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An Assessment of the Risk Associated with the Movement Turkeys to Market Into, Within, and Out of a Control Area During a Highly Pathogenic Avian Influenza Outbreak in the United States

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A Collaboration between the Turkey Sector Working Group, the University of Minnesota's Secure Food Systems Team, and USDA:APHIS:VS:CEAH

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Contents

1 Abbreviations and Definitions

AC	Antigen capture (as in "AC testing")
AI	Avian influenza
APHIS	Animal and Plant Health Inspection Service (USDA:APHIS)
BWG	Broiler Working Group
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
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
IAV-S	Influenza A virus of swine
ICS	Incident Command System
ILT	Infectious laryngotracheitis
IP	Infected premises
IVPI	Intravenous pathogenicity index
LPAI	Low pathogenic avian influenza
NA	Neuraminidase
NAHLN	National Animal Health Laboratory Network
NAHMS	National Animal Health Monitoring System (USDA)
NPIP	National Poultry Improvement Plan
NVSL	National Veterinary Services Laboratory (USDA)
OIE	World Organization for Animal Health (formerly Office International des Epizooties)
PBA	Perimeter Buffer Area
PM	Particulate matter

PMIP	Pre-Movement Isolation Period
PPE	Personal protective equipment
PRRSV	Porcine reproductive and respiratory syndrome virus
RH	Relative humidity
rRT-PCR	Real-time reverse transcription polymerase chain reaction
SAHO	State animal health official
SPF	Specific Pathogen Free
TWG	Turkey Working Group
U.S.	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet light
VS	Veterinary Services (USDA:APHIS:VS)
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% bird infectious dose. One BID_{50} unit is the amount of virus that will infect 50% of inoculated birds.

Biosecurity

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

Breeder farm

A farm with breeder flocks that produce hatching eggs. The hatching eggs from a breeder farm are transported to a hatchery.

Broiler Sector Working Group (BWG)

A working group, which is made up of representatives from the broiler industry, academia, SAHOs, and USDA:APHIS, to support permits for the movement of broiler hatching eggs, chicks, or birds during an HPAI outbreak.

Brooder premises

Premises with facilities that raise poults (young turkeys) during the first few weeks of production. Day-old poults from a hatchery are transported to a brooder farm, some of which may also include grow-out barns.

Buffer zone

The zone immediately surrounding the infected zone; the buffer zone and the infected zone constitute the Control Area.

CID_{50}

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

Contact Rate

The rate at which susceptibles meet infecteds, measured as individuals per unit time.

Control Area

A Control Area, consisting of an infected zone and a buffer surveillance zone, will be established to ensure the rapid and effective containment of the disease. The potential modes of transmission of HPAI are considered when determining the minimum size and shape of a Control Area. Movement control—through the use of permits—should be maintained until the disease is eradicated.

Downtime for Visitors

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

EID_{50}

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

ELD_{50}

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

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 part of the Free Area.

Free Premises

Poultry premises that are not in an HPAI Control Area and are not Contact or Suspect premises.

Hatchery

A commercial establishment that produces day-old poults from hatching eggs. Commercial hatcheries receive hatching eggs from off-site breeder farms and produce day-old poults that are shipped to brooder operations.

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 of time 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.

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 which have an increased likelihood for disease transmission with proximity to infected flocks.

Movement permit

A VS Form 1-27, a state-issued permit, or a 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 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.

Observation period

The time interval between moving birds from a brooder house to the placement of new poults into the brooder house during an HPAI outbreak where the previously raised flock is observed (in the grow-out barn) for clinical signs of HPAI. The purpose of observation time is to gain confidence that birds previously raised in the brooder house were not infected.

Perimeter Buffer Area (PBA)

The perimeter buffer area on a poultry premises is a functional zone surrounding the poultry houses or poultry raising area that separates them from areas unrelated to poultry production on that site and/or adjoining properties. It is composed of the poultry houses and poultry raising areas as well as nearby structures and high-traffic areas involved in the daily function of the poultry farm. This would usually include, but not be limited to, such things as feed bins, manure sheds, composting areas, egg rooms, generators, pump rooms, etc.

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). PPE in the event of an HPAI outbreak 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.

Poult

A young turkey.

Premises

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

Pre-Movement Isolation Period (PMIP)

The PMIP is a critical biosecurity component that involves a defined period of greatly intensified biosecurity for an entire premises prior to permitted movement of live poultry within, into, or out of a regulatory Control Area during an HPAI outbreak. The PMIP starts a specified number of days prior to the scheduled movement date and ends when load-out begins (i.e., the hours or days of load-out are not considered part of the PMIP).

Secure Broiler Supply (SBS) Plan

A science-based plan that is 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 poultry product movement in an HPAI outbreak.

Secure Turkey Supply (STS) Plan

A strategic plan, containing science-based outbreak measures and protocols 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 an HPAI Control Area.

Standard Operating Procedure (SOP)

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

Turkey Sector Working Group (TWG)

A working group, which is made up of representatives from the turkey industry, academia, SAHOs, and the USDA:APHIS, to support permits for the movement of turkey hatching eggs, poults, or birds during an HPAI outbreak.

$TCID_{50}$

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

Zoonosis

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

2 Executive Summary

In the event of a highly pathogenic avian influenza (HPAI) outbreak in the United States, poultry industry, local, state, and federal authorities will implement a foreign animal disease emergency response. In these circumstances, permit requests to move poultry and poultry products must be supported by risk assessments which demonstrate that the risk of HPAI spread associated with the movement is acceptable. Performing the risk assessments prior to an HPAI outbreak can enhance emergency response and facilitate timely movement permitting decisions during an outbreak. This document assesses the risk that the movement of market-age turkeys to processing (i.e., turkeys to market), during an HPAI outbreak, from a premises located within the Control Area, will result in HPAI virus spread to a virus-free commercial poultry population (e.g., another poultry farm or birds remaining on a multi-age premises). 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 live turkeys (for meat) to slaughter.

This risk assessment is a joint effort of the Secure Turkey Supply (STS) Working Group, which is made up of representatives from the turkey industry, academia, State Animal Health Officials (SAHOs), and the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS), to support permits for the terminal movement of turkeys to processing during an HPAI outbreak. This assessment is applicable to intensively raised commercial or contract grow-out turkey premises that do not have other poultry on the premises, that participate in the USDA National Poultry Improvement Plan (NPIP), and that follow the STS Plan in the event of an HPAI outbreak. The STS Plan contains science-based outbreak measures developed by the STS working group to mitigate the risk of HPAI spread associated with the terminal movement of live birds to market.

This risk assessment assumes that applicable current industry practices and biosecurity measures (e.g., the NPIP) as well as outbreak-specific measures stipulated within the STS Plan are followed. The main categories of outbreak measures outlined in the STS Plan for turkey premises that wish to move birds to slaughter from a Control Area include:

- Establishing all criteria needed to meet the definition of an avian influenza (AI) Monitored Premises
- Active surveillance (e.g., rRT-PCR [real-time reverse transcription polymerase chain reaction] testing, detection of abnormally high mortality)
- Observing the greatly enhanced biosecurity measures of the Pre-Movement Isolation Period (PMIP)
- Following specific infection mitigation measures pertaining to load-out vehicles, crews, and equipment as determined by the duration of load-out process

The PMIP is a critical biosecurity component that involves a defined period of greatly intensified biosecurity for an entire premises prior to permitted movement of live poultry within, into, or out of a regulatory Control Area during an HPAI outbreak. The PMIP starts a specified number of days prior to the scheduled movement date and ends when load-out begins (i.e., the hours or days of load-out are not considered part of the PMIP). The PMIP duration to move turkeys to market is set at 8 days and includes the following stipulations:

- No live or dead poultry will be moved onto or off the premises.

- Only critical operational visits to the premises will continue.
- Manure, litter, and garbage will not be removed from the 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.
- Enhanced biosecurity will be implemented for people, vehicles, and equipment entering the premises; no off-site equipment will be pre-staged on-site.

The length of the PMIP decided upon by the STS working group is 8 days, which is expected to provide a high probability (i.e., ≥95%) of detection for nearly all HPAI strains, though the possibility exists that an 8-day PMIP may not be sufficient either due to an especially problematic HPAI strain or reduced contact rate (rate of within-house spread).

We assume that movement of infected turkeys to processing would pose a high likelihood of HPAI spread to susceptible poultry and have high 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 turkeys to market, this risk assessment evaluated the possible pathways for virus transmission to turkey 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, vehicles, or equipment; and (3) load-out processes. Local area spread refers to risk pathways which cause an increased likelihood of disease transmission with proximity to infected poultry flocks. If, due to a lapse in PMIP biosecurity practices or other unforeseen events, turkeys are moved from the finishing barn 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 market-age turkeys 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 in order to evaluate the overall risk of spread associated with movement of turkeys to processing. The pathways and their corresponding likelihood and risk ratings are described below. The overall finding and conclusion qualitatively integrates the results from the pathway assessments and the estimation of likelihood of detection, taking into account the assumed high consequences.

