolving product-specific risk assessment that will be reviewed and updated as necessary before and during an outbreak to incorporate the latest scientific information and preventive measures. If the Incident Command System (ICS) is activated in response to an HPAI outbreak, APHIS (and State veterinarians and subsequent staff) will review this risk assessment with respect to the situation to assess industry requests for movement of upland gamebirds to release.

Overall Finding and Conclusion

The risk that movement of upland gamebirds from a premises outside of a Control Area to release on a hunting preserve into, within, and out of a State with an active HPAI outbreak, resulting in the infection of susceptible poultry, is ***low***, provided that all applicable preventive measures from the Secure Upland Gamebird Supply Plan (SUGS Plan), in particular the Pre-Movement Isolation Period, are strictly followed.

3 Introduction

During a highly pathogenic avian influenza (HPAI) outbreak in the U.S., poultry industry, local, State, and Federal authorities will implement a foreign animal disease emergency response. This response consists of a control and eradication strategy utilizing depopulation, quarantine, and movement control measures within a Control Area to prevent further spread of HPAI virus. State and/or Federal authorities may also issue official permits to allow movement of birds and their products from not-known-to-be HPAI infected premises within the Control Area to promote business continuity. A request for a movement permit must be supported by a risk assessment (or some scientifically based logical argument) to demonstrate that the risk of HPAI spread associated with the movement of the product in question is acceptable; whether the assessed risk level is acceptable will be determined by regulatory authorities and industry. Similar processes have been utilized in the present risk assessment to evaluate the risk of moving upland gamebirds located outside of a Control Area but located within a State with an active HPAI outbreak to demonstrate to regulatory authorities of other States receiving upland gamebirds whether the associated risk level is acceptable.

Completing these types of risk assessments in a timely manner during an outbreak can be challenging due to the fast-paced flow of animals into the market. Within the upland gamebird industry, individually operating producers precisely manage their own operations, raising thousands of birds, to coincide with the hunting seasons of the upland gamebirds they raise. These operations have extensive order lists that require an efficient flow of birds through the market. Proactive risk analysis identifies areas of risk and incorporates mitigation steps that minimize the spread of infection. Evaluating risk before an outbreak occurs facilitates timely emergency response and movement permitting decisions and minimizes unintended disruptions to business continuity.

Previous assessments within the Secure Poultry Supply Plan have explored the risk of HPAI infection or contamination during movements of egg products, hatching eggs, day-old chicks, and live birds in the broiler, egg laying chicken, and turkey poultry industry sectors. As of 2020, there have been no risk assessments for movements of live birds or other movements related to the commercial upland gamebird industry.

The purpose of this assessment is to provide regulators with an objective and defensible method of assessing the disease risk associated with the movement of upland gamebirds to a hunting preserve for release. As upland gamebirds are generally marketed between 16 and 28 weeks of age depending on the species, HPAI infection early in the brood or grow period would likely be detected before movement. However, it is less likely that HPAI would be detected by the time of movement if the upland gamebirds became infected during load-out or in the days leading up to movement, due to a delay between infection and the manifestation of clinical signs or increased mortality.

To evaluate the risk of movement of upland gamebirds that are located outside of a Control Area, but in an HPAI-infected State to release, plausible pathways were identified for the spread of HPAI infection. This analysis focused on pathways for HPAI infecting an upland gamebird flock in the days leading up to movement (entry assessment of HPAI virus onto upland gamebird farms at or before scheduled time of movement to a hunting preserve) as well as the pathways by which this movement of upland gamebirds could infect another flock in the area (exposure assessment of HPAI as the result of moving an infected but undetected upland gamebird flock).

Each pathway may consist of combinations of several activities and has been grouped into one several categories: 1) local area spread; 2) people, vehicles, or equipment; and 3) load-out.

Local area spread refers to risk pathways that pose an increased likelihood for infection due to proximity to an infected premises. The components of local area spread considered in this analysis include:

- bio-aerosols generated from neighboring infected poultry or upland gamebird flocks;
- transmission of HPAI virus through insects, rodents, predatory mammals, or wild birds (aquatic and nonaquatic);
- mechanical or biological transmission from dead bird disposal via wildlife; and
- fomite-mediated transmission from poultry live-haul routes.

Other pathways considered in this analysis include transmission through:

- feed delivery;
- vehicles associated with essential visitors;
- fomites associated with visitors or grower premises employees who may have had contact with infected poultry or poultry waste; and
- personnel and equipment used during load-out

This assessment applies only to the movement of upland gamebirds off premises located outside of a Control Area, but in an HPAI-infected State to release on a hunting preserve. This assessment considers current industry practices and biosecurity measures as well as outbreak-specific measures applicable for the movement of upland gamebirds to a hunting preserve in the risk evaluation. Specific biosecurity measures may vary widely by farm and geographic area. Categories of outbreak-specific measures from the Secure Upland Gamebird Supply (SUGS) Plan considered here include a Pre-Movement Isolation Period (PMIP) for flocks prior to movement to a hunting preserve. Other measures include:

- Limiting visitors to critical operations visits
- Specific feed truck and driver biosecurity measures
- Biosecurity measures for farm personnel and other essential visitors
- Load-out truck and crew biosecurity, including truck routing

If the Incident Command System (ICS) is activated in response to an HPAI outbreak, U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA:APHIS), the State Veterinarian of the State of premises origin, and the State Veterinarian of the State receiving the shipment of upland gamebirds will review this risk assessment regarding the situation in order to assess industry requests for movement of upland gamebirds to release at a hunting preserve. However, the ICS will not be involved in issuing permits for movements under the scope of this risk assessment, given that no birds under the scope of this risk assessment will be moving out of, into, or through a Control Area.[1]

4 Scope

This section describes the scope of the assessment regarding the type of movements addressed, the facilities covered, and additional considerations related to the hosts and pathogens covered within this assessment.

4.1 Facilities Covered under this Risk Assessment

This risk assessment is applicable to commercial upland gamebird facilities producing mature, flight-ready upland gamebirds that meet all the criteria listed below:

- Are raising upland gamebirds for the purpose of release (e.g., primarily ring-necked pheasants, partridge such as chukar, red-legged, and/or similar varieties, and bob-white quail)
- Are in a U.S. State that has an active HPAI infection
- Are NOT located within an HPAI Control Area (i.e., are not within 10km of a poultry or upland gamebird farm known to be infected with HPAI)
- Do not participate in activities related to live bird markets
- Participate in the USDA APHIS National Poultry Improvement Plan (NPIP) as stated in 9CFR145 subpart J and 9CFR146 subpart J and in conjunction with biosecurity principles approved at the 44th NPIP Biennial Conference
- Implement the SUGS Plan in the event of an HPAI outbreak
- Do not have conventional poultry (e.g., chicken or turkeys) on the premises
- Do not have ANY type of waterfowl (e.g., domestic or game species) on the premises

4.2 Types of Movements Addressed under this Risk Assessment

This risk assessment will address only the pathways that potentially affect the movement with the following criteria:

- Type of bird/product: Mature flight-ready upland gamebirds
- Destination premises: Hunting preserve
- Moving within or out of a U.S. State with an active HPAI outbreak
- Origin premises AND destination premises are not in a Control Area

4.3 Other Considerations

- The assessment focuses on the risk that movement of upland gamebirds to release will result in the spread of HPAI to other susceptible poultry. Although the risks to humans or wildlife associated with the production or movement of live upland gamebirds are critical concerns that should be addressed, they are outside the scope of this assessment. The Highly Pathogenic Avian Influenza Response Plan has personnel safety measures designed to mitigate risks to humans.
- This assessment does not evaluate the risk of transmitting poultry diseases other than HPAI. Risk management decisions for poultry diseases other than HPAI are not directly supported by this work.

5 Overview of Data Analysis Approaches

This assessment follows the general qualitative risk assessment principles recommended by the World Organisation for Animal Health (WOAH) import risk analysis guidelines.[2] However, the risk assessment organization has been modified from that proposed in the WOAH import risk analysis handbook as appropriate for the movement of mature, flight-ready upland gamebirds to hunting preserves. As noted in the introduction, many of the described pathways may play a role in both entry assessment (i.e., entry of HPAI virus onto upland gamebird farms at or before the scheduled time of movement to release) and exposure assessment (i.e., spread of HPAI to an upland gamebird flock as a result of the movement of an infected but undetected flock to release). Consequences of the movement of upland gamebirds to release are assumed to be less severe than the movement of birds to a processing plant or a premises with conventional poultry activities, based on the differences between volume and density of birds present onsite, the number of farms making deliveries to the site, and opportunities for cross contamination at processing plants in comparison to hunting preserves. However, a complete consequence assessment is outside the scope of this risk assessment.

The assessment utilizes an evaluation approach that rates the likelihood of individual pathways on a qualitative scale. The likelihood for each pathway was assessed and categorized using the descriptive scale (see **Table 1**). The qualitative ratings for the pathways were determined using multiple data sources and evaluation approaches, such as literature review, expert opinion, quantitative simulation model predictions, and past outbreak experiences. In cases where expert opinion is utilized, the influence of bias, uncertainty, and inconsistency between subject matter experts is considered as an important limitation to such data and information. Quantitative simulation model results from previously completed proactive risk assessments were used to estimate the prevalence of infectious birds in potentially infected but undetected poultry flocks located near the grow-out facility. Steady-state aerosol dispersion models recommended by the U.S. Environmental Protection Agency (EPA) were used to partially inform the risk of aerosol spread from infected and undetected farms, along with other approaches. To determine the rating for pathways involving a chain of events in which all must occur for the pathway to be completed, relatively more weight was given to events with the lowest likelihood in the chain.

Table 1. Descriptive scale to estimate the likelihood for an event to occur.

Likelihood Rating	Description
Extremely High	The event is almost certain to occur
High	There is more than an even chance that the event will occur
Moderate	The event is unlikely but does occur
Low	It is very unlikely that the event will occur
Very Low	There is a remote chance that the event will occur
Negligible	The likelihood that the event will occur is insignificant, not worth considering

The descriptive rating scale specific to the hazard (HPAI) in this assessment is provided **Table 2.**

Table 2. Descriptive rating scale specific to the hazard (HPAI) in this assessment.

Likelihood Rating	Description
Extremely High Risk	HPAI spread to other susceptible poultry through the risk pathway is almost certain to occur.
High Risk	There is more than an even chance that HPAI spread to other susceptible poultry through the risk pathway will occur
Moderate Risk	HPAI spread to other susceptible poultry through the risk pathway is unlikely but does occur.
Low Risk	HPAI spread to other susceptible poultry through the risk pathway is very unlikely
Very Low Risk	HPAI spread to other susceptible poultry through the risk pathway is remote
Negligible Risk	HPAI spread to other susceptible poultry through the risk pathway is insignificant or not worth considering

Uncertainty within the likelihood/risk estimations was accounted for by using a range defined by the terms in the descriptive rating scale. A risk estimate of *negligible* to *low* includes the true risk, which is not deterministically known, where the interval between the two ratings represents the uncertainty in the analysis. For example, a *negligible* to *low* rating if the premises is located 1.5 km from an infected but undetected poultry farm was used with regard to aerosol transmission where there is considerable uncertainty in the aerosol dose-response relationship in individual birds and the particle size distribution of aerosols generated in flock houses or pens depends on the ventilation, production type, and age of the birds. Other areas of uncertainty were handled similarly during the analysis.

The overall risk estimate for the movement of upland gamebirds to release was determined by qualitatively combining the likelihoods of the individual pathways assuming that all applicable preventive measures from the SUGS Plan, particularly the PMIP, are strictly followed (see **Figure 1** below).

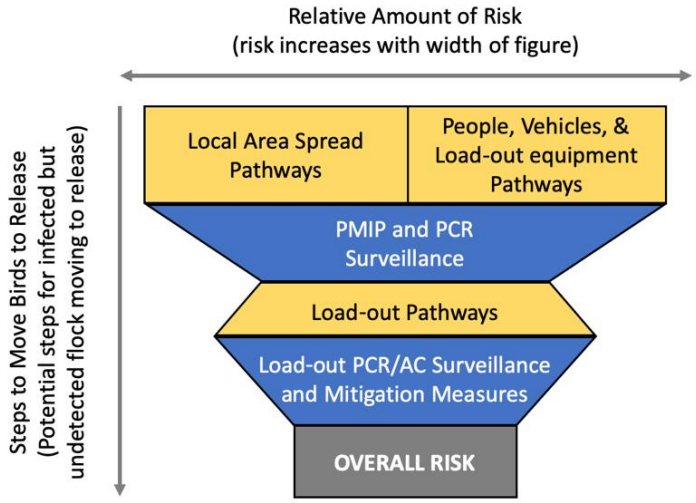


Figure 1: Diagrammatic representation of the overall assessed risk. The overall risk assessment is based on consideration of the steps needed to move upland gamebirds to release and the pathways that could lead to infection of a flock, the subsequent likelihood of detection of the infected flock, and potential movement of an infected but undetected flock.

6 Significant Assumptions Used in the Risk Assessment

This assessment is proactive in nature and cannot address the specific circumstances surrounding an outbreak in detail. Therefore, we must make some assumptions to establish context and applicability. These assumptions are that:

- An HPAI outbreak has been detected, APHIS is implementing the HPAI Response Plan, and some degree of planning has taken place at other levels. The APHIS HPAI Response Plan is intended to complement regional, State, and industry plans. APHIS recommends their continued development.
- Upland gamebird farms may have HPAI infection in their flocks, but it has not yet been detected. If there were absolute certainty that an upland gamebird shipment arrives at a preserve without evidence of HPAI infection, there would be no risk of HPAI spread from movement of birds from an upland gamebird farm. On the other hand, if HPAI infection has been detected on the premises, it is assumed that Incident Command would quarantine the premises. If infection was detected, the movement of upland gamebirds to release would not be allowed (and the premises would be depopulated, cleaned, and disinfected before resuming production).
- Movement of infected but undetected upland gamebirds to release could potentially spread HPAI to susceptible poultry. While a complete consequence assessment is outside the scope of this risk assessment, compared to movements of infected but undetected live conventional poultry (e.g., transfer movements or terminal movements of commercially-raised chickens and turkeys) where consequences of movements are assumed to be high due to contamination of poultry processing plants and other poultry farms with or without live poultry, consequences of movements of infected but undetected upland gamebirds to release are assumed to be lower. This assumption is based on the following:
    - After upland gamebirds are released at the hunting preserve, their risk to surrounding susceptible poultry premises is similar to wild birds (with key differences in wild bird versus captive-raised bird behavior listed in **Appendix 1 Table 2**). The role of HPAI spread to a conventional poultry flock in a control area via wild non-aquatic birds (including wild or free roaming ring-necked pheasants) in farm vicinity is thoroughly assessed in other live bird movement Secure Poultry Supply risk assessments. The likelihood of HPAI entry via wild non-aquatic birds for is rated as *low* for layer pullet farms, broiler farms, and turkey grow-out farms, per their corresponding risk assessments.
    - Findings from **Appendix 1**: Pen-Raised Upland Gamebird Survival Post-Release which summarizes the published literature as well as expert opinion regarding pen-raised upland gamebird dispersal and survival post-release as well as causes of mortality outside of human harvest.
- The movement of upland gamebirds to release is in accordance with the SUGS Plan, and all relevant preventive measures from the SUGS Plan are strictly followed. The assessment does not evaluate the risk that preventative measures are incorrectly implemented either intentionally or unintentionally. If preventative measures, as outlined in the SUGS Plan and the corresponding permit guidance derived from this risk assessment are not implemented correctly, the risk rating presented within this risk assessment is assumed to be higher than what is reported.

- Other mechanisms outside of the SUGS Plan may be utilized for HPAI control at the discretion of the Incident Commander. Such mechanisms may aid in reducing the risk rating for the movement reported within this risk assessment; however, without formal evaluation of these mechanisms, the effect of such actions is uncertain.
- The upland gamebird premises only contains the upland gamebird types that are outlined within the scope of the risk assessment. If upland gamebird premises contain species outside the scope of this risk assessment, the risk rating associated with movements from those premises will differ (i.e., could be higher) than the risk rating reported in the present assessment.
- The risk assessment applies to HPAI virus strains that cause clinical infection and increased mortality in infected upland gamebirds (e.g., gallinaceous birds). The risk assessment may not apply to strains that do not cause clinical signs representative of HPAI infection (e.g., AI strains that are classified as highly pathogenic on a molecular basis only). For such strains, this risk assessment would have to be revised to reflect the biological characteristics of the virus. As such the risk rating of moving upland gamebirds from premises that fall under the scope of this assessment would likely be higher than is reported in the present assessment if there is an outbreak of an HPAI virus strain that does not cause clinical infection and increased mortality in infected upland gamebirds due to the impact on the efficacy of surveillance protocols as described in this assessment.
- The disinfectants used to implement various cleaning and disinfection (C&D) measures in the SUGS Plan during an outbreak have been approved by the Incident Command and are applied according to the manufacturer's label directions or recommended procedures. If disinfectants are applied in a manner that is alternate to manufacturer's label directions or recommendations, the risk rating of moving upland gamebirds from premises that fall under the scope of this assessment would likely be higher than is reported in the present assessment.
- Experimental studies and case reports, which inform this risk assessment, are reported as they have been written. Measures, like Ct values, which may vary based on primer design, specimen type, and materials and equipment used, should not be interpreted to be specific quantitative estimates or taken out of context.
- Mathematical simulation models, by definition, represent a simplification of biological disease transmission processes. Some variability in the actual outcomes (which could increase or decrease the actual risk of product movement) is expected due to factors such as production practices, strain variability, bird genetics, and environmental conditions. Therefore, the model predictions are approximate, and the results may vary for specific outbreak scenarios. Nevertheless, mathematical simulation models are critical to inform animal health emergency management and have been widely applied for various aspects such as surveillance design, risk assessment, and evaluation of outbreak control strategies. Moreover, the only other alternative is to conduct field experiments, which are a higher-consequence endeavor.
- Of note, no upland gamebird premises under the scope of this risk assessment will be within 10 km of a known-to-be-infected poultry premises, thus only risks of known-to-be-infected premises greater than 10 km away and of infected but undetected premises

are considered when looking at farm distance-dependent pathways. If upland gamebird premises *within* 10 km of an infected but undetected premises (i.e., within an HPAI Control Area) were considered within the scope of this risk assessment, the likelihood of introduction via distance-dependent would differ (i.e., potentially be higher) than the likelihoods reported in the present risk assessment.

Of note, if any of the assumptions listed above are not met, the more uncertainty would be introduced to the movement scenario, thus changing the risk rating as reported within this assessment.

7 Background

7.1 Definition of Upland Gamebird Species

Commercial raised-for-release upland gamebirds are defined as birds in the order of Galliformes, including the species of wild turkeys, partridges, pheasants, and quail, specifically excluding waterfowl, doves, and pigeons, that are raised for release onto a preserve or into the wild for the purposes of hunting.[3] Within Title 9 CFR 145, NPIP upland gamebirds are defined as: domesticated fowl such as pheasants, partridge, quail, grouse, and guineas, but not doves and pigeons. Upland gamebirds are formally included within the 9 CFR 145 definition of poultry. However, while formally classified as poultry, they are also considered a "wildlife crop."[4]

Various types of pheasant, quail of the genus *Colinus*, chukar partridges, and wild turkey are the most prevalent upland gamebird varieties raised in the United States,[5] with the different species and subspecies within each type having almost identical husbandry requirements and production set ups [3, 4] (Secure Upland Gamebird Supply Working Group, personal communication, August 2016).

7.2 Definition of the Maturation and Harvest Process

On a commercial upland gamebird farm, production of live flight-ready birds sold for release coincides with hunting seasons, which are generally from early or mid-autumn to mid-winter, depending on the species and State regulations.[3] Hatching of chicks begins in mid-March and continues through mid-August. Birds are moved to brooder buildings, similar to those used in the conventional poultry industries, starting in April. Brooding of multiple batches goes on until as late as September or October.[3] When birds leave the brooders, they are referred to as started birds and are moved into large, sectioned pens covered with netting. Birds are kept in outdoor pens until they are considered flight ready; i.e., birds that have reached adult weight and plumage and are ready for release.[3] Birds selected as breeders are placed in pens to overwinter until the next production cycle in the following spring, with some birds starting to lay as early as December[6] (Secure Upland Gamebird Supply Working Group, personal communication, August & September 2016).

This risk assessment specifically focuses on the movement of mature, flight-ready upland gamebirds from upland gamebird farms to hunting preserves.

7.3 Overview of Commercial Raised-for-Release Upland Gamebird Production in the United States

The commercial upland gamebird industry started in the 1940s[3,7,8] when operations moved beyond hobby production. This small but substantial sector of commercial poultry production has grown into a niche industry of considerable value to numerous communities in the United States.[9] Nationally, in 2003, the upland gamebird industry directly contributed more than $1.6 billion to the economy.[9]

Hunting preserves and private hunters annually purchase roughly 5 million pheasants and close to 3 million chukars for the purposes of release and recreational hunting. The top pheasant producing States include Kansas, Minnesota, Pennsylvania, North Dakota, South Dakota, and Wisconsin, while the top bobwhite quail producing States include Alabama, Mississippi, Georgia, North and South Carolina, and Texas.[7,10]

7.3.1 Integration

Most of the individual upland gamebird production premises in the United States possess facilities where birds are bred, hatched, brooded, and grown to maturity by a single establishment[3, 4, 6, 8] (Secure Upland Gamebird Supply Working Group, personal communication, July 2017).

7.3.2 Service Technicians and Poultry Health Monitoring

Service technicians are not used in the upland gamebird industry, with day-to-day health monitoring of the flock performed by the farm owner and employed flock caretakers of the farm[6] (Secure Upland Gamebird Supply Working Group, personal communication, July 2018). Farm owners schedule chick arrivals, feed deliveries to farms, and final load-out for transport. Farms will employ contracted veterinarians to assess flock health on an *ad hoc* basis.[11]

7.3.3 Upland Gamebird Distribution and Logistics

Mature birds are sold in accordance with the hunting seasons of the region and species, with deliveries beginning a week or two before the start of the season and the majority of the birds delivered during the actual dates of the hunting season. Most of the larger farms will contract with a hunting preserve or client on an annual basis (for the entire hunting season of that year). Farms are typically paid either by cash on delivery or a net payment due within 30 days. While not common, some contracts will last for multiple years. Farms take a pre-order of a specified number of birds and dates of drop off are pre-determined or birds are delivered on an as needed basis. If dates are pre-determined, a certain level of flexibility of dates is required based on external situations such as weather. While the practice of setting pre-determined dates versus delivery on an as-needed basis varies regionally, the proportion of on-demand delivery is observed to be about 70 to 80 percent. If customers do not pre-order birds, typically only surplus birds are available for purchase (Secure Upland Gamebird Supply Working Group, personal communication, February 2019).

Because orders are spread out over an entire hunting season, upland gamebirds are usually sold in small batches and pens are not always cleared out all at once[12] (Secure Upland Gamebird Supply Working Group, personal communication, August 2016; Observed in Ssematimba et al. (2019) study's unpublished mortality data). For example, one producer reportedly ships as many as 4,000 ring-necked pheasant roosters in one load or up to 6,500 ring-necked pheasant hens or 7,500 partridges per load since the latter two are smaller than roosters.[13]

Upland gamebird growers typically move birds regionally within the United States;[3] however, groups of birds can travel on average anywhere from 160 to 1609 km, depending on the location of the farm and destination site, and interstate movements are not uncommon[6] (Secure Upland Gamebird Supply Working Group, personal communication, May 2018). In some cases, flight-ready upland gamebirds can also be picked up at the production premises by customers. Regardless of how birds are transported, growers aim for birds to reach their destination within 48 hours of load-out,[3] with delivery within 24 hours being the most typical (Secure Upland Gamebird Supply Working Group, personal communication, May 2018).

7.4 Overview of Major Steps in Production of Upland Gamebirds during Routine Operations

7.4.1 Upland Gamebird Facility Operations

Generally, farms encompass all steps of production on a single premises. That is, breeding, hatching, brooding, and grow-out usually occur on the same site, and birds are typically moved in small batches off the farm due to the demands of the hunting preserve markets.[8,14] It is common practice for producers to produce more than one species of upland gamebird on a farm.[3]

Co-mingling of species grown on a single site is not recommended nor generally practiced, and raising non-upland gamebird species (such as chickens, ducks, waterfowl) onsite is discouraged.[15]

The major steps in upland gamebird production and finishing during normal operations are described in the following sections. Biosecurity compliance can be variable, similar to other livestock sectors.

7.4.1.1 Upland Gamebird Outdoor Pen and Indoor Housing Preparations

Downtime is the period after all the birds are removed and before the pen is filled again. Upland gamebird farms operate on a seasonal production schedule. If pen segments are only used once per season, downtime for the majority of upland gamebird farms is between 6 and 8 months[14] (Secure Upland Gamebird Supply Working Group, personal communication, May 2018; Secure Upland Gamebird Supply Working Group personal communication, July 2018). The long downtime period is due to the seasonal demands of the market. During the downtime period, cover crops in the pens are tilled or mowed and regrown for the next season. Using pen segments twice in one season occurs only if the growing seasons begin early and the market demand allows for it. If a pen segment is used more than once, the downtime between emptying and refilling of the pen is about one week (Secure Upland Gamebird Supply Working Group, personal communication, May 2018; Secure Upland Gamebird Supply Working Group, personal communication, July 2018).

Cover crops are an important component of production pens; they provide enrichment and protection from flock members to individual birds.[4] They also serve as an additional source of food, shelter, and shade for birds.[4] Producers choose which cover crop to use based upon climate, season of use, the species of upland gamebird they raise, and access to irrigation or rainfall. Typically, if the cover crops become too dense and impede the mobility of the workers or birds, the rows are cut.[4] For breeder upland gamebirds, pens are devoid of cover crops so that workers can locate eggs easily.[3]

Pens are covered with regularly maintained netting and fencing to aid in wild bird and rodent exclusion and control[14] and to keep birds from escaping confinement.

7.4.1.2 Grow-out Period Management

7.4.1.2.1 Chick Production

Chicks are usually hatched onsite within the producer's own hatchery[14] (Secure Upland Gamebird Supply Working Group, personal communication, July 2018). Personnel follow the farm's biosecurity guidelines, wear clean boots and uniforms, and maintain lines of separation and workflow patterns from dirty to clean areas (Secure Upland Gamebird Supply Working Group, personal communication, July 2018). Ventilation systems are in place to reduce backflow contamination into clean areas. On some larger operations, there are hatchery-specific personnel,

but generally farm employees work in all aspects of production, including in the hatchery[14] (Secure Upland Gamebird Supply Working Group, personal communication, June 2018). Chicks are transferred from hatcheries to brooder barns by hand or using boxes carried from hatcheries to brooders. Once empty, the chick boxes should be removed and returned to the hatchery for washing and disinfection (Secure Upland Gamebird Supply Working Group, personal communication, June 2018).

7.4.1.2.2 Brooding

Like in conventional poultry, upland gamebirds are artificially brooded in facilities that maintain optimal environmental conditions for the chicks. In brooder barns, light intensity is low to reduce aggression among chicks.[7] Producers typically have the brooding facility onsite alongside with the other components of production (e.g., breeding, hatching, growing).[8] Upland gamebird growers typically utilize one of two common styles of brooder facilities, the first of which is a Room A/Room B brooding facility (Secure Upland Gamebird Supply Working Group, personal communication, June 2018). The Room A/Room B facility can accommodate the brooding of two batches of chicks of different age groups in the same facility. Each room grows a single batch of birds until they are ready to be moved outside to the pens. The second brooder facility type is the single room facility. In a single room facility, a single age group (i.e., a single batch of chicks) is grown and emptied in an all-in, all-out manner (Secure Upland Gamebird Supply Working Group, personal communication, June 2018).

Upland gamebird producers typically use either cool-room or warm-room brooding. Cool-room brooding provides isolated heat sources that chicks can move to and from to self-regulate body temperature, while warm-room brooding maintains the entire room at uniform temperature.

Heat sources include radiant or hover type in open floor brooding set ups, specifically different types of heat lamps, hot water pipes, or stoves are used to provide heat. In cold-room set ups, ambient heat provided by heat sources should be around 95 degrees F, with a slightly higher temperature for quail and chukar species. In warm-room setups, the temperature of the room should start at around 90 degrees F. As chicks mature, the temperature can be gradually reduced.[4]

Ventilation in brooding houses is controlled to maintain good air quality. Because chicks are susceptible to air quality problems and drafts, maximum ammonia levels and air speed need to be monitored.[4] Although chukar chicks are brooded in the same way as pheasants, they are better off raised on wire (instead of straw as done for pheasants) after 2 to 3 weeks of age, due to their high sensitivity to excess moisture and fecal-borne pathogens.[7]

7.4.1.2.3 Grow-out

Grow-out, the second stage of upland gamebird growth, begins when started birds are moved to outdoor pens. The age at which upland gamebirds are moved into outdoor pens varies by species. Specifically, pheasants are moved at 5 to 8 weeks, chukar partridge are moved at 6 to 8 weeks, and bobwhite quail are moved at 5 to 6 weeks, though bobwhite quail are most often raised entirely indoors.

Once birds are moved to outdoor or grow pens, the environmental control is diminished, and birds are subjected to natural changes in temperature, precipitation, and air quality. While outdoors, severe weather such as heavy rains, late spring snow storms, hail, winds, and predators may lead to bird losses.[7] Providing adequate shelter, accessible but protected food and water sources, and proper cover crops helps to ensure that birds can cope with changing environmental

conditions. It is widely assumed that since upland gamebirds are a wildlife crop,[4] they are better suited for outdoor pens, and maintaining upland gamebird "wild" behavior is desirable. By the time started birds are moved into the pens, they are hardy enough to resist disease or changes in temperature, compared to younger birds.

Pheasants: Started pheasants are moved to outdoor pens at 5 to 8 weeks of age and their finishing period begins at around 22 weeks of age, with flight-ready birds marketed at 22 to 28 weeks of age. Typically, at five weeks of age, pheasants are fitted with specs (short for spectacles and also called peepers), a small piece of plastic that obscures the bird's direct center vision in a process called peeping. In addition to proper cover crops and adequate shelters, specs reduce aggression between pen mates[3] (Secure Upland Gamebird Supply Working Group, personal communication, June 2018). In case the ground in the pens gets muddy due to heavy rain, straw is put down to keep the birds out of the mixture of feces and mud.[13]

Chukar partridges: Started chukar are moved to outdoor pens at 6 to 8 weeks of age and are marketed as flight-ready birds at 15 to 20 weeks of age.[3]

Bobwhite quail: Bobwhite quail are brooded until 5 to 6 weeks of age and are raised until 18 to 20 weeks of age when they are marketed. They are predominantly grown indoors in confinement set ups on floors with wood shavings similar to those in conventional poultry, though they could also be immediately moved and grown in flight pens after brooding. These indoor confinement set ups have resulted in improved livability, reduced feed consumption, and minimized disease issues.[3] Aggression between birds is reduced by maintaining low light intensity or by practicing beak trimming.[3] When raised in flight pens, shelter and dense vegetation cover allow quail to escape bird to bird aggression (Secure Upland Gamebird Supply Working Group, personal communication, September 2016).

7.4.1.3 Load-out

Flock caretakers (i.e., general employees of the farm) perform the catching and load-out, thus requiring no outside crews (Secure Upland Gamebird Supply Working Group, personal communication, May 2018). Birds are typically caught in the morning. Catching birds in the morning allows birds to be caught in cooler temperatures to help reduce stress and overheating and allows delivery trucks to have the maximum amount of time to travel to the delivery destination (Secure Upland Gamebird Supply Working Group, personal communication, February 2019). Evening or night catches are done if overnight travel is needed to get the birds to their destination during daylight hours the following day. Workers performing the load-out will cut and remove specs by hand during the load-out process. Methods of catching birds and transferring to crates varies slightly among the industry, with the most common methods including:

- Birds are herded into driving lanes outside of the pen and held in catching pens the night prior to load-out. The following morning, birds are caught by hand. Birds are provided with *ad libitum* water and food to ensure that they are ready for transport to preserves. Catching pens most often have fiberglass or cloth walls and are at least two feet tall. To prevent birds from piling in the corners, crates are placed in the corners of the catching pens. Workers will catch and hold five to six birds at a time before placing them in the crates once peepers have been removed. Workers will move as quickly as possible to ensure efficiency and reduce the amount of stress on the birds (Secure Upland Gamebird Supply Working Group, personal communication, June 2018).

- Birds are caught in their original pens using landing nets and transferred to crates by hand. Birds are taken out of the nets and have specs removed before being placed into crates one by one. This method limits the ability to efficiently sort by sex and quality of bird and can be slightly more stressful for the birds (Secure Upland Gamebird Supply Working Group, personal communication, June 2018).

Factors considered during load-out include weather (e.g., heat, precipitation, humidity), bird density in each crate, and ventilation depending on season (Secure Upland Gamebird Supply Working Group, personal communication, June 2018).

7.4.1.4 Transportation of Upland Gamebirds to Hunting Preserves and Awaiting Release

The type of crates and vehicles used for bird load-out varies from farm to farm depending on the scale of the operation and resources available. Most often, straw-lined plastic or wooden crates or disposable cardboard boxes hold birds during transport. Recently, more farms have acknowledged the importance of adopting plastic crates due to ease of cleaning and reduced chance of sustaining invasive micro-organisms in the crate material. In colder months, larger crates are used to haul birds. Roughly 10 rooster pheasants can fit into larger crates without damaging tail feathers. In warmer weather, smaller crates with wire sides are used for better ventilation. These smaller crates can hold roughly five rooster pheasants, 10 pheasant hens, or 15 partridges. Crates are loaded onto vehicles by hand.[13]

Farms use either their own or leased vehicles which are specific to their premises[13] (Secure Upland Gamebird Supply Working Group, personal communication, February 2019), and mostly include small trucks that do not require a commercial driver's license to operate with custom trailers with the top producing farms using semi-trucks and trailers[3] (Secure Upland Gamebird Supply Working Group, personal communication, February 2019). Truck capacities can vary depending on the type and size of truck and specifics of the order but loads of birds transported at one time vary between 500 to 4000 for pheasants, with the maximum load numbers being higher for the smaller species such as chukar and quail. Typically, shipments of chukar and quail are added to trucks already delivering loads of pheasants, but maximum capacity for trucks doing quail-only shipments have been reported to be as high as 15,000 birds in one shipment. Large loads of quail are more commonly seen in southern States where this species is more heavily produced (Secure Upland Gamebird Supply Working Group, personal communication, February 2019).

Although delivering to multiple hunting preserves in one trip is discouraged,[3] this practice is viewed as necessary in the industry because of how bird orders and deliveries are structured.

Onsite, birds may be released directly from crates into the field but are more often held in pens or buildings until needed for restocking the field. Delivery drivers will unload crates of birds into or just outside of the pens, empty the crates of birds into the pens, and then reload crates back onto the truck. Sizes and numbers of holding pens or buildings varies depending on the operations of the hunting preserves. Larger hunting preserves located in regions with colder autumn temperatures may have larger numbers and sizes of pens. Some preserves that operate part-time (e.g., only on weekends) will have smaller and fewer pens. The practice of direct release into the field varies based on region and individual hunting preserve (Secure Upland Gamebird Supply Working Group, personal communication, February 2019). Upon release into the field (either from the pens or crates), if they are not killed by hunters, birds typically do not survive to the next hunting season on preserves because of predation, starvation, or mechanical

injuries[16] (see **Appendix 1**: Pen-Raised Upland Gamebird Post-Release Survival & Dispersal Range for more details).

7.5 Overview of Current Disease Prevention and Biosecurity Efforts in Upland Gamebird Production

Biosecurity involves procedures that reduce the probability of disease outbreaks and includes two components: (1) bioexclusion (keeping pathogens out) prior to an outbreak, and (2) biocontainment (keeping pathogens from leaving a flock) after an outbreak occurs. Farms with poor biosecurity are vulnerable to diseases, which have the potential to ruin an entire flock. Loss of income from disease can be an enormous financial burden to upland gamebird and other poultry growers, so the importance of biosecurity cannot be overstated.[7,17]

In the upland gamebird industry, despite inabilities of outdoor production systems to maintain perfect bioexclusion because of direct exposure to the environment, farms possess an observed potential for strong biocontainment during non-outbreak time periods. Upland gamebird farms are shown to be more geographically isolated, providing them with strong conceptual biosecurity. Additionally, upland gamebird farms are less likely to be involved in production-related networks (i.e., delivering birds to a shared poultry processor, using shared crews for load-out or vaccination, etc.).[8,14]

7.5.1 Current Disease Prevention and Containment Measures in Grow-out Operations during Normal (non-outbreak) Situations

The NPIP is a cooperative industry-State-Federal program focused on preventing disease in poultry and promoting safety of poultry products throughout the country. Participation in NPIP provides breeders and hatcheries with standardized guidelines for poultry and egg management, as well as biosecurity practices.

NPIP Provisions 9 CFR 145 and 9 CFR 147 are pertinent to poultry facilities and contain various C&D and biosecurity measures for production. Some of the typical preventive biosecurity measures practiced in the participating industries currently include: (1) monitoring the health status of flocks, (2) C&D of reusable materials, and (3) segregation of setting, hatching, and chick-processing operations.

Participation of upland gamebird producers in the biosecurity auditing program set outline in the NPIP is becoming commonplace (i.e., close to 95 percent), with members of North American Gamebird Association pushing for commercial operations to participate.[17,18] At the 44th Biennial NPIP Conference, Subpart J of the 9 CFR 145 was approved, which outlines provisions specifically for raise-for-release birds, which are defined as “birds grown under confinement for the primary purpose of producing eggs, chicks, started, or mature birds for release on game preserves or in the wild.”[19]

Minimum biosecurity standards for growers of all industries were approved at the 44th Biennial NPIP Conference and are listed under 9 CFR § 53.11. According to NPIP, the biosecurity program should include a designated line of separation (LOS) and perimeter buffer area (PBA), and provisions to address personnel biosecurity practices; control of wild birds, rodents, and insects; equipment and vehicle management; mortality disposal; manure and litter management; water supplies and feed; and replacement litter management. How individual producers meet these guidelines is variable, depending on farm layout and resources.

Other biosecurity plans and standards are often guided by individual producers, industry organization recommendations, and flock veterinarian recommendations.

7.5.2 Biosecurity

7.5.2.1 Conceptual and Structural Biosecurity

Conceptual and structural biosecurity includes planning and building poultry grower sites in a way that limits disease transfer.[20] Some key concepts employed in the upland gamebird industry include:

- Locating upland gamebird farms so they are geographically isolated from other premises with domestic poultry[8]
- Locating all aspects production on one premises (e.g., locating breeding, hatching, brooding, growing)[3]
- Avoiding raising upland gamebirds on the same site as captive waterfowl raised for release or any other commercial purpose[15]
- Avoiding raising different species of birds in pens together. Given the severity of the disease, and the resulting loss of birds, it is simply prudent not to expose one species of upland gamebird to another[3,15]
- Building pens on soil with appropriate drainage to reduce the amount of standing water and mud in pens in order limit pathogen-sustaining environments that birds have contact with[21]

7.5.2.2 Operational Biosecurity

Operational biosecurity involves management decisions and routine procedures intended to prevent introduction of disease agents.[7] To prevent disease introduction and subsequent transmission to other premises if infection occurs, sanitation and biosecurity measures are used at all farms, to varying degrees.

The North American Gamebird Association (NAGA) guidelines presented as part of their avian influenza resources for producers[11] and the standard operating procedures as suggested by Secure Upland Gamebird Supply Working Group (Secure Upland Gamebird Supply Working Group, personal communication, June & July 2018), include those listed below. Other industry standards are individually cited with specific resources and reports.

7.5.2.2.1 Secured Farm Entry and Visitor Protocols

- Farms should limit visitors to only those who are essential (such as veterinarians and repairmen).
- Farms should keep a record of all visitors and their previous farm visits.
- All visitors should wash their hands and put on protective outer clothing, including clean boots and head gear, before working with the flocks.
- Any visitors that own backyard or farmed poultry, upland gamebirds, or waterfowl should not be allowed across the LOS; i.e., not allowed inside the pens.
- Signs should be posted at farm entry. Entrances of each pen should announce that the area is a biosecure zone and unauthorized entry is prohibited.

- A PBA, an outer control boundary around the poultry houses, should be clearly delineated such that non-essential vehicles do not enter into it and personnel do not leave it in the course of their daily tasks.[3,4]

7.5.2.2.2 Producers and Farm Personnel

- Farm workers should change into clothes and properly disinfect and clean footwear before entering pens.[15]
- Producers and farm workers should change into other clothes before leaving the premises.[17]
- Footbaths and protective boot covers should be used if maintaining dedicated footwear for pens and buildings is not feasible.[17]
- Farm workers who engage in activities of hunting, fishing, biking, hiking, and camping should not wear the clothes or footwear worn during these activities to work.[17]
- For farm workers who engage in hunting activities, especially waterfowl hunting: 1) clothing worn during hunting or cleaning birds on hunting trips should be laundered in hot water and detergent; 2) shoes should be disinfected and farm workers should shower and change clothes before caring for birds; and 3) any personal vehicles that farm workers use to go waterfowl hunting offsite should be cleaned after the hunting trip and before driving close to the pens.[22]
- Whenever employees engage in any activities near water (e.g., boating, trapping, and/or fishing), caution should be exercised and mitigation measures should be taken. Employees who visit shorelines and/or parks (especially those with lakes and ponds) should clean and disinfect shoes and vehicle floorboards, at a minimum.[22]
- Farm workers should not work on multiple areas of the farm (e.g., in the hatchery, brooder, and pens);[22] however, depending on the scale of the operation, this is not always feasible and appropriate biosecurity measures are instituted (Secure Upland Gamebird Supply Working Group, personal communication, June 2018).
- Employees who work in specific farm areas (e.g., hatchery, brooder, pens) should change clothes, shower, and wait 24 hours before working in another farm area.
- If farm workers work with multiple age groups on the farm, they should progress through chores going from youngest birds to oldest birds, when possible.
- Farm workers should work on only one farm (e.g., gamebird, poultry).[23]
- Personnel or any visitors entering the PBA should shower and change into clean clothes before arriving on site.[7]
- Disposable items used during flock visits should be bagged and left on the farm.[7]
- Farm staff should wash hands with soap, water, and a disinfectant before entering bird areas and handling birds.[7]
- After returning from a location where birds are present, including a feed store, all equipment, truck tires, clothing, and shoes should be cleaned and disinfected.[7]
- Farm workers should not own any birds of any kind.
- No visitors should enter the hatchery or bird facilities.

- Biosecurity training should occur at hire of new employees, in addition to annual biosecurity training.
- Visitors should not visit livestock operations (including cattle and pigs) prior to coming onto the upland gamebird farm.

7.5.2.2.3 Feed Delivery

- Feed delivery drivers should wear disposable protective foot coverings and spray off the tires of their vehicles with disinfectant.
- Farms should have their own feed trucks, if possible.

7.5.2.2.4 Sanitation Facilities on Farm

- Work-specific clothing and footwear should not leave the premises.[23]
- Farms should have separate washing and drying facilities and separate bins for dirty and clean clothes. Pen entrances should have bins for dirty clothes.[23] A Danish entry system should be used.
- Facilities to wash hands and hand sanitizer should be readily available.[15]
- Regularly maintained disinfectant trays or foot mats should be at every entrance to the barns, offices, and break rooms.

7.5.2.2.5 Other Operational Procedures:

- Waterfowl hunting does not happen onsite. In rare instances where waterfowl hunting takes place nearby (e.g., on adjacent hunting locations), there should be separation from the upland gamebird farm (Secure Upland Gamebird Supply Working Group, personal communication, January 2023).

7.5.2.3 Cleaning & Disinfection (C&D)

7.5.2.3.1 Vehicles and Drivers

- After dropping birds off for a client, drivers making bird deliveries should stop at a local car wash and pressure wash tires and vehicles.[17]
- Before entering and leaving a premises, drivers delivering chicks or birds to customers should spray their vehicle's wheels.
- If the driver gets out of the truck to load or unload, the driver should always wear protective foot coverings and coveralls.
- When leaving the customer's premises, the delivery driver should disinfect the foot pedals using disinfectant spray.
- Tires on all vehicles should be sprayed off before vehicles enter the farm.
- Trucks and trailers should be washed at the end of every delivery day.
- Farms should stock their delivery vehicles with disinfectant sprayers, coveralls, disposable foot coverings, and an aerosol can of disinfectant.

7.5.2.3.2 Equipment

- Growers should not allow farm equipment (e.g., tractors, front end loaders, shovels, etc., that have been used in fields or other areas of the farm) into pens or buildings housing upland gamebirds unless thoroughly washed and disinfected.[22]
- Equipment or supplies (e.g., shovels, screwdrivers, saws, rakes, mowers, skid loaders, etc.) should not be shared with other poultry premises. (Secure Upland Gamebird Supply Working Group, personal communication, June 2018). Tool sets and small equipment should remain onsite and employing a color-coding system will ensure they stay in designated spots.[23]
- Large equipment that must leave its designated locale to be used elsewhere should be thoroughly cleaned and disinfected before leaving its home premises and thoroughly cleaned and disinfected before returning.[23]
- Equipment should be effectively sanitized between uses; sharing of equipment between premises areas or departments should be minimized. Tractors should be washed at the end of every activity day.[7]
- Organic matter such as manure, litter, debris, and feathers should be removed with soap and water before disinfecting equipment.[7]

7.5.2.3.3 Water Supplies

- Surface water (e.g., water from ponds, lakes, or streams) should not be used to water birds.[18,24]
- If water comes from a surface source for use in cleaning, cool cells (e.g., evaporative cooling systems), or drinking, experts in water treatment should be consulted on how to continuously treat water to eliminate viable virus (USDA APHIS).

7.5.2.3.4 Housing Area

- Growers should routinely inspect netting and fencing for maintenance. Repairs should be done promptly to minimize predator and pest entry into pens.[21]
- At the end of the season, cover crops within pens should be mowed and allowed to sit for the duration of the off-season.

7.5.2.3.5 Load-out

- Growers should work with customers to determine off-farm locations to deliver and unload birds. Additionally, growers should clean and disinfect delivery trucks prior to returning to their own farms.[22]

7.5.2.3.6 Animal, Pest, and Insect Control

- Nets and fencing around pens should be regularly maintained to keep out wild birds, nuisance mammals, and other potential predators.[15,21,22] Nets should be kept tight to eliminate the chance of upland gamebirds flying up and getting caught in the nets.
- Windbreaks in the form of trees and shrubbery should be used to act as a physical barrier between upland gamebird pens and bodies of water that could attract waterfowl.[23]
- If possible, growers should drain ponds that are next to pens or cover smaller ponds with netting to discourage the presence of wild waterfowl.[22]

- Growers should attempt to control starlings through use of noise cannons or population management when appropriate.[25] Poison may be used under direction of the Fish and Wildlife Service.
- Dogs and cats should never be allowed entry into brooder buildings or flight pens. Other animals should be kept off site, but if dogs are on the property, they should be kenneled far away from the brooder and flight pens. Ideally, the caretaker of these dogs should not have direct contact with live upland gamebirds.[23]
- Two-way door systems into and out of pens should be implemented to keep predators out of pens while caregivers are entering or exiting.
- Sight barriers and an electric fencing should be used to keep predators out of pens. Pen fencing should be buried and flared out to prevent predators from digging their way into the pens.
- Spilled feed should be kept to a minimum and immediately cleaned up to not attract any wild birds or rodents.
- Predators should be removed via trapping. Growers should partner with trappers and falconers to remove predatory mammals and birds of prey from the premises.
- Gravel should be placed around the perimeter of pens. Growers should mow and trim grass between pens and the tree line or fence line to reduce cover for predators.
- Flock caretakers should regularly patrol pen perimeters for escaped birds to reduce the attraction of predators.

7.5.2.3.7 Dead Bird Disposal

Disposal of dead birds is regulated by local and State governments to control the impact of carcass disposal on air quality, water quality, and the spread of disease. Disposal of mortality is a daily necessity since dead birds can harbor pathogenic microorganisms, with potential transmission to other poultry. Cost of supplies, labor reliability, maximum anticipated daily mortality, and degree of biosecurity associated with each method must be assessed.[7]

- If compost piles are used, proper conditions should be maintained to protect both the compost pile and environment. Composting should be done on a concrete floor and under a roof. Temperature of the pile should be monitored to ensure it is hot enough to destroy pathogens.[4]
- If incineration is used, local air pollution standards must be maintained. Ash must be disposed of properly.[4]
- If burial is used, the location should prevent ground water contamination and flies should be managed.[4]
- Disposal methods should avoid the potential for cross-contamination with dead birds from other facilities.[7]
- If possible, farms should have designated staff that will move dead birds to the composter and do not return to work with live birds.
- Dead birds should be regularly picked up to reduce predators and pests.

7.5.2.3.8 Manure and Litter Management

Upland gamebird operations typically only use litter in brooder buildings, except for operations raising bobwhite quail indoors.

- Manure and spent litter should be removed in a manner that prevents exposure of susceptible birds, either on or off the farm of origin, to disease agents.[7]
- Fresh litter should be stored and handled so it cannot be contaminated by insects, wild birds, or rodents.[7]
- Upon removal at the end of the seasonal brooding cycle, generally, litter is immediately spread on managed fields (Secure Upland Gamebird Supply Working Group, personal communication, January 2023).

8 Hazard Identification: HPAI Overview

Hazard identification consists of listing the pathogenic agents associated with the species from which a commodity is derived and whether the agents can be classified as hazards for further consideration in the risk assessment.[2] For movement of raised-for-release upland gamebirds to a hunting preserve, the pathogenic agent of concern is HPAI virus. Properties of HPAI viruses, including environmental persistence, transmission characteristics, and physical and chemical inactivation, have been extensively reviewed in comprehensive texts.[26] This section is a brief summary of the key properties of HPAI viruses from published scientific literature and expert opinion, with emphasis on the variability between HPAI virus strains and transmission characteristics in poultry, including upland gamebird species.

8.1 Agent

AI viruses are negative sense, segmented, ribonucleic acid viruses of the family Orthomyxoviridae. The Orthomyxoviridae family includes several segmented viruses, including the Type A, B, C, and D influenza viruses. The Type A influenza viruses, which include all AI viruses, can infect a wide variety of animals, including wild waterfowl, chickens, turkeys, pheasants, partridge, quail, pigs, horses, mink, seals, bats, and humans. The type B and C viruses primarily infect humans and occasionally pigs.[27–30] Type D have mainly been isolated from cattle and pigs.

Two surface glycoproteins of the influenza virus, hemagglutinin (HA) and neuraminidase (NA), are the most important antigenic sites to produce protective immunity in the host; however, these proteins also have the greatest genetic variation. For AI viruses in birds, there are 16 known different subtypes of HA (H1 to H16), 9 known different subtypes of NA (N1 to N9), and 144 different HA:NA combinations.[30–32]Although relatively few of the 144 subtype combinations have been isolated from mammalian species, all subtypes, in the majority of combinations, have been isolated from avian species.[33]

8.1.1 Definition of Highly Pathogenic Notifiable Avian Influenza

For disease control programs and international trade in domestic poultry products, HPAI is defined in the Code of Federal Regulations, Title 9, Section 53.1 as (2016):

(1) Any influenza virus that kills at least 75 percent of eight 4- to 6-week-old susceptible chickens [or 6 out of 8 birds], within 10 days following intravenous inoculation with 0.2 mL of a 1:10 dilution of a bacteria-free, infectious allantoic fluid;

(2) Any H5 or H7 virus that does not meet the criteria in paragraph 1 of this definition, but has an amino acid sequence at the hemagglutinin cleavage site that is compatible with HPAI viruses; or

(3) Any influenza virus that is not an H5 or H7 subtype and that kills one to five [out of eight inoculated] chickens and grows in cell culture in the absence of trypsin.

The WOAH Terrestrial Animal Health Code Article 10.4.1 defines HPAI viruses to be AI viruses that “have an IVPI [intravenous pathogenicity index] in six-week-old chickens greater than 1.2 or, as an alternative, cause at least 75 percent mortality in four-to eight-week-old chickens infected intravenously. H5 and H7 viruses which do not have an IVPI of greater than 1.2, or cause less than 75 percent mortality in an intravenous lethality test, should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the haemagglutinin molecule (HA0); if the amino acid motif is similar to that observed for other

high pathogenicity avian influenza isolates, the isolate being tested should be considered as high pathogenicity avian influenza virus."[34]

All H5 or H7 virus isolates of both low and high pathogenicity, and all HPAI virus isolates regardless of subtype, are reportable to State and national veterinary authorities and to WOAH.[35] Although other low pathogenicity avian influenza (LPAI) viruses may cause considerable morbidity and production losses, they are not reportable diseases to WOAH, but may be reportable in some States.

8.1.2 Host Range

Wild waterfowl are considered the natural reservoirs of LPAI viruses, but the role of wild birds as reservoirs for most HPAI viruses responsible for high mortality in domestic birds is not fully elucidated.[36] Recent surveillance and phylogenetic analyses, however, suggest that migratory waterfowl are important in the maintenance, reassortment, and spread of HPAI viruses.[37–39] The phrase "highly pathogenic for chickens" does not indicate or imply that the AI virus strain is highly pathogenic for other bird species, especially wild ducks or geese (Anseriformes). However, if a virus is highly pathogenic for chickens *(Gallus domesticus*), the virus will usually be highly pathogenic for other birds within the order Galliformes, family Phasianidae, such as turkeys (*Meleagris sp*.), pheasants (*Phasianus colchicus*), and chukar (*Alectoris chukar*). Also, experimentally, quail (including *Coturnix sp*. and *Colinus sp*.) are suggested to be "...susceptible to infection with goose/Guangdong/1996 (gs/GD/96) lineage H5N1 HPAI viruses.[40] The gs/GD/96 lineage of H5 HPAI viruses is the most widespread HPAI virus in wild birds worldwide and frequently transmits to domestic poultry. It is endemic in poultry in parts of Africa, Asia, and the Middle East.

Most HPAI viruses are generally non-pathogenic or minimally pathogenic for ducks and geese in experimental studies.[28] However, the lethality of HPAI viruses has changed since the re-emergence of the gs/GD/96 H5N1 HPAI viruses in Hong Kong in 2002, as some strains have become highly lethal in some naturally and experimentally infected waterfowl.[36] For example, the 2017 H5N6 HPAI outbreak on a domestic meat duck commercial farm in the Netherlands was associated with high mortality.[41] The evolving H5 HPAI viruses spread throughout Asia and Europe between 2005 and 2014.[42] In late 2014, the gs/GD/96 H5 clade 2.3.4.4 viruses were detected in North American wild birds,[38,43,44] reassorted with American AI viruses, and similar gs/GD/96 American HPAI H5 viruses were identified during the domestic poultry outbreak in 2015 in the United States.[45]

Characterization of the gs/GD/96 American HPAI H5 viruses found in wild birds was done through inter-agency collaborations, including the U.S. Department of the Interior, U.S. Geological Survey National Wildlife Health Center, and USDA APHIS.[42] Researchers suggest identifying these HPAI H5 viruses as intercontinental group A clade 2.3.4.4 gs/GD/96 lineage (icA) to differentiate this changing subset of viruses from other Asian H5N1 HPAI.[44] Some wild birds—including ducks and geese—that were found to be positive for icA H5N8 and icA H5N2 exhibited morbidity or mortality at the time of sample collection.[46] Experimentally, both strains—H5N8 (A/GF/WA/14) and H5N2 (A/NP/WA/14)—led to some mortality in domestic geese (Chinese geese) but not in domestic ducks (Pekin).[47] Numerous wild duck species can be infected, but clinical signs are not apparent.[48–50]An icA HPAI H5N2 strain isolated from infected turkeys in Minnesota in 2015 (A/Tk/MN/12582/2015) was experimentally inoculated into mallard ducks (*Anas platyrhynchos*) and caused mortality in individual birds in each group at medium (10^4) and high (10^6) inoculation doses, with a mean death time of 9 days.[51]

Additionally, minor gallinaceous species (specifically Japanese quail, bobwhite quail, chukar partridge, ring-necked pheasant, and pearl guinea fowl (*Numida meleagris*)) experimentally inoculated with icA H5N8 and icA H5N2 varied in their mean death time, mean bird infectious dose, and their mean bird lethal dose.[27] Their mortality rate and mean time to death also varied in an experiment with A/chicken/Hong Kong/220/97 (H5N1).[40] Thus, the avian host range affected by icA H5 viruses is broad and the clinical signs in each host are variable.

HPAI strains are known to emerge in poultry after the introduction of LPAI viruses from wild birds and after circulation of virus for varying lengths of time in domestic poultry.[52] This likely occurred in the U.S. turkey industry in early 2016 when the first HPAI case caused by an H7N8 virus, A/turkey/Indiana/2016, was detected in commercial turkeys. Subsequent detections of H7N8 LPAI occurred on other turkey premises; all HPAI and LPAI viruses were found to be of North American wild bird lineage.[53] In 2017, a similar situation occurred in Tennessee when an H7N9 HPAI outbreak emerged following the circulation of an H7N9 LPAI virus in commercial poultry in the same area. Based on chicken host 28S ribosomal RNA, it is suggested the circulation LPAI virus mutated to an HPAI virus during replication in the poultry.[54] The 2008 identification of an H5N2 virus with an HPAI genotype—with evidence of non-lethal infection in wild waterfowl and without evidence of prior extensive circulation in domestic poultry—suggests that some AI strains with potential high pathogenicity for poultry could be maintained in a wild waterfowl community prior to introduction.[36]

Host adaptation is a key determinant of the ability of an HPAI virus to maintain transmission within domestic poultry. Once adapted to gallinaceous birds, most HPAI viruses are unlikely to circulate again among wild birds.[55] However, the emergence of gs/GD/96 HPAI H5 strains has led to increased uncertainty regarding the role of wild birds as reservoirs in the maintenance of HPAI viruses in nature.[37,56] Pantin-Jackwood et al. (2016) demonstrated that viruses of gs/GD lineage H5 HPAI can replicate to higher virus titers in ducks than H5 and H7 viruses of other lineages, which is suggested to impact the ability of gs/GD lineage viruses to circulate in wild waterfowl.[57] Prior to the outbreak of gs/GD/96 H5N1 HPAI virus in Europe, Asia, and Africa starting in late 2003, HPAI viruses had only rarely been isolated from wild birds—usually associated with outbreaks in domestic poultry—with one exception: An outbreak of HPAI H5N3 (A/Tern/South Africa/1961) in South Africa in 1961 that was observed in a population of terns (*Sterna hirundo*).[58] Now, Eurasian HPAI H5 strains have been isolated from multiple species of wild birds, both from healthy birds and from sick, moribund, or dead birds.[39,59] Despite extensive global wildlife surveillance efforts, infection with gs/GD/96 H5N1 HPAI viruses has not been detected in healthy wild birds, except for a few isolated cases.[58] The significance of wild birds as a source of infection and their influence on the epidemiology of HPAI viruses are yet to be fully established.[36,39]

Additional hosts also may play a role in the epidemiology of these viruses as they continue to spread and reassort. Experimental studies have shown that various LPAI and HPAI viruses can infect and replicate in multiple mammalian species (e.g., cats, ferrets, mink, pigs, rabbits, raccoons, and skunks).[60–63] Several species of concern (e.g., wild animals that may have contact with commercial poultry premises such as rabbits, skunks, and raccoons) have been shown to be capable of shedding AI virus, and in some cases, of experimentally transmitting the virus to ducks via indirect contact under conditions meant to simulate contact in a natural environment.[62,64] These species may serve as mechanical vectors, but to what extent is unclear.

8.2 Geographic Distribution of H5 and H7 HPAI

- The current list of all confirmed affected countries with H5 or H7 infection in animals is maintained by WOAH at https://www.woah.org/en/disease/avian-influenza/[65]
- In a graphical display of the HPAI virus, H5 subtype, outbreaks that occurred in the United States in 2014–2015, both in relation to time and to poultry distribution and wild bird migratory patterns in **Figure 2** and in the video: https://www.youtube.com/watch?v=gZcCKT9SvZM.[66]

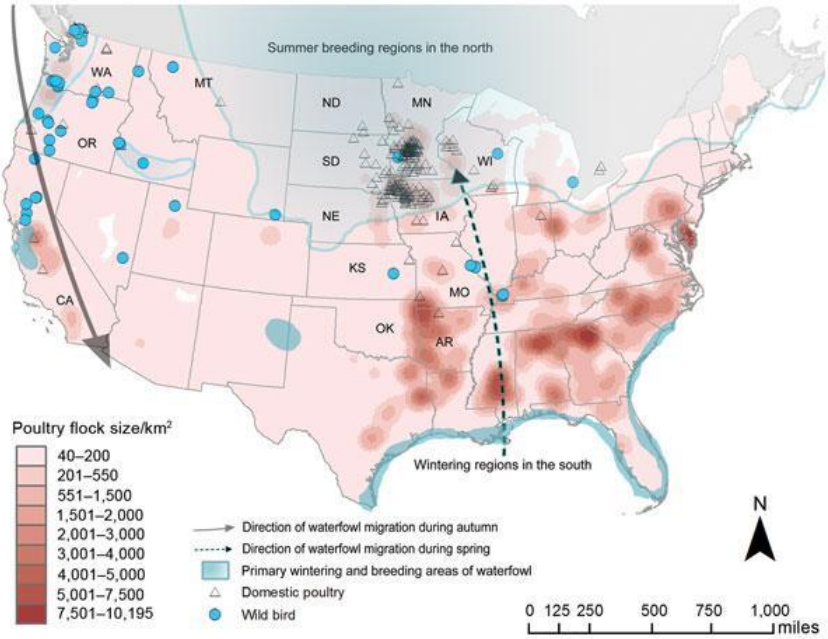


Figure 2. "Distribution of outbreaks caused by highly pathogenic avian influenza (HPAI) virus, subtype H5, in domestic poultry compared with domestic poultry flock density and direction of wild waterfowl migration. Triangles represent outbreaks caused by HPAI virus, subtype H5, in domestic poultry and blue circles represent HPAI virus, subtype H5 outbreaks in wild birds. Blue shading indicates migratory waterfowl wintering and breeding regions, and arrows represent general direction of seasonal movements. Pink shading indicates density of domestic poultry holdings, with darker shades representing areas where flock densities are higher." Source: https://wwwnc.cdc.gov/eid/article/22/1/15-1053-f1[66]

- Finally, migrating waterfowl and gulls are hypothesized to distribute HPAI viruses across longitudinal flyways, particularly from moving viruses intercontinentally from Europe to North America, as evidenced by European and American lineage viruses being found independently in migrating waterfowl in Iceland.

8.3 Virus Shedding

HPAI viruses have been isolated from respiratory secretions, blood, feces, and feathers, as well as eggshell surfaces, albumen, yolk, meat, and other tissues (e.g., spleen and lung) from infected poultry. Upland gamebirds species, including various types of quail and partridge have been documented to shed virus via oral secretions, feces, and feather pulp.[68,69] In naturally infected Japanese quail, 78 percent (7/9) of oviductal, 72 percent (13/18) of tracheal, and 86 percent

(12/14) of rectal tissue samples were found to be indirect immunofluorescence assay-positive for H5N1 HPAI virus.[70]

Estimates of HPAI virus concentrations in chicken and turkey secretions, feces, feathers, and other tissues generally range between 10^3 and10^7 EID_{50} per gram or per milliliter, although higher concentrations have been observed in some cases.[71–79] A quantity of $10^{4.7}$ EID_{50} was found in feces of experimentally infected pheasants for at least 15 days.[80]

Experimentally inoculated red-legged partridge have demonstrated viral shedding of HPAI H7N1 (A/Chicken/Italy/5093/1999) virus via the oropharyngeal route, starting just one day post inoculation until the end of the experiment, via the cloacal route between days 2 and 8 post-inoculation and in feather pulp between days 2 and 8 post-inoculation.[69] Virus concentrations ranged from 4 and 10 log 10 viral RNA copies/sample with the highest concentrations occurring between days 2 and 8 post-inoculation in the feather pulp.[68]

Bertran et al. (2013) demonstrates similar findings in inoculated and contact European Quail (*Coturnix c. coturnix*) with HPAI H7N1 (A/Chicken/Italy/5093/1999) and HPAI H5N1 (A/Great crested grebe/Basque Country/06.03249/2006) viruses. For the HPAI H7N1 virus, viral shedding was observed via the cloacal route, oropharyngeal route, and in feather pulp, all starting on day one post inoculation until death, with the highest virus concentrations demonstrated in oropharyngeal excretions. Contact quail added to the pens 4 hours after inoculating quail in the study exhibited similar findings, but with a 2-day delay. Quail inoculated with the HPAI H5N1 virus demonstrated the highest shedding via feather pulp, then oropharyngeal route, then through the cloacal route. Similar to contact-exposed quail infected with the H7 virus, contact-exposed quail infected with the H5N1 virus exhibited similar shed patterns to the inoculated quail.[68]

Humberd et al. (2006) and Makarova et al. (2003) experimentally assessed the replication and transmission of 15 LPAI viruses in upland gamebird species. It was found that pheasants shed the viruses longer than chukar partridge and Japanese quail. For example, inoculated pheasants shed A/Duck/Hokkaido/447/00 (H5N3) virus for 14 days and A/Mallard/Netherlands/12/03 (H7N3) virus for 20 days while contact-infected pheasants shed these viruses for 20 days and 16 days, respectively. Chukar partridges in contact with the inoculated chukar shed the H5 virus for close to 10 days and the H7 virus for at least 7 days. These two studies[81,82] further revealed that pheasants and quail shed similar amounts of virus. In both chukar partridges and Japanese quail, all 15 viruses tested replicated in the respiratory tract and for approximately the same duration. In this study, replication predominantly occurred in the gastrointestinal tract in pheasants.

Pheasants appeared to be long-term shedders of other LPAI viruses (e.g., A/Duck/Hong Kong/562/79 (H10N9)) for which cloacal titers ranged from $10^{2.5}$ EID_{50}/mL to $10^{5.5}$ EID_{50}/mL. Atypical patterns of replication were observed with a peak in titers from the cloaca occurring on day 5 post inoculation, with undetectable virus on day 12, only to appear again on day 14 (cloacal swab of $10^{4.75}$ EID_{50}/mL of virus).[81] Titers for the A/Mallard/Netherlands/12/03 (H7N3) and the A/Duck/Hokkaido/447/00 (H5N3) viruses in water samples from pheasant pens were respectively $10^{6.5}$ EID^{50}/mL and $<10^1$ EID^{50}/mL and $10^{3.75}$ EID_{50}/mL and 10^2 EID_{50}/mL in chukar pens.[81] In Japanese quail, virus titers in tracheal samples at 3 days post-inoculation (dpi) for the two experiments ranged from $10^{2.5}$ to $10^{4.8}$ EID_{50}/mL for A/Mallard/Alberta/271/88 (H5N3) (H5N3) and $10^{2.3}$ to $10^{6.5}$ EID_{50}/mL for the A/Mallard/Alberta/24/01 (H7N3) virus.[82]

In Jeong et al. (2009), birds were intranasally inoculated with $10^{6.5}$ EID_{50} of A/Chicken/Korea/IS/06. Japanese quail shed virus for up to 6 dpi with a maximum dose of 5.0 ±

2.1 Log_{10} $TCID_{50}$ per 0.1 mL, while chickens shed for 3 dpi with a maximum titer of 3.6 ± 1.8 Log_{10} $TCID_{50}$ per 0.1 mL. The virus titers were higher in oropharyngeal swabs than cloacal swabs.[83]

H5N2 HPAI (A/chicken/Pennsylvania/1370/1983) viruses have been isolated from the eggshell surface, yolk, and albumen of eggs laid by experimentally inoculated chickens,[84] naturally infected chickens,[85] and H5N1 HPAI virus in eggs of naturally-infected Japanese quail.[86] Italian HPAI H7N1 (A/chicken/Italy/445/99) viruses have also been isolated from eggs laid by infected hens.[87] In experimental studies, H5N2 HPAI viruses were not recovered from eggs laid on the first day post-inoculation of hens. This may have been because the developing egg was protected from exposure in the shell gland (uterus) during the later stages of eggshell formation (about 15 hours), combined with the latent infected period (eclipse period) of at least 6 hours in individual birds in this study. In contrast, HPAI virus was recovered from the yolk and albumen of eggs forming in the oviduct of dead chickens at postmortem, 35 to 37 hours after being experimentally infected with an HPAI virus strain (Dutch East Indies) isolated from chickens.[88]

In an experimental study, the concentration of H5N2 HPAI (A/chicken/Pennsylvania/1370/1983) virus ranged from 0.97 to $10^{5.9}$ EID_{50}/eggshell sample; from 0.97 to $10^{6.1}$ EID_{50}/mL in albumen; and from 0.93 to $10^{4.8}$ EID_{50}/mL in yolk of eggs laid by infected hens[84] and H5N1 HPAI virus titers of $10^{4.6}$-$10^{6.2}$ ELD_{50}/mL were directly measured from the internal content of infected eggs of naturally infected Japanese quail.[86]

AI viruses in sexually mature turkeys demonstrate a relatively high degree of affinity for oviductal tissue.[89] A predilection for replication within these tissues may explain the precipitous drops in egg production reported in turkey breeder hen flocks during natural outbreaks.[90–93]Narayan et al. (1969) recovered AA5-turkey/Ontario 7732/66 HPAI virus from the yolks of each of three eggs laid by 30-week-old turkey hens that were infected through contact with a hen experimentally infected with an HPAI virus.[94] In turkey breeder hens experimentally inoculated with swine-origin LPAI H3N2 (A/turkey/Ohio/313053/04), virus was recovered from eggshells and egg contents.[89] In this study, the percentage of viral detection on shell surfaces was significantly higher ($P<0.005$) than in albumen, when shell-less eggs were excluded from the analysis. In the Bertran et al. (2011) study exploring HPAI H7N1 (A/Chicken/Italy/5093/1999) infection in red legged partridge, while virus concentration in egg contents or eggshells was not assessed, single positive cells for HPAI H7N1 were observed on 8 dpi within the epithelial cells of the oviduct.[69]

8.4 Chemical and Physical Inactivation

AI viruses are inactivated by physical factors such as heat, extremes of pH, hyper-isotonic conditions, and dryness; however, their infectivity can be maintained for several weeks under moist, low-temperature conditions.

Due to their lipid envelope, AI viruses are relatively sensitive to disinfection agents and inactivation by lipid solvents such as detergents. The EPA maintains a list of disinfectants with label claims for AI viruses. These products include halogens, aldehydes, quaternary ammoniums, phenols, alcohols, peroxides, and some detergents.[95–97] To ensure effective disinfection, appropriate operational conditions as recommended by the manufacturer have to be maintained. Operational conditions such as disinfectant concentration, temperature, contact time, pH, and organic load may impact the degree of virus inactivation.

8.5 Persistence of HPAI Virus in Manure and other Media

Persistence of AI viruses at various humidity levels and temperatures and on various substrates is summarized in **Appendix 2**: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates. The HPAI virus shed by infected birds may be protected in the environment by accompanying organic material, like mucus or feces, which shields the virus particles from physical and chemical inactivation. Specific environmental conditions such as cool and moist conditions increase survival times (i.e., the ability to detect any live virus) in organic media and on surfaces. For example, H5N2 HPAI virus (A/chicken/Pennsylvania/1370/1983) remained viable in wet poultry manure in a barn up to 105 days following bird depopulation in the Pennsylvania 1983–1984 outbreak (presumably in winter under freezing conditions). Experimentally, an HPAI strain from this outbreak survived for at least 35 days under moist conditions, but only 9 to 21 days under dry conditions at 4 °C (39°F).[98,99] H5N1 HPAI virus was viable in allantoic fluid for 10 days at 25 to 32°C (77 to 90°F) when kept out of direct sunlight, but was killed within 30 minutes of exposure to sunlight (32 to 35°C; 90 to 95°F).[100] Microbial digestion can affect virus survival times unpredictably in organic material.[101]

8.6 Transmission

Contact with migratory waterfowl, water birds, or shore birds is a risk factor for introduction of AI virus into domestic poultry populations.[102] Because AI virus can be isolated in large quantities from the feces and respiratory secretions of infected birds, an important mode of transmission is the mechanical transfer of infectious feces.[26] Note that for influenza viruses, fecal-oral and aerosol routes of transmission are predominantly associated with virus replication in gastrointestinal and respiratory tracts, respectively.