2.1 Likelihood of Turkey Flock Becoming Infected with HPAI via Components of Local Area Spread Resulting in Infected but Undetected Movement to Market

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

following is a breakdown for the likelihood of HPAI spread to a turkey flock via insect transmission:

	Composite likelihood rating (insects)		
	Distance from source (km)		
Source premises type	>1.5	>2	>3
Known infected premises	*Negligible to moderate*	*Negligible to low*	*Negligible*
Infected but undetected premises	*Negligible to low*	*Negligible to low*	*Negligible*

- **Aerosols**. The likelihood of a turkey premises becoming infected with HPAI virus via bio-aerosols varies with distance and 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. The following is a breakdown for the likelihood of HPAI spread to a turkey flock via bio-aerosol transmission:

	Composite likelihood rating (aerosols)		
	Distance from source (km)		
Source premises type	0.5	1	>1.5
Known infected premises	*High*	*Moderate*	*Low*
Infected but undetected premises	*Low to moderate*	*Low*	*Negligible to low*

- **Wild birds.** The likelihood of HPAI virus spread to a turkey grow-out premises via wild birds depends upon the type of wild birds and exposure to the wild birds. Aquatic species and larger non-aquatic species have not been known to gain entry to poultry barns, while passerine birds may access the inside of a turkey grow-out barn. With an effective PMIP including increased barn-to-barn biosecurity and the use of house-specific footwear, the likelihood of HPAI infection via wild aquatic birds and via non-passerine non-aquatic birds may decrease, as they and their waste are unlikely to access or be tracked into a turkey grow-out barn. Given that passerine birds may access the inside of turkey grow-out barns (even during a PMIP) and have been shown to be capable of shedding the virus, the likelihood of HPAI spread to a turkey flock 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 (passerine-type)	*Low to moderate*
Non-aquatic wild birds (non-passerine)	*Low*

- **Live-haul routes.** The risk of HPAI virus spread to turkey grow-out 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 premises within the Control Area may originate from within or outside the Control Area, the following risk ratings are provided:

Risk 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*

2.2 Likelihood of Turkey Flock Becoming Infected with HPAI via Movements of People, Vehicles, or Equipment, Resulting in Infected but Undetected Movement to Market

- **Feed and critical operational visits.** Critical operational visits will be limited during a PMIP; however, delivery of feed during this period is likely and the potential for emergency maintenance visits and service visits for bird health also exists. Provided the biosecurity stipulations of the PMIP are in place and strictly followed, the likelihood of a turkey flock becoming infected with HPAI via feed and critical operational visits during a PMIP was assessed as follows:

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

- **People and Their Vehicles.** During the PMIP, vehicle and visitor traffic to a poultry premises may include only critical visitors, employees, and growers. Provided the STS PMIP measures for people are strictly followed (e.g., use of farm-specific clothing and barn-specific footwear), we rate the likelihood of a turkey 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 poultry barns	*Low*
Persons not entering poultry barns	*Very low*

- **Shared Equipment (other than load-out equipment).** Previous poultry disease outbreaks demonstrate a known risk for virus spread as a result of movement of contaminated and shared equipment. Equipment that is brought onto a poultry premises may contaminate the ground or personnel who work with poultry, or if used inside a barn, may come into direct contact with live poultry. During the PMIP, no off-site equipment will be pre-staged and only equipment associated with critical operational visits may be brought to the premises. Provided the biosecurity stipulations of the PMIP are in place and strictly followed, the likelihood of a turkey flock becoming infected with HPAI virus via shared machinery or equipment during the PMIP is *low.*

Pathway	Composite likelihood rating (shared equipment)
Shared equipment	*Low*

- **Dead Bird Disposal.** The risks of HPAI introduction associated with off-site dead bird disposal methods, such as rendering, are well documented, and off-site disposal of dead birds must be discontinued during a PMIP. Nevertheless, the risky practice of off-site dead bird disposal may still occur in the Control Area on other premises, and on a turkey premises in the days leading up to a PMIP. Off-site dead bird disposal methods prior to a PMIP result in possible premises contamination, although the implementation of a PMIP does reduce the likelihood that such contamination will be tracked inside a grow-out barn during the PMIP. Additionally, many scavenger species can biologically or mechanically carry HPAI virus and have home ranges of adequate size to contain adjacent poultry farms. As a result, access to any on-farm dead bird storage container or disposal method represents a pathway for HPAI spread. Provided the STS PMIP measures—specifically discontinuing any off-farm mortality disposal and utilizing barn-specific footwear—are strictly followed, we rate the likelihood of a turkey flock becoming infected with HPAI via dead bird disposal as *moderate.*

Mortality disposal practice	Composite likelihood rating (dead bird disposal)
Likelihood of a turkey flock becoming infected via the mechanical or biological transfer of HPAI virus from on-farm dead bird disposal during PMIP	*Moderate*
Likelihood of a turkey 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	*Moderate*

- **Garbage Management.** Multiple types of potentially contaminated items have been reported to be disposed of in garbage on poultry operations, and there is potential for HPAI virus associated with garbage management to be tracked into the turkey house. During a PMIP, no off-site movement of garbage is allowed. Provided the STS PMIP measures (specifically discontinuing any off-farm garbage disposal and utilizing barn-specific footwear) are strictly followed, we rate the likelihood of a turkey 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 Turkey Flock Becoming Infected with HPAI via Load-out Crews, Vehicles, or Equipment

- **Load-out.** Previous outbreaks have implicated contaminated load-out crews and equipment in the spread of AI. If a flock were infected via contaminated load-out crews or equipment, shortening the time between premises load-out and slaughter limits the time that the virus may spread within the flock. Furthermore, depopulation of the entire premises (i.e., no "split" or "partial load-outs" permitted as might happen on a multi-age premises) leaves no susceptible hosts on-site, as load-out results in a terminal movement. Given that PMIP enhanced biosecurity and testing measures are strictly implemented, and that additional load-out mitigation measures are in place commensurate with the duration of the premises-wide load-out process, the risk of a turkey flock becoming infected with HPAI virus via load-out operations and resulting in an infected but undetected movement to market is estimated to be *low to moderate.*

  However, on multi-age turkey premises, birds that remain on the premises after a load-out (i.e., younger birds) are at a higher risk for infection due to proximity and potential for HPAI-contaminated load-out equipment or crew introducing virus to the premises and into the barns. Given that premises-wide load-out mitigation measures are in place, specifically a prohibition of load-out crews entering other turkey houses on the same

farm, the risk of remaining turkeys on a multi-age premises becoming infected with HPAI virus via load-out operations on that premises is estimated to be *moderate to high*.

Pathway	Composite risk rating (load-out)
Load-out operations result in infected but undetected birds moving to market	*Low to moderate*
Load-out of a portion of a multi-age premises results in HPAI spread to remaining birds on the same premises	*Moderate to high*

This assessment aids, but does not replace, the judgment of on-scene 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 is activated in response to an HPAI outbreak, APHIS (and Incident Command staff) will review this risk assessment with respect to the situation in order to assess industry requests for movement of turkeys to market.

Overall Finding and Conclusion

The risk that movement of turkeys to market into, within, and out of a Control Area during an HPAI outbreak results in the infection of susceptible poultry **on other premises** is *moderate*, provided that all applicable preventive measures from the Secure Turkey Supply Plan (STS Plan), in particular the Pre-Movement Isolation Period (PMIP), are strictly followed.

The risk that movement of turkeys **from a multi-age premises** to market into, within, and out of a Control Area during an HPAI outbreak results in the infection of susceptible poultry **on the same premises** is *moderate to high*, provided that all applicable preventive measures from the STS Plan, in particular the PMIP, are strictly followed.

3 Introduction

In the event of 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 premises not known to be infected 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; ultimately, whether or not the assessed risk level is acceptable will be determined by regulatory authorities and industry.

Completing these types of risk assessments in a timely manner during an outbreak can be challenging. Integrated poultry systems precisely manage grow-out facilities to maximize carcass value and minimize cost of turkey meat production. Extended movement restrictions may result in delays to processing, increased cost of production, and loss of carcass value. Proactive risk analysis identifies areas of risk and incorporates mitigation steps in order to 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 have explored the risk of HPAI infection of turkey day-old poults at the hatchery via horizontal transmission from breeder premises and the risk of HPAI infection of day-old poults due to local area spread (See Turkey Hatching Eggs risk assessment and Turkey Day-Old Poults risk assessment). These pathways were evaluated to be *negligible to low* when the outbreak measures specified in the SPS Plan are implemented.

The purpose of this assessment is to provide regulators and industry with an objective and defensible method of assessing the disease risk associated with the movement of live turkeys (for meat) to slaughter. As turkeys are generally slaughtered between 14 and 20 weeks of age, HPAI infection early in the grow-out period would likely be detected before movement. However, it is less likely that HPAI would be detected by the time of movement if they 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 mortality.

In order to evaluate the risk of movement of turkeys in a Control Area to market, plausible pathways were identified for the spread of HPAI infection. This analysis evaluated pathways for HPAI infecting a turkey flock in the days leading up to movement (entry assessment of HPAI virus introduction onto turkey farms at or before scheduled time of movement to slaughter) as well as the pathways by which this movement of turkeys to market could infect another flock in the area (exposure assessment of HPAI spread as the result of moving an infected but undetected turkey flock). Each pathway may consist of combinations of several activities. These pathways have been grouped into three 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 disease transmission due to proximity to an infected premises. The components of local area spread considered in this analysis include transmission of HPAI virus via:

- Insects;

- bio-aerosols generated from neighboring infected flocks;
- wild birds (aquatic and non-aquatic); and
- fomite transmission from poultry live-haul roads.

Other pathways considered in this analysis include transmission of HPAI virus through:

- feed delivery;
- people and vehicles associated with critical operational visitors;
- fomites associated with people (e.g., visitors or grower premises employees who may have had contact with infected poultry or poultry waste);
- shared machinery or equipment;
- mechanical or biological transmission from dead bird disposal via wildlife;
- garbage collection and disposal; and
- equipment and crews used for load-out.

This assessment applies only to the movement of turkeys off premises located in the Control Area to slaughter either inside or outside the Control Area. This assessment considers current industry practices and biosecurity measures as well as outbreak-specific measures applicable for the movement of turkeys to market in the risk evaluation. Specific biosecurity measures may vary widely by farm and geographic area. Categories of outbreak-specific measures from the STS Plan considered here include a Pre-Movement Isolation Period (PMIP) for flocks prior to movement to market. Other measures include:

- Limiting visitors to critical operations visits
- Specific feed truck and driver biosecurity measures
- Biosecurity measures for grow-out farm personnel and other essential visitors
- Measures for persons collecting surveillance samples
- Load-out truck and crew biosecurity, including truck routing
- Following PMIP, specific downtime measures

This assessment 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, U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA:APHIS) and Incident Command staff will review this risk assessment regarding the situation in order to assess industry requests for movement of broilers to market.

4 Scope

This section describes the scope of the assessment regarding the type of movements addressed and the facilities covered.

4.1 Facilities Covered under this Risk Assessment

This risk assessment is applicable to intensively raised commercial or contract grow-out facilities producing market-weight turkeys that meet all of the criteria listed below:

- Are in an HPAI Control Area
- Participate in the USDA APHIS National Poultry Improvement Plan (NPIP) as stated in 9CFR145 and 9CFR147 and in conjunction with biosecurity principles approved at the 43rd NPIP Biennial Conference[1,2]
- Implement the STS Plan in the event of an HPAI outbreak
- Do not have other poultry species on the premises.

4.2 Types of Movements Addressed under this Risk Assessment

This risk assessment will address only the pathways that may potentially affect movement of market-age turkeys within the Control Area directly to commercial USDA-inspected slaughter facilities inside or out of the Control Area.

5 Overview of Data Analysis Approaches

This assessment follows the general qualitative risk assessment principles recommended by the World Organisation for Animal Health (OIE) import risk analysis guidelines.[3] However, the risk assessment organization has been modified from that proposed in the OIE import risk analysis handbook as appropriate for the movement of finished turkeys to slaughter facilities. As noted in the introduction, many of the described pathways may play a role in both the entry assessment (i.e., entry of HPAI virus onto turkey farms at or before the scheduled time of movement to slaughter) and exposure assessment (i.e., spread of HPAI to a turkey flock as a result of the movement of an infected but undetected flock to slaughter). A consequence assessment was assumed to be high as the risk of moving infected turkeys could have considerable adverse consequences with regard to HPAI spread.

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 in **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. 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 overall risk rating for pathways involving a chain of events in which all have to occur for the pathway to be completed, relatively more weight was given to events with 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 below.

Negligible risk: HPAI spread to other susceptible poultry through the risk pathway is insignificant or not worth considering.

Very low: HPAI spread to other susceptible poultry through the risk pathway is remote.

Low risk: HPAI spread to other susceptible poultry through the risk pathway is very unlikely.

Moderate risk: HPAI spread to other susceptible poultry through the risk pathway is unlikely but does occur.

High risk: There is more than an even chance that HPAI spread to other susceptible poultry through the risk pathway will occur.

Extremely high risk: HPAI spread to other susceptible poultry through the risk pathway is almost certain to occur.

Uncertainty within the likelihood/risk estimations was accounted for by using a range defined by the terms in the descriptive rating scale provided in **Table 1**. 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 turkeys and the particle size distribution of aerosols generated in poultry houses depends on the ventilation design, 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 turkeys to market was determined by qualitatively combining the likelihoods of the individual pathways and the likelihood of detection assuming that all applicable preventive measures from the Secure Turkey Supply Plan (STS Plan), in particular the Pre-Movement Isolation Period, are strictly followed (see **Figure 1** below).

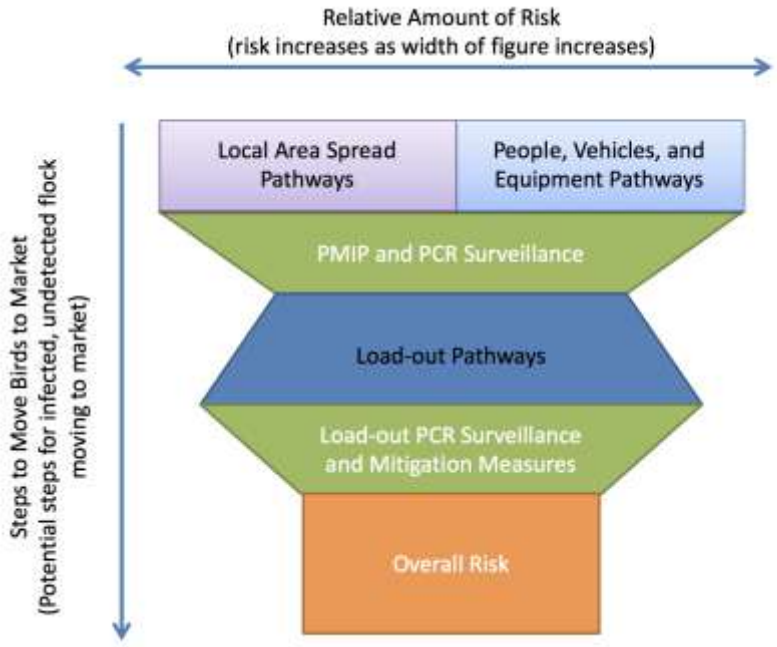


Figure 1: Diagrammatic representation of the overall assessed risk with the relative amount of risk increasing as the width of the figure increases (the overall risk of component parts is not to scale). The overall risk assessment is based on consideration of the steps needed to move live birds to market 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 form of local and state planning has taken place. The APHIS HPAI Response Plan is intended to complement regional, state, and industry plans. APHIS recommends their continued development.
- Turkey farms may have undetected HPAI infection in their flocks. If there were absolute certainty that a turkey flock arrived at slaughter without HPAI, there would be no risk of HPAI spread from movement of birds from a turkey 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 were detected, the movement of turkeys to market would not be allowed (and the facility would be depopulated, cleaned, and disinfected before resuming production).
- Movement of infected turkeys to processing would have a high likelihood of spreading HPAI to susceptible poultry and have high adverse consequences, and therefore we rated the risk according to the likelihood of moving infected and undetected birds.
- The movement of turkeys to market in the Control Area is in accordance with the STS Plan, and all relevant recommended preventive measures from the STS Plan are strictly followed. The assessment does not evaluate the risk that the preventive measures are incorrectly implemented either intentionally or unintentionally.
- Other mechanisms outside of the STS Plan may be utilized for HPAI control at the discretion of the Incident Commander. Risks associated with movement of birds to slaughter at a date earlier than usually marketed (i.e., early marketing) in order to decrease the number of susceptible species within the Control Area falls outside the scope of this Risk Assessment.
- The assessment evaluates the risk that movement of turkeys to market will result in the spread of HPAI to other susceptible poultry. Although the risks to humans, wildlife, and other livestock associated with the production or movement of live poultry 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 the risk to humans.
- The turkey grow-out premises is a standalone facility without other poultry species on the premises. It is also assumed that the perimeter buffer area (PBA) excludes any other livestock species.
- The adverse consequences of movement of infected turkeys are assumed to be high. Hence, the risk rating was determined on the basis of the likelihood of HPAI spread, and the consequences of the event were not evaluated.
- The risk assessment applies to HPAI virus strains that cause clinical infection and increased mortality in infected turkeys. The risk assessment may not apply to strains that

do not cause clinical signs representative of HPAI infection (i.e., 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.