Data regarding AI virus transmission in upland gamebird species is scarce. For LPAI viruses, experiments assessing the replication and transmission of 15 hemagglutinin subtypes (H1 through H15) in ring-necked pheasants, chukar partridges,[81] and Japanese quail[82] were conducted. Most of the 15 subtypes transmitted to naïve contact pheasants, primarily via the fecal-oral route. Given the high viral titers measured in water samples in the pens, it was hypothesized that spread via water drinker may have been at least one route of transmission. Moreover, Makarova et al. (2003) concluded that since the Japanese quail placed in aerosol contact with infected birds showed no evidence of infection, contact birds may have been infected through the drinking water.[82]

Additionally, in one experiment,[103] authors assessed transmission of the human isolate H7N9 A/Anhui/1/2013 virus between challenged chickens and contact quail (Coturnix *sp*.) and contact ring-necked pheasants in a stacked cage formation (See **Figure 3**). Results demonstrated transmission from challenged chicken to contact exposed quail (located in a cage underneath the infected chickens), with quail shedding virus at a maximum of $10^{3.7}$ PFU/mL via oral swabs. Quail shed virus as early as 3 days post contact (DPC) and as late as 11 DPC. None of the pheasants showed indications of infection through swab test results, serology, or clinical signs.

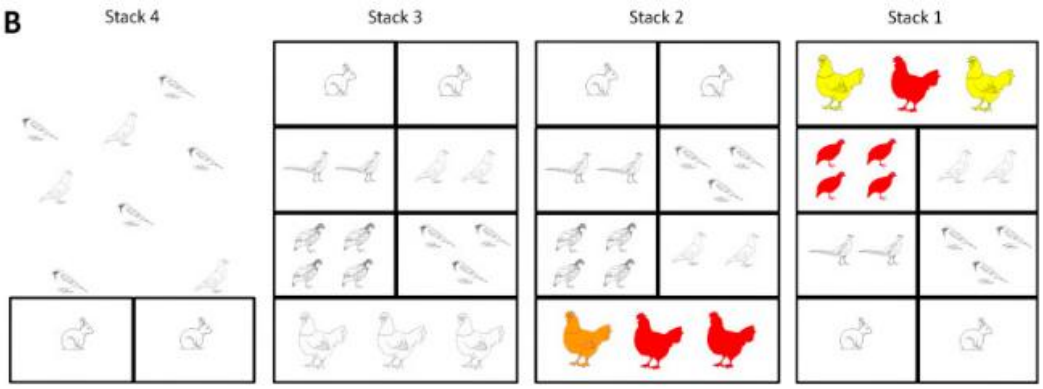


Figure 3. From https://www.ncbi.nlm.nih.gov/pubmed/27236304: "Observed transmission within four separate stacks of cages in a simulated H7N9 live animal market experiment. Shapes with red fill indicate animals that shed virus and seroconverted. Shapes with yellow fill represent animals that seroconverted but did not shed virus. Shapes with orange fill indicate animals that shed small amounts of virus (on a single day's post inoculation) but did not seroconvert. Shapes with white fill represent animals that neither shed virus nor seroconverted."[103]

All 15 HA subtypes replicated in pheasants,[81] while 14/15 of those studied by Marakov et al. (2003) replicated in Japanese quail. Chukar partridges were found to be less susceptible to infection in general than quail and pheasants. For LPAI H5 and H7 viruses in this study, by day 5 pi, A/Duck/Hokkaido/447/00 (H5N3) and A/Mallard/Netherlands/12/03 (H7N3) had each transmitted to 1/2 and 2/2 contact pheasants and chukar partridges, respectively.[81]

Japanese quail experimentally infected with the HPAI virus Turkey/Ontario/7732/66 (H5N9) showed no signs of disease. While birds remained asymptomatic, replication occurred in the respiratory tract, reproductive organs, and pancreas, and transmission between quail occurred without evidence of clinical signs. Serial intratracheal passaging of the original virus yielded a variant that eventually (i.e., after 3 passages) caused disease in and became lethal for European quail (*Coturnix coturnix*) and both viruses were highly pathogenic for chickens.[104]. A different study noted, however, that quail are more susceptible to experimental infection with goose Guangdong H5N1 influenza viruses from southeastern China than are chickens.[105]

In one study by Alexander et al. (1986), three HPAI viruses were able to transmit to in-contact Japanese quail.[106] The fractions of in-contact quail, turkey and chickens that became infected are listed in the table below:

Virus Strain	Species	Fraction of in-contact birds that became infected (%)
A/tern/South Africa/61 (H5N3)	Japanese Quail	20%
	Turkey	20%
	Chickens	0%
A/chicken/Pennsylvania/1370/83 (H5N2)	Japanese Quail	40%
	Turkey	100%
	Chickens	100%
A/turkey/Ireland/1378/83 (H5N8)	Japanese Quail	50%
	Turkey	70%

	Chickens	0%

In experimental settings, groups of European quail inoculated with HPAI H7N1 (A/Chicken/Italy/5093/1999) virus or HPAI H5N1 (A/Great crested grebe/Basque Country/06.03249/2006) virus effectively transmitted virus to naïve quail, with 4/4 contact birds rRT-PCR-positive for the HPAI H7N1 virus by 4 dpi and 4/4 contact birds positive for the HPAI H5N1 virus by 5 dpi. Hypothesized routes of transmission suggested by Bertran et al. (2013) included oral-oral through drinkers and feather picking between birds.[68]

Similar findings were reported in experiments assessing HPAI viruses in red legged partridges. Inoculated red legged partridge transmitted HPAI H7N1 (A/Chicken/Italy/5093/ 1999) virus to naïve contact partridges. Given that inoculated birds exhibited only oropharyngeal shedding on 1 dpi, coupled with detection of virus in contact birds at 2 dpi, and the oral-oral route (hypothesized to be through drinkers) is likely.[63] In the same experiment, shedding via feather pulp suggests shedding via feather follicles and subsequent feather picking from other birds as a possible route of transmission.[69] HPAI virus seems to cause systemic infection in partridge similar to other gallinaceous birds.

Once introduced into a flock, AI virus can spread directly from flock to flock by movement of infected birds and indirectly via contaminated equipment, vehicles, and people. Windborne transmission may occur when farms are closely situated and appropriate air movement exists.[107,108] Wild animals such as raccoons and foxes have also been implicated in local area spread; some wild animals, specifically skunks and cottontail rabbits, have been shown to be experimentally capable of transmitting virus to birds via indirect contact through shared environments.[64,109]AI introduction and transmission pathways for upland gamebirds may differ from those in turkey and chicken poultry sectors.[8,14] For example, it is hypothesized that during the 1999-2000 AI epidemics in Italy, the observed difference in spread between caged and litter-raised birds related to the amount of infected feces in direct contact with the birds.[110] Other mechanisms of transmission are outlined below.

8.6.1 Vertical Transmission

Evidence of vertical transmission of AI virus from infected hens to day-old chicks or turkey poults has been lacking thus far, as most strains are lethal to embryos.[111–114] Groups of turkey hens in egg production, with no clinical evidence of influenza A virus infection, were inoculated intravenously, or intratracheally, or were inseminated with semen contaminated with two influenza A viruses (T/Calif/meleagrium/64, T/Calif/5142/66), and virus was not recovered from poults hatched from eggs laid by exposed turkey hens.[115] Chicks hatched from eggs produced by two broiler breeder flocks infected with HPAI H7N3 (A/Chicken/Canada/AVFV2/04) tested negative for AI during an outbreak in British Columbia in 2004. The outbreak report of the Canadian Food Inspection Agency states, “Because avian influenza does not survive long at incubator temperatures, day-old chicks are not a likely source of infection for broiler growers.”[116] In the 1983 Pennsylvania HPAI H5N2 (A/chicken/Pennsylvania/1370/1983) outbreak, eggs from four severely infected layer breeder flocks were incubated and assayed for AI virus. None of the dead embryos yielded HPAI virus in this study.[117] Also, the 214 chicks hatched from these eggs showed no sign of AI disease and had not developed AI antibodies.[117] In experimental studies with H5, H7, and H9 LPAI viruses, low quantities of virus can be detected on eggshells laid by experimentally-infected chickens (E. Spackman, Personal communication,

July 2021,[118]). Higher quantities of eggs were contaminated externally and internally with an H7N8 HPAI viruses.[118]

Transmission of HPAI or LPAI viruses from infected breeder flocks to day-old poults via hatchery dissemination has not been observed in previous outbreaks. Turkey industry veterinarians and AI experts have stated that although there have been several LPAI outbreaks in the United States, vertical transmission or hatchery transmission has not been observed.[29] In a small-scale survey conducted by the University of Minnesota, turkey industry representatives provided reports of 26 flocks that had undergone avian and other influenza A virus infections and where eggs from those flocks were set and not removed from incubation.[29] There was no evidence of horizontal or vertical transmission of AI within the hatchery to day-old poults in any of these instances. Additionally, for upland gamebirds, most farms are not vertically integrated, implying that companies hatch their own eggs thus eliminating potential avenues for hatchery cross contamination and limiting epidemiological links between farms.[8,14]

8.7 Dose Response

8.7.1 Dose Response in Upland Gamebirds

An experimental study by Bertran et al. (2017) in which Japanese quail, bobwhite quail, pearl guinea fowl, chukar partridges, and ring-necked pheasants were challenged with A/Northern pintail/Washington/40964/2014 (H5N2) or A/Gyrfalcon/ Washington/40188-6/2014 (H5N8) viruses reported mean bird infectious doses (BID_{50}) ranging from $<10^2$ to $10^{3.7}$ BID_{50}.[27] Variability in susceptibility of bobwhite quail, chukar partridges, and ring-necked pheasants to both viruses was evident. Bobwhite quail and chukar partridges respectively required an infectious dose of $<10^2$ and $10^{3.6}$ BID_{50}, while the pheasants required $10^{3.4}$ and $10^{3.0}$ BID_{50} for H5N2 and H5N8 viruses, respectively.[27] These species were more susceptible than chickens ($10^{4.4}$ BID_{50})[119] or turkeys ($10^{5.0}$ BID_{50})[120] experimentally inoculated with the same virus isolates. In experiments with LPAI viruses, turkeys were more susceptible than chickens[89,121] and a similar trend has been reported in the poultry industry manual.[7]

Slemons and Easterday (1972) performed experiments involving intranasal inoculation of different avian species with influenza viruses.[122] For LPAI virus, A/turkey/On tario/7732/66 (H5N9), they reported EID_{50} ranges as 3.1×10^4 - 2.2×10^5 for turkey, 3.1×10^3 - 2.2×10^4 for Japanese quail, and 1.7×10^4 - 1.1×10^5 for ring-necked pheasant and concluded that the virus was highly pathogenic for turkey but less so for quail and pheasants. With the LPAI A/turkey/Wisconsin/68 (H5N9) virus, reported EID_{50}s were 3×10^4 for turkey, 7.5×10^4 for pheasants, and 1×10^4 for quail. [122]

Note that experimental challenge studies frequently do not approximate field conditions due to challenge doses being inflated, challenge methods are often artificial, many extraneous variables are controlled for, and viruses may be serially passaged, lab adapted and grown in cell culture. Additionally, study cohorts are often very small, providing lack of power to studies and there is a lack of study design continuity with some study designs being limited in their robustness. All of these factors can confound any interpretations that can be made regarding the collective findings of all of these studies, which is a limitation within this risk assessment.

8.7.2 Route of Entry used in this Assessment

In poultry, the choanal cleft (palatine fissure; located on the roof of the mouth) is a papillae-lined, narrow slit that connects the oral and nasal cavities. During mastication or drinking,

contents of the oral cavity may pass through this slit and contact the mucosal surfaces lining the nasal cavity. Because of the variability in the efficiency of different inoculation routes for infection with HPAI virus (intranasal vs. intragastric) observed in laboratory inoculation and experimental feeding trials, there is considerable uncertainty as to the infectious dose needed for natural exposure via feeding of contaminated materials. The route of entry impacts the dose-response parameters in the exposure assessment.

We obtained expert opinion regarding the route of entry (intranasal or intragastric) and associated infectious dose that best represents oral exposure in chickens, given the limited data on this topic.[123] Experts stated that it is reasonable to assume that transmission may occur if contaminated food or water were to pass through the choanal cleft into the nasal cavity. Therefore, due to the limited studies on exposure via natural feeding of contaminated materials and the associated uncertainty, we conservatively assumed that transmission of HPAI viruses through consumption of contaminated materials might occur with exposure to doses infectious for the intranasal route, in turkeys, chickens and upland gamebirds.

8.8 Mean Time to onset of Signs, Mean Time to Death, Latently Infected and Infectious Periods in Upland Gamebirds

In individual birds, incubation period is dependent on the dose, route of exposure, species susceptibility and individual host susceptibility. At the flock level, detection is highly dependent on the level of clinical signs and the ability of the grower to detect them.[124] For trade purposes, WOAH defines the flock incubation period for HPAI as 21 days.[125]

For bobwhite quail, chukar partridges, and ring-necked pheasants among others the mean times to death (MDT) were estimated in an experimental study by Bertran et al. (2017) with A/Northern pintail/Washington/40964/2014 (H5N2) or A/Gyrfalcon/ Washington/40188-6/2014 (H5N8) HPAI viruses at three different challenge doses (10^2, 10^4, 10^6 EID_{50}) via intrachoanal inoculation. At the highest challenge dose (10^6 EID_{50}), there was 100 percent mortality for both viruses in bobwhite quail, chukar, and pheasants, and the reported MDTs were 4.7, 4.1, and 3.4 days for H5N2 and 4.9, 5.2, and 4.8 days for H5N8, respectively. At lower challenge doses, mortality was lower and the MDT was slightly longer for both viruses in the three species.[27]

Perkins and Swayne (2001) experimentally investigated the pathobiology of A/chicken/Hong Kong/220/97 (H5N1) HPAI virus in seven gallinaceous species that were inoculated with 0.05 or 0.1 mL of inoculum containing $10^{5.8}$ to $10^{6.2}$ EID_{50} of the virus intranasally. They reported 100 percent mortality within 10 days in all species investigated except chukar partridges, which had 75 percent mortality after 10 days. Reported mean time to death and ranges included chicken: 1.5 (1.5–2.0); Japanese quail: 2.0 (1.5–2.5); Bobwhite quail: 2.25 (2.0–3.5); turkey: 2.5 (2.0–2.5); pheasants: 3.25 (2.5–4.0); and chukar: 4.5 (4.0–6.5) days.[40]

In a study[106] of avian influenza H5 subtype viruses, three of the six HPAI viruses were transmitted to in-contact Japanese quail: A/tern/South Africa/61 (H5N3), A/chicken/Pennsylvania/1370/83 (H5N2), and A/turkey/Ireland/1378/83 (H5N8). For these three viruses, the mean time to onset of clinical signs and mean time to death (and mean time to death in brackets) for contact infected animals were respectively reported in the following table:

Virus Strain	Species	Mean time to onset of clinical signs (Mean time to death)

A/tern/South Africa/61 (H5N3)	Japanese Quail	8.5 (10.0)
	Turkey	7.0 (8.0)
	Chickens	none
A/chicken/Pennsylvania/1370/83 (H5N2)	Japanese Quail	8.5 (8.8)
	Turkey	6.6 (7.7)
	Chickens	8.0 (9.2)
A/turkey/Ireland/1378/83 (H5N8)	Japanese Quail	5.6 (8.8)
	Turkey	5.8 (6.6)
	Chickens	none

Van der goot et al. (2007) conducted an experiment in which pheasants were each inoculated both intranasally and intratracheally with 0.1 mL of 10^6 EID_{50}/mL of A/Chicken/Netherlands/621557/03 H7N7 HPAI virus. Among unvaccinated pheasants, 80 percent of inoculated and 40 percent of the contact pheasants developed clinical signs and died. A latent period of one day was assumed and the infectious period was estimated to be 12.2 days (95 percent CI: 7.7–16.7).[126]

The mean time to death of 2-month-old European quail that were contact-infected with either A/Chicken/Italy/5093/1999 (H7N1) or A/Great crested grebe *Podiceps cristatus*/Basque Country/06.03249/2006 was estimated at 7 and 6 days, respectively.[68]

From an experimental study, Isoda et al. (2006) reported that all Japanese quail inoculated with either A/chicken/Yamaguchi/7/04 (H5N1) or A/duck/Yokohama/aq-10/03 (H5N1) HPAI viruses died between 2 to 3 dpi and between 3 to 4 dpi, respectively, while for the same viruses, inoculated chickens died on day 2 and between 2 to 4 dpi, respectively. In another study involving inoculation of birds with A/chicken/Korea/IS/06, all the contact-infected chicken and Japanese quail died and the mean time to death was 5.3 and 7.5 dpi, respectively.[83]

From an experiment with four strains of HPAI viruses of the H5N1 subtype—A/chicken/Suphanburi/1/2004, A/quail/Angthong/71/2004, A/duck/Angthong/72/2004, and A/chicken/Yamaguchi/7/04, Saito et al. (2009) reported mortality of 100 percent in both inoculated chickens and Japanese quail. For chickens, the mean times to death were respectively 2.3, 1.9, 1.4, and 2.0 dpi, respectively, for each of the viruses, while for quail they were 1.4, 1.1, 1.0, and 3.4 dpi, respectively.[127]

8.9 Clinical Signs

8.9.1 Clinical Signs in Chickens and Turkeys

The presence and severity of clinical signs of HPAI infection depend on the virus strain and bird species affected.[55] Infected wild and domestic ducks may be have asymptomatic infections, whereas clinical signs in gallinaceous poultry are usually severe, resulting in high mortality.[128] In chickens and turkeys, the clinical signs associated with HPAI infection include marked lethargy with ruffled feathers, lack of appetite, neurological signs (e.g., tremors, torticollis, opisthotonos, etc.), decreased egg production, soft-shelled or misshapen eggs, watery diarrhea, sudden, unexpected death, and/or, on occasion, respiratory signs (coughing and sneezing).[29,128] Mature chickens frequently have swollen, cyanotic combs and wattles, and edema surrounding the eyes.[128] In turkeys, a cessation in flock vocalization ("cathedral syndrome") often accompanies infection.[129] Progressive somnolence, reduction of normal vocalization, swollen sinuses,

oculonasal discharge, edema of the face, and hemorrhages on the shanks are other clinical signs observed in turkeys.[124,130,131]

The mortality rate in an infected flock can reach 100 percent.[132] In mature birds, gross lesions on necropsy may consist of subcutaneous edema of the head and neck; fluid in the nares, oral cavity, and trachea; congested conjunctivae and kidneys (urates); and petechial hemorrhages that cover the abdominal fat, serosal surfaces, peritoneum inside the proventriculus, and surface under the keel.[29,128] Albeit, one study found that there was little virus replication in capillary endothelial cells at any clinical stage, meaning there was a lack of severe edematous and hemorrhagic lesions.[133] In layers, the ovary may be hemorrhagic or inactive and necrotic.[29,110,134,135] Hemorrhagic lesions are less common in turkeys than other gallinaceous species.[136]

8.9.2 Clinical Signs in Pheasants (*Phasianus colchicus*)

Species of upland gamebirds exhibit similar clinical signs as chickens and turkeys when infected with HPAI viruses. During a recent outbreak of HPAI H5N2 in Washington State in 2015, ring-neck pheasants on an upland gamebird farm displayed reluctance to move, torticollis, ruffled feathers, depression, and drooping heads.[137] In a 1999 outbreak of HPAI H7N1 in Italy, infected pheasants displayed similar clinical signs to turkeys and chickens, including tremors, incoordination, anorexia, and depression.[110] In experimental infection of pheasants with HPAI H5N2 (A/Chicken/Pennsylvania/83), 61 percent of pheasants had asymptomatic infections, with the remaining birds presented with lethargy and dragging wings.[80] In Bertran et al.'s (2017) experimental infection with HPAI H5N8 and HPAI H5N2, inoculated pheasants showed non-specific listlessness.[27]

Mortality rates in pheasant have been observed at 10 percent mortality overnight for a flock infected with HPAI H5N2 in a Washington State farm.[137] However, during the 1999–2001 HPAI H7N1 outbreak in Italy, while pheasant flocks experienced high mortality, they experienced lower mortality in comparison to turkeys, chickens, and guinea fowl (*Numididae*).[110] Common gross pathology findings during necropsy of pheasants infected with HPAI viruses include moderate to severe congestion of meningeal blood vessels, enlargement and mottling of the spleen, histological lesions in the brain, heart, spleen, pancreas, and liver, and vasculitis of the meninges,[137] in addition to muscle hemorrhages and enlargement of the kidneys.[133] However, the same authors found in a later study that there was a lack of severe edematous and hemorrhagic lesions in pheasants infected with HPAI H5N8 and HPAI H5N2.[133]

8.9.3 Clinical Signs in Quail (*Coturnix sp*. and *Colinus sp.)*

There is limited information on the clinical signs of HPAI infections in quail, and as such, we have included studies on both quail genera: *Coturnix*, which are not commonly a species released for hunting in the United States, and *Colinus*, which are a common American upland gamebird.

Coturnix sp. of quail have demonstrated onset of clinical signs during HPAI infection; however, with observable variation. European quail challenged with HPAI H7N1 and groups challenged with HPAI H5N1 presented with non-specific clinical signs such as lethargy, anorexia, ruffled feathers, and severe neurological signs such as tremors, incoordination, circling, head tilts, and opisthotonus.[68] Similar to upland gamebirds, European quail infected with HPAI H7N1 (including both inoculated and contact birds) exhibited enlargement and mottling of the spleen and gross lesions on the pancreas.[27] European quail infected with HPAI H5N1 (A/Great crested grebe/Basque Country/06.03249/2006) or HPAI H7N1 (A/Chicken/Italy/5093/1999), experienced atrophy of thymus, minor bleeding of the mucosa around the proventriculus and

gizzard, and histological lesions on the pancreas, heart, and brain were also observed, in addition to the gizzard, cecal tonsil, and spinal cord.[68]

A study assessing HPAI H5N1(A/chicken/Korea/IS/06) virus in Japanese quail reported similar findings to studies evaluating clinical signs in European quail, with infected Japanese quail showing depression and decreased food consumption.[83] In Bertran et al.'s (2017) study, Japanese quail inoculated with HPAI H5N2 or HPAI H5N8 exhibited listlessness within the first 24 hours and only one of the quail infected showed neurological signs such as head tremors and leg paralysis.[47] In Alexander et al.'s (1986) experiments, Japanese quail infected with HPAI H5N1 A/chicken/Scotland/59 and HPAI H5N9A/turkey/Ontario/7732/66 showed no clinical signs prior to death.[106] A similar lack of clinical signs before sudden death was found in Saito et al.'s (2009) study assessing Thai strains of HPAI H5N1.[127] Field observations of caged Japanese quail infected during the 1999 HPAI H7N1 outbreak demonstrated quail exhibiting severe depression.[110] Bertran et al. (2019) found in a later study a lack of severe edematous and hemorrhagic lesions in Japanese Quail infected with the HPAI H5N8 and HPAI H5N2 viruses used in previous studies (2013 and 2017).[133] Bertran et al. found in previous studies (2013 and 2017) that Japanese quail infected with the same viruses exhibited enlargement and mottling of the spleen and gross lesions on the pancreas.[27,68]

Regarding HPAI-induced mortality in quail (*Coturnix sp*.), mortality rates of experimentally infected European quail in Bertran et al.'s (2013) study were found to be between 60 and 100 percent for the viruses HPAI H7N1 and HPAI H5N1 used, including inoculated and contact birds.[68] In Perkins and Swayne's (2001) study, Japanese quail inoculated with HPAI H5N1 (A/chicken/Hong Kong/220/97) yielded 100 percent mortality (26/26).[40]

Limited research focusing on bobwhite quail show similar HPAI-induced clinical signs. In Bertran et al.'s (2019) study, bobwhite quail exhibited similar clinical signs to Japanese quail, with HPAI H5N8- and HPAI H5N2-affected quail demonstrating low amounts of severe edematous and hemorrhagic lesions when compared to European quail.[133] Bobwhite quail infected with HPAI H5N2 or HPAI H5N8 exhibited similarly enlarged and mottled of the spleen compared to Japanese quail as well as similar gross lesions on the pancreas.[27,68] Researchers note that bobwhite quail exhibit lethargy when infected with HPAI H5N8 or HPAI H5N2; however, the clinical period prior to death is incredibly short, so lethargy is only noticed just before death (Erica Spackman, personal communication, July 2021). Mortality caused by HPAI in bobwhite quail is similar to other quail species based on experimental evidence with Perkins and Swayne (2001) observing 100 percent mortality for bobwhite quail inoculated with HPAI H5N1 (A/chicken/Hong Kong/220/97).[40]

8.9.4 Clinical Signs in Partridge (*Alectoris chukar* and *Alectoris rufa*)

In Bertran et al.'s (2013) study assessing HPAI H7N1 in red-legged partridge (*Alectoris rufa*), both inoculated and contact birds displayed clinical signs starting 3 dpi, which included depression, apathy, and ruffled feathers. As in other upland gamebird species, some of the surviving birds exhibited more severe clinical signs, including incoordination, paralysis (wings and legs), head tremors, and opisthotonos, in addition to impaired respiration, diarrhea, and torticollis.[68] In Bertran et al.'s (2017) study assessing pathogenesis in minor gallinaceous species, chukar partridges challenged with either HPAI H5N8 or HPAI H5N2 exhibited listlessness.

Partridges experimentally infected with HPAI H7N1(A/Chicken/Italy/5093/1999) exhibited gross findings of hemorrhaging fasciae in leg muscle, atrophy of the thymus, gross lesions of kidneys, congestion in the brain, severe histological lesions on the kidney, adrenal gland, feather follicles, and central nervous system (CNS; brain and spinal cord), and less severe histological lesions on the intestines, liver, pancreas, myocardium, breast muscle, bursa of Fabricius, and respiratory tract.[69] Chukar partridge in Bertran et al.'s studies challenged with HPAI H5N2 and HPAI H5N8 also exhibited muscle hemorrhaging and kidney lesions.[27] However, in a later study, a lack of severe edematous and hemorrhagic lesions were found in chukar partridges infected with HPAI H5N8 and HPAI H5N2.[133]

In most of the experiments documenting the gross and histological lesions of infected birds, lesions and other physiological findings began to appear 2 to 3.5 dpi on average.[27,68,138]

8.10 Diagnosis

HPAI is a differential diagnosis to be considered in any flock in which marked lethargy, inappetence, or a drastic decline in egg production are followed by sudden death. In the United States, confirmation of a presumptive positive H5 or H7 test by polymerase chain reaction (PCR) is made by the National Veterinary Services Laboratories (NVSL) in Ames, Iowa. Upon positive confirmation of HPAI, subsequent samples from premises inside the established Control Area may be sent to approved laboratories that are part of the National Animal Health Laboratory Network (NAHLN).[139] Acceptable tests for surveillance testing in the United States include serological tests (Agar gel immunodiffusion [AGID] or USDA-licensed influenza A enzyme-linked immunosorbent assay [ELISA] in conjunction with a confirmation of antibody to H5 or H7 by hemagglutination inhibition [HI], antigen test [Antigen capture immunoassays (ACIA)]). Samples must be taken from clinically ill or dead birds with molecular confirmation by PCR, or virus isolation. Virus isolation includes tracheal/oropharyngeal and cloacal swabs, fresh feces from live or dead birds, or samples from organs pooled by organ system.

The reference standard for diagnosis of viable AI virus is virus isolation—confirming the presence of a virus that could infect other birds.[140] In the laboratory, 9- to 11-day-old embryonated chicken eggs are inoculated with swab or tissue specimens. Additional tests on fluids from the egg are required to confirm the presence of AI virus and determine HA and NA subtype.[26]

The application of molecular methods for detection of viral nucleic acid and whole genome sequencing for viral genes have become important tools in recent years. The rRT-PCR has advantages for outbreak surveillance such as speed, scalability for high throughput, high sensitivity, and high specificity.[26]

Antigen detection immunoassay kits have also been used in prior outbreaks and have advantages of speed (15 to 20 minutes), simplicity, and good specificity. While the low analytical sensitivity (detection limit greater than 10^4 EID_{50}) is a limiting factor, birds with clinical signs of AI, or that died of AI infection, generally shed adequate virus antigen for detection with these kits. In contrast, the assays are not recommended for screening of apparently healthy poultry, due to the lower level of shedding before the disease is clinical.[26]

8.11 Differential Diagnosis

HPAI can resemble several other avian diseases, including Newcastle disease (of the highly pathogenic type), infectious laryngotracheitis, mycoplasmosis, infectious coryza, fowl cholera,

aspergillosis, and *Escherichia coli* infection. It also must be differentiated from heat exhaustion, toxicoses, and severe water deprivation.

9 Risk Evaluation

9.1 Pathways for an Upland Gamebird Flock Becoming Infected with HPAI virus via Local Area Spread Components other than those Involving Movements of People, Vehicles, and Equipment

9.1.1 Role of Local Spread Components in Previous AI Outbreaks

Local area spread refers to mechanisms whereby the transmission likelihood increases with decreasing proximity to infected farms. The implementation of a Control Area (minimum 3 km infected zone plus 7 km buffer zone) is based on potential for local spread. A review of past outbreak experiences indicates that most local area spread of AI virus between farms can be attributed to the movement of people and equipment. We evaluated the likelihood of local spread occurring via wild birds, predatory, mammals, rodents, insects, and aerosols in this chapter.

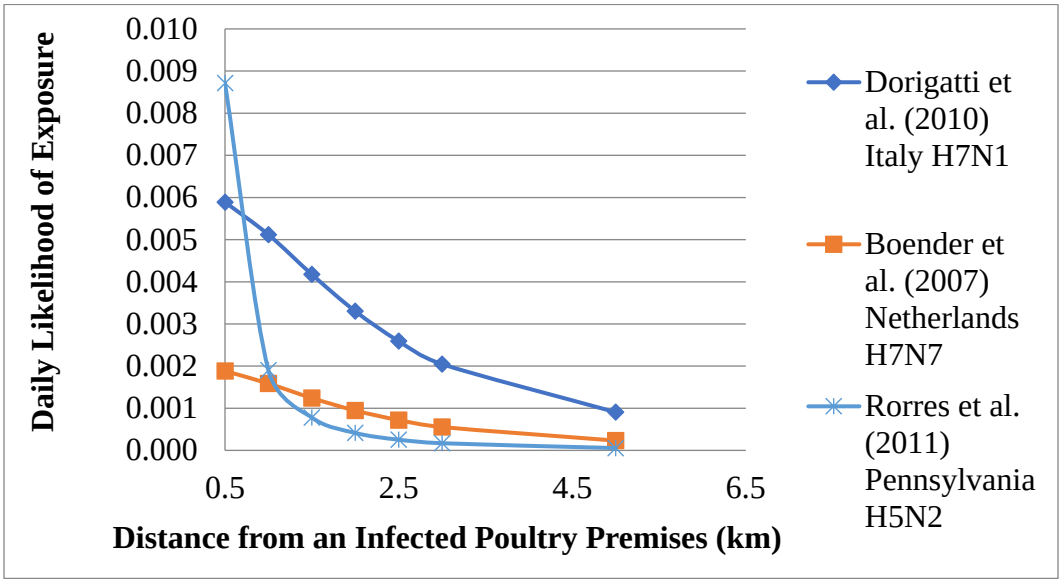


Figure 4. Relationship between the daily likelihood of exposure and distance from infected premises estimated from past HPAI outbreak data (also called a "transmission kernel"). Note that all these transmission kernels are not "mechanism-specific" and include the movements of people, vehicles, and equipment between farms as possible transmission mechanisms.[141–143]

Several HPAI outbreak studies have evaluated proximity as a risk factor in general without differentiating between component mechanisms. Spatial and risk-factor analysis from HPAI outbreaks in the Netherlands and Italy indicates a considerable decrease in the chances of infection with distance from infected premises. For example, in Busani et al. (2009), farms within 1.5 km of an infected premises had a 4 to 5 times greater chance of infection relative to farms located more than 4.5 km away.[144]

Figure 4 above shows the relationship between the daily likelihood of infection and distance from infected premises, based on transmission equations estimated from different HPAI outbreaks. The predicted likelihood of exposure steadily decreases with increasing distance in all curves. The specific mechanisms by which the transmission likelihood increases with proximity is ambiguous based on these studies (see **Appendix 3**: Literature Review on the Role of Local Area Spread in Previous Outbreaks for a summary of past outbreak studies on proximity).

Nevertheless, the transmission likelihood estimates from these studies can be considered as a conservative (upper bound or maximum) estimate of the spread that occurs due to mechanisms not associated with movement of people, vehicles, and equipment.

Apart from the above spatial analyses, most other AI outbreak observations indicate limited spread of AI among poultry premises by local spread mechanisms such as via insects, aerosols, and wildlife. For example, in a 2008 HPAI outbreak in the United Kingdom, there was no spread to 78 other farms within 3 km of an infected farm.[145] There are several instances where spread did not occur to other houses even on the same premises. (See **Appendix 3**: Literature Review on the Role of Local Area Spread in Previous Outbreaks for a summary of past outbreak studies on proximity).

9.1.2 Role of Aerosol Transmission of HPAI Virus

Aerosol spread of AI virus between premises has been implicated in some outbreaks, although most considered it to have had a limited role.[107,108,146–151] Aerosol transmission of AI is an active research area with considerable data gaps. We used a combination of approaches, including literature review of past outbreak experiences and experimental studies, exploratory dispersion models, and expert opinion to evaluate the role of aerosol transmission.

9.1.2.1 Aerosol Transmission of AI Virus in Past Outbreaks

- The limited role of local area spread through all mechanisms not involving movements of people and equipment in most previous AI outbreaks indicates a limited role for aerosol spread.
    - In several AI outbreaks, such as the LPAI H7N2 outbreak in Virginia, the geographic distribution of affected farms did not show a specific pattern, suggesting that aerosols were not a primary mode of transmission.[93] In an HPAI H5N1 outbreak in the United Kingdom, there was no transmission to 78 other farms within 3 km of an infected turkey farm. The authors concluded that there was no evidence of local area spread beyond 1 km.[145] **Appendix 3**: Literature Review on the Role of Local Area Spread in Previous Outbreaks summarizes the literature on the role of local spread in previous outbreaks.
    - Ypma et al. (2012) estimated the contribution of a possible wind-mediated mechanism to the total amount of spread during the 2003 HPAI H7N7 outbreak in the Netherlands to be around 18 percent. This estimate was based on the observed correlation between the wind direction and the direction of the spread of disease, estimated through phylogenetic and epidemiological data. The possibility of the direction of spread coinciding with the wind direction by chance was also accounted for in their statistical analysis. We note that this outbreak occurred in a region of very high poultry density (~4 farms per km^2), which may increase the likelihood of spread over short distances.[108]
    - Additionally, for the Dutch 2003 H7N7 HPAI epidemic, Ssematimba et al. 2012 used a dispersion modeling approach to assess the possible contribution of the windborne route to the transmission of the virus between farms. They concluded that the windborne route alone was insufficient to explain the observed spread although it could contribute substantially to the spread over short distance ranges.[107]

- Aerosol transmission between poultry barns that were in close proximity was suspected as a possible means of spread in the 2004 HPAI H7N7 outbreak in British Columbia. In this outbreak, there were anecdotal reports of some of the infected farms being in close proximity and downwind of other infected flocks.[147] Some of these anecdotal reports were associated with depopulation methods used early in the outbreak, such as grinding carcasses outside the barn or bringing birds outside the barn to depopulate. Although it was suspected, there is no conclusive evidence that aerosol transmission played a major role in this outbreak.[152]
- A case study of a multi-species upland gamebird farm in Utah in 2010 affected by LPAI H5N8, found that only pheasant pens and ducks pens that shared a fence line were found to have active shedding and/or serologically positive birds. Chukars in open-sided pens elsewhere on the premises of 2.3 ha (0.02 km^2) were negative suggesting no viral transmission due to aerosol or wind mediation at that point.[153]
- In a case-control study of infected layer facilities in Iowa and Nebraska in the 2014–2015 HPAI outbreak, the authors were not able to determine if aerosol transmission was responsible for infection at a facility.[154]
- A plume analysis model of infected farms in the 2014–2015 HPAI outbreak in Minnesota found that farms located 7 to 15 km from an infected farm were at low to moderate risk of infection via aerosol transmission; however, wind speed and direction may impact the distance at which transmission can occur. Farms located within 5 km of an infected premises were at increased risk regardless of wind conditions.[154]

- Activities that can generate AI virus-contaminated dust or aerosols very close to susceptible poultry have been implicated as a transmission mechanism.
  - Live haul trucking of birds actively infected with AI virus within 200 meters of a susceptible flock can pose a risk for aerosol transmission[155] (D. Halvorson, personal communication, July 2016).
  - Depopulation activities up to 400 yards (366 meters) upwind from a susceptible flock can present a risk for aerosol transmission.[154] In an LPAI H7N2 outbreak in Pennsylvania, aerosols generated by stirring up organic materials during depopulation were considered a potential mechanism of spread to farms within 1.61 to 2 km.[156] Depopulation methods used early in the 2004 HPAI outbreak in Canada, such as grinding carcasses outside the barn or bringing birds outside the barn to depopulate, were implicated in spread of HPAI.[152]
  - Spreading of non-composted contaminated litter on adjacent fields was suspected as a transmission mechanism during the 1983 HPAI H5N2 AI outbreak[155] (D. Halvorson, personal communication, March 2016). Spread of non-composted manure from infected farms approximately 2 km from susceptible poultry was suspected to have resulted in transmission in one instance during an LPAI H7N2 outbreak in Pennsylvania in 1996–1998.[156]
  - A 2015 survey of HPAI-infected turkey farms in the Midwest highlighted anecdotal evidence of aerosol spread related to recent nearby bird transport, blowing sawdust, and depopulation of nearby farms.[154] [160]

- Some studies have reported air-sampling results from or around HPAI-infected houses during previous outbreaks. These studies demonstrate the effect of dilution on aerosol concentration with increasing distance from the generating source.
    - High-volume air sampling was conducted in and near an infected layer flock that had high mortality during the HPAI H7N7 outbreak in Canada. Inside the barn, a viral titer of 292 $TCID_{50}/m^3$ was detected in air samples.[2a] Air sampling at a command post outside the barn showed a much lower viral load of 12.5 $TCID_{50}/m^3$ based on quantitative PCR; however, no viable virus was recovered. Low concentration and inactivation of virus by sunlight was hypothesized as a possible explanation for the apparent absence of viable virus in these samples.[157]
    - In the 1983 H5N2 HPAI outbreak in Pennsylvania, 5 of 6 samples taken 3 to 6 meters downwind of affected flocks on six farms were positive by virus isolation, whereas only 1 of 12 samples taken 45 to 85 meters downwind of affected flocks on eight farms was virus-positive; the positive sample was taken 45 meters downwind.[155]
    - During the 2015 H5N2 HPAI outbreak in the Midwest, the USDA/APHIS veterinarians in collaboration with researchers from College of Veterinary Medicine, School of Public Health and College of Science and Engineering from the University of Minnesota, and poultry industry veterinarians conducted air and environmental sampling of three turkey flocks in Minnesota and three layer flocks in Iowa and Nebraska. Air samples were collected inside and immediately outside (~5 meters from the exhaust fans) of affected barns, and at extended distances ranging from approximately 70 to 1,000 meters downwind from the barns.
        - Analysis of the results in the 2015 USDA epidemiological report, note that five of the six flocks had at least one air sample test positive. Roughly 23 percent of all the air samples came back positive via RT-PCR (based on Ct values of 35 or greater); however, only 2 percent of samples that were taken 70 meters or greater downwind from the barn came back positive.[154]
        - Torremorrel et al. (2016) found that HPAI viral RNA was detected inside infected barns and up to 1000 meters from infected facilities. Virus was isolated from air samples collected inside, immediately outside, up to 70 meters from infected facilities, and in aerosol particles larger than 2.1 μm.[158]
        - Alonso et al. (2017) reports five confirmed positive flocks (including three turkey flocks in Minnesota and one layer flock each in Iowa and Nebraska) and testing the samples for HPAI virus. They found the virus was detectable in association with aerosolized particles in 61 percent of the samples. The airborne virus concentration was found to be 4.53 ± 0.97 $\log_{10}$ RNA copies/m^3 of air and higher numbers of RNA copies were associated with larger particles.[159]
    - Scoizec et al. (2018) investigated the plausibility of airborne transmission during the 2016–2017 HPAI H5N8 outbreak in southwestern France by collecting air samples inside, outside, and downwind from infected duck and chicken facilities. They detected virus RNA in all samples collected inside poultry houses, at external

[2a] $TCID_{50}$ refers to the 50% tissue culture infectious dose. The MDCK cell line was used for the tissue culture.

exhaust fans and at 5 meters from poultry houses. For three of the five flocks studied, viral genomic RNA was detected in the sample collected at 50 to 110 meters. The measured viral air concentrations ranged between 4.3 and 6.4 $\log_{10}$ RNA copies per m^3.[151]

9.1.2.2 Experimental Studies of Aerosol Transmission of AI Virus

Besides factors such as the viral strain, species of birds, and other environmental factors that may influence the ability of AI viruses to spread,[58,160] the amount of virus released from the respiratory or intestinal route by infected birds also plays a role.[161]

Several experimental studies indicate that airborne transmission of HPAI infection between turkeys and chickens in adjacent pens or cages is possible but inefficient. These studies also suggest that aerosols may not be a primary route of transmission within a flock.

- In several experimental studies, aerosol transmission of HPAI (H7N7 A/chicken/Victoria/85 and H5N1A/Chicken/Hong Kong/258/97) was not observed between groups of inoculated and susceptible chickens housed in adjacent cages or chambers with direct airflow.[28,72,162] Similarly, for Japanese quail, there was no evidence of virus transmission to birds placed in aerosol contact at 30 cm.[82]
- In other studies, inefficient transmission or low transmission of AI was observed between groups of inoculated and susceptible chickens housed in adjacent cages or chambers with direct airflow.
  - LPAI H9N2 A/turkey/Wisconsin/66 virus was transmitted via aerosols between groups of 400 turkeys in different compartments of a building. In this experiment, AI virus was transmitted to one out of three exposed groups of turkeys in different compartments. Infection was detected based on serology and hemagglutination inhibition (HI) titer, and no virus was recovered from tracheal swabs.[163]
  - Three out of six strains of LPAI H9N2 viruses (A/chicken/Shanghai/F/1998, A/chicken/Shanghai/7/2001, and A/chicken/Shanghai/1/2002) were transmitted via aerosol from a cage with four infected chickens to chickens in an adjacent cage 100 cm away.[164]
  - For chickens housed in cages 10 cm apart, airborne transmission of HPAI H5N1 A/chicken/Yamaguchi/7/04 virus occurred inefficiently when one to two chickens were infected, but efficiently when four to eight chickens were infected.[165] With likely similar distances, Yee et al. (2009) found the aerosol route to be an important mode of AI virus transmission among chickens in a simulated live bird market setting (i.e., stacked cages) using LPAI H6N2 A/chicken/California/1772/02 virus.[160]
  - For HPAI H5N1 A/turkey/Turkey/1/2005, Spekreijse et al. (2011 & 2013) estimated a transmission rate of 0.10 new infections per infectious bird per day for chickens housed one meter away.[166,167]
- Experimental studies indicate that variability between strains can impact transmissibility via aerosols. For example, Zhong et al. (2014) found different strains of LPAI H9N2 virus to have markedly different aerosol transmissibility between chickens. The study proposed that the influenza virus genes HA and PA are important in determining aerosol transmissibility.[168]
- Several studies have indicated efficient transmission of HPAI H5N1 (A/Chicken/Kurgan/05/2005) and LPAI H9N2 (A/Ck/HN/1/98) viruses to chickens by

aerosols that were mechanically generated by nebulizing virus containing stock fluid to very small particle sizes (2 to 5 μm).[169,170]

- Several studies have found that influenza A viruses at higher temperature and relative humidity have decreased survivability in aerosols.[171,172]

Note that quail (*Coturnix sp*.) are very receptive to influenza A virus strains of waterfowl origin,[173] and infection in quail (*Coturnix sp.*)[82,105,173,174] and chukar partridge (*Alectoris chukar*)[81] with AI viruses is almost unequivocally established in the respiratory tract, and thus transmitted by aerosol. For pheasants, replication mainly occurs in the gastrointestinal tract,[81] rendering the oral-fecal route of infection more effective.

9.1.2.3 Other Studies of Aerosol Transmission of AI Virus

- A study in Australia involving elicitation of expert opinion reported that the probability of AI infection was higher for free range-raised birds than for cage- and barn-raised birds. Introduction of infection via aerial dispersion of feces was less likely to occur when compared with pathways such as indirect contact via fomites or via a contaminated water source. However, aerial dispersion was implicated among the most likely pathways of between-shed virus spread. For between-farm spread, it was believed that long distance aerosol transmission was only possible in poultry dense areas.[175]
- AERMOD plume models used in other Secure Poultry Supply (SPS) risk assessments[176,177] that focus on live bird movements demonstrate a measure of interest was HPAI virus concentration.
    - In *"An Assessment of the Risk Associated with the Movement of Broilers to Market Into, Within, and Out of a Control Area during a Highly Pathogenic Avian Influenza Outbreak in the United States,"* Cardona et al. (2018) utilize dispersion models that estimate the risk of transmission to a house of near market-weight broilers 20,000 birds using three scenarios (A through C).[177]
        - In a scenario in which a house of 25,000 broilers was infected, aerosol concentration was predicted to be highest downwind from the infected flock; concentration of virus was predicted to fall sharply as distance increases. In this model, infectious dose was estimated at $10^{5.44}$ EID_{50}/m^3, meteorological parameters and particle size were accounted for, and the predicted concentration of aerosolized virus farther than 2.5 km from the infected premises was considered to be low.[177]
            - When the infectious dose was lowered to 10^4 EID_{50}/m^3, the AERMOD model predicted that transmission likelihoods are much higher at longer distances.[177]
        - In an alternate scenario involving multiple different variables (the source of infection was a somewhat smaller turkey flock and weather conditions were from a different geographic area), the predicted HPAI virus concentration at a given distance from the infected source was greater than when broilers were the source flock, and transmission likelihoods increased somewhat as well.[177]
    - In *"An Assessment of the Risk Associated with the Movement of Turkeys to Market Into, Within, and Out of a Control Area during a Highly Pathogenic Avian Influenza Outbreak in the United States,"* Cardona et al. (2018) utilize dispersion model scenarios that estimate the risk of transmission to a house of 14,000 turkey hens assumed to weigh 15.53 lbs (7 kg).[176]

- In the two scenarios where the source flock was a 25,000-bird infected broiler house, aerosol concentration was predicted to be highest downwind from the infected flock; concentration of virus was predicted to fall sharply with increasing distance. In these models, two different infectious doses for the exposed turkey house were estimated (10^4 EID_{50} and $10^{3.2}$ EID_{50}, respectively), meteorological parameters and particle size were accounted for, and the predicted concentration of aerosolized virus farther than 2.5 km from the source infected premises was considered to be low (scenarios A and C).[176]
- The predicted probability of exposure of the turkey house in 1 day is substantial for both scenarios. However, it must be noted that there is considerable uncertainty in the aerosol dose response relationship in turkeys and that the particle size distribution of aerosols generated in poultry houses depends on the ventilation design, production type, and age of the birds.[176]
- With the lower infectious dose ($10^{3.2}$ EID_{50}), the AERMOD model predicted probabilities of exposure are significantly higher at all distances modeled. These results indicate that the likelihood of aerosol transmission in turkeys is very sensitive to the aerosol infectious dose for turkeys and warrant further studies to decrease uncertainty in the turkey aerosol dose.[176]
    - In an alternate scenario where multiple different variables were used (the source of infection was a 14,000-bird turkey flock, weather conditions were from a different geographic area, aerosol source emission rates were approximated using data from the 2015 HPAI outbreak, etc.) with the higher infectious dose of 10^4 EID_{50}, the predicted HPAI virus concentration at a given distance from the infected source was greater than when broilers were the source flock, and transmission likelihoods increased as well when compared with scenario A, which used the same infectious dose (scenario B).[176]

- These results highlight differences between epidemiological analysis in previous AI outbreaks (where an association between aerosol exposure and the case status of a premises was not found) and the higher transmission likelihoods from dispersion model predictions. However, we note that there is considerable uncertainty in some of the key dispersion modeling parameters. For example, there is little data on the decay rate for HPAI virus in aerosols under various environmental conditions. In addition, variations in AI virus strain characteristics and laboratory procedures may impact modeling calculations on the viable virus concentration in aerosols. In particular for distances within 0.5 km from an infected source, there is too much uncertainty and too many other possible risk factors to adequately address risk from aerosol transmission alone.[176]

9.1.2.4 Expert Opinion

- We obtained expert opinion from 12 experts on aerosol spread as a risk factor. Experts consisted of upland gamebird industry veterinarians and regulatory veterinarians, as well as aerosol experts who have done previous work involving aerosol spread during AI outbreaks in poultry. Experts rated this risk factor on a categorical scale ranging from negligible to extremely high (see **Appendix 4**: Expert Polling on Aerosol Transmission Routes for details of the questionnaire and the complete data set). In a scenario in which depopulation activities *were not* taking place, most experts (9 out of 12) rated the likelihood of aerosol transmission from a known infected premises 10 km away from a

susceptible upland gamebird farm as negligible. In a scenario in which depopulation activities *were* taking place, most experts (8 out of 12) rated the likelihood of aerosol transmission from known infected premises 10 km away from a susceptible upland gamebird farm as negligible. In the case of aerosol transmission from infected but undetected farms, most experts rated the likelihood of transmission to a susceptible farm that is:

1 km away – *Low* (as ranked by 6 out of 12 experts)

5 km away – *Negligible* (as ranked by 7 out of 12 experts)

10 km away – Negligible (as ranked by 8 out of 12 experts)

15 km away – *Negligible* (as ranked by 11 out of 12 experts)

9.1.2.5 Qualitative Analysis

We considered the following factors in evaluating this pathway:

- Most ready-for-release upland gamebirds (except bobwhite quail) are raised in outdoor pens and would be considered closer to free range or pasture-raised birds. In their 2017 study in Australia involving elicitation expert opinion, Singh et al. (2018) reported that probability of infection was higher for free range-raised birds than for cage- and barn-raised birds.[175] However, the scope of the upland gamebird farms included within this risk assessment (e.g., farms that are outside of a Control Area) should be kept in mind when synthesizing this information into the final risk rating.
- Factors such as infectivity, susceptibility, amount of virus transferred during contact, contact rate, and the number of flocks that make contact are known to influence AI transmission.[178] The probability of an airborne virus-laden particle causing an infection depends on its infectious potential and its ability to resist the stress of aerosolization and through conducting epidemiological studies and/or by analyzing the microbiological content of air samples, this probability can be determined.[148]
- The birds under study are at the least 10 km away from a known candidate infecting source since only upland gamebird flocks outside of a Control Area are included within the scope of this risk assessment.
- Transmission via the aerosol pathway involves many constantly changing variables.
- Virus viability may change with temperature, humidity, and UV exposure, as increased temperature, humidity, and UV exposure may or may not cause virus inactivation.[171,172,179–181]
- Weather conditions (temperature, humidity, wind speed and direction) vary widely by season and geography. Dispersion of particulate matter and virus from an infected premises may not be consistent over time.
- To date, all exploratory models have assumed the source to be a static premises (i.e., infected poultry house). Other sources of infection, such as proximity to trucking routes or road traffic, have not been investigated.

9.1.2.6 Likelihood Rating and Conclusion

9.1.2.6.1 Likelihood of HPAI Spread to an Upland Gamebird Flock in a Control Area via Aerosol Transmission from a Known HPAI-Infected Flock

While there is higher predicted prevalence of infectious birds in known infected flocks, given the scope of risk assessment, the minimum distance a susceptible upland gamebird farm would be from a known infected poultry farm is 10 km. Thus, ratings strongly factor in that based on literature review and most previous outbreak reports indicating that local area spread and aerosol transmission were not an important factor at distances more than 1.5 km from an infected flock. Based on these findings in addition to insights provided by expert opinion and exploratory dispersion modeling results the risk of HPAI infection via aerosol from a known-to-be-infected poultry farm is ***negligible*** (see **Table 3**).

9.1.2.6.2 Likelihood of HPAI Spread to an Upland Gamebird Flock in a Control Area via Aerosol Transmission from an Infected but Undetected Flock

In this case of infected but undetected poultry flocks, susceptible upland gamebird farms have the possibility of being within 10 km of these farms. While literature provides a less clear predictive picture of this scenario, based on the limited literature in addition to expert opinion ratings and dispersion modeling risk would be higher based on proximity. We rated the risks of upland gamebirds becoming infected with HPAI via aerosols from an infected but undetected poultry flock depending upon the distance from the infected premises as ranging from ***low to negligible*** (see **Table 3**).

Table 3. Likelihood of an upland gamebird premises becoming infected with HPAI virus via aerosol transmission based on qualitative analysis and expert opinion.

Source premises type	Composite likelihood rating Distance from source (km)			
	1 km	5 km	10 km	15+ km [a]
Infected but undetected premises	*Low*	*Negligible*	*Negligible*	*Negligible*
Known-to-be-infected premises	*Not applicable*	*Not applicable*	*Negligible*	*Negligible*

[a] 15.42 km is the average distance an upland gamebird farm is located in relation to a poultry farm or other upland gamebird farm in the State of MN[14]

9.1.2.6.3 Conclusion

The risk of exposure of an upland gamebird flock from bioaerosols ranges from ***low to negligible,*** depending on the distance from, and prevalence of virus in, the source flock. The assessed risk is highest for flocks located within 1 km from an infected but undetected poultry farm. We estimate the risks of exposure of an upland gamebird flock to be *negligible* if the premises is located 10 km from an infected but undetected poultry farm, and *negligible* if the premises is a known infected poultry farm.

9.1.3 Role of Insects in the Transmission of HPAI Virus

Houseflies (*Musca domestica*) are reservoirs and vectors of a wide variety of pathogenic organisms affecting poultry.[182] Insect or fly transmission of AI virus has been suspected in previous HPAI outbreaks based on anecdotal reports;[155,183] however, there are no quantitative epidemiological studies establishing transmission via flies. Some biosecurity plans and

guidelines for AI control recommend fly control to minimize the spread of AI because of the existing uncertainty about fly transmission of HPAI.[184,185]

The most found insects in upland gamebird pens include houseflies (*Musca domestica*) and grasshoppers (e.g., *Schistocerca americana*) (personal communication, Secure Upland Gamebird Supply Working Group, August 2019). Additionally, based on studies of wild pheasant, various beetle species (order Coleoptera), crickets (e.g., *Gryllus pennsylvanicus*), and grasshoppers (e.g., *Schistocerca americana*) that are out during the warmer months, are observed to be the preferred insects for consumption by pheasants.[186] While blowflies (family Calliphoridae) are common on poultry farms, because they are a result of improper disposal of mortality in a poultry operation, [182] they are not prevalent on upland gamebird operations (personal communication, Secure Upland Gamebird Supply Working Group, August 2019).

Below is a summary of the literature from previous outbreaks implicating insects in the transmission of HPAI, survivability of AI viruses in and on flies, dispersion likelihood, and transmission of HPAI to a flock.

9.1.3.1 Literature Review

9.1.3.1.1 Transmission of AI via insects in previous outbreaks

- Insects are considered more of a potential influenza A virus transmission pathway between farms for free-range (i.e., outdoor) operations based on expert veterinary opinions. Polled veterinarians from one study suggested that insects have the potential to act as mechanical vectors that could spread AI infection between farms and pose a higher risk on free range farms than enclosed farms for both broiler and layer chickens.[175]
- During the H5N2 HPAI outbreak in Pennsylvania in the 1980s, roughly 300 pools of insects from 15 different species were collected from 42 affected premises for the purpose of virus isolation attempts. Virus was isolated from 25 pools (7.7 percent) of houseflies (spp. not specified), 9 pools (2.8 percent) of black garbage flies (spp. not specified), and 8 pools (2.5 percent) of small dung flies (spp. not specified). Flies were suggested to be a probable source of infection for several flocks in Pennsylvania.[155]
- Blowflies (*Calliphora nigribarbis* and *Aldrichina grahami*) were considered a potential mechanism of transmission in the 2004 HPAI H5N1 outbreak in Japan.[187,188] In this outbreak, the detection of H5 virus genes was highest in blowflies collected 600 to 700 meters from the infected farm (35 percent of total flies [7/40 total flies; *Calliphora nigribarbis*, 5/20; *Aldrichina graham*, 2/20]). HPAI virus gene-positive flies (10 percent [1/10], *Calliphora nigribarbis*) could be detected up to two kilometers from the infected premises. Authors determined that the virus isolated from the *Calliphora nigribarbis* flies (A/blow fly/Kyoto/93/ 2004) was recognized as the same strain of the virus (A/chicken/Kyoto/3/2004) infecting chickens in the area. Additionally, the authors estimated that 5 percent of the *Calliphora nigribarbis* flies around the epidemic area had viable virus.[188]

9.1.3.1.2 Survivability of AI viruses in and on flies

- Flies (spp. not specified) were collected as part of environmental sampling from the enclosed housing of white storks (*Ciconia ciconia*) in a German zoo that had infection of

HPAI H5N8 clade 2.3.4.4**.** All environmental RT-qPCR tests done on the flies came back negative.[189]

- Tsuda et al. (2009) proposed a mechanism of transmission whereby poultry directly feed on HPAI-infected blowflies.[190] However, feeding dead flies (*Calliphora nigribarbis*) contaminated with H5N1 virus did not result in transmission (unpublished data; personal communication, Yoshio Tsuda, 2012) and it is unclear how such data would translate to upland gamebird species. Additionally, in the context of upland gamebirds, there is no evidence that pheasant, chukar, or bobwhite quail prefer flies as a dietary choice while housed in pens (personal communication, Secure Upland Gamebird Supply Working Group, August 2019).
- Habibi et al. (2018) found that of 90 flies (spp. not specified) collected from campus of School of Veterinary Medicine, Shiraz University, Iran, 18 samples subjected to RNA extraction were found to have 450 base pairs of M1 of avian influenza based on published primers. Authors infer that such findings suggest flies are capable of transmitting viruses either by way of on the body surface and/or via actively ingesting infectious materials.[191]
- Wanaratana et al. (2013) evaluated the potential of the housefly (*Musca domestica*) to serve as a mechanical vector of the H5N1 virus. H5N1 virus could survive within the body of the housefly and remain infective for up to 72 hours post-exposure.[192]
    - Viral titers in housefly (*Musca domestica*) homogenate varied between $10^{5.43}$ EID_{50}/mL at 6 hours post-exposure to 10^{2} EID_{50}/mL at 72 hours post-exposure.[192]
    - In this study, the potential for virus transmission via virus on the fly body was also investigated. At 24 hours post-exposure, the virus concentration was 1.9 log ELD_{50}/mL (the concentration at time 0 was 4.7 log ELD_{50}/mL), whereas virus could not be recovered by 48 hours post-exposure.[192]
    - Authors demonstrated that chickens fed fly homogenate via oral drop with a pipette one day after exposure to $10^{8.5}$ ELD_{50} experienced virus transmission from the homogenate. Based on the timing of virus shedding,[3] between 1 and 3 chickens of 10 appeared to have been directly exposed from the fly homogenate in this study.[192] However, in upland gamebirds, specifically equivalent data is not available.
- Experimental studies indicate that flies (*Musca domestica* and *Calliphora nigribarbis*) can ingest influenza A virus and that there is a steady decrease in viable virus titer over time.[193–195]
- Tyasasmaya et al. (2016) found that influenza A virus H5N1 remained in the gastro-intestinal tracts of houseflies (*Musca domestica*) for at least 24 hours post-exposure based on RT-qPCR results.[196]
- In Nielsen et al. (2011) experimental study, low-pathogenic avian influenza viruses of the H7N1 and H5N7 subtypes were isolated from the alimentary tract of houseflies (*Musca domestica*) for at least 24 hours after ingestion. External variables such as temperature, incubation period post-ingestion, and load of ingested virus were shown to have a role in

[3] Only 3 birds of 10 were shedding by day 2 post-inoculation. In experimental studies in the literature, most HPAI strains had a mean latent infection period of less than 1.5 days.

viral persistence, however, overall virus was observed to decline at all concentrations and temperatures over time. Only one out of the 36 groups (3 percent) tested after 24 h at 25 °C and 35 °C were found to be positive.[193]

- Sawabe et al. (2009) evaluated the survivability of H5N1 virus in blowflies (*Calliphora nigribarbis*) after experimental exposure. Viable virus was recovered in the crop and intestine up to 24 hours post-exposure. However, there was a steady decrease in viral titers from gut contents over time. Most of the flies had viral titers below the level of detection for the assay (0.50 log $TCID_{50}$/0.05 mL of fly homogenate) at 24 hours. All of the flies had viral titers below the level of detection at 48 hours post-exposure.[194]

9.1.3.1.3 Fly dispersal

- Fly dispersal behavior varies by species and environmental conditions. Houseflies (*Musca domestica*) remain close to their breeding site as long as they find suitable food, breeding sites, and shelter. Additionally, the dispersal rate of flies decreases in temperatures below 11.7 °C and increases during premises cleanout or litter spreading.[197] A summary of fly dispersal rates appears in **Table 4**.

Table 4. Reported dispersal rates for types of flies implicated in the mechanical transmission of H5N1 HPAI.

Common name	Reported dispersal rates	Reference
Housefly	1-3 km/day	*198*
Housefly	Generally, range less than 3.2 km; range in a radius of 328 to 1,640 feet from breeding site if suitable food available; only 8 to 30% disperse beyond a poultry facility	*199*
Housefly	Up to 11.8 km within 24 h	*200*

- During the Pennsylvania outbreak of H5N2 in 1983, flies (spp. not specified) were observed to congregate in vehicles that were parked by poultry houses[155] implying there was a potential to transfer insects from one premises to another in vehicles.
- Beetles (e.g., *Alphitobius diaperinus*) have also been implicated as a possible vector for transmitting AI viruses in three studies;[201–203] however, there are minimal data on the experimental transmission of AI via beetles. In the 1983 HPAI H5N2 outbreak in Pennsylvania, the testing of 144 pools of beetles (various Coleoptera spp.) yielded only two positive pools. One of the positive pools consisted of darkling beetles (family Tenebrionidae), and the second of hide beetles (family Dermestidae).[183]
    - Beetles (order Coleoptera) are rarely apparent in extensive numbers on upland gamebird farms unless brooder bedding is poorly managed which is outside standard

practice (personal communication, Secure Upland Gamebird Supply Working Group, August 2019).

9.1.3.2 Expert Opinion

- We obtained expert opinion from 12 experts on insect spread as a risk factor. Experts consisted of upland gamebird industry and regulatory veterinarians as well as local area spread experts who have done previous work involving local area spread during AI outbreaks in poultry. Experts rated this risk factor on a categorical scale ranging from negligible to extremely high (see **Appendix 5**: Expert Polling on Insect Transmission Routes for details of the questionnaire and the complete data set). Most experts (10 out of 12) rated the likelihood of transmission via insects from a known infected premises 10 km away from a susceptible upland gamebird farm as negligible. In the case of transmission via insects from infected but undetected farms, most experts rated the likelihood of transmission to a susceptible farm that is:

  1 km away – *Low* (as ranked by 7 out of 12 experts)

  5 km away – *Negligible* (as ranked by 7 out of 12 experts)

  10 km away – *Negligible* (as ranked by 10 out of 12 experts)

  15 km away – *Negligible* (based on 11 out of 12 experts)

9.1.3.3 Qualitative Analysis

We considered the following factors in evaluating this pathway:

- While houseflies (*Musca domestica*) and other insects have been proposed as a possible mechanism for spread of HPAI, local area spread components (other than mechanisms involving movement of people, vehicles, and equipment) have historically played a minimal role in most AI outbreaks. (See **Section 9.1.1**, Role of Local Spread Components in Previous AI Outbreaks, for more detail.)
- Although chickens have been shown to ingest live and actively flying houseflies (*Musca domestica*)[194] upland gamebirds have been reported to generally ignore flies (Secure Upland Gamebird Supply Working Group, 2019, [186]) and there has been no experimental evidence of chickens, turkeys, or upland gamebirds becoming infected with AI virus through feeding on contaminated whole flies in previous outbreaks. Infection was achieved experimentally in chickens using fly homogenate administered via pipette, which likely approximates the oral or possibly nasal/choanal route of infection.[192] We hypothesized that HPAI transmission via feeding of whole flies as opposed to homogenate would have a low likelihood for the following reasons:
- For HPAI virus encapsulated in the fly body (e.g., virus ingested by a fly), the most likely inoculation route to the chicken is intragastric. As gallinaceous birds (including chickens, turkeys, pheasants, chukar, and bobwhite quail) do not grind or masticate their food within the oral cavity,[204] the likelihood that fly gut contents would contact the choanal cleft during ingestion is decreased. Intragastric infectious dose (CID_{50}) estimates are quite high at $10^{5.2}$ EID_{50} to $10^{6.2}$ EID_{50} based on two studies done in chickens[170,205] (equivalent data for upland gamebird species not available).
- Wanaratana et al. (2013) have found a considerable decrease in the external HPAI virus concentration on an exposed fly (*Musca domestica*) within 24 hours.[192] While HPAI virus

is inactivated at a slower rate in fly gut content, and after 24 hours persistence of virus in gut content is reduced,[193] the likelihood of infection due to the virus encapsulated in the fly gut would be reduced because of the higher infectious dose needed for the intragastric route.

- Contamination of fly perching surfaces with virus from the fly body, vomit, or feces is a possibility. However, available experimental studies indicated that there would be a considerable reduction in the virus concentration in fly body, vomit, or feces by 6 to 24 hours post-exposure of the fly to virus (See **Section 9.1.3.1**, Summary of Literature on Insect Transmission). The relatively rapid inactivation of virus present externally on flies would result in reduced likelihood of transmission at greater distances.
    - In addition, the oral infectious dose for HPAI virus in chickens (data unavailable for upland gamebird species) is also relatively high compared with intranasal (or choanal) exposure (estimates range from $10^{3.9}$ to $10^{6.7}$ for HPAI H5N1 and 10^{8} for LPAI H9N2).[169,170,205]
- While a proportion of flies around an infected premises can harbor virus, previous outbreaks sampling flies at infected premises and uninfected premises show that flies containing virus only occurred on infected premises and not uninfected premises.[206] Dispersal behavior may vary depending on environmental conditions and fly species, and dispersal is hypothesized to increase during outbreak activities such as premises depopulation. With this in mind, other dispersal considerations include:
    - Flies have been observed to be less concentrated in pasture/field environments where manure is more dispersed in comparison to confined poultry houses with high concentrations of birds and consequently higher concentrations of manure.[207] With broiler, turkey, and layer stocking densities being much higher than upland gamebird stocking densities,[14] it is assumed manure is much more dispersed than other species. Observations additionally point to flies having a higher likelihood of having breeding areas in these unsanitary conditions.[207] If the assumption that manure is more dispersed in upland gamebird pens than in barns housing other poultry species does not hold, fly levels would be roughly equivalent to those on other types of poultry premises.
    - Observations regarding fly concentrations on premises producing higher and more concentrated manure have been made between conventional poultry commodities. In the period leading up to load-out, the inside of a broiler house likely contains a large amount of manure and other environmental conditions that may attract flies. Winpisinger et al. (2005) found the number of houseflies (*Musca domestica*) was significantly higher near (within 3.2 km) large (>2 million) caged layer operations, compared with background fly levels in rural areas.[208] However, dispersal may depend on outdoor environmental and other factors. The number of flies caught at a distance of 0.8 km (3 to 22 percent of the mean value at layer farm) and 1.6 km (2 to 8 percent of the mean value at layer farm) was much lower than the number of flies trapped at the layer facilities.
    - In relation to dispersal, due to the criteria of the movement being assessed (see **Section 4.1** Facilities Covered Under this Risk Assessment) the birds under study are

at least 10 km away from a candidate infecting source (as is inherent to the established Control Area).

- Additionally, upland gamebird premises have been found to be on average 15 km away from any other premises with poultry,[14] negating the risk of infected but undetected farms and fly dispersal ranges evidenced in **Table 4.**
- However, it is important to note that extended dispersal ranges due to flies congregating in vehicles on poultry sites is possible as was observed by Brugh & Johnson (2003).[155] Even so, upland gamebird farms are observed to have less shared vehicle traffic,[14] thus less opportunity to receive stow away flies from infected premises.

9.1.3.4 Likelihood Rating and Conclusion

We rated the likelihood of an upland gamebird premises becoming infected with HPAI virus via insect transmission to vary with distance as described in **Table 5**. Of note, no upland gamebird premises under the scope of this risk assessment will be within 10 km of a known-to-be-infected premises, thus assessment of the likelihood of infection via insects traveling from known-to-be-infected premises greater than 10 km away and insects from infected but undetected premises are considered when determining the likelihood of rating.

Table 5. Likelihood of an upland gamebird premises becoming infected with HPAI virus via insect transmission based on qualitative analysis and expert opinion.

Composite likelihood rating				
Source premises type	**Distance from source (km)**			
	1 km	**5 km**	**10 km**	**15+ km[a]**
Infected but undetected premises	*Low*	*Negligible to low*	*Negligible*	*Negligible*
Known-to-be-infected premises	*Not applicable*	*Not applicable*	*Negligible*	*Negligible*

[a] 15.42 km is the average distance an upland gamebird farm is located in relation to a poultry farm or other gamebird farm in the State of Minnesota. [14]

9.1.4 Role of Rodents in the Transmission of HPAI Virus

The role of rodents in perpetuating and spreading influenza A virus is a reoccurring area of question for investigators of AI. Anecdotal reports and epidemiological investigations[209] point to the possibility of rodent participation in outbreaks and the possible role of rodents as a bridge species.[210] Some investigators have built a theoretical framework for rodent involvement in poultry AI outbreaks which is based on literature review, however there is acknowledgement that more evidence is needed.[211] In an expert elicitation study, Singh et al. (2018) reported that rats and snakes (species not specified) were identified as mechanical vectors that could spread the influenza A viruses between farms in Australia.[175]

Observational evidence paired with straightforward reasoning creates a potential argument for rodent involvement in outbreak dissemination. Some species of mammals are known to be susceptible to infection and may spread various influenza A viruses depending on the subtype

and strain.[212] The potential susceptibility and ability to shed virus of some mammals is then important when considering that some wildlife species adapt—and regularly habituate—to livestock operations due to abundant access to food and shelter.[64] Types of rodents, such as rats including the species of black rats (*Rattus rattus*) and brown rats (*Rattus norvegicus*), and mice including house mice (*Mus musculus*),[211,213–215] are considered synanthropic species. However, this is not to say that other minor species found on or near poultry farms, such as deer mice (*Peromyscus maniculatus*), voles (*Zapus hudsonius*), and shrew (*Blarina brevicauda*), could not be involved in influenza A virus transmission without appropriate investigation.

Focusing specifically on rat and mouse populations, the species listed above have been observed to be incredibly widespread and infiltrative, with estimated numbers, accounting for pest control mitigations, reflecting one rat per three to four chickens on farms.[213]

Because of rodents' habitat utilization and distribution, rodents often closely share their environments with *both* wild birds and domestic poultry[211] and there is reasonable speculation that rodents have the potential to act as a bridging species[210] for influenza viruses either as fomites or actively shedding hosts. If rodents are able to travel farm to farm, they may spread the virus, and rodents, like brown rats (*Rattus norvegicus*), that have the capacity to travel between wetland environments to poultry structures could bring virus with them.[211]

Below is a summary of the literature from previous outbreaks implicating rodents in transmission of HPAI, efficacy of rodent control measures in previous outbreaks, experimentally determined susceptibility of rodents, experimentally determined transmission of influenza A viruses from rodents, survivability of AI viruses on rodents (i.e., capacity for mechanical transmission), and dispersion likelihood of rodents.

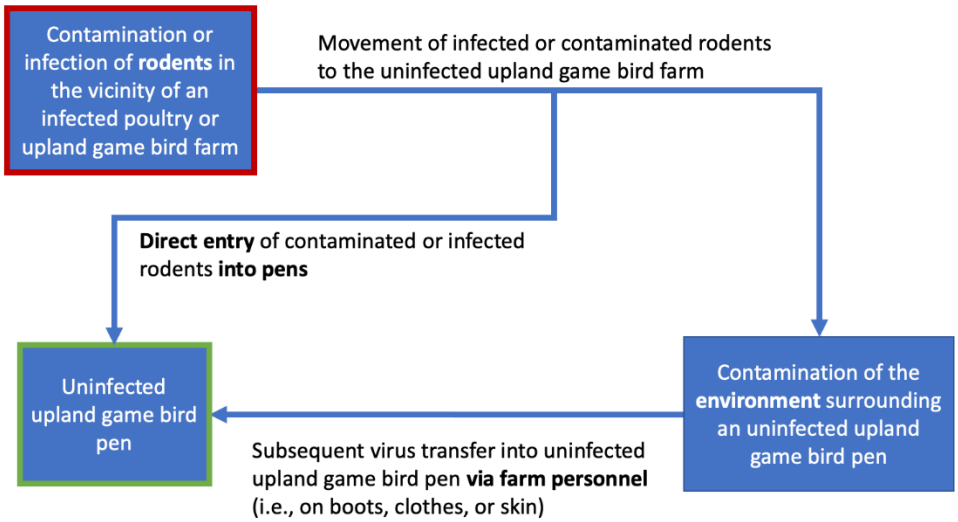


Figure 5. Pathway of HPAI virus transmission through rodents.

9.1.4.1 Literature Review

9.1.4.1.1 Prevalence of AI in rodents in previous outbreaks of AI involving poultry

- Lung tissue samples and toes (for purposes of external swabbing) from mice (*Mus musculus*) (n=245) and rats (*Rattus norvegicus*) (n=9) were taken for virus isolation from

farms in a quarantine zone in the Pennsylvania during the 1983–1984 HPAI H5N2 outbreak. No virus was isolated from any of the samples.[216]

- During the 2014–2015 H5N2 HPAI outbreak, Shriner et al. (2016) investigated the presence of virus in and on wild mammals, including rodents such as house mice (*Mus musculus*) and deer mice (*Peromyscus maniculatus)* populations around five infected and five uninfected farms in Iowa. All oral swabs, nasal swabs and washes, external swabs, serum, and tissues (e.g., lung and/or trachea) samples were negative for presence of virus and antibodies based on RRT-PCR and antigen testing respectively. However, investigators noted that sampling occurred post-depopulation for four of the five infected farms and that sampled wildlife were most often juveniles meaning they were born after the outbreak.[209]
- Rats (spp. not specified) from live poultry markets during the H5N1 outbreak in Hong Kong SAR in 1997, were sampled via fecal swabs and no virus was isolated. However, sera from the collected rats (spp. not specified) did demonstrate haemagglutination inhibiting activity.[217]
- In 2008, an upland gamebird farm in Idaho had an outbreak of HPAI H5N8 and was subsequently depopulated. Shriner et al. (2012) found that of the six house mice (*Mus musculus*), one harvest mouse (*Reithrodontomys megalotis*), one deer mouse (*Peromyscus maniculatus*), and six brown rats (*Rattus norvegicus*) sampled via oral swabs and serologically on the farm, no influenza A virus viral RNA was detected. However, sera samples from the six house mice (*Mus musculus*) were positive for influenza A virus antibodies via indirect ELISA. [153,218]
- Vermin (e.g., mice and rats, species not specified) that could have been contaminated with bird feces were considered and assessed as mechanical transmitters between premises in 2016 during an outbreak of HPAI H5N8 in northern Germany. However, investigators determined the risk of rodent movements as an introductory mechanism for influenza A virus onto a premises to be low to negligible.[219]
- In LPAI H7N2 outbreak of 1996–1997 in Pennsylvania, field investigators collected 141 house mice (*Mus musculus*) from 18 houses of 10 infected premises between the months of June and September. Forty-six pools of lung and intestinal tissue samples were taken from the collected house mice (*Mus musculus*), all of which were negative for influenza A virus by virus isolation.[157]
- One field study conducted by Grear et al. (2017) in Wisconsin in September 2015 (roughly five months after the HPAI H5N2 outbreak in the Midwest U.S.) sampled mammal populations on previously infected poultry premises, unaffected poultry premises and natural areas via sera collection and oral swabs. Mammals sampled on poultry premises were all rodents including the following species: Eastern chipmunk (*Tamias striatus*), masked shrew (*Sorex cinereus*), meadow vole (*Zapus hudsonius*), house mouse (*Mus musculus*), deer mouse (*Peromyscus spp.*), and short-tailed shrew (*Blarina brevicauda*), with deer mice (*Peromyscus maniculatus*) making up the majority of the sample size in each group (49/67, 45/48, and 63/81, respectively). None of the mammals sampled yielded positive results for viral detection via PCR using oral swabs. Of the 47 rodents sampled on the previously infected farms only one of 45 was positive for influenza A virus antibodies via ELISA. Only one of 45 sampled on unaffected

poultry farms was positive for antibodies and none of the 67 sampled in natural areas were positive.[220]

- In a field study conducted by Houston et al. (2017) that examined influenza A virus prevalence in wild birds and mammals in natural areas of Iowa following the 2015 H5N2 outbreak, the following rodent species were sampled at poultry sites and wetland sites: deer mouse (*Peromyscus maniculatus*; n=3 and n=109, respectively), house mouse (*Mus musculus*; n=19 and n=1, respectively), northern short-tailed shrew (*Blarina brevicauda*; n=5 and n=6, respectively), meadow vole (*Microtus pennsylvanicus*; n=2 and n=2, respectively), and Norway rat (*Rattus norvegicus*; n=0 and n=1, respectively). All individual rodents had oropharyngeal and cloacal/anal swabs and blood samples taken. All swabs came back negative via PCR and the serology showed no antibody activity for any of the rodents.[221]
- In the USDA's epidemiological report on the Tennessee HPAI H7N9 outbreak under "Sampling for Avian Influenza Virus in Synanthropic Wildlife", over a 4-day period in March 2017, 53 house mice (*Mus musculus*) and three white-footed mice (*Peromyscus leucopus*) were sampled. There no positives for viral RNA via RT-PCR and no positives for antibodies via serology among the mice sampled.[54]

9.1.4.1.2 Prevalence of AI in rodents outside of active HPAI outbreaks in poultry

- In an exploratory study by Cummings et al. (2019) brown rats (*Rattus norvegicus*) were sampled within the metropolitan city of Boston, Massachusetts via oronasal swabs and lung tissue extraction—9 of 161 rats (*Rattus norvegicus*) sampled were RT-PCR positive for influenza A viruses via oronasal samples and 2 of 108 were RT-PCR positive for influenza A viruses in lung tissue samples.[222]
- In the El-Sayed et al. (2013) field study examining the presence of influenza A viruses in Egypt, investigators sampled rats (species not specified) (n=72) from the Nile-Delta area serologically and found that only two rats were positive using the hemaglutination inhibition test (one with titer 4 and one with titer >4). It was not determined if any of the rats sampled were ELISA positive.[223]

9.1.4.1.3 Efficacy of rodent control to mitigate risk in previous outbreaks

- During the 2002 LPAI outbreak in Virginia, a case-control study assessed the impact of rodent control differences in relation to a farm's infection status.[109] McQuiston et al., (2005) surveyed 147 infected farms and 197 non-infected farms and found insignificant, marginal differences between the frequency of rodent control on infected farms and non-infected farms including rodent traps checked every six weeks (119/147 [81 percent] compared to 162/197 [82 percent]), traps checked less than every six weeks (28/147 [19 percent] compared to 35/197 [18 percent]), and no rodent control (0/0 [0 percent] for both).
- A cross-sectional study examining the use of pest control practices in seropositive and non-seropositive flocks in Maryland found sampled flocks that were seropositive for influenza A virus antibodies were 2.5 times less likely to implement pest control practices on-site. A questionnaire administered to premises owners showed that that 87 percent (13/15) of non-seropositive flocks used pest control methods, while only 66 percent (14/21) seropositive flocks had used pest control methods.[224]

- In the Duvauchelle et al. (2013) study looking at risk factors associated with seroprevalence in French breeder duck flocks, pest control from an outside firm was considered a risk factor in introduction of influenza A virus onto farms. However, Duvauchelle et al. (2013) attributes the risk more to the opportunity for outside crews bringing virus on farm via persons and vehicles rather than related to the elimination of pests.[225]
- During the 2014–2015 HPAI H5N2 outbreak in the United States, 104 HPAI-infected premises were surveyed via an epidemiological questionnaire and 92.3 percent responded that rodent bait stations were utilized and were actively checked every 6 weeks.[226]
- Fasina et al. (2011) found that in a case-control study, that case poultry farms infected with HPAI compared to controls had no significant or substantially observable differences in rodent control, with 17/31 (55 percent) of cases and 52/78 (67 percent) (p=0.26) of controls experiencing problems with rodent control onsite.[227]
- Wakawa et al. (2012) surveyed 64 farms in Nigeria, 32 which were affected by the HPAI H5N1 outbreak during 2006–2008 and 32 farms that were unaffected. Investigators found that 71.9 percent of unaffected farms compared to 62.5 percent of affected farms prevented rodents and wild birds from accessing feed. The results were found to be significant (p=0.024) with an odds ratio of 3.65.[228]

9.1.4.1.4 Experimentally determined susceptibility of rodents to AI viruses

- Hiono et al. (2016) found, in an experimental study assessing multiple synanthropic species, that black rats (*Rattus rattus*) play a negligible roll in transmission of multiple influenza A viruses and were less susceptible to influenza A viruses than sparrows (*Passer montanus*) or crows (*Corvus macrorhynchos*). Rats (*Rattus rattus*) intranasally inoculated with one of an HPAI H5N2 virus, and HPAI H5N8 virus an HPAI H7N9 virus and four different HPAI H5N1 viruses, all survived and seroconverted, yielded HI titers in serum ranging between >2 and 64, and only one of 28 rats (*Rattus rattus*) exhibited any virus titers in its internal organs.[215]
- Another study by VanDalen et al. (2019) that examined influenza A viruses in brown rats (*Rattus norvegicus)* found that rats inoculated individually with one of LPAI H6N2 (A/CK/CA/S0408793/04), LPAI H4N8 (A/CK/AL/75), LPAI H4N6 (A/mallard/CO/P66F1-5/08), LPAI H3N8 (A/wildbird/CA/18771826/08) demonstrated some level of viral replication over the 14-day study period post-inoculation. Replication of virus observed in tissue samples of rats were classified as extremely low for the H4N8 virus, minimal for the H6N2 virus, and moderate for the H3N8 and H4N6 viruses, with the highest tissue viral load ranging observed at 5.45 log10 PCR EID_{50} equivalents/mL.[212]
- A study by Blanco et al. (2013) using cotton rats (*Sigmodon hispidus*) intranasally inoculated with LPAI H3N2 (A/duck/Hong Kong/375/1975), LPAI H9N2 (A/guinea fowl/Hong Kong/WF10/1999), HPAI H5N1 (A/Vietnam/1203/2004), or pandemic H1N1 (A/California/04/2009) showed that the rats possess both types of receptors (α2,3-linked and α2,6-linked sialic acid receptors) that enable susceptibility to influenza A viruses and human influenza viruses. The cotton rats were inoculated with 10^2 to 10^7 EID_{50}/rat of the HPAI H5N1 A/Vietnam/1203/2004, with mortality occurring at 100 percent for cotton rats in the 10^7 EID_{50} group by day 1 post-inoculation, 75 percent for the

cotton rats in the 10^6 EID_{50} by dpi 3, and no mortality for 10^5 EID_{50} and below for the entire study length. Virus replication was evident with viral titers in the lungs present for inoculations as low as 10^4 EID_{50} at over 10^7 $TCID_{50}$/g. Clinical signs of disease such as hunching and substantial weight loss were observed.[229]

- In a study by VanDalen et al. (2019) brown rats (*Rattus norvegicus*) were intranasally inoculated with 10^5 EID_{50} delivered in 100 µL of one of the following LPAI viruses: H6N2 A/CK/CA/S0408793/04, H4N8 A/CK/AL/75, H4N6 A/mallard/CO/P66F1-5/08, or H3N8 A/wildbird/CA/187718-26/08. Fecal and oral swabs were all negative for viral RNA for the 94 brown rats inoculated (24 per virus subtype). However, 12 of the 94 nasal swabs collected were positive for viral RNA, including 5 positives coming from H3N8 inoculated rats, 4 from H4N6 rats, 3 from H6N2 rats, and none from H4N8. The mean viral RNA across all virus subtypes was 3.32 log10 PCR EID_{50} equivalents/mL. Detection of virus RNA for all subtypes was found in all tissues sampled (e.g., nasal turbinates, caudal lung sections, cranial lung sections, and trachea) with the exceptions of no H6N2 virus RNA was found in the trachea of any of the inoculated rats (n=23) and no H4N8 virus RNA found in caudal lung, cranial lung, or trachea samples.[212]
- In an experimental study simulating the multi-species transmission conditions of a farm environment, Achenbach & Bowen (2011), assessed Sprague Dawley rats (*Rattus norvegicus domestica*) for influenza A virus transmission. Contact Sprague Dawley rats (*Rattus norvegicus domestica*) that shared an environment with ducks inoculated with LPAI H5N2 or LPAI H7N3 virus, did not display any clinical signs for disease and had no virus isolated from oropharyngeal swabs. The contact Sprague Dawley rats (*Rattus norvegicus domestica*) showed no seroconversion based on ELISA and HAI test results for the H5N2 virus. For the H7N3 virus, while there were no positive HAI test results, 6 of 7 Sprague Dawley rats (*Rattus norvegicus domestica*) had positive ELISA test results, indicating seroconversion. The shared environment included drinking out of the same water source as infected birds and traveling over the same floor space.[230]
    - In the same study, Achenbach & Bowen (2011), performed experiments directly inoculating a group of Sprague Dawley rats (*Rattus norvegicus domestica*) intranasally, with the same viruses at 10^6 PFU in 0.1 mL, having each rat separately caged. Results from the direct inoculation experiments revealed that 100 percent of the Sprague Dawley rats (*Rattus norvegicus domestica*) for each virus type and seroconversion test type were positive for seroconversion (with the exception of only 4 out of 5 Sprague Dawley rats (*Rattus norvegicus domestica*) being positive for H7N3 seroconversion via HAI test).[230]
- In an experimental study, Romero Tejeda et al. (2015) assessed the susceptibility and the transmissibility of voles (*Myodes glareolus*) to influenza A viruses. Voles were intranasally inoculated with $10^{3.75}$ and $10^{4.4}$ EID_{50}/0.1 mL of HPAI H7N1 A/ostrich/Italy/2332/2000 and H5N1 A/turkey/Turkey/1/2005 viruses, respectively. The H7N1-inoculated voles showed no clinical signs, however viral shedding via nasal washes was observed in one out of three samples with a viral load peaking at 7.9×10^7 viral copies/µL, and virus isolation was achieved from the nasal wash of only one vole. One of the twelve H5N1-inoculated voles displayed clinical signs (e.g., mild depression) with viral shedding via nasal washes peaking at 3.70×10^9 viral copies/µL, however no virus could be isolated from nasal washes.[231] Of the experiments assessing infection in

contact animals serving as sentinels, one of the two sentinel voles for the H7N1 virus was positive via RRT-PCR in the nasal wash and lung tissue samples, with virus successfully isolated from the lung tissue sample. One of the two sentinel voles for the H5N1 virus experiments was positive via RRT-PCR in the nasal wash and had successful virus isolation.[231]

9.1.4.1.5 Experimentally determined transmissibility of AI viruses by rodents

- In VanDalen et al.'s (2019) study using brown rats (*Rattus norvegicus*), virus replication observed in fecal, oral, and nasal swabs was classified as minimal across all viruses used in the study. Of note, the two wild-bird origin viruses demonstrated the highest viral RNA replication.[212]
- In Achenbach and Bowen's (2011) study involving Sprague Dawley rats (*Rattus norvegicus domestica*), no viral shedding via oropharynx route from rats intranasally inoculated with either LPAI virus used (H5N2 or H7N3) was observed despite evidence of seroconversion.[230]
- In Romero Tejeda et al.'s (2015) study using voles (*Myodes glareolus*), one of the two sentinel voles for the H5N1 virus experiments was positive via RRT-PCR in the nasal wash and had successful virus isolation demonstrating transmission between rodents.[231]

9.1.4.1.6 Survivability of AI viruses on rodents

- AI virus survivability in fur has been suggested based on the ability of the influenza A viruses to survive in host feathers which has been proven in previous studies.[211] One study demonstrated that HPAI virus H5N1 can survive on—and spread via contact with—feathers for 15 to 160 days at 4 °C to 20 °C.[232]
- However, in Shriner et al., (2016)'s study, 185 house mice (*Mus musculus*) were collected from infected poultry premises during the 2015 HPAI H5N2 outbreak in Iowa. Between 24 and 26 mice were externally swabbed, with all external swabs tested via RT-qPCR, all of which were negative.[209]
- Swabs of rodent toes (spp. not specified) were taken for virus isolation from farms in a quarantine zone in Pennsylvania during the 1983–1984 HPAI H5N2 outbreak. No virus was isolated from any of the samples.[216]
- In a wildlife surveillance study[221] looking at small mammals that are on poultry premises and in wetland environments, researchers sampled deer mouse (*Peromyscus maniculatus*; n=3 and n=109, respectively), house mice (*Mus musculus*; n=19 and n=1, respectively), black rats (*Rattus rattus*; n=4 and n=0, respectively), Northern short-tailed shrew (*Blarina brevicauda*; n=5 and n=6, respectively), and meadow voles (*Microtus pennsylvanicus*; n=2 and n=2, respectively). Three sample types were taken per individual animal including external swabs on feet and fur, oropharyngeal and cloacal/anal swabs, and blood samples. All samples from individual rodents sampled were negative.[221]
- In a study by Cummings et al. (2019) looking at urban rat (*Rattus norvegicus*) populations, investigators found that of the 161 rats sampled via swabbing of the paw pads, 9 were positive when tested via RT-PCR.[222]

- One review study[233] of influenza A viruses in pigeons (spp. not specified) concluded that influenza A viruses can readily survive on feet and plumage and allow the birds to act as mechanical vectors. Such findings may translate to the feet and fur of rodents.

9.1.4.1.7 Rodent dispersal

- Houston et al.'s (2017) wildlife surveillance study sampling small mammals for influenza A viruses found that house mice (*Mus musculus*), deer mice (*Peromyscus maniculatus*), Northern short-tailed shrews (*Blarina brevicauda*), and meadow voles (*Microtus pennsylvanicus*) habituated in both wetland environments and poultry premises.[221]
- Reperant et al.'s (2009) review acknowledges that rodents are often likely to scavenge and prey on infected poultry and wild birds, creating an influenza A virus exposure opportunity for rodents.[234]
- In an Argentinian field survey [235] assessing rodent populations in poultry sheds (broiler breeders), interphase areas between farms and perimeters, and perimeter areas, investigators concluded that house mice (*Mus musculus*) are more likely to be found in poultry sheds compared to surrounding environments, with trap success (TS) ([number of captures/number of trap nights]*100) for n = 16 farms showing poultry sheds as 3.3 TS, perimeters 0.6 TS, and interphase 2.5 TS. Additionally, populations were observed as steady across seasons suggesting colonization and no natural migration between poultry farms, however, the possibility of accidental human-facilitated movement of house mice between farms was acknowledged as plausible.[235]
- Another study[236] assessing the distribution of house mice (*Mus musculus*) in relation to poultry farms and other natural environments (specifically including human houses, crop fields, pastures, crop field and pasture borders, riparian habitats, railway embankments, and woodlots) found that house mice were significantly more likely to be found on poultry farms than any other environment. Investigators of the study conclude that house mice populations are restricted to poultry houses without sustained populations in surrounding natural areas, and when poultry houses are emptied of poultry, the house mice populations dissipate.[236] However, it should be acknowledged that depending on climate and dynamics with other native species of other rodents this restriction of house mice populations to farms may vary.
- TN H7N9 2017 USDA investigation report: "A relatively high rodent burden was noted on the infected farm. Of interest, the barns farthest from the infected barn had the highest densities of mice, many of which were observed primarily utilizing exterior walls of the barns for cover."[54]

9.1.4.2 Qualitative Analysis

We considered the following factors in evaluating this pathway:

- The primary species of rodents that were collected and sampled in the field during or shortly after HPAI (and LPAI) outbreaks were rats (*Rattus norvegicus*) and mice (namely *Mus musculus* and *Peromyscus spp.*).
- There were no instances of virus being isolated or detected in any field samples taken from rodents, however, there were a few instances of antibody activity in both mice and rats. These data suggest that the possibility of infected rodents can have exposure to influenza A viruses but are unlikely to play a large role in transmission in an outbreak.

However, sampling was not always done during the active outbreak or immediately after depopulation, so it is difficult to determine the extent of infection that occurred in rodent populations at the time, given that some may have occurred based on antibody activity.

  - Additionally, the efficacy of HI tests that may be originally intended for use in avian species is hindered when used on mammalian species such as mice (*Mus musculus*) and rats (*Rattus norvegicus*).[237]

- In instances where rodents were assessed for influenza A virus prevalence outside of outbreak scenarios, natural rat (*Rattus spp.*) populations were examined. Outside of outbreaks (in urban areas), field studies suggest that there is some activity of circulating influenza A viruses. More investigation is required to determine the implications of such findings needed to understand the exact origins of circulating viruses.
- Based on studies utilizing surveys and epidemiological questionnaires, there was some evidence that rodent control was related to reduced risk of exposure, but also evidence that found rodent control did not matter substantially. Given the marginal differences between exposure on farms with and without various degrees of rodent control, the risk of exposure via rodent based on control methods (or lack thereof) is not of great importance regarding AI infection prevention.
- Both rats (*Rattus norvegicus*) and mice (*Mus musculus*) (see the literature review above for exact species) used in the experimental studies exhibited infection upon inoculation with varying strains of virus. Consistent clinical signs such as weight loss and depression were observed across studies. Replication of HPAI virus was often observed in tissues including lung, intestine, and brain. It is important to note that in some studies, the mice and rat species and breeds were not those that would be found in the wild, but those that are specifically bred for utilization in experiment, thus results from these studies may not be directly applicable to those rodent species found in the wild. Additionally, the experimental study assessing voles (*Myodes glareolus*) illustrated influenza A viruses can infect minor species of rodents.
- While rats (*Rattus norvegicus*) and mice (*Mus musculus*) (see the literature review above for exact species) demonstrated ability to shed virus via oral or fecal routes based on nasal, oropharyngeal, or rectal swabs, experiments where rodents demonstrated the capacity to infect other animals were limited. In Romero Tejeda et al.'s (2015) study, the voles (*Myodes glareolus*) demonstrated the ability to infect contact voles (*Myodes glareolus*), however, sample size was limited.
- Viruses demonstrate the ability to survive on fur, feathers, and skin based on field studies. However, in most studies where rodents were sampled in the field in areas that were within proximity to poultry outbreaks temporally and spatially, very few of the external swabs came back positive. An instance where influenza A virus was found via external swab on a rat found was in urban or rural-urban interface areas of Egypt. More evidence is needed to determine the actual role of rodent as fomites.
- Rats (see the literature review above for exact species) have been shown to persist in rural environments, however, while house mice (*Mus musculus*) have been shown to heavily congregate within or near poultry barns, they have been shown to vacate a premises after poultry are removed and human activity ceases. It is unlikely rats or mice

(see the literature review above for exact species) move between farms unless otherwise transported via human activity.

  - Such likelihood of dispersal from farms should be considered in conjunction with the fact that premises within the scope of this risk assessment are outside of a control area, meaning they are at least 10 km away from an infected farm. Meaning the likelihood of rodents traveling from a known-to-be-infected farm to an upland gamebird farm is incredibly small.

- Finally, the upland gamebird premises in the scope of this assessment have an outdoor production system and thus rodent populations can never be fully eliminated from the premises or pens.

9.1.4.3 Likelihood Rating and Conclusion

While rodents have proven unlikely to play an important role in the transmission of HPAI virus in poultry outbreaks, uncertainty remains as to their potential as vectors (particularly mechanical vectors), and because upland gamebird are housed in pens, the presence of rodents cannot be fully eliminated. However, given that the premises within the scope of this assessment are at least 10 km away from the nearest farm, the likelihood of an infected or contaminated rodent traveling from an infected farm to a susceptible upland gamebird farm is unlikely. Additionally, because upland gamebird premises have limited sharing of vehicles and resources with other farms of any kind, it is unlikely human activity would move infected or contaminated rodents onto an upland gamebird farm. Thus, the likelihood of HPAI infection via rodents in the farm vicinity is ***very low.***

9.1.5 Role of Predatory Mammals in the Transmission of HPAI Virus

Except for some bobwhite quail operations, the majority of commercial upland gamebirds are raised in outdoor pens, a set-up which leads to attention from both avian and mammalian predators (personal communication, Secure Upland Gamebird Supply Work Group, August 2019). While the ability for each type of predator to access pens varies depending on the species of the predator and on the construction and upkeep of pens, most upland gamebird producers inevitably deal with predators encountering their birds. Mammalian species that have been reported to pose predatory risk to upland gamebirds in pens include mink (e.g., *Neogale vison*), foxes (e.g., *Vulpes vulpes*), coyotes (*Canis latrans*), raccoons (*Procyon lotor*), domestic cats (*Felis catus*) and domestic dogs (*Canis lupus familiaris*), with variation existing on a regional basis (personal communication, Secure Upland Gamebird Supply Work Group, August 2019). In the case of avian predators, for a complete summary of the influenza A virus transmission risk that avian predators may pose to upland gamebird flocks see **Section 9.1.7** Role of HPAI Virus Spread to an Upland Gamebird Flock via Wild Non-Aquatic Birds in Farm Vicinity.

Pathways for virus transmission from mammalian predators to upland gamebird flocks include the direct contact between upland gamebird flocks and predators through the netting or inside the pen if predators slip through or around barriers. Predators can also contaminate personnel during situations where personnel are attempting to control on-farm predator presence. Contaminated personnel can subsequently track virus into pens.

Soundly constructed fencing and netting around flocks is the primary method to prevent contact between upland gamebirds and mammalian predators, however, persistent predators are dealt with in a variety of ways such as the use of scare tactics, employment of traps, or elimination and removal of the predators using lethal means, when appropriate licensure is in place. Producers

and employees that handle the predators they trap or kill on the premises have the potential to bring virus into pens.

Below is a summary of the literature from previous outbreaks implicating the role of predatory mammals in the spread of HPAI, experimentally determined susceptibility of predatory mammals to influenza A viruses, experimentally determined transmission of influenza A viruses from predatory mammals, survivability of influenza A viruses on mammals (i.e., capacity for mechanical transmission), and range dispersion likelihood of predatory mammals. **Figure 6** demonstrates the potential on-farm influenza A virus transmission pathways that exist due to predatory mammals.

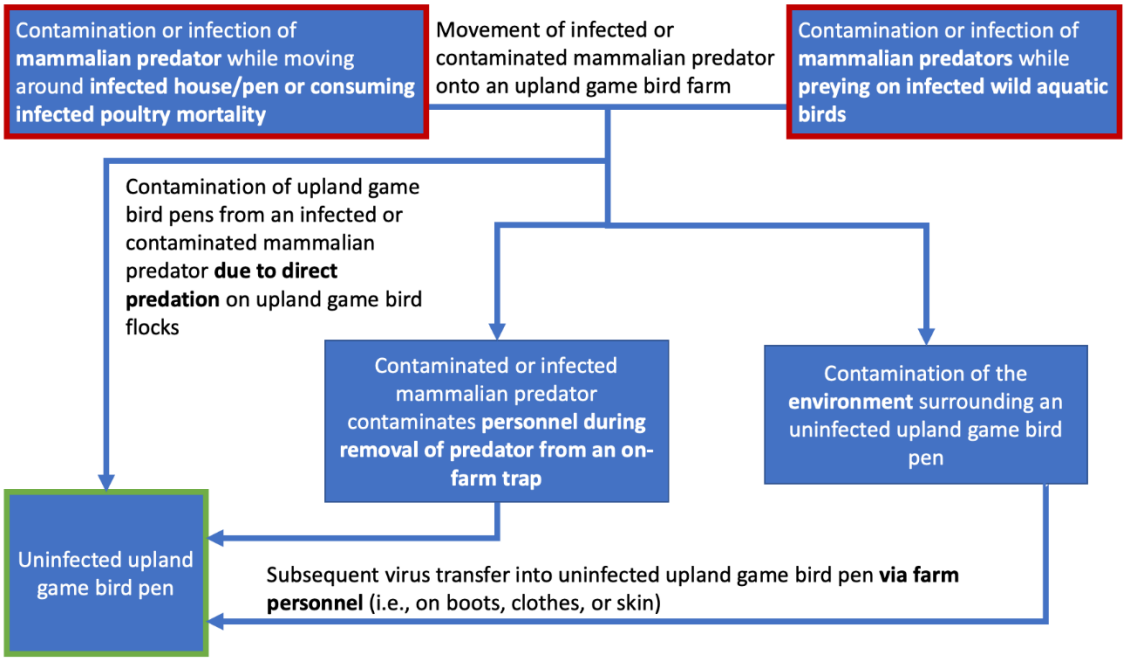


Figure 6. Pathway of HPAI virus transmission through predatory mammals.

9.1.5.1 Literature Review

9.1.5.1.1 Prevalence of AI in mammalian predators in previous outbreaks of AI involving poultry

While not often assessed during outbreaks of poultry, medium-sized mammal activity in relation to LPAI and HPAI outbreaks has been documented.

- In the 2002 LPAI H7N2 outbreak in Virginia, a multivariate analysis determined that the presence of foxes (e.g., *Vulpes Vulpes*), raccoons (*Procyon lotor*), and opossums (*Didelphis virginiana*) was an approximately two-fold increase in risk of infection.[109]
- Organ samples from three cats (*Felis catus*) in South Korea were positive via RT-PCR for A/feline/Korea/H646-1/2016(H5N6) and A/feline/Korea/H646-2/2016(H5N6), which were genetically similar to the HPAI H5N6 circulating in poultry at the time.[238] Lee et al. (2018) hypothesize the cats became infected by feeding on wild birds, however they also note that H5N6 affected poultry premises were located 1 km away from the households where the cats lived.[238] However, there was no evidence of the cats spread the disease to other farms.

- Songserm et al. (2006) describe a fatal HPAI H5N1 infection in a dog (*Canis lupus familiaris*) following ingestion of infected duck (Family, *Anatidae*; spp. not specified) carcasses during an outbreak in Thailand in 2004.[100] However, there was no evidence that dogs were involved in spreading AI.
- The case-control study by Shriner et al. (2016) assessing the role of synanthropic mammals on farms that had been infected and uninfected in Iowa during the 2014–2015 HPAI H5N2 outbreak sampled three raccoons (*Procyon lotor*) via rRT-PCR swabs and blood samples for serology from an infected farm. No animals were available for testing from infected farms. All three animals were negative on both rRT-PCR and antibody tests.[209]

9.1.5.1.2 Prevalence of AI in mammalian predators outside of active HPAI outbreaks in poultry

- In 2016, an outbreak of LPAI H7N7 occurred in cats (*Felis catus*) in a New York animal shelter with widespread transmission among cats, however, no transmission to dogs (*Canis lupus familiaris*) housed in the same facility was observed.[239]
- Field data from raccoons (*Procyon lotor*) sampled from numerous wild populations in the States of California, Texas, Louisiana, Maryland, Wyoming, and Colorado demonstrated that 17 of the 730 raccoons sampled were positive for AI antibodies. AI antibodies found were for influenza A virus subtypes including H1, H3, H4, and H10.[62]
- Results from 1,088 serology sampled wild raccoons (*Procyon lotor*) revealed ten individual animals that tested positive for having antibodies for H5N1.[240]
- Yamaguchi et al. (2014) found similar results with 12 of 634 samples being positive for various influenza A viruses via serology tests over a three-year period including two raccoons (*Procyon lotor*) with antibodies for influenza A virus H5N1. Raccoons from different regions have anti-bodies for different virus subtypes. Additionally, of the 131 nasal swabs and 129 rectal swabs taken from the racoon populations, none came back positive for virus isolation.[241]
- Bakken et al. (2020) found 2 of 139 samples taken from predatory mammals (including red fox (*Vulpes vulpes*), racoon (*Procyon lotor*), and coyote (*Canis latrans*) positive for either H1N1 human pandemic virus or the 2007 human seasonal H1N1 virus. The positive samples came from one raccoon and one coyote.[242]

9.1.5.1.3 Experimentally determined susceptibility of predatory mammals to AI viruses

- Following experimental gastrointestinal HPAI H5N1 infection, cats (*Felis catus*) became systemically infected and viral shedding was detected (via RT-PCR) in pharyngeal and rectal swabs. Pharyngeal shedding occurred in both cats with gastrointestinal exposure, beginning 2 dpi. Rectal shedding was observed in only one of the cats, and only 2 dpi.[243]
- Spread between cats (*Felis catus*) was demonstrated in an experimental setting based on Hatta et al.'s (2018) results where cats inoculated with 10^6 PFU of viruses in 0.5 mL of phosphate-buffered saline demonstrated spread of the feline H7N2 subtype to other exposed cats via direct contact (3/3) and respiratory droplets (2/3).[244]
- Ferrets (*Mustela furo*) and foxes (*Vulpes vulpes*) fed HPAI H5N1-infected chicken meat developed respiratory and/or digestive infections, demonstrating mammalian potential to shed HPAI virus after consuming HPAI virus-tainted meat.[245,246]

- Lipatov et al. (2009) measured presence of viral antigen in ferret (*Mustela furo*) tissue, not actual viral shedding.[245]
- Reperant et al. (2008), however, demonstrated pharyngeal shedding in red foxes (*Vulpes vulpes*) for 3 to 7 days, peaking at $10^{3.5}$ to $10^{5.2}$ $TCID_{50}$/mL following intratracheal inoculation. Pharyngeal shedding peaked at $10^{4.2}$ to $10^{4.5}$ $TCID_{50}$/mL and lasted for 3 to 5 days after feeding infected carcasses. Rectal shedding was detected in one of three foxes inoculated intratracheally at approximately 10^{2} $TCID_{50}$/mL, only at 2 dpi and in one of three foxes fed infected meat, at approximately 10^{1} $TCID_{50}$/mL, on 1 dpi only. All foxes were euthanized at 7 dpi, and virus isolation was negative from all organs sampled from foxes fed infected carcasses.[246]

- In another experiment by Lyoo, et al. (2017) dogs (*Canis lupus familiaris*) in each treatment group were intranasally inoculated with $10^{6.0}$ EID_{50} in 2-mL sterile PBS of each of the following HPAI viruses per dog based on the treatment group: H5N1virus A/chicken/VN/ KienGiang/P140082/201, H5N1virus A/duck/VN/QuangTri/P140164/2014, and H5N6 virus /chicken/VN/LangSon/P140450/2014. Two out of three (2/3) inoculated dogs and zero out of three (0/3) contact dogs exhibited seroconversion for the H5N1 chicken virus, one out of three (1/3) inoculated dogs, and zero out of three (0/3) contact dogs exhibited seroconversion for the H5N1 duck virus, and two out of three (2/3) inoculated dogs and zero out of three (0/3) contact dogs exhibited seroconversion for the H5N6 virus.[247]
- Both striped skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*) have been shown to shed LPAI H4N8 and H4N6, respectively, following experimental nasal inoculation with those strains. For most of the skunks, nasal shedding of H4N8 peaked at 8 dpi at an average $10^{5.65}$ PCR EID_{50} [4] equivalents/mL, and oral shedding at 7 dpi at an average $10^{4.82}$ PCR EID_{50} equivalents/mL. Nasal shedding of H4N6 in the raccoons (*Procyon lotor*) varied from 1 to 6 days of shedding and between $10^{0.02}$ and $10^{1.1}$ EID_{50} equivalents/mL. Both species (plus cottontail rabbits [*Sylvilagus spp.*]) also have been shown to shed novel avian-origin H7N9 (A/Anhui/1/2013) influenza virus at more than 10^{5} PFU/mL nasal flush.[248]
- When experimentally fed carcasses of LPAI H4N6-inoculated mallards (*Anas platyrhynchos*) or H4N6-spiked and coated chicken eggs, raccoons (*Procyon lotor*) failed to subsequently shed AI virus RNA. While this study does not support predatory mammals as a source of LPAI biological transmission, the authors propose that HPAI virus may be more likely to be shed by predatory mammals because of its ability to cause more disseminated infection.[249]

9.1.5.1.4 Experimentally determined transmissibility of AI viruses by predatory mammals

- In a study assessing mammalian transmission, experimentally infected striped skunks (*Mephitis mephitis*) successfully transmitted LPAI H4N6 to mallard ducks (*Anas platyrhynchos*) through contact with shared resources (i.e., through contaminating the environment).[250]

[4]PCR EID_{50} equivalent is a measure based on comparing the viral load in the experimental samples with the viral load in samples with known virus titers, as measured by rRT-PCR

- In an experiment by Yuk et al. (2017), one of four (1/4) dogs (*Canis lupus familiaris*) became infected with A/baikal teal/Korea/K14-E016/2014 after contact exposure to dogs that were intranasally inoculated with 10^7 EID_{50} of HPAI H5N8 virus.[251]

9.1.5.1.5 Survivability of AI viruses on mammals (e.g., fur and foot pads)

- A summary of studies demonstrating the survivability of influenza A viruses on fur, feet, and toes of rodents is described in **Section 9.1.4** Role of Rodents in the Transmission of HPAI Virus. Such findings translate directly to the potential of mechanical transmission of influenza A virus by other mammalian species.

9.1.5.1.6 Predatory mammal range dispersion

Home territory ranges of predatory mammals are important to consider for determining how far such animals might carry disease between farms.

- Red foxes (*Vulpes vulpes*) generally have a home range of up to 8 km, being largest in the winter.[252]
- Raccoons (*Procyon lotor)* generally have a home range of 1.8 to 3 km.[253]
- Opossums (*Didelphis virginianis*) generally have a home range of 1.3 and 2 km.[254]
- Striped skunks (*Mephitis mephitis*) generally have a home range 2.2 to 2.5 km in diameter.[255]
- Coyotes (*Canis latrans*) have variable home ranges depending on the geographic region as well as an individual's sociality. **Table 6** below summarizes average home ranges of coyotes in rural-natural settings.

  Table 6. Estimates of home ranges of coyotes (*Canis latrans*) in the United States

State	Home range size (km^2) and diameter (km) estimate of resident coyotes	Home range size (km^2) and diameter (km) estimate of transient coyotes	Study
South Texas	Mean home range size of 4.3 and 4.7 km^2 depending on sex of coyotes.	Mean home range size of 31.2 to 42.8 km^2 depending on sex of coyotes.	[256]
Washington	Home range size varied between 1.5 and 313.3 km^2 of with a mean 92.4 km^2		[257]
Georgia & South Carolina	Home ranges sizes varied between 5.4 km^2 to 39.2 km^2, with a mean home range size of 13.5 km^2		[258]
Minnesota	Mean home range size of 10.1 and 41.9 km^2 depending on sex of coyotes.		[259]
Arkansas	Mean home range size of 33.2 and 13.2 km^2 depending on sex of coyotes.		[260]

9.1.5.2 Qualitative Analysis

We considered the following factors in evaluating this pathway:

- Many of the same species of mammals that act as predators for penned upland gamebirds (personal communication, Secure Upland Gamebird Supply Work Group, August 2019) have also been reported to visit compost piles of poultry farms,[261] which is an important

consideration when thinking about the risk they bring to a susceptible upland gamebird farm.

- For an assessment on how predators can become contaminated or infected via compost piles see **Section 9.2.4** Role of Role of HPAI Virus Spread to an Upland Gamebird Flock via Dead Bird Disposal.
- Additionally, studies demonstrate that there is a general trend that predators in ecosystems that naturally eat birds are more likely to display higher prevalence for influenza A viruses.[262] Thus, predators of upland gamebirds might be more apt to have influenza viruses.

- Based on previously published literature, there is little assessment of the role that mammalian predators play in poultry AI outbreaks. The limited work includes a few studies demonstrating AI infection in domestic mammalian species (such as dogs and cats) that pose a predatory and/or scavenger risk for consuming infected poultry carcasses. Only one field study assessed the presence of predators contributing to an increased risk of contamination. The authors' analysis did yield an almost two-fold increase in risk of infection for farms that noted the presence of certain species of predatory mammals.[109]
- Because there is possible direct contact between predators and penned upland gamebird flocks, transmissibility between predatory mammalian species and avian species is important.
  - The susceptibility of mammalian predators has been shown in field and experimental settings, with evidence pointing to some susceptibility to numerous LPAI viruses and HPAI viruses including H5N1 (see Literature Review above for exact data from studies).
    - However, the evidence from field studies is built primarily on serologic sampling results. Thus, just as Root (2020) suggests, while mammalian predators (particularly raccoons [*Procyon lotor*]) appear to have been exposed to various influenza A viruses, the mechanisms of exposure to influenza A viruses remain largely undetermined.[263]
    - Experimental studies indicate susceptibility of some predatory mammalian species primarily through the route of the ingestion of infected carcasses or through consumption of contaminated water (see Literature Review above for exact data from studies). Such findings implicate that if predatory mammalian species are scavenging infected mortality piles, they could become infected.
  - The ability for infected mammalian predators to shed virus that could be transmitted to susceptible upland gamebirds (i.e., the amount of virus shed, the route and the duration of shedding) varies depending on the species based on the limited available literature.
    - In the studies in which rectal shedding following consumption of HPAI-infected meat was studied, it was short-lived and occurred inconsistently.[243,246]
    - Additionally, HPAI H5N1 strains that replicate mostly in the lower respiratory tract may not be readily excreted via the upper respiratory system of mammals.[234]

The role of other excretory systems, such as the gastrointestinal and urinary tracts, as portals of viral exit is unknown at this time.

- Upon entry of a pen, an actively influenza A virus-shedding predatory mammal can directly contaminate the environment leading to subsequent infection of birds within the pen.
  - One experimental study[250] demonstrated the ability of shedding, experimentally infected striped skunks (*Mephitis mephitis*) transmitting LPAI through contaminating a shared environment.
- While active shedding from an infected predator is of concern, the capacity of a contaminated, rather than infected, predatory mammals coming into direct contact with upland gamebirds is also of critical consideration.
  - Literature as outlined in **Section 9.1.4** Role of Rodents in the Transmission of HPAI Virus and **Appendix 2**: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates, suggest there is substantial potential for influenza A viruses to survive on fur, skin, and footpads of mammals.
- The pathway of indirect transmission from predatory mammal to upland gamebird through personnel acting as a fomite is also of important consideration. This pathway can involve varying steps, depending upon when the mammal was actively shedding the virus or acting as a mechanical vector.
  - If the predatory mammal becomes infected with and subsequently sheds HPAI virus on the grounds outside the uninfected upland gamebird pen, there are only two contact steps: from the contaminated grounds to personnel boots, and from the boots to the ground within the pen. The transfer of virus would largely depend on how much virus the mammal shed (details reported in the above text).
  - If, however, the predatory mammal was acting as a mechanical vector the indirect pathway of: *Infected undetected carcass→scavenger→ground area on uninfected premises→farm personnel boots→upland gamebird pen* involves four contact steps. In general, the chances of the pathway resulting in virus transmission decreases with the number of contact steps that need to occur. Furthermore, even if the transfer steps occur, the virus concentration transferred to the final step would likely be low. This is because only a fraction of the virus (6 to 27 percent) on a donor surface is transferred to the recipient surface in each direct contact.[123] The ground traveled by the scavenger between the carcass and the uninfected upland gamebird premises would further lessen the amount of virus present on the scavenger for transmission once at the premises.
    - If a predatory mammal were contaminated by an infected carcass, we would expect virus may be transferred via feces, bodily fluids, or feathers of that carcass. One gram of organic matter from a poultry carcass may contain 10^6 EID_{50}/g.[264]
      - Additionally, the level of contamination can depend upon the source of contamination, such as a mammalian predator ingesting an infected/contaminated wild or domestic bird from a mortality storage site. Again, the impact of the composting process of the infectiousness of carcass

material is depicted in **Section 9.2.4** Role of HPAI Virus Spread to an Upland Gamebird Flock via Dead Bird Disposal.

- For perspective, using a mid-range viral transfer concentration, if 15 percent of virus is transferred at each contact step described above, enough virus particles remain after four steps to infect five birds (assuming an infectious dose of 10^2 EID_{50}) if only a single gram of feathers, fluid, or feces is present at the first step of the pathway.

- Other plausible pathways where fewer contact steps are involved include those where the grower or other poultry farm personnel directly contacts an infected or contaminated scavenger species:
  - An infected or contaminated predatory mammal is trapped and/or killed on an uninfected farm. The grower or employee disposes of the predator and then enters an upland gamebird pen, introducing virus to the flock.
  - A domesticated mammalian predator (e.g., dog or cat) is infected or contaminated on an infected neighboring farm. The grower or employee touches the pet and then enters an upland gamebird pen, introducing virus into the flock.
- The enhanced biosecurity required during the PMIP applies only to farms participating in the Secure Poultry Supply Plans, being either located in a Control Area (in the case of broiler, turkey, and layer premises) or in States with an active outbreak (in the case of upland gamebird premises) that wish to move birds off the premises. While it is assumed that biosecurity practices may be elevated in an outbreak situation, it is assumed that there may be marked variation in the practices on farms within or outside the Control Area that are not currently adhering to a PMIP.
- Finally, the distance between farms (i.e., the distance a predatory mammal must travel between encountering an infected carcass and an uninfected upland gamebird farm), including upland gamebird farms and poultry farms, also impacts the likelihood of HPAI transmission via the contaminated and/or infected mammal. The infected carcass and the uninfected farm must be within the likely range of the predatory mammal for transmission to potentially occur. Based on knowledge of mammalian predator ranges, the likelihood of this scenario is not likely for some mammalian predator species and plausible for other mammalian species (e.g., coyotes [*Canis latrans*]) given that upland gamebird farms within the scope of this risk assessment will be at least 10 km from a known-to-be-infected farm and upland gamebird farms have been reported to be on average 15.48 km away from other commercial premises with poultry or upland gamebirds.[14]

9.1.5.3 Likelihood Rating and Conclusion

While predatory mammals have very little documented evidence to support that they play a significant role in the transmission of HPAI virus in poultry outbreaks (including outbreaks that involved outdoor penned or free-range farms) uncertainty remains as to their potential as vectors (particularly mechanical vectors). Because upland gamebirds are housed in pens, contact with predatory mammals is possible and the risk cannot be eliminated even with mitigation measures. However, given that the premises within the scope of this assessment are at least 10 km away from the nearest known-to-be infected farm in conjunction with reported home ranges of predatory mammalian species, the likelihood of an infected or contaminated predatory mammal

traveling from and infected farm to a susceptible upland gamebird farm is unlikely. Thus, the likelihood of HPAI infection via rodents in the farm vicinity is ***low.***

9.1.6 Role of HPAI Virus Spread to an Upland Gamebird Flock via Wild Aquatic Birds in the Farm Vicinity

Wild aquatic birds (defined for these purposes as waterfowl (e.g., order *Anseriformes*), shorebirds and gulls (e.g., order *Charadriiformes*), and other wading birds found in wetlands such as egrets and herons (e.g., order *Pelecaniformes*, specifically, family Aredeidae) are the observed reservoir of influenza A viruses in nature. Wild aquatic birds have demonstrated to harbor all 16 (H1–H16) HA and all 9 (N1–N9) NA subtypes of AI across their population.[265] Most of the isolates from aquatic birds have been LPAI virus, which generally do not cause disease in the wild population. One of the exceptions is the 2016 HPAI H5N8, clade 2.3.4.4 group B (H5N8B) that caused a series of outbreaks in Europe, causing high mortality in waterfowl and domestic birds,[266–269] the H5N6 HPAI virus that led to an exponential increase in daily mortality in a duck barn in the Netherlands in 2017.[41,269] It is understood that the virus circulates continuously in the wild population, but often at low levels.[270]

To understand the ecology of influenza A virus in wild waterfowl, Nolting et al. (2019) examined Ohio's nearly year-round sampling data spanning 2008 to 2016 and involving 3,645 cloacal samples from mallard ducks (*Anas platyrhynchos*). They found that both viral recovery and subtype diversity varied between seasons and varied by age of the duck. They report that in August the frequency of viral recovery is 29.8 percent, with isolates representing at least 47 HA/NA combinations while in November, influenza A virus isolation drops to 6.2 percent, with only 25 HA/NA combinations.[271]

Various orders, families, and species of wild aquatic birds are implicated in the maintenance of AI viruses:

- Wild waterfowl are considered to be the primary source of new H5 or H7 LPAI outbreaks in poultry, particularly in poultry raised in semi-intensive or extensive (free-range) conditions.[272] Wild ducks have been found to carry a higher prevalence of virus during their southern migration in the fall (22.2 percent) than during their spring northerly migration (0.3 percent). This difference may be due to the increased number of susceptible young birds during the fall migration.[270]
- Anecdotally, during the 2014–2015 HPAI outbreak in the Midwest, snow geese were observed in the proximity of poultry houses that later became infected with H5N2 HPAI.
- A higher percentage of shorebirds have also been found to carry influenza A viruses than ducks during the spring migration.[270]
- Gulls (*Larinae*) are susceptible to HPAI viruses[273] and are a known reservoir of influenza A viruses.[274,275] Gulls are suspected to have been the source of a 2002 outbreak in the Chilean poultry industry. In this instance, the HPAI virus likely arose from an LPAI strain through mutation.[276] The role of gulls in the transmission of AI is likely twofold because of their susceptibility to infection and their opportunistic nature when they scavenge for food. Gulls are susceptible to AI and thus can contract but transmission from gulls to other species is less clear.[273] Because they are opportunists, gulls (*Larinae*) are likely to be present near poultry barns and may come into contact with dead birds. In this case, gulls may act as fomites in the dispersal of influenza A viruses onto upland

gamebird farms (more in-depth analysis of the role of scavengers can be found in the "Dead bird disposal" chapter of this Risk Assessment). However, gulls (*Larinae*) are regarded fairly uncommon on U.S. upland gamebird farms (personal communication, Secure Upland Gamebird Supply Working Group, August 2019), most likely due to complete overhead netting and the absence of attractants such as spilled feed or warmth from barn roofs that may be present on commercial poultry farms (personal communication, David A. Halvorson, July 2019).

- Cattle egrets (*Bubulcus ibis*) have been implicated as being able to carry IAVs, with observed instances of IAV viral RNA detected in cattle egrets (*Bubulcus ibis*) by rRT-PCR as well as observed instances of virus isolation.[265] Similarly, white ibises (*Eudocimus albus*) have been demonstrated to be competent reservoirs for IAVs, with 70.4 percent serum samples (n=574) from white ibises (*Eudocimus albus*) in south Florida positive by bELISA.[277]

Influenza A viruses have been shown to affect all types of domestic birds, and apart from susceptibility of the poultry species of concern, the primary infection partly depends on the degree of contact with wild birds, the ability of that species to shed and transmit the virus, and the pathogenicity of the virus, itself. As mentioned in **Section 9.1.1**., Role of Local Spread Components in Previous AI Outbreaks, secondary spread usually results from human activities that transfer infective feces to susceptible birds.[278] Potential pathways of HPAI virus transmission through wild aquatic birds in the farm vicinity are illustrated below in **Figure 7.**

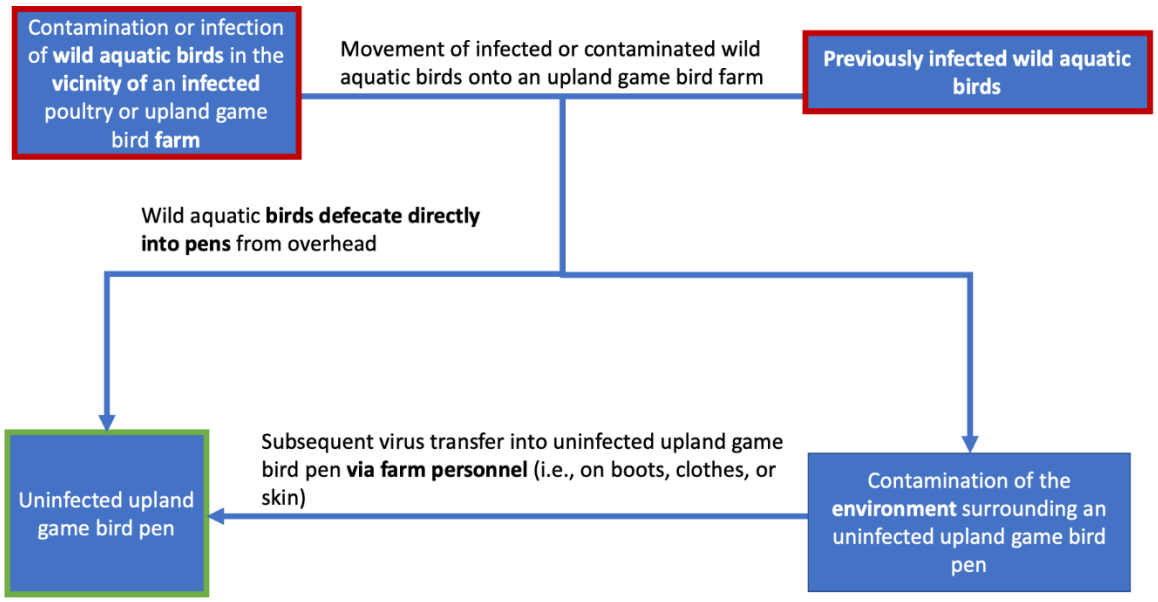


Figure 7. Pathway of HPAI virus transmission through wild aquatic birds

9.1.6.1 Literature Review

Historically, HPAI viruses rarely have been isolated from wild birds. Where HPAI viruses were identified, they were usually from isolates obtained from dead wild birds found in the vicinity of HPAI-infected poultry farms.[270,279] In Minnesota wild bird surveillance efforts involving monitoring of wild bird morbidity and mortality during the 2015 outbreak, personnel sampled 104 birds and found the only positive mortality in counties with no positive poultry premises.[280]

Studies have shown that HPAI viruses are present in populations of different wild aquatic bird species covering wide geographical areas globally.

- In a survey conducted in China from 2004 to 2007, 14,472 wild bird samples (cloacal swabs, organ tissues, or fresh excrement) were collected from 10 bird orders. The samples from Anseriformes had the highest prevalence of H5N1 virus. The positive samples were collected from nine species of ducks, geese, and swans.[281]
- HPAI outbreaks in migratory water birds from 2005 to 2011 in Mongolia, a country with very few domestic poultry (fewer than 100,000 birds), provided strong evidence that wild birds can carry HPAI virus over at least moderate distances, but may not be competent as indefinite reservoirs.[282]
- A large-scale surveillance program detected HPAI H5N2 in healthy birds of two wild waterfowl species sampled in Nigeria and genetically-related LPAI H5N2 in Eurasian domestic poultry.[36]
- HPAI H5N8 was identified in poultry in South Korea in January 2014, and closely related strains subsequently appeared in Japan, China, and Europe. Several reassortant H5 HPAI viruses recently isolated in North America show 99 percent similarity to the Korean H5 strains.[283,284]
- Wild bird sampling activities in the Netherlands between November 2014 and February 2015, following H5N8 virus outbreaks in poultry, detected HPAI H5N8 virus in two samples (out of 4,018 birds sampled) from ducks of the Eurasian wigeon species.[285]
- Between December 2014 and February 2015, Eurasian/North American reassortant HPAI H5N1, H5N2, and H5N8 were found in several species of wild ducks, as well as wild raptors, in the States of Washington, Oregon, California, Utah, Idaho, and Nevada. After February 2015, new H5N2 cases in wild aquatic birds and raptors were also detected in Minnesota, Wisconsin, Michigan, Wyoming, Kansas, and Kentucky (see **Table 7).**[46]

Table 7. H5N2 cases in U.S. aquatic birds, December 2014 to June 2015[46]

Bird Species	Number	State	Cause of death
Canada goose ***Branta canadensis***	5 1 1 1	Michigan Wyoming Kansas Washington	Morbidity/mortality
Lesser snow goose ***Anser caerulescens caerulescens***	1 2	Kentucky Montana	Morbidity/mortality
Ring-necked duck ***Aythya collaris***	1	Kentucky	Morbidity/mortality
American green-winged teal ***Anas crecca***	1 1	Idaho Oregon	Hunter harvest
Mallard ***Anas platyhrynchos***	2 5 3	Idaho Washington Oregon	Hunter harvest

Northern pintail ***Anas acuta***	2 1	Oregon Washington	Hunter harvest
Northern shoveler ***Anas clypeata***	3	Oregon	Hunter harvest
Wood duck ***Aix sponsa***	3	Oregon	Hunter harvest

- During the 2014–2015 H5N2 outbreak in the midwestern United States, sampling of wildlife took place on five infected and five uninfected farms. Out of 419 individual birds sampled, killdeers were the only species that may be aquatic collected, and none tested positive for HPAI. It should be noted, however, that the samples were collected 2 to 4 weeks after clinical signs of HPAI were observed in the poultry flocks, and while depopulation was complete at some infected farms, it was ongoing at others.[154]
- Froberg et al. (2019) performed a study in Minnesota in which 1346 ring-billed gulls were captured and sampled during 2016–2017. They did not detect HPAI virus in any of the samples and 301 oropharyngeal swabs were positive for AI viruses.[275]
- Among the wild bird species sampled for virus detection during the 2017–2018 outbreaks of HPAI H5 viruses in Europe, virus was detected in two Great Black-backed Gulls (*Larus marinus*), one Black-headed Gull (*Chroicocephalus ridibundus*) and ten Eurasian Wigeons (*Anas penelope*) in the Netherlands, and in a Mallard (*Anas platyrhynchos*, 28 January 2018), and an Armenian Gull (*Larus armenicus*) in the Republic of Georgia.[269]
- Interestingly, the role of wild aquatic birds in perpetuating HPAI viruses remains unresolved. AI researchers have examined current and historical aquatic bird influenza A virus surveillance and outbreaks of HPAI H5 viruses in poultry in the United States and Canada dating back 43 years prior to the 2014–2015 outbreak. This analysis failed to detect HPAI viruses in wild aquatic birds before or after the resolution of that outbreak, suggesting that there are yet undetermined mechanisms preventing wild aquatic birds from perpetuating HPAI viruses.[286]

Experimental studies suggest that while most aquatic bird species show minor or no clinical signs after being infected with HPAI viruses, some can efficiently transmit the viruses to their contacts. **Table 8** summarizes the results of several studies on HPAI virus in wild and domesticated aquatic birds.

Table 8. Summary of experimental studies of HPAI virus in wild and domesticated aquatic birds.

HPAI virus	Bird species	Inoculation	Findings	Reference
H5N8 (A/chicken/ Netherlands/emc-3/2014)	Common pochard, Mallard, Common teal, and Eurasian wigeon	3 mL containing 10^4 $TCID_{50}$, 1.5 mL into the trachea, and 1.5 mL into the esophagus of each bird	Excretion was highest in Eurasian wigeons Virus infection was subclinical in all four species Note, virus caused systemic disease and high mortality in chicken	287
H7N3 (A/chicken/Chile/184240-1/02)	Chiloé wigeon and cinnamon teal	10^6 EID_{50} (intranasal)	No ducks developed disease or died Oral and/or cloacal shedding in all virus-inoculated cinnamon teals and oral shedding in 2/8 Chiloé wigeons at day 2 post-inoculation Virus efficiently transmitted to cinnamon teal contacts, not to Chiloé wigeon contacts	288
H5N1 (A/chicken/Scotland/59) H5N2 (A/chicken/Pennsylvania/1370/83) H5N2 (A/chicken/Pennsylvania/1/83) H5N9 (A/turkey/Ontario/7732/66)	Khaki-Campbell duck	0.1 mL of diluted infectious allantoic fluid (intramuscular and intranasal routes, and contact with inoculated ducks)	No infection and no shedding established	106

Table 8. Summary of experimental studies of HPAI virus in wild and domesticated aquatic birds, cont.

H5N8 (A/turkey/Ireland/83) H5N8 (A/duck/Ireland/113/84)	Khaki-Campbell duck	0.1 mL of diluted infectious allantoic fluid (intramuscular and intranasal routes and contact with inoculated ducks)	Virus shedding in cloaca and trachea and transmission to in-contact ducks No clinical signs or deaths	106
H7N7 (A/Chicken/Netherlands/621557/03)	Ringed teal	0.2 mL of tenfold diluted allantoic fluid (intravenous)	All unvaccinated ringed teals became infected and rapidly transmitted to all contact teals Shedding through cloaca and trachea in all animals 2/10 developed conjunctivitis; no clinical signs in others	289
H5N2 (A/chicken/Pennsylvania/1/83)	Ring-billed gull	10^8 EID_{50} (intranasal/ intraocular)	Virus detected in the intestine, lung, and spleen No transmission to in-contact birds	80
H5N1 (A/Whooper Swan/ Mongolia/244/05) H5N1 (A/Duck Meat/ Anyang/01)	Mallard, Northern pintail, Blue-winged teal, Redhead, wood duck, and nestling Laughing gulls.	0.1 mL of diluted allantoic fluid from inoculated eggs diluted in brain-heart infusion (intranasal)	Wood ducks were the only species of duck to exhibit illness or death after inoculation with either of the HPAI viruses. Severe clinical signs appeared in all the inoculated gulls. In both species virus was isolated from internal organs. Viral titers were higher in oropharyngeal swabs than in cloacal swabs	273

Table 8. Summary of experimental studies of HPAI virus in wild and domesticated aquatic birds, cont.				
H5N8 (A/Gyrfalcon/Washington/41088/2014) H5N2 (A/Northern Pintail/Washington/40964/2014)	(1) White Chinese Goose (2) Pekin duck (3) Mallards	10^6 EID_{50}	Geese: few clinical signs, some mortality Pekin duck: no mortality Mallards: no mortality or clinical signs, but lower body weight and elevated body temperature	[47]

A study of several H5 and H7 HPAI virus strains in Mallard ducks further illustrates the variability in shedding and transmission to contacts, depending on the virus strain.[47] These findings are summarized in **Table 9**.

Table 9. Shedding and transmission results of experimental infection of Mallard ducks with H5 and H7 HPAI virus at10^6 EID_{50} intranasally.[47]

Virus Strain	Shedding (days)	OP vs. CL	Trans. to contacts	> Chicken BID_{50} log10
H7N3 A/chicken/Chile/184240-1/2002	14	CL	3/3	na
H7N3 A/chicken/Canada/314514-2/2005	14	CL	3/3	na
H7N3 A/chicken/Jalisco/CPA1/2012	14	CL	3/3	na
H7N7 A/chicken/Victoria/1985	11	CL	3/3	>2.9
H7N7 A/chicken/North Korea/7916/2005	11	CL	3/3	na
H7N7 A/chicken/Netherlands/1/2003	11	=	3/3	na
H7N1 A/turkey/Italy/4580/1999	11	=	3/3	>2
H5N2 A/chicken/Pennsylvania/1370/1983	14	=	3/3	>3
H5N2 A/chicken/Queretaro/14588/1995	4	OP	1/3	>3
H5N8 A/turkey/Ireland/1378/1983	11	OP	2/3	<4.7
H5N3 A/tern/South Africa/1961	14	=	1/3	>3.4

OP: primarily oropharyngeal shedding; CL: primarily cloacal shedding; =: equal OP and CL shedding.
BID_{50}: 50% bird infectious dose. One BID_{50} unit is the amount of virus that will infect 50% of inoculated birds.

The evidence that connects wild birds to infected farms is divergent. In a case-control study of layer and pullet premises in Iowa and Nebraska in the 2015 HPAI outbreak, no consistent association was observed between infected or control farm status and wild bird sightings.[290] In other cases, evidence has been found linking wild birds to infected premises.

- Observations of wild bird activity in two provinces in Canada showed seven species of wild aquatic birds—Canada goose (*Branta candensis*), Mallard (*Anas platyrhynchos*), Ring-billed gull (*Larus delawarensis*), Glaucous-winged gull (*Larus glaucescens)*, Mew gull (*Larus canus*), Killdeer (*Charadrius vociferus*), and Trumpeter swan (*Cyngnus buccinator*)—were seen in the immediate barn area at least twice. They were most frequently observed near feed silos. No wild aquatic birds were observed entering the poultry houses.[291]

Additional evidence for outbreaks resulting from possible introduction of HPAI virus into domestic birds from wild aquatic birds.

- A North American outbreak of HPAI with H5 of Eurasian lineage began on December 1, 2014, and H5N2 HPAI was detected in 11 commercial poultry farms, including seven broiler breeder, one table egg layer, and three turkey farms in British Columbia by December 17, 2014. In addition, the Canadian Food Inspection Agency confirmed HPAI H5N1 on a noncommercial poultry farm on February 7, 2015.[292] Influenza viruses had been previously isolated from wild and domestic ducks in British Columbia.[293]

- Eurasian H5N8 was confirmed in a backyard mixed poultry flock in Oregon on December 19, 2014, followed by Eurasian/North American reassortant H5N2 outbreaks in backyard flocks in Washington, Oregon, and Idaho in January and February 2015.[294,295]
- Various positive aquatic birds were found during the outbreak, as shown in **Table 10**, cementing the possibility of introduction from wild aquatic birds.

Table 10. Hunter-harvested wild bird surveillance for HPAI virus H5 intercontinental A (icA) results for AI matrix gene, Pacific Flyway, December 2014 through February 1, 2015, as reported in.[295]

Species	n	HPAI virus icA positive
Mallard, *Anas platyrhynchos*	1,410	15
Northern shoveler, *Anas clypeata*	555	3
Green-winged teal, *Anas crecca*	724	4
American wigeon, *Anas americana*	777	31
Northern pintail, *Anas acuta*	460	5
Cinnamon teal, *Anas cyanoptera*	67	0
Wood duck, *Aix sponsa*	27	3
Gadwall, *Anas strepera*	185	1
Canvasback, *Aythya valisineria*	68	0
Ruddy duck, *Oxyura jamaicensis*	46	0
Bufflehead, *Bucephala albeola*	35	0
Canada goose, *Branta canadensis*	148	1
Cackling goose, *Branta hutchinsii*	33	0
Lesser scaup, *Aythya affinis*	14	0
Ring-necked duck, *Aythya collaris*	65	0
Common goldeneye, *Bucephala clangula*	39	0
All other species sampled	76	0

- Commercial turkey flocks in Stanislaus County, California, were infected with a novel Eurasian HPAI H5N8 in January 2015, and the outbreak is considered related to the HPAI events in wild birds. This novel AI virus of Eurasian origin (EA-H5N8 clade 2.3.4.4) spread rapidly along wild bird migratory pathways during 2014.[296] On February 12, 2015, Eurasian H5N8 was also confirmed in a commercial chicken flock in Kings County, California.[294]
- Between March and June of 2015, an outbreak of H5N2 was observed in the Midwest; turkey barns were the most impacted in Minnesota and chickens were more involved in Iowa.[154] Although 3,139 waterfowl fecal samples were tested during this outbreak, HPAI virus was not isolated from any aquatic bird fecal samples.[280]

- In the 2017 outbreak of North American wild bird lineage H7N9 IAV in broiler breeder farms in Tennessee, factors such as the presence of rodents and other wild mammals and waterfowl near barns, the condition of the housing, and breaches in biosecurity protocols were determined to be environmental risk factors.[297]
- In the context of spillover from wild waterfowl to upland gamebird flocks specifically, a study by Ramey et al. (2016) documented a genetically equivalent virus observed in pen-reared pheasants and in wild bird populations within the vicinity at the time. Besides the Ramey et al. (2016) study, all other case studies documenting introductions of virus into upland gamebird flocks via wild birds are based on epidemiological field investigation and the elimination of other potential pathways of introduction.[298]
    - From Frame & Simmunich's (2011) case study: "Although not definitively proven, it is highly likely that the initial introduction of AI subtype H5N8 occurred through the intermingling of wild and captive ducks."[153]
    - From Karunakaran et al.'s (1981) report: "Although the source of the infection was not determined for either outbreak [on the pheasant farms], the authors speculate that wild waterfowl may have introduced the influenza A virus isolates onto both farms."[299]
    - From Dhillon & Wallner-Pendleton's (1986) report: "The pheasants brought and added to this flock from southern California could not be considered to have brought infection, as those birds had been introduced approximately 8-10 months previously. It is very likely that wild waterfowl possibly infected the white Pekin ducks (*Anas platyrhynchos domesticus*) and at a later date avian influenza virus invaded the pheasants."[300]
    - From Aijthdoss et al.'s (2017) case study: "Exposure to the migrating waterfowl was suspected as the source of infection for the outbreak… In the farm of the present report, the upland gamebirds were at risk of developing influenza A virus infection from exposure to migrating waterfowl, as they were raised in mesh-covered outdoor runs."[137]
- Additionally, most authors point out the heightened risk of wild bird exposure due to using an open water source (e.g., a nearby river) as the source of drinking water for the captive raised birds. Of other interest is the observation that no chukars onsite were infected, possibly due to less farm personnel foot traffic in pens and that pens have wire mesh floors raised off the ground, possibly limiting contamination.[137]

9.1.6.2 Qualitative Analysis

We considered the following factors in evaluating this pathway:

Experimental studies suggest that the possibility of HPAI infection in wild aquatic birds varies, and their ability to transmit viruses depends on the combination of virus strain and host demonstrated in the findings shown in **Tables 7** and **8**

- The probability of aquatic wild birds coming onto an upland gamebird farm without any obvious attractants is low thus limiting the risk of contamination of the environment or the direct infection from waterfowl.

  - If ponds are present on an upland gamebird premises, waterfowl have been observed to stop at them during migration, but if no ponds or surface water access is available, waterfowl have not been observed as typically landing on upland gamebird premises (personal communication, Secure Upland Gamebird Supply Working Group, August 2019).
- To prevent attracting wild waterfowl which if infected may contaminate the environment, generally, upland gamebird producers maintain feed bins on-farm and promptly clean up feed spills.
- Additionally, in most documented cases of influenza A virus infection on upland gamebird premises, the premises was raising captive ducks (i.e., the infected premises in those studies fall out of the scope of this risk assessment).
- The spread of HPAI viruses via migratory waterfowl routes is far less likely to occur in poultry farms with bird-proof confinement.[301] Spread of virus due to waterfowl coming onsite may occur due to the potential difficulties in preventing contamination of bird raising areas with waterfowl feces (indoor confinement systems can easily exclude waterfowl vs free-range systems that allow waterfowl to directly enter the bird raising areas).[302] However, pen-rearing systems allow much more limited direct contact between waterfowl and birds in the pens and the bird raising areas. The netting and fencing on the pens greatly limit any possibility for co-mingling of waterfowl with penned birds and direct contamination of the pen.
- Anecdotally, there have been reports of suspected movement of LPAI virus between flocks of free-range turkeys (Mahesh Kumar, personal communication, November 1995), but these free-range flocks have a higher degree of exposure because they may directly co-mingle with waterfowl as result of not being kept in uncovered pens. Once the viruses move from wild birds to poultry, it is assumed that human activities—especially movement of personnel and equipment from farm to farm—are responsible for transferring infective materials from infected to susceptible birds.[161] Secondary spread caused by wild birds between poultry premises should be considered possible but only in rare instances.
- Wood et al. (1985) demonstrated little to no fecal shedding of HPAI H5N2 in wild Ring-billed gulls (family Laridae) and domestic Pekin ducks (*Anas platyrhynchos domesticus*), suggesting these birds were unlikely to transmit virus from farm to farm in the 1983 Pennsylvania outbreak.[80]
- None of the HPAI-infected wild ducks (*Anas platyrhychos*) (H5N2, H5N1, and H5N8) found in the 2014-2015 U.S. outbreak have been implicated in transferring the virus from one poultry farm to another.[154,284,296,303]
- In the above-mentioned HPAI H5N8 outbreak in commercial California turkeys, other houses on the premises remained negative, and spread of the disease within the Control Area did not occur.[283]
- Studies on the introduction and spread dynamics of influenza A virus in both commercial upland gamebirds and conventional poultry sectors (e.g., broilers, turkeys, and layers)[8,14] report similar trends in the prevalence of virus

introductions onto each farm-type. Limited differences in proportion of introductions could be reflective of similarities in biosecurity levels and other risk mitigation measures. If there are sector-specific differences, then some of the practices seem to compensate for the other risks that are enhanced by outdoor raising of most mature upland gamebirds (e.g., presence of spilled feed or presence of sources of warmth such as the roofs of poultry houses densely filled with birds).