- The disinfectants used to implement various C&D measures in the STS Plan during an outbreak have been approved by the Incident Command and are applied according to the manufacturer's label directions or recommended procedures.
- 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.

7 Background and Industry Characterization: Turkeys to Market

7.1 Definition of the Grow-out and Harvest Process

The commercial turkey industry in the United States (U.S.) raises turkeys, both females (hens) and males (toms), for meat.

Commercial hatcheries (establishments dedicated to the hatching of eggs for the production of poults—young turkeys) receive hatching eggs from off-site breeder farms, incubate and hatch the eggs, process the poults, and then ship the day-old poults to commercial turkey farms for the brooding and grow-out periods (**Figure 2**). Poults hatched from eggs originating from primary breeder flocks are used to supply primary or multiplier breeder turkey flocks. Poults hatched from eggs originating from multiplier breeder flocks are used for commercial turkey (meat) production. Turkeys are kept in brooder barns (for the brooding period in which the birds require and receive supplemental heat) until they are moved to grow-out, or finishing, barns at around 4 to 7 weeks of age. The grow-out period continues until the desired market live weight for the turkeys (e.g., 14 to 15 lb for light hens, 18 to 20 lb for heavy hens, or 40 to 45 lb for toms) is obtained between 14 and 20 weeks of age. Commercial turkeys are then loaded onto live-haul trucks and transported to processing.

This risk assessment specifically evaluates the movement of commercial turkeys from turkey grow-out barns to processing plants/slaughter facilities.

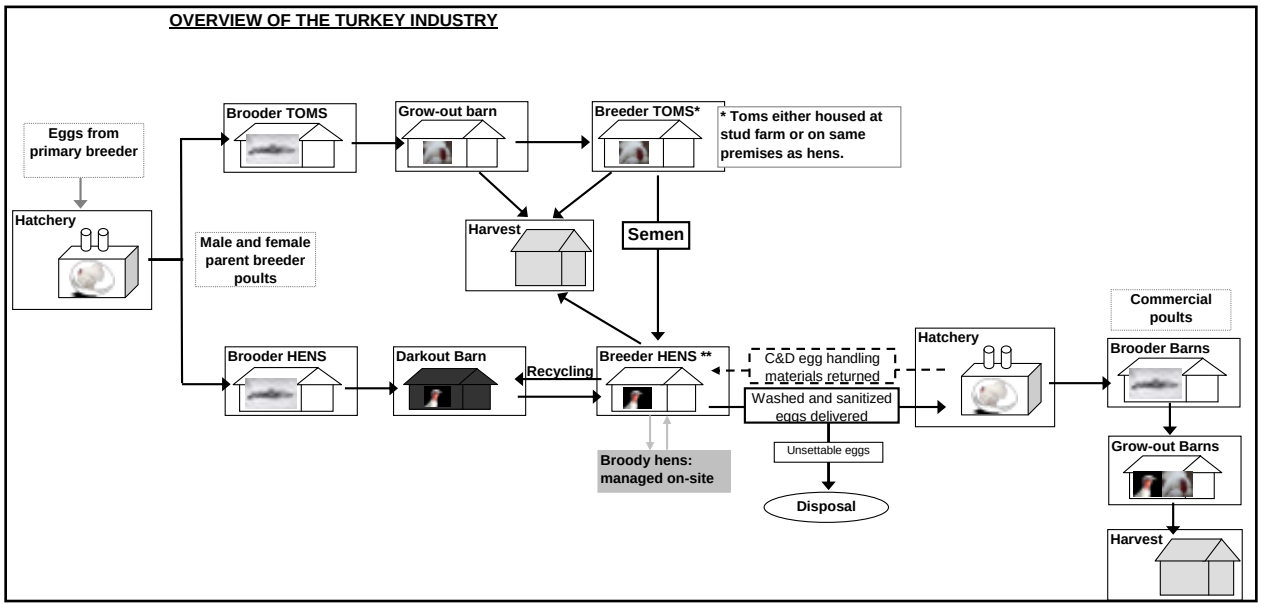


Figure 2. Overview of the U.S. turkey industry. Image courtesy of USDA APHIS Poultry Industry Manual Overview of the Turkey Grow-Out and Harvest Process in the United States

The commercial turkey industry in the U.S. produces over 230 million birds annually with most production facilities located in the North-central, Central, Mid-east, and Mid-Atlantic regions (see **Figure 3**).[4-6] The majority of turkeys raised intensively for commercial meat production in the U.S. are Broad-breasted White turkeys, which come from two genetic strains (Aviagen turkeys and hybrid turkeys). Other turkey breeds in the U.S. are generally raised only to preserve specific breeds (e.g., "heritage turkeys") or for specialty niche markets or direct sale.

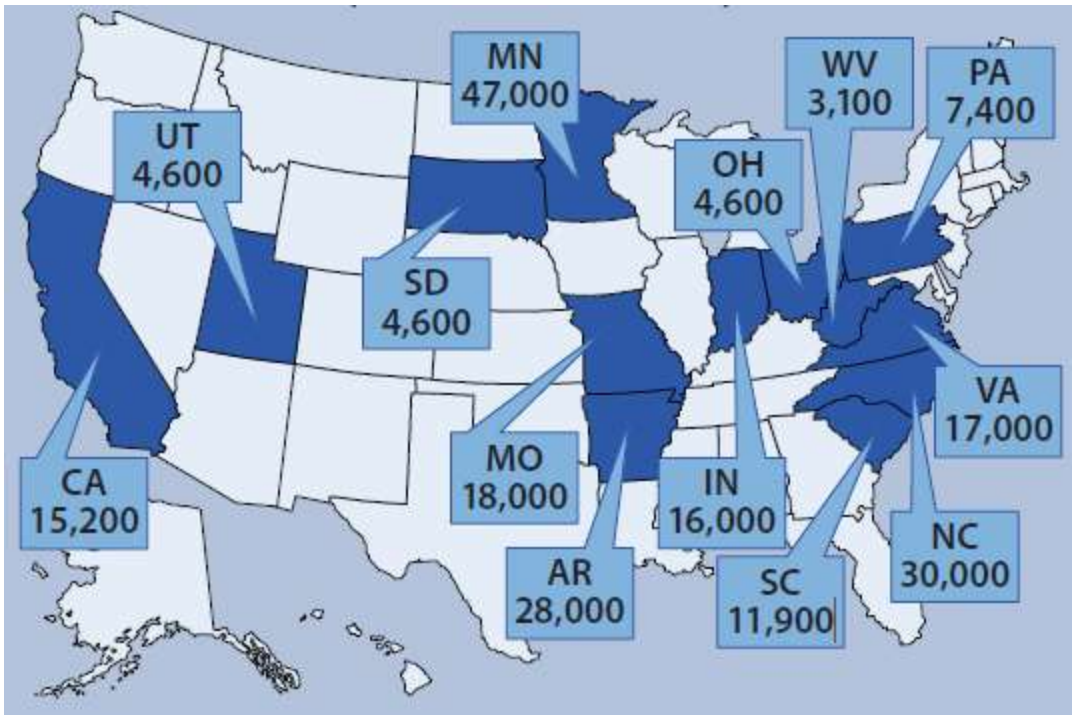


Figure 3. Number of turkeys raised in the U.S., 2010 (in thousands of head). For more recent statistics, see the USDA Poultry-Production and Value 2015 Summary (April 2016)[6]

7.1.1 Vertical Integration

Most turkey producing companies in the U.S. are vertically integrated. Some turkeys are still produced by cooperatives formed by owners of the production facilities; however, both companies and cooperatives typically own or control various steps in the turkey production cycle and thus are vertically integrated.[4]

Vertical integration facilitates synchronization of scheduling to meet product demand and plan for future markets, removes middlemen, and maximizes financial returns by reducing costs over the entire production process. The degree of vertical integration varies among companies. A complex consists of a vertically integrated unit that usually includes breeders, hatchery, brooding, grow-out/finishing, feed mill, and processing with varying numbers of birds produced by a single complex depending upon the company and resource utilization.[4] While an entire complex may involve more than 10 million birds, according to a National Animal Health Monitoring System (NAHMS) report on U.S. poultry from 2010, three quarters of individual turkey farms (excluding turkey breeders) had fewer than 50,000 birds at maximum capacity.[7]

Larger companies may have complexes located in different geographic areas or different states. Some integrated turkey companies may also raise other livestock such as pigs, cattle, or broiler or layer chickens[4] (TWG, personal communication, November 2016). How much a company actually owns in the integration and how much it controls through contractual arrangements differs among companies. According to the 2011 NAHMS report, the majority of turkey production farms (67.3%) were supplied with poults produced by multiplier flocks owned by an independent operator or another company.[7] Some contract producers provide the facilities and

labor in return for a contract price (which may include bonuses for superior livability, growth, and feed conversion) when the flock is marketed, while the company owns the birds and provides feed, health care, and technical expertise.[4] Other producers grow their turkeys independently and have a marketing contract with a processor.[4]

7.1.2 Service Technicians and Poultry Health Monitoring

Service technicians, who are employed by the company and usually have formal post-secondary education or long-term experience raising turkeys, oversee the turkeys through regular visits to the flocks during which they monitor the flock's progress and advise on management and health.[4] Sometimes service technicians also have their own farm and flocks, which they grow under contract for the company, though they generally are not permitted to have other types of poultry or contract flocks with another company, and additional constraints may be implemented in such situations during an HPAI outbreak[4] (TWG, personal communication, November 2016).