9.1.6.3 Likelihood Rating and Conclusion

While wild aquatic birds are natural reservoirs for influenza A viruses (possibly including several strains of HPAI virus) and could cause a spillover of disease to domestic poultry, primary infection in domestic poultry and captive upland gamebirds depends upon the degree of contact with wild birds. While environmental contamination from waterfowl is elevated in comparison to barn-confined poultry and direct contact with waterfowl is not completely eliminated, upland gamebird flocks are still able to mitigate some of the potential risk of exposure to wild waterfowl. Properly constructed pens with secure netting in addition to limiting on-farm attractants such as waterfowl minimizes any incentive for wild waterfowl to come onsite. Additionally, practical biosecurity measures limit possible infection via environmental contamination due to fomites that had contact with waterfowl. Despite these measures, upland gamebirds housed in pens are exposed to flying waterfowl that may pass overhead, and biosecurity measures may not be completely maintainable throughout the growing season for penned birds, therefore, we conclude that the likelihood of HPAI infection in upland gamebirds via wild aquatic birds in the farm vicinity is ***low.***

9.1.7 Role of HPAI Virus Spread to an Upland Gamebird Flock via Wild Non-Aquatic Birds in Farm Vicinity

An AI virus was first identified in wild birds in 1961 when HPAI H5N3 was isolated from common terns (*Sterna hirundo*) in South Africa.[304] A compilation of more recent surveys of wild birds describes an overall AI virus prevalence of 15.2 percent in Anseriformes (waterfowl), 2.9 percent in Passeriformes (perching birds), and 2.2 percent in Charadriiformes (waders, gulls, and auks).[161] Since its appearance, HPAI H5N1viruses of the goose Guandong lineage have demonstrated the unique ability among HPAI viruses to infect a wide variety of species, including wild birds outside of aquatic species. Influenza A viruses are primarily spread from wild birds to domestic poultry through the mechanical transfer of infective feces, usually via human activity.[161] Non-aquatic wild birds are often attracted to poultry farms if feed is accessible[291] (e.g., if feed is kept in an insecure way, if feed is regularly spilled, or if feeders are accessible). Such species of wild non-aquatic birds that are attracted to feed include birds in the orders of Passeriformes (perching birds) and Columbiformes (pigeons and doves). However other species of wild bird such as scavenging and/or predatory species (e.g., Accipitriformes [hawks, eagles, and New World vultures], Strigiformes [owls], and specific scavenging Passeriformes under the family of Corvidae [crows]) are attracted to premises if live birds and/or poultry mortality are accessible or perceived as accessible. In the case of upland gamebird farms, where outdoor netted pens are employed, the upland gamebirds within the pens can attract the predatory species of birds. These scavenging and predatory birds could potentially act as infected carriers or mechanical fomites if they encounter HPAI

virus-infected birds or HPAI virus-infectious mortality and then subsequently visit an upland gamebird farm. Additionally, as observed above, in some instances there are omnivorous wild bird species (e.g., American crows [*Corvus brachyrhynchos*]) that may be attracted to both accessible poultry feed and poultry mortality. Below, the aforementioned orders of non-aquatic wild birds are assessed based on their propensity to fit into specific disease pathways (e.g., being attracted to feed on an infected premises or directly contacting HPAI virus-infected or -contaminated birds). For a thorough review of pathways associated specifically with aquatic bird species, see **Section 9.1.6** Role of HPAI Spread to an Upland Gamebird Flock in a Control Area via Wild Aquatic Birds in the Farm Vicinity.

9.1.7.1 Likelihood of Infection via Non-Aquatic Wild Birds in Farm Vicinity That are Attracted to Feed (i.e., Passeriformes, Columbiformes, and Wild Galliformes)

Small perching birds of the order Passeriformes (passerines) commonly frequent poultry farm areas and thus have the potential to serve as biological or mechanical vectors of H5N1, or as so-called bridge species in its transmission.[305] This group includes commonly encountered species such as sparrows, swallows, and starlings. Other potential bridge species include the Columbiforme birds, which include pigeons and doves.[305] The potential pathways for HPAI transmission via passerine or Columbiforme birds include infection or contamination of the wild bird on an infected poultry or upland gamebird farm or premises contaminated with infected wild bird feces, with subsequent primary or secondary transmission into an uninfected upland gamebird farm. The distances that some wild bird species travel depend on the availability of food supply and weather. For example, starlings and blackbirds disperse as far as 24 to 40 km on average, with some individuals traveling up to 80.5 km daily from roost to their feeding grounds.[306] Any of these movements provide an individual bird the opportunity to contact and disseminate AI viruses.

Additionally, wild Galliforme species should also be considered as fomites and sources of infection for any commercial premises raising upland gamebirds or poultry.

- In a survey of infected turkey farms during the 2014–2015 outbreak in Iowa, Minnesota, North Dakota, South Dakota, and Wisconsin (n=81), 26 percent reported seeing wild turkeys, pheasants, and quail around their poultry barns.[45]
- The presence of wild upland gamebirds on commercial upland gamebird farms is minimal based on reports of producers. The type of wild upland gamebird that may happen upon a farm varies by region and habitat in the area surrounding the farm (personal communication, Secure Upland Gamebird Supply Working Group, August 2019).

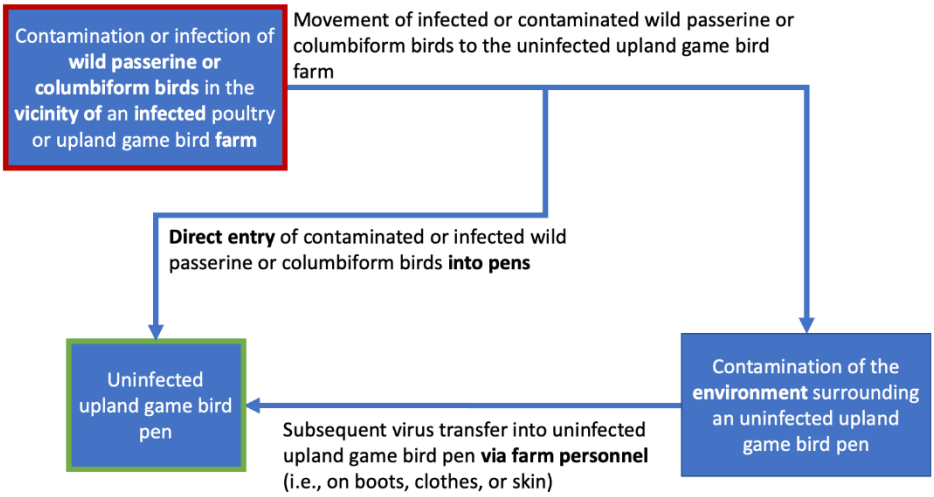


Figure 8. Pathway for exposure of an upland gamebird farm via wild Passerine or Columbiforme birds

9.1.7.1.1 Literature Review

9.1.7.1.1.1 Passeriformes (excluding family Corvidae)

9.1.7.1.1.1.1 Prevalence of AI in Passeriformes (excluding the family Corvidae) in previous AI outbreaks involving poultry

- In a 1985 H7N7 HPAI outbreak in chickens in Australia, an antigenically closely related strain was isolated from starlings on the affected farm, and serologic evidence of H7N7 infection was found in sparrows (*Passer domesticus*) as well.[307]
- In a 1995 survey to establish disease freedom for poultry operations during an outbreak of HPAI H5N2 virus in Mexico, serologic evidence of infection of three passerine birds (species not specified) to an H5N2 serotype was reported.[308] However, an LPAI H5N2 virus had been circulating in poultry in 11 Mexican states prior to the outbreak; it is ambiguous as to which virus resulted in the exposure.
- In Jalisco, Mexico, in 2012, 81,000 general surveillance samples in an H7N3 outbreak region yielded one positive common grackle (*Quiscalus quiscula*) and one positive barn swallow (*Hirundo rustica*).[138]
- A chickadee (*Poecile atricapillus*) recovered in metropolitan Ramsey County, Minnesota, and delivered on June 10, 2015, to a wildlife rehabilitation center later tested positive for AI by immunohistochemical stains of fixed brain tissues.[316] No virus was isolated, but the chickadee tissues were positive by the H5 inter-continental A (icA) molecular assay, which targets the Eurasian H5 clade 2.3.4.4 viruses. However, hemagglutinin gene sequencing attempts were negative. Where the bird may have become exposed to icA H5 is unknown since complete information about submission circumstances was unavailable.[309]

- As part of a case-control study of layer flocks in northwest Iowa in 2015, wild birds and mammals around the flocks were sampled.[297]
    - Of over 1,600 wild bird samples collected—caught using a mist-net around a nest built in a walkway between two poultry barns on an infected premises—a single sample of lung tissue from a juvenile European starling (*Sturnus vulgaris*) was positive for Eurasian H5 (icA).[297]
    - Additional serological evidence of positives for icA H5 were found in a house sparrow (*Passer domesticus*), another European starling, and two American robins (*Turdus migratorious*) sampled around the same positive farm.[297]
- Passerines (European Starlings [*Sturnus vulgaris*; n=508], House sparrows [*Passer domesticus*; n = 534]) sampled from infected farms during the 1983–84 HPAI H5N2 avian influenza epizootic in domestic poultry in Pennsylvania, New Jersey, Maryland, and Virginia were all negative for virus.[207]
- Additionally, European starlings (*Sturnus vulgaris*; n=2) sampled during the 1996–1998 LPAI H7N2 outbreak in Pennsylvania demonstrated no infection based on virus isolation.[156]
- In Russia, during an avian influenza epizootic in 2007, one out of the five European starlings (*Sturnus vulgaris*) were positive for influenza virus A/H5 via rRT-PCR.[265,310]
- Passerine species of importance (including American robins [*Turdus migratorius*; n=20; 4.5 percent], Red-winged blackbirds [*Agelaius phoeniceus*; n=13; 2.9 percent] House sparrows [*Passer domesticus*; n=44; 9.8 percent], and European starlings [*Sturnus vulgaris*; n=5; 1.1 percent]) sampled in wetlands and on poultry farms during wildlife surveillance after the 2015 outbreak yielded no virus based on rRT-PCR or antibodies based on ELISA.[221]
- Of the 73 and 18 peridomestic birds (including passerines such as House sparrows [*Passer domesticus*] and Red-winged black birds [*Agelaius phoeniceus*]) on farms that were infected and unaffected, respectively, during the 2015 H5N2 outbreak in Wisconsin, none were positive for virus via rRT-PCR or antibodies via ELISA.[261]

9.1.7.1.1.1.2 Prevalence of AI in Passeriformes (excluding the family Corvidae) outside of AI outbreaks involving poultry

- In a summary of three studies from 1979 to 1980, in which a total of 11 passerine species were tested, AI virus isolation was reported from 17 out of 586 (0.029 percent) individual birds.[300]
- In a study surveying overwintering starlings (*Sturnus vulgaris*) in Israel in 1979, of the 42 starlings sampled via cloacal swabs, 1 (0.024 percent) yielded virus isolate (A/starlin~/Kinneret,Israel/78/(HavlNeq 1)).[311]
- In Canada in 1980 from July to August, nine species of Passeriformes were sampled for IAVs. Of the 262 samples, 15 samples (0.057 percent) were positive for IAVs via virus isolation.[312]
- No influenza virus was isolated from 83 cloacal swabs collected from 4 adult and 79 juvenile reed warblers (*Acrocephalus scirpaceus*) in 1993, despite proximity to aquatic habitats of known AI reservoir species.[272]

- A 1999 field study conducted in Ohio sampled 7 Passeriforme species from 38 different field sites all located either on or within 1 mile (1.6 km) of an agricultural premises (e.g., poultry, swine or dairy farms). Of the 1,709 serum samples collected, none (0 percent) were positive for IAV antibodies, including the 868 samples from European starlings (*Sturnus vulgaris*) and the 373 samples from House sparrows (*Passer domesticus*).[313]
- In a survey of passerine birds in the State of Georgia from 1999 to 2009, 0 of 234 birds (0 percent) of 25 different species tested positive for AI antibodies.[314]
- On Helgoland Island in the North Sea in 2001, 543 migrating passerine birds of different species all tested negative for AI virus subtypes H5 and H7.[315]
- From a total of 670 cloacal swabs from 37 different species of migratory passerine birds in Slovenia from 2004 to 2006, there was one (0.001 percent) positive rRT-PCR in the only common starling (*Sturnus vulgaris*) tested, but virus isolation was unsuccessful.[316]
- In China, from 2004 to 2007, RT-PCR on 7,320 cloacal, tissue, or fecal samples from 33 Passeriforme species identified 0.36 percent to be H5N1-positive; 1.09 percent of tree sparrows (*Passer montanus*) were positive.[281]
- During active surveillance of Passeriformes for HPAI H5N1 in Mongolia from 2005 to 2011, 0 of 80 (0 percent) live-bird, fecal, and sick-bird samples were positive.[282]
- In 2006, out of 8,961 Passeriformes sampled tested via RT-PCR in Europe, one (0.0001 percent) was H5N1 positive and 8 (0.0009 percent) were LPAI positive.[305]
- In a 2007 study in Slovakia, 30 percent of 155 passerine birds of 12 species were AI virus-positive via RT-PCR on cloacal and/or oropharyngeal samples, including 3 of 6 swallows (*Hirundo rustica*). Influenza A virus subtypes observed with the positives RT-PCR results included 10 different haemagglutinin subtypes and 4 different neuraminidase subtypes. The authors speculate that the higher than typically reported prevalence may be due to the increased sensitivity of *nested* RT-PCR used in this study.[317]
- In 2007, a field study from Iraq yielded no positives via ELISA or HI tests of the serum samples from 60 European starlings (*Sturnus vulgaris*).[318]
- In 2008, 50 cloacal samples from European starlings (*Sturnus vulgaris*) located around a piggery in Australia were all negative for AIV via RT-PCR.[319]
- Rectal samples from 1,300 tree sparrows (*Passer montanus*) in China in 2011 yielded no AI virus, while 94 of 800 (0.118 percent) were serologically positive for H5N1, and 0 of 800 (0 percent) were seropositive for H7.[320]
- Peridomestic species sampled (n = 82) from natural areas in Dane and Jefferson counties of Wisconsin between September 10th and 29th of 2015 were all found negative for virus and antibodies.[261]
- Nine out of 453 (0.020 percent) samples taken from passerine birds during wild bird surveillance in Ohio during 2015 were positive for influenza A virus via RRT-PCR, however no virus was isolated. The PCR-positive species included: Swanison's Thrush (*Catharus ustulatus*), Gray Catbird (*Dumetella carolinensis*), Common Yellow Throat (*Geothlypis trichas*), Black-capped Chickadee (*Poecile*

atricapillus), House Wren (*Troglodytes aedon*), and White-throated Sparrow (*Zonotrichia albicollis*).[321]

9.1.7.1.1.1.3 Experimentally determined susceptibility of Passeriformes to AI viruses

- American Robins (*Turdus migratorius*) experimentally infected with various clade 2.3.4.4. HPAI viruses from the U.S. 2014–2015 outbreak demonstrated shedding of all three viruses and positive serology. Most of the shedding was oral with virus titers reaching $10^{4.3}$, $10^{4.3}$ and $10^{4.8}$ PFU/mL for each virus strain. Cloacal shedding was observed for one robin infected with HPAI H5N8 A/gyrfalcon/Washington/41088-6/2014, with virus titers reaching $10^{3.5}$ PFU/mL.[322]
- In an experiment with sparrows (*Passer domesticus*) and European Starlings (*Sturnus vulgaris*) inoculated with HPAI H5N2 A/Northern pintail/Washington/40964/2014, HPAI H5N2 A/turkey/ Minnesota/9845-4/2015, or HPAI H5N8 A/gyrfalcon/Washington/41088-6/2014, mortality was observed in sparrows (5/24), but not in any of starlings (0/24). In the case of starlings, almost no clinical signs of any kind were observed in inoculated birds. Additionally, depending on the HPAI virus, shedding was observed ranging from 1 to 5 dpi for sparrows, with highest virus titer observed being 10^3 PFU/mL. No shedding was observed for starlings in the study.[323]
- Boon et al. (2007) found that sparrows (*Passer domesticus*) experimentally inoculated with one of four different H5N1 strains (A/duck/Thailand/144/2005, A/quail/Thailand/551/2005, magpie/Hong Kong/645/2006, and A/Japanese white-eye/Hong Kong/1038/2006) experienced mortality of 66 to 100 percent and actively shed virus, with oropharyngeal and cloacal titers as high as 4.7 and 4.1 $\log_{10}$ EID_{50}/mL, respectively, at 4 dpi.[328] Mortality was 0 percent in European starlings (*Sturnus vulgaris*) with no obvious clinical signs. Almost no shedding via cloacal or oropharyngeal routes were observed for starlings between 1 and 6 dpi except for one bird that shed a maximum cloacal titer was 3.8 $\log_{10}$ EID_{50}/mL at 2 dpi. While extremely limited shedding was observed, 96 percent of the starlings seroconverted.[324]
- Eighteen European starlings (*Sturnus vulgaris*) were inoculated with one of three different amounts of HPAI H7N9 A/Anhui/1/2013 virus (six birds per inoculation dose) with six starlings acting as negative controls. Starlings in the control group (no inoculation) and in the two lower inoculum groups ($10^{1.9}$ and $10^{3.9}$ EID_{50}/100 µL) no birds demonstrated infection based on RNA amounts detected and no seroconversion was observed. Starlings in the group inoculated with the most virus ($10^{5.9}$ EID_{50}/100 µL) yielded substantial Ct values. Six of the eight starlings in the $10^{5.9}$ EID_{50}/100 µL group excreted detectable amounts via the oropharyngeal route, with excretion of virus occurring as early as 1 dpi for one starling and lasting until 8 dpi for two starlings. Oropharyngeal shedding titers ranged from 9.28×10^2 to 1.483×10^6 EID_{50}/100 µL.[325]
- Two studies with the HPAI H5N1 strain A/chicken/Hong Kong/220/97 resulted in no mortality and infrequent occurrence of histopathologic lesions in house sparrows (*Passer domesticus*) and European starlings (*Sturnus Vulgaris*).[330,331]

While mortality among house finches (*Carpodacus mexicanus*) averaged 44 percent, histopathologic lesions were absent to mild and viral antigen rare in the nasal cavity and gastrointestinal tract. The authors were not able to draw any definitive conclusions regarding the role of these species as biological vectors.[326,327]

- In a study by Forrest et al. (2010), house sparrows (*Passer domesticus*) (n=8) intranasally inoculated with A/duck/Laos/25/06 H5N1 shed virus both via oropharyngeal and cloacal routes beginning in on 1 dpi until 5 dpi, with maximum virus titers measured at ~4 and ~3.5 $\log_{10}EID_{50}$/mL, respectively.[328]
    - Additionally, eight contact house sparrows were provided 1 L of water that contained 1 mL resuspended fecal material ($10^{6.5}$ EID_{50}/mL) procured from chickens inoculated with 10^2 EID_{50}/mL of the same virus via instranasal, intratracheal, and eye crop routes 24 hours after inoculation. Virus was transmitted to two of the contact sparrows which died 5 and 10 days post contact with contaminated water. The mean virus titers shed by the two infected sparrows were 3.88 $\log_{10}EID_{50}$/mL via oropharyngeal route and 4.25 $\log_{10}EID_{50}$/mL.[328]
    - Authors suggested that house sparrows are unlikely to be infected from chickens under normal field conditions in an HPAI outbreak.[328]
- In Gutierrez et al.'s (2011) experimental study, 4 groups of Eurasian tree sparrows (*Passer montanus*), consisting of 35 total sparrows, were inoculated with 10^6EID_{50}/50 µL of H5N1 HPAI A/Chicken/Cambodia/LC1AL/2007 per bird via oral, nasal, and cloacal routes. Inoculated sparrows exhibited 97 percent (34/35) mortality with mean time of death ranged between 3.7 to 4.1 dpi across the four groups. For inoculated sparrows, the mean virus titer of cloacal and oropharyngeal samples taken daily after inoculation was $10^{7.2}$ viral RNA copies per mL of VTM and the mean virus titer in feathers taken daily after inoculation was $10^{9.50}$ viral RNA copies per gram.[329]
    - Additionally, in the same experiment, 6 of Eurasian tree sparrows were freely housed in an isolator with 40 ducks (Specific pathogen free; Species not specified; local Cambodian breed) inoculated with 10^6EID_{50}/500 µL of H5N1 HPAI A/Chicken/Cambodia/LC1AL/2007 via oral, nasal, and cloacal routes. Fifty percent of the exposed sparrows (3/6) died due to HPAI exposure, with the mean time of death being 9 days post-contact. For exposed sparrows, the mean virus titer of cloacal and oropharyngeal samples taken daily after inoculation was 1.936×10^6 viral RNA copies per mL of VTM and the mean virus titer in feathers taken daily after inoculation was 2.596×10^8 viral RNA copies per gram.[329]
- In Eurasian tree sparrows (*Passer montanus*) inoculated with HPAI H5N1A/chicken/Miyazaki/K11/2007 and A/chicken/Shimane/1/2010, mortality was 100 percent within 11 days (mean >6 days), with oral swabs positive from 1 to 8 dpi and maximum titers of $10^{6.5}$ to $10^{7.3}$ EID_{50}/mL.[330]
- Nestorowicz et al. (1987) infected house sparrows (*Passer domesticus*) (n=10) and European starlings (*Sturnus Vulgaris*) (n=12) with 10^5 log EID_{50} of an isolate

of an HPAI H7N7 virus from chickens (A/Chicken/Victoria/1/85) via the oral/tracheal and nasal cleft route. Three of the 10 sparrows died due to the disease. The three sparrows died within 2 dpi with mean virus titers across all tissue samples taken from the deceased sparrows being within ranging between $10^{2.2}$ to $10^{5.5}$ ElD_{50}/g homogenate. All (12/12) starlings died from disease within 2 dpi with mean virus titers across all tissue samples in ranging between $10^{1.2}$ to $10^{5.2}$ ElD_{50}/g homogenate and 50 percent of the birds having tissue virus titers between 10^{3} to 10^{5} ElD_{50}/g homogenate.[307]

- Twenty-three of 23 stonechats (*Saxicola torquata*) inoculated oculo-oronasally with $10^{6.0}$ ElD_{50}/0.25 mL of A/Cygnus cygnus/Germany/R65/2006 H5N1 died within 3 to 7 days, most with no clinical signs. Oropharyngeal shedding peaked at 10^{3} to 10^{4} $TCID_{50}$/mL on 3 to 6 dpi.[331]
- In European starlings (*Sturnus vulgaris*) and house sparrows (*Passer domesticus*) that were inoculated with LPAI virus strain A/wild bird/California/08 (H3N8) via ocular, nasal, and oropharyngeal routes, 35 of 36 starlings and 19 of 36 sparrows exhibited viral shedding between dpi 1 and 5, with an average duration of oropharyngeal viral shedding being 3.3 days for starlings and 1.5 days for sparrows. Only three oropharyngeal swab samples and one cloacal from sparrows were positive via virus isolation. Virus titers from oropharyngeal samples ranged from $10^{2.2}$ to $10^{3.1}$ EID_{50}/mL and the virus titer from the cloacal swab was $10^{4.3}$ EID_{50}/mL. Oropharyngeal swabs from six starlings were positive via virus isolation, with virus titers ranging from $10^{2.2}$ to $10^{3.4}$ EID_{50}/mL. However, no clinical signs were observed in any of the birds aside from one sparrow mortality at 14 dpi.[332]
- Of 24 European starlings (*Sturnus vulgaris*), 12 of which were intranasally inoculated with A/chicken/OH/494832/2007 H2N3 (LPAI) and the other 12 with A/Northern Shoveler/OH/28926-3/2007 H4N2 (LPAI), no starlings showed neither clinical signs nor mortality and average titers from all time points (dpi 2, 4, and 7) across both strains were less than $10^{2.76}$ EID_{50} per 0.2 mL.[333]

9.1.7.1.1.1.4 Experimentally determined transmissibility of AI viruses by Passeriformes (excluding family Corvidae)

- In Boon et al.'s (2007) experiment (see additional details in the previous section), inoculated sparrows (*Passer domesticus;* n = 3*)* were housed with uninfected contact sparrows (*Passer domesticus*; n = 3*)* starting at 1 dpi. No same-species transmission was observed at point during the study period for any of the four experiments, each using a different strain of H5N1 HPAI. Similarly, inoculated European starlings (*Sturnus vulgaris*; n = not specified) were housed with uninfected contact starlings (*Sturnus vulgaris*; n = not specified) at a 1:1 ratio. Same-species transmission between starlings was observed in the experiment involving A/CM/HK/645/06 virus, but not in the other two experiments where starlings were inoculated with A/DK/TH/144/05 or A/JW/HK/1038/06.[324]
    - The authors suggest that sparrows may act as intermediate hosts for transmission to both poultry and mammals, but the lack of contact transmission and high mortality which preclude them from being considered reservoir species for H5N1 HPAI.[328] Additionally, starlings

only experienced one unduplicated instance of contact transmission. While European starlings may also act as intermediate hosts, the authors conclude the low contact transmission rate to starlings likely indicates they should not be considered as an influenza A virus reservoir.[324]

- Forrest et al. (2010) (see additional details in the previous section), experimentally found that no chickens offered 3L of a 1:3 dilution of water from trough of inoculated house sparrows (*Passer domesticus)* within the same experiment became infected via the contaminated water.[328]
- Gutierrez et al. (2011) (see additional details in the previous section), conducted two experiments exploring same-species HPAI transmission between Eurasian tree sparrows (*Passer montanus*) as well as two different experiments exploring the propensity of sparrows (*Passer montanus*) to transmit infection to chickens. In all experiments contact birds were introduced to the environment with inoculated sparrows on 1 dpi. More detailed experimental design and the results for each experiment are as follows:
    - When contact sparrows (n = 14) were freed in the same isolator as inoculated sparrows (n = 16), no transmission of infection to other sparrows was observed based on absence of clinical signs, mortality, and virus from swab samples in contact sparrows. However, 14 percent of contact sparrows seroconverted.[329]
    - When contact sparrows (n = 22) were confined in the same cage as inoculated sparrows (n = 20), no transmission of infection to other sparrows was observed based on absence of mortality and virus from swab samples in contact sparrows. However, 36 percent of contact sparrows seroconverted.[329]
    - When contact chickens (n = 40) were free within an isolator with inoculated sparrows (n = 24) that were free flying within the same isolator, no transmission of infection to chickens was observed based on the absence of mortality, symptoms, viral RNA, and seroconversion of chickens in the isolator.[329]
    - Finally, when contact chickens (n = 12) were free within an isolator while inoculated sparrows (n =10) were caged within the same isolator, transmission of infection to chickens did occur. Chickens exhibited 100 percent mortality with a mean time of death of 6.5 days post-exposure.[329]
- In Yamamoto et al.'s (2013) experimental study (see additional details in the previous section), Eurasian tree sparrows (*Passer montanus*) inoculated with HPAI H5N1A/chicken/Miyazaki/K11/2007 and A/chicken/Shimane/1/2010, while there was no intraspecies transmission among sparrows, 10 of 16 (62.5 percent) contact chickens died when housed with infected sparrows.[329]
    - Due to the prolonged viral shedding observed in this study, the authors suggest that tree sparrows have the potential to serve as biological vectors of HPAI.[329]

- In Nestorowicz et al.'s (1987) experimental study (see additional details in the previous section), uninfected Passeriforme birds were placed in contact with infected birds of the same species. Transmission to starlings (*Sturnus vulgaris*) was observed. More details from the experiment are provided in **Table 11** below.

Table 11. Summary of the experimental transmission of H7N7 HPAI virus in house sparrows and European starlings by Nestorowicz et al. (1987).[314]

Common name (*Latin name*)	Mortality	Virus isolation	Transmission
European Starlings (*Sturnus vulgaris*)	100%; All inoculated birds died within 48 hrs. post-inoculation	Not reported	Contact birds (n = 2) died within 4 days of being placed with infected birds (n = 6)
House Sparrows *(Passer domesticus)*	30% mortality rate	Isolated from all tissues from birds that died within 2 days post-inoculation	No transmission between infected birds (n = 10) uninfected contact birds (n = 8)

- Finally, in Nemeth et al.'s (2010) study (see additional details in the previous section), two H5N3 LPAI (A/wild bird/California/08)-inoculated house sparrows (*Passer domesticus*) were housed with two sham-inoculated sparrows of the same speices, and separately, two LPAI-inoculated European Starlings (*Sturnus vulgaris*) were housed with two sham-inoculated starlings of the same species. In both the sparrow and starling experiments, no cage-mate transmission occurred for either species through 28 dpi.[332]

9.1.7.1.1.1.5 Passeriformes (excluding family Corvidae) behavior and dispersal

Some species or populations of passerines could be termed synanthropic, as they occupy a distinct ecological niche in and around human agricultural activities. Species such as black birds (*Agelaius phoeniceus*) and starlings (*Sturnus vulgaris*) may rest in large groups on netting of pens on upland gamebird farms.[3] Additionally, passerines are observed to be attracted to poultry (and by extension, upland gamebird) feed.[291] For more information on specific risks of feed contamination if passerines breach biosecurity at feed mills or on farms, see USDA–APHIS's Poultry Feed Risk Assessment.[334] The behavioral characteristics of passerines that may contribute to their ability to play a role in the transmission of AI to domestic poultry and upland gamebirds are summarized in **Table 12**. Additionally, Forrest et al. (2010) inferred that in their experimental transmission study between sparrows (*Passer domesticus*) and chickens, the behavior of infected house sparrows may be a determining factor in their potential to be intermediate HPAI hosts because of viral shedding into drinking water.[328] Small passerine species including European starlings (*Sturnus vulgaris*) and small sparrow species have been observed to be able to slip through upland gamebird pen netting (Secure Upland

Gamebird Work Group, personal communication, August 2019), potentially allowing access to feed.

Table 12. Behavioral characteristics of several members of the order Passeriformes that may impact their roles in HPAI virus transmission in farm and poultry houses as well as upland gamebird pen environments.

Common name (species)	Migration	Habitat	Nesting behavior	Food
Common Grackle (*Quiscalus quiscula*)	Resident or short-distance migrant	Agricultural fields, feedlots, woodland, forest edges, marshes	Nearly always in scattered trees, rarely in barns	Omnivorous; seeds (agricultural grains)
House Sparrow (*Passer domesticus*)	Resident	Closely associated with people and their buildings	Prefer structures; eaves or walls of buildings	Grains and seeds (livestock feed)
European Starling (*Sturnus vulgaris*)	Resident or short-distance migrant	Countryside near human settlements; feed in fields	Trees, buildings, structures	Focus on insects and invertebrates; also, fruits, berries, grains (livestock feed)
House Finch (*Haemorhous mexicanus*)	Resident or short-distance migrant	Farms, parks, urban centers, backyards	In or near buildings; trees	Plant materials almost exclusively; millet, milo, etc.

Table from USDA–APHIS Poultry Feed Risk Assessment.[334]

9.1.7.1.1.2 Columbiformes

While Columbiformes, such as pigeons (*Columba livia domestica*), are regular visitors to and inhabitants of commercial poultry farms, they are not commonly reported on upland gamebird farms (personal communication, Secure Upland Gamebird Supply Working Group, August 2019). Even so, their abundance in rural landscapes warrants some investigation into the role of Columbiforme dynamics regarding HPAI viruses. Thorough two thorough reviews[265,335] have been published on the prevalence of HPAI viruses within Columbiformes based on field data as well as the experimental susceptibility of Columbiformes to HPAI viruses.

The reviews observe the following as it relates to the prevalence of HPAI viruses within Columbiformes:

- Based on published field data dating back to 1985, Abolnik (2016) catalogs that in 17 HPAI outbreaks (including outbreaks of H5N1, H7N9, H7N3, and H7N1) involving in poultry and wherein Columbiformes within the vicinity were tested for HPAI, of the 2,035 of samples taken from Columbiformes and tested for HPAI via chicken egg isolation in only 12 samples (0.006 percent) was virus

detected. Such results suggest limited direct and/or indirect transmission from infected poultry or other wild birds to Columbiformes during HPAI outbreaks in poultry.[335]

- Abolnik (2016) summarized 26 experimental studies in which Columbiformes were inoculated with HPAI viruses of numerous strains (see Abolnik, 2016 for exact strains) and deduced the general conclusions that while Columbiformes have a "low to medium" infectious dose, they are "ineffective propagators and disseminators of the virus." Additionally, Abolnik (2016) reported that given the available literature, "minute quantities" of virus, both choanally and via feces and viral titers shed by Colubiformes, are observed to be "below the minimum threshold require to infect other species."[335] In Shriner & Root's (2020) review, the authors summarized an additional nine experimental studies demonstrating similar trends "that pigeons can generally become infected with a variety of IAVs [and] usually shed limited viral quantities for brief periods of time and rarely transmit to naïve contacts." However, Shriner & Root (2020) suggest that certain strains and individuals could still be candidates for virus transmission and that Columibformes should not be completely dismissed as potential vectors.[265]
- Abolnik's (2016) suggests the most prominent role that Columbiformes would play during an HPAI outbreak would be that of a mechanical vector via spreading virus on their feet or plumage.[335] Such mechanical transfer is not outside the realm of possibility given that influenza A viruses to survive in host feathers which has been proven in previous studies,[336] with one study demonstrating that HPAI virus H5N1 can survive on—and spread via contact with—feathers for 15 to 160 days at 4 °C to 20 °C.[337]

9.1.7.1.1.3 Non-Domestic Galliformes

Additionally, non-domestic Galliforme species should also be considered as mechanical vectors and potential sources of infection for any commercial premises raising upland gamebirds.

- In a survey of infected turkey farms during the 2014–2015 outbreak in Iowa, Minnesota, North Dakota, South Dakota, and Wisconsin (n=81), 26 percent reported seeing wild turkeys, pheasants, and quail around their poultry barns.[45]
- The presence of wild upland gamebirds on commercial upland gamebird farms is minimal based on reports of producers. The type of wild upland gamebird that may happen upon a farm varies by region and habitat in the area surrounding the farm (personal communication, Secure Upland Gamebird Supply Working Group, August 2019). However, an equivalent exposure risk to consider are upland gamebirds that escape from outdoor pens. Escaped birds are an occurrence that ranges from common to rare on upland gamebirds, with most upland gamebird farms returning escaped birds to the pens (personal communication, Secure Upland Gamebird Supply Working Group, January 2023).

Wild upland gamebirds are known to be susceptible to influenza A viruses, however there is no evidence of these birds playing a role in past outbreaks. For a more in-depth look at the susceptibility and transmissibility of influenza A viruses in upland gamebird species

see **Section 8** Hazard Identification: HPAI Overview. Noting that captive upland gamebirds differ slightly from upland wild gamebirds genetically.

- Experimentally infected wild pheasants (order Galliformes) shed the virus in their feces for up to 15 days, demonstrating the potential to transmit HPAI H5N2 (A/Chicken/Penn./1370/83). However, surveillance of wild pheasants in quarantine areas did not support that this actually occurred.[80]

Surveillance of wild Galliformes birds for AI virus has demonstrated zero to low prevalence in the wild.

- Peterson et al. (2002) found a 0 percent prevalence of AI virus in wild turkeys (*Melleagris gallopavo*) in a survey of 70 turkeys in Texas.[338]
- Another study of wild captured or hunter-harvested wild bobwhite quail (*Colinus virginianus*) in Texas found prevalence of 1.4 percent using rRT-PCR; however, no virus could be isolated.[339]
- De Marco et al. (2005) found that 27 of 219 free-living pheasants sampled between 1995 and 2002 in the Emilia Romagna region of Italy were positive via ELISA for antibodies.[340] While not specific to North America, such field data demonstrate the propensity for wild pheasants to carry infection.

9.1.7.1.2 Qualitative Analysis

We considered the following qualitative factors for evaluating this pathway:

- There are limited instances of Passeriforme, Columbiforme, and wild Gallinaceous birds becoming infected with HPAI during outbreaks of HPAI involving poultry based on available field data (see additional details in the Literature Review).
- Similarly, prevalence of HPAI (as evidenced by serology, rRT-PCR, and virus isolation surveillance results) in wild Passeriforme, Columbiforme, and wild Gallinaceous wild bird species regardless of active HPAI outbreaks in surrounding populations is incredibly limited (see specific details in the Literature Review).
- To date, HPAI viruses of various strains have proven to be able to infect a variety of Passeriforme, Columbiforme, and Gallinaceous wild bird species based on experimental data. However, there is variability between species as noted in the literature.
    - Additionally, there are mixed results from experimental evidence for species-specific susceptibility to HPAI viruses based on the subtype, strain, and inoculum dose of the virus for two common passerines found on poultry and upland gamebird farms: European starlings (*Sturnus vulgaris*) and house sparrows (*Passer domesticu*s).
- In five of the studies exploring intraspecies transmission of HPAI viruses in Passeriformes (specifically European starlings (*Sturnus vulgaris*) and/or sparrows (*Passer domesticu*s and *Passer montanus*), only one study observed HPAI transmission between European starlings.[307,324,329,330,332] Such results demonstrate

a limited capacity for Passeriformes to incubate HPAI viruses within their own populations.

- Additionally, two studies demonstrated chickens and ducks do have the capacity to infect Passeriformes (specifically *Passer domestica*s and *Passer montanus*) with HPAI when in close contact.[328,329] Implicating it is possible for infected poultry to infect Passeriformes in close contact scenarios.
    - Albeit, one study suggests that house sparrows are unlikely to become infected with HPAI from chicken in field conditions.[328]
- Of the three studies experimentally testing the ability of Passeriformes to infect chickens with HPAI viruses, there were mixed findings.
    - Two studies did not observe transmission. Gutiérrez et al. (2011) observed that small passerines free flying in environments had a limited propensity to spread virus to gallinaceous birds on the ground.[329] Additionally, Forrest et al. (2010) experimentally found that no chickens offered 3L of a 1:3 dilution of water from trough of inoculated sparrows within the same experiment became infected via the contaminated water.[328]
    - However, two studies did observe transmission. Gutiérrez et al. (2011) also observed that caged passerines within an isolator with chickens transmitted virus to susceptible chickens.[329] In addition, Yamamoto et al. (2013) demonstrated transmission between passerines and chickens housed together.[330]
- The BID_{50} for HPAI H5N2 and HPAI H5N8 infection via intrachoanal inoculation for upland gamebird species (Bobwhite quail, ring necked pheasant, and chukar) are estimated to range between $<10^2$ to $10^{3.7}$ BID_{50} depending on the species (see **Section 8.7.1**, Dose Response in Upland Gamebirds).
    - The studies summarized in literature demonstrate variability in oropharyngeal and cloacal HPAI virus titers in passerines, depending on the bird species and the strain, but when shed titers were measured, some studies indicate there is for the amount of virus shed to meet the infectious dose for gallinaceous birds like upland gamebirds.
- Biosecurity guidelines dictate measures to prevent wild bird access to upland gamebird barns and pens. Additionally, maintenance of feed bins such that wild birds are prevented from frequenting them reduces farm visits (**Section 7.5.2.3.6** Animal, Pest and Insect Control).[344,345]
    - Proper feed management, especially the minimization of spilled feed, ensures that as few feed attractants are available to wild birds, however in pens, it is impossible to limit the attractant of open feed troughs. Small passerine species and small dove species can slip through the nets of upland gamebird pens and gain access to feed (personal communication, Secure Upland Gamebird Supply Working Group, August 2019).
    - Even in the case of poultry buildings intended to be bird proof, Burns et al. (2012) observed wild birds frequenting and entering poultry barns on premises where the producers were “highly involved in poultry industry

management" and, the authors note, may have thus been practicing more stringent biosecurity than other producers.[291]

- For more information on the potential for contamination of finished feed products by passerine birds, see USDA–APHIS's Poultry Feed Risk Assessment.[334]

- Published literature suggests that sparrows (*Passer spp.*) could play a role in AI virus transmission in an outbreak, most likely via contamination of the environment and feed due to their predominantly oropharyngeal shedding. And while some poultry species may scavenge dead small passerines, upland gamebirds have exhibited no interest in picking at small passerine mortality (personal communication, Secure Upland Gamebird Supply Working Group, August 2019).
- Secondary transmission of HPAI (via feces or mechanical transfer by external surfaces) from a small passerine bird outside an upland gamebird pen is potentially possible but unlikely.
  - As potential biological vectors, passerine birds shed lower cloacal viral titers, and their fecal volume is small.
  - However, it should be noted that even if peridomestic passerines shed small amounts of virus and infection is not prevalent among passerines, their large flock sizes and frequent visits to poultry farms increase their potential for a role in transmission. For example, European starlings (*Sturnus vulgaris*) can mass in very large numbers, thus it is speculated that the sheer size of a flock congregating around one resource on a farm (e.g., perching area on netting, puddles or waterers for thirst, and/or being attracted to spilled or open feed access and/or cover crops) could still pose an opportunity for contamination.
    - However, the risk of AI transmission is much lower from a single infected bird than from a population of birds in which infection is established. And both field and experimental evidence suggests that starling flocks would likely not sustain a large amount of infectious birds at any one time.
  - There also is the possibility of mechanical transmission of HPAI virus if plumage or feet were to become contaminated. Preliminary results from a survey of 419 passerine birds on 5 farms infected with HPAI H5N2 virus and 5 uninfected farms in Iowa indicates that mechanical transmission through external contamination of passerine birds is a possibility, although the likelihood is very low (only one external surface swab was positive by matrix gene rRT-PCR and submitted for further testing).[334] As potential mechanical vectors, Passeriformes, due to their small size, can only carry a small volume of contaminated feces from an infected premises.
    - In surveillance sampling during the 1983–1984 HPAI H5N2 avian influenza epizootic in domestic poultry in Pennsylvania, New Jersey, Maryland, and Virginia all external samples of starling

(*Sturnus vulgaris*), sparrow (*Passer spp.*), and pigeon (*Columba livia domestica*) feet came back negative.[216]

- Given the preponderance of passerine birds in poultry and upland gamebird settings, more disease spread out of Control Areas in previous outbreaks would be expected to have occurred if these birds played an important role in the transmission of HPAI.
    - As discussed in **Section 9.1.1**, Role of Local Spread Components in Previous AI Outbreaks, most studies indicate limited spread of AI between poultry premises via mechanisms that do not involve the movement of people, vehicles, or equipment.
- Finally, upland gamebirds that are being produced by the farm can escape from and be returned to pens. Escaped and returned birds can feasibly possess a similar risk to penned birds as wild birds entering the pen. There is variation as to how often birds escape and how long they are exposed to the unnetted environment (Secure Upland Gamebird Supply Working Group, personal communication, January 2023).
    - Depending on the frequency of netting inspection and repair, the ability for upland gamebirds to escape pens varies, with birds escaping regularly (e.g., on a daily basis) on some farms, but only occasionally or rarely throughout the grow-out season on other farms (Secure Upland Gamebird Supply Working Group, personal communication, January 2023).
    - Given that gallinaceous birds raised in flocks have demonstrated the tendency for social reinstatement when separated from their flocks,[341] it is unsurprising that escaped upland gamebirds are generally observed to roughly stay within ~91 m of the pens. However, some producers note that on rare occasions birds can stray up to a 1.6 km, although birds that stray such distances are typically killed by predators (Secure Upland Gamebird Supply Working Group, personal communication, January 2023).
    - Escaped birds are normally captured within 1 to 2 days of their escape when regular patrols around pens are conducted; however, on very rare occasions they can outside of the pen for up to a week (Secure Upland Gamebird Supply, personal communication, January 2023).

9.1.7.1.3 Likelihood Rating and Conclusion

Columbiformes are unlikely to play a major role in influenza A virus transmission onto upland gamebird farms given their inherent absence on farms and the unclear picture of their ability as vectors based on current literature. Additionally, Passeriformes (excluding the family Corvidae) have demonstrated a limited prevalence for HPAI viruses during and outside of HPAI outbreaks in poultry based on field data. However, because Passeriformes 1) are experimentally susceptible to HPAI viruses, 2) have experimentally demonstrated the ability to potentially shed the mean bird infectious dose for various gamebird species, and 3) some Passeriforme species have experimentally transmitted HPAI infection to gallinaceous birds, they remain potential vectors, especially given Passeriformes have demonstrated the ability to enter upland gamebird pens. Experimental results point to free flying passerines as unlikely to transmit infection to gallinaceous birds on the ground; however, if these birds are regularly accessing feed within and

congregating on top of the pens the likelihood of transmission is greater. Thus, the likelihood of HPAI infection via passerine birds in the farm vicinity is ***moderate to low***.

9.1.7.2 Likelihood of Infection via Raptor and Scavenging Bird Species in Farm Vicinity

Other non-aquatic wild avian species that can be present on poultry and upland gamebird farms include Accipitriformes (e.g., hawks, eagles, and New World vultures), Strigiformes (e.g., owls), and specific scavenging Passeriformes under the family of Corvidae (e.g., crows). These birds of prey and/or scavenger species vary greatly in number and behavior around poultry farms, however, with outdoor systems, such as upland gamebird farms, it is not uncommon for such birds to be attracted to pens. Unlike non-Corvidae Passeriforme or Columbiforme species, birds of prey and/or scavenger species are unlikely to be able to enter barns or pens, however upland gamebirds that manage to get tangled in pen netting or that are near the permitter of pens can be preyed upon by such species. How such contact would affect the rest of the flock is uncertain. In terms of if such birds can contribute to environmental contamination of farms, review studies have suggested that birds flying between geographic areas can act as fomites and transport viruses such as HPAI H5N1.[56] These birds might have contact with manure stored outside infected poultry houses or manure that is land-applied. Although the quantity of manure that wild birds can carry is unknown, as well as the host adaptability of other HPAI virus strains to different wild bird species, for this risk assessment it was conservatively (and hypothetically) assumed that wild birds will carry HPAI-contaminated manure if they have contact with it. Additionally, a predatory bird or scavenger may become contaminated with feathers or body fluids of infected prey.

Common predator and scavenging wild birds take a variety of short- and long-distance trips to search for food and cover. These include daily movements to and from hunting/feeding and roosting areas, post-fledging dispersal, and seasonal movements.[56] Scavenger species may be attracted to premises with improperly secured carcasses removed from pens or barns. Species known to scavenge avian carcasses in the United States considered in this assessment include vultures (turkey vultures [*Cathartes aura*] and black vultures [*Coragyps atratus*]), some hawks (*Buteo spp.*) and eagles (*Haliaeetus leucocephalus*), crows (*Corvus brachyrhynchos*), ravens (*Corvus corax*), and magpies (*Pica hudsonia*). Some gull species that may scavenge are covered separately in **Section 9.1.6**, Role of HPAI Spread to an Upland Gamebird Flock via Wild Aquatic Birds in the Farm Vicinity.

Finally, while not considered a primary predator or scavenger of upland gamebirds and not considered to consume feed on spilled poultry feed, Falconiformes are considered in this section of the risk assessment due to some species presence in prairie habitats (e.g., American kestrels [*Falco sparverius*], Merlins [*Falco columbarius*], and Prairie falcons [*Falco mexicanus*]) and their potential to contaminate the upland gamebird farm environment.

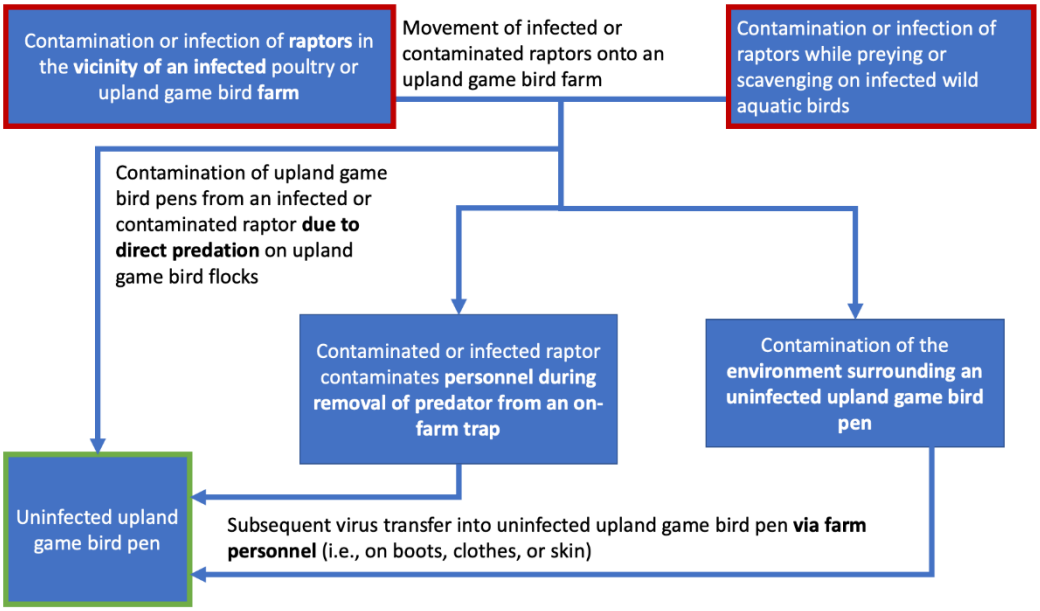


Figure 9. Pathway for exposure of an upland gamebird farm via scavenging birds or raptor species. A similar conduit would apply to wild gallinaceous birds.

9.1.7.2.1 Literature Review

Scavenging birds and raptors have not been directly implicated in the spread of HPAI in previous outbreaks, and few birds of this type have tested positive for AI in the vicinity of outbreaks in poultry or wild waterfowl. However, various types of raptors and scavenging birds have been involved in past HPAI outbreaks (that occurred in either or both poultry and wild bird populations). The presence of these birds on captive upland gamebird premises, particularly hawks and large owls, is common and interactions between the flocks and large predatory birds are a regular occurrence (personal communication, Secure Upland Gamebird Supply Working Group, August 2019).

9.1.7.2.1.1 Accipitriformes

9.1.7.2.1.1.1 Prevalence of AI in Accipitriformes in previous AI outbreaks involving poultry

Various species of Accipitiformes have been involved in past HPAI outbreaks that occurred in poultry and/or wild bird populations.

- In Japan, a mountain hawk-eagle (*Nisaetus nipalensis*) tested positive for HPAI H5N1 only 9 days before an outbreak of HPAI of the same viral strain was reported on a chicken farm in a neighboring prefecture. Three subsequent farms became positive within 3 weeks. Authors hypothesize the mountain eagle hawk became infected by scavenging infected poultry mortality.[342]
- Raptors found dead during an H5N1 outbreak in wild water birds in Germany in 2006 revealed evidence of H5N1 infection in common buzzards (*Buteo buteo*) and peregrine falcons (*Falco peregrinus*).[343] The authors hypothesize that in this H5N1 outbreak in wild water birds, exposure and mortality likely occurred more often in species that hunt or scavenge sick or dead medium-sized prey birds.

- The highest concentration of H5N1 was found in brain tissue and air sacs, with marked encephalitis as a common finding on histopathology.
- The suspected main cause of death in H5N1-positive raptors was encephalitis.
- No infection was found in other Accipitiformes tested, including Eurasian sparrow hawk (*Accipiter nisus*), White-tailed sea eagle (*Haliaeetus albicilla*), undetermined species buzzard (*Buteo sp*.), undetermined species raptor, Red kite (*Milvus milvus*), Rough-legged buzzard (*Buteo lagopus*), Western marsh-harrier (*Circus aeruginosus*), and Goshawk (*Accipiter gentilis*).

- Turkey vultures (*Cathartes aura*) may visit poultry farms to feed on dead birds. Turkey and Black vultures (*Coragyps atratus*) both belong to the order Accipitriformes, family Cathartidae.
  - During the 1983–1984 HPAI H5N2 outbreak in Pennsylvania, Virginia, and Maryland, 8 turkey vultures (*Cathartes aura*) and 22 black vultures from the quarantine zones were tested for H5N2 and none were positive.[344]
  - However, during an HPAI H5N1 outbreak in intensively raised poultry in the west African country of Burkina Faso in 2006, three hooded vulture samples were found to be positive for HPAI H5N1 similar genetically to that circulating in poultry. Authors hypothesized that likely route of transmission being the vultures feeding on infected poultry carcasses on nearby farms.[345]
- 10.5 percent of wild birds testing positive during the 2006 HPAI H5N1 outbreak in Germany were birds of prey, including common buzzards.[350]
  - The buzzards reportedly displayed severe central nervous system infection without systemic virus distribution (unpublished data).
- A wild bird outbreak of HPAI H5N8 occurred in the autumn and winter of the 2016–2017 seasons in the Netherlands with a mass die off of roughly 13,600 birds. Of those 8,882 birds where the avian family was identified, 119 were identified as Accipitridae (order Accipitriformes). Of those, two Accipitridae birds were reported by WOAH as a positive HPAI H5N8 case. **Table 13** below summarizes exact species.[266]

Table 13. Scavenger and predatory families of bird carcass counts and HPAI case reports from Kleyheeg et al. (2017)

Family	Species	Number of Dead sampled	Positive HPAI cases reported by WOAH
Accipitridae	Eurasian sparrowhawk *(Accipiter nisus)*	12	0
	Northern goshawk *(Accipiter gentilis)*	9	0
	Hen harrier *(Cirus cyaneus)*	4	0
	White-tailed eagle (*Haliaeetus albicilla)*	1	1
	Common Buzzard *(Buteo buteo)*	86	1
	Unidentified Hawk Species	7	0
Falconidae	Common Kestrel *(Falco tinnunculus)*	4	0
	Merlin *(Falco columbarius)*	1	0

	Peregrine falcon *(Falco peregrinus)*	16	1
	Unidentified Falcon Species	2	0
Corvidae	Eurasian jay *(Garrulus glandarius)*	3	0
	Eurasian magpie *(Pica pica)*	27	1
	Western jackdaw *(Coloeus monedula)*	16	0
	Carrion crow *(Corvus corone)*	9	0
	Rook *(Corvus frugilegus)*	2	0
	Common raven *(Corvus corax)*	1	0
	Unidentified Corvid Species	30	1

- Seventeen White-tailed sea eagles (*Haliaeetus albicilla*) were found positive for HPAI H5N8 (14 found dead, 3 found alive and subsequently euthanized) between November 2016 and April 2017 during a wild bird HPAI H5N8 outbreak in Germany. The eagles (family Accipitridae) displayed clinical signs that mainly included mild to severe neurological symptoms, with lead poisoning being ruled out as a comorbidity.[346]
- An HPAI H5N1-positive common buzzard carcass found in Bulgaria in 2010 during an HPAI outbreak contained no gross pathological lesions, suggesting the bird died shortly after infection and likely would not have served as a reservoir of infection.[347]
- The U.S. Interagency Steering Committee on Avian Influenza in Wild Birds has compiled all U.S. wild bird cases of HPAI H5 from December 2014 to June 2015.[348] Of 100 positive birds, only 7 were from non-passerine non-aquatic species, with 5 being Accipitriformes (see **Table 14**).

Table 14. HPAI H5-positive samples from non-passerine non-aquatic species collected from December 2014 to April 2015 in the United States.[291,353]

Date	**Species**	**Lineage**	**Sampling type (location)**
N/A	Great horned owl (*Bubo virginianus*)	EA/AM H5N2	N/A
4/14/15	Cooper's hawk (*Accipiter cooperii*)	EA/AM H5N2	Mortality (MN)
4/13/15	Snowy owl (*Bubo scandiacus*)	EA/AM H5N2	Mortality (WI)
1/20/15	Bald eagle (*Haliaeetus leucocephalus*)	EA H5N8	Mortality (ID)
1/9/15	Red-tailed hawk (*Buteo jamaicensis*)	EA/AM H5N2	Mortality (WA)
12/31/14	Red-tailed hawk (*Buteo jamaicensis*)	EA/AM H5N2	Mortality (WA)
12/29/14	Cooper's hawk (*Accipiter cooperii*)	EA/AM H5N2	Mortality (WA)
12/29/14	Peregrine falcon (*Falco peregrinus*)	EA H5N8	Mortality (WA)

- Within the State of Minnesota, wild bird surveillance efforts involving monitoring of wild bird morbidity and mortality during the 2015 outbreak, personnel sampled 27 birds of prey (summarized in **Table 15**), with 24 birds being from order Accipitriformes.[280] Of these, only one Cooper's hawk was found positive (see **Tables 14** and **15**).

Table 15. From: Collected and sampled relevant predatory and carrion wild bird morbidity and mortality for highly pathogenic avian influenza virus screening through Minnesota Department of Natural Resources sampling efforts, Minnesota, March 9–June 4, 2015.[349]

Order	Species	Number of Dead sampled
Accipitriformes	Turkey vulture (*Cathartes aura*)	1
	Bald eagle (*Haliaeetus leucocephalus*)	5
	Sharp-shinned hawk (*Accipiter striatus*)	8
	Cooper's hawk (*Accipiter cooperii*)	6
	Broad-winged hawk (Buteo platypterus)	1
	Red-tailed hawk (*Buteo jamaicensis*)	3
Strigiformes	Great horned owl (*Bubo virginianus*)	3

9.1.7.2.1.1.2 Prevalence of AI in Accipitriformes outside of AI outbreaks involving poultry

- An infectious disease survey done in Oklahoma assessing wild birds of prey admitted to a local zoo and wildlife rehabilitation clinic found from the 45 Accipitriformes sampled, only 1 Red-tailed hawk (*Buteo jamaicensis*; n=34) tested positive for type A influenza antibodies.[350]
    - Other Accipitriformes that were sampled but did not test positive for type A influenza antibodies included a Ferruginous hawk (*Buteo regalis*; n=1), Red-shouldered hawks (*Buteo lineantus*; n=2), Rough-legged hawks (*Buteo lagopus*; n=2), Marsh hawks (*Circus cyaneus*; n=3), and Cooper's hawks (*Accipter cooperii*; n=3).[350]
- Nestling (4 to 8 weeks) White-tailed sea eagles (*Haliaeetus albicilla*) that were sampled as part of an influenza A virus monitoring program in Sweden revealed a low prevalence of the virus in wild populations while no active outbreak was occurring. None of the RT-PCR results of sampled eagle (family Accipitridae) (n=181) nestlings were positive for viral RNA, and none of the serologically tested eagles (family Accipitridae) (n=123) were positive for antibodies.[351]
- A serological survey of wild birds from 2011 to 2016 in South Korea found A virus antibodies in the following Accipitriformes: White-tailed Sea eagle (*Haliaeetus albicilla*) (1/2, 50 percent H5N1 [2.3.2.1c] and H9N2) and cinereous vulture (*Aegypius monachus*) (1/6, 16.6 percent H5N1 [2.3.2.1c]). Seropositivity was determined via influenza A virus NP-specific competitive ELISA, with subtypes determined via hemagglutination inhibition assay of ELISA-positive samples.[352]
    - Other Accipitriformes that were sampled in the same study but not found to be seropositive for influenza A viruses included non-specific Buteo species (*Buteo spp.*; n=15) and Northern Goshawks (*Accipiter gentilis*; n=6).[352]
- A survey of 616 raptors admitted to two U.S. wildlife rehabilitation centers found relatively low prevalence of antibodies to influenza A viruses (subtypes not

described) in a variety of raptor species, including Accipitriformes. Results of the survey are summarized in **Table 17**.[353]

- Antibodies to influenza A (subtyping not possible due to low HI ratio in sera) were found in Bald eagles (*Haliaeetus leucocephalus*) and Cooper's hawks (*Accipiter cooperii*), but no influenza A antibodies were detected in turkey vultures (*Cathartes aura*) or black vultures (*Coragyps atratus*). See **Table 17** for exact numbers.[353]

Table 17. Serologic evidence of influenza A in raptors admitted to two U.S. wildlife rehabilitation centers.[353]

Species	Number tested	Number positive	Percent positive
Bald eagle (*Haliaeetus leucocephalus)*	406	22	5.1
Peregrine falcon (*Falco peregrinus*)	472	1	0.2
Great horned owl (*Bubo virginianus*)	81	1	1.2
Cooper's hawk (*Accipiter cooperii*)	100	1	1.0
Turkey vulture (*Cathartes aura*)	21	0	0
Black vulture (*Coragyps atratus*)	8	0	0

- A 2010 survey of antibodies to influenza A virus in wild birds revealed 0 positives out of 184 black vultures (*Coragyps atratus*) sampled in Mississippi.[314] The authors note that nearly all species of terrestrial birds tested in this study were negative for influenza A virus antibodies.

Isolated incidents of captive Accipitriformes testing positive for HPAI have been observed.

- Two Crested eagles (*Morphnus guianensis*) were confiscated after an attempt was made to smuggle them from Thailand to Belgium and tested positive for HPAI H5N1 via virus isolation. The isolate is named A/crestedeagle/Belgium/01/2004.[354]
- A German risk assessment looking at captive birds of prey used in the sport of falconry (n=54). Of the falconry birds sampled, 40.8 percent (n=24) were species in the Accipitriformes order (specifically Harris Hawks (*Parabuteo unicinctus*; n=9) and Northern Goshawks (*Accipiter gentilis*; n=13). All Accipitriformes sampled were negative for viral RNA and antibodies for any influenza A virus.[355]

9.1.7.2.1.1.3 Experimentally determined susceptibility of Accipitriformes to AI viruses

No literature on experimental susceptibility of Accipitriformes to HPAI viruses is available for review at the time of assessment; thus, there is a degree of uncertainty regarding susceptibility for these species.

9.1.7.2.1.1.4 Accipitriformes dispersal range

- Red tailed hawks (*Buteo jamaicensis*) have an average home range size of 1.06 km^2 to 2.12 km^2 depending on the sex and the season.[356] Based on data from Wisconsin populations.

- Resident Bald eagle (*Haliaeetus leucocephalus)* breeding pairs have an average home range of 22 km^2 during both the breeding or non-breeding seasons.[357] Based on data from Washington and Oregon populations.
- New World vulture (turkey vultures [*Cathartes aura*] and Black vultures [*Coragyps atratus*]), average home range sizes are reported in **Section 9.2.4** Role of HPAI Virus Spread to an Upland Gamebird Flock due to Dead Bird Disposal.

9.1.7.2.1.2 Corvidae Passeriformes

9.1.7.2.1.2.1 Prevalence of AI in Corvidae Passeriformes

- A wild bird outbreak of HPAI H5N8 occurred in the autumn and winter of the 2016–2017 seasons in the Netherlands with a mass die-off of roughly 13,600 birds. Of those 8,882 birds where the avian family was identified, 106 were identified as from the family Corvidae. Of those, two birds (Eurasian magpie [*Pica pica*] and Unidentified Corvid Species) were reported by WOAH as a positive HPAI H5N8 case. **Table 13** above summarizes exact species of all corvids sampled.[266]
- In Pakistan in 2007, four wild crows (exact species not reported) were found to be H5N1-positive following outbreaks in backyard poultry and zoo birds.[358]
- In Hong Kong in 2009, among 22 birds found dead, including chickens, 1 large-billed crow (*Corvus macrorhynchus*) was found to be infected with H5N1.[358]
- House crows (*Corvus splendens*) sampled in areas of Bangladesh that were endemic with H5N1 in poultry populations were found to have high seroprevalence to the virus in comparison to other passerine species sampled. Authors hypothesize the high prevalence is related to the large amounts of offal from live bird markets that these crows in the areas consume.[359]

9.1.7.2.1.2.2 Experimentally determined susceptibility of Corvidae Passeriformes to AI viruses

Experimental susceptibility of Corvidae Passeriformes to HPAI viruses is relatively unstudied, but the limited studies available suggest susceptibility at high inoculation doses.

- Jungle crows (*Corvus macrorhynchos*) intranasally inoculated with $10^{6.0}$ EID_{50} of one of seven HPAI viruses: showed varying degrees of susceptibility to the viruses depending on the specific virus. A total of 28 crows were included in the study, with four birds each inoculated with the same virus.[215]
    - At least two out of the four crows (*Corvus macrorhynchos*) in the groups inoculated with A/muscovy duck/Vietnam/OIE-559/2011 (H5N1), A/whooper swan/Hokkaido/4/2011 (H5N1), A/chicken/Kumamoto/1-7/2014 (H5N8), A/chicken/Taiwan/0502/2012 (H5N2), A/Anhui/1/2013 (H7N9), and A/duck/Hokkaido/Vac-1/2004 (H5N1) survived for the entire 14-day study period post-inoculation. All four crows in the groups inoculated with A/whooper swan/Hokkaido/4/2011 (H5N1), A/chicken/Taiwan/0502/2012 (H5N2), A/Anhui/1/2013 (H7N9) and A/duck/Hokkaido/Vac-1/2004 (H5N1) survived the whole study period. All four crows inoculated with A/peregrine falcon/Hong Kong/810/2009 (H5N1) were dead by 7 dpi.[215]

- Clinical signs varied depending on the virus, with crows (*Corvus macrorhynchos*) presenting depression, anorexia, and neurological signs immediately prior to death (within 1 day) when inoculated with A/peregrine falcon/Hong Kong/810/2009 (H5N1) while presenting just depression when inoculated with A/muscovy duck/Vietnam/OIE-559/2011 (H5N1) and no clinical signs when inoculated with the remaining viruses. HI titers in serum of crows inoculated with A/duck/Hokkaido/Vac-1/2004 (H5N1) measured at 14 days post-inoculation indicated that the birds did not seroconvert, and authors suggest the birds did not even become infected.[215]
- Recovered virus amounts based on virus titers from oral and cloacal swabs ranged from 1.6 to 2.4 log PFU/mL and 1.8 to 3.6 log PFU/mL, respectively, with detectable virus only being found in crows (*Corvus macrorhynchos*) inoculated with A/peregrine falcon/Hong Kong/810/2009 (H5N1), A/chicken/Kumamoto/1-7/2014 (H5N8), and A/Anhui/1/2013 (H7N9).[215]

9.1.7.2.1.2.3 Corvidae Passeriformes dispersal

- American crows have an average home range size of 6.4 km^2 to 9.6 km^2 depending on age, based on data from Illinois.[360]

9.1.7.2.1.3 Strigiformes

9.1.7.2.1.3.1 Prevalence of AI in Strigiformes in previous AI outbreaks involving poultry

- Of the wild birds that tested positive during the 2006 HPAI H5N1 outbreak in Germany, 36 (10.5 percent)were birds of prey, including one species of Strigiformes, the Eurasian eagle owl.[361]
- During an outbreak of HPAI H5N1 in poultry and wild birds that occurred 2010–2011 in South Korea, not only were cases of wild waterfowl reported, but a number of cases in wild Strigiformes were reported as well. Notably, between late January and mid-February of 2011, four Eurasian eagle owls (*Bubo bubo*) were reported to be positive for HPAI H5N1.[362]
- Of the 100 positive HPAI H5 wild bird cases identified in the United States between December 2014 to June 2015, only 1 was from a species of Strigiformes (Snowy owl [*Bubo scandiacus*]) (see **Table 14**).[348] However, Shearn-Bochsler et al. (2019) subsequently reported an additional case in a wild Great horned owl (*Bubo viriginianus*) (see **Table 14**).[363]
- Within the State of Minnesota, wild bird surveillance efforts involving monitoring of wild bird morbidity and mortality during the 2015 outbreak, personnel sampled three birds from the order Strigiformes (summarized in **Table 15**).[280] No Strigiformes were found to be positive in MN (see **Table 14** and **Table 15**).[280,348,363]
- During the 2015 HPAI H5 outbreak in the United States, there was only one case of HPAI confirmed in captive Strigiformes, specifically a Great horned owl (*Bubo viriginianus*) located in Idaho (see **Table 16**).[364]

Table 16. HPAI-positive samples from captive wild birds in the United States.[364]

Date	Species	Lineage	Sample location
3/27/15	Captive gyrfalcon (*Falco rusticolus*)	EA/AM H5N2	MT
3/27/15	Captive (hybrid)	EA/AM H5N2	MO
1/29/15	Captive gyrfalcon (2) (*Falco rusticolus*)	EA H5N8	ID
1/16/15	Captive falcons, Great horned owl (*Bubo viriginianus*)	EA/AM H5N2	ID
12/14/14	Captive gyrfalcon (*Falco rusticolus*)	EA H5N8	WA

9.1.7.2.1.3.2 Prevalence of AI in Strigiformes outside of AI outbreaks involving poultry

- An infectious disease survey done in Oklahoma assessing wild birds of prey admitted to a local zoo and wildlife rehabilitation clinic found from 36 Strigiformes (including Barred owls [*Strix varia*; n=9], Great horned owls [*Bubo viriginianus*; n=21], and Screech owls [*Otus asio*; n=6], none were positive influenza A virus antibodies.[350]
- A serological survey of wild birds from 2011 to 2016 in South Korea found influenza A virus antibodies in the following Strigiformes species: Eurasian eagle owl (*Bubo bubo*; 7/93, 7.5 percent, H5N2 and H5N1 (2.3.2.1c)).[352]
    - Other Strigiformes that were sampled in the same study but not found to be seropositive for influenza A viruses included Eurasian Scops owls (*Otus scops*; n=7), Brown-Hawk owls (*Ninox scutulata*; n=14), Tawny owls (*Strix aluco*; n=4), and Indian scops owls (*Otus bakkamoena*; n=2).[352]
- A survey of antibodies to influenza A viruses in 616 raptors admitted to two U.S. wildlife rehabilitation centers found relatively low prevalence of influenza A virus antibodies in Strigiformes. Results of the survey are summarized in **Table 17**.[353]
    - Antibodies to influenza A (subtyping not possible due to low HI ratio in sera) were found in only 1 out of 86 Strigiformes (Great horned owl [*Bubo viriginianus*]) that were sampled.[353]

9.1.7.2.1.3.3 Experimentally determined susceptibility of Strigiformes to AI viruses

No literature on experimental susceptibility of Strigiformes to HPAI viruses was available for review at the time of assessment; thus, there is a degree of uncertainty regarding susceptibility for these species.

9.1.7.2.1.3.4 Strigiformes dispersal

- Great horned owls (*Bubo viriginianus*) have an average home range size of between 1.13 km^2 and 3.76 km^2 based on season, sex, and establishment of successful pair-bonds.[356]

9.1.7.2.1.4 Falconiformes

9.1.7.2.1.4.1 Prevalence of AI in Falconiformes in previous AI outbreaks involving poultry

- Of the wild birds testing positive during the 2006 HPAI H5N1 outbreak in Germany were birds of prey, including two species of Falconiformes, specifically peregrine falcons (*Falco peregrinus*) and kestrels (*Falco tinnunculus*).[361]
- A wild bird outbreak of HPAI H5N8 occurred in the autumn and winter of the 2016–2017 seasons in the Netherlands with a mass die-off of roughly 13,600 birds. Of those 8,882 dead birds sampled during the 2016–2017 HPAI H5N8 in the Netherlands, 23 were identified as being from the order Falconiformes. Of those, only one Peregrine falcon (*Falco peregrinus*) was reported by WOAH as a positive HPAI H5N8 case. **Table 13** above summarizes exact species.[266]
- During an outbreak of HPAI H5N1 in poultry and wild birds that occurred 2010–2011 in South Korea, not only were cases of wild waterfowl reported, but also one case in wild Falconiformes. Specifically, one Common Kestrel (*Falco tinnunculus*) was reported to be positive for HPAI H5N1 by virus isolation.[362]
- The U.S. Interagency Steering Committee on Avian Influenza in Wild Birds has compiled all U.S. wild bird cases of HPAI H5 from December 2014 to June 2015. Of 100 positive birds, 1 was from the family Falconiformes (Peregrine falcon [*Falco peregrinus*]) (see **Table 14**).[348]

9.1.7.2.1.4.2 Prevalence of AI in Falconiformes outside of AI outbreaks involving poultry

There have also been cases of HPAI confirmed in captive Falconiformes.

- At least six captive falcons (including captive gyrfalcons [*Falco rusticolus*; n=4] and other undescribed species/ hybrid species; n=~2) tested positive for H5 HPAI during the 2014–2015 outbreak in the United States(see **Table 16**).[364]
- An outbreak of H5N1 clade 2.3.2.1c in captive falconry birds in Dubai and avian prey species at a breeding facility included mortality in HPAI infected gyrfalcons (*Falco rusticolus*) and hybrid gyr/Peregrine falcons (*Falco rusticolus* x *Falco peregrinus*).[365]

Surveillance of Falconiformes birds for AI virus has demonstrated low prevalence in the wild.

- An infectious disease survey done in Oklahoma assessing wild birds of prey admitted to a local zoo and wildlife rehabilitation clinic found from five American Kestrels (*Falco sparverius*) sampled, none were positive influenza A virus antibodies.[350]
- A German risk assessment looking at captive birds of prey used in the sport of falconry (n=54) found that all 32 Falconiformes falconry birds were negative for viral RNA and antibodies for any influenza A virus. Specific species of Falconiformes sampled in the study included Peregrine falcons (*Falco peregrinus*; n=28), gyrfalcons (*Falco rusticolus*; n=2), Barbary falcon (*Falco pelegrinoides*; n=1), and Lanner falcon (*Falco biarmicus*; n=1).[355]
- Nestling (4 to 8 weeks) Peregrine falcons (*Falco peregrinus*) sampled as part of an influenza A virus monitoring program in Sweden revealed a low prevalence of the virus in wild populations while no active outbreak was occurring. None of the

RT-PCR results of sampled falcon nestlings (n=168) were positive for viral RNA, and none of the serologically tested falcons (n=6) were positive for antibodies.[351]

- A serological survey of wild birds from 2011 to 2016 in South Korea found no influenza A virus antibodies in the following Falconiformes species: Peregrine falcons (*Falco peregrinus*; n=1), Eurasian hobby (*Falco Subbuteo*; n=1), and Common Kestrel (*Falco tinnunculus*; n=18).[352]
- A survey of antibodies to influenza A viruses in plasma from migrating peregrine falcons (*Falco peregrinus*; n=472) caught at a banding station, found a very low prevalence of antibodies (subtypes not described), with only 1 out of 472 (0.2 percent) falcons (*Falco*) positive for antibodies (**Table 17**).[353]

Isolated incidents of naturally infected Falconiformes falconry birds from Saudi Arabia have been reported, demonstrating captive falcon (*Falco*) propensities for infection. Such case reports illustrate the susceptibility of falcon species to HPAI viruses.