7.2 Overview of Major Steps in the Production and Processing of Turkeys for Market During Routine Operations

Day-old poults are received at brooder houses where they remain, and receive supplemental heat and special care, for 4 to 7 weeks before being moved to grow-out houses (for detailed information on the production and processing of turkey hatching eggs, see the Turkey Hatching Egg risk assessment[8] and the USDA Industry Manual[4]). Where on-farm brooding is practiced, young turkeys are brooded on the same premises where they are grown and finished. As of 2010, 43% of turkey-grower farms brooded poults on the same premises.[7] Off-farm brooding has become more standard, especially for tom turkeys, primarily to prevent disease transmission from grower to brooder flocks on the same premises. In an effort to better prevent disease transmission, grower-only farms may follow an "all-in, all-out" principle, that is, individual sites manage birds of a single age and load all birds out for slaughter at the same time (i.e., within a matter of days). However, grower-only farms may also manage birds of differing ages (on "multi-age premises"), with some birds being ready for market, and thus loaded out, weeks before younger birds on the same premises.

Following are the major steps in turkey production and processing during normal operations considered in the proactive risk assessment for movement of live turkeys to market.

7.2.1 House Preparation

Brooder barns are prepared for the next flock of turkeys as soon as birds from the previous flock are removed. Litter and all organic material are removed and insecticides or other control measures are applied; the house, entry way and surrounding areas, equipment, water lines, feed lines, and feed bins are thoroughly cleaned and disinfected; and fans, timers, lighting, alarms, curtains or air inlets, and other equipment are inspected to ensure they are in good working order. After cleaning, the house is closed and left vacant. The sooner the house is prepared, the longer the down time before the new flock arrives. A minimum down time of 10 days is recommended, but longer times are preferred to permit microbes in the house to die.

Grow-out barns or finishing houses are prepared for the arrival of each new flock of turkeys from the brooding house, but typically they are completely cleaned and disinfected only if there was a disease outbreak in the previous flock, or depending on the company they may be cleaned annually. Equipment is checked and repaired, and structural repairs are done as needed. Water

lines are flushed and disinfected. Feed lines are emptied and cleaned; feed bins are inspected for residual caked feed and cleaned if indicated. Entryways and areas around the house are inspected and cleaned. Vegetation along the sides of the house is sprayed with herbicide, and rodent bait stations are replenished.

7.2.1.1 Litter Management Practices

In brooder houses, fresh, clean, dry litter is spread evenly over the floor to a depth of 3 to 4 inches (7.6 to 10.2 cm). A spray may be applied to the surface of the litter to help reduce fungal spores and other contaminants prior to poult placement. During brooding, litter needs to be raked daily or removed to prevent buildup of cake along feed lines and around drinkers.

In grow-out barns, in between flocks, caked litter is removed and the remaining litter worked so that it is friable. Litter is occasionally composted by moving it into one or more raised "wind-rows" the length of the house. Heat from composting significantly reduces infectious agents in the litter. If the floor is exposed, rock salt, at the rate of 75 to100 lb (34 to 45.5 kg) per 1000 ft^2 (93 m^2), may be applied to dehydrate and kill coccidia oocysts and helminth eggs. The litter is then spread evenly throughout the house to a minimum depth of 4 inches (10 cm). Litter from empty brooding houses may be added to "top dress" litter in grow-out barns. After the turkeys are in the house, caked litter that builds up along feed lines and around drinkers should be removed and replaced.

7.2.2 Brooder and Grow-Out Period Management

Poults are usually restricted in brooding rings during the first few days of life and then are usually allocated at least 1 ft^2 (0.1 m^2) per bird in brooder houses. Pancake or pan brooders (heating equipment) are usually set at 24 inches (61 cm) above the litter but may be raised or lowered slightly to obtain optimal litter temperatures. Radiant tube heating systems mounted on the ceiling are used in some newer brooder housing. They rely less on heating circulating air than traditional pan brooders. Regardless of the type of brooding equipment, it needs to provide even, appropriate house temperatures at the level of the poults. Temperatures of the litter under the brooders and at the edge of brooding rings should be checked with an infrared gun to be sure they are in the range of 100-115°F (38-46°C) and 75-85°F (24-29°C), respectively. It is important to have a 20-30°F (9-15°C) gradient between the center and edge of the ring to allow each poult to locate its own comfort zone.

Poults need to be checked frequently the first few days in the brooder house, and yet initial conditions in the house need to remain as quiet and calm as possible to ensure that poults begin drinking and eating. Poults must drink or they will not eat. During the next few days, drinkers are adjusted (to compensate for litter compaction and average poult size), cleaned, and kept filled. A common practice is to combine two brooding rings together after 3 to 5 days and remove them altogether by 7 days. Cardboard that formed the rings is discarded. Supplemental drinkers and feeders are gradually removed as the poults learn to use the equipment that will remain in the house. Temperatures under the brooder and in the house are reduced by approximately 5°F (2.8°C) weekly until they match ambient temperatures, and ventilation is increased to control litter moisture, ammonia, and dust levels in the house. Poults grow rapidly during the remainder of the brooding period. It is important to check them at least twice a day, and to adjust feeders and drinkers to the correct shoulder height and depth for the poults every few days.

Turkeys are generally moved from brooder to grow-out barns at 5 to 6 weeks of age, though this may range from 4 to 7 weeks. Hens and toms are provided with at least 2 ft^2 (0.2 m^2) and 3 ft^2 (0.3 m^2) of floor space, respectively. Stocking densities of 6 to10 lb/ft^2 (29 to 49 kg/m^2) are recommended and should not exceed 15 lb/ft^2 (73 kg/m^2) when environment and management are ideal.

For a few days after the turkeys arrive from the brooder house, it helps for the grower to keep feeders full to encourage them to eat, and to frequently walk the birds so they can quickly adjust to their new surroundings. The turkeys will not need this much attention when they become accustomed to their new environment, and the amount of feed in feeders can be reduced to minimize spillage.

During finishing, turkeys should be checked at least twice daily, more frequently if there is a problem. Time spent with the flock provides added benefit, because turkeys socialized by frequent contact with people are healthier and more productive than turkeys that are not well socialized. Adult tom turkeys may not be checked as frequently prior to processing because of aggressive behavior towards people.

As in brooding, feeders and drinkers need to be adjusted to the average bird's shoulder height every 2 to 3 days because of the turkeys' rapid growth. Dirty drinkers are cleaned, but daily cleaning of all drinkers is not practiced under routine conditions. Any waterers that are leaking are repaired or replaced. Grossly contaminated or caked feed is removed and discarded.

Drinkers can be rotated to prevent heavy caking of litter by moving them in a three- (triangle) or four-point (square) pattern using extra hooks from which the drinkers can be suspended. If litter around a drinker is excessively wet, the height of the drinker and depth of water in the drinker lip is checked and adjusted. Soaked litter needs to be removed and replaced as quickly as possible.

An average hen turkey will consume approximately 35 lb of feed and reach 14 to 20 lb (live weight) in 12 to 14 weeks, while toms average 90 lb of feed consumption and take 16 to 19 weeks to reach 35 to 42 lb.[9]

7.2.2.1 Ventilation

Proper ventilation minimizes ammonia and dust and helps control litter moisture. Ideal litter moisture is between 25 and 40%, but it varies widely depending on the location in the house. Litter moisture below 25% predisposes to dusty conditions and aspergillosis, while ammonia and flies increase when levels are above 40%. Excessively moist conditions provide an ideal environment for the survival of enteric pathogens including bacteria, viruses, and coccidia. Ventilation can be adjusted during daily flock checks. Turkeys are more susceptible to high heat and humidity than are chickens and must be kept as cool as possible during periods of excess heat. Fans are adjusted to achieve recommended temperatures and air exchanges.

7.2.3 Load-Out

Current industry practices for load-out of market age turkeys involve manual labor and a "turkey loader" machine to transfer turkeys from the grow-out barn onto a live-haul truck. The duration of load-out varies depending upon the size of the farm (i.e., the number of birds and barns to be loaded out) and may range from 1 evening to 10 days (TWG, personal communication, January 2016). Load-out (or catching) crews normally consist of 7 to 10 persons. Most farms do not own a loader, so the machine travels from farm to farm.