- Cases of HPAI in captive falconry birds in Dubai suggests that these Falconiformes were likely infected through consumption of infected farmed or wild prey.[365]
- A Peregrine falcon (*Falco peregrinus*) from the United Arab Emirates tested positive for HPAI H7N3 via virus isolation. No active outbreaks of HPAI H7N3 were occurring at the time in the United Arab Emirates. However, authors hypothesized the route of infection was via consumption of infected waterfowl.[366]
- During an HPAI H5N1 outbreak in a flock of Houbara bustards (*Chlamydotis undulata*) used as falconry quarry, falcons that either ate or were fed carcasses of infected of Houbara bustards (*Chlamydotis undulata*) became infected with the virus based on RT-PCR and virus isolation, with a resultant mortality of falcons being 10 out of 16.[367]

9.1.7.2.1.4.3 Experimentally determined susceptibility of Falconiformes to AI viruses

- Juvenile captive-reared gyr-saker (*Falco rusticolus* x *Falco cherrug*) hybrid falcons experimentally infected with HPAI H5N1 A/Great crested grebe/Basque Country/06.03249/2006 virus via nasochoanal inoculation (10^6 EID_{50}) and feeding of whole oculonasally-inoculated chicks, exhibited mean oropharyngeal Ct values of 28 to 35 and 26 to 32, respectively, between dpi 1 and 7. Such results demonstrate the susceptibility of falcons to HPAI H5N1 virus through the most likely natural route of infection: ingestion of infected prey. All falcons (n=17), regardless of route of infection ceased shedding by dpi 7.[368]
- Experimentally infected American kestrels (*Falco sparverius*) with H5N1 HPAI (A/whooperswan/Mongolia/244/05) demonstrated 100 percent mortality within 7 days of inoculation.[369]
    - The American kestrels (*Falco sparverius*) shed virus oropharyngeally, and to a lesser extent, cloacally. Infectious virus was not detected in cloacal samples although viral RNA was.[369]
    - Seroconversion occurred by dpi 4 to 5.[369]

- The most consistent histopathological lesions occurred in brain and pancreas; all infected birds had some evidence of both meningitis and encephalitis.[369]

9.1.7.2.2 Qualitative Analysis

We considered the following qualitative factors in evaluating this pathway:

- The risk of AI transmission is much lower from a single infected bird than from a population of birds in which infection is established. Additionally, solitary living patterns, and apparent rapid mortality in raptors make risk of spread within these predatory species less likely as demonstrated by low circulating antibodies.
- Predatory birds of primary concern that would be in contact with captive upland gamebird flocks due to predation would mostly be Accipitriformes and Strigiformes with the propensity to prey upon upland gamebirds[356,370] (personal communication, Secure Upland Gamebird Supply Working Group, August 2019). Accipitriformes of most concern include Red-tailed hawks (*Buteo jamaicensis*), Red-shouldered hawks (*Buteo lineatus*), Coopers hawks (*Accipiter cooperii*), Northern goshawks (*Accipiter gentilis*), and Bald eagles (*Haliaeetus leucocephalus*) as well as Strigiformes such as Great horned owls (*Bubo virginianus*) and Snowy owls (*Bubo scandiacus*). These birds of prey, particularly Red-tailed hawks (*Buteo jamaicensis*), are strongly attracted to upland gamebird farms, requiring trapping and removal regularly (personal communication, Secure Upland Gamebird Supply Working Group, August 2019).
- Species that would be considered most likely to contribute to environmental contamination of upland gamebird farms because of their attraction to carrion (upland gamebird flock mortality) include the previously mentioned New World vulture species (e.g., turkey vultures [*Cathartes aura*] and black vultures [*Coragyps atratus*]), Bald eagles (*Haliaeetus leucocephalus*), and Corvidae Passeriformes. Additionally, while falcons (*Falco spp.*), particularly Peregrine falcons (*Falco peregrinus*), prey singularly on avian species and have the ability to kill various upland gamebird species, because of predatory behavior of hunting only birds that are in flight, the likelihood of their contact with penned upland gamebirds is low.
- While raptors or crows (*Corvus spp.*) picking up and carrying infected prey items or carrion to an upland gamebird premises is possible, the likelihood of such behavior contributing to environmental contamination could be assumed to be negligible because raptors typically only carry food items directly to a nest or a short distance (not greater than 10 km) to a place of cover). If such an assumption doesn't hold and prey (specifically prey infected within or contaminated by HPAI virus) are carried distances greater than 10km, the likelihood of virus introduction would increase.
- As noted in the literature review, surveillance of birds of prey and scavenging birds have demonstrated that these types of birds have a low prevalence of AI virus, including the more pervasive H5N1 HPAI viruses. However, prevalence of disease in wild populations of birds is difficult to completely capture due to sampling bias (e.g., birds that have died as the result of the disease are unlikely to be captured).

- Redig & Goyal's (2008) study assessing influenza A virus prevalence in birds of prey admitted to a wildlife rehabilitation facility may come the closest to accounting for this bias given that namely clinical and downed (e,g., birds struggling to or that cannot fly) birds are most likely to be admitted. Thus, birds in a more severe clinical stage were likely sampled. However, there are limitations to this study given that influenza A virus prevalence was only assessed via presence of antibodies based on blocking.[353]
- Given that some scavenger species may have relatively large home ranges, spread beyond the Control Areas in previous outbreaks would have been expected if these birds played an important role in the transmission of HPAI.
    - Further discussion of avian scavenger species, home ranges, and factors for likelihood of transmission can be found **Section 9.2.4** Role of HPAI Virus Spread to an Upland Gamebird Flock via Dead Bird Disposal).
    - As discussed in **Section 9.1.1**, Role of Local Spread Components in Previous AI Outbreaks, most studies indicate limited spread of AI between poultry premises via mechanisms that do not involve the movement of people, vehicles, or equipment.
- Biosecurity guidelines dictate measures to prevent wild bird access to upland gamebird pens, managed dead bird disposal, and maintenance of feed bins such that wild birds are neither frequenting nor accessing upland gamebird premises (see **Section 7.5.2.3.6** Animal, Pest and Insect Control).
    - While various species of Falconiformes were shown to be highly susceptible to influenza A viruses in field cases and experimental studies, there are few studies on Accipitriformes and Strigiformes. The variability in susceptibility to infection among species, families, and orders is not well understood.

9.1.7.2.3 Likelihood Rating and Conclusion

Predatory and scavenging bird species have the potential to become infected with HPAI virus and some species have home ranges of adequate size to contain upland gamebird farms as well as infected poultry premises where they potentially may access contaminated carcasses, manure, or other infectious material. While such bird species may have contact with captive upland gamebirds, they are unlikely to have direct contact with commercial poultry flocks if standard biosecurity measures are in place. Additionally, their ability to shed virus has not been studied in many species, but there is a lack of evidence suggesting their contribution to spread of previous outbreaks. For the above reasons, the likelihood of HPAI infection via predatory and scavenging bird species in the farm vicinity was rated as ***low.***

9.1.8 Role of HPAI Virus Spread to an Upland Gamebird Premises near Poultry Live-Haul Routes via Feathers, Feces, and Other Fomites

The evaluation of the risk of HPAI virus transmission to an upland gamebird premises in a State with HPAI near poultry live-haul routes assumes the release of potentially HPAI virus-contaminated material from live-haul trailers along roadways and transportation routes near an upland gamebird premises. The birds in transit may originate from

premises inside or outside a Control Area in a State with HPAI. The likelihood that birds originate from inside of a Control Area depends on the size and scope of the HPAI outbreak; however, during an outbreak, routing of live poultry movements is planned in a manner that avoids poultry [and by extension upland gamebird] farms if the incident Management Team is using the Secure Poultry Supply plan (view permit guidances available at https://securepoultrysupply.umn.edu/). This evaluation is adapted for upland gamebird premises, and some of the concepts have been previously developed in the live broiler- and turkey- to-market risk assessments [176,177] and can be translated across the other live-bird movements.

As a requirement of the Secure Poultry Supply Plans, the Pre-Movement Isolation Period (PMIP) decreases the likelihood of infected but undetected flocks from or within a Control Area or, in the case of upland gamebirds, a State with HPAI. Additionally, upland gamebird premises in a State with HPAI requesting permitted movement can adhere to the greatly intensified biosecurity of the PMIP, which minimizes the likelihood of exposure to virus in the days leading up to movement (see **Appendix 6**: Pre-Movement Isolation Period).

9.1.8.1 Likelihood of HPAI Virus Transmission to an Upland Gamebird Premises near Poultry Live-haul Routes

The transport of an infected but undetected flock near an upland gamebird facility represents a potential pathway for local area spread. HPAI virus transfer to premises near the live-haul route could occur via HPAI virus-contaminated feathers, feces, and other fomites, which may contaminate an upland gamebird premises close to the route and may subsequently be tracked into upland gamebird pens. The two specific pathways identified are: (1) HPAI virus-contaminated fomites from a live-haul truck blow into or are tracked onto an upland gamebird premises and bring virus to flocks in pens, and (2) a contaminated live-haul road contaminates a vehicle that enters the upland gamebird premises and subsequent virus transfer into a pen. **Figure 10** diagrams the exposure pathway.

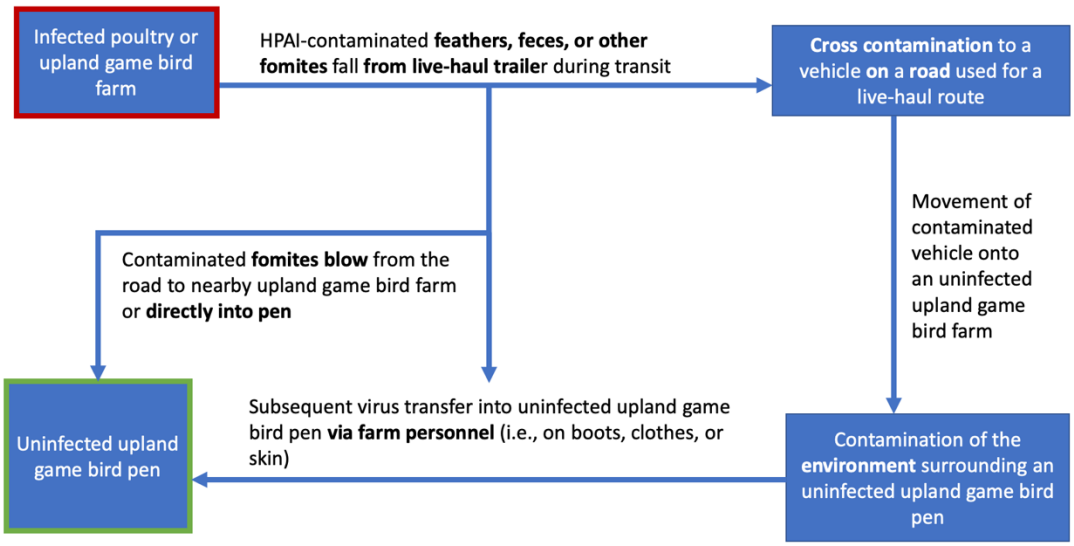


Figure 10. Pathway for exposure of an upland gamebird premises via fomites originating from a nearby live-haul route.

9.1.8.2 Literature Review

If infected poultry are transported to processing or any other destinations, the extent of virus contamination available to infect an upland gamebird flock near the live-haul route is affected by the virus shedding by the transported birds, virus persistence in the environment, and the efficiency of the virus transfer steps.

- Estimates of HPAI virus concentrations in feathers, feces, and blood from HPAI-infected poultry generally range between 10^3 and 10^7 EID_{50} per gram or per milliliter of tested substrate (see data summary in the bullets below). Various units of measure are used.
    - Feather follicles: In an inoculation study with three H5N1 HPAI viruses given $10^{7.4}$ EID_{50}, $10^{8.4}$ EID_{50}, and $10^{5.7}$ EID_{50} per duck and $10^{7.0}$ EID_{50}, $10^{8.0}$ EID_{50}, and $10^{5.3}$ EID_{50} per chicken, Nuradji et al. (2017) found that in ducks viral antigen was mainly detected in the epidermal layer of feather follicles and feathers. In chickens, viral antigen was mostly found in the dermis of these structures and that abundant antigen was found in nearly all of the chicken feathers examined.[371]
    - Immature feathers: In chicken feathers, the median viral titers for three HPAI H5N1 virus strains (A/duck/Sleman/BBVW-1003- 34368/2007, A/duck/Sleman/BBVW-598-32226/2007, and A/Muscovy duck/Vietnam/453/2004) tested were ~10^5 $TCID_{50}$/0.1mL, ~10^6 $TCID_{50}$/0.1mL, and ~$10^{5.7}$ $TCID_{50}$/0.1mL for immature pectorosternal feathers, immature flight feathers, and immature tail feathers, respectively, after feather samples were ground with a mortar and pestle.[372] From chicks inoculated with an HPAI H7N1 strain (A/Chicken/Italy/5093/99) at 15 days of age, viral RNA load was higher in feather pulp than in oropharyngeal and cloacal swabs for most days tested post-inoculation.[373] Feather pulp was obtained by squeezing the calamus (the feather quill).[373] In detached feather quills from ducks, HPAI viral titers were $10^{5.5}$ EID_{50}/mL and $10^{6.3}$ EID_{50}/mL at day 10 at 4 °C for two H5N1 virus strains (A/chicken/Miyazaki/ K11/2007 and A/whooper swan/Akita/1/2008) tested, respectively, when 4-week-old ducks were inoculated with 10^7 EID_{50}.[232]
    - Mature feathers: In chickens, viral antigen was detected in feather stromal cells and feather epidermal cells in 7- and 8-week-old chickens inoculated with Ck/Miya/K 11/07 or Ws/Akita/1/08.[374] In ducks, 3.8 percent of mature pectorosternal feather samples were positive post-challenge and, in the virus-positive feathers, titers ranged from ~$10^{0.6}$ to ~$10^{4.5}$ $TCID_{50}$/0.1 mL.[372] From 24-week-old Pekin ducks inoculated with A/duck/Nigeria/1071-23/2007, 31.25 percent of breast and tail feather calami and 37.5 percent of wing feather calami were positive by rRT-PCR at 3 dpi[375]
    - On virus survival in feathers, Karunakaran et al. (2019) conducted a simulation study to analyze the effect of preen oil on the survivability of

HPAI virus (H5N1) on duck feathers. Feathers were spiked with H5N1 virus at initial concentrations of 10^4 EID_{50} and 10^6 EID_{50} per mL, stored at either 37 °C, 25 °C or 10 °C and tested at regular intervals. Survival increased as temperatures decreased and starting dose increased. For the naturally preened duck feathers spiked with 10^6 EID_{50}, mean virus persistence was 73.3 ± 3.04 days at 10 °C and 29.7 ± 0.304 days at 37 °C. In contrast, feathers those spiked with 10^4 EID_{50}, mean survival was 55.8 ± 1.402 and 19.8 ± 0.495 days for storage at 10 °C and 37 °C, respectively.[376] Yamamoto et al. (2017) investigated the survival of virus in feather tissues collected from six chickens experimentally infected with HPAI H5N1 virus and found that viral survived 30 days and 240 days in samples stored at 20 °C and 4 °C, respectively.[377]

- Feces: In chicken feces, HPAI viral titers were greater than 10^9 ELD_{50}/g when chickens were inoculated with 1983 Pennsylvania H5N2 (SEPRL-PA isolate).[117] In feces from turkeys infected with 2015 HPAI H5N2 viruses (A/turkey/MN/12528/2015 and A/chicken/IA/13388/2015), HPAI viral titers were estimated to be between 10^3 and 10^5 EID_{50}/mL (interpolated from cloacal swab data (E. Spackman, personal communication, May 2016,[120])
- Blood: In blood from turkeys inoculated with 10^6 EID_{50} of an H7N1 virus strain (A/chicken/Italy/1067/1999), HPAI viral titers ranged from 10^1 to $10^{5.8}$ EID_{50}/0.1 mL at 1-3 dpi.[74]

- Once the virus is outside a live host, it remains viable for a varying amount of time depending on viral strain and environmental conditions, such as humidity and temperature. Virus persistence is generally longer at cooler temperatures and in more humid conditions.
  - For virus persistence data in a range of conditions and on substrates relevant to this pathway, such as feathers, feces, and water, see **Appendix 2**: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates.
- This transmission pathway is likely multi-step, and Mulatti et al. (2018) report a potential virus transmission via this route in recent outbreaks in Italy.[378] The available literature suggests virus concentration decreases with increasing numbers of transfers between surfaces. Mechanical transmission of an enveloped virus has been modeled after multiple contact steps has occurred.[379]
- Virus transfer efficiency between surfaces for non-AI viruses ranges from undetectable to 46 percent of the starting amount transferred,[380] with viral transfer efficiency between two surfaces defined as the ratio of the amount of virus transferred to a virus-free surface via direct contact, to the amount of virus present on the original contaminated surface. For more detailed information regarding the specific studies see "Appendix 5. Exploratory Scenario Analysis of HPAI Viral Titers on Surfaces via Successive Virus Transfers" in Goldsmith et al.'s (2015) *Risk Assessment of the Movement of Broiler Hatching Eggs During a HPAI Outbreak.*
- Mechanical transmission via a multiple-step pathway was documented using porcine reproductive and respiratory syndrome virus (PRRSV) in 1 out of 10

replicates by virus isolation and in 8 out of 10 replicates by RT-PCR at less than 0 °C in a swine industry-like setting.[379]

- Similar to HPAI virus, PRRSV is an enveloped virus shed in feces, urine, semen, aerosolized respiratory secretions, and other bodily fluids.
- Experimental design simulated a four-step transmission pathway: PRRSV-inoculated (field strain MN 30-100) carrier attached to undercarriage of vehicle and driven 50 km→ Contact between PRRSV-inoculated carrier and driver's boots→ Driver re-entered vehicle and drove 50 km→ Driver's boots entered farm anteroom→ Contact between farm anteroom floor and containers of four surface types (cardboard, Styrofoam, metal, and plastic).
- PRRSV RNA was detected by PCR in 8 out of 10 replicates on three of the container surface types (Styrofoam, metal, and plastic) and 7 out of 10 replicates on a cardboard container after the final transmission step at less than 0 °C.[379]
- At 10 to 16 °C, infectious PRRSV RNA was detected by PCR in 2 out of 10 replicates on the farm anteroom floor.[381]

Findings from previous disease outbreaks suggest virus transmission to a poultry premises near a live-haul route is possible.

- In the 2002–2003 outbreak of ILT on Mississippi broiler farms, mean distance of the nearest live-haul road to case farms was 0.64 km, while distance of the nearest live-haul road to control farms was 1.6 km (distance to nearest live-haul road [km]: Odds Ratio = 0.54; P-value = 0.0392; univariate analysis).[382]
- In the 1995 outbreak of LPAI H9N2 in Minnesota, visualization of the locations of positive premises on a satellite map suggested exposure to the live-haul route used to transport a known infected turkey flock that was sent to slaughter was a risk for premises infection (eight of nine premises within 250 meters of live-haul route became infected) (D. Halvorson, personal communication, June 2016).
- Close proximity to an infected premises has been associated with an increased risk of infection.[107,114,142,144,383,384] As a function of distance, the pathway of infection is not clear. For a detailed examination of the literature on local area spread in AI outbreaks, see **Appendix 3**: Literature Review on the Role of Local Area Spread in Previous Outbreaks.

If the virus is transferred into a pen, the likelihood of infection is dependent not only on the amount transferred but also the infectious dose of the virus. Mean infectious doses vary with poultry species and virus strain.[385]

- In Bobwhite quail and chukar partridges, the mean bird infectious doses (BID_{50}) were $<10^2$ for A/Northern pintail/Washington/40964/2014 (H5N2) virus and $10^{3.6}$ for A/Gyrfalcon/ Washington/40188-6/2014 (H5N8) virus. The pheasants required $10^{3.4}$ and $10^{3.0}$ BID_{50} for the H5N2 and H5N8 viruses respectively.[27]

9.1.8.3 Qualitative Analysis

We considered the following factors in evaluating this pathway:

- In a study analyzing 2015 HPAI outbreak in Minnesota, Ssematimba et al. (2019) reported that 1) on average, upland gamebird premises are 15.42 km from the nearest premises with birds compared to 3.74 km for turkey premises, 2) the average poultry farm density in a radius of 10 km of an upland gamebird premises was less than half when compared to turkey premises, and, 3) turkey premises were 3.8 times more likely to fall within a control area than were upland gamebird premises.
- While this risk assessment is limited to evaluating risk of HPAI infection on premises located outside the Control Area but within a State with HPAI, the epidemiologically relevant poultry transport on routes passing close to the premises of interest may include flocks originating inside or outside of a Control Area, which have different movement requirements.
- Permitted terminal and transfer movements of live poultry originating from a Control Area (for non-upland gamebirds) likely will require movement from a Monitored Premises (e.g., adherence to a PMIP, and rRT-PCR testing in the days preceding movement). The duration of PMIP may vary by sector and type of movement but is determined in part to provide a 95 percent probability or greater of detection in flocks exposed to HPAI virus before the PMIP begins, given a 100 percent effective PMIP.[176,177] As an example of movements originating from inside a Control Area, **Table 18** shows simulation results for the detection probability for broilers and turkeys with SPS pre-movement testing and PMIP. **Table 18** shows the simulation results for upland gamebirds with SUGS pre-movement testing and PMIP. This modeling assumed a 100 percent effective PMIP, which prevents flock exposure to virus during the PMIP. For modeling with a PMIP that is not 100 percent effective, see **Appendix 10** in the Secure Broiler Supply and Secure Turkey Supply Plans. [176,177]
- Movement of poultry from premises located outside a Control Area may not be subject to permitted movement and because the mitigations within **Appendix 6**: Pre-Movement Isolation Period (PMIP) will only apply to those upland gamebird farms that actively choose to participate in the SUGS Plan. There is no guarantee that all upland gamebird shipments originating from premises in a State with HPAI are participating in the SUGS Plan. There may be variation in pre-movement testing as State or Incident Command may require testing for poultry movements from premises in the Free Area[386] but if not, these premises may not be subject to pre-movement testing requirements beyond routine NPIP surveillance for LPAI. There is also likely variation among biosecurity practices in the Free Area. Biosecurity measures may be heightened in an outbreak scenario, but implementation may differ markedly between premises.
- For this analysis, the pre-movement surveillance modeled as the method to detect infection prior to movement from outside the Control Area for other poultry and from non-participating upland gamebird farms consists of rRT-PCR testing of 2 pools of 11 swabs and a mortality trigger of 3 birds per 1,000 for broilers and turkeys, and testing of one pooled sample of 11 swabs at the start of an 8-day 100 percent effective PMIP together with continued mortality monitoring and AC testing of 3 pooled samples of 5 swabs at day of movement for upland gamebirds.

Simulation results are shown in **Table 18** for broilers and turkeys and in **Table 19** for upland gamebirds. Viral characteristics and transmission parameters will determine when expected mortality is exceeded.[12,387] In the models for movements originating outside a Control Area (in the case of broilers and turkeys) or for movements of an upland gamebird flock not participating in the SUGS Plan, the flock could be exposed 1 to 10 days prior to movement since a PMIP is not implemented. Introduction close to movement is more likely to go undetected, and, if infection is not detected, there may be fewer infected undetected birds at movement.

- During the 2014–2015 HPAI outbreak in the United States, approximately one third (36/103) of the positive commercial premises in Minnesota were located outside a Control Area at the time of detection ((P. Bonney, personal communication, September 2016).[14]

Table 18. Detection probabilities for HPAI in broilers and turkeys using three biosecurity and surveillance protocol scenarios*

Biosecurity and Surveillance Protocol	**Detection Probability**	
	Broilers	**Turkeys**
Scenario A rRT-PCR testing of a pooled sample of 11 swabs each on 2 consecutive days with a 5- or 8-day 100% effective PMIP. Second test within 24 hours of movement.	0.98[b]	0.98[a]
Scenario B rRT-PCR testing of a pooled sample of 11 swabs each on 2 consecutive days. Second test within 24 hours of movement. No PMIP implemented.	0.74	0.60
Scenario C Detection under mortality trigger of 3 birds per 1,000 only. No PMIP implemented.	0.54	0.32

*Probabilities estimated from 6,000 simulation iterations using EA/AM HPAI H5N2 strain characteristics and considering virus exposure within 10 days of movement.
[a] 8 days PMIP
[b] 5 days PMIP

Table 19. Detection probabilities for pheasants using three biosecurity and surveillance protocol scenarios*

Biosecurity and Surveillance Protocol	Detection Probability
Scenario A	
Detection by rRT-PCR testing of one pooled sample of 11 swabs at the start of an 8-day 100% effective PMIP together with continued mortality monitoring and AC testing of 3 pooled samples of 5 swabs at day of movement.	0.98
Scenario B	
Detection by rRT-PCR testing of one pooled sample of 11 swabs 8 days prior to movement together with continued mortality monitoring and AC testing of 3 pooled samples of 5 swabs at day of movement. No PMIP implemented.	0.68
Scenario C	
Detection by rRT-PCR testing of one pooled sample of 11 swabs 8 days prior to movement together with continued mortality monitoring and AC testing of 3 pooled samples of 5 swabs at day of movement. No AC testing and no PMIP implemented.	0.45

*Probabilities estimated from 10,000 simulation iterations using A/chicken/NL/621557/03 (H7N7) HPAI strain characteristics and considering virus exposure within 12 days of movement.[a]

[a]Detection by mortality trigger of 1.5 birds per 1,000 on two consecutive days, PCR se =86.5% and AC se =50%

- If infected poultry are transported to processing, the initial contamination for this pathway is dependent on HPAI VIRUS-contaminated material falling from the live-haul trailer. Feathers, feces, and other potential fomites fall from live-haul trailers because they are not enclosed, as shown in **Figures 11-14** (D. Halvorson, personal communication, July 2016), with the trailer set up for upland gamebirds being similar to those used for the conventional poultry live bird movements, as shown in **Figure 14**. Day-old chicks and poults are transferred in different vehicles and are totally enclosed.
- Netting systems to contain feathers in the live-haul trailer typically are not used because they are ineffective and create an additional biosecurity issue as nets are difficult to clean. Thus, nets were not used on live-haul trucks during the 2014–2015 or 2016 U.S. HPAI outbreaks.[176] The likelihood of this contamination reaching a premises and infecting the flock may depend on the distance of the premises from the live-haul road, weather conditions, natural barriers/landscape, and virus transfer steps.

Figures 11-14 show the crates used for live haul in the broiler, turkey, and upland gamebird industry.

Figure 11. Crates filled with broilers to be loaded onto a live-haul truck (Photo courtesy of GNP Company).

Figure 12. Live-haul trailer of turkeys after load-out (Photo: Anonymous)

Figure 13. Live-haul trailer of turkeys (Photo: Jill Nezworski.)

Figure 14. Live-haul trailer of pheasants (Photo courtesy of Tim Zindl of Oak Ridge Pheasant Ranch, Inc.)

- Close proximity of pens to township roads is observed in the upland gamebird industry, however, upland gamebirds are not often near poultry premises.
    - Upland gamebird farms, specifically production pens, can be located close to public roads, with variation reported among industry representatives, (e.g., anywhere between a couple hundred feet to a quarter of a mile) (personal communication, Secure Upland Gamebird Supply Working Group, August 2019).
    - The roads with the closest proximity to upland gamebird pens are typically Township roads, which are inherently unlikely to be used by poultry haulers based on reports (personal communication, Secure Upland Gamebird Supply Working Group, August 2019). Such reports are supported with the documented geographic isolation that upland gamebirds have in relation to poultry slaughter facilities and other poultry premises, especially compared to conventional poultry premises (e.g., turkey, broiler, or layer premises).

- During the 2014–2015 HPAI outbreaks in the United States, live-haul routing did not require approval for permitted movement in Minnesota (Minnesota Board of Animal Health, personal communication, October 2016) and were not mandated by Incident Command in 2016 in Indiana.[176] However, the distance between the live-haul roads and poultry premises may be efficiently maximized by strategic routing, when possible or based on company requests. Poultry live-haul routes are determined by individual bird growers based on timing and bird welfare.[176,177] Large upland gamebird producers engage with State poultry industry groups as well as with State agencies to have up to date information and participate in routing determinations, however, engagement and participation varies between producers and States (Secure Upland Gamebird Supply Working Group, personal communication, August 2019).
- Poultry companies near outbreaks have communicated frequently and shared locations of premises; although knowledge of the locations of other poultry premises by a particular company or veterinarian varies.[176,177] Again, upland gamebird producers have varied engagement with conventional poultry industries, especially outside of communication facilitated by State poultry organizations or State agency-driven communication (Secure Upland Gamebird Supply Working Group, personal communication, August 2019).
    - In geographic areas with many poultry production premises, routing may take on increased importance due to the density of susceptible birds near a route. However, upland gamebird premises are less likely to be located in high density areas with the average distance between upland gamebird premises and any other commercial premises in Minnesota is 15.42 km.[14]
    - For permitted movement from premises in an HPAI Control Area, both the Secure Broiler Supply (SBS) and Secure Turkey Supply (STS) Plans recommend live-haul route approval from the Incident Command team or routes selected in consultation with a poultry veterinarian or production manager.[388,389]
- In the management of ILT outbreaks, geographic information system (GIS)-assisted live-haul route planning has been used to minimize the number of farms within a specified distance along the route to processing from a broiler premises in a Biosecurity Zone.[390] The transmission steps of this pathway could be affected by weather conditions, natural barriers/landscape, and C&D.
- Since feathers are lightweight, transmission to the premises via feathers over short distances might be a possibility. Weather conditions such as wind and precipitation as well as natural barriers/landscape between the live-haul route and upland gamebird premises may affect whether virus arrives on-farm. As most upland gamebird pens are outdoor, feathers could blow directly into a pen but given the distances of pens from State highways and decreased likelihood that live haul trailers will travel through areas where upland gamebird farms are located, feathers blowing into pens is not a likely event.
- Virus transmission from a live-haul trailer to a premises close to the road represents a multi-step transmission pathway. With each virus transfer step, virus

concentration is likely to decrease. Among the potential pathways identified, blowing of HPAI VIRUS-contaminated fomites from a live-haul trailer to an upland gamebird premises, with subsequent transfer into the pen, involves fewer transfer steps compared to a vehicle bringing virus to an upland gamebird premises from a contaminated live-haul road, followed by transfer into the pen.

  - The minimum biosecurity guidelines for poultry premises participating in the NPIP and the intensified biosecurity of the PMIP upland gamebird premises in a State with HPAI that wish to follow the guidance of the SUGS Plan during an outbreak are designed to reduce the likelihood that contamination which reaches the premises would subsequently infect the flock.

- Standardized biosecurity in the poultry industry, including the upland gamebird industry, such as rules about entering the perimeter buffer area, crossing lines of separation, and managing vehicle access, are intended to prevent flock exposure to disease agents.[391]
- For upland gamebird premises in a State with HPAI that wish to participate in the SUGS Plan and move live birds, the enhanced biosecurity of the PMIP minimizes the chances of a flock being exposed to HPAI. The PMIP reduces the likelihood of a vehicle contaminated from a live-haul road bringing virus to an upland gamebird operation, as all vehicles will be cleaned and disinfected before entering the premises. A requirement to use pen-specific footwear to enter the pen (and barn-specific footwear for brooder barns) during the PMIP minimizes introduction of virus via tracking into the pen on the boots of personnel. The pertinent biosecurity guidelines of the PMIP are:
  - Limiting visits to the premises to critical operational visits
  - Requiring specific biosecurity for those critical visits (see **Appendix 6**: Pre-Movement Isolation Period)
- Vehicles and any equipment arriving on an upland gamebird premises may be difficult to disinfect thoroughly, especially during harsh winter conditions. Thus, the virus may remain on vehicles contaminated from the live-haul route, despite C&D.
- Previously, 10 experienced poultry veterinarians evaluated the risk of infecting susceptible poultry flocks via the microbial load from 2 truckloads of turkeys shedding a generic pathogen at varying distances (results shown in **Table 20)**.[392]

Table 20. Perceived qualitative risk posed by two truckloads of turkeys at varying distances from susceptible poultry based on expert opinion, as reported in Halvorson and Hueston (2006).[392]

Distance to susceptible poultry	**10 m**	**100 m**	**1,000 m**	**10,000 m**
Risk rating*	Intolerable	Intolerable	Low	Negligible

*Risk rating scale of negligible, low, moderate, high, and intolerable.

- The results of the veterinarian survey were strongly correlated (P<0.01) with the values calculated with an exposure risk index, which took into account mass of contaminant, percentage of the pathogen available for transmission, initial titer of the pathogen, age of contaminant/half-life of virus, and distance to susceptible poultry.[392]
- Given that the susceptible poultry above had fully enclosed flocks in mind, the risk ratings are not directly translatable to the risk that would be posed to upland gamebird flocks.

9.1.8.4 Likelihood Rating and Conclusion

9.1.8.4.1 Likelihood of HPAI Transmission to an Upland Gamebird Premises in a State with HPAI near Route of Live-Haul Trailer

Literature review and expert opinion indicate a potential for increased risk when a poultry premises is located close to live-haul routes used for transporting infectious birds. This risk is most likely elevated if birds are in outdoor pens. The guidance for the SPS plans, specifically implementing an effective PMIP, increase the likelihood of detection prior to scheduled movements that originate in a Control Area (in the case of broilers, turkeys, and layers) or from a State with HPAI (in the case of upland gamebirds). Vehicles transporting live poultry from a Monitored Premises following SPS plan guidance (PMIP, PCR, AC testing) are less likely to represent an infected but undetected movement than if the PMIP and testing are not in place. As presented in **Section 9.4** Likelihood of Detecting HPAI in an Infected Upland Gamebird Pen, it is also unlikely that flocks moved after a PMIP and testing would contain large numbers (e.g., 50 or more) of clinically infected birds.

During the 2014–2015 HPAI outbreak in the United States, infected premises were identified both inside and outside Control Areas at the time of detection. It is expected that biosecurity may be heightened during an outbreak scenario; however, there may be variation in biosecurity and pre-movement testing from the Free Area (unless they are upland gamebird farms following SUGS Plan guidance). With the use of a mortality trigger alone or pre-movement testing without implementing a PMIP, the likelihood of detecting HPAI virus in a flock before movement is estimated to be substantially lower than the detection probability with a PMIP in place.

9.1.8.4.2 Conclusion

Considering the above factors, if the preventive measures specified in the SPS plans are strictly followed when moving live poultry and given that live-haul vehicles passing a premises in a State with HPAI may originate from within or outside a Control Area, the following likelihood ratings are provided:

The likelihood of HPAI infection at an upland gamebird located in a State with HPAI due to HPAI-infected poultry or contaminated live-haul vehicles passing on a nearby road is rated:

Likelihood rating at given distance (between live-haul road and poultry premises)

Characteristics of live-haul vehicle	<100 meters	100-1000 meters	>1000 meters
Truck hauling birds that had no PMIP and no tests	*High*	*Moderate*	*Low*
Truck hauling birds that had less than optimum PMIP and tests (80% effective PMIP; delayed testing; or load-out >24 hours)	*Low*	*Very Low*	*Negligible*
Truck hauling birds that had a PMIP & rRT-PCR negative birds (100% effective PMIP; two tests within 24 hours of move and completion within 24 hours)	*Very Low*	*Negligible*	*Negligible*

9.2 Pathways for an Upland Gamebird Flock Becoming Infected with HPAI via Movements of People, Vehicles, or Equipment

9.2.1 Role of Movements of People, Vehicles, or Equipment in Previous AI Outbreaks

Movements of people, vehicles, and equipment may transfer potentially infectious or contaminated materials between farms. A review of past outbreak experiences indicates that the majority of spread of AI virus between farms can be attributed to the movement of people and equipment.[393] In this chapter, we evaluated the likelihood of spread due to the movement of relevant fomites involved in specific processes and contexts including movement of growers and employees and their vehicles, critical operation visits, dead bird disposal, and garbage management. While other Secure Poultry Supply Plan risk assessments explore pathways associated with shared equipment, the pathway is excluded from analysis in this chapter due to the practice of sharing equipment not being relevant in the upland gamebird industry (personal communication, Secure Upland Gamebird Work Group, August 2019).[14]

9.2.2 Role of HPAI Virus Spread to an Upland Gamebird Flock via Critical Operational Visits during PMIP

Routine operational visits to an upland gamebird farm include feed delivery, propane delivery, shavings delivery, and visits from flock veterinarians, meter readers, repairmen, customers, and others. The SUGS Plan requires most operational visits to be halted or occur outside of the PBA during the PMIP before moving upland gamebirds. However, some critical operational visits, such as feed delivery, would need to continue during the PMIP. Feed delivery for upland gamebirds varies depending upon the size of the farm as well as season. On average, one pheasant will eat one pound of feed per week, increasing amounts as the temperature decreases,[10] however the frequency of deliveries varies and will be heavier between August and October when the hunting season opens and hunting preserves and hunt clubs are looking to populate their grounds with flight-ready. At the peak of growing season feed deliveries can occur multiple times per week depending on the size of the farm and may taper off to biweekly or monthly as mature birds are sold (personal communication, Secure Upland Gamebird Work Group, August 2019).

Other deliveries such as propane and shavings vary based on season. Typically, propane deliveries can range from every other week to every few months depending on if the farm is enduring a cold winter season. Shaving shipments occur once annually to every six weeks during the spring/early summer brooding season (personal communication, Secure Upland Gamebird Work Group, August 2019). Visitors providing services such as veterinarians, repairmen, meter readers, and inspection personnel have varied frequencies for their visits depending on the needs of the farm. Unlike in conventional poultry industries such as broiler, turkey, and/or layer industries, company service personnel, multi-premises farm managers, critical mechanical equipment repair personnel, vaccination crews, and contracted load-out crews are not utilized in the upland gamebird industry (personal communication, Secure Upland Gamebird Work Group, August 2019). Pit inspectors do visit quail farms every three to four months (personal communication, Doug Anderson, August 2019).

9.2.2.1 Likelihood of Infection via Feeds

During the 2015 HPAI outbreak in Minnesota and Iowa, risk managers were concerned about biosecurity practices related to storage of feed ingredients and finished feed. Specifically, the observation of corn piles stored on the ground at feed mills and contaminated with wild bird feces raised concerns about the possibility that contaminated corn might be a pathway for HPAI virus introduction and spread. Additional concerns include the chance that finished feed could become contaminated by wild birds through breaches in biosecurity at the feed mill or feed storage bins on a farm.[394] Feedback from the Secure Upland Gamebird Supply Working Group indicated that feed spilled on upland gamebird farms (outside the pen) would not be fed to the birds (personal communication, Secure Upland Gamebird Supply Work Group, August 2019).

Feed is specifically formulated at mills for upland gamebirds and resembles poultry feed, most often being supplied by feed mills that supply other poultry or livestock farms (personal communication, Secure Upland Gamebird Supply Work Group, August 2019). The Feed Risk Assessment assessed the risk of HPAI transmission to poultry fed contaminated feed in a variety of scenarios listed in **Table 21**. Further information can be found in the Feed Risk Assessment.[394]

Table 21. Risk ratings for various types of poultry feed products.[394]

Pathway	Risk
Potential that corn stored on ground is contaminated with feces from wild migratory birds	*Low to very low**
Potential that pelleted feed made with contaminated corn transmits HPAI to poultry flock	*Negligible*
Potential that untreated mash feed made with contaminated corn transmits HPAI to poultry flock	*Low to very low*
Potential that formaldehyde-treated mash feed made with contaminated corn transmits HPAI to poultry flock	*Negligible*
Potential that finished feed contaminated by perching birds at feed mill or storage bins on farm transmits HPAI to poultry flock	*Low to very low*

*Under fall and spring seasonal conditions

9.2.2.2 Likelihood of Infection via Feed Delivery or Other Critical Operations Visits

Under normal operations, feed vehicles may deliver to multiple farms the same day (a range of 0 to 5 deliveries per day was used in Dorea et al. [2010]).[395] The possible pathways for transmission via feed delivery involve contamination of the vehicle or driver at an infected but undetected farm, and subsequent cross-contamination of a virus-free upland gamebird premises. During the PMIP, only the following critical operational visits to the premises are allowed:

- Feed delivery in a dedicated truck directly from a stand-alone feed mill
- Veterinary visits to address changes in bird health

Additionally, during the PMIP, the feed truck delivering feed to upland gamebirds under a PMIP should not also enter a Control Area. In addition to feed delivery, other critical operations visits (e.g., veterinary visits) are assumed to offer a similar potential pathway to that of feed trucks.

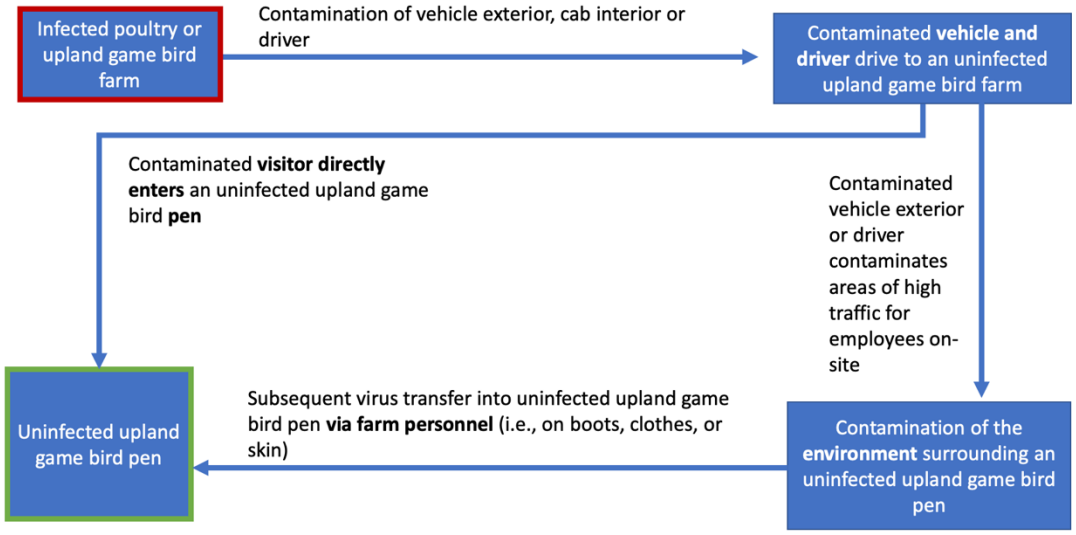


Figure 15. Pathway for exposure of an upland gamebird farm via a feed delivery or critical visitor vehicle

9.2.2.2.1 Literature Review

- In a Monte Carlo simulation model based off of results from a survey of contract broiler growers in the United States, feed delivery accounted for 74 percent of total point estimates of risk for farms using the same integrator as index farm.[396] Of note, this model considered all vehicle/visitor traffic to a farm, even activities that would not be allowed under PMIP, and did not account for differences in magnitude of virus contamination in different types of visitor contacts.
- Similarly, a stochastic model by Dorea et al. (2010) predicted that off-farm spread of HPAI by visitors is most frequently associated with feed trucks and company personnel.[395]

  - Of the reviewed HPAI and LPAI outbreaks in the U.S., feed delivery or contaminated feed was implicated in only the 1983–1984 Pennsylvania outbreak (mixed LPAI/HPAI).[393]

- In a model of risk for ILT infection during an outbreak, farms with more visits per month by feed trucks were associated with higher risk for ILT (OR=1.18; P=0.0099).[382]
- From the data collected during the 2003 H7N7 HPAI epizootic in the Netherlands, Ssematimba et al. (2012) estimated the probabilities of virus transmission as 0.0414 per feed delivery contact and 0.133 per other-professional contact, causing an estimated 2.63 and 0.94 percent of all infections, respectively.[107] For the same epidemic, another study calculated an upper estimate for the probability of transmission by a person per visit as 0.037.[397]
- For the 2016–2017 H5N8 HPAI epidemic in Italy in which 83 poultry farms (16 and 67 in first and second epidemic wave respectively) were infected, movement of feed trucks was the most abundant information available (n = 314), although only nine contacts (2.87 percent) occurred directly between infected farm pairs.[378]
- During the 2017 Tennessee H7N9 LPAI outbreaks, the six commercial farms involved five different integrated poultry complexes suggesting unique sources of feed, among other supplies for most of the cases.[54]

9.2.2.2.2 Qualitative Analysis

We considered the following factors in evaluating this pathway:

- Feed truck visits and feed delivery are likely to occur on most, if not all, upland gamebird operations during the PMIP.
- While feed truck visits will be the most frequent type of contact during PMIP, they are subject to specific biosecurity guidelines outlined in the SUGS Plan (see SUGS PMIP recommendations for a full list of biosecurity requirements relevant to feed trucks and drivers).[389]
  - Feed trucks delivering feed must not have entered a Control Area, prior to delivering feed to the upland gamebird premises.
  - Feed truck drivers may not enter the upland gamebird pen or brooder house and must put on disposable boots and gloves before exiting the truck cab onto the premises.
  - Feed truck drivers will sanitize or wash hands before leaving and upon re-entering the cab, and will spray the cab interior floors, pedals, and bottoms of feet after every stop with disinfectant.
- The SUGS Plan also outlines biosecurity practices for other critical visitors (e.g., veterinarians).
  - Personnel who have contact with upland gamebirds or poultry on other premises must shower and change clothes before entering the premises and wear necessary protective clothing and footwear as described in appropriate biosecurity protocols.
  - All vehicles and equipment will be C&D prior to entering premises.

- Critical visitors other than those associated with feed delivery may be required to enter an upland gamebird pen to complete their necessary tasks (e.g., bird health inspection by a veterinarian).
- Visitors who enter upland gamebird pens during PMIP may contact birds directly, thus decreasing the number of steps in the potential pathway to infection diagrammed above.

9.2.2.2.3 Likelihood Rating and Conclusion

Critical operations visits will be limited during PMIP; however, delivery of feed during this will continue and there is potential for veterinary visits as needed. Assuming all requirements for biosecurity during PMIP are followed, the likelihood of introducing HPAI virus to an upland gamebird flock by feed, feed delivery, and critical visits during PMIP is as follows:

Pathway	Likelihood
Contaminated feed	*Negligible*
Feed delivery (driver and/or vehicle)	*Low*
Other critical visitors (veterinary personnel and/or vehicle)	*Low to Moderate*

9.2.3 Role of HPAI Virus Spread to an Upland Gamebird Flock via Growers or Employees and their Vehicles Entering the Premises

Off-site movements of poultry growers, their families, and their employees have been implicated as risk factors for disease transmission in previous outbreaks of avian influenza,[109,382] with such risks being translatable to upland gamebird growers, their families, and their employees. While already a common practice outside of outbreak scenarios for most upland gamebird farms, growers and employees of susceptible upland gamebird farms following the SUGS Plan will not be permitted to visit poultry farms or other upland gamebird farms during the PMIP. However, off-site social contacts with other growers may still occur, albeit this is reportedly a rare occurrence for upland gamebird growers outside of growers attending annual industry conventions and conferences (personal communication, Secure Upland Gamebird Supply Working Group, August 2019). Additionally, during a PMIP, all non-critical visitors are prohibited from entering upland gamebird premises, and thus, vehicle and personnel traffic is likely to include only growers, employees, and critical visitors. For a discussion on critical operations visitors and their vehicles, see **Section 9.2.2**, Role of HPAI Virus Spread to an Upland Gamebird Flock via Critical Operational Visits During PMIP.

9.2.3.1 Likelihood of Infection via Movement of Growers and Full-Time Employees

The possible pathways for transmission via social contacts between growers and/or employees involve contamination of the growers' or employees' clothes, shoes, hands, or vehicle at a meeting place with a person from an infected but undetected poultry or upland gamebird farm, and subsequent cross-contamination of a virus-free upland gamebird premises. These pathways are shown below in **Figure 16**.

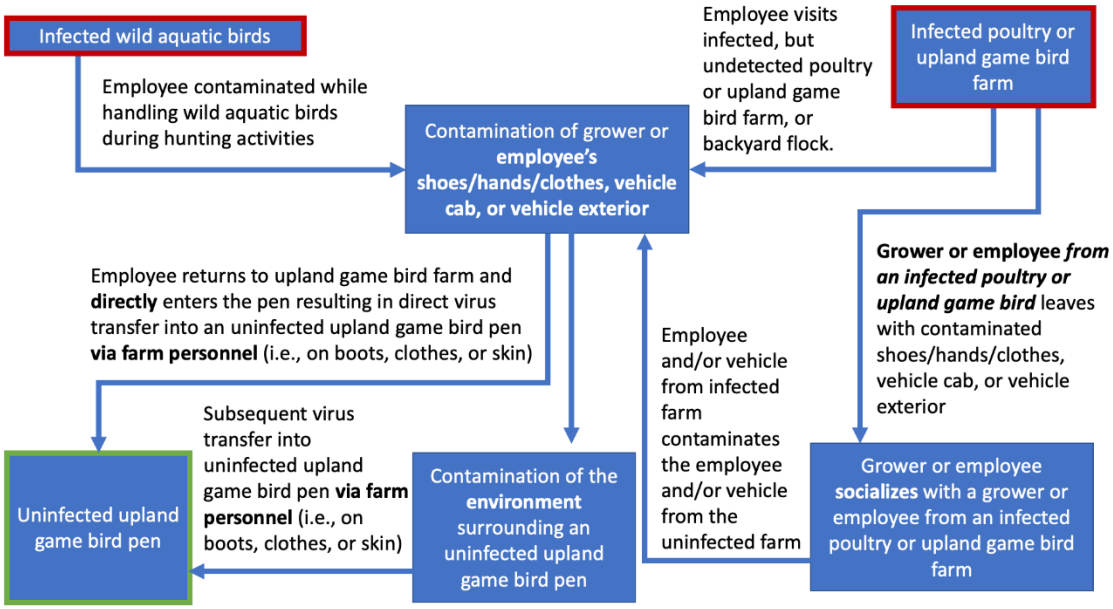


Figure 16. Pathway for exposure of an upland gamebird premises due to virus introduction by grower or employee.

9.2.3.1.1 Literature Review

- HPAI virus has the potential to be transmitted via feces-contaminated shoes or vehicle tires, depending on ambient temperature, humidity, and elapsed time. For additional information on virus survival on various surfaces and under various conditions, see **Appendix 2**: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates.
    - At low ambient temperatures of 4.0 to 6.7 °C and low to moderate relative humidity (15.2 to 46.3 percent), HPAI H5N1 (A/Vietnam/1203/2004) in chicken feces remained viable until day 13.[398]
    - However, at temperatures closer to summer conditions in the United States (22.4 to 23.7 °C and 89.1 - 91.2 percent relative humidity), the same HPAI H5N1 virus strain in chicken feces was inactivated at day 4.[398]
    - On two rubber surfaces (gumboot and tire) at an unspecified room temperature, LPAI H13N7 was below the detectable limit by day 6.[399]
- Glanville et al. (2010) used modeling to predict the average probability of HPAI H5N1 virus transmission via contaminated shoes from a house *in which an infection is beginning* into a house on another farm (if shoes are not cleaned and disinfected) to be P= 0.039 to 0.15 per transfer event.[400]
    - The model was based on a small-scale broiler farm in Indonesia, and model parameters were estimated from survey data, literature review, and expert opinion.[400]

  - Variables affecting the risk estimation include viral concentration on shoes after arriving at the second broiler farm, as well as the proportion of fecal matter (and virus) transferred from the shoes.[400]
  - In the same study, imposing a mandatory 24-hour downtime between farms decreased the predicted probability of transmission to P=0.0016 in this exploratory model.[400]

- The probability of human-mediated HPAI H7N7 virus spread between farms during the 2003 epidemic in the Netherlands was quantified as 0.0011 per crisis organization (e.g., visits representing organizations that aimed to control the outbreak) contact and 0.133 per other-professional contacts, respectively, accounting for 0.13 percent and 0.94 percent of all secondary spread cases.[401] For the same epidemic, another study calculated an upper estimate for the probability of transmission by a person per visit as 0.037.[397]
- Respiratory viruses can be transmitted via human hands, though studies with HPAI virus are lacking.
  - As detailed in Appendix 6 of the *Risk Assessment of the Movement of Broiler Hatching Eggs During an HPAI Outbreak*, several studies have determined the transfer rate for various non-AI viruses between different surfaces, including from fingerpad to fingerpad.[380] Depending on the virus, percentage transferred via fingerpads ranged from undetectable to 23 percent.
  - Ansari et al. (1991) demonstrated that 20 minutes after deposition on donor fingertips, 0.7 percent of human rhinovirus transferred to recipient fingertips.[402] On the other hand, transfer of human parainfluenza virus was undetectable at 20 minutes post-deposition. Both parainfluenza and rhinovirus are enveloped, single-stranded RNA viruses similar to influenza.
  - Assuming a virus transmission efficiency of 0 to 20 percent, and based on data extrapolation from other viruses (including the above study), modeling by Glanville et al. (2010) demonstrated an average 5 percent chance of a bird being infected with HPAI H5N1 virus via hand contact with someone who *directly* handled an infected bird at another farm.[400] This estimate applies only to the first susceptible bird handled and incorporates the effect of estimated travel time—specific to the study locale in Indonesia—on virus decay.

9.2.3.1.2 Qualitative Analysis

We considered the following qualitative factors for evaluating this pathway:

- Movement of people, including temporary staff, shared personnel, company supervisors, and part-time employees, has been implicated in the spread of poultry viruses in previous outbreaks, although such personnel types are not common in the upland gamebird industry.
  - In the epidemiological questionnaires and interviews conducted during the 2015 HPAI H5N2 outbreak on pullet and layer premises in Iowa and

Nebraska, nine out of the 28 (32 percent) producers from HPAI case farms suggested potential virus spread via the movement of supervisors or employees who visited multiple company premises. Additionally, authors observed that visits from company service personnel within the last 14 days put premises significantly more at risk for infection, with 50 percent of HPAI case farms (n=28) having visits from service personnel compared to 19 percent of control farms (n=31) (odds ratio = 5.0, $p < 0.001$, AF = 15.0 percent).[403]

- Researchers studying the 1999-2000 H7N1 outbreaks in Italy, which included LPAI and HPAI outbreaks in turkeys, broilers, layers, and other poultry types, have suggested that temporary staff on larger farms may have contributed to the identification of larger farm size as a risk factor for infection.[144]

- In the 2002–2003 infectious laryngotracheitis (ILT) outbreak in Mississippi, farms whose workers visited other chicken farms daily were significantly more likely to be infected with ILT virus than those with less frequent visits (OR = 13.75; multivariate analysis).[382]
- Alexander stated that the dominant route of secondary spread in domestic poultry has been via people and that farm owners and caretaker staff have been implicated in the spread of AI.[58]

However, the frequency and types of people moving between upland gamebird farms is very different from movements of people in conventional poultry industries.

- In Australia, the type and frequency of horizontal contacts between upland gamebird farms is substantially different from those in the commercial chicken industry,[404] and studies in the United States report roughly similar findings.[8,14] Generally, the frequency of people, stock, and equipment moving between upland gamebird farms is much lower than that occurring in the bigger integrated poultry industries in Australia[404] and in the United States.[8,14]
- Social contacts between growers have been evaluated as a risk in disease transmission in a poultry producer setting based on social science data combined with stochastic disease modeling[396] and field experiences including in the H7N2 LPAI in the 2001–2002 Pennsylvania outbreak in broiler chickens.[405]

However, analytical studies on disease transmission resulting from off-farm social contact between poultry growers are lacking.

- Additionally, while studies assessing social contact and disease risk in conventional poultry industries exist, there are no comparable studies available in the commercial upland gamebird industry, and frequency of contacts has not been studied.
- There is the potential for growers, members of their households, or employees to have regular social or other contacts with other upland gamebird or poultry growers or employees. During the PMIP, however, these contacts will occur off the upland gamebird premises.
- Growers or household members who may potentially become contaminated via social contacts should, however, change clothes and shoes before encountering birds on their premises.

- PMIP measures state that for the duration of PMIP, growers must wear clothing dedicated to the farm and shoes dedicated to the pen before entering upland gamebird pens or brooder barns. See **Appendix 6**: Pre-Movement Isolation Period.
- The level of contamination on the person a grower is meeting, however, may be variable.
    - Other growers whose premises are operating under heightened PMIP biosecurity may represent a lower risk as they will have taken measures to remove any potential virus contamination before departing the premises.
        - SUGS measures state that growers participating in the SUGS Plan should shower and change to clean clothes before leaving the farm during PMIP (See **Appendix 6**: Pre-Movement Isolation Period).
        - As detailed in Appendix 6 of the Broiler Hatching Eggs Risk Assessment,[380] several studies have demonstrated the effectiveness of showering and changing clothes in preventing the transmission of infectious diseases.
    - There are no cleaning or disinfection stipulations for other poultry and upland gamebird growers and their employees who are not observing a PMIP. While it is reasonable to assume that biosecurity may be heightened in the face of an HPAI outbreak (especially for poultry farms within a Control Area or upland gamebird farms located in a State with HPAI), the practices utilized on individual commercial or noncommercial poultry premises will likely vary.
- As outlined above, virus may survive days to weeks, depending on weather conditions and type of contaminated surface.
    - A grower with contaminated boots, hands, or clothing may drive on his or her premises (for example, from working in an upland gamebird pen to residence on the same premises) without any C&D step. This contamination may remain in the cab of a vehicle, thus re-contaminating an individual who uses that vehicle to drive off-site to meet with another grower or employee of a poultry or upland gamebird farm.
- The potential pathways involve multiple virus transfer steps between contact surfaces. In general, the chances of the pathway resulting in virus transmission decrease with the number of contact steps that need to occur. Furthermore, even if the transfer steps occur, there would likely be a substantial reduction in the virus concentration transferred with each contact step. This is because only a fraction of the virus (6 to 27 percent) on a donor surface is transferred to the recipient surface in each direct contact event.[380]
- Viral contamination on the exterior of a vehicle on an infected and undetected farm, already reduced by dilution outside the pen/house (depending on the type of commercial farm), would undergo multiple transfer steps each with a reduction in viral load *(e.g., vehicle tires→ travel to social meeting place→ ground surrounding social meeting place→ tires of vehicle from uninfected farm→ travel to uninfected farm→ ground surrounding uninfected pens→ grower's boots→ uninfected pen).*

- If, however, the social contact was directly contaminated and the grower contaminated the interior of the vehicle, which is not cleaned or disinfected before use on farm, fewer contact steps are needed *(e.g., contaminated grower colleague→ grower→ vehicle→ re-contamination of grower hands/clothes→ uninfected pen).*
  - In this scenario, contamination in the interior of a vehicle serves as a point of re-contamination even if a grower were to change clothes and boots before working with poultry.
- In the period before the PMIP begins, growers may visit other upland gamebird farms or poultry farms, thus decreasing the number of transfer steps needed to bring virus onto the premises, where it may be tracked into the pen during PMIP.

- Biosecurity measures such as wearing PPE, dedicated work clothing, pen-dedicated footwear, showers, and hand hygiene further reduce the likelihood of virus transmission. In an outbreak situation, it is expected that biosecurity measures may be heightened on many premises in addition to those undergoing the PMIP.[176,177]
  - Appendix 6 of the Broiler Hatching Eggs Risk Assessment details the effectiveness of PPE and hand hygiene in mitigating the transmission of infectious diseases.[380]
  - Post-outbreak questionnaire data from case turkey premises (n = 81) in the 2015 outbreak in the Upper Midwest showed that 25.2 percent of surveyed premises had a changing area where poultry workers took a shower; at 71.8 percent of surveyed premises, poultry workers wore dedicated laundered coveralls before entering each house; and at 98.1 percent of surveyed premises, poultry workers wore rubber boots or boot covers in poultry houses.[226]

9.2.3.2 Likelihood Rating and Conclusion

Although some contact may be unavoidable, it is recommended that growers and their employees minimize unnecessary contact with other growers or employees of other upland gamebird or poultry farms during the PMIP and restrict travel to poultry premises or other upland gamebird premises during the entire grow period. Still, social and other non-business contacts have the potential to occur between growers, members of their families, or employees. During the PMIP, vehicle and visitor traffic to susceptible upland gamebird premises will be decreased to include only critical visitors, employees, and growers. The prevention of HPAI virus transmission by growers and employees during the PMIP is dependent on close adherence to the biosecurity measures outlined in the PMIP.

Provided the SUGS PMIP measures for growers and employees are strictly followed, the likelihood of HPAI transmission during the PMIP is as follows:

Personnel type	Likelihood Rating
Critical operations visitors and vehicles	See **Section 9.2.2** Role of HPAI Virus Spread to an Upland Gamebird Flock via Critical Operational Visits during PMIP
Growers and employees entering upland gamebird pens during PMIP	*Low*
Employees who may contact other birds (not entering barns during PMIP)	*Very low*

9.2.4 Role of HPAI Virus Spread to an Upland Gamebird Flock via Dead Bird Disposal

The process of dead bird disposal in this risk evaluation relates to normal mortality on an upland gamebird premises, as opposed to mortality from known infected premises (i.e., not including FAD-related depopulation). Processes described are recommended within the SUGS Plan and the PMIP document (see **Appendix 6**: Pre-Movement Isolation Period).

Dead upland gamebirds must be regularly collected and removed from pens in a biosecure manner and moved to an on-site location that is as far away from the pens and brooder barns as possible; containers (dumpsters) for dead upland gamebirds should never leave the farm although best practice is to place the outside the perimeter buffer area. Under normal operations, upland gamebird premises primarily employ on-site disposal methods, namely composting or incineration. Off-site disposal methods are usually only employed in quail operations which may dispose of carcasses via landfill (personal communication, Secure Upland Gamebird Supply Working Group, August 2019). The SUGS Plan restricts off-site transportation of carcasses for the duration of the PMIP (i.e., the duration of an active outbreak), eliminating any mortality management that may vary from the typical on-site disposal methods of composting and incineration.

9.2.4.1 Dead Bird Disposal Using On-Site Disposal Methods (i.e., Disposal Methods Allowed During the PMIP)

Due to the potential spread of HPAI via carcass disposal, the PMIP measures restrict off-site carcass transportation for disposal during the PMIP. Dead bird disposal is limited to secure on-site storage or disposal during the PMIP, as outlined in the SUGS Plan. Secure on-site storage or disposal options include industry-typical composting and incineration. Because the methods of individual burial, pit burial, refrigerator/freezer storage, and carcass fermentation are not widely used in the upland gamebird industry, this risk evaluation will focus on the more common on-site practices of composting and incineration.

Composting

Composting (controlled decomposition under thermophilic and aerobic conditions) is the most widely used method of carcass disposal in the upland gamebird industry (personal communication, Secure Upland Gamebird Supply Working Group, August 2019). Under conditions of routine mortality, carcasses are composted together in piles or bins to which a supplemental carbon source, such as litter or sawdust, is added. Under good composting practices, the carcasses are positioned and layered within the carbon source in a manner

optimal for complete and odor-free composting. The resulting product is humus-like, with only feathers and small bone fragments remaining, and the process is generally able to deactivate many pathogens due to the high temperatures (130 to 150°F) achieved. Composted carcasses may be used as fertilizer, soil amendments, or as sources of organic material for composting additional material.[406]

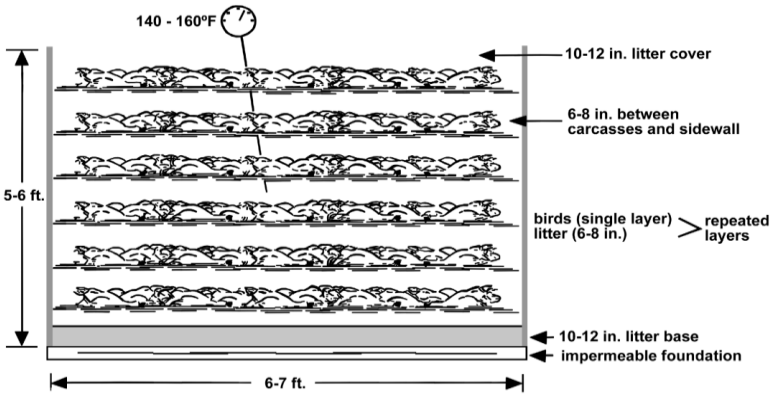


Figure 17. Mortality composter profile (Ritz & Worley, 2012)

Mortality composters are typically constructed on a concrete slab to prevent nutrient leaching and vermin entrance (**Figure 17**). They typically are three-sided and have an overhead roof.[407] Multiple peridomestic species have been shown to access poultry carcass compost piles (**Figure 18**), including raccoon (*Procyon lotor*), opossum (*Didelphis virginiana*), striped skunk (*Mephitis mephitis*), and domestic cats (*Felis catus*).[220] Upland gamebird farms have a much lower volume of mortality compared with commercial poultry operations, and do not report significant scavenger attraction to compost piles (personal communication, Secure Upland Gamebird Supply Working Group, August 2019).

Figure 18. Wild mammals accessing poultry mortality compost piles. Photos courtesy of USGS.

Incineration

Incineration is a commonly used method for upland gamebird carcass disposal and one of the most biosecure methods. Complete carcass combustion occurs in the incinerator unit and the resultant residue does not attract animal or insect pests.[408]

9.2.4.2 Dead Bird Disposal Using Off-site Disposal Methods (i.e., Possible Methods Used Before the PMIP)

The vast majority of upland gamebird farms utilize on-farm mortality disposal methods under normal operating conditions and thus should refer to protocols and procedures listed in **Section 9.2.4.1** Likelihood of an Upland Gamebird Flock Becoming Infected via On-farm Dead Bird Disposal and Scavengers during PMIP. However, there are some upland gamebird facilities that utilize off-site disposal methods during normal operating situations. The only offsite method reported by upland gamebird producers is mortality disposal through landfill disposal (e.g., throwing mortality in the garbage) (personal communication, Secure Upland Gamebird Supply Working Group, August 2019). While other poultry sectors use other off-site disposal methods such as rendering or transportation of mortality for use as feed for other carnivore-raising operations, these methods are not practiced in the upland gamebird sector and are not applicable to this risk assessment. Off-site methods are prohibited during the PMIP; however, it is important to assess the risk that these practices may pose prior to implementation of the PMIP. Given that the only off-site dead bird disposal could method used in the upland gamebird industry is landfill disposal, likelihood of an upland gamebird flock becoming infected as a result of HPAI virus introduction to the flock (before or during the PMIP) is assessed and reported in **Section 9.2.5** Role of HPAI Virus Spread to an Upland Gamebird Flock due to Garbage Management.

9.2.4.3 Likelihood of an Upland Gamebird Flock Becoming Infected via On-farm Dead Bird Disposal and Scavengers During PMIP

Carcass disposal on a farm presents an opportunity for vermin and scavengers to access infected wildlife or poultry carcasses and transmit the HPAI virus to a neighboring susceptible upland gamebird pen or mortality disposal site, either mechanically or via virus shedding. On-site disposal sites on susceptible farms serve as an attractant to scavenger species. The virus could subsequently be transmitted into the pen via farm personnel or other mechanisms. **Figure 19** illustrates the transmission pathway from scavengers to penned upland gamebirds. Proper management of mortality disposal or storage as well as mortality volume impact the degree to which on-farm morality sites serve as a scavenger attractant.

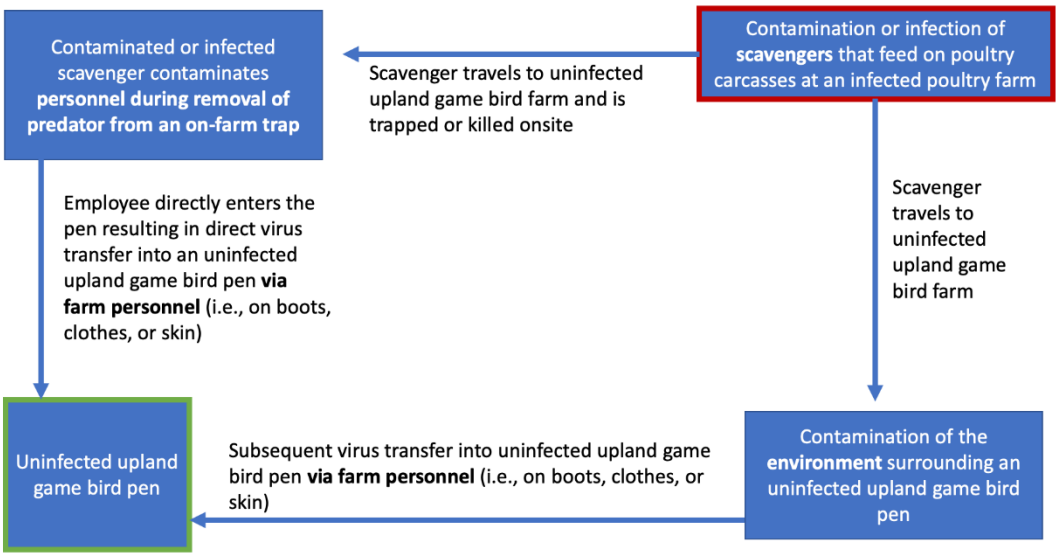


Figure 19. Pathway for exposure of an upland gamebird farm via dead bird disposal on-site

9.2.4.3.1 Literature Review

- Several studies have evaluated the impact of composting on HPAI virus:
  - Using a small-scale duplicate of a typical on-farm compost bin (depicted above, **Figure 17**), Senne et al. (1994) composted HPAI H5N2-infected chicken carcasses for 20 days at 22°C (72°F) ambient temperature, with compost turning at day 10.[406]
    - Peak composting temperatures were 57.3° and 58.3°C (135° and 137°F) during the first and second phases of composting, respectively, for the upper layer of carcasses, and 41.5° and 42.8°C (107° and 109°F), respectively, for the lower layer.
    - Despite the lower temperatures in the lower carcass layer, no HPAI virus was detected from any of the carcasses at 10 and 20 days, including from carcasses placed at the periphery of the bin, within 15 to 20 cm (6 to 8 inches) of the walls.
  - Elving et al. (2012) composted HPAI H7N1, a strain with known prolonged survival in manure at 5° to 22°C.[408] In laboratory-scale reactors at 35°, 45°, and 55°C (95°, 113°, and 131°F), they found a 12-log viral load reduction within 6.4, 1.7, and 0.5 hours, respectively, in a manure/straw mixture, and within 7.6, 9.8, and 0.5 hours, respectively, in a manure/straw/embryonated egg mixture.[408]
    - They recommend:
      - No turning of compost pile during the first phase of composting, to avoid aerosolization of HPAI virus

        - An insulating top layer on the compost to maintain adequate temperature
        - Monitoring of the surface temperature as a parameter for HPAI virus inactivation
    - Ahmed et al. (2012) could no longer isolate an H5N1 virus strain by day 15 from a closed composter used to dispose of infected birds and their wastes, with temperatures reaching 60ºC (140ºF).[409]
    - Using a static pile passive aeration composting system, Guan et al. (2009) demonstrated inactivation of H6N2 virus in chicken tissue samples and embryonated eggs by day 10 at 61.5ºC (143ºF) at the top and 50.3ºC (123ºF) at the bottom of the bin.[410] While still detectable at day 10, viral RNA was degraded in all samples by day 21.
    - In the 2004 LPAI H7N2 outbreak on the Delmarva Peninsula in Delaware, in-house windrow composting was the method of carcass disposal.[411] AI virus was undetectable in all samples from the compost and house environment upon compost turning at days 14 to 19 and again upon compost removal at 4 to 5 weeks.
        - In this case, as an additional measure, the houses were heated to 37.8ºC (100ºF) for three consecutive days after windrow formation and again after compost turning.
        - The outbreak was contained to three farms in a dense poultry production area, which the authors attribute largely to on-site composting, as opposed to off-site disposal, for carcass disposition.
    - As previously noted, due to the lower levels of mortality observed in upland gamebird flocks[412] under normal conditions in comparison to conventional broiler, turkey, or laying hen operations,[413] scavengers are reported to be uncommon around compost piles (personal communication, Secure Upland Gamebird Supply Working Group, August 2019). However, compost piles, even if secure, act as potential attractants to scavengers and in other poultry sectors the observation of scavengers near poultry houses has been identified as a risk factor for AI transmission.[109] Multiple studies have demonstrated the susceptibility of mammals, including scavenger species that have the potential to visit compost piles on farms. Such species include raccoons, skunks, foxes, mink/ferrets, domestic cats, and domestic dogs.
    - The same types of mammals that scavenge on mortality piles on farms often attempt to prey on penned upland gamebird flocks. For a detailed assessment of susceptibility and pathogenicity in mammalian predator species please see **Section 9.1.5** Role of Predatory Mammals in the Transmission of HPAI Virus.
- Turkey vultures (*Cathartes aura*) may visit poultry farms to feed on dead birds. Turkey and black vultures (*Coragyps atratus*) both belong to the order Accipitriformes, family Cathartidae. While a review of the literature revealed a paucity of studies of AI in turkey vultures and other Cathartidae, other birds of

prey in the order Accipitriformes, such as the common buzzard (*Buteo buteo*), have become infected in previous HPAI H5N1 outbreaks.[347]

- For a detailed assessment of susceptibility and pathogenicity in avian scavenger species please see **Section 9.1.7** Role of HPAI Virus Spread to Upland Gamebird Flock via Wild Non-Aquatic Birds in Farm Vicinity.

9.2.4.3.2 Qualitative Analysis

We considered the following qualitative factors for evaluating this pathway:

- Scavengers must gain access to the infected carcass at the source farm to contact and transmit HPAI virus.
  - As described above, it may be unlikely for scavengers to access carcasses in incinerators since the chambers are designed to prevent animal entrance. Additionally, disposal methods such as refrigerator/freezer storage and carcass fermentation that are used in other poultry sectors are quite secure and unlikely to be accessed by scavengers.
    - However, we assume that some industry variation exists in frequency of mortality collection, volume of mortality, and type of storage container used to gather carcasses from the time they are removed from the poultry house or upland gamebird pen to the point when they are moved to the disposal site. Such variation could impact the degree to which scavenging animals are attracted to premises.
    - Intermediate transport or storage containers should also prevent access by scavengers on premises observing PMIP (i.e., premises in a Control Area or in a State with HPAI for upland gamebirds).[177]
    - Additionally, if composting is done improperly or burial is poorly set up, scavengers may gain access to carcasses in disposal sites on poultry farms.
  - While most often constructed on a concrete slab, in part to prevent vermin access, compost bins typically are not completely enclosed. The top layer of litter or sawdust, however, is at a depth of 10 to 12 inches and designed to prevent odor production that would attract scavengers and rodents.[407]
    - When a carcass is surrounded by a sufficient carbon source and the proper moisture level is maintained, odorous gases enter an aerobic zone and are degraded to CO_2 and water.[414]
    - Reports vary on the prevalence of vermin and scavengers with a properly managed composter.[261,407]
    - In their univariate analysis, McQuiston et al. (2005) found that uninfected farms were significantly more likely to dispose of dead birds via composting than infected farms (77.9 percent versus 63.9 percent, P = 0.008).[139]
- Pathways that are factored into the risk associated with on-farm disposal included the involvement of one or more virus transfer steps between scavengers and contact surfaces. For example:

- If a scavenger is acting as a mechanical vector, the pathway: *infected undetected carcass→scavenger→ground area on uninfected premises→farm personnel's boots→upland gamebird pen* which involves four contact steps.
- If the scavenger becomes infected with and subsequently sheds HPAI virus on the grounds outside the uninfected upland gamebird pen, that pathway is *scavenger→ ground area on uninfected premises→farm personnel's boots→upland gamebird pen,* and there are only two contact steps.
  - In general, the chances of the pathway resulting in virus transmission decreases with the number of contact steps that need to occur. Furthermore, even if the transfer steps do occur, the virus concentration transferred will likely decrease substantially with each contact step.
  - The complete details involved with these pathways are examined in depth in the **Section 9.1.5** Role of Predatory Mammals in the Transmission of HPAI Virus.

- Additionally, the distance between farms, including upland gamebird farms and poultry farms, (i.e., the distance a predatory mammal must travel between encountering an infected carcass and an uninfected upland gamebird farm), also impacts the likelihood of HPAI transmission via a contaminated and/or infected mammal.
  - A summary of different mammalian scavenger ranges is covered in **Section 9.1.5** Role of Predatory Mammals in the Transmission of HPAI Virus.
- Finally, the enhanced biosecurity required during the PMIP applies only to farms following the Secure Poultry Supply Plan guidance, being either located in a Control Area (in the case of broiler, turkey, and layer premises) or in States with an active outbreak (in the case of upland gamebird premises) that wish to move birds off the premises. While it is assumed that biosecurity practices may be elevated in an outbreak situation, it is assumed that there may be marked variation in the practices on farms within or outside the Control Area that are not currently adhering to a PMIP.

9.2.4.3.3 Likelihood Rating and Conclusion

Employing best practices for exclusive on-site carcass disposal, SUGS Plan biosecurity measures, and the extremely low mortality produced in upland gamebird pens are factors which decrease the likelihood of attracting scavenger species to upland gamebird mortality on an upland gamebird farm during an outbreak and subsequent PMIP. While it is known that mammalian and avian scavengers have the potential to biologically or mechanically carry HPAI virus, most of the relevant scavenger species do not have home ranges of adequate size to contain both an infected poultry farm and a susceptible upland gamebird farm. This is in due part that upland gamebird farms are generally located 15 or more km away from any commercial poultry operation and that any farms in the scope of this risk assessment will be at least 10 km away from a known-to-be-infected farm due to the size of a Control Area. Given that a susceptible upland gamebird farm is located at least 10 km from an infected farm (to be eligible for movement under SUGS guidance), and that a PMIP is in place, the likelihood of HPAI introduction to an upland gamebird

farm during the PMIP via scavengers is ***very low.***

9.2.5 Role of HPAI Virus Spread to an Upland Gamebird Flock due to Garbage Management

Garbage is typically removed from upland gamebird premises by contracted garbage management services, driven to landfills by premises employees, or incinerated on site (personal communication, Secure Upland Gamebird Supply Working Group, August 2019). In the 2015 U.S. HPAI outbreak, garbage trucks near the barns were a significant risk factor for infection in a case-control study of egg layer flocks in two midwestern States.[403] This evaluation considers the possible ways an upland gamebird flock could become infected with HPAI virus before movement to a hunting preserve due to garbage management practices.

9.2.5.1 Likelihood of HPAI Virus Infection via Garbage Management

Garbage management represents a potential pathway for HPAI virus infection of an upland gamebird flock, as multiple poultry premises may share a common disposal site (e.g., landfill), trash collection provider, or trash collection site (e.g., shared dumpster for multiple premises). HPAI virus may enter an upland gamebird premises via contaminated garbage trucks or drivers. **Figure 20** diagrams the transmission pathway.

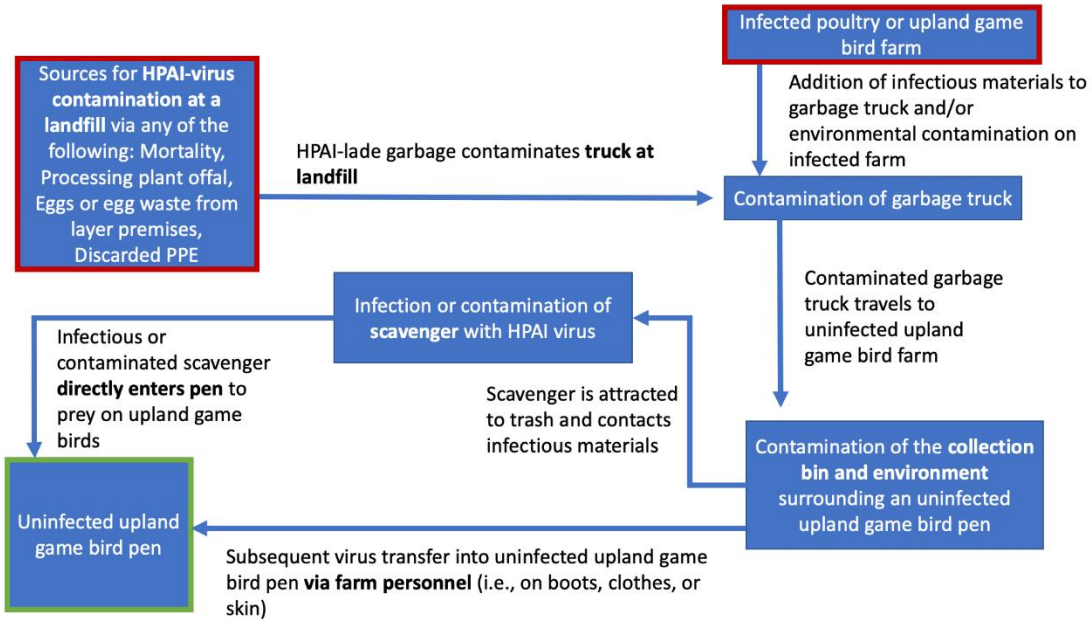


Figure 20. Pathway of HPAI virus infection of an upland gamebird flock via garbage management.

9.2.5.2 Literature Review

- Due to the small number of HPAI or LPAI outbreaks documented in the upland gamebird industry,[8] the following literature focuses on outbreaks related to garbage management on conventional poultry farms because of similar garbage

management practices (personal communication, Secure Upland Gamebird Supply Working Group, August 2019).

- In the 2014–2015 HPAI outbreak, garbage management was identified as a novel risk factor for disease spread.[403]
    - In the 2014–2015 outbreak of HPAI H5N2 in the United States, a case-control study with multivariable analysis of infected egg layer flocks in Nebraska and Iowa identified garbage trucks coming near the barns as a risk for infection at the farm level (OR = 14.7; P < 0.001). This practice occurred at 61 percent of case farms and 23 percent of control farms.[403]
        - The univariate analyses (of factors considered for the farm-level multivariable model) showed that 39 percent of control farms had garbage trucks come to the perimeter of the premises; this did not occur at case farms (P = 0.003). The frequency of garbage trucks entering the farm but not nearing barns was reported to be comparable among case and control farms (case farms, 21 percent; control farms, 26 percent).[403]
    - The frequency with which garbage trucks visited the farms in this study is not known.
- Prior to 2015, epidemiologic trace-back questionnaires in AI outbreaks did not specifically identify garbage management services as a risk factor. However, previous studies have assessed the risk related to non-company visitors that, similar to garbage collectors, do not typically need to access the poultry house arrive in their private vehicles, and may visit or contract with multiple poultry premises in an area.
- Using data collected during the 2003 H7N7 HPAI outbreak in Netherlands, Ssematimba et al. (2012) quantified the probability of virus transmission as 0.133 per other-professional contact, including veterinarians, dealers, advisors, technicians, and ‘unspecified-others’ and 0.246 per rendering contact (e.g., routine pick up of dead birds).[107]
    - In the 2002–2003 outbreak of ILT virus on Mississippi broiler farms, each gas supplier visit to the farm per month increased the likelihood of infection (gas suppliers per month: OR = 6.89; P = 0.0132; multivariate model, matched controls).[382]
        - The authors suggest gas suppliers may have contributed to viral spread by transporting contaminated material between farms.
    - Based on a stochastic model predicting the spread of HPAI virus between Georgia broiler farms in low- and high-poultry-density regions, gas delivery and utility management visitors contributed minimally (approximately 2 to 4 percent) to off-farm transmission.[395]
        - The models estimated the percent contribution to off-farm transmission. Visitor activities in high-poultry-density region (1.45 farms/12.9 km^2) and low-poultry-density region (0.49 farms/12.9 km^2) were calculated separately.

- Additionally, disposing of poultry carcasses in premises garbage dumpsters as a means of mortality disposal has been documented in a survey of commercial poultry operations.[416]
- Walz et al. (2018) note that their survey respondents represented a convenience sample of individuals with knowledge of garbage practices (e.g., veterinarians and other managers in the poultry industry) in various poultry sectors and that statistical analyses (including prevalence of different disposal practices) were not conducted for these data.[416]
- In the 1983–1984 LPAI and HPAI H5N2 outbreak in Pennsylvania, contaminated transport trucks and coops, and movement of dead (and live) birds, were some of the factors implicated in spread of the virus,[393] implicating the spread of virus through vehicles carrying potentially infectious or contaminated materials.
- In many areas, noncommercial poultry operations (e.g., live poultry markets and backyard flocks) may employ the same garbage management contractors as commercial poultry farms. On noncommercial poultry operations, disposal of mortality in garbage has been identified as a risk factor for AI.
    - In an evaluation of risk factors for live bird markets in New York, New Jersey, Pennsylvania, and New England, markets that disposed of dead birds and offal in the trash were 2.4 times more likely to have a repeated presence of LPAI H5 and H7 viruses (OR, 2.4; 95 percent CI, 1.8 - 3.4).[417]
    - In an analysis of risk factors associated with H5N1 in backyard poultry in Egypt from 2010 to 2012, disposing of mortality and poultry feces in garbage piles outside was significantly correlated in the regression model ($F = 15.7$; $P < 0.0001$).[418]
- Landfills may serve as a potential site of cross-contamination as multiple contractors or employees of poultry premises may transport garbage to the same landfill. This risk likely increases if landfills are used as an off-site disposal method for positive depopulated flocks, which has been reported in previous LPAI outbreaks.[93,405]
    - In the 2002 LPAI H7N2 outbreak in Virginia, disposal of depopulated flocks transported in sealed, leak-proof trucks that were cleaned and disinfected on-farm and at the landfill mainly occurred at “mega-landfills.”[93]
    - During the 2001–2002 Pennsylvania H7N2 LPAI outbreak, some euthanized case flocks were disposed of at landfills after being transported in closed containers.[405]
- Garbage trucks which visit poultry or upland gamebird operations may transport infectious material between premises. Many studies have demonstrated high titers and the persistence of HPAI virus in various poultry tissues and fluids (including muscle, organs, feathers, and feces) (see **Table 22**) that can be found on items which might be carried by trucks.

Table 22. Viral titers in infectious materials that may be present on garbage trucks that have visited poultry sites.

Species	Exposure type with volume of virus type	Tissues/Material type	Viral titer in tissue	Source
Turkeys	Oro-nasally inoculated with 100 µl of 10^6 EID_{50} of HPAI H7N1	Muscle tissue	$>10^4 EID_{50}$/ g of tissue	[78]
Turkeys	Experimentally infected with A/turkey/Italy HPAI H7N1	Blood	$10^{6.8}$ EID_{50}/mL of blood	[419]
Chicken	Experimentally infected with EA/AM HPAI H5N2	Organ tissues (spleen and lung)	10^7 to 10^8 EID_{50}/g of tissue	[119]
Chicken	Experimentally infected with HPAI H5N1	Muscle tissue (thigh muscle)	$10^{7.5}$ EID_{50} /g of tissue	[73]
Turkey	EA/AM HPAI H5N2	Feces	10^3 to 10^5 EID_{50}/mL of feces	(E. Spackman, personal communication, May 2016)[125]
Chicken	Experimentally infected with 1983 Pennsylvania HPAI H5N2 strain	Feces	~10^9 ELD_{50}/g of feces	[117]
Turkey	Experimentally infected with HPAI H5N1	Feather (tip pools)	$10^{4.168}$ to $10^{5.79}$ EID_{50}/mL per pool	(M. Slomka, personal communication, January 2014)
N/A	Experimentally infected with Indiana HPAI H7N8	Feather (root)	$10^{5.9}$ EID_{50}/mL per root sample	(M. Pantin-Jackwood and E. Spackman, personal communication, May 2016)
Chicken (chicks)	Intratracheally inoculated with	Organ tissue (liver, lung,	$10^{6.3}$ to $>10^{9.3}$	[246]

	2.5×10^4 $TCID_{50}$ of HPAI virus (H5N1)	kidney, and brain homogenates)	$TCID_{50}$/g of tissue	
Ducks	Experimentally infected with HPAI H5N1	Feather	$10^{4.0}$ to $10^{5.5}$ EID_{50}/mL, depending on temperature	[232]

9.2.5.3 Qualitative Analysis

We considered the following qualitative factors in evaluating this pathway:

- The types of potentially infectious or contaminated material disposed of in garbage vary by sector of the poultry industry.[416] However, many potentially contaminated or infectious materials have been reported to be routinely disposed of in the trash, according to survey responses from representatives of the different sectors of the poultry industry.
  - In the broiler, turkey, and layer sectors, a survey found a large distribution of potentially infectious discarded items as listed in **Table 22**.[416]
  - Similar to the conventional poultry sectors, upland gamebird industry representatives report items such as egg products, disposable egg or day-old chick boxes, used PPE, used diagnostic materials (e.g., gauze, needles, etc.), and, in the quail industry, mortality may go into the garbage (personal communication, Secure Upland Gamebird Supply Work Group, August 2019).
  - A premises with frequent garbage pickups or transport events has increased opportunities to contact a contaminated truck or contents relative to less frequent transport or pickup schedules.
  - On upland gamebird premises, the frequency of garbage pickup is most often weekly or every other week, based on survey responses from representatives of the upland gamebird industry (personal communication Secure Upland Gamebird Working Group, August 2019).

Table 23. Survey results[416] concerning material disposed of in garbage on premises in the broiler, turkey, and layer industries.[a]

Item	Broiler sector (n=8 respondents)	Turkey sector (n=15 respondents)	Layer sector (n=39 respondents)
Dead wildlife/wild birds	Yes (1/8)	Yes (5/15)	Yes (1/39)
Rodents	Yes (3/8)	Yes (5/15)	Yes (10/39)
Mortality or poultry carcasses	No (0/8)	Yes (1/15)	Yes (9/39)
Eggs or egg products[b]	Yes (1/8)	Yes (1/15)	Yes (8/39)

Item	Broiler sector (n=8 respondents)	Turkey sector (n=15 respondents)	Layer sector (n=39 respondents)
Manure	No (0/8)	No (0/15)	Yes (1/39)
Spilled feed	Yes (2/8)	Yes (8/15)	Yes (7/39)
Disposable chick transport boxes[b]	Yes (4/8)	Yes (4/15)	Yes (24/39)
Used needles/syringes/diagnostic supplies that have contacted birds[b]	Yes (1/8)	Yes (5/15)	Yes (14/39)
PPE (boot covers, gloves, coveralls, etc.)	Yes (8/8)	Yes (14/15)	Yes (36/39)
Feathers	No (0/8)	Yes (2/15)	Yes (4/39)
Offal	No (0/8)	No (0/15)	No (0/39)
Equipment or supplies from inside barns[c]	Yes	Yes	Yes (22/39)
Household garbage from farm manager or any other residence[c]	--	Yes	Yes (20/39)
Trash associated with waterfowl hunting[c]	--	--	No (0/39)
Garbage from processing operation[c]	--	--	Yes (23/39)
Lunchroom and restroom garbage[c]	--	--	Yes (37/39)

[a]Yes indicate materials disposed of in the garbage by one or more survey respondents within each industry. In parenthesis, numerator indicates number of survey respondents reporting disposal of item and denominator indicates total number of respondents.

[b]Language of selection choice modified in survey distributed to representatives of layer industry.

[c]Item explicitly asked only in survey distributed to representatives of layer industry. Yes in the broiler and turkey industries for these items indicates at least one respondent manually reported disposing of that item in the garbage.

- Of potential HPAI-contaminated or infectious material reported to be disposed of in the garbage on poultry premises (e.g., dead wildlife, poultry carcasses, egg shells, and potentially contaminated materials that have contacted poultry) (see **Table 23**), the hypothetical expected virus concentration on each type of item varies.[416]
    - The amount of viral persistence and titer volume of HPAI VIRUS that can occur in various poultry tissues and fluids based on previous literature is substantial. HPAI virus has been recovered in many tissues of poultry carcasses, such as muscle, liver, kidney, brain, spleen, and blood (see **Table 22**) A conservative compilation of these results indicates that 1.0 g of tissue or 1.0 mL of feather pulp could contain a minimum 10^4 EID_{50} of HPAI virus.
        - Assuming a relatively low infectious dose of 10^2 viral particles, based on findings discussed in **Section 8.7.1** Dose Response in Upland Gamebirds, only 1.5 ounces (~44 mL) of carcass fluid contains enough viral particles to infect approximately 4,400 birds.
    - Additionally, while fecal material containing high viral loads may be quickly diluted in the environment, contaminated feathers may persist as solid materials in the field and could be transferred from farm to farm via garbage trucks if poultry carcasses are thrown away by producers.

- There are reports of disposing of dead wildlife in trash on commercial poultry premises.
  - Evidence of AI virus infection of multiple mammalian species, such as ferrets, foxes, cats, dogs, skunks, raccoons, and mink, has been demonstrated by virus isolation, antigen detection, and PCR. For a detailed description on mammalian susceptibility, see **Section 9.1.5** Role of Predatory Mammals in the Transmission of HPAI Virus.
  - Evidence of AI virus infection of rodents has been demonstrated by virus isolation, antigen detection, and PCR in some instances. Additionally, it has been demonstrated that rodents carry the potential to be mechanical carriers of virus. See **Section 9.1.4** Role of HPAI Virus Spread to an Upland Gamebird Flock via Rodents.
  - Wild and domesticated bird species can be infected with HPAI virus. For a detailed description of experimental studies in wild and domesticated aquatic birds, see **Section 9.1.6** Role of HPAI Spread to an Upland Gamebird Flock in a State with HPAI via Wild Aquatic Birds in the Farm Vicinity. For a detailed review of HPAI detections, prevalence, and susceptibility of passerine birds and non-passerine non-aquatic birds, see **Section 9.1.7** Role of HPAI Virus Spread to an Upland gamebird Flock via Wild Non-Aquatic Birds in Farm Vicinity.
- Eggs from infected hens have tested positive for HPAI virus, including shells, albumen, and yolk. Measured concentrations have varied. See the Secure Egg Supply Egg Shell Risk Assessment for more details.[420]

- Influenza virus survival varies depending on strain and environmental conditions, such as humidity and temperature. Virus persistence is generally longer at cooler temperatures and in more humid conditions. For virus persistence data on materials that may be disposed of in the garbage, such as poultry carcasses, feathers, eggshells, egg trays, wood, steel, glass, and PPE, see **Appendix 2**: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates.
- In Walz et al.'s (2018) survey results and in reports from upland gamebird industry representatives (personal communication, Secure Upland Gamebird Supply Work Group, August 2019), garbage from broiler, turkey, layer, and upland gamebird premises is collected via third party companies and transported to offsite disposal locations (e.g., municipal landfills), facilitating the possibility for farms (conventional poultry or upland gamebird) to be on the same garbage pick-up route or have trucks coming onto the farm that contain or are contaminated with infectious materials.
  - Transport trucks may become contaminated at municipal landfills; it has been noted that upon arrival at landfills, garbage management vehicles may drive over previously deposited garbage (D. Halvorson, personal communication, June 2016).

        - The CFR provides standards for design and operation of landfills.[421] For municipal solid waste landfills, these include 6 inches of covering on disposed solid waste each day or as necessary, disease vector control, and access requirements.[421]
    - Garbage management contractors used by some turkey and broiler premises have been reported to visit multiple poultry premises on one route before depositing a load at the landfill; thus, HPAI-virus-contaminated garbage from an undetected premises may be present on the truck when it shares a garbage route with and arrives on an upland gamebird farm.
        - The types of potentially contaminated trash from other types of poultry operations (e.g., backyard poultry, processing facilities, live bird markets, etc.) are not known, but are assumed to include materials similar to those reported in garbage from commercial poultry operations.
            - A shared dumpster or common trash collection point for farms represents an additional site for potential cross-contamination between operations, however upland gamebirds typically have garbage picked up directly onsite or drive it directly to the landfill;[14] making shared garbage sites outside of municipal landfills unlikely.
    - The risk of upland gamebird farms being on the same garbage route as other poultry premises is lower than other poultry types given the more prominent geographic isolation of upland gamebird farms in comparison to other types of poultry premises such as turkeys.[14]
- Garbage trucks and drivers typically do not contact live poultry or upland gamebirds while completing contracted duties on a poultry premises. Biosecurity recommendations and site-specific biosecurity plans may not stipulate specific measures for garbage management drivers, but it is recommended that visitors follow procedures to cross the PBA and LOS.[391]
    - In a qualitative evaluation of potential AI transmission pathways on broiler and layer premises in the Netherlands, Ssematimba et al. (2013) proposed an exposure risk classification of "medium" for the majority of contacts assessed that access only the premises and have no contact with live poultry.[114] The analysis considered contact frequency, biosecurity practices, and risk category.
- Virus introduction into upland gamebird pens via garbage management may involve one or more virus transfer steps. Although there would likely be reduction in the virus concentration (6 to 27 percent) between a donor surface and recipient surface in each direct contact,[380] the virus concentration potentially tracked into the pen may still exceed the infectious dose. This depends on the initial viral load and infectious dose of that virus strain in upland gamebirds.
    - It is assumed that the ground traveled by the vehicle between the time of contact with infected garbage and the upland gamebird premises may lessen the amount of virus present for transmission once at the premises. However,

mechanical transmission of a similar type virus (e.g., PRRSV) has been demonstrated experimentally in a swine industry-like setting.[381]

- The transfer of infected and undetected carcasses or other organic material from the dumpster into the garbage truck at a neighboring farm can result in feathers and bodily fluids contaminating the truck's lift arms, the outside of the truck bed, and the ground surrounding the truck. When the same truck collects a load from an upland gamebird premises, the lift arms could contaminate the dumpster there, and the truck tires could contaminate the ground near the dumpster.
- If the garbage truck bed is not securely covered or the disposed morality or other organic material in the garbage truck is not securely bagged, feathers and other material may escape and result in contamination along the truck's route, with the potential for subsequent transfer into other poultry houses or upland gamebird pens along the route.
- Additionally, even if a truck were covered, feathers or other material may still escape at driving speeds.
- Alternatively, if an infected load of garbage is in the truck at the time of arrival on an upland gamebird premises, fewer transfer steps are required than if just the truck, itself, was contaminated and not carrying infectious material.
- Dumpsters may not be consistently or securely covered, allowing potential access to scavengers.
- As discussed in other sections of this risk assessment (concerning visitors/people, wild non-aquatic birds, and on-farm disposal during PMIP), inconsistently covering dumpsters presents the opportunity for mechanical or biological transfer of HPAI virus via scavengers from infected and undetected carcasses onto the surrounding grounds. This practice could potentially result in cross-contamination of the garbage truck tires and personnel boots, with subsequent contamination of other premises and upland gamebird pens.

- The enhanced biosecurity required during a PMIP applies only to broiler, turkey, and layer farms located in a Control Area and to upland gamebird farms located in a State with HPAI for operations that wish to move birds off the premises during an outbreak. It is assumed that there may be marked variation in the biosecurity and garbage practices on farms that are not currently adhering to a PMIP, despite a likely elevation of biosecurity during an outbreak.
- If garbage management activities and visits occur outside of the PBA (as is required for those farms participating in the SPS plans within a Control Area and for those upland gamebird farms participating in the SUGS Plan in a State with HPAI), there is a decreased likelihood of cross-contamination between contaminated garbage trucks/personnel/stray garbage and personnel, equipment, or other potential fomites that may access the upland gamebird pen.
  - Additionally, based on reports from representatives of the upland gamebird industry, it is common practice for the dumpster or trash collection point to be located at the entrance or perimeter of the farm. Industry representatives state

the garbage pickup distances range from 100 ft to 250+ ft from their pens, but this distance varies (personal communication Secure Upland Gamebird Working Group, August 2019).

- Also, in accordance with the PMIP requirements for upland gamebird farms participating in the SUGS Plan, all growers and farm employees who are entering a farm must change into pen-specific boots prior to entering the pen. The change of footwear/use of disposable protective foot coverings will likely reduce potential transfer of virus from around the garbage dumpster into a pen.
- As is true with other third-party contractors, upland gamebird producers may find it difficult to control or influence certain practices by garbage haulers, including C&D of garbage trucks, pickup routing, and landfill practices.

9.2.5.4 Likelihood Rating and Conclusion

9.2.5.4.1 Likelihood of an Upland Gamebird Flock Becoming Infected with HPAI Virus due to Garbage Management when a PMIP is not Implemented

Garbage management was identified as a novel risk factor for HPAI virus introduction in the 2014–2015 outbreak in the United States. Epidemiological studies of past outbreaks have not specifically investigated garbage as a potential route for HPAI virus entry onto a poultry premises, but a recent survey identified a number of items disposed of in trash across poultry industry sectors that could be potentially infectious or contaminated by HPAI virus, and upland gamebird producers appear to have similar practices in garbage management and items disposed in the trash. There is potential for HPAI virus associated with garbage management to be tracked into an upland gamebird pen, albeit this risk is dependent on the proximity of upland gamebird farms to poultry or other upland gamebird premises. Additionally, because upland gamebird farms in the scope of this risk assessment are outside a Control Area, the likelihood of a garbage truck visiting a known-to-be-infected farm prior to coming onto an upland gamebird farm is almost completely eliminated. Given the preceding evidence, the likelihood of an upland gamebird flock becoming infected with HPAI virus due to garbage management without a PMIP is ***moderate.***

9.2.5.4.2 Likelihood of an Upland Gamebird Flock Becoming Infected with HPAI Virus due to Garbage Management when a PMIP is Implemented

During the PMIP, garbage collection sites are required to be located outside of the established PBA limiting garbage trucks and potentially infectious trash from coming near pens. The greatly intensified biosecurity measures of the PMIP, such as using footwear specific to each upland gamebird pen (e.g., pen-specific footwear), should decrease the likelihood that virus is tracked into pens (see **Appendix 6**: Pre-Movement Isolation Period). Provided on-farm biosecurity measures are strictly followed during a PMIP, the likelihood of an upland gamebird flock becoming infected with HPAI virus due to garbage management during PMIP is ***low.***

9.3 Pathways for an Upland Gamebird Flock Becoming Infected with HPAI via Load-Out Operations

Movements of load-out equipment and crews have been implicated in AI transmission in previous outbreaks. According to Poss et al. (2003), load-out crews (such as contract crews used in broiler and turkey industries), which may load-out more than one flock within 12 hours, have been associated with the spread of AI.[422] Several large LPAI outbreaks in turkeys in Minnesota, such as the 1978 and 1995–1996 LPAI outbreaks, were attributed in part to potentially contaminated load-out crews and equipment or processing trucks coming into close contact with birds that remained on the farms after partial flock removals.[98,423] During the 1986 LPAI H5N2 outbreak in Pennsylvania, restricting farm access to only sanitized load-out trucks and crates interrupted infection transmission.[424] In the case of these instances the primary source of contamination stemmed from load-out equipment, crews, and vehicles being used for multiple flocks on multiple premises. Within the upland gamebird industry load-out equipment, crews, and vehicles are all owned (or employed) by the producer and thus not shared between premises.[14] Instead, concern of contamination comes from the equipment, crew (e.g., farm employees), and vehicles coming into contact with virus that may be present on equipment before it returns from a delivery to a hunting preserve.

In this chapter we are assessing the likelihood that an upland gamebird flock becomes infected during the load-out process, resulting in movement of infected but undetected birds to a hunting preserve. Pathways considered include contaminated load-out equipment (e.g., crates) and vehicles and/or farm employees that are returning from a drop off premises that are subsequently involved with load-out processes.

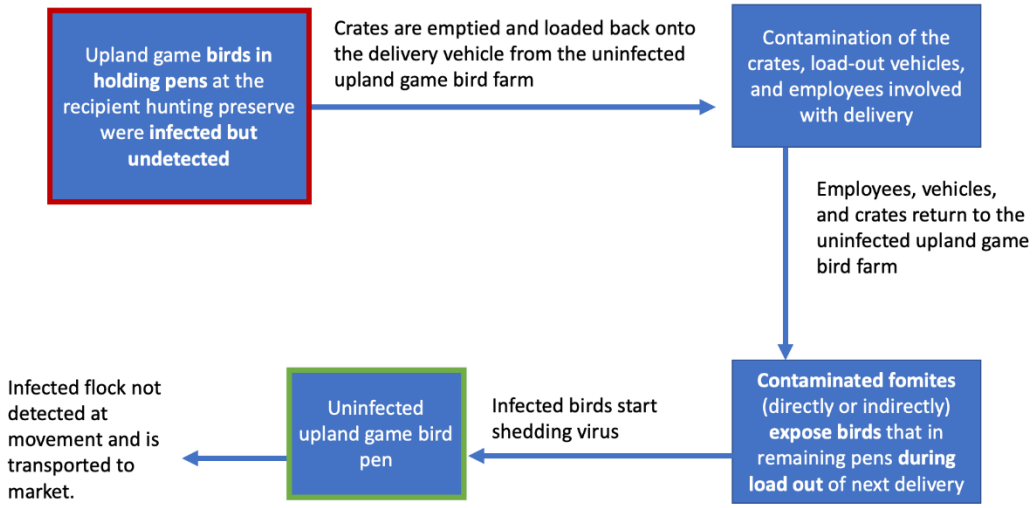


Figure 21. Pathway for exposure for an upland gamebird flock during load-out operations.

9.3.1 PMIP Measures for Moving Upland Gamebirds to Hunting Preserves

For premises that are in a State with an active AI outbreak, but not within a Control Area that wish to move upland gamebirds to hunting preserves, a PMIP is defined that limits non-critical visits and personnel on the farm, while biosecurity and flock disease

surveillance is increased (see **Appendix 6**: Pre-Movement Isolation Period).[389] Adherence to enhanced biosecurity principles during this isolation period prior to scheduled movement minimizes the likelihood that the flock will become exposed to HPAI via contact with people, vehicles, or equipment that may be contaminated with HPAI during an active outbreak occurring within the premises' State. Similarly, decreasing the likelihood of late introduction of virus to a flock will increase the sensitivity of surveillance and sampling performed during the PMIP. For further information on the likelihood of detecting infection close to movement, see **Section 9.4.2.4.2** Estimated Overall Likelihood of not Detecting HPAI in an Upland Gamebird Pen Prior to the Start of Load-out.

9.3.1.1 Load-out Mitigation Measures for Movement of Upland Gamebirds to Release

Load-out begins when the first piece of load equipment (e.g., crates) are brought into the pen and ends when the load of birds departs the premises. If birds are infected by contaminated crates, employees, or vehicles coming onto the premises, they have the potential to shed virus up until the time of delivery, which may vary from a minimum of 2 hours up to 48 hours (Secure Upland Gamebird Supply Working Group, personal communication, January 2020). Viral contamination may be tracked into occupied upland gamebird pens which are still awaiting load-out, or into pens that will not be loaded out until later in the season (which could be within a few days or in over a month). Such partial loadouts extend the period for HPAI virus to replicate and spread through the flock and includes any time the flock remains in the pen until load-out, in addition to transit time. Load-outs and transit times of longer duration pose an increased risk of transporting a considerable number of infected but undetected birds to market.

To meet the permit guidance criteria for movement from a premises within a State with an active infection (but not within a Control Area), all upland gamebird premises (regardless of load-out time) should adhere to mitigation measures for the entire duration of any active infection within their State. Measures include load-out crew stipulations and live-haul routing requirements, as well as mitigations that occur during delivery and prior to returning to the premises and sanitation procedures for crates when moving into, out of or within the State. Additionally, movement of birds into a Control Area is prohibited. The biosecurity and sampling stipulations pertinent to the load-out of upland gamebirds are outlined in **Appendix 6**: Pre-Movement Isolation Period.

Emphasis is placed on diligent biosecurity between pens to minimize spread between upland gamebird pens in the event of a virus introduction during load-out. Crates must be adequately cleaned and disinfected or delivery procedures must occur in a fashion that minimizes contact of crates, delivery personnel, and vehicles with surfaces on the delivery site as well as personnel and vehicle decontamination prior to returning to the premises.

Further detail on load-out mitigations recommended for upland gamebird premises to complete the load-out and transport to the hunting preserve are outlined in **Appendix 6**: Pre-Movement Isolation Period (also available on the Secure Poultry Supply Plan website). Results of modeling simulations to support the increased biosecurity and other

PMIP measures prior to and during load-out are detailed in **Section 9.4** Likelihood of Detecting HPAI in an Infected Upland Gamebird Pen.

9.3.1.2 Literature Review

If personnel, equipment, and/or vehicles that are returning from previous deliveries carry virus back onto the farm and take part in the next load-out process, viral persistence requires consideration. Viral persistence depends on substrate, temperature, and humidity, among other factors. Virus may persist for days to weeks or longer in a climate like that of the continental United States.

- Kurmi et al. (2013), Beard et al. (1984), and Wood et al. (2010) reported that HPAI virus strains were inactivated in poultry (chicken) feces in less than 5 days in warm temperatures (21.7 ° to 25 °C) and persisted nearly 2 to 8 weeks in cooler temperatures (4 ° to 7.8 °C).[98,398,425] In these experimental studies, when temperature was constant, time to virus inactivation in feces usually increased as moisture level increased.[98,398] On substrates that may be found in vehicles or poultry transport crates (translatable to crates used to haul upland gamebirds), an LPAI virus strain (A/Herring gull/Delaware 471/86 [H13N7]) was below detectable limit at day 6 on tires, steel, and plastic, and at hour 72 on wood.[399] On glass and soil in cool temperatures (4 ° to 7.8 °C), an HPAI H5N1 strain (A/Vietnam/1203/2004 [H5N1 clade 1]) was recovered at day 13 in low relative humidity and day 9 in high relative humidity.[398]
- For further data on viral persistence on different substrates and in varying environments, see **Appendix 2**: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates.

Findings from previous disease outbreaks suggest that virus transmission to poultry premises near live haul routes is possible. For a review of literature on infection of premises near live haul routes in past outbreaks, see **Section 9.1.8**, Role of HPAI Virus Spread to Upland Gamebird Premises near Poultry Live-Haul Routes Via Feathers, Feces, and Other Fomites.

9.3.1.3 Qualitative Analysis

We considered the following qualitative factors for evaluating this pathway:

The load-out process and time from beginning of load-out to delivery of live birds to a hunting preserve for the upland gamebird industry varies, however there are some consistencies that allow for an effective assessment of risk.

- The time required to load-out a shipment of upland gamebirds on a premises depends on size of the shipment, crew and equipment logistics, species of upland gamebird, and variation in bird collection processes by the premises. Given the factors associated with load-out and their corresponding variation, typically the load-out process can range between 1 to 8 hours (Secure Upland Gamebird Supply Working Group, personal communication, January 2020).
- Transport time from farms to hunting preserves represents additional time for potential viral shedding within the flock being delivered. The transportation time for commercial upland gamebird systems in the United States varies but is

generally between 4 hours to sometimes beyond 24 hours for long distance deliveries (Secure Upland Gamebird Supply Working Group, personal communication, January 2020).

- Industry representatives report that for most shipments, producers and their employees can complete the cumulative process of load-out and transit time amount in under 48 hours more often than not. This timeline is optimized to minimize transit mortality and maintain bird well-being and value, however time to completion is dependent upon where customers are located (Secure Upland Gamebird Supply Working Group, personal communication, January 2020).
- Load-out crews used in the upland gamebird industry are only involved in load-out processes on the farm by which they are employed and may participate in the delivery process to hunting preserves that might have other upland gamebirds in holding pens but rarely work on other upland gamebird farms[14] (Secure Upland Gamebird Supply Working Group, personal communication, August 2019). Under ideal PMIP mitigations, wholesale shipments of upland gamebirds to other upland gamebird farms would not occur.
    - Load-out crews never consist of third party contracted crews and all employees involved never work for or visit poultry farms. Utilizing farm-employed personnel rather than third party crews is in contrast to poultry industry sectors such as in turkeys, broilers and layers. For such industries, in past LPAI outbreaks (including outbreaks occurring in 1978,[393] 1986,[424] and 1995–1996[423]) load-out equipment and crews have been implicated as a means of virus spread between farms, especially those involving partial flock removals and movement of load-out crews between premises. Due to the use of internal crews, the risk of contracted crews bringing virus onto the farm from poultry farms is substantially minimized. Additional considerations regarding upland gamebird load-out personnel and disease spread include:
- During an outbreak, upland gamebird farms electing to follow the highest level of PMIP biosecurity will only allow a maximum of four personnel who are not live-in residents of the upland gamebird farm to be involved in loadouts. All other personnel involved with the load-out process must be live-in residents of the farm. Both mitigations aid in limiting the amount of exposure employees have to potential environmental contamination before involvement with the load-out process (See **Appendix 6**: Pre-Movement Isolation Period).
- During an outbreak, all upland gamebird farms following the PMIP will have all personnel follow personnel biosecurity mitigations when coming onto the farm as described in **Appendix 6**: Pre-Movement Isolation Period.
- During an outbreak, all upland gamebird farms following the PMIP will only involve one farm employee (acting as a driver) to perform deliveries post-load-out of birds. The assigned driver will follow truck and driver biosecurity as described in **Appendix 6**: Pre-Movement Isolation Period.
- Interaction between farm employees involved in load-out and other poultry industry and upland gamebird industry activities is addressed in **Section 9.2**

Pathways for an Upland Gamebird Flock Becoming Infected with HPAI via Movements of People, Vehicles, or Equipment.

- Equipment and vehicles can also act as fomites for disease if moved between premises during an outbreak as demonstrated during previous poultry disease outbreaks (LPAI, HPAI, and ILT).[185,393] However, upland gamebird premises typically own all of their own equipment and vehicles.[14] Thus, the only load-out equipment leaving the upland gamebird premises would be premises-owned crates. Such crates transport birds produced by the premises that owns the crates to hunting preserves. Additionally, during an active HPAI outbreak, upland gamebird producers will institute biosecurity mitigations for crates as described in **Appendix 6**: Pre-Movement Isolation Period.
- Vehicles used for deliveries of upland gamebirds are usually farm-owned and premises owners are in control of the biosecurity surrounding these vehicles. During an active HPAI outbreak, upland gamebird producers will have all personnel follow the vehicle mitigations listed in **Appendix 6**: Pre-Movement Isolation Period.
- The load-out processes in all poultry sectors, including upland gamebirds, inherently places crews, vehicles, and equipment in close contact with live birds, bird feces, and bird feathers.
- While there is no specific data available for upland gamebird species, they are thought to be similar to that found in other poultry. Estimates of HPAI virus concentrations in chicken secretions, feces, feathers, and other tissues generally range between 10^3 and10^7 EID_{50} per gram or per milliliter, although higher concentrations have been observed in some cases.[72,73,79]
- For further information on viral load on substrates related to live-bird movement, see **Section 9.1.8** Role of HPAI Virus Spread to Upland Gamebird Premises near Poultry Live-Haul Routes via Feathers, Feces, and Other Fomites and **Appendix 2**: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates.
- Unlike other poultry sectors, upland gamebird farms use their own crews, vehicles, and equipment thus limiting exposure of these load-out components with live birds, feces, and feathers from other premises where birds (e.g., poultry or upland gamebirds) are produced or slaughtered.
- Personnel involved with load-out do not work for other upland gamebird premises or poultry premises and are not going onto other bird producing sites. During an outbreak, all personnel involved with load-out will follow the biosecurity mitigations as described in **Appendix 6**: Pre-Movement Isolation Period prior to beginning the load-out process.
- Crates are farm-owned and will only be stocked with birds that are produced by that farm. Throughout the duration of an active outbreak, upland gamebird farms in the PMIP follow crate-specific biosecurity measures pre- and post-delivery of birds as described in **Appendix 6**: Pre-Movement Isolation Period.

As discussed in **Section 9.4.3** Likelihood of Moving Infectious but Undetected Upland Gamebirds Following Exposure During Load-out, the likelihood of an upland gamebird pen group becoming infected with HPAI in the days leading up to movement is lower

when PMIP enhanced biosecurity measures are implemented, and the premises is located far enough from infected premises. Increased biosecurity and greater distance help reduce the chances of moving birds that are infectious because of exposure to HPAI during the PMIP. In the scope of this risk assessment, all upland gamebird premises are at least 10 km away from known-to-be-infected poultry premises since they are not in a Control Area. Additionally, personnel, vehicles, and crates are not allowed to enter a Control Area during an outbreak.

- It is possible that farm-owned crates, drivers, and vehicles used during load-out could be contaminated in previous deliveries, posing a risk for cross-contamination of pens that house birds that have yet to be marketed. However, these risks are mitigated as outlined in **Appendix 6**: Pre-Movement Isolation Period.
- If birds are infected during the load-out process, they have the potential to shed virus after the completion of the latent period up until the time of delivery. This includes load-out and transit time before release. A longer cumulative duration of load-out and transport time thus pose an increased risk of transporting a considerable number of infected but undetected birds to a hunting preserve. In the event of a single point-source infection, **Table 30** in **Section 9.4** Likelihood of Detecting HPAI in an Infected Upland Gamebird Pen shows the estimated number of birds on a truck which may be infected, depending on duration of time between infection and release (i.e., load-out and transit time)
- In the absence of a disease emergency, crates are not routinely cleaned and disinfected between movements in the upland gamebird industry. Feces, feathers, bedding in the crates and possible contaminants may remain on surfaces that will contact a subsequent flock.
- While upland gamebird specific data is limited, the mean and standard deviation of latent period based on the distribution of HPAI latent periods for chickens is 0.64 days ± 0.68 days (see **Table 24** for more details; Estimated from[73,167,426–428]), albeit the period varies with virus strain and infectious dose. Thus, considering both the latent period of similar gallinaceous species (in this case, chickens) and adequate contact rate among upland gamebirds in the event of exposure to HPAI virus, the number of infectious upland gamebirds shedding virus in a flock at the end of a 48-hour combined load-out and transit period would be low (**Table 30** in **Section 9.4.3**).
    - Greater variation in infectious period and mean time to death has been reported, with data specific to upland gamebird's species available. For bobwhite quail, chukar, and pheasants, an experimental study reported mean times to death as 4.7, 4.1, and 3.4 days for H5N2 HPAI and 4.9, 5.2, and 4.8 days for H5N8 HPAI for, respectively.[27] At the lower challenge doses, mortality was lower and the MDT was slightly longer for both viruses in the three species.
    - For a more detailed review of experimental studies of latency period, infectious period, and mean time to death from AI infections in upland gamebirds and relevant gallinaceous birds, see **Section 8**, Hazard Identification: HPAI Overview.

- Pen-group to pen-group biosecurity measures should be implemented to limit likelihood of contaminating pens still occupied by upland gamebirds during load-out, such as utilizing pen-specific footwear and farm-specific clothing and handwashing (see **Appendix 6**: Pre-Movement Isolation Period).
- Flocks which are infected during the load-out process may not be detected by clinical signs or a mortality trigger alone.
    - The PCR testing of birds occurs every 8 days and antigen capture testing occurs during load-out as outlined in the **Section 9.4** Likelihood of Detecting HPAI in an Infected Upland Gamebird Pen on a premises should increase the probability of detecting infections that occurred because of the load-out process.
    - For further information on load-out testing and surveillance protocols and sensitivity analysis of such protocols, see **Section 9.4** Likelihood of Detecting HPAI in an Infected Upland Gamebird Pen.

9.3.1.4 Likelihood Rating and Conclusion

Previous outbreaks have implicated contaminated load-out crews and equipment in the spread of AI in conventional poultry sectors such as turkeys and layers. In the United States, commercial upland gamebird industry, load-out crews consist of farm employees that do not work on any other upland gamebird or poultry premises essentially eliminating spread that could originate from poultry farms or other upland gamebird farms. Additionally, during an outbreak, PMIP measures include cleaning and disinfection of vehicles and crates used to complete deliveries to hunting preserves that may or may not contain other upland gamebirds. These protocols are implemented in conjunction with strict personnel biosecurity mitigations.

Given that PMIP enhanced biosecurity on farm and implemented during deliveries are occurring, the associated testing protocols outlined in the permit guidance and **Section 9.4** Likelihood of Detecting HPAI in an Infected Upland Gamebird Pen are being implemented, and that the premises is not located within a Control Area, we estimate the likelihood of an upland gamebird flock becoming infected with HPAI VIRUS via load-out operations and resulting in an infected but undetected movement to release to be ***very low.***

Upland gamebirds remaining on a premises represent a susceptible host population at increased risk of exposure to HPAI-contaminated crates, vehicles, or crews due to proximity. Given that PMIP and load-out mitigation measures are in place, the likelihood of the remaining upland gamebirds on the premises becoming infected with HPAI virus via load-out operations on that premises is estimated to be ***low.***

9.4 Likelihood of Detecting HPAI in an Infected Upland Gamebird Pen

9.4.1 HPAI Surveillance Measures

9.4.1.1 Current Measures

Current routine influenza surveillance measures involve testing of raised-for-release flocks for H5/H7 subtypes of AI for birds on premises participating in the U.S. H5/H7 Avian Influenza Monitored program of the NPIP (see 9 CFR part 146.53b for further information).

9.4.1.2 Outbreak Measures

Active Surveillance by rRT-PCR Testing and Antigen Capture Testing

The active surveillance protocol option outlined in the SUGS Plan involves testing one pooled sample of swabs from 11 freshly dead birds via rRT-PCR at National Animal Health Laboratory Network (NAHLN) labs. rRT-PCR testing of samples from each pen on the premises must done every 8 days and antigen capture testing should be done on day of load-out for upland gamebirds.

Current USDA:APHIS HPAI emergency response plans assume same-day turnaround for submitted rRT-PCR samples. For example, the results of samples collected and submitted to NAHLN labs for rRT-PCR testing in the morning are assumed to be available to the Incident Command at the end of the same business day. However, this may not always be feasible for premises following the guidance of the SUGS Plan given that they are not only not infected, but also outside of a Control Area, giving them limited priority in the lab testing queue. In this case, earlier sample collection times for rRT-PCR tests may be needed on a case-by-case basis. Collecting rRT-PCR samples earlier may reduce the likelihood of detecting HPAI prior to the load-out start. Thus, for improved detection, we recommend that additional samples be collected and tested by antigen capture on the day of load-out. It is important to note that this alternate testing protocol is outside the scope of preferred testing protocols as outlined in other SPS Plans.

Detection through Trigger for High Mortality

If daily mortality is abnormally high (more than 1.5 per 1,000 birds in a pen, excluding culls depending on the farm on two consecutive days)[12] immediately prior to a scheduled movement, upland gamebirds should not move until diagnostic sampling and testing steps have been initiated and HPAI has been ruled out as the cause of elevated mortality.

9.4.2 Quantitative Methods for Estimating the Likelihood of HPAI Detection prior to the Start of Load-out on a Premises

The likelihood of detecting HPAI in an upland gamebird pen prior to the start of load-out is estimated via simulation. The approach consists of a stochastic disease transmission model, which simulates the spread of HPAI within a pen, and an active surveillance model, which uses the output from the disease transmission model to simulate the probability of detection under a given active surveillance protocol. A technical description of the simulation model algorithms can be found in Weaver et al. (2015).[429]

These simulation models from Weaver et al. have been reparametrized for upland gamebirds for use in the current analysis.[429] A summary of the input parameters is given in **Table 24,** and details on their estimation are given in **Appendix 7**: Modeling Technical Details. A brief overview of the disease transmission and active surveillance models is given below.

9.4.2.1 Overview of Disease Transmission and Active Surveillance Models

The likelihood of detecting HPAI depends on the following factors:

- The HPAI spread dynamics within a pen, which impacts the rate of mortality and morbidity rises over time. The HPAI spread dynamics depend on parameters such as the length of latent infection and infectious periods in individual birds, the "contact rate" between infectious and susceptible upland gamebirds on the farm, and the susceptibility of the upland gamebirds on the farm.
- The variability in the steps of the detection process, given an active surveillance protocol option. Factors such as the normal mortality (mortality not related to HPAI) and HPAI mortality rates impact the chances of including a virus-positive swab in the test sample (either tested with rRT-PCR or antigen capture). The chances of detecting a virus-positive sample depend on the diagnostic sensitivity of the test.

HPAI spread dynamics within a pen are simulated by the disease transmission model. Disease states included in the model are susceptible (S), latently infected (L), infectious (I), and removed (R). The number of upland gamebirds in each disease state is updated at 0.1-day intervals. Transitions from the latent to the infectious state and the infectious to removed state are determined by latent and infectious period distributions estimated based on data from experimental studies. Once a bird is in the removed state, it is deceased and remains in that state for the remainder of the simulation. The transition from the susceptible to the latently infected state is determined by the adequate contact rate and number of infectious birds in the current time period. The adequate contact rate (β) is defined as the mean number of birds each bird encounters per unit time such that the contact is adequate to transmit infection. Higher adequate contact rates result in a higher likelihood of infection. Similarly, as the number of infectious birds increases, the likelihood of infection increases.

Table 24. Parameter estimates for the HPAI transmission model for upland gamebird pens.

Parameter name	Parameter description	Distribution/Value	Sources
Latent period distribution	Distribution of the length of latent period of HPAI	Gamma: shape = 0.89, scale = 0.72 (i.e., mean = 0.64 days, standard deviation = 0.68 days)	Estimated from data in [73,167,426–428]
Infectious period distribution	Distribution of the length of infectious period of HPAI	Gamma: shape = 4.38, scale = 2.21 (i.e., mean = 9.68 days, standard deviation = 4.63 days)	[126]

Adequate contact rate	Distribution of the number of contacts per unit time that a bird has with others that are sufficient to transmit HPAI	Gamma: shape = 8.69, scale = 0.36 (i.e., mean = 3.13 per day, standard deviation = 1.06 per day)	[126]
Number stocked in pen	Distribution for the number of birds per pen	Generalized beta distribution with shape parameters: alpha = 1.89, beta = 8.74, minimum = 0 and maximum = 10,354 (Range in raw data: 406 to 5420 birds)	[12]
Disease mortality	Proportion of HPAI infected birds that dies due to the disease	Fixed: 100%	[106,126,128]
Daily normal mortality fraction distribution	Distribution for the proportion of dead birds per pen per day	Beta distribution with shape parameters: alpha = 0.113, beta = 74.35 truncated at minimum = 0 and maximum = 0.016	[12]
rRT-PCR sensitivity	Rate of true positive test results by rRT-PCR	Fixed: 86.5%	[429]
AC sensitivity	Rate of true positive test results by AC	50%[176] , 71%[430]	[176,430]

The variability in the detection process is simulated by the active surveillance model. Detection of HPAI in the surveillance model occurs through either diagnostic testing or heightened mortality. Samples for diagnostic tests are randomly selected from the normal and disease mortality available on the test day. The normal mortality is simulated based on industry-provided daily mortality, while the disease mortality is drawn from the transmission model output. Provided at least one infected bird is present in the test sample, detection occurs according to a Bernoulli trial with probability equal to the test sensitivity. Detection via heightened mortality occurs if the total mortality exceeds the trigger level on the days prior to the start of load-out.

9.4.2.2 Model Scenarios

The likelihood of detecting HPAI in an upland gamebird pen prior to movement is evaluated under scenarios where infection with the A/chicken/NL/621557/03 (H7N7) HPAI occurs in a pen. The length of latent and infectious period distributions can impact the time to detection: for example, HPAI strains with long mean times to death—the combined length of the latent and infectious periods—will generally take longer to detect via active surveillance due to the slower rise in mortality. Because latent and infectious periods are virus strain-specific and can vary, evaluating results based on multiple strains is important for developing robust risk management strategies. However, because of limited availability of upland gamebird-specific data, in the current analysis, the likelihood of detection can only be estimated for latent and infectious period distributions based on A/chicken/NL/621557/03 (H7N7) HPAI.

9.4.2.3 Estimated Likelihood of Detection under a Pre-Movement Isolation Period (PMIP)

As discussed previously, a PMIP involves the implementation of heightened biosecurity to minimize the chances of a pen becoming exposed to HPAI close to the start of load-out. **Table 25** gives the detection probabilities for a pen one to ten days following exposure to HPAI under the active surveillance protocol of one sample of 11 swabs taken for rRT-PCR testing 8 days prior to move and AC at load-out with daily mortality monitoring throughout.

If a pen was exposed to HPAI two days prior to the start of load-out, the estimated probability of detection is 10 percent and this probability increases to 95 percent if exposure is 8 days prior. In this example, the probability of detection improves as the number of days post-exposure increases. This is due to the continual rise in mortality that occurs as HPAI moves through the pen, which increases the likelihood of including at least one bird dead from HPAI in the pooled sample taken for diagnostic testing or total mortality that exceeds the threshold amount. Thus, by reducing the chances of exposure to HPAI close to the start of load-out, the PMIP decreases the risk of releasing infected but undetected birds by allowing sufficient time for the infection to spread within the pen.

Table 25 can be used to inform the length of the PMIP under an assumption that the PMIP is 100 percent effective in preventing exposure to the pathogen. In these scenarios, it is conservatively assumed that the pen is infected immediately prior to implementation of the heightened biosecurity of PMIP. For example, under a 4-day PMIP, a pen is assumed to have been infected 4 days before the start of load-out, just prior to the start of the PMIP. The detection probability in this case, is estimated to be 47 percent. Subsequently, the scenario under an eight-day PMIP is estimated to result in a 95 percent likelihood of detection. The length of the PMIP decided on by the SUGS Workgroup is 8 days, which generally achieves high probabilities of detection.

Table 25. Simulation model results showing the predicted probability of HPAI detection for a pheasant pen infected some given number of days prior to the start of load-out in the pen. Virus strain is A/chicken/NL/621557/03 (H7N7) HPAI [a]

	Number of days prior to movement on which exposure to HPAI occurs										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Predicted probability of HPAI detection	0.04	0.10	0.24	0.47	0.68	0.81	0.90	0.95	0.98	0.99	1.00

[a] The detection probabilities are estimated from 10000 simulation iterations. The active surveillance protocol consists of one sample of 11 swabs taken for rRT-PCR testing 8 days prior to move and AC at load-out with mortality monitoring throughout.

Table 26 reports results estimating the effect of AC testing on the detection probability. Three protocols were evaluated at two different AC testing sensitivities. Protocols consisted of one pooled sample of 11 swabs taken for rRT-PCR 8 days prior to

movement with the addition of one, two, or three samples of 5 swabs each taken for AC at 50 percent sensitivity and 71 percent sensitivity immediately prior to the start of load-out. The detection probabilities and mean with the 5th and 95th percentile of the number of infectious birds present in an undetected flock at the time of movement, are given in **Table 26** under the assumption that exposure occurred between 8 to 12 days prior to movement due to a 100 percent effective eight-day PMIP. The estimates are obtained from 10,000 iterations of the simulation model.

The results demonstrate that rRT-PCR testing with any of the suggested protocols, with the exception of the protocol utilizing only one sample of five pooled swabs for AC testing at 50 percent sensitivity, gave a probability of detection over >95 percent. Such findings suggest that even if sensitivity is compromised (e.g., dead or sick birds are not available), the number of pools helps keep the probability of detection at a high.

Table 26. Likelihood of AI detection and mean number of infectious undetected birds (5th, 95th percentile) for different active surveillance protocols. A 100% effective 8-day PMIP is assumed to have been implemented.

AC sensitivity	**Active surveillance protocol[a]**		
	Mortality trigger; PCR; 1 sample of 5 swabs for AC	Mortality trigger; PCR; 2 samples of 5 swabs for AC	Mortality trigger; PCR; 3 samples of 5 swabs for AC
	Predicted detection probability [b] **Mean number of infected undetected birds in pen (5th, 95th percentile)**		
AC se = 50%	0.94 1437 (198, 3311)	0.97 1417 (75, 3273)	0.98 1437 (65, 3119)
AC se = 71%	0.96 1372 (103, 3475)	0.99 1316 (77, 3715)	0.99 983 (14, 3337)

[a] Samples taken for rRT-PCR testing consist of 11 swabs at start of 8-day PMIP and samples for AC testing consist of pools with five swabs taken at the same time immediately prior to the start of load-out.
[b] Probabilities are estimated from 10,000 simulation iterations.

Table 27 compares the probability of detection under four different active surveillance and PMIP strategies. Under the scenarios with no PMIP, exposure is assumed to occur between one and twelve days prior to the start of load-out. Under the scenario with an 8-day, 100 percent effective PMIP, meaning the PMIP guarantees the pen is not infected during its implementation, exposure is assumed to occur sometime between eight and twelve days prior to the start of load-out. Exposures occurring earlier than 12 days prior to load-out are not considered since infection is almost certain to be detected via diagnostic testing and monitoring of mortality, so the risk of moving infected but undetected upland gamebirds would be minimal in such cases.

The results in **Table 27** indicate that performing active surveillance without implementing a PMIP is insufficient (with <95 percent chance) for detecting HPAI in an upland gamebird pen. Results also show that including antigen capture testing involving three pooled samples of five swabs from birds at load-out substantially improves the likelihood of detecting HPAI in the pen prior to movement. We also observe that when a PMIP is not implemented, exposures occurring within 12 days of load-out are hard to detect despite testing and when the exposures occurring close to the time of movement are prevented through the 8-day PMIP, the disease is detected with a high degree of

confidence. In the absence of AC testing on the day of movement, if a PMIP is in place, acceptable levels of HPAI detection can only be attained if rRT-PCR testing is performed on samples collected less than 36 hours before the move.

Also included in **Table 27** is the mean number of infectious birds at the start of load-out in the pens that go undetected, along with the 5th and 95th percentile. The mean number of infectious birds at the start of load-out in pens that went undetected is higher under the scenario of diagnostic testing with an 8-day PMIP, because the infection is present in the pen for at least 8 days, which leads to more birds becoming infected. Diagnostic testing with no PMIP, on the other hand, allows for infections to occur within eight days of the start of load-out, which provides less time for large numbers (e.g., 50 or more) of infectious birds to accumulate. The amount of mortality due to HPAI will also be lower when infections occur within 8 days of the start of load-out.

As HPAI is less likely to be detected when mortality is low, exposures close to the time of load-out have a higher probability of going undetected; therefore, they represent a greater proportion of the cases with infectious but undetected birds and lead to the lower mean dead bird number. While the mean number of infectious birds in undetected pen is higher under the scenario using both diagnostic testing and PMIP, the likelihood of detecting the infection is relatively high. Thus, this scenario poses the lowest risk for HPAI spread.

Table 27. Likelihood of detecting HPAI in a pheasant pen prior to the start of load-out on the premises followed by the mean and the 5th and 95th percentile number of infectious birds in an undetected pen at the time of movement. A/chicken/NL/621557/03 (H7N7) HPAI

PCR Scenario	Active surveillance and PMIP scenario varying by status and effectiveness[a]			
	Mortality trigger with no PMIP and no AC	**AC testing and mortality trigger and no PMIP[b]**	**No AC testing, with mortality trigger and, 100% effective 8-day PMIP[c]**	**AC testing and mortality trigger and 100% effective 8-day PMIP[c]**
	Likelihood of detection **Mean number of infectious upland gamebirds**			
PCR at 1 day	0.56 246 (1, 1255)	0.69 77 (0, 371)	0.96 1199 (79, 3119)	0.99 724 (29, 2445)
PCR at 1.5 days	0.54 298 (1, 1464)	0.69 98 (0, 542)	0.96 1041 (128, 2666)	0.99 768 (25, 2008)
PCR at 2 days	0.50 342 (1, 1630)	0.69 106 (0, 630)	0.94 1154 (143, 2900)	0.99 804 (41, 2422)
PCR at 4 days	0.46 471 (1, 2199)	0.68 136 (0, 835)	0.89 1442 (194, 3346)	0.98 1312 (47, 3100)
PCR at 8 days	0.45 480 (1, 2143)	0.68 150 (0, 935)	0.88 1442 (164, 3232)	0.98 1285 (64, 3103)

[a]Days indicated are days prior to movement when samples are collected for testing. Parentheses indicate the 5th and 95th percentiles estimated from 10000 iterations and RT-PCR involves one pool of 11 swabs while AC involves 3 pools of 5 swabs.

[b]Pen is assumed to be infected sometime within 1 to 12 days of the start of load-out with no PMIP.

[c]Pen is assumed to be infected sometime within 8 to 12 days of the start of load-out with a PMIP.

9.4.2.4 Overall Likelihood of not Detecting HPAI in an Upland Gamebird Pen Prior to the Start of Load-out on the Premises

The overall probability of not detecting HPAI in an infected upland gamebird pen by the start of load-out considers two events: the probability a susceptible pen becomes infected based on its distance from an infectious premises, and the probability that the infection is not detected in the pen prior to the start of load-out. The probability that a susceptible premises located a given distance from an infectious premises also becomes infected is estimated via a spatial transmission kernel, which is discussed below in **Section 9.4.2.4.1** Estimation of the Probability of Infection via a Spatial Transmission Kernel. The probability that infectious birds are not detected by the start of load-out, given that the pen has been infected, is estimated using the transmission and active surveillance simulation models discussed in the previous sections. The two probabilities are combined into an overall likelihood using a method described in Weaver et al. that considers the 12 days prior to the start of load-out.[429]

9.4.2.4.1 Estimation of the Probability of Infection via a Spatial Transmission Kernel

A spatial transmission kernel uses outbreak data to estimate the hazard rate, or infection risk, posed by an infectious premises a given distance away from a susceptible premises. The spatial transmission kernel theoretically averages the risk over all transmission pathways at the given inter-premises distance, therefore providing a summary view of outbreak spread. The current analysis considers a transmission kernel estimated from the 2015 HPAI H5N2 outbreak in Minnesota, specifically data from infected turkey premises.[384] The Minnesota transmission kernel was estimated using the maximum likelihood method from Boender et al. (2007) with an additional parameter added to the force of infection, which is the cumulative hazard rate faced by a susceptible premises on a given day.[142] The force of infection on susceptible premises *i* on day *t*, $\lambda_i(t)$, is given in Boender et al. (2007) as

$$\lambda_i(t) = \sum_{j \neq i} h(d_{ij}) 1\{j \; is \; infectious\}$$

where $h(d_{ij})$ represents the spatial transmission kernel as a function of the distance between susceptible premises *i* and infectious premises *j*.[142]

The force of infection as defined above assumes all spread to be lateral, dependent only on the number of infectious premises on day *t*. Due to phylogenetic evidence of primary introductions occurring concurrently with lateral spread in the Minnesota outbreak, an additional parameter, *k*, was added to the force of infection equation used to estimate the spatial transmission kernel for Minnesota, giving the following expression[142]:

$$\lambda_i(t) = \left[\sum_{j \neq i} h(d_{ij}) 1\{j \; is \; infectious\} \right] + k$$

The additional parameter represents a constant, distance-independent hazard primarily expressing the infection risk posed by distance-independent environmental factors—note that *k* does not depend on the number of infectious premises—such as wild birds. For more details on the estimation of the spatial transmission kernel for the Minnesota HPAI H5N2 outbreak, see **Appendix 7**: Modeling Technical Details.

The force of infection is used to estimate the probability that susceptible farm *i* is infected on day *t*, called $q_i(t)$. The expression for $q_i(t)$ is defined below:

$$q_i(t) = 1 - e^{-\lambda_i(t)}$$

As the force of infection increases, the probability of infection increases. **Figure 22** is a comparison of the Netherlands HPAI H7N7 and Minnesota HPAI H5N2 transmission kernels under the mean maximum likelihood estimates. Both transmission kernels indicate that infection risk was primarily distance-dependent during their respective outbreaks.

As the mean hazard rate for the Minnesota outbreak is higher and persists over longer distances relative to the Netherlands outbreak, the probability of infection will also be higher and remain elevated at larger distances under the Minnesota transmission kernel. As the overall probability of not detecting HPAI in a house (translated to "pen" for the purposes of the SUGS Plan) prior to the start of load-out is derived using the transmission-kernel-based probability of infection, it is expected to exhibit similar behavior.

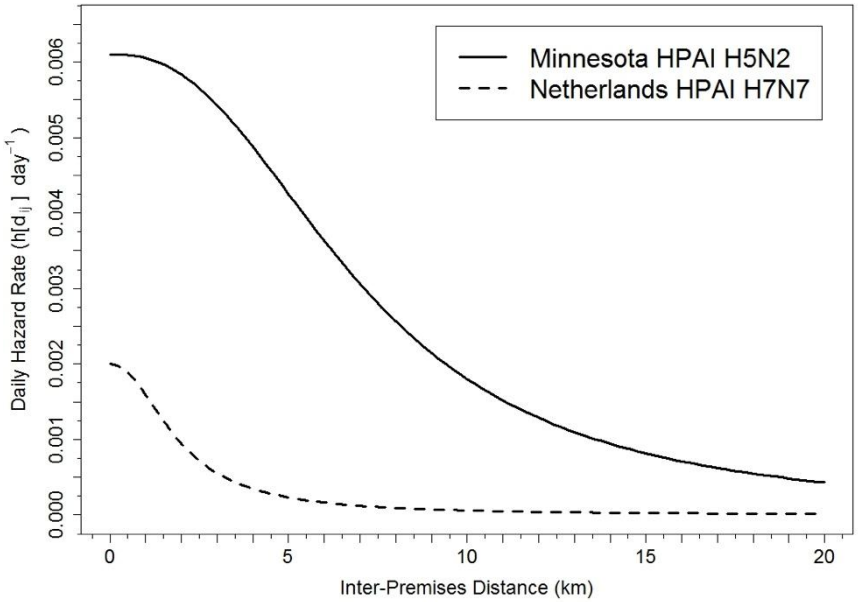


Figure 22. Spatial transmission kernels estimated from the 2003 HPAI H7N7 outbreak in the Netherlands by Boender et al. (2007)[142] and the 2015 HPAI H5N2 outbreak in Minnesota by Bonney et al. (2018).[384]

9.4.2.4.2 Estimated Overall Likelihood of not Detecting HPAI in an Upland Gamebird Pen Prior to the Start of Load-out

Estimates for the overall likelihood of not detecting HPAI in an upland gamebird pen prior to the start of load-out are given in **Table 27**. The overall likelihood is the combined probability of a pen first being exposed to HPAI and then HPAI going undetected in the pen prior to load-out following exposure. The probability that a susceptible premises is infected with HPAI by an infectious premises located a specific distance away is estimated using the Minnesota HPAI H5N2 spatial transmission kernels. It is important to note that estimates are conservative given that the application of the Minnesota HPAI H5N2 spatial transmission kernels to upland gamebird premises. The conservative application is attributed to the kernels being based on data representing turkey premises which are from an industry that operates in a more integrated fashion and has different production activities and set ups when compared to the commercial upland gamebird industry. The probability the infection goes undetected in the pen is estimated using the active surveillance simulation model under a diagnostic testing protocol of one pooled sample of 11 swabs taken for rRT-PCR testing 8 days before load-out (i.e., at start of PMIP, with continued mortality monitoring and AC on day of load-out).

The overall likelihood is estimated under three scenarios varying by the effectiveness of the PMIP at preventing exposure during the 8 days prior to the start of load-out. Premises did not institute a PMIP during the outbreak. Since the heightened biosecurity during the PMIP should result in lower likelihoods of exposure, the spatial transmission kernels estimated from these outbreaks likely overestimate the infection risk during this time. The baseline scenario in **Table 28** assumes the daily probability of exposure does not change during the PMIP, which would be expected if no additional biosecurity measures were implemented. The second scenario assumes the PMIP is 80 percent effective at preventing exposure, which means the daily probability of infection during the PMIP is reduced to one-fifth of the probability prior to the PMIP. The last scenario considers a 100 percent effective PMIP, which means the daily probability of exposure during PMIP is zero.

The estimates given in **Table 28** provide evidence that limiting exposure close to the time of movement through a PMIP reduces the overall likelihood of infection; even a partially effective PMIP leads to a considerable reduction. The overall likelihood decreases as distance from the infectious premises increases, due to the distance dependence exhibited by the spatial transmission kernel. Biosecurity and distance from an infectious premises both play a critical role in preventing exposure to HPAI and thereby limiting the risk of not detecting the infection in a pen prior to the start of load-out.

This risk can be further reduced by implementing a sound active surveillance protocol. **Table 28** indicates that the heightened biosecurity during the PMIP combined with an active surveillance protocol of an active surveillance protocol of one pooled sample of 11 swabs taken for rRT-PCR testing 8 days before load-out (i.e., at start of PMIP, with continued mortality monitoring and AC testing of three pooled samples of five swabs on day of load-out) is a viable strategy for reducing the overall likelihood, yielding low likelihoods of moving infected and undetected birds even at the edge of a Control Zone (e.g., 10 km) and under the higher hazard rates of the Minnesota transmission kernel.

Table 28. Predicted percent likelihood of a pheasant pen being exposed to HPAI from an infected premises at a specific distance and is undetected prior to the start of load-out following exposure; under three PMIP scenarios varying by biosecurity effectiveness.