Typically on the day the flock is to be removed from the farm, the loader is hauled to the farm and is placed just inside a door of the grow-out barn where transfer of the turkeys will take place. The other end is placed alongside the coops on the live-haul truck when it arrives. Chutes constructed with movable panels and stakes funnel the turkeys into a preloader in the house. Turkeys are gently driven into the chute where they move onto the conveyer belt of the loader, which takes them to the coops on the truck. Individuals of the load-out crew stand on either side of the loader and manually move the turkeys into the coops. Coordinating movement of the truck and loader makes it possible to move the turkeys into coops with minimum handling. The number of turkeys per coop depends on turkey type and size, size of the coop, environmental conditions, and distance to the processing plant. On a typical live-haul truck, there are 5 to7 levels of coops, ranging in height from 14 to19 inches (36 to 48 cm).

7.2.3.1 Transportation of Turkeys to Processing

Once the live-haul truck is loaded, it travels to the processing plant without unnecessary or prolonged stops; the transport time will vary depending upon location of the grow-out premises but generally ranges from less than 1 hour to 6 hours (TWG, personal communication, January 2017). Mortality during the process of catching and transportation should not exceed 0.1%. Trucks used for transporting turkeys to the processing plant for slaughter are usually dedicated for this purpose only and owned by the company. According to the STS Plan recommended biosecurity measures (both prior to and following an outbreak of HPAI), trucks (which includes coops) and load-out equipment are cleaned and disinfected before entry onto a farm[10]; ordinarily this C&D takes place at the processing plant.

7.2.3.2 Awaiting Processing

At the processing plant, trailers with coops loaded with turkeys are placed into a holding shed where air is circulated by large fans. Misting may be used if temperatures are high. Trailers are pulled into the plant when it is time for the birds to be processed. To unload the birds, the location and height of the trailer is periodically adjusted to allow turkeys to be manually removed from the coops and either stunned with CO_2 and then hung by their feet in shackles that are attached to a moving chain, or hung by their feet to be stunned electrically. The area is kept darkened and a bar contacts the breast of the birds to help keep them quiet, reduce wing flapping, minimize distress, and reduce parts condemnations due to hemorrhages, bruising, or broken wings.

7.3 Overview of Current Disease Prevention and Biosecurity Efforts in Turkey Production

To prevent disease introduction, as well as onward transmission to other premises if infection occurs, sanitation and biosecurity measures are addressed at all farms, though to varying degrees. Conceptual biosecurity involves evaluating the potential location of new poultry operations including regional poultry density, proximity to other poultry/animal facilities, flyways, prevailing winds, potential for flooding or other adverse weather events, and movement of poultry in the region. Structural biosecurity is achieved through the physical construction and maintenance of a facility and is the most reliable. However, it may require extensive changes to existing premises and therefore may take time to execute. Operational biosecurity, in which standard operating procedures (e.g., pest control, farm/barn access requirements, C&D, etc) are implemented, can readily be updated, but relies on compliance and is thus less consistent.

Following the 2015 H5N2 outbreak, many operational biosecurity recommendations were updated, and a biosecurity officer or coordinator is now recommended for all commercial operations.[1] The biosecurity coordinator is responsible for the development, implementation, maintenance, and ongoing effectiveness of the biosecurity program and should be knowledgeable in the principles of biosecurity.

7.3.1 Current Disease Prevention and Containment Measures in Grow-Out Operations During Normal (Non-Outbreak) Situations

The National Poultry Improvement Plan (NPIP) is a cooperative industry-state-federal program focused on disease prevention in poultry and safety of poultry products throughout the country. It operates through a memorandum of understanding with each of the 50 states and includes an H5/H7 LPAI program. Participating meat turkey slaughter plants are monitored for H5/H7 LPAI and audited at least once yearly to ensure compliance with the relevant NPIP provisions. Each state administers its own H5/H7 LPAI plan for grower flocks affiliated with NPIP monitored slaughter plants, with biosecurity recommendations outlined in each state's plan. NPIP minimum biosecurity standards for growers were approved at the 43rd Biennial NPIP Conference.[1] These practices and principles are designed to prevent the introduction and spread of infectious disease. According to NPIP, the biosecurity program should include a designated Line of Separation (LOS) and Perimeter Buffer Area (PBA), and provisions on personnel biosecurity practices; control of wild birds, rodents and insects; equipment and vehicle management; mortality disposal; manure and litter management; replacement poultry practices; water supplies; feed and replacement litter management; morbidity and mortality reporting, and regular biosecurity auditing.

Biosecurity guidelines have been written by poultry industry representatives (STS Plan; National Turkey Federation [NTF] 2012 & 2015), and the following sections describe some of these recommended biosecurity measures. How individual producers meet these guidelines varies depending on farm layout and resources. Biosecurity is generally heightened during outbreak situations.

7.3.2 Structural Biosecurity: Secured Farm Entry

- "No Admittance – Biosecurity Zone" signs are posted at the farm entrance and at all turkey house entrances, to warn people not to enter the farm or any of its buildings.[10]
- Locks and fences are also useful in preventing unwanted visitors from entering the farm or houses.[4]
- A PBA, an outer control boundary around the poultry houses, should be clearly delineated such that nonessential vehicles do not enter it and personnel do not leave it in the course of their daily tasks.[1]
    - If personnel must enter or exit the PBA, a specified entrance is used.
    - Vehicles entering the PBA must be cleaned and decontaminated (via disinfectant or heat) before entering.
    - Personal vehicles of employees and visitors should be parked in a designated area outside the PBA.

- Non-essential visitors are limited and usually allowed onto a farm only with authorization.
- Vehicles should be parked away from turkey houses as much as possible, and in locations not exposed to air from turkey houses.[10]
- A logbook for all people entering the farm helps to ensure that they meet requirements for not having been in contact with other poultry within a specified time.[4]
- Only cleaned and sanitized footwear, disposable footwear, or footwear dedicated to a turkey house shall be worn.[10]

7.3.3 Operational Biosecurity

7.3.3.1 People

- Biosecurity training stresses the importance of not owning, and avoiding contact with, other birds not owned by the business, including birds at live markets, pet birds, domestic chickens, fighting chickens, ducks, geese, waterfowl, exotic birds, quail, partridges, or pheasants. In the event of contact with any of the above, employees agree that they will comply with a 24-hour waiting period before returning to work.[10]
- Showering and changing into clean clothes and footwear immediately prior to or upon arrival at the premises is recommended.[1]
- An LOS, normally the walls of the poultry house plus a marked line in the entry room to the house, should be established to isolate the poultry from potential sources of HPAI virus.[1]
    - The LOS should be clearly marked with paint, tape, or a low physical barrier, and should have appropriate signage.
    - All equipment and supplies crossing the LOS must be C&D.
    - Personal items, such as cell phones and jewelry, should not cross the LOS.
    - Site-specific coveralls/clothing and footwear are provided at the LOS.
    - Handwashing facilities, or hand sanitizer, and instructional signage should be present at the LOS entrance.
- Clean clothes or disposable coveralls and hair covering are recommended.[4]

7.3.3.2 Feed Delivery

- Feed delivery is one of the most common types of visits at turkey premises, occurring at least weekly at roughly 80% of premises. On larger premises, feed deliveries likely occur several times per week. Therefore, distances between farms and the feed mill must be kept at a minimum, while still allowing adequate separation of production sites for disease control and manure management purposes.
- Feed delivery routes should be selected in consultation with a poultry veterinarian to minimize contact with and proximity to live poultry and poultry products.[10]

- Feed bins are secured to prevent contamination by wild birds or rodents, and any spilled feed is cleaned up promptly to prevent attracting such pests.[10]

7.3.3.3 Sanitation Facilities on Farm

- A clean parking area for visitors at the farm entry drive that is away from the turkey houses will minimize on-farm traffic. It can provide a place for visitors and service persons to put on protective clothing before walking onto the farm and a place to leave contaminated clothing before leaving.[11]
- Disposable boots, masks, and hairnets should be deposited in an appropriate disposal container prior to exiting the farm.[10]
- Rubber boots should be washed and disinfected before wearing on another farm. Wash water should be dumped and the bottom of the bucket cleaned with a brush.[10]
- Gloves and/or facilities for employees to clean their hands (sanitizer or sink) are recommended.[4]
- Clean outer clothing and footwear should be worn, and there should be a cleanup area for all personnel and equipment at the entrance and exit to each barn.[11]

7.3.3.4 Cleaning and Disinfection (C&D)

7.3.3.4.1 Vehicles and Drivers

- A decontamination area for vehicles and equipment that must enter the farm and buildings should be available.[11]
- Live-haul trucks and vehicles that have been to a rendering plant must be cleaned and disinfected before entering or returning to the turkey farm.[10]
- Drivers are prohibited from entering turkey houses.[10]
- Delivery vehicle drivers who cross the PBA should remain in their vehicles.[1]
    - If they must exit the vehicle, they should put on PPE or new disposable footwear and use disposable gloves or hand sanitizer.