Distance from an infected premises (km)	Scenario for the daily likelihood of exposure during 8-day PMIP[b]		
	Baseline-no PMIP	80% effective PMIP	100% effective PMIP
	Predicted likelihood		
10	0.79%	0.17%	0.0076%
15	0.43%	0.09%	0.0041%
30	0.19%	0.04%	0.0017%
200	0.12%	0.03%	0.0012%

[b]In all scenarios, an active surveillance protocol of one pooled sample of 11 swabs taken for rRT-PCR testing 8 days before load-out (i.e., at start of PMIP, with continued mortality monitoring and AC of three pooled samples of five swabs on day of load-out). Likelihood estimates expressed as a percent.

9.4.3 Likelihood of Moving Infectious but Undetected Upland Gamebirds Following Exposure during Load-out

Contaminated employees set to perform load-out and crates used to haul birds entering a pen pose an infection risk. As discussed in **Section 9.3** Pathways for an Upland Gamebird Flock Becoming Infected with HPAI VIRUS via Load-Out Operations, the number of infectious birds can increase rapidly in pens infected during or shortly before the load-out and transportation process, which could pose significant consequences if these birds were to be transported from the premises. Additional diagnostic testing during the load-out period can decrease the likelihood of moving large numbers (e.g., 50 or more) of infectious birds following exposure to HPAI during the load-out process. The estimated likelihood of detection for a single pen 2 to 10 days following exposure to HPAI under the active surveillance protocol decided upon by the SUGS Working Group is in **Table 29.**

The protocol is evaluated under four scenarios varying by the number of birds assumed to be initially infected, which represents increasing levels of contamination on the employees loading birds out and crates. This model uses the A/chicken/NL/621557/03 (H7N7) HPAI virus. The testing protocol decided upon by the SUGS Workgroup involves the options of either:

- Protocol 1: rRT-PCR testing of one pooled sample of 11 swabs of birds at least 36 hours prior to movement of birds off farm, or
- Protocol 2: rRT-PCR testing of one pooled sample of 11 swabs of birds every 8 days with AC testing of three pooled samples of 5 swabs each immediately prior to movement of birds.

As expected, the likelihood of detection increases as the number of days since exposure increases. Similarly, the likelihood of detection increases as the number of initially infected birds increases, since more infectious birds results in faster growth of the

infection within the pen. When the initial number of infected birds is one, the probability of infection exceeds the 95 percent threshold nine days post-exposure. On the other end of the scale, when the initial number of infected birds is 100, the 95 percent threshold is estimated to be exceeded at five days post-exposure. The low detection probabilities for pens exposed close to the time of movement can be improved using AC testing.

Table 29. The likelihood of detecting HPAI in a pen prior to the transportation of pheasants to a hunting preserve for different number of days post-exposure and numbers of initially infected birds, meant to represent the possibility of contiguous pens infecting the pen of interest

	Days post-exposure								
Initial no. of birds infected	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	Predicted Detection Probability for Protocol 1[a] and (Protocol 2)[b]								
1	0.00 (0.06)	0.00 (0.10)	0.04 (0.16)	0.18 (0.29)	0.48 (0.58)	0.77 (0.82)	0.91 (0.94)	0.98 (0.98)	0.99 (1.00)
5	0.00 (0.06)	0.02 (0.11)	0.16 (0.27)	0.56 (0.64)	0.89 (0.91)	0.98 (0.99)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)
10	0.00 (0.07)	0.04 (0.13)	0.29 (0.38)	0.76 (0.80)	0.96 (0.97)	0.99 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)
100	0.01 (0.07)	0.32 (0.38)	0.87 (0.88)	0.98 (0.99)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)

[a]Protocol 1: rRT-PCR testing of one pooled sample of 11 swabs of birds at least 36 hours prior to movement of birds off farm
[b]Protocol 2: rRT-PCR testing of one pooled sample of 11 swabs of birds every 8 days with AC testing of on three pooled samples of five swabs immediately prior to movement of birds.

Despite the low probabilities of detection of three to four days prior to testing, the likelihood of sending large numbers (e.g., 50 or more) of infectious but undetected upland gamebirds to release for most shipments of upland gamebirds (e.g., roughly 500 birds delivered within 24 hours [unpublished data, Secure Upland Gamebird Supply Working Group]) is negligible. Given in **Table 30** is the predicted percent probability of not detecting HPAI in a pen where the number of infectious but undetected upland gamebirds is at least 50 birds or 100 birds, given exposure occurred during load-out, some number of hours prior to arrival at the hunting preserve. The percent probabilities are estimated from the A/chicken/NL/621557/03 (H7N7) HPAI virus under the active surveillance protocol of rRT-PCR testing consist of 11 swabs at start of 8-day PMIP and samples for AC testing consist of pools with five swabs taken at the same time immediately prior to the start of load-out.

The results in **Table 29** suggest that the risk of sending many infectious but undetected upland gamebirds to release (e.g., in numbers of 50 or more) can vary greatly depending on the distance travelled. For the average load size of 500 within the average delivery time within 24 hours, the probability is 0.00 percent regardless of 1 or 5 infectious birds being present at the beginning on transit. **Table 30** demonstrates that both the difference in load size and the difference in time elapsed since the completion of the load-out process does not have a substantially large impact on the risk of delivering infectious birds. The use of relevant biosecurity to prevent contamination of load-out crates and

personnel and prevent contaminants from entering the pens is essential in eliminating the possibility for any infectious birds to be present before or during load.

Recommended practices during an active outbreak for loadouts include cleaning and disinfecting crates prior to use on the premises. This measure prevents the crates from being highly contaminated, making the scenario where only one bird is initially infected more likely than having many infected birds initially infected. In addition, heightened pen-to-pen biosecurity, such as pen-specific footwear, is recommended, which limits the likelihood of HPAI entering a populated pen before load-out begins in that pen. This may keep HPAI virus from infecting a pen for multiple days. Considering these recommended exposure mitigations, the likelihood of sending at least 50 infectious but undetected upland gamebirds to release is expected to be low.

It should be noted that results in **Table 30** apply to the circumstance of delivery to the first premises, the percent probabilities for subsequent deliveries at *additional* premises would be adjusted given the changing number of birds and additional potential exposures.

Table 30. The estimated percent probability of delivering more than 50 or 100 HPAI infected undetected pheasants to a hunting preserve following exposure during load-out.

Initial number of birds infected[a]	Load size[b]	Time elapsed since loading completion		
		12 hours	24 hours	36 hours
		Predicted probability [%] of at least the following amount of infectious pheasants delivered at the preserve: 50 pheasants (100 pheasants)[c]		
1	500	0.00 (0.00)	0.00 (0.00)	0.04 (0.00)
	1000	0.00 (0.00)	0.00 (0.00)	0.05 (0.00)
5	500	0.00 (0.00)	0.00 (0.00)	2.72 (0.06)
	1000	0.00 (0.00)	0.01(0.12)	2.86 (0.12)

[a]The initial number of birds infected is a proxy for the level of contamination present on the load-out equipment crew and equipment.
[b]Total number of birds loaded onto a truck intended for delivery to the end destination.
[b]Percent probabilities are estimated from 10,000 simulations.

Upon completion of the load-out process (i.e., when the truck filled with a shipment of birds leaves the premises), if exposure occurs during load-out, depending on the transit duration, the number of infectious birds can increase during transit. **Table 31** estimates, based on the number of birds in a shipment (i.e., birds on truck), the average number of infectious birds that will be present upon delivery of the first drop off site and the probability of at least one bird arriving dead upon delivery due to HPAI-induced mortality. Most deliveries of upland gamebirds occur within 2 to 24 hours (personal communication, Secure Upland Gamebird Working Group, January 2020) and shipments average 500 birds (unpublished data, Secure Upland Gamebird Supply Working Group). Based on this delivery range and shipment average, results from **Table 31** estimate if infection happens at load-out, transmission among birds within the shipment for the majority of deliveries of upland gamebirds would on average lead to two infectious birds on the truck if one pre-infectious bird (a bird in the eclipse period) was present at the completion of load-out, and the probability of at least one HPAI-induced mortality upon arrival at the first delivery site being 0.01 percent. On the extreme end of the spectrum of

shipments, **Table 31** estimates that for a shipment of 7,500 birds being transported over a 36-hour duration, the number of infectious birds in the shipment upon arrival to the first delivery site would be on average 4 (with a range of 0 to 13 birds based on the simulations performed), and the probability of having at least one HPAI-induced mortality is 0.12 percent.

The results from **Table 31** demonstrate the impact of the spread of disease that can take place within a shipment during transit is negligible. Results depicting the probability of at least one bird resulting from an HPAI-induced mortality upon arrival suggest that the number of mortalities upon arrival would not be useful in determining the presence of disease.

Table 31. The estimated average number of infectious birds on a truck, probability of having at least one dead bird having died from disease over time based on load size with one latently infected bird at the beginning of transit, and range of HPAI-induced deaths.

Load size[a]	Duration in transit		
	12 hours	**24 hours**	**36 hours**
	Average number of infectious birds on truck (5th and 95th percentiles of the number infectious birds)[b]		
	Percentage of 10,000 simulations in which at least one bird died due to HPAI while in transit [%]		
	Minimum and maximum number of HPAI-induced deaths [Minimum, Maximum]		
10	1 (0, 2)	1 (0, 4)	3 (0, 6)
	0.00	0.02	0.10
	0, 0	0, 1	0, 1
50	1 (0, 2)	2 (0, 5)	4 (0, 11)
	0.00	0.01	0.17
	0, 0	0, 1	0, 1
500	1 (0, 2)	2 (0, 5)	4 (0, 13)
	0.00	0.01	0.12
	0, 0	0, 1	0, 1
1000	1 (0, 2)	2 (0, 5)	4 (0, 13)
	0.00	0.02	0.15
	0, 0	0, 1	0, 1
7500	1 (0, 2)	2 (0, 5)	4 (0, 13)
	0.00	0. 02	0.12
	0, 0	0, 1	0, 1

[a]Total number of birds loaded onto a truck intended for delivery to the end destination.

[b]Numbers are rounded off to nearest integer. In all simulated scenarios, disease mortality was zero on average.

9.4.4 Conclusions

An effective PMIP increases the probability of detection by preventing exposure close to the time of load-out, which allows a longer time for HPAI to spread within the pen. This leads to higher levels of disease mortality and increases the likelihood that the total mortality exceeds the trigger level or that a swab from an HPAI-infected dead bird is included in the diagnostic test sample. An 8-day PMIP generally yields high probabilities of detection, though it may not be entirely robust for all HPAI strains and within-pen

spread scenarios. Given the load-out biosecurity and active surveillance measures in place, if an infected but undetected movement were to take place, a movement containing large numbers (e.g., 50 or more) of infectious birds would be unlikely.

Assuming that an effective PMIP is implemented, and that both mechanisms for active surveillance outlined in the SUGS Plan (trigger for elevated mortality, rRT-PCR mortality testing every 8 days, and AC testing at load-out) are utilized as described, and that load-out biosecurity measures are implemented, the likelihood of HPAI in an infected upland gamebird pen going undetected is rated as follows:

- The overall likelihood of HPAI-infected but undetected upland gamebirds in a pen at the conclusion of PMIP and prior to the start of load-out on the premises is estimated to be ***very low*** at a distance of 10 km or more from an infected premises.
- The likelihood of HPAI-infected but undetected upland gamebirds in a pen at the conclusion of load-out, resulting transmission of virus during transit and the movement of large numbers of infectious birds ($\geq 50\ or\ \geq 100$) to release, is estimated to be ***very low*** if delivered before 36 hours.

Overall Conclusion

The objective of this assessment was to estimate the risk that the movement of mature upland gamebirds to release (mature, flight-ready birds to a hunting preserve), from a premises that is not located within a Control Area, but is located within a U.S. State with an active HPAI outbreak, resulting in the introduction of HPAI infection onto a poultry or upland gamebird premises (e.g., poultry farm or another upland gamebird farm).

The assessment considered relevant current industry practices and current biosecurity measures as well as outbreak-specific measures from the SUGS Plan, particularly the PMIP. The assessment focused on the risk pathways for HPAI infection of mature, flight-ready upland gamebirds on an upland gamebird farm located outside of an HPAI Control Area but within a State with HPAI via components of local area spread, people and vehicles, and load-out processes. Many of these pathways do not involve the movement of live birds and rather relate to the likelihood of infection of live birds that will move and potential for a missed detection prior to movement. Qualitatively compiling the assessed risks and likelihoods of the pathways analyzed yields the overall risk of HPAI spread to susceptible poultry due to the movement of upland gamebirds to release at a hunting preserve **(Figure 23).**

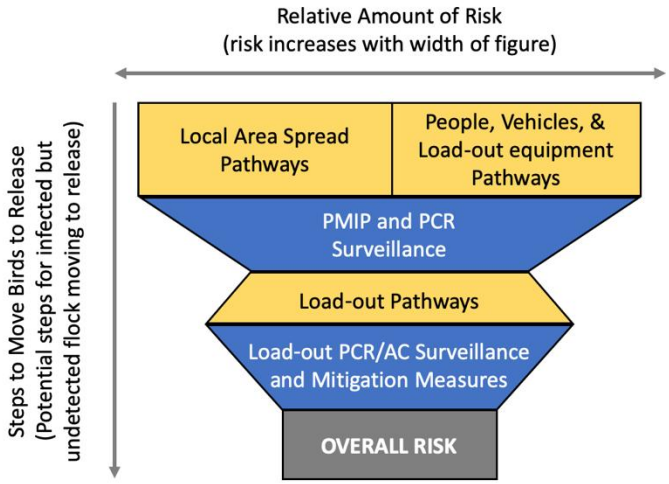


Figure 23: Diagrammatic representation of the overall assessed risk. The overall risk assessment is based on consideration of the steps needed to move live birds to release and the pathways that could lead to infection of a flock, the subsequent likelihood of detection of the infected flock, and potential movement of an infected but undetected flock.

The evaluation of the major risk pathways identified resulted in the following conclusions:

9.5 Local Area Spread Pathways

- **Aerosols.** The likelihood of an upland gamebird premises becoming infected with HPAI virus via bioaerosol transmission varies with distance and with viral load at the source premises. Literature review and most previous outbreak reports indicated that aerosol transmission was not an important factor at distances more than 1.5 km from an infected flock. However, there is some evidence of aerosol transmission over shorter distances. Thus, the likelihood of an upland gamebird premises becoming infected via bioaerosol transmission is rated as follows:
  - ***Low to negligible*** if >1 km from an infected but undetected premises depending upon distance.
  - ***Negligible*** if >10 km from a known-to-be-infected premises located in a Control Area.
- **Insects.** The likelihood of an upland gamebird premises becoming infected with HPAI virus via insect transmission varies with distance from the infected premises. For premises located closer than 1 km to an infected flock, there are too many variables to accurately assess the likelihood of becoming infected with HPAI via insect transmission.
  - ***Low to negligible*** if > 1 km from an infected but undetected premises depending upon distance.

- ***Negligible*** if >10 km from a known-to-be-infected premises located in a Control Area.

- **Rodents.** While rodents have proven unlikely to play an important role in the transmission of HPAI virus in poultry outbreaks, uncertainty remains as to their potential as vectors (particularly mechanical vectors), and because upland gamebird are housed in pens, the presence of rodents cannot be fully eliminated. However, the given that the premises within the scope of this assessment are at least 10 km away from the nearest known-to-be-infected farm, the likelihood of an infected or contaminated rodent traveling from an infected farm to a new farm is unlikely. Additionally, because upland gamebird premises have limited sharing of vehicles and resources with other farms of any kind, it is unlikely human activity would move infected or contaminated rodents onto an upland gamebird farm. Thus, the likelihood of HPAI infection via rodents in the farm vicinity is ***very low.***
- **Predatory Mammals.** While predatory mammals have very little documented evidence to support that they play a significant role in the transmission of HPAI virus in poultry outbreaks (including outbreaks that involved penned or free-range farms) uncertainty remains as to their potential as vectors (particularly mechanical vectors). In regard to conventional poultry farms (e.g., commercial turkey, broiler, and egg laying chickens), predators will have no access to potentially infected birds in barns, however, predatory species have the potential to scavenger from mortality piles. Because upland gamebirds are housed in pens, contact with potentially contaminated or infected predatory mammals is possible and the risk cannot be completely eliminated even with mitigation measures. However, adequate predatory mitigations and proper pen-to-pen biosecurity, specifically wearing pen-specific footwear and handwashing after handling a trapped or dispatched predatory mammal onsite, reduces the possibility of transmission. Finally, while many predatory species can biologically or mechanically carry HPAI virus, the home ranges of these mammal were typically smaller than the minimum distance between a known-to-be-infected farm and an upland gamebird premises following the SUGS Plan, thus the likelihood of an infected or contaminated predatory mammal traveling from and infected farm to a susceptible upland gamebird farm is ***low.***
- **Wild Birds.** The likelihood of HPAI virus spread to an upland gamebird premises via wild birds depends upon the type of wild birds and exposure to the wild birds. However, because there are limited wild bird attractants on upland gamebird farms, barriers including fencing and netting protect the upland gamebirds, the likelihood of wild birds visiting infected poultry farms prior to coming to an upland gamebird premises, and effective PMIP mitigations, the likelihood of HPAI infection via wild aquatic and non-aquatic birds including scavenger and passerine birds via either direct contact or indirectly is ***low to moderate.***
- **Proximity to Live-haul Routes.** The likelihood of HPAI virus spread to an upland gamebird premises near poultry live-haul routes via feathers, feces, and other fomites depends on both distance and source flock. For trucks hauling birds that had an effective PMIP and negative rRT-PCR test results, the likelihood is

estimated to be ***negligible to low*** no matter the distance. In contrast, for trucks hauling infected but undetected birds that had no PMIP and no diagnostic tests (e.g., from premises outside the Control Area), the likelihood ranges from ***low to high,*** with premises within 100 meters of the live-haul route at highest likelihood.

9.6 People, Vehicles, and Equipment Movement Pathways

- **Feed and Critical Operational Visits.** Critical operation visits will be limited during PMIP; however, feed delivery during this period is likely, and the potential for emergency veterinary visits also exists. The likelihood of an upland gamebird flock becoming infected with HPAI via critical operational visits during PMIP was assessed as *negligible to moderate,* as follows:
    - ***Negligible*** via contaminated feed
    - ***Low*** via feed delivery (e.g., contaminated driver and/or vehicle)
    - ***Low to moderate*** via other critical operational visits (e.g., personnel or vehicle)
- **Growers, Employees, and their Vehicles.** Provided PMIP measures for people are strictly followed and people wear farm-specific clothing and pen-specific footwear, we rate the likelihood of an upland gamebird flock becoming infected with HPAI via people and their vehicles entering the premises during the PMIP as ***low*** for people entering the bird pens and ***very low*** for people who do not enter the bird pens.
- **Dead Bird Disposal.** For on-farm dead bird disposal methods used in the upland gamebird industry, risks associated with scavenger species were assessed. While many scavenger species can biologically or mechanically carry HPAI virus, the home ranges of these scavengers were typically smaller than the minimum distance between a known-to-be-infected farm and an upland gamebird premises participating in the SUGS Plan, thus we assessed the likelihood of HPAI introduction to an upland gamebird farm during the PMIP as ***very low.*** While off-site dead bird disposal methods prior to a PMIP may result in premises contamination, because the only common off-site disposal method used in the upland gamebird industry is landfill disposal, the associated likelihood of introduction is equivalent to that associated with garbage management (see below).
- **Garbage Management.** There is potential for HPAI virus associated with garbage management to be tracked into an upland gamebird pen especially if the garbage dumpster is located within the perimeter buffer area, and thus we assessed the likelihood of an upland gamebird flock becoming infected with HPAI virus due to garbage management outside of a PMIP to be ***moderate***. During a PMIP, garbage pick-up is outside the perimeter buffer area and pen-specific footwear will be employed, and thus we assessed the likelihood of an upland gamebird flock becoming infected with HPAI virus due to garbage management during a PMIP as ***low.***

9.7 Load-out Pathways

- **Load-out.** Assuming PMIP enhanced biosecurity and testing measures are strictly implemented, and that additional load-out mitigation measures are in place and commensurate with the duration of the premises-wide load-out process, the likelihood that a broiler flock will become infected with HPAI virus via load-out operations and that this will result in an infected but undetected movement to market is estimated to be ***very low*** to ***low*.**

9.8 Likelihood of Detecting HPAI Prior to Movement of Upland Gamebirds off a Farm

- Assuming that an effective PMIP is implemented, and that both mechanisms for active surveillance outlined in the SUGS Plan (trigger for elevated mortality, rRT-PCR mortality testing every 8 days, and AC testing at load-out) are utilized as described, and that load-out biosecurity measures are implemented, the likelihood of HPAI in an infected upland gamebird pen going undetected is rated as follows:
    - The overall likelihood of HPAI-infected but undetected upland gamebirds in a pen at the conclusion of PMIP and prior to the start of load-out on the premises is estimated to be ***very low*** at a distance of 10 km or more from an infected premises.
    - The likelihood of HPAI-infected but undetected upland gamebirds in a pen at the conclusion of load-out, resulting transmission of virus during transit and the movement of large numbers of infectious birds (≥ 50 *or* ≥ 100) to release, is estimated to be ***very low*** if delivered before 36 hours.

9.9 Overall Risk

It is concluded that the overall risk of HPAI spread to susceptible poultry associated with the movement of mature upland gamebirds to a hunting preserve outside of a Control Area is ***low*** provided that all applicable preventive measures from the SUGS Plan, in particular the PMIP, are strictly followed and suggested pre-movement surveillance testing results are negative

Note: The consequences of the movement of mature upland gamebirds to a hunting preserve as they impact susceptible poultry are assumed to be lower than consequences of other live bird movements in the broiler, turkey, and layer industries. While a complete consequence assessment is out of the scope of this risk assessment, the assumptions related to the consequences of the movement as outlined in **Section 6**: Significant Assumptions Used in the Risk Assessment are factored into the overall risk rating.

In using the results of this risk assessment, it should be remembered that:

- This assessment is based on information, data, and literature published or obtained as of *November 2020* and will need to be reviewed and revised as circumstances warrant.
- The assessment does not replace the judgment of on-scene officials with first-hand knowledge of the outbreak situation and the premises

Appendix 1: Pen-Raised Upland Gamebird Post-Release Survival & Dispersal Range

Appendix 1 Table 1 descriptively summarizes the published literature assessing the dispersal ranges of, survival of, and documented causes of mortality for non-bagged (i.e., that were not collected via hunter-harvest) pen-raised (i.e., raise-for-released) upland gamebirds included within the scope of this risk assessment (i.e., Ring-necked pheasant [*Phasianus colchicus*], chukar and red-ledgged partridge [*Alectoris chukar* and *Alectoris rufa*], and Bobwhite quail [*Colinus virginianus*]). Note that studies assessing the same outcomes for relocated wild upland gamebirds were excluded from the literature summary because they are not relevant to the scope of the risk assessment. Additionally, only studies carried out in the United States were included, and studies before 1960 were excluded due to limitations with availability and access. Finally, an upland gamebird private hunting preserve subject matter expert panel comprised of key members from the North American Gamebird Association (NAGA) was consulted for their expert opinion on the released birds' dispersal ranges, post-release survival, and non-hunting related causes of mortality. Although, it should be noted that bias and uncertainty is introduced to data and insights provided by any expert panel.

Authors across 10 of the 19 identified studies observed that pen-raised upland gamebirds released into the wild engaged in limited travel from the release site. Documented dispersal ranges of "most"[5a] birds within each study varying between 0.11 km and 3.2 km. However, May & Haugen (1973) observed isolated incidents where individual banded, released birds had traveled substantially farther (e.g., 10 km).[431] Additional, estimations from expert opinion indicate that "most" pen-raised birds that are released stay within 2.4 km, on average established. Although, the expert panel also notes that in rare instances, dispersion could be farther, with the possibility that birds could travel up to 4.8 km (NAGA expert panel, personal communication, August 2020). The mean and median dispersal ranges for "most" birds across the studies[6a] were 0.94 km (SD = 0.93) and 0.52 km (IRQ = 1.12), respectively, when including the expert opinion estimation and 0.83 km (SD = 0.86) km and 0.50 km (IRQ = 0.71), respectively, when excluding the expert opinion. Burger (1964) suggests that seasonality may impact dispersal ranges of birds given that previous studies[7a] found similar dispersal ranges during hunting seasons (i.e., during early fall to early winter months, albeit there is variability between States), but that wider dispersal ranges consistently existed during spring months.[432] For details from specific studies related to dispersal ranges post-release, see **Appendix 1 Table 1** below.

General trends from the literature suggest that most non-bagged released pen-raised upland gamebirds do not survive to the next season, but there is some degree of variation. Survival of released pen-raised upland gamebirds is likely impacted by numerous factors based on available literature, including the season that birds were released,[433,434] the environment they were released into,[435] predator presence in the environment, and subjection to hunter harvest (see **Appendix 1 Table 1**). Additionally, studies varied in how many days post-release that survival was measured as well as in which methods were utilized to measure and report survival. Given such variation across studies, average survival rates across the literature were not calculated and the specific

[5a] "Most" as being used verbatim by some authors or the subject matter expert panel; Otherwise, quantifiable definitions are reported for individual studies in Appendix 1 Table 1.
[6a] Not considering the number of birds represented in each study.
[7a] Data inaccessible and, thus, not captured in this literature review.

details for each study are presented independently in **Appendix 1 Table 1.** Like post-release dispersal range, survival of released pen-raised birds may depend on the season. Krauss et al. (1987) observed that pen-raised birds that survived beyond 35 days post-release were released in June rather than in March and April, and authors hypothesized that associated seasonal changes such as cover availability and cover use could have been attributed to the success of birds released in June.[433]

Expert opinion also suggests that access to appropriate cover dictates the adequacy of habitat for upland gamebirds and that land with adequate habitat has declined over time. Experts hypothesize that, consequently, wild populations of certain upland gamebird species, such as pheasants, in specific geographical areas "have not been established since the 1980's" due to loss of habitat (North American Gamebird Association expert panel, personal communication, August 2020). Additional literature supports such claims, recognizing that pheasant populations in Midwest United States peaked in the mid twentieth century[436] and started declining in the 1970's and 1980's due to land being converted for agriculture purposes.[436–438] While numbers of wild pheasants only observed a mild uptick in the 1990's,[439–441] these improvements occurred after concerted efforts to maintain suitable habitat were made. Albeit outcomes of the continuation of such efforts during the 2000's are not as well understood.[442] In addition, retrospective studies looking at pheasant population data in the Western United States observed similar declines in pheasant populations due to habitat loss.[443,444] Moreover, given that 15 out of the 19 studies within the literature summary are older than 10 years old at the time of the present risk assessment's publication, it is possible that the data represented in these studies trend towards higher survival rates, especially those studies published in during peak wild population levels, such as in the 1960's. Ultimately, it is suggested that for pheasant populations to successfully establish, the land needs to be intentionally managed as pheasant habitat,[442] which agricultural land is not. Other upland gamebird species exhibit similar population trends based on expert opinion and available literature. Expert opinion suggests that wild populations of bobwhite are still established and maintained for hunting purposes (NAGA expert panel, personal communication, August 2020); however, literature reports that populations of bobwhite quail across the United States have been declining since the 1960's due to habitat loss and pesticide presence as a result of land being transitioned for agriculture use.[445,446] Additionally, Oakely et al. (2002) observed that bobwhite quail released have better survival rates on land that is maintained for wildlife versus land that is transitional between agricultural land and naturally maintained land, demonstrating habitat quality and cover availability is also important for bobwhite quail survival.[435] Less is known about established populations of partridge species used for hunting.[447]

Fourteen out of 19 studies observed predation by avian and/or mammalian predators as the leading cause of mortality for non-bagged released birds. Of those studies that reported proportions of recovered mortality as killed by mammalian and/or avian predators, the proportions[8a] ranged from 20 percent to 100 percent, with the mean and median proportions of non-bagged bird mortality attributed predation being 73.9 percent (SD = 27.8) and 88.2 percent (IRQ = 48.3), respectively. Additionally, estimations from expert opinion were that roughly that 33 percent of released birds will succumb to predation if they are not bagged by hunters. Thus, the mean and median proportions of non-bagged bird mortality attributed predation when including expert opinion estimation were 71.0 percent (SD = 28.9) and 88.1 percent (IRQ=49.7),

[8a] Not considering the number of birds represented in each study.

respectively. For details from specific studies related to the outcomes discussed, see **Appendix 1 Table 1** below.

Finally, additional observations related to how behavioral tendencies specific to pen-raised upland gamebirds may impact post-release survival and dispersal were detailed within 13 of the 19 reviewed studies. Authors commenting on behavior noted that pen-raised birds potentially lacked a strong predator avoidance response in comparison to wild upland gamebirds as evidenced by their response to the physical presence of study observers. Similar observations were made in an experimental study assessing the escape behavior of pen-raised red-legged partridge (*Alectoris rufa*).[448] Such observations may account for heavy predation and poor overall survival and as supported by other studies, with Perez et al. (2002) directly making the hypothesis that captive-raised upland gamebirds are more susceptible to predators.[449] Other studies have explored this hypothesis experimentally, with Perkins et al. (2018) providing preliminary data for raptor preference of captive-raised upland gamebirds over wild upland gamebirds;[450] however, Reyna and Newman (2018) note that there were no pronounced differences in escape reactions to simulated predator attacks observed between captive-raised and wild upland gamebirds.[450] **Appendix 1 Table 2** summarizes these observations within the literature.

Appendix 1 Table 1. Summary of pen-raised upland gamebird dispersal and survival as well as reasons for non-bagged bird mortality, based on field studies conducted in the United States and expert opinion.

Reference	Species	Location of study	Month birds were released	Findings on post-release range of birds	Findings on post-release survivability	Survival impacted by hunter harvest (Y/N/UNK)	Findings on causes of mortality for non-bagged birds
Carter, (2015)[451]	Bobwhite quail	Kentucky	April	N/A	Survival of radio tagged pen-raised birds was 0% (0/31) by 34 days post-release. On release site 1, 2, and 3 the number of days until survival rates were reported as 0 were 9, 4, and 9 days, respectively. Estimated mean survival duration was 6.7±6.4 days in Year 1 and 3.0±2.6 days in Year 2.	UNK	Predators killed 84% (26/31) of recovered pen-raised quail. Specifically, raptors killed 39% (12/31) quail, coyotes killed 29% (9/31), and mesopredators killed 16% (5/31).
DeVos & Speake, (1995)[452]	Bobwhite quail	Alabama	Early November and mid-December	N/A	Roughly 90% (~720/800) of pen-raised birds survived 7 days post-release, 60% (480) of pen-raised birds survived to roughly day 30 post-release, and roughly 25% (~200/800) of pen-raised birds survived to the end of the hunting season (~100 days post release).	Y/N[c]	"Predation was implicated in the majority of bobwhite deaths, with raptors and mammals as the principal predators. Avian predators were responsible for 67.1% (537/800) of *known predation* for [...] pen-raised mortalities... Mammals caused [...] 32.9% (312/800) of known mortalities for pen raised post release bobwhites.
Fies et al. (2000)[453]	Bobwhite quail	Virginia	October and March	N/A	0% (0/60) of pen-raised birds survived to day 9 post-release when birds were released in the fall. 0% (0/60) of pen-raised birds survived to day 19 post-release when birds were released in the spring.	UNK[d]	55.8% of pen-raised birds were killed by mammalian predators, 32.5% were killed by avian predators, 0.08% were killed by unknown predators, 0.02% were killed by stress, and 0.02% were killed by other means.

Appendix 1 Table 1. Summary of pen-raised upland gamebird dispersal and survival as well as reasons for non-bagged bird mortality, based on field studies conducted in the United States and expert opinion.

Reference	Species	Location of study	Month birds were released	Findings on post-release range of birds	Findings on post-release survivability	Survival impacted by hunter harvest (Y/N/UNK)	Findings on causes of mortality for non-bagged birds
Hutchins, (2003)[454]	Bobwhite quail	Texas	August and September	Mean home range of pen-raised quail post-release was 0.21km^2 when Year 1 and Year 2 were pooled.	"Pen-raised bobwhites experienced a 0.0% (0/800) survival rate from November 2000 to February 2001."	Y	Predators killed 40% (12/30) of released pen-raised quail when data from Year 1 and Year 2 were pooled. Specifically, avian predators killed 13% (4/30) and mammalian predators killed 27% (8/30) of pen-raised quail.
Oakley et al., (2002)[435]	Bobwhite quail	Maryland	September	Estimated home range areas for pen-raised] bobwhite coveys ranged from 0.002 km^2 to 0.66 km^2 with a mean home range of 0.24±0.035 km^2	Estimated survival rate of pen-raised birds was 0.0 (n = 177) by roughly day 133 post-release.	Y	"Predators were the primary cause of mortality (88%), followed by other causes including unknown causes (7%), stress (2%), hunting (2%), and roadkill (1%). Predation accounted for 97% of the mortalities in buffer strip areas and 78% in non-buffer strip areas. Avian predators were responsible for most mortalities in buffered (42%) and non-buffered (41%) areas. Mammalian predation accounted for 27% and 12% of mortalities in buffered and non-buffered areas, respectively. While unknown predation accounted for 26% in buffered areas and 25% in non-buffered areas."

Appendix 1 Table 1. Summary of pen-raised upland gamebird dispersal and survival as well as reasons for non-bagged bird mortality, based on field studies conducted in the United States and expert opinion.

Reference	Species	Location of study	Month birds were released	Findings on post-release range of birds	Findings on post-release survivability	Survival impacted by hunter harvest (Y/N/UNK)	Findings on causes of mortality for non-bagged birds
Perez, et al. (2002)[449]	Bobwhite quail	Texas	November	N/A	"Game-farm [...] quail reached 50% (23/46) mortality in 9 [...] days." Survival rate of pen-raised birds was 0.0 by roughly day 66 post-release.	Y	26.3% (12/46) of pen-raised birds were killed by avian predators, 61.9% (28/46) were killed by mammalian predators, 9.5% (4/46) were killed by unknown means, 0% (0/46) were killed via being shot, and 2.3% (1/46) had their collar come off.
Reyna et al. (2021)[434]	Bobwhite quail	Texas	April	In Year 1, mean *movement* distances from the release site were 0.614±0.174 km (n = 29) and 0.521±0.071 km (n = 34) for captive-raised quail release in areas with and without existing resident quail populations, respectively. Additionally, mean *recovery* distances from the release site were 0.536±0.082 km (n = 37) and 0.431± 0.032 km (n = 45) for captive-raised quail release in areas with and without existing resident quail populations, respectively. In Year 2, mean *movement* distances from the release site were 0.266±0.025 km (n = 109) and 0.314±0.052 km (n = 46) for captive-raised quail release in areas with and without existing resident quail populations,	Mean survival duration for captive-raised birds (n = 95) into an area without a resident bobwhite quail population was ~2.2 weeks post-release during Year 1 and ~1.8 weeks post-release during Year 2. Mean survival duration for captive-raised birds (n = N/A) into an area with an existing resident bobwhite quail population was ~ 4.2 weeks post-release during Year 1 and ~ 1.4 weeks post-release during Year 2. Additionally, "Only ~8% (~8/95) of [captive-raised] birds with radios were known to be alive after 60 days." And "Only 1.9% (n = N/A) of our captive-reared birds survived to 16 weeks post-release..."	UNK	"All of our transmitter recovery sites showed signs of predation. However, no conclusive evidence could be obtained based on transmitter appearance and recovery location"

Appendix 1 Table 1. Summary of pen-raised upland gamebird dispersal and survival as well as reasons for non-bagged bird mortality, based on field studies conducted in the United States and expert opinion.

Reference	Species	Location of study	Month birds were released	Findings on post-release range of birds	Findings on post-release survivability	Survival impacted by hunter harvest (Y/N/UNK)	Findings on causes of mortality for non-bagged birds
				respectively. Additionally, mean *recovery* distances from the release site were 0.183±0.018 km (n = 52) and 0.298±0.044 km (n = 45) for captive-raised quail release in areas with and without existing resident quail populations, respectively.			
Roseberry et al., (1987)[455]	Bobwhite quail	Illinois	Late November to early December	During Year 1, 64% (55/86) of released pen-raised birds recovered with 0.25 km of their release sites. The average distance from the release site in which release pen-raised birds were recovered was 0.16m. In Year 2, 50% (42/83) of released pen-raised birds recovered with 0.05 km of their release sites. The average distance from the release site in which release pen-raised birds were recovered was 0.11 km.	40.7% (35/86), 8.1% (7/86), and 2% (2/86) of pen-raised quail survived 45, 90, and roughly 104 days post-release in Year 1 and 56.6% (47/83), 20.5% (17/83), and 0% (0/83) of pen-raised quail survived 33 days, 90 days, and to the next season post-release in Year 2, respectively.	N	N/A
Webb & Nelson, (1971)	Bobwhite quail	South Carolina	Between November and March	"The recovery data showed that considerable movement took place after the quail were released regardless of where or when the quail were released within the study area. Of the banded quail released in six of the nine hunt areas and recovered	Harvested pen-raised quail represented approximately 35% (1,068/3,049) quail released on the study area.	Y	N/A

Appendix 1 Table 1. Summary of pen-raised upland gamebird dispersal and survival as well as reasons for non-bagged bird mortality, based on field studies conducted in the United States and expert opinion.

Reference	Species	Location of study	Month birds were released	Findings on post-release range of birds	Findings on post-release survivability	Survival impacted by hunter harvest (Y/N/UNK)	Findings on causes of mortality for non-bagged birds
				during the study, over 50 percent were killed in areas other than the one in which they were released." Note: The maximum length of hunt areas was ≤ 2.4 km.			
Woods, (2013)[456]	Bobwhite quail	Texas	July	N/A	Survival rate of pen-raised birds was 58% (28/48) by 72 days post-release in Year 1 and 21% (10/49) by 41 days post-release.	Y/N[e]	In Year 1, 27% (13/48) were killed by predators within 7 days post-release, and in Year 2, 49% (24/49) were killed by predators within 7 days post-release.
Slaugh et al. (1992)[457]	Chukar partridge	Utah	May, August, and September	N/A	Total survival probability for pen-raised chukar was 0.4 at week 1 and 0.0 by week 12 (n = 156)	UNK[d]	Authors noted "Captive-reared birds remained at lower elevations and sought cove the sparse vegetation, where they suffered mortality." However, specific cause of death was not specified.
North American Gamebird Association, (personal communica-tion, August, 2020)	Mixed	Pennsylvania	Not specified	Estimate that birds stay within roughly 2.4 km of their release site on average with rare outliers dispersing farther than 3.2 to 4.8 km.	Estimate that around 1% of birds make it to the next season.	Y	Estimate that predator presence has a "huge impact" on non-bagged birds, with predators responsible for roughly 33% of mortality outside of hunter harvest.
Anderson, (1964)[458]	Pheasants	Illinois	January and February	N/A	28% (168/600) of released pen-raised pheasants in Year 1 (February 1960) survived to May 1, 1960 (93 to 95 days), and 18% (55/300) of released pen-raised pheasants survived	UNK	"Precise factors that caused deaths of [recovered] birds were undetermined in most cases."

Appendix 1 Table 1. Summary of pen-raised upland gamebird dispersal and survival as well as reasons for non-bagged bird mortality, based on field studies conducted in the United States and expert opinion.

Reference	Species	Location of study	Month birds were released	Findings on post-release range of birds	Findings on post-release survivability	Survival impacted by hunter harvest (Y/N/UNK)	Findings on causes of mortality for non-bagged birds
					in Year 2 (January 1961) to May 1, 1961 (91 to 92 days).		
Burger, (1964)[432]	Pheasants	Wisconsin	Mid-October to Mid-January	"[...] a fairly rapid and relatively uniform dispersal during the first 2 weeks after release, averaging 1/3 mile [(0.48 km) dispersion] [...]" Additionally, "84% of the 2,400 recover[ed birds] came from within 1/4 mile [(0.4 km)] of the site of release, and 95.5% were within 1 mile [(1.6 km) of the release site] [...]"	"Of 5,441 pheasants released in 3 years, 50% were harvested by preserve hunters and 13% were found dead. Each spring an estimated average of 8% of the previous season's releases survived on the preserve or outside within 2 miles [3.2 km] of the release sites." Additionally, "[o]f 220 banded remains for which... [an exact death date was fixed], 65% (143/220) had died within 1 week of release, and a total of 73 and 82% had died within 2 and 3 weeks, respective."	Y	Causes of death of collected non-bagged birds with identifiable causes of death [n=226] included "release shock (28%) [63/226], shot wounds (29%) [65/226], and predation (42%) [108/226] [...]"
Diefenbach et al., (2000)[459]	Pheasants	Pennsylvania	October and November	N/A	Estimated survival rates for stocked pheasants 7 days post-release ranged between 27.3% to 51.3% and ranged between 0.4% and 5.7% 30 days post-release. These rates are likely due to high harvest rates (31% to 61%) and may have been "biased low" due to lack of distinguishing between birds moving out of the area or	Y	N/A

Appendix 1 Table 1. Summary of pen-raised upland gamebird dispersal and survival as well as reasons for non-bagged bird mortality, based on field studies conducted in the United States and expert opinion.

Reference	Species	Location of study	Month birds were released	Findings on post-release range of birds	Findings on post-release survivability	Survival impacted by hunter harvest (Y/N/UNK)	Findings on causes of mortality for non-bagged birds
					dying via non-harvest means within the analysis.[b]		
Hessler et al., (1970)[16]	Pheasants	Minnesota	August and September	"[...] little dispersion of pen reared pheasants from the release sites during the first 28 days following release [was observed], and most remained within 1 mile [(1.6 km)] of the release site."	25% (15/60) of successfully tracked released pheasants survived to 28 days post-release.	N	"Predators were responsible for 90 percent [54/60] of the mortality observed in the 28-day post-release period. Red-tailed hawks and great-horned owls were the principal avian predators. Fox and mink were the principal mammalian predators. In this study, avian predators were the most serious threat to survival of released gamefarm birds."
Krauss et al., (1987)[433]	Pheasants	Pennsylvania	March to June	Commercially raised birds (n = 16) had a mean dispersal distance of 0.5 km ± 0.06 km seven days post-release in Year 1 of the study and a mean dispersal distance of 0.5 km ± 0.04 km seven days post release in Year 2 of the study. Additionally, in both study years, birds (including commercially raised) that survived beyond 2 weeks tended to "localize their movements" with >90% of	Roughly 35% (9/26) of commercially raised birds survived 50 days post-release in Year 1 of the study and roughly 10% (1/12) of commercially raised birds survived 50 days post-release in Year 2. Note: This experimental study was conducted outside of pheasant hunting season, thus released birds were not subjected to hunter harvest	N	In Year 1 of the study, 93% (14/15) of commercially raised birds that were released were killed by predators. Red foxes (*Vulpes vulpes*), dogs, and avian predators, specifically great horned owls *(Bubo viriginianus)*, common barn owls *(Tyto alba)* and red-tailed hawks *(Buteo jamaicensis)*, were identified as the preparators of predation.

Appendix 1 Table 1. Summary of pen-raised upland gamebird dispersal and survival as well as reasons for non-bagged bird mortality, based on field studies conducted in the United States and expert opinion.

Reference	Species	Location of study	Month birds were released	Findings on post-release range of birds	Findings on post-release survivability	Survival impacted by hunter harvest (Y/N/UNK)	Findings on causes of mortality for non-bagged birds
				birds in Year 1 and >80% of birds in Year 2 ultimately staying localized.	(i.e., no mortality was a result from hunting by humans)		
Leif, (1994)[460]	Pheasants	South Dakota	April	"All 9 pen-reared hens that survived reproductive periods were located ≤ 1 km of release sites."	7.8% (12/159) of pen-reared female birds survived 181 days post-release.	N	90.3% of pen-reared birds were killed by predators. Red fox (*Vulpes vulpes*), coyote (*Canis la- trans*), mink (*Mustela vison*), American badger (*Taxidea taxus*), raccoon (*Procyon lotor*), and avian predators were identified as the preparators of predation. Other causes of mortality included vehicle and farm machinery collisions and drowning in wetlands.
Musil & Connelly, (2009)[461]	Pheasants	Idaho	March and April	N/A	0% (0/17) of pen-reared male pheasants survived 30 days post-release, with only 14% (2/17) to 50% (9/17) surviving within seven days post-release. 4% (4/89) to 8% (7/89) of pen-reared female pheasants survived 60 days post-release. Note: This experimental study did not allow the released birds (n = 38) to be hunted (i.e., no mortality was a result from hunting by humans)	N	99% (88/89) of pen-reared hen pheasants' mortality was attributed to predation.

Appendix 1 Table 1. Summary of pen-raised upland gamebird dispersal and survival as well as reasons for non-bagged bird mortality, based on field studies conducted in the United States and expert opinion.

Reference	Species	Location of study	Month birds were released	Findings on post-release range of birds	Findings on post-release survivability	Survival impacted by hunter harvest (Y/N/UNK)	Findings on causes of mortality for non-bagged birds
May & Haugen, (1973)[431]	Pheasants[a]	Iowa	September and October	"The 25 sections in the release area were searched during this 2-month post-release period, but most of the birds were seen within 2 miles [3.2 km] of the release site. Locations of sightings by the senior author and farmers in the area indicated that the birds initially dispersed along waterways."	1.8% [43/2,406] of the net number of pheasants released were recovered as mortality within the first two months, so it is assumed that 98.2% of pheasants survived.	N	"Avian predators caused four deaths, and foxes were responsible for three others. Three of the 35 dead birds showed no indications of injury and could possibly have died from shock or a lingering internal injury associated with handling and release. For 25 of the 35 carcasses found, the cause of death could not be clearly determined because most of the carcasses had been at least partly consumed and were partly decomposed."

[a] Pheasants released were F1 progeny of wild-trapped pheasants from populations local to southeastern Iowa.
[b] Based on a survival rate estimation model (see Diefenbach et al., 2000[459] for exact details and parameters of the model).
[c] Limited hunting allowed on one of the release sites where 600 pen-raised bobwhite quail were released, while no hunting was allowed on a second release site where 200 pen-raised bobwhite quails were released.
[d] Authors note that no birds were killed by hunters during the study period, but do not specify whether the study areas permitted hunting.
[e] Birds harvested during the hunting season were censored from the survival rate analysis.

Appendix 1 Table 2. Field study observations on behavior of released, pen-raised upland gamebirds with hypothesized connections to post-release survivability and dispersal

Reference	Species	Specific observations related to behavior that were hypothesized by authors to impact survivability
Hessler, (1970)[16]	Pheasants	"Birds used in this investigation appeared very reluctant to fly for several weeks after release. This may have been due to the weight or to the psychological effect of the transmitter, but Wilcomb (1956) also called attention to this behavioral characteristic of non-transmitter-equipped pen-reared birds released into the wild. The striking behavioral characteristic of pen-reared birds was that they were unwary and it was not unusual to see them standing and walking in roads for prolonged periods of time. They were easily identified and could be heard. For at least 3 weeks, it was possible to walk up to and even stand among them without visibly creating excessive nervousness. It was also possible, in this interval, to recapture them with landing nets. During the first week, they showed no tendency to hide or seek cover and they readily showed themselves in cover in which they might have hidden, by extending their heads high in the air."[a]
Krauss et al. (1987)[433]	Pheasants	"Compared to wild birds, both State and commercial game-farm birds exhibited a low avoidance response to the observer."[a]
Leif, (1994)[460]	Pheasants	"As noted from radio signal wild hens avoided approaching researchers by running or hiding in herbaceous cover. Pen reared hens exhibited these behaviors to a lesser extent but tended to crouch or stand upright in habitat openings."[a]
Roseberry et al., (1987)[455]	Bobwhite quail	"Even after 5-10 weeks post-release, pen-reared bobwhites were often reluctant to fly when approached and could be observed on the ground in relatively open cover. When released following recapture, pen-reared quail often flew slower and not as far as their wild cohorts, especially early in the season. As time passed, game farm and semi- wild birds became less 'tame' and their flight capabilities improved noticeably. Even during late winter, however, they could often be distinguished by their behavior, especially the relative ease with which they could be approached." [a]
Fies et al. (2000)[453]	Bobwhite quail	"Game farm and Fl bobwhites in our study survived poorly because they appeared to lack behavioral skills necessary to escape predation. Even the "genetically wild" Fl birds were easily killed by predators, suggesting that the influences of being raised in captivity had a greater impact on survival than innate genetic programming"
DeVos & Speake, (1995)[452]	Bobwhite quail	"Although insignificant, the higher incidence of mammalian predation in the pen- raised bird groups was probably due to the reluctance of pen-raised bobwhites to flush during the first few weeks post- release. For approximately 2 weeks, released birds walked away from an approaching observer or dog. However, by 1 month post-release, pen-raised bobwhites held tight or moved to thicker cover upon the approach of a dog."
Perez, et al. (2002)[449]	Bobwhite quail	"Other observational information includes behavioral traits of captive-reared birds and integration of these birds into wild coveys. Game-farm birds showed little fear of humans, rarely flushing or not flushing very far. Avian predators took more GF birds (28%) than any other group. They were frequently found at the same daily location, usually under a mature mesquite, which may have improved avian predator efficiency."

[an] Only anecdotally mentioned

Appendix 2: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates.

Appendix 2 Tables 1-6 summarize the results of studies documenting survival and persistence of AI viruses at various humidity levels, at various temperatures, and on various substrates. The trend in persistence and survival times in the environment for AI viruses appears to be decreased survival in lower moisture and higher temperature conditions. Virus survival and persistence in the environment has also been reported to be longer near neutral pH, in low salinity, and without UV exposure.[398,462–465]

These tables are compiled to describe virus survival and persistence across a range of conditions. Of note, multiple methodologies were used to determine virus survival or persistence; readers should consult the studies listed to evaluate all parameters and methods utilized in experimental studies, as definitions of these terms are not uniformly applied. In compiling data from the literature for these tables, studies where HPAI virus was utilized were given preference over LPAI studies. Where information on AI virus was not available, data on other influenza A viruses are included as indicated. Virus inactivation was prioritized as a time point in the summary tables below. In studies in which virus remained viable for all time points measured, the last reported time when virus was measured (and detected) is included in the tables for comparison.

These summary tables focus on conditions that may be similar to those encountered on commercial poultry operations and climatic parameters similar to those of the continental United States. Further summaries of virus inactivation times in eggs and egg products can be found in the WOAH Terrestrial Animal Health Code (Article 10.4.25),[126] and inactivation times at high temperatures have been summarized by USDA documents on parameters to inactivate HPAI virus using heat treatment.[466]

Appendix 2 Table 1. Summary of experimental studies on survival of AI viruses in feces and manure by increasing temperature.

Substrate[a]	Temperature	Humidity (as described by study authors)	Sub-type	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Duck feces	0 °C (32 °F)	Moist germ carrier: feces in closed 50-mL plastic tubes	LPAI H5N1	A/Teal/Wv632/ Germany/05	-	T_{90}[b] value of 75 days	467
Wet Chicken feces	4 °C (39.2 °F)	Closed vial	HPAI H5N2	#1370 isolate	Viable virus through 35 days (last time point tested)	-	98
Commercial chicken manure (field house)	4 °C 39.2°F)	Manure-virus mixture in a 50-mL sterile tube	LPAI H7N2	A/chicken/PA/3779-2/ 97AIV	Remained activated at 20 days	-	468
Wet chicken feces	4 °C (39.2 °F)	Capped vials	HPAI H5N1	A\Ck\Sikkim\151466\2008	-	0% infectivity at week 7	425
Dry chicken feces	4 °C (39.2 °F)	Capped vials	HPAI H5N1	A\Ck\Sikkim\151466\2008	-	0% infectivity at week 8	425
Duck feces	4 °C	About 60% relative humidity	LPAI H6N2	Not specified	-	Virus not detected at day 18	469
Chicken feces	4.0-6.7 °C (39.2–44.06 °F)	15.2–46.3% relative humidity	HPAI H5N1	A/Vietnam/1203/ 2004v	-	Virus not detected at day 13	398
Chicken feces	6.7-7.8 °C (44.06–46.04 °F)	79.0–96.9% relative humidity	HPAI H5N1	A/Vietnam/1203/ 2004	Day 13 (last time point tested)	-	398

Duck feces	10 °C (50 °F)	Moist germ carrier: feces in closed 50-mL plastic tubes	LPAI H6N8	A/Mute Swan/Germany/R2927/07	-	T_{90} value of 14 days	[467]
Duck feces	15 °C	About 60% relative humidity	LPAI H6N2	Not specified	-	Virus not detected at day 8	[469]
Commercial chicken manure (field house)	15–20 °C (59–68 °F)	Manure-virus mixture in a 50-mL sterile tube	LPAI H7N2	A/chicken/PA/3779-2/ 97AIV	Remained activated at 2 days	-	[468]
Field commercial turkey bedding material and feces	19–22.5 °C (66.2–72.5 °F)	Tightly sealed container	HPAI H5N8	A/Chicken/California/15-004912/2015	-	Virus not detected at hour 60	[470]
Field commercial broiler bedding material and feces	19–22.5 °C (66.2–72.5 °F)	Tightly sealed container	HPAI H5N8	A/Chicken/California/15-004912/2015	-	Virus not detected at hour 60	[470]
Field commercial egg-layer Manure	19–22.5 °C (66.2–72.5 °F)	Tightly sealed container	HPAI H5N8	A/Chicken/California/15-004912/2015	Still detected at hour 96	-	[470]
Field commercial turkey bedding material and feces	19–22.5 °C (66.2–72.5 °F)	Tightly sealed container	LPAI H6N2	A/Chicken/California/2000	-	Virus not detected at hour 24	[470]
Field commercial broiler bedding material and feces	19–22.5 °C (66.2–72.5 °F)	Tightly sealed container	LPAI H6N2	A/Chicken/California/2000	-	Virus not detected at hour 24	[470]
Field commercial egg-layer Manure	19–22.5 °C (66.2–72.5 °F)	Tightly sealed container	LPAI H6N2	A/Chicken/California/2000	-	Virus not detected at hour 24	[470]

Duck feces	20 °C (68 °F)	Moist germ carrier: feces in closed 50-mL plastic tubes	LPAI H4N6	A/Mallard/Wv1732-34/03	-	T_{90} value of 4 days	[467]
Duck feces	22 °C	About 60% relative humidity	LPAI H6N2	Not specified	-	Virus not detected at day 4	[469]
Fecal material	22 °C (71.6 °F)	Capped glass vials	LPAI H3N6	A/Duck/Memphis/546/74	-	Infectious virus not detected at day 13	[471]
Chicken feces	22.0–22.7 °C (71.6–72.86 °F)	30–42% relative humidity	HPAI H5N1	A/Vietnam/1203/2004	-	Virus not detected at day 2	[398]
Chicken feces	22.4–23.7 °C (72.32–74.66 °F)	89.1–91.2% relative humidity	HPAI H5N1	A/Vietnam/1203/2004	-	Virus not detected at day 4	[470]
Wet chicken feces	25 °C (77 °F)	Closed vial	HPAI H5N2	#1370 isolate	-	No viable virus at day 3	[98]
Field commercial chicken manure	28–30 °C (82.4–86 °F)	Manure-virus mixture in a 50-mL sterile tube	LPAI H7N2	A/chicken/PA/3779-2/97AIV		Inactivated at hour 12	[468]
Duck feces	30 °C (86 °F)	Moist germ carrier: feces in closed 50-mL plastic tubes	LPAI H4N6	A/Mallard/Wv1732-34/03	-	T_{90} value of 2 days	[467]
Dry chicken feces	37 °C (98.6 °F)	Capped vials	HPAI H5N1	A\Ck\Sikkim\151466\2008	-	0% infectivity at hour 30	[425]
Wet chicken feces	37 °C (98.6 °F)	Capped vials	HPAI H5N1	A\Ck\Sikkim\151466\2008	-	0% infectivity at hour 30	[425]
Field commercial chicken manure	37 °C (98.6 °F)	Manure-virus mixture in a 50-mL sterile tube	LPAI H7N2	A/chicken/PA/3779-2/97AIV	-	Inactivated at hour 24	[468]

Dry chicken feces	42 °C (107.6 °F)	Capped vials	HPAI H5N1	A\Ck\Sikkim\151466\2008	-	0% infectivity at hour 24	[425]
Wet chicken feces	42 °C (107.6 °F)	Capped vials	HPAI H5N1	A\Ck\Sikkim\151466\2008	-	0% infectivity at hour 24	[425]
Field commercial chicken manure	56 °C (132.8 °F)	Manure-virus mixture in a 50-mL sterile tube	LPAI H7N2	A/chicken/PA/3779-2/97AIV	-	Inactivated at minute 15	[468]

[a]Microbial digestion likely plays a role in manure over time, although it is not considered here because it has rarely been measured experimentally.
[b] T_{90} value: time required for 90% loss of virus infectivity

Appendix 2 Table 2. Summary of experimental studies on survival of AI viruses in compost by increasing temperature

Substrate	Temperature	Humidity (as described by study authors)	Sub type	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
0.1:1:2 parts of straw, chicken carcasses, and manure	Temperature when sampled 30–33 °C (86–91.4 °F) Peak temperatures reached: Upper layer of dead bird compost: 57 °C (134.6 °F); Lower layer of dead bird compost: 41 °C (105.8 °F)	Dialysis bags held infected chicken carcass parts that were tested. Moisture/humidity not reported.	HPAI H5N2	A/CK/PA/1370/83		No virus isolated at day 10 (1st time point tested)	406
Compost material consisting of manure mixed with straw	35 °C (95 °F)	1.5L compost reactors; humidity 65%	HPAI H7N1	A/turkey/Italy/1387/00	-	Time to 12-log_{10} reduction reported to be 6.4 hours	408
Compost material consisting of manure, straw, and embryonated eggs	35 °C (95 °F)	1.5L compost reactors; humidity 58%	HPAI H7N1	A/turkey/Italy/1387/00	-	Time to 12-log_{10} reduction reported to be 7.6 hours	408
Compost material consisting of manure mixed with straw	45 °C (113 °F)	1.5L compost reactors; humidity 65%	HPAI H7N1	A/turkey/Italy/1387/00	-	Time to 12-log_{10} reduction reported to be 1.7 hours	408

Appendix 2 Table 2. Summary of experimental studies on survival of AI viruses in compost by increasing temperature

Substrate	Temperature	Humidity (as described by study authors)	Sub type	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Compost material consisting of manure, straw, and embryonated eggs	45 °C (113 °F)	1.5L compost reactors; humidity 58%	HPAI H7N1	A/turkey/Italy/1387/00	-	Time to 12-log_{10} reduction reported to be 9.8 hours	408
Cage layer manure in middle of compost	Peak recorded 46 °C (114.8 °F)	Nylon mesh bag; 65% moisture content of compost	LPAI H6N2	A/Tky/Mass/3740/65	-	Virus below detectable limit at day 3 (1st time point tested)	410
Used litter in middle of compost	Peak recorded 46 °C (114.8 °F)	Nylon mesh bag; 65% moisture content of compost	LPAI H6N2	A/Tky/Mass/3740/65	-	Virus below detectable limit at day 3 (1st time point tested)	410
Breast muscle in abdominal cavity of chicken carcass at bottom of compost	Peak recorded 50.3 °C (122.54 °F)	Plastic netting; 65% moisture content of compost	LPAI H6N2	A/Tky/Mass/3740/65	-	Virus below detectable limit at day 10	410
Embryonated chicken eggs at bottom of compost	Peak recorded 50.3 °C (122.54 °F)	Plastic mesh baskets; 65% moisture content of compost	LPAI H6N2	A/Tky/Mass/3740/65	-	Virus below detectable limit at day 10	410
Compost material consisting of manure mixed with straw	55 °C (131 °F)	1.5L compost reactors; humidity 65%	HPAI H7N1	A/turkey/Italy/1387/00	-	Time to 12-log_{10} reduction reported to be 29 minutes	408
Compost material consisting of manure, straw, and embryonated eggs	55 °C (131 °F)	1.5L compost reactors; humidity 58%	HPAI H7N1	A/turkey/Italy/1387/00	-	Time to 12-log_{10} reduction reported to be 30 minutes	408

Appendix 2 Table 3. Summary of experimental studies on survival of AI viruses in water by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Surface water (Lake Constance)	-10 °C (14 °F)	-	LPAI H6N8	A/mute swan/ Germany/R2927 /07	-	T_{90} value of 395 days	[472]
Surface water (Lake Constance)	0 °C (32 °F)	-	LPAI H5N1	A/teal/Germany/ Wv632/05	-	T_{90} value of 208 days	[472]
Contaminated fecal material in river water	4 °C (39.2 °F)	-	LPAI H3N6	A/Duck/Memph is/546/74	Viable for all contact times (32 days)	-	[471]
City pond water (Gdansk-Oliwa, Poland)	4 °C (39.2 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 60+ days	[473]
River mouth water (Gdansk-Oliwa, Poland)	4 °C (39.2 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 60+ days	[473]
Sea water (Gdansk Bay, Baltic Sea)	4 °C (39.2 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 28–39 days depending on viral dose	[473]
Filtered sea water (Gdansk Bay, Baltic Sea)	4 °C (39.2 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 60+ days	[473]
Distilled water	4 °C (39.2 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 60+ days	[473]
Sea water (Black Sea)	5–6 °C (41–42.8 °F)	-	LPAI H6N2	Not specified	-	No infective virus detected at day 7	[474]
Sea water (Black Sea)	5–6 °C (41–42.8 °F)	-	LPAI H11N6	A/duck/England / 56	-	No infective virus detected at day 9	[474]

Appendix 2 Table 3. Summary of experimental studies on survival of AI viruses in water by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Surface water (Koprinka dam)	5–6 °C (41–42.8 °F)	-	LPAI H6N2	Not specified	-	No infective virus detected at day 16	[474]
Surface water (Koprinka dam)	5–6 °C (41–42.8 °F)	-	LPAI H11N6	A/duck/England / 56	-	No infective virus detected at day 18	[474]
Surface water (Lake Constance)	10 °C (50 °F)	-	LPAI H4N6	A/mallard/Germany/Wv1732-34/03	-	T_{90} value of 85 days	[472]
City pond water (Gdansk-Oliwa, Poland)	10 °C (50 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 38–56 days depending on viral dose	[473]
River mouth water (Gdansk-Oliwa, Poland)	10 °C (50 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 42–60+ days depending on viral dose	[473]
Sea water (Gdansk Bay, Baltic Sea)	10 °C (50 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 24–39 days depending on viral dose	[473]
Filtered sea water (Gdansk Bay, Baltic Sea)	10 °C (50 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 42–60+ days depending on viral dose	[473]
Distilled water	10 °C (50 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 60+ days	[473]
Surface water (Ovcharitsa dam)	10–12 °C (50–53.6 °F)	-	LPAI H6N2	Not specified	-	No infective virus detected at day 1	[474]
Surface water (Ovcharitsa dam)	10–12 °C (50–53.6 °F)	-	LPAI H11N6	A/duck/England / 56	-	No infective virus detected at day 1	[474]

Appendix 2 Table 3. Summary of experimental studies on survival of AI viruses in water by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Distilled water	17 °C (62.6 °F)	-	LPAI H3N8	A/gadwall/LA/17G/87	-	Estimated duration of infectivity of 194 days	[475]
Distilled water	17 °C (62.6 °F)	-	LPAI H4N6	A/blue-winged teal/LA/44B/87	-	Estimated duration of infectivity of 207 days	[475]
Distilled water	17 °C (62.6 °F)	-	LPAI H6N2	A/mottled duck/LA/38M/87	-	Estimated duration of infectivity of 176 days	[475]
Distilled water	17 °C (62.6 °F)	-	LPAI H12N5	A/blue-winged teal/LA/188B/87	-	Estimated duration of infectivity of 126 days	[475]
Distilled water	17 °C (62.6 °F)	-	LPAI H10N7	A/green-winged teal/LA/169GW/87	-	Estimated duration of infectivity of 146 days	[475]
Distilled water	17 °C (62.6 °F)	-	HPAI H5N1	A/WhooperSwan/Mongolia/244/05	-	Predicted persistence of 158 days	[465]
Distilled water	17 °C (62.6 °F)	-	HPAI H5N1	A/chicken/Hong Kong/220/1997	-	Predicted persistence of 16–41 days depending on salinity	[476]
Distilled water	17 °C (62.6 °F)	-	HPAI H5N1	A/environment (goose pen)/Hong Kong/485.3/2000	-	Predicted persistence of 22–48 days depending on salinity	[476]

Appendix 2 Table 3. Summary of experimental studies on survival of AI viruses in water by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Distilled water	17 °C (62.6 °F)	-	HPAI H5N1	A/goose/Vietnam/113/2001	-	Predicted persistence of 32–69 days depending on salinity	[476]
Distilled water	17 °C (62.6 °F)	-	HPAI H5N1	A/Vietnam/1203/2004	-	Predicted persistence of 24–66 days depending on salinity	[476]
Distilled water	17 °C (62.6 °F)	-	HPAI H5N1	A/egret/Hong Kong/757.2/2002	-	Predicted persistence of 71–78 days depending on salinity	[476]
Distilled water	17 °C (62.6 °F)	-	HPAI H5N1	A/duck/bac lieu/NCVD 07-09/2007	-	Predicted persistence of 22–40 days depending on salinity	[476]
Distilled water	17 °C (62.6 °F)	-	HPAI H5N1	A/West Java/PWT-WIJ/2006	-	Predicted persistence of 26–33 days depending on salinity	[476]
Distilled water	17 °C (62.6 °F)	-	HPAI H5N1	A/chicken/Nigeria/-228-10/2006	-	Predicted persistence of 20–27 days depending on salinity	[476]
Distilled water	17 °C (62.6 °F)	-	HPAI H5N1	A/duck/Vietnam/201/2006	-	Predicted persistence of 43–50 days depending on salinity	[476]
Distilled water	17 °C (62.6 °F)	-	HPAI H5N1	A/muscovy/Ha Nam/NCVD 07-84/2007	-	Predicted persistence of 38–46 days depending on salinity	[476]
Distilled water	17 °C (62.6 °F)	-	HPAI H5N1	A/chicken/Korea/ES/2003	-	Predicted persistence of 26–43 days depending on salinity	[476]
Surface water (Lake Constance)	20 °C (68 °F)	-	LPAI H4N6	A/mallard/Germany/Wv1732-34/03	-	T_{90} value of 23 days	[472]
City pond water (Gdansk-Oliwa, Poland)	20 °C (68 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 14–21days depending on viral dose	[473]

Appendix 2 Table 3. Summary of experimental studies on survival of AI viruses in water by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
River mouth water (Gdansk-Oliwa, Poland)	20 °C (68 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 21–32 days depending on viral dose	[476]
Sea water (Gdansk Bay, Baltic Sea)	20 °C (68 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 10–14days depending on viral dose	[476]
Filtered sea water (Gdansk Bay, Baltic Sea)	20 °C (68 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 10–60 days depending on viral dose	[476]
Distilled water	20 °C (68 °F)	-	HPAI H5N1	A/Mute swan/305/06	-	Predicted persistence of 60+ days	[476]
Non-chlorinated demineralized water	20–22 °C (68–71.6 °F)	-	LPAI H9N2	*A/chicken/India/ 50438/2007*	-	Virus not detected at day 14	[477]
Raw pond water (from Anowara, Bangladesh)	22 °C (71.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 4 hours	[478]
Raw pond water (Chandanaish, Bangladesh)	22 °C (71.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 4 hours	[478]
Raw pond water (Banshkhali, Bangladesh)	22 °C (71.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	Virus viable for full 4 hours of testing period	-	[478]
Raw pond water (Hathazari, Bangladesh)	22 °C (71.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	Virus viable for full 4 hours of testing period	-	[478]
Raw pond water (Rangunia, Bangladesh)	22 °C (71.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	Virus viable for full 4 hours of testing period	-	[478]

Appendix 2 Table 3. Summary of experimental studies on survival of AI viruses in water by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Boiled and filtered pond water (from Anowara, Bangladesh)	22 °C (71.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	Virus viable for full 4 hours of testing period	-	[478]
Boiled and filtered pond water (Chandanaish, Bangladesh)	22 °C (71.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	Virus viable for full 4 hours of testing period	-	[478]
Boiled and filtered pond water (Banshkhali, Bangladesh)	22 °C (71.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	Virus viable for full 4 hours of testing period	-	[478]
Boiled and filtered pond water (Hathazari, Bangladesh)	22 °C (71.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	Virus viable for full 4 hours of testing period	-	[478]
Boiled and filtered pond water (Rangunia, Bangladesh)	22 °C (71.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	Virus viable for full 4 hours of testing period	-	[478]
Contaminated fecal material in river water	22 °C (71.6 °F)	-	LPAI H3N6	A/Duck/Memphis/546/74	-	Virus not detected at day 7	[471]
Distilled water	28 °C (82.4 °F)	-	HPAI H5N1	A/DuckMeat/Anyang/01	-	Predicted persistence of 30 days	[465]
Distilled water	28 °C (82.4 °F)	-	HPAI H5N1	A/chicken/Hong Kong/220/1997	-	Predicted persistence of 16–41 days depending on salinity	[476]

Appendix 2 Table 3. Summary of experimental studies on survival of AI viruses in water by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Distilled water	28 °C (82.4 °F)	-	HPAI H5N1	A/environment (goose pen)/Hong Kong/485.3/2000	-	Predicted persistence of 5–8 days depending on salinity	[476]
Distilled water	28 °C (82.4 °F)	-	HPAI H5N1	A/goose/Vietnam/113/2001	-	Predicted persistence of 9–14 days depending on salinity	[476]
Distilled water	28 °C (82.4 °F)	-	HPAI H5N1	A/Vietnam/1203/2004	-	Predicted persistence of 5–16 days depending on salinity	[476]
Distilled water	28 °C (82.4 °F)	-	HPAI H5N1	A/egret/Hong Kong/757.2/2002	-	Predicted persistence of 6–9 days depending on salinity	[476]
Distilled water	28 °C (82.4 °F)	-	HPAI H5N1	A/duck/bac lieu/NCVD 07-09/2007	-	Predicted persistence of 2–5 days depending on salinity	[476]
Distilled water	28 °C (82.4 °F)	-	HPAI H5N1	A/West Java/PWT-WIJ/2006	-	Predicted persistence of 5–10 days depending on salinity	[476]
Distilled water	28 °C (82.4 °F)	-	HPAI H5N1	A/chicken/Nigeria/-228-10/2006	-	Predicted persistence of 7–10 days depending on salinity	[476]
Distilled water	28 °C (82.4 °F)	-	HPAI H5N1	A/duck/Vietnam/201/2006	-	Predicted persistence of 11–21 days depending on salinity	[476]
Distilled water	28 °C (82.4 °F)	-	HPAI H5N1	A/muscovy/Ha Nam/NCVD 07-84/2007	-	Predicted persistence of 6–12 days depending on salinity	[476]
Distilled water	28 °C (82.4 °F)	-	HPAI H5N1	A/chicken/Korea/ES/2003	-	Predicted persistence of 5–9 days depending on salinity	[476]
Distilled water	28 °C (82.4 °F)	-	LPAI H3N8	*A/gadwall/LA/17G/87*	-	Estimated duration of infectivity of 66 days	[475]

Appendix 2 Table 3. Summary of experimental studies on survival of AI viruses in water by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Distilled water	28 °C (82.4 °F)	-	LPAI H4N6	A/blue-winged teal/ LA/44B/87	-	Estimated duration of infectivity of 80 days	475
Distilled water	28 °C (82.4 °F)	-	LPAI H6N2	*A/mottled duck/LA/38M/ 87*	-	Estimated duration of infectivity of 98 days	475
Distilled water	28 °C (82.4 °F)	-	LPAI H12N5	A/blue-winged teal/LA/188B/ 87	-	Estimated duration of infectivity of 30 days	475
Distilled water	28 °C (82.4 °F)	-	LPAI H10N7	*A/green-winged teal/LA/169G W/87*	-	Estimated duration of infectivity of 102 days	475
Surface water (Lake Constance)	30 °C (86 °F)	-	LPAI H4N6	A/mallard/Germany/Wv1732-34/03	-	T_{90} value of 14 days	472
Raw pond water (from Anowara, Bangladesh)	30 °C (86 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 4 hours	478
Raw pond water (Chandanaish, Bangladesh)	30 °C (86 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 3 hours	478
Raw pond water (Banshkhali, Bangladesh)	30 °C (86 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 3 hours	478
Raw pond water (Hathazari, Bangladesh)	30 °C (86 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 3 hours	478