7.3.3.4.2 Equipment

- Any equipment entering the PBA should be C&D before entering.[1]
- Sharing equipment between turkey farms is not recommended. In the event that equipment must be shared, effective cleaning and disinfecting must take place between uses.[1,10]
- When cleaning surfaces, vehicles, and equipment, it is important to remove organic material before disinfection, as most disinfectants do not work well in the presence of organic material.[4]

7.3.3.4.3 Water Supplies

- Water sources must be secure and inaccessible to free-flying birds or rodents, or else water sources must be disinfected.[10]

7.3.3.4.4 Housing Area

- After the flock is removed, unused feed is removed from the feed system and might be reprocessed or delivered to another farm (unless associated with a disease situation, in which case the feed is destroyed).[4]
- Grow-out barns are usually cleaned out annually, or cleaned between flocks as indicated, and the litter spread onto fields.[4]

7.3.3.4.5 Load-out

- Transport trucks and load-out equipment are cleaned and disinfected prior to entry onto the farm.[10]

7.3.3.5 Animal, Pest, and Insect Control

- No domestic birds are to be maintained on premises outside turkey houses.[10]
- Control measures to discourage the presence of wild and migratory birds on the premises should be in place.[10]
- All doors and ventilation openings on each barn must be screened to prevent wild birds from entering the buildings.[11]
- Doors and other ground-level openings around the entire perimeter of the building must fit tightly and have coverings to prevent wildlife and other animals from coming into contact with the turkeys.[11] Dogs and cats are not allowed in turkey houses.[10]
- Bait stations are used to control rodents and insects; clearing vegetation around the house for at least 18 inches (0.5 m) is useful for preventing rodents.[4]
- Feed bins must be secured to prevent contamination by wild birds or rodents.[10]
- Spilled feed is cleaned up promptly to prevent attracting wild birds and rodents.[10]
- Water sources must be secure and inaccessible to free-flying birds or rodents, or water must be disinfected.[10]
- Biosecurity measures to protect poultry from wild birds, rodents, and insects should cover three categories: clean, exclude, and control.[1]
    - Clean: Feed spills should be cleaned up immediately; trash should be regularly removed; dead birds should be removed promptly; moisture should be kept low in manure and litter; feed should be protected; and standing water should be removed.
    - Exclude: Doors, windows, vents, and holes larger than ¼ inch should be sealed or screened to exclude birds, rodents, and insects, and vegetation near poultry houses should be kept short.
    - Control: Waterfowl may be harassed to discourage them from frequenting water near poultry premises; for rodent control, rodenticides and trapping are recommended; and biological or chemical control programs will minimize insect populations.
- Delivery vehicle windows and doors should be kept closed as much as possible (to prevent insect access), and cab insect spray should be available if needed.[1]

7.3.3.6 Dead Bird Disposal

- Dead birds from all houses should be disposed of or held on-site in a biosecure manner. Disposal of dead turkeys should not expose turkeys in other houses or other farms to potential pathogens. There is a dedicated on-site location for mortality that is as far away from the barns as possible.[10]
- Multiple poultry farms should not share initial collection sites for dead poultry.[10]
- Containers for dead turkeys (dumpsters) should never leave the farm.[10]
- Dead birds should be placed in a leak-proof container within the LOS during the day; they are transferred to a collection container located on the edge of the PBA, such that it can be emptied daily from outside the PBA.[1]
    - Employees delivering dead birds to the collection container should always use the biosecure entry system when crossing the LOS to re-enter the poultry house.
    - Re-usable containers should be C&D before being returned to the house.
- Onsite disposal should be located outside the PBA and designed to prevent disease transmission.[1]
- Collection vehicles or equipment for off-site disposal should remain outside the PBA.[1]

7.3.3.7 Manure and Litter Management

- Manure trucks should never go from one poultry farm to another on the same day. However, if this is required, the manure trucks must be washed with detergent and disinfected prior to arrival at the next farm.[10]
- At the end of a production cycle, empty buildings should temporarily be treated as outside the PBA, such that crews and equipment may repeatedly enter the building to remove manure and litter.[1]
    - Once the building is C&D, the LOS and PBA around the building are restored.

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.[12] For movement of turkeys to market, 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.[13] 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.

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, and C influenza viruses. The Type A influenza viruses, which include all AI viruses, can infect a wide variety of animals including wild ducks, chickens, turkeys, pigs, horses, mink, seals, bats, and humans. The type B and C viruses primarily infect humans and occasionally pigs.[13-15]

Two surface glycoproteins of the influenza A virus, hemagglutinin (HA) and neuraminidase (NA), are the most important antigenic sites for the production of protective immunity in the host; however, these proteins also have the greatest variation. For AI viruses there are sixteen known different subtypes of HA (H1 to H16), nine known different subtypes of NA (N1 to N9), and 144 different HA:NA combinations (H17N10 and H18N11 were only recently isolated from bats).[13,15] 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.

8.1.1 Definition of Highly Pathogenic Notifiable Avian Influenza

For the purpose of disease control programs and international trade in domestic poultry products, HPAI is defined in the Code of Federal Regulations, Title 9, Section 53.1[16] as:

1) Any influenza virus that kills at least 75% 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 1 to 5 [out of 8 inoculated] chickens and grows in cell culture in the absence of trypsin.

The World Organization for Animal Health (OIE) Terrestrial Animal Health Code Article 10.4.1 defines HPAI viruses to be AI viruses that "have an IVPI [intravenous pathogenicity index] in 6-week-old chickens greater than 1.2 or, as an alternative, cause at least 75% mortality in 4- to 8-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% 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."[17]

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 the OIE.[18] Although other low-pathogenic avian influenza (LPAI) viruses may cause considerable morbidity and production losses, they are not reportable diseases to the OIE (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.[19] Recent surveillance and phylogenetic analyses, however, suggest that migratory waterfowl are important in the maintenance, reassortment, and spread of HPAI viruses.[20-22] 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, the virus will usually be highly pathogenic for other birds within the order Galliformes, family Phasianidae, such as turkeys and Japanese quail.

Most HPAI viruses for chickens are generally non-pathogenic for ducks and geese in experimental studies.[14] However, the lethality of HPAI viruses has changed since the re-emergence of H5N1 HPAI viruses in Hong Kong in 2002, as some strains have become highly lethal in some naturally and experimentally infected waterfowl.[19] The evolving H5 HPAI viruses spread throughout Asia and Europe between 2005 and 2014.[23] In late 2014, the Eurasian H5 clade 2.3.4.4 viruses were detected in North American wild birds[21,24,25] and reassorted with American AI viruses, and similar Eurasian/American HPAI H5 viruses were identified during the domestic poultry outbreak in 2015 in the United States.[26]

Characterization of the Eurasian/American HPAI H5 viruses found in wild birds was done by the National Wildlife Health Center and USDA National Veterinary Services Laboratory. Researchers at these agencies suggest identifying these HPAI H5 viruses as intercontinental group A (icA) to differentiate this changing subset of viruses from other Asian H5N1 HPAI.[24] Some wild birds—including ducks and geese—that were found to be positive for icA H5N8 and icA H5N2 exhibited morbidity/mortality at the time of sample collection.[27] 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) (Pantin-Jackwood, personal communication, August 2016). An icA HPAI H5N2 strain isolated from infected turkeys in Minnesota in 2015 (A/Tk/MN/12582/2015) was experimentally inoculated into mallard ducks 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.[28] Thus, the 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.[29] This is likely what occurred in the U.S. turkey industry in early 2016 when the first HPAI caused by an H7N8 virus (in any species), A/turkey/Indiana/2016, was detected in commercial turkeys.[30]

Subsequent detections of H7N8 LPAI occurred on additional turkey premises; all HPAI and LPAI viruses were found to be of North American wild bird lineage. 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.[19]

Host adaptation is a key determinant of the ability of an HPAI virus to maintain transmission within domestic poultry. Once adapted to gallinaceous birds, HPAI viruses are unlikely to circulate again among wild birds because they are adapted to poultry.[31] However, the emergence of Asian-origin HPAI H5 strains has led to increased uncertainty regarding the role of wild birds as reservoirs in the maintenance of HPAI viruses in nature.[20,32] Prior to the outbreak of HPAI H5N1 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 was observed in a population of terns.[33] 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.[22,34,35] However, despite extensive global wildlife surveillance efforts, infection with H5N1 HPAI viruses has been detected in healthy wild birds in only a few isolated cases.[33,35] 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.[19,22]