Appendix 2 Table 3. Summary of experimental studies on survival of AI viruses in water by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Raw pond water (Rangunia, Bangladesh)	30 °C (86 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 4 hours	[478]
Boiled and filtered pond water (from Anowara, Bangladesh)	30 °C (86 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 4 hours	[478]
Boiled and filtered pond water (Chandanaish, Bangladesh)	30 °C (86 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 4 hours	[478]
Boiled and filtered pond water (Banshkhali, Bangladesh)	30 °C (86 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 4 hours	[478]
Boiled and filtered pond water (Hathazari, Bangladesh)	30 °C (86 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 4 hours	[478]
Boiled and filtered pond water (Rangunia, Bangladesh)	30 °C (86 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	Virus viable for full 4 hours of testing period	-	[478]
Raw pond water (from Anowara, Bangladesh)	37 °C (98.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 3 hours	[478]

Appendix 2 Table 3. Summary of experimental studies on survival of AI viruses in water by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Raw pond water (Chandanaish, Bangladesh)	37 °C (98.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 1 hour	[478]
Raw pond water (Banshkhali, Bangladesh)	37 °C (98.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 3 hours	[478]
Raw pond water (Hathazari, Bangladesh)	37 °C (98.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 2 hours	[478]
Raw pond water (Rangunia, Bangladesh)	37 °C (98.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 3 hours	[478]
Boiled and filtered pond water (from Anowara, Bangladesh)	37 °C (98.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 4 hours	[478]
Boiled and filtered pond water (Chandanaish, Bangladesh)	37 °C (98.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 2 hours	[478]
Boiled and filtered pond water (Banshkhali, Bangladesh)	37 °C (98.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 4 hours	[478]
Boiled and filtered pond water (Hathazari, Bangladesh)	37 °C (98.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	-	Virus not detected at 4 hours	[478]

Appendix 2 Table 3. Summary of experimental studies on survival of AI viruses in water by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Boiled and filtered pond water (Rangunia, Bangladesh)	37 °C (98.6 °F)	-	LPAI H9N2	Unspecified strain from Bangladesh	Virus viable for full 4 hours of testing period	-	[478]

Appendix 2 Table 4. Summary of experimental studies on survival of AI viruses in poultry carcass (meat, liver, muscle, feather) by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Duck feathers	4 °C (39.2 °F)	Placed in incubator	HPAI H5N1	A/chicken/Miyazaki/K11/2007 A/WhooperSwan/Akita/1/2008	-	Negative for virus isolation at day 200	374
Breast muscle in abdominal cavity of chicken carcass	3.9–7.9 °C (39–46.2 °F)	Plastic netting outside compost bin	LPAI H6N2	A/Tky/Mass/3740/65	Virus detected at all times tested (21 days)	-	410
Liver in abdominal cavity of chicken carcass	4.0–7.9 °C (39.2–46.2 °F)	Plastic netting outside compost bin	LPAI H6N2	A/Tky/Mass/3740/65	-	Virus not detected at day 7	410
Duckling feathers	10 °C (50 °F)	Screw-capped vials	HPAI H5N1	A/crow/India/11TI16/2011	-	Mean number of days of survivability for virus reported at 31.7 ± 0.962 days	376
Duck feathers treated to remove preen oil	10 °C (50 °F)	Screw-capped vials	HPAI H5N1	A/crow/India/11TI16/2011	-	Mean number of days of survivability for virus reported at 35 ± 1.17 days	376
Duck feathers	10 °C (50 °F)	Screw-capped vials	HPAI H5N1	A/crow/India/11TI16/2011	-	Mean number of days of survivability for virus reported at 55.8 ± 1.402	376

Appendix 2 Table 4. Summary of experimental studies on survival of AI viruses in poultry carcass (meat, liver, muscle, feather) by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Duck feathers	20 °C (68 °F)	Placed in incubator	HPAI H5N1	A/WhooperSwan/Akita/1/2008	-	Negative for virus isolation at day 20	374
Duckling feathers	25 °C (77 °F)	Screw-capped vials	HPAI H5N1	A/crow/India/11TI16/2011	-	Mean number of days of survivability for virus reported at 14.3 ± 0.384	376
Duck feathers treated to remove preen oil	25 °C (77 °F)	Screw-capped vials	HPAI H5N1	A/crow/India/11TI16/2011	-	Mean number of days of survivability for virus reported at 16 ± 0.408	376
Duck feathers	25 °C (77 °F)	Screw-capped vials	HPAI H5N1	A/crow/India/11TI16/2011	-	Mean number of days of survivability for virus reported at 30.7 ± 0.56	376
Duckling feathers	37 °C (98.6 °F)	Screw-capped vials	HPAI H5N1	A/crow/India/11TI16/2011	-	Mean number of days of survivability for virus 7	376
Duck feathers treated to remove preen oil	37 °C (98.6 °F)	Screw-capped vials	HPAI H5N1	A/crow/India/11TI16/2011	-	Mean number of days of survivability for virus reported at 9.7 ± 0.384	376
Duck feathers	37 °C (98.6 °F)	Screw-capped vials	HPAI H5N1	A/crow/India/11TI16/2011	-	Mean number of days of survivability for virus reported at 19.8 ± 0.495	376
Chicken meat	57.8 °C (136.04 °F)	PCR tubes in PCR thermocycler heating block	HPAI H5N1	A/chicken/Korea/ES/2003	-	Predicted 11-log EID_{50} reduction at 39.6 minutes	479

Appendix 2 Table 5. Summary of experimental studies on survival of AI viruses in allantoic fluid and embryonated chicken eggs by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Embryonated chicken eggs	3.9–7.9 °C (39–46.2 °F)	Plastic mesh baskets outside compost bin	LPAI H6N2	A/Tky/Mass/3740/65	Virus detected at all times tested (21 days)	-	[410]
Allantoic fluid	4 °C (39.2 °F)	Sealed in incubation tubes	LPAI H9N2	A/ck/Gshor/1525/10/12/06	-	Viability decay time of virus estimated to be 327.6 days	[480]
Allantoic fluid	20 °C (68 °F)	Sealed in incubation tubes	LPAI H9N2	A/ck/Gshor/1525/10/12/06	-	Viability decay time of virus estimated to be 85.29 days	[480]
Allantoic fluid	37 °C (98.6 °F)	Sealed in incubation tubes	LPAI H9N2	A/ck/Gshor/1525/10/12/06	-	Viability decay time of virus estimated to be 4.67 days	[480]
Allantoic fluid	37 °C (98.6 °F)	Sealed in incubation tubes	LPAI H9N2	A/ty/Shadmot Dvora/1567/06/01/04	-	Viability decay time of virus estimated to be 2.86 days	[480]
Allantoic fluid	37 °C (98.6 °F)	Sealed in incubation tubes	LPAI H9N2	A/ty Givat Haim/965/17/03/02	-	Viability decay time of virus estimated to be 3.62 days	[480]
Allantoic fluid	55 °C (131 °F)	Capped centrifuge tubes	HPAI H5N1	A/chicken/Chonburi/Thailand/CU-7/04, A/chicken/Nakorn Patom/Thailand/CU-K2/2004, A/chicken/Ratchaburi/Thailand/CU-68/04	Infective at all contact times (60 minutes)	-	[481]

Appendix 2 Table 5. Summary of experimental studies on survival of AI viruses in allantoic fluid and embryonated chicken eggs by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected (if viable for all contact times)	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Allantoic fluid	56 °C (132.8 °F)	Thermocycler tubes in heating block	LPAI H7N9	A/Anhui/1/2013, A/Shanghai/1/2013	-	Virus not infective at minute 30	[482]
Allantoic fluid	60 °C (140 °F)	Capped centrifuge tubes	HPAI H5N1	A/chicken/Chonburi/ Thailand/CU-7/04, A/chicken/Nakorn Patom/Thailand/CU-K2/2004, A/chicken/Ratchaburi/ Thailand/CU-68/04	-	Virus not infective at minute 60	[481]

Appendix 2 Table 6. Summary of experimental studies on survival of influenza A viruses on additional substrates by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Galvanized metal, glass, soil	4.0–6.7 °C (39.2–44.06 °F)	15.2–46.3% relative humidity	HPAI H5N1	A/Vietnam/1203/ 2004	Virus detected at all times tested (13 days)	-	398
Galvanized metal	6.7–7.8 °C (44.06–46.04 °F)	89.5–96.9% relative humidity	HPAI H5N1	A/Vietnam/1203/ 2004	-	Virus below detectable limit at day 9	398
Glass, soil	6.7–7.8 °C (44.06–46.04 °F)	79.0–96.9% relative humidity	HPAI H5N1	A/Vietnam/1203/ 2004	-	Virus below detectable limit at day 13	398
Window glass, unvarnished oak	17–21 °C (62.6–69.8 °F)	23–24% humidity	H1N1	A/PuertoRico/8/34 (PR8)	-	Virus not detected at hour 4	483
Stainless steel, plastic control	17–21 °C (62.6–69.8 °F)	23–24% humidity	H1N1	A/PuertoRico/8/34 (PR8)	-	Virus not detected at hour 24	483
Cotton	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/Bris/59/07/	-	Viable virus not detected at 1 week	484
Cotton	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/Cal/4/09/	-	Viable virus not detected at 1 week	484
Cotton	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/Cal/7/09/	-	Viable virus not detected at 1 week	484
Cotton	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/PR/8/34	-	Viable virus not detected at 2 weeks	484

Appendix 2 Table 6. Summary of experimental studies on survival of influenza A viruses on additional substrates by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Cotton	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/Sol/3/06	-	Viable virus not detected at 1 week	484
Microfibre	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/Bris/59/07/	-	Viable virus not detected at 1 week	484
Microfibre	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/Cal/4/09/	-	Viable virus not detected at 1 week	484
Microfibre	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/Cal/7/09/	-	Viable virus not detected at hour 24	484
Microfibre	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/PR/8/34	-	Viable virus not detected at 2 weeks	484
Microfibre	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/Sol/3/06	-	Viable virus not detected at 1 week	484
Stainless steel	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/Bris/59/07/	-	Viable virus not detected at 3 weeks	484
Stainless steel	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/Cal/4/09/	-	Viable virus not detected at 3 weeks	484
Stainless steel	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/Cal/7/09/	-	Viable virus not detected at 3 weeks	484

Appendix 2 Table 6. Summary of experimental studies on survival of influenza A viruses on additional substrates by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Stainless steel	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/PR/8/34	-	Viable virus not detected at 3 weeks	[484]
Stainless steel	19.5–19.7 °C (67.1–67.5 °F)	10.2–10.5% humidity (dark environment)	LPAI H1N1	A/Sol/3/06	-	Viable virus not detected at 2 weeks	[484]
Cotton	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/Bris/59/07/	-	Viable virus not detected at 1 week	[484]
Cotton	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/Cal/4/09/	-	Viable virus not detected at 1 week	[484]
Cotton	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/Cal/7/09/	-	Viable virus not detected at 1 week	[484]
Cotton	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/PR/8/34	-	Viable virus not detected at 1 week	[484]
Cotton	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/Sol/3/06	-	Viable virus not detected at 1 week	[484]
Microfibre	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/Bris/59/07/	-	Viable virus not detected at 1 week	[484]
Microfibre	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/Cal/4/09/	-	Viable virus not detected at hour 24	[484]

Appendix 2 Table 6. Summary of experimental studies on survival of influenza A viruses on additional substrates by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Microfibre	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/Cal/7/09/	-	Viable virus not detected at 1 week	484
Microfibre	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/PR/8/34	-	Viable virus not detected at 1 week	484
Microfibre	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/Sol/3/06	-	Viable virus not detected at 1 week	484
Stainless steel	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/Bris/59/07/	-	Viable virus not detected at 3 weeks	484
Stainless steel	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/Cal/4/09/	-	Viable virus not detected at 2 weeks	484
Stainless steel	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/Cal/7/09/	-	Viable virus not detected at 3 weeks	484
Stainless steel	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/PR/8/34	-	Viable virus not detected at 3 weeks	484
Stainless steel	19.5–19.7 °C (67.1–67.5 °F)	55.2–55.6% humidity (light environment)	LPAI H1N1	A/Sol/3/06	-	Viable virus not detected at 2 weeks	484
Steel, tile, gumboot, tire, eggshell, plastic	Unspecified room temperature	In 14-mL round-bottom tubes and stored in a drawer	LPAI H13N7	A/Herringgull/ Delaware 471/86	-	Virus below detectable limit at day 6	399

Appendix 2 Table 6. Summary of experimental studies on survival of influenza A viruses on additional substrates by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Latex, feather	Unspecified room temperature	In 14-mL round-bottom tubes and stored in a drawer	LPAI H13N7	A/Herringgull/ Delaware 471/86	Virus detected at day 6 (last time point tested)	-	[399]
Wood	Unspecified room temperature	In 14-mL round-bottom tubes and stored in a drawer	LPAI H13N7	A/Herringgull/ Delaware 471/86	-	Virus below detectable limit at hour 72	[399]
Egg tray, polyester fabric	Unspecified room temperature	In 14-mL round-bottom tubes and stored in a drawer	LPAI H13N7	A/Herringgull/ Delaware 471/86	-	Virus below detectable limit at hour 24	[399]
Cotton fabric	Unspecified room temperature	In 14-mL round-bottom tubes and stored in a drawer	LPAI H13N7	A/Herringgull/ Delaware 471/86	-	Virus below detectable limit at hour 48	[399]
Stainless steel	22 °C (71.6 °F)	50–60% relative humidity	H1N1	A/PR/8/34	Viable virus at hour 24 (last time examined)	-	[485]
Galvanized metal, glass	22.7–23.4 °C (72.86–74.12 °F)	32–38% relative humidity	HPAI H5N1	A/Vietnam/1203/ 2004	-	Virus below detectable limit at day 1	[398]
Soil	22.0–23.4 °C (71.6–74.12 °F)	30–42% relative humidity	HPAI H5N1	A/Vietnam/1203/ 2004	-	Virus below detectable limit at day 2	[398]
Galvanized metal, glass	22.4 °C (72.32 °F)	89.1% relative humidity	HPAI H5N1	A/Vietnam/1203/ 2004	-	Virus below detectable limit at day 1	[398]
Soil	22.4–23.4 °C (72.32–74.12 °F)	89.1–90.4% relative humidity	HPAI H5N1	A/Vietnam/1203/ 2004	-	Virus below detectable limit at day 2	[398]

Appendix 2 Table 6. Summary of experimental studies on survival of influenza A viruses on additional substrates by increasing temperature.

Substrate	Temperature	Humidity (as described by study authors)	Subtype	Strain	Last time point detected	Time to virus inactivation (experimental, estimated, or predicted based on regression analysis)	Reference
Rubber glove, N95 particulate respirator, surgical mask (non-woven fabric), gown (Dupont Tyvek), coated wooden desk, stainless steel	25.2 °C (77.36 °F)	55% relative humidity	H1N1	A/PR/8/34	Virus detected at hour 24 (last time point tested)	-	[486]
Plastic	27.8–28.3 °C (82.0–82.9 °F)	35–40% relative humidity	H1N1	A/Brazil/11/78-like	Virus detected at ~$10^1$$TCID_{50}$/0.1 mL at hour 48 (last time point tested)	-	[71]
Stainless steel	27.8– 28.3 °C (82.0– 82.9 °F)	35–40% relative humidity	H1N1	A/Brazil/11/78-like	-	Virus below detectable limit at hour 72	[71]
Stainless steel	55 °C (131 °F)	50% relative humidity	H1N1	A/PR/8/34	Minute 60 (last time point tested)	-	[487]
Stainless steel	60 °C (140 °F)	50% relative humidity	H1N1	A/PR/8/34	-	Virus below detectable limit at minute 30	[487]
Stainless steel	65 °C (149 °F)	50% relative humidity	H1N1	A/PR/8/34	-	Virus below detectable limit at minute 15 (1st time point tested)	[487]

Appendix 3: Literature Review on the Role of Local Area Spread in Previous Outbreaks

Appendix 3 Table 1 below summarizes the results from studies (including modeling) on the influence of local area spread in AI transmission during previous outbreaks.

Appendix 3 Table 1. Previous AI outbreak investigations and results associated with local area spread.

AI strain (Location)	Year of outbreak (species involved)	Study approach	Key findings	Source
HPAI/LPAI H7N9 (Southeast United States)	2017 (broiler breeders)	Case series, expert elicitation (case-control), waterfowl and wildlife surveillance	The case series identified no conclusive factors of spread without controls to compare to. In the expert elicitation study where experts were comparing case farms with matched control farms revealed that environmental factors such as intrusion of mesopredators and rodents as well as the high density of poultry farms within the location of case farms may have played significant roles in spread of LPAI, given the lack of integrator connections (i.e., feed, pullets, males, egg transport trucks, and crews for most of the cases). Additionally, types of sectors (e.g., broilers and egg laying industry) with premises not involved in the outbreak were hypothesized to have little involvement due to their lack of abundance in the affected area and their limited rodent presence on farm.	348
HPAI/LPAI H7N8 (Indiana)	2016 (turkeys)	Geospatial analysis; case-control (9 cases, 30 controls)	The geospatial analysis showed a likely association between infected premises and a common driving route. The case-control study identified risk factors more common on case farms and in case barns than on control farms and in control barns such as shorter distance to dead bird disposal and litter compost area, presence of wild mammals, and visitors entering barns.	455
HPAI H5N2 (Iowa)	2015 (layer chickens)	Case-control (28 cases, 31 control) with multivariate logistic regression	Farm-level analysis indicated that location in an existing control zone (10 km perimeter beyond the closest infected premises) was highly associated with infection status.	290

HPAI H5N8 (South Korea)	2014–2016 (broiler ducks)	Case-control (43 cases, 43 controls); Geospatial analysis	Proximity to nearest other farms (poultry farms located within 500m of farm) was indicated to be a risk factor based on a multivariate analysis of risk factors as well as from geospatial analysis. Farms having equal to or greater than seven flocks, farm owner experience, and not using feces removal services were also demonstrated risk factors.	[488,489]
LPAI H9N2 (Pakistan)	2009–2010	Case-control (133 cases, 133 controls)	Distance to the nearest infected farm of ≤1 km was identified as a risk factor, demonstrating a strong association with an increased risk of influenza A viruses based on the multivariate model of the case-control comparison. Farm location of ≤0.5 km of major roads and distance to the nearest commercial farm (regardless of infection status) was another identified risk factor.	[490]
HPAI H5N1 (England)	2007 (turkeys)	Outbreak observation, spatial simulation model	Spread to 3 houses on the same premises. No transmission to 78 other farms within a 3-km protection zone or 70 farms within a 10-km surveillance zone. Simulation showed no evidence of local transmission above 1 km.	[130,145]
HPAI H5N1 (Romania)	2005 (primarily backyard chickens)	Case-control (155 cases, 155 controls); Geospatial analysis	Villages being less than 5 km from a major road was a risk factor for poultry populations within villages. Additional risk factors identified included proximity to river/stream and regularly flooded areas.	[491]
LPAI H5N2 (Japan)	2005 (layer chickens)	Case-control (37 cases, 36 controls) with multivariate logistic regression. Biosecurity factors controlled for.	Distance up to 1.5 km from infected premises identified as a risk factor for egg layer farms in Japan. Equipment sharing and visitor biosecurity were also identified as risk factors.	[383]
HPAI H5N2 (Texas)	2004 (broiler chickens)	Outbreak observation	No area spread. Samples were collected from 368 premises (39 in the 8-km affected zone, 167 in the surveillance zone [16 km], and 162 in the buffer zone [50 km]).	[492]
HPAI H7N7 (Netherlands)	2003 (multiple poultry species)	Spatial transmission model with distance and infectious period at premises level as factors	Exposure increased with proximity to infectious farm. Farms ≤1 km from an infected premises were at least 8 times more likely to become infected than farms ≥5 km.	[142]

LPAI H7N2 (Virginia, West Virginia, North Carolina)	2002 (chickens and turkeys)	Outbreak observation	Spread mainly by people and fomites, including equipment, rendering especially high risk. Very little evidence for airborne spread.	[493]
LPAI H7N2 (Pennsyl-vania)	2001–2002 (broiler breeders and broiler chickens)	Outbreak observation	Local spread within 1 mile. Likely mechanisms were family ties, business connection, social contact, etc.	[405]
HPAI H7N1 (Italy)	1999–2000 (turkeys [meat and breeder], chickens [breeders, layers, and broilers], geese, quail, ostriches, guinea fowl, pheasants) (cont.)	Multivariable Cox regression: people and equipment flow not controlled for in model.	Flocks ≤1.5 km from an infected premises were estimated to have a Hazard ratio of 7.9. Poultry species and farm size also were identified as risk factors.	[494]
		Multivariable Cox regression: people and equipment flow not controlled for in model.	Flocks ≥4.5 km from infected premises had lower risk. Flocks ≤1.5 km from infected premises had highest risk (hazard ratio 4.6 in comparison to flocks >4.5 km from an infected premises). Poultry species, type of production, and farm size also were identified as risk factors.	[144]
HPAI H7N1 (Italy) (cont.)	1999–2000 (turkeys [meat and breeder], chickens [breeders, layers, and broilers], geese, quail, ostriches, guinea fowl, pheasants) (cont.)	Spatial transmission model with distance and infectious period at premises level as factors	Proximity to infectious farms increased the risk of infection, e.g., probability of infection estimated to be 2.5 times higher for susceptible farms 1 km from an infectious farm than for farms 3 km away. Control measures such as culling of infected farms and banning restocking were identified through simulation to reduce infection spread.	[141]
LPAI H5N3 (California)	1984 (turkeys)	Outbreak observation	Spread associated with insemination at 5 breeder premises across 177 km, linked to one company and insemination crew. No spread to 193 other turkey premises or >800 chicken premises in the State.	[129]

LPAI H6N1 (Minnesota)	1978 (layer chickens)	Outbreak observation	No spread to 1 of 4 houses on the same layer premises; the unaffected house was across a road from the 3 affected and interconnected houses. No spread to epidemiologically linked layer farms or neighboring premises.	[495]
LPAI A/T/Minn./67 (Minnesota)	1967 (turkeys)	Outbreak observation	Spread between houses on the same premises and between premises. Spread between premises appeared associated with insemination; some houses on severely infected premises were not infected.	[496]

Appendix 4: Expert Polling on Aerosol Transmission Route

A panel of twelve experts in the poultry industry with field experience managing AI as well as experts serving as regulatory veterinarians with upland gamebird experience were anonymously surveyed between February 28, 2020, and June 9, 2020, on the risk of HPAI transmission via multiple routes local area of infection. Surveys were administered through the online polling service Qualtrics. Experts were asked to provide their opinion, based on previous experience and subject matter expertise, of perceived risk for given scenarios. Qualitative risk rating definitions were provided and match those used in this risk assessment (with the exception that the survey did not include a "very low risk" option; see **Section 5** Overview of Data Analysis Approaches, for risk rating definitions). Below is the subset of questions that pertain to spread by aerosol transmission under two scenarios: with and without depopulation activities happening at source farm. Associated expert responses to these questions are shown in **Appendix 4 Tables 1-3** and **Appendix 4 Figures 1-3**.

Q1: Please qualitatively rate the risk of AI transmission via aerosol from a known infected poultry flock to a susceptible upland gamebird flock located at distances specified below. In this scenario, there are NO depopulation activities happening at source flock. Please complete the following table, selecting a risk rating for each scenario as negligible, low, moderate, high, or extremely high, for each distance based on your expert opinion.

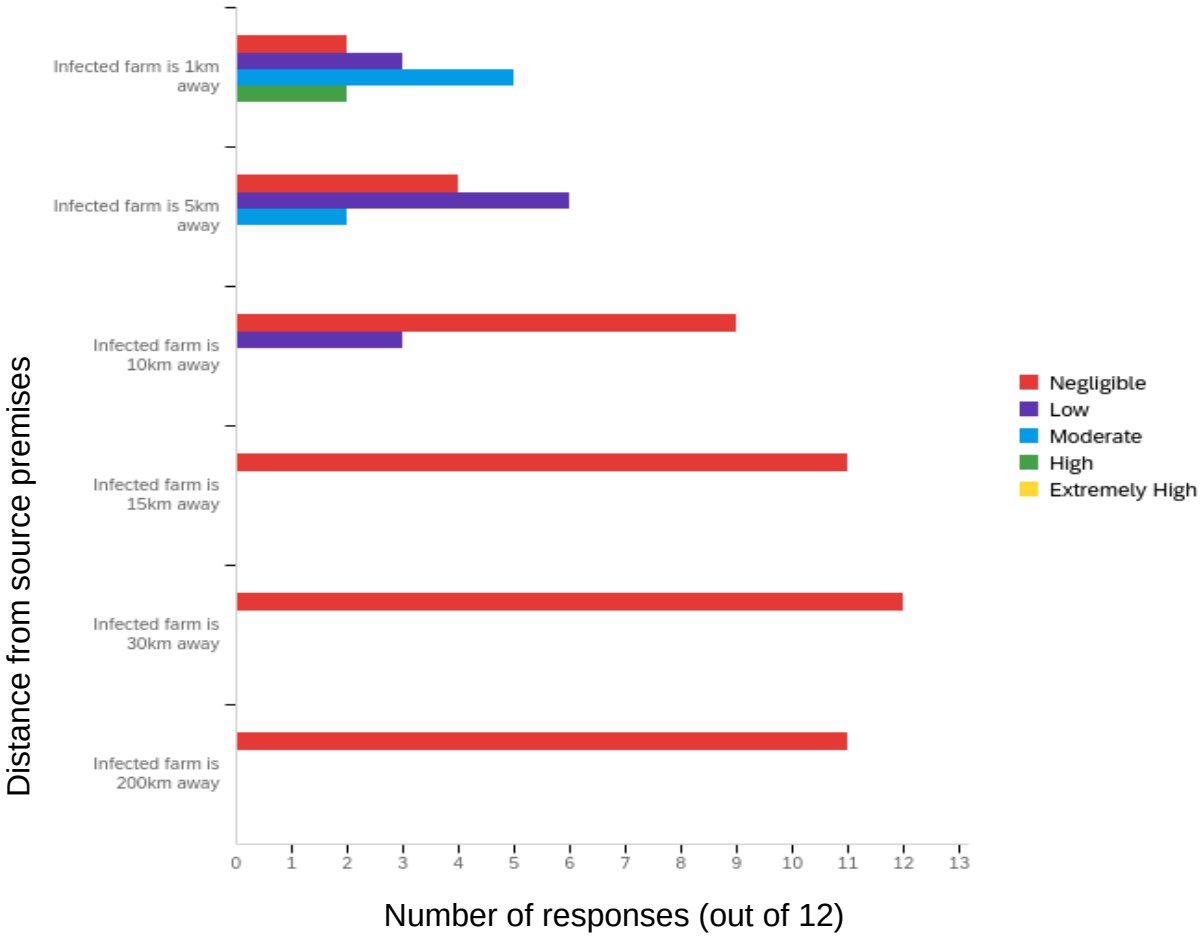
Q2: Please qualitatively rate the risk of AI transmission via aerosol from a known infected poultry flock to a susceptible upland gamebird flock located at distances specified below. In this scenario, there ARE depopulation activities happening at source flock. Please complete the following table, selecting a risk rating for each scenario as negligible, low, moderate, high, or extremely high, based on your expert opinion.

Q3: Please qualitatively rate the risk of AI transmission via aerosol from an infected but undetected flock to a susceptible upland gamebird flock located at distances specified below. Please complete the following table, selecting a risk rating for each scenario as negligible, low, moderate, high, or extremely high, based on your expert opinion.

Appendix 4 Table 1. Expert responses (n=12) to the question of the likelihood of AI transmission from a known infected flock to a susceptible upland gamebird flock at specified distances when no depopulation activities are happening at source flock (Question 1).

Distance from source flock	Risk Rating				
	Negligible	Low	Moderate	High	Extremely high
1 km	2	3	5	2	0
5 km	4	6	2	0	0
10 km	9	3	1	0	0
15 km [a]	11	0	0	0	0
30 km	12	0	0	0	0
200 km [a]	11	0	0	0	0

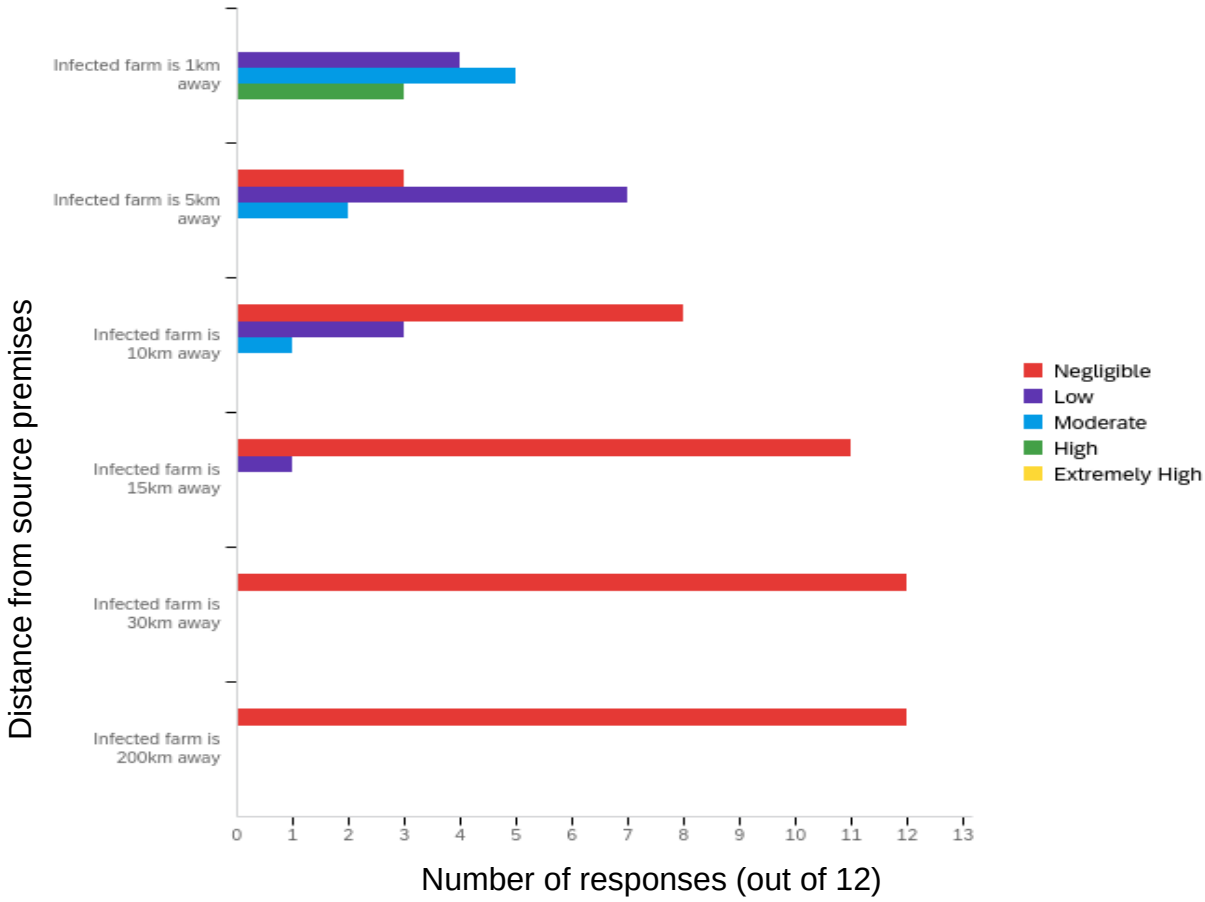
[a] Missing response from one respondent.



Appendix 4 Figure 1. Expert responses (n=12) to the question of likelihood of AI transmission from a known infected flock to a susceptible upland gamebird flock at specified distances when no depopulation activities are happening at source flock (Question 1)

Appendix 4 Table 2. Expert responses (n=12) to the question of the likelihood of AI transmission from a known infected flock to a susceptible upland gamebird flock at specified distances where depopulation activities are happening at source flock (Question 2).

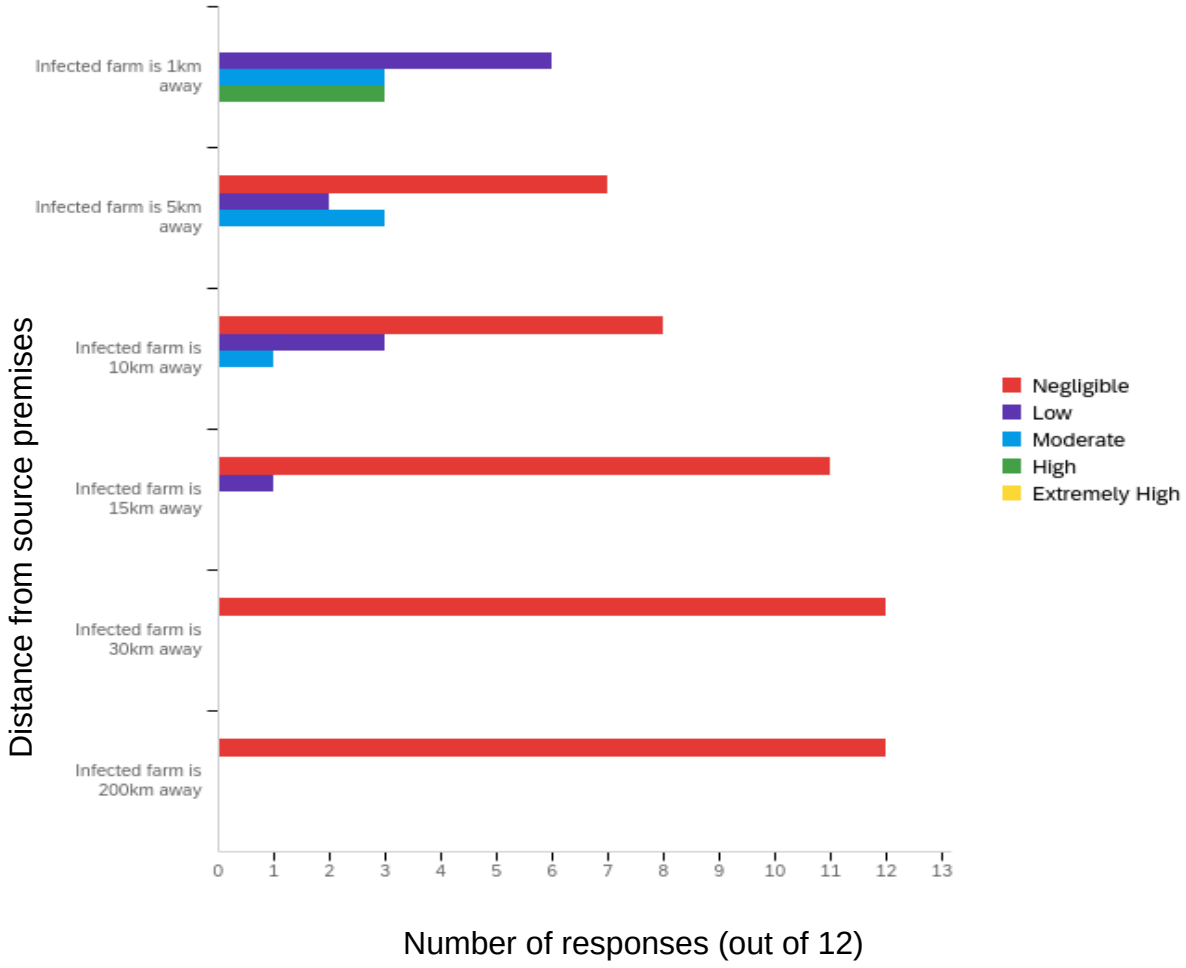
Distance from source flock	Risk rating				
	Negligible	Low	Moderate	High	Extremely high
1 km	0	4	5	3	0
5 km	3	7	2	0	0
10 km	8	3	1	0	0
15 km	11	1	0	0	0
30 km	12	0	0	0	0
200 km	12	0	0	0	0



Appendix 4 Figure 2. Expert responses (n=12) to the question of likelihood of AI transmission from a known infected flock to a susceptible upland gamebird flock at specified distances where depopulation activities are happening at source flock (Question 2).

Appendix 4 Table 3. Expert responses (n=12) to the question of likelihood of AI transmission from an undetected but infected flock to a susceptible upland gamebird flock at specified distances where depopulation activities are happening at source flock (Question 2).

Distance from source flock	Risk rating				
	Negligible	Low	Moderate	High	Extremely high
1 km	0	6	3	3	0
5 km	7	2	3	0	0
10 km	8	3	1	0	0
15 km	11	1	0	0	0
30 km	12	0	0	0	0
200 km	12	0	0	0	0



Appendix 4 Figure 3. Expert responses (n=12) to the question of likelihood of AI transmission from an infected but undetected flock to a susceptible upland gamebird flock at specified distances where depopulation activities are happening at source flock (Question 2).

Appendix 5: Expert Polling on Insect Transmission Routes

A panel of twelve experts in the poultry industry with field experience managing AI as well as experts serving as regulatory veterinarians with upland gamebird experience were anonymously surveyed between February 28th, 2020 and June 9th, 2020 on risk of HPAI transmission via multiple routes local area of infection. Surveys were administered through the online polling service Qualtrics. Experts were asked to provide their opinion, based on previous experience and subject matter expertise, of perceived risk for given scenarios. Qualitative risk rating definitions were provided and match those used in this risk assessment (with the exception that the survey did not include a "very low risk" option) (see **Section 5** Overview of Data Analysis Approaches, for risk rating definitions). Below is the subset of questions that pertain to spread by aerosol transmission under two scenarios: with and without depopulation activities happening at source farm. Associated expert responses to these questions are shown in **Appendix 5 Tables 1 and 2** and **Appendix 5 Figures 1 and 2**.

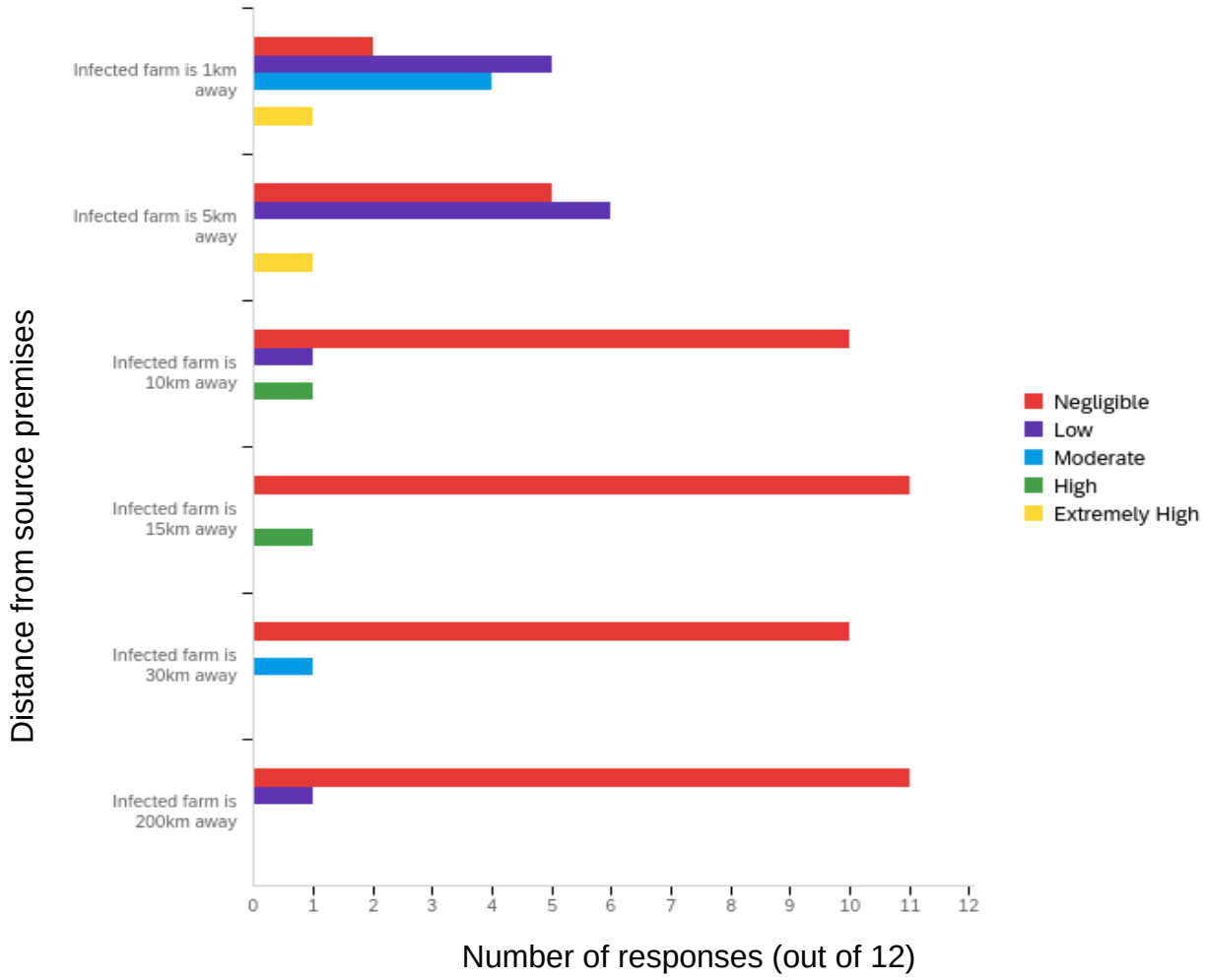
Q1. Please qualitatively rate the risk of AI transmission via insects from a known infected flock to a susceptible upland gamebird flock located at distances specified below. Please complete the following table, selecting a risk rating for each scenario as negligible, low, moderate, high, or extremely high, for each distance based on your expert opinion.

Q2. Please qualitatively rate the risk of AI transmission via insects from an infected but undetected flock to a susceptible upland gamebird flock located at distances specified below. Please complete the following table, selecting a risk rating for each scenario as negligible, low, moderate, high, or extremely high, for each distance based on your expert opinion.

Appendix 5 Table 1. Expert responses (n=12) to the question of likelihood of AI transmission from a known infected flock to a susceptible upland gamebird flock via insects at specified distances (Question 1).

Distance from source flock	Risk rating				
	Negligible	Low	Moderate	High	Extremely high
1 km	2	5	4	0	1
5 km	5	6	0	0	1
10 km	10	1	0	1	0
15 km	11	0	1	0	0
30 km[a]	10	0	1	0	0
200 km	11	1	0	0	0

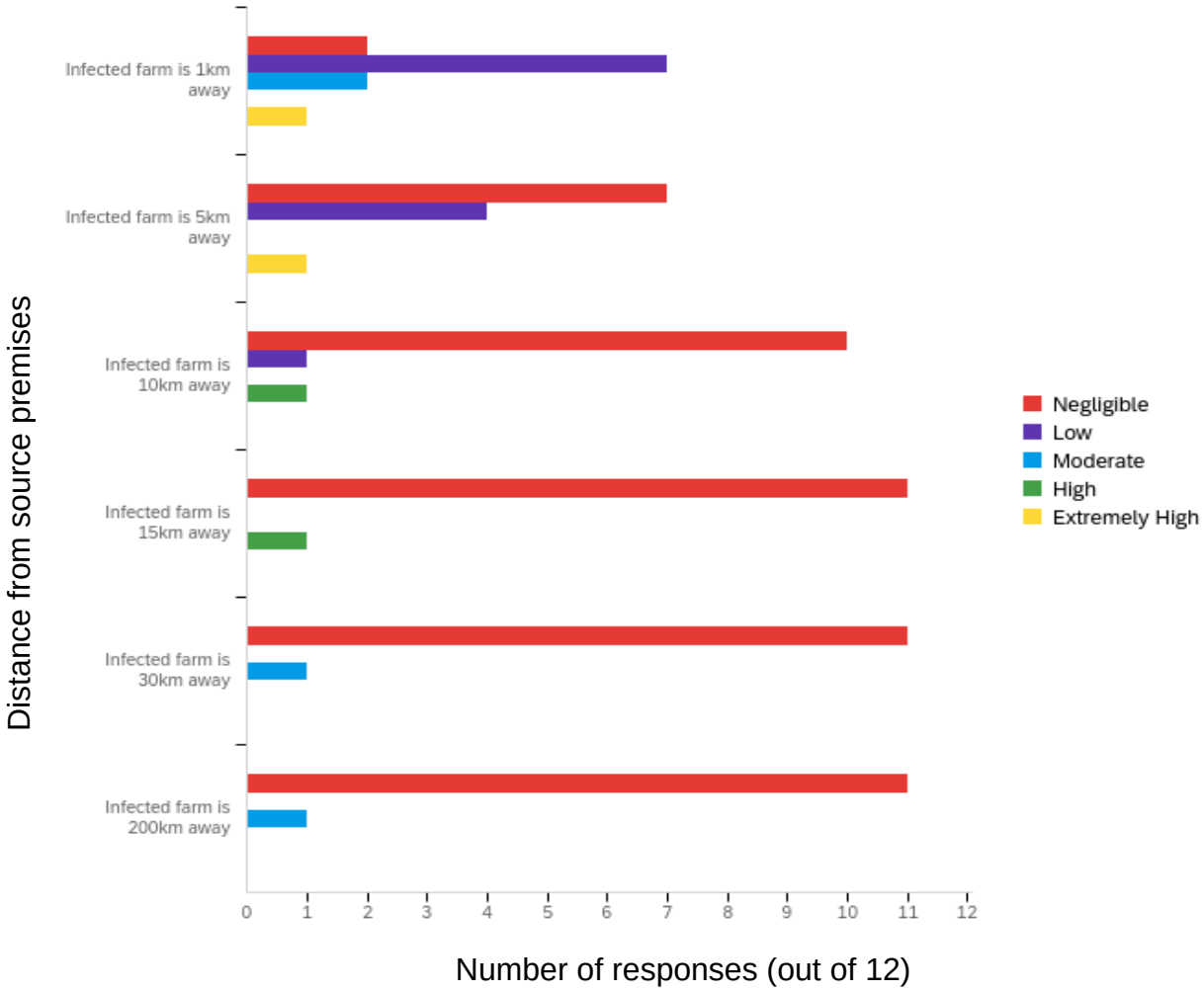
[a] Missing response from one respondent.



Appendix 5 Figure 1. Expert responses (n=12) to the question of likelihood of AI transmission from a **known infected** flock to a susceptible upland gamebird flock via insects at specified distances (Question 1)

Appendix 5 Table 2. Expert responses (n=12) to the question of likelihood of AI transmission from an infected but undetected flock to a susceptible upland gamebird flock via insects at specified distances (Question 1).

Distance from source flock	Risk rating				
	Negligible	Low	Moderate	High	Extremely high
1 km	2	7	2	0	1
5 km	7	4	0	0	1
10 km	10	1	0	1	0
15 km	11	0	0	1	0
30 km	11	0	1	0	0
200 km	11	0	1	0	0



Appendix 5 Figure 2. Expert responses (n=12) to the question of likelihood of AI transmission from an **infected but undetected** (lower prevalence) flock to a susceptible upland gamebird flock via insects at specified distances (Question 2)

Appendix 6: Pre-Movement Isolation Period

TO MOVE UPLAND GAMEBIRDS DURING AN HPAI OUTBREAK, PRODUCERS NEED TO AGREE TO A PRE-MOVEMENT ISOLATION PERIOD (PMIP) PRIOR TO MOVEMENT OF BIRDS OUT OF A STATE WITH HPAI.

- Activities associated with lateral virus transmission are prohibited.
- Only critical operational visits to the premises will continue.
- Specific biosecurity measures are implemented, depending on the acceptable level of risk.

GOAL: For producers to actively and effectively implement enhanced biosecurity procedures in the critical time period before live upland gamebirds are moved, thus reducing the risk of lateral HPAI transmission.

Prohibited activities during PMIP:

The following activities have a risk for lateral transmission of HPAI virus and are prohibited during the PMIP:

- Off-farm disposal of mortality is prohibited, if not already implemented. Risks associated with dead bird disposal on-site must be managed.
- Off-farm removal of manure or litter is prohibited, if not already implemented. Risks associated with manure or litter movement on-site must be managed.
- Garbage pick-up sites on the farm must be located outside of the perimeter buffer area (PBA). Garbage pick-up vehicles and personnel should not cross the PBA at any time during the PMIP.
- Visiting other poultry, upland gamebird, or waterfowl farms is prohibited for people who work on gamebird farms.
- All non-critical visitors are prohibited from entering farms (i.e., crossing the PBA). All non-critical, routine, or operational visits must be replaced by telephone communication or must be scheduled outside of the PMIP.
- Entering a gamebird pen or brooder barn is prohibited unless the person is wearing clothing dedicated to the farm and footwear dedicated to the pens or barns.
- Non-critical equipment (e.g., yard maintenance equipment, etc.) from off-site is prohibited from being moved on-site.
- Moving live upland gamebirds or poultry onto the premises is prohibited.
- Moving any type of upland gamebird product or live bird to any type of premises (e.g., hunting preserve, other upland gamebird farm, backyard farm, etc.) located within a Control Area is prohibited.
- Movement of product, equipment, people, and vehicles to a premises with ducks onsite or that engages in live bird market sales is prohibited.

Critical operational visits during PMIP require specific biosecurity measures:

- Feed delivery in a dedicated truck directly from a stand-alone feed mill (no poultry on-site at feed mill).
- Veterinary visits to address changes in bird health.

Specific biosecurity measures during PMIP:

In addition to standard biosecurity protocols, as described in the Secure Upland Gamebird Supply Plan, the following enhanced biosecurity measures must be implemented during the PMIP:

Personnel and vehicles mitigations required during the PIMP:

- All people who are going to enter a pen or barn must shower and change clothes and also wear necessary protective clothing dedicated to the farm and footwear dedicated to the pen group or barn as described in appropriate biosecurity protocols.
- All vehicles and equipment entering the premises will be cleaned and disinfected as approved by regulatory personnel prior to entering premises.
    - Driver must mitigate the risk of moving insects on and off the farm.
    - Driver must mitigate the risk of a contaminated vehicle interior due to exiting and re-entering the vehicle.
    - Driver must mitigate the risk of contaminated hands.

Product movement-specific mitigations required during the PMIP:

Movement of mature upland gamebirds to a hunting preserve

- All of the following preventative mitigations are **required** to be in place:

Mitigation serving to **LIMIT** contamination	Effect of mitigation
The minimum necessary number of non-resident personnel (e.g., those farm workers who DO NOT have living quarters onsite), up to a maximum of four, are involved with load-out procedures prior to birds leaving the farm premises. No limit on the number of resident personnel (e.g., those farm workers that have living quarters onsite) involved in load-out procedures.	Reduces the number of possible fomites (e.g., potentially contaminated clothing, shoes, or skin of farm personnel) birds come into contact prior to load-out.
Only one farm worker serving as the truck driver performs bird deliveries to other premises.	Reduces the number of possible fomites (e.g., potentially contaminated clothing, shoes, or skin of farm personnel) returning to the farm from a delivery premises.
Crates used to deliver birds contain no bedding.	Eliminates the possibility of bedding acting as fomites.

	Allows easier and more efficient cleaning and disinfection of crates.
Crates used to deliver birds do not touch the ground or enter a holding pen. ● Tarps must be used as a barrier between ground at the delivery and crates. Tarps must be disposed of at the delivery premises and not come back onto the delivery vehicle. ● Crates cannot cross the line of separation. Birds are required to be transferred into the pens by hand or gently dumped into pens.)	Reduces the level of contact that crates have with potentially contaminated surfaces at the delivery premises.
Disposable crates or boxes are used if proper disinfection procedures of reusable crates cannot be achieved (See *Recommended Crate Cleaning and Disinfecting Protocol* below). Note: Wooden crates cannot be completely disinfected unless a disinfectant with active ingredients of NaDCC or Glutaraldehyde is used.[466]	Eliminates any possibility of returning crates that would act as a fomite.

b. The following reducing mitigations are **required** to be in place:

Mitigation serving to **REDUCE or ELIMINATE** virus	Effect of mitigation
Crates are cleaned and disinfected using an appropriate procedure. (See *Recommended Crate Cleaning and Disinfecting Protocol* below).	Reduces organic material potentially harboring virus and kills virus present on crate surfaces.
The following biosecurity protocols for the **delivery truck** must be followed: ● Vehicle windows should be always rolled up while on the poultry farm to prevent flies from getting into the vehicle. ● Insecticide should be sprayed inside trucks as needed to eliminate the transporting of flies from farm to farm during warm months of the year. ● Floors, pedals, and bottoms of feet should be sprayed with disinfectant after every stop.	Reduces organic material potentially harboring virus and kills virus present on surfaces on the outside or inside of the vehicle or on fomites such as insects.

• The outside of all vehicles should be cleaned and disinfected using a biosecure truck wash or commercial car wash.	
The following biosecurity protocols for the **delivery driver** must be followed: • If the driver gets out of the vehicle, the cab interior must be cleaned and disinfected, and the driver must wear protective clothing, such as disposable boots and gloves, and remove them before getting back in the cab. • The driver should use a hand sanitizer before leaving and after re-entering the cab. • The driver should shower and change clothes prior to returning to the farm (prior to crossing the farm's perimeter buffer area).	Reduces organic material, potentially harboring virus and kills virus present on driver-related fomites (e.g., skin, clothes, or shoes).

Recommended Crate Cleaning and Disinfecting Protocol

C&D Step	**Specifics for C&D Step**
Step 1: Dry clean crates to remove any organic material and gross contamination.	Use a pressure washer to initially dislodge and remove all visible organic material.
Step 2: Wash crates with an appropriate detergent to continue the breakdown of organic material. Rinse crates once the wash procedure has removed all organic material.	Spray crates inside and outside with a detergent and let sit for 10-15 min. Then use a pressure water with a barrel wand to rinse the inside of crates, spraying in all directions and it all crevices of the crates. If any organic material remains, repeat the wash procedure as needed (with a reduced sitting time for applied detergent).
Step 3: Ensure that crates are completely dried.	Set crates to dry in a clean area (not where they were washed and rinsed). During the time crates are drying, the area where crates were washed and rinsed could be cleaned of dirt and sprayed down.
Step 4: Apply disinfectant to inside and outside of crates.	An EPA-registered disinfectant suitable for avian influenza viruses and appropriate for the crate material is required (including those listed on the EPA's *Potential Pesticides to Use Against the Causative Agents of Selected Foreign Animal Diseases in Farm Settings* document). Using a pressure washer, disinfectant should be applied as a foam to cover the maximum amount of

	crate surface area. Allow crates to dry completely or until the needed contact (dependent upon disinfectant) time.

NOTE: The type and number of mitigations applied under sections 1a and 1b should be considered in scenarios where other birds are present on hunting preserve sites within holding pens or elsewhere onsite. The degree of potential environmental contamination could vary depending upon the presence of other birds onsite.

- **Movement of mature upland gamebirds to populate an upland gamebird farm for wholesale purposes**
  - Movements of mature upland gamebirds to an upland gamebird farm for wholesale purposes should be halted completely if mitigations in 1a and 1b are not completely met during mature bird movements to upland gamebird farms AND hunting preserves.

- **Movement of started upland gamebirds to an upland gamebird farm**
  - Movements of started upland gamebirds to an upland gamebird farm will be halted completely if mitigations in 1a and 1b are not completely met during started bird movements to upland gamebird farms hunting preserves AND during movements of started upland gamebird movements to upland gamebird farms.

- **Movement of hatching eggs**
  - All movements of hatching eggs are required to be conducted through an offsite nationally recognized parcel courier or mail service (e.g., USPS, UPS, or FedEx). Deliveries to premises that reside within Control Areas are restricted. Direct deliveries of hatching eggs to other premises are restricted.

- **Movement of day-old chicks**
  - Movements of day-old chicks will be conducted through an offsite nationally recognized parcel courier or mail service (e.g., USPS, UPS, or FedEx). If a courier service is not feasible, deliveries of day-old chicks should occur either at a neutral location with the buyer (i.e., not at either the premises of origin or destination premises) or chicks can be delivered to the delivery premises if the mitigation measures below and the delivery truck and driver biosecurity protocols from 1c are followed. Deliveries to premises that reside within Control Areas are restricted. Direct deliveries of day-old chicks to other premises are restricted.

Mitigation serving to **LIMIT** contamination	Effect of mitigation
Disposable boxes are used to transport chicks. No transport or boxing material returns to the premises of origin.	Eliminates any possibility of returning boxes to act as a fomite.
Truck and driver should not cross the perimeter buffer area of the delivery premises.	Reduces the opportunity for contamination of clothing, shoes, or skin of farm personnel and/or wheels of vehicles, thus reduces number of fomites that could return to the farm.
The single driver is the only personnel from the premises of origin involved with delivery.	Reduces the number of possible fomites (e.g., potentially contaminated clothing, shoes, or skin of farm personnel) returning to the farm.

- **Movement of mature birds to off-site processing location**
    - All movements of live birds to off-site processing plants that process commercial poultry (e.g., chickens or turkeys) are prohibited.

PMIP mitigations occur for as long as an active outbreak is occurring within the State from which upland gamebirds will be moved.

Appendix 7: Modeling Technical Details

This appendix provides the technical details for the methods applied in estimating the detection probabilities evaluated in **Section 9.4** Likelihood of Detecting HPAI in an Infected Upland Gamebird Pen. The probability of detection before the start of load-out and the probability of detection prior to movement to processing are estimated from simulation models consisting of a stochastic disease transmission model and active surveillance model. A description of the transmission and surveillance model algorithms can be found in Weaver et al. (2016)[429] and Ssematimba et al (2019).[12] The models from Weaver et al. (2016) were reparametrized to upland gamebirds facts and assumptions for use in the analyses presented in this risk assessment.[12,429] The derivation of the upland gamebird-specific parameters is detailed in the section following the introduction.

The probability of detection prior to the start of load-out as estimated from the simulation models is a critical component in estimating the overall likelihood of not detecting HPAI in a flock prior to the start of load-out. The overall likelihood combines the probability of two events: First, the probability that a susceptible flock is infected given it is some distance from an infectious premises; and second, the probability the infection is not detected in the flock prior to the start of load-out, transit, and delivery. As previously mentioned, the second probability is estimated using the simulation models. The first probability, that a susceptible premises a given distance from an infectious premises is itself infected, is estimated using a spatial transmission kernel, which estimates the hazard rate posed by an infectious premises to a susceptible premises at a given distance. The two probabilities are combined into the overall likelihood following a method outlined in Weaver et al. (2016).[429] The transmission kernel estimated from data on the 2015 HPAI H5N2 outbreak in Minnesota was used to estimate the overall likelihood.[384] Details on the kernel estimation are given following an explanation of the estimation of disease transmission model parameters used in the simulation. It is important to note that spatial transmission kernels use poultry premises data not including any upland gamebird premises, meaning applicability of the kernels must be interpreted conservatively.

Estimation of Transmission Model Parameters

Adequate Contact Rate

The distribution for the adequate contact rate was estimated based on the reported results from transmission experiments with unvaccinated pheasants by van der Goot et al. (2007)[126] and has been used in Ssematimba et al. (2019)'s transmission modeling.[12] A parametric distribution for the contact rate for use in simulation models was not provided in the article although the most likely value and the 95 percent (CI) for the contact rate were reported. We estimated a Gamma distribution for the contact rate by minimizing the sum of squared difference between the reported distribution characteristic from the article (mean and 95 percent interval) and the corresponding value for the estimated Gamma distribution using the R package Optim. The shape parameter was estimated to be 8.69 and the scale parameter was estimated to be 0.36 giving a mean of 3.13 per day and a standard deviation of 1.06 per day.[12]

Latent and Infectious Period Distributions

Latent period duration. Currently, the only available source of precise data on influenza A virus transmission dynamics in pheasants is the van der Goot et al. (2007) study,[126] but their design is

not permissive to fitting the latent period distribution. Thus, we used data available in relevant literature[73,167,426–428] involving H5N1 HPAI in chickens. During this fitting process, the latent period was assumed to begin once the bird was inoculated and end sometime between the last negative and first positive test for that particular bird.

Let t_a be the time of the last negative test and let t_b be the time of first positive test, so the transition from the latent to the infectious period occurs in $(t_a, t_b]$. The probability of observing the transition in this time period is given by F(t_b) - F(t_a), where F is the distribution of the latent period, here assumed to be gamma distributed.

Let t_c be the sampling time. The probability that the transition from the latent to the infectious period occurred prior to t_c in birds for whom the test is positive is F(t_c), while the probability the transition occurs after t_c in birds testing negative is 1 – F(t_c). Parameters for the gamma distribution were estimated by maximizing the cumulative likelihood of the observed transition from the latent to the infectious period in each inoculated chicken in each of the cited experiments. The likelihood was maximized using the "nlminb" algorithm, a bounds-constrained quasi-Newton method in R's "optimx" function.[497–499] The shape parameter was estimated to be 0.89 and the scale parameter was estimated to be 0.72 giving a mean of 0.64 days and a standard deviation of 0.68 days.[12]

Infectious period duration. We used raw data from the transmission experiments with unvaccinated pheasants from the van der Goot et al. (2007) study.[126] The estimation procedure uses data from both the inoculated and contact-infected birds, and accounts for the censored nature of the data, leading to an assumption that the transition to the infectious state occurred within the day of the first positive test result. The shape parameter was estimated to be 4.38 and the scale parameter was estimated to be 2.21 giving a mean of 9.68 days and a standard deviation of 4.63 days.[12]

Number of Birds per Pen

The distribution of the number of birds stocked in a pen was estimated from the raw data collected by Ssematimba et al. (2019) upon considering various plausible candidate distributions.[12]

Estimation of Active Surveillance Model Parameters

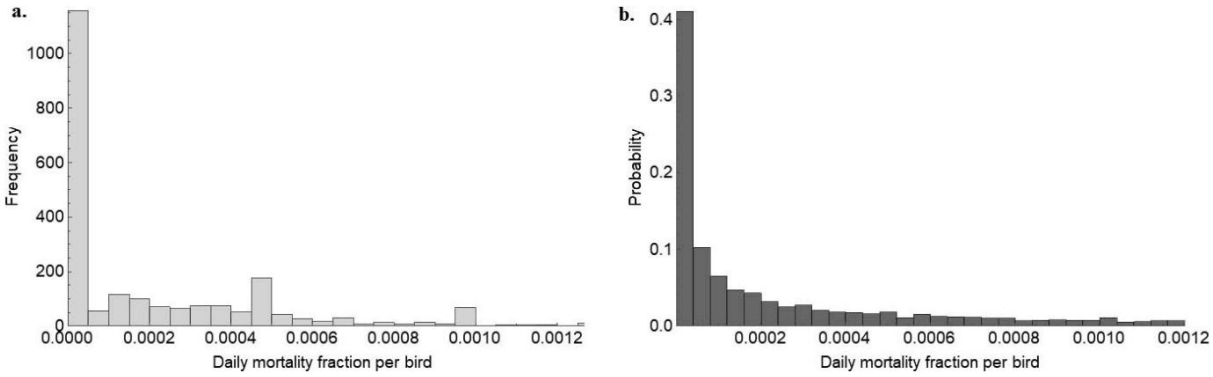
Daily Mortality

This study used daily mortality data to determine normal trends in mature upland gamebirds. Pen-level daily normal mortality data were collected by selected producers from ready-for-release pheasant pens in the United States. The producers were conveniently selected to cover the dominant upland gamebird producing regions to correct for possible regional variations in seasons. The data were collected during the high hunting activity period in the commercial upland gamebird industry (September 2017 through January 2018). To capture elements of seasonality, the data were gathered in batches of 30 days and the recorded fields included the type and number of birds stocked, the date of stocking, and the daily number of dead, culled and sold birds.

Data spanning approximately 30 days to the day of bird release were obtained electronically as spreadsheets in Microsoft Excel (Microsoft Corporation, Redmond, WA) from 40 pheasant pens on 5 commercial raised-for-release upland gamebird farms. This data was used to obtain

descriptive statistics as well as to test the daily pen mortality counts for autocorrelation to assess independence of daily counts using the software Mathematica 11.1.1 (Wolfram Research, Inc.).

The daily counts were standardized to daily proportions by calculating the ratio of the current day's number dead to the total number of birds in the pen on the previous day. The standardized data were then used for the assessment of false alarm rates and time to detection for the different trigger types. We generated a sizeable mortality dataset of 10,000 entries that is equivalent to the collected field data by simulating 30 days normal daily mortality proportions in 10,000 flocks. The mortality rate used in this simulation was randomly drawn from a distribution fitted to the collected daily mortality proportions. Data were obtained from forty pheasant pens, and overall, 66 percent of the field-recorded days had zero deaths. The calculated mean number stocked per pen was 1841 birds (ranging from 406 to 5420 birds), the mean bird age was 139 days (ranging from 78 to 214 days), and the mean normal mortality per day was 0.6 birds (ranging from 0.1 to 4.9 birds). Beta distribution with shape parameters: alpha = 0.113, beta = 74.35 truncated at minimum = 0 and maximum = 0.016.[12] The daily counts were standardized to daily proportions by calculating the ratio of the current day's number dead to the total number of birds in the pen on the previous day. The standardized data were then used for the assessment of false alarm rates and time to detection for the different trigger types. We generated a sizeable mortality dataset of 10,000 entries that is equivalent to the collected field data by simulating 30 days normal daily mortality proportions in 10,000 flocks. The mortality rate used in this simulation was randomly drawn from a distribution fitted to the collected daily mortality proportions. **Appendix 6 Figure 1** depicts histograms for the collected and simulated daily mortality proportions data scaled down to a per-bird level. To get total number of dead birds on a given day, the per-bird rate was multiplied by the prevailing flock size. Panel (a) depicts the daily mortality proportions obtained directly from the collected field data. Panel (b) shows the summary of daily mortality proportions from simulated data of 10000 flocks. The simulation was based on a truncated beta distribution with shape parameter =0.113 and scale parameter=74.35 and minimum = 0 and maximum = 0.016 parameterized by fitting to the collected data shown in panel (a). This data was then used in pre-movement surveillance scenario analyses.



Appendix 6 Figure 1. Histogram of standardized (a) and simulated (b) daily mortality in a mature ready-for-release pheasant pen in the 30 days prior to start of release.[12]

Diagnostic Test Sensitivity

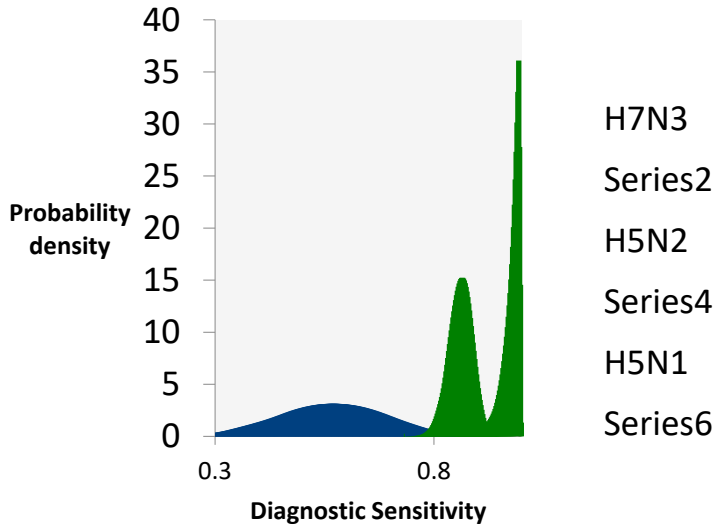
The sensitivity of the rRT-PCR test is estimated to be 86.5 percent, meaning there is a 13.5 percent chance the infection will not be detected even when the pooled sample contains an

HPAI-positive swab.[500] AI experts noted this sensitivity estimate is conservative considering recent enhancements to test protocols.[501]

In the main testing protocol, with rRT-PCR testing done a few days earlier, antigen capture immune assays using lateral flow devices are utilized at the day of load-out. These tests require high virus concentrations to detect AI virus (detection limit is between 10^4 and 10^6 EID_{50}).[502–504] The diagnostic sensitivity of these tests therefore depends on the clinical status of the infectious birds, which impacts the level of virus shedding.

A study performed at the USDA Southeast Poultry Research Laboratory (SEPRL) was undertaken to provide data on antigen capture (AC) test performance in dead birds infected with HPAI viruses. AC test sensitivity was estimated for two strains separately using a Bayesian approach from swabs taken from 14 and 46 dead chickens following exposure to HPAI H7N3 Jalisco and Pennsylvania HPAI H5N2, respectively. In addition, the AC test sensitivity was estimated for HPAI H5N1 (several clades) from a literature review. The resulting posterior distributions are given in **Appendix 6 Figure 2**. The estimated means and 95 percent credibility intervals for the AC test sensitivities are 57 percent (33 to 80 percent) for the HPAI H7N3 Jalisco strain, 86 percent (80 to 91 percent) for the HPAI H5N1 strain, and 97.9 percent (92 to 99.9 percent) for the HPAI H5N2 strain.[505] The wider credibility interval in the case of HPAI H7N3 is due to the smaller sample size and correspondingly greater uncertainty.

The estimated AC test sensitivities suggest that there is considerable between-strain variation, which is likely due to the variation in the levels of virus shedding between different strains, which affects detection because of the low analytic sensitivity of the AC test. AC test sensitivities for LPAI as identified through a literature review were generally lower than the estimates for HPAI, with an average of about 50 percent. Given the uncertainty and variance surrounding the estimates for AC test sensitivity, a conservative estimate of 50 percent is chosen for this analysis.



Appendix 6 Figure 2. Statistical distributions for the diagnostic sensitivity of antigen capture immunoassays for different HPAI strains.

Estimation of the 2015 HPAI H5N2 Minnesota Outbreak Spatial Transmission Kernel

Spatial Transmission Kernel Model

Due to phylogenetic evidence of primary introductions occurring concurrently with lateral spread Bonney et al. (2018)[384] adapted the transmission kernel in Boender et al. (2007)[142] by introducing an additional parameter to the force-of-infection equation.

The Boender et al. (2007)[142] transmission kernel is given below as a function of distance between susceptible premises *i* and infectious premises *j*:

$$h(d_{ij}) = \frac{h_0}{1 + \left(\frac{d_{ij}}{r_0}\right)^{\alpha}}$$

h_0, r_0, and α are constants to be estimated from outbreak data, where h_0 is the maximum daily hazard rate (occurring when the inter-premises distance is zero), and r_0 and α determine the decline in the hazard rate as inter-premises distance increases from zero.

The force of infection describes the overall hazard faced by susceptible premises *i* at time *t*, and in Boender et al. (2007) it depends solely on the number of infectious premises.[142] The force of infection from Boender et al. (2007)[142] is given below as a function of *t*:

$$\lambda_i(t) = \sum_{i \neq j} \quad h(d_{ij}) 1\{j \; is \; infectious\}$$

Bonney et al. modified this equation for use in the Minnesota outbreak through the addition of a parameter, *k*, allowing for infection to occur independently of the number of infectious premises:

$$\lambda_i(t) = \left(\sum_{i \neq j} \quad h(d_{ij}) 1\{j \; is \; infectious\}\right) + k$$

Note that *k* is constant and distance-independent in addition to not being reliant on the number of infectious premises at time *t*. Therefore, *k* largely expresses the risk posed by distance-independent environmental factors such as wild birds.

Estimation of the Spatial Transmission Kernel Parameters

The four parameters, h_0, r_0, α, and *k*, were estimated following the maximum likelihood method approach described in Boender *et al*. (2007).[142] The method depends only on inter-premises distance and premises-level infection status. As the exact days on which the infectious period of a case premises started and ended are unknown, a number of simplifying assumptions must be made. For the Minnesota outbreak, case premises are assumed to be infected eight days prior to the detection date. The infectious period is assumed to begin three days later, five days prior to the detection date. The infectious period lasts up to and including the day on which disposal of the depopulated poultry carcasses begins. The mean parameter estimates and 95 percent confidence intervals under these assumptions regarding infection status are given in **Appendix 6 Table 1**.

Appendix 6 Table 1. Mean estimates and 95% confidence intervals of spatial transmission kernel model parameters estimated from HPAI outbreaks in Minnesota.

Description	h_0	r_0	α	$k(10^{-4})$

Minnesota 2015 HPAI H5N2: Case premises are infected 8 days prior to detection; infectious period starts 5 days prior to detection and lasts up to and including compost start date.	0.0061 (0.0025, 0.0137)	7.02 (3.07, 16.16)	2.46 (1.80, 4.38)	3.2 (1.6, 5.2)

Estimation of the Probability of Infection

The spatial transmission kernel is used to estimate the probability that a susceptible premises becomes infected given it is some distance from an infectious premises through the force of infection. The probability that a susceptible premises *i* becomes infected on day *t*, $q_i(t)$, is given below:

$$q_i(t) = 1 - e^{-\lambda_i(t)}$$

The mean parameter estimates estimate the probability of infection applied in the estimation of the overall probability.

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