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, skunks).[36-39] 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).[38,40]

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 the OIE at http://www.oie.int/en/animal-health-in-the-world/update-on-avian-influenza/2015/.[16]
- A Centers for Disease Control and Prevention (CDC) publication graphically displayed the outbreaks of HPAI virus, H5 subtype, that occurred in the United States in 2014-2015 both in relation to time and to poultry distribution and wild bird migratory patterns; the maps can be viewed at http://wwwnc.cdc.gov/eid/article/22/1/15-1053_article#tnF1.[41]
- The Global Early Warning System for Major Animal Diseases Including Zoonosis (GLEWS)—a joint effort of the Food and Agriculture Organization of the United Nations (FAO), OIE, and the World Health Organization (WHO)—provides a regular update on global H5N1 HPAI events in the Global Animal Disease Intelligence Report, which can be viewed at http://www.glews.net/.[42]

8.3 Virus Shedding

HPAI viruses have been isolated from respiratory secretions, blood, feces, and feathers, as well as the eggshell surface, albumen, yolk, meat, and other tissues (e.g., spleen and lung) from infected poultry. 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.[43-51]

H5N2 HPAI (A/chicken/Pennsylvania/1370/1983) viruses have been isolated from the eggshell surface, yolk, and albumen of eggs laid by experimentally inoculated hens.[52] In these experimental studies, H5N2 HPAI viruses were not recovered from eggs laid on the first day post-inoculation of hens. This may have been due to the developing egg being protected from exposure in the shell gland (uterus) during the later stages of eggshell formation (about 15 hours), in combination with the latently infected 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.[53] Italian HPAI H7N1 (A/chicken/Italy/445/99) viruses have also been isolated from eggs laid by infected hens.[54]

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, 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.[52]

As compared to chickens, AI viruses in turkeys demonstrate a relatively high degree of affinity for oviduct tissue, relative to respiratory and digestive tissue.[55] A predilection for replication within these tissues may explain the precipitous drops in egg production reported in turkey breeder hen flocks during natural outbreaks.[56-59] Narayan et al.(1969) recovered AA 5-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.[60] In turkey breeder hens experimentally inoculated with swine-origin LPAI H3N2 (A/turkey/Ohio/313053/04), virus was recovered from eggshells and egg contents.[55] 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.

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.[61-63] 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 1: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates. The HPAI virus shed by infected birds may be protected environmentally by accompanying organic material that shields the virus particles from physical and chemical inactivation. Specific environmental conditions such as cool and moist conditions increase survival times in organic media and on surfaces. For example, H5N2 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 4ºC (39ºF).[64,65] H5N1 HPAI virus was viable in allantoic fluid for 10 days at 25 to 32ºC (77 to 90ºF) when kept out of direct sunlight, but was killed within 30 minutes of placing the sample in sunlight (32 to 35ºC; 90 to 95ºF).[66]

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.[67] Because AI virus can be isolated in large quantities from feces and respiratory secretions of infected birds, an important mode of transmission is the mechanical transfer of infective feces.[13] Once introduced into a flock, AI virus can spread directly from flock to flock by movement of infected birds and indirectly via contaminated equipment, egg flats, feed trucks, off-site mortality disposal, garbage trucks, service crews, or other means. Windborne transmission may occur when farms are closely situated and appropriate air movement exists.[68,69] 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 experimentally capable of transmitting virus to birds via indirect contact through shared environments.[40,70] Other mechanisms of transmission are outlined below.

8.6.1 Vertical Transmission in Chickens and Turkeys

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.[71-74] 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 LPAI viruses (T/Calif/meleagrium/64, T/Calif/5142/66), and virus was not recovered from poults hatched from eggs laid by exposed turkey hens.[75] 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."[76] 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.[77] Also, the 214 chicks hatched from these eggs showed no sign of AI disease and had not developed AI antibodies.[77]

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.[78] 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.[78] There was no evidence of horizontal or vertical transmission of AI within the hatchery to day-old poults in any of these instances.

8.6.2 Transmission via Artificial Insemination in Turkeys

As compared to chickens, there is an additional risk in turkeys of viral transmission via the artificial insemination process. It is not anatomically or practically possible to collect semen without the collection device touching the cloaca; semen could also be contaminated during the semen preparation process in the laboratory. Contaminated fomites, such as hands or equipment of insemination crews and contaminated turkey semen, have been implicated in the spread of AI viruses between commercial turkey breeder operations and to commercial turkeys from humans.[79-82] Although semen was implicated in the spread of AI in field outbreak investigations, isolation of AI virus from tom turkey semen was not reported in these studies.

AI virus has previously been isolated from tom turkey semen, but titer levels were not reported.[83] It was unclear whether this virus came from the semen *per se*, or from the cloaca contaminated by fecal material.[84] Other studies have demonstrated that AI viruses can be transmitted to turkey breeder hens through artificial insemination with semen experimentally contaminated with AI virus on the day of collection.[85] Pantin-Jackwood et al. (2010) transmitted pandemic H1N1 (A/Chile/3536/2009) virus to hens by intracloacal or intrauterine inoculation, demonstrating that transmission is possible through contamination of these mucosal surfaces by semen or fomites.[86]

HPAI virus antigen has been observed in testes, suggesting that virus could be present in semen.[87] In a 2013 study, tom turkeys were inoculated intranasally with 10^6 $TCID_{50}$/0.5ml of triple-reassortant H3N2 influenza A virus of swine (IAV-S) A/Turkey/OH/313053/2004.[88] Low viral titers were detected in the reproductive tract (testicles, epididymis, vas deferens, and phallus) and semen by rRT-PCR, but virus isolation was unsuccessful. The authors suspect that the low virus titers and/or the seminal environment may have adversely affected virus isolation. Nonetheless, based on the presence of viral RNA in the reproductive tract and semen, there remains a potential for venereal transmission of influenza virus in turkeys.

8.7 Dose Response

8.7.1 Dose Response in Turkeys

Both intraocular and intranasal inoculation were used in an experimental study of infectious and lethal doses of two HPAI strains in turkeys.[89] In this study, turkeys were inoculated with H5N1 (A/turkey/Turkey/1/05) and H7N1 (A/ostrich/Italy/984/00) strains, and all birds shown to be infected died. The ID_{50} and LD_{50} were thus equal; the median was 10^{1} EID_{50} (or less) for H5N1 and $10^{2.2}$ EID_{50} for H7N1. Turkeys were found to be more susceptible than chickens by over 200-fold for both H5N1 and H7N1.

In another study, turkeys were inoculated with different doses of A/ostrich/Italy/984/2000 H7N1 HPAI by a combined intranasal/intraocular route.[90] Although ID_{50} and LD_{50} were not explicitly measured, the latter can be extrapolated from their data and was shown to be both dose- and time-dependent. There was no mortality with 10^{1} EID_{50} by 7 days post-inoculation (PI), but there was greater than 50% (4/5) mortality with 10^{6} EID_{50} at 48 hours PI. At 72 hours PI, the LD_{50} was 10^{3} EID_{50}, and it was 10^{2} EID_{50} by 96 hours PI.

In their studies using a highly poultry-adapted LPAI strain (A/turkey/Ohio/313053/04), Pillai et al. (2010) demonstrated a markedly lower ID_{50} for turkeys ($10^{1.4}$ EID_{50}) than for chickens ($10^{2.6}$ EID_{50}).[55] They cautioned that virus strain as well as genetic make-up of the study birds may affect the minimum infectious dose, such that it may not be possible to generalize results from a few isolates in a certain breed of turkey.

As stated above, the infectious dose for turkeys through intranasal inoculation for HPAI viruses (H5N1 and H7N1) has been found to be 2 to 3 logs lower than that for chickens.[89] Given a 50% chicken infectious dose of 5 to 6 log EID_{50} for aerosol transmission from the dose-response models, it is possible that the turkey infectious dose is between 3 and 4 log EID_{50}. Transmission of LPAI (A/turkey/Wisconsin/1966) to turkeys has been demonstrated via an estimated aerosol dose between 3 and 4 log EID_{50}.[91] Data from this experimental study suggests that the 50% aerosol infectious dose is close to or less than 3 to 4 log EID_{50}.

HPAI infection via the gastric route is not well-documented in turkeys. In one small study, 50-day-old turkeys were inoculated by the direct esophageal route with A/turkey/Italy/4580/1999 HPAI H7N1 in a dose of 2 g of $10^{3.6}$ EID_{50}/0.1g infective meat homogenate (for a total dose of $10^{4.9}$ EID_{50}).[49] Tracheal and cloacal swabs collected up to day 7 remained negative, as did serum samples up to day 21, and no clinical signs were observed. These results imply that the infective dose for HPAI via esophageal inoculation is likely more than 20 times $10^{3.6}$ $EID_{50.}$ However, since the choanal cleft was bypassed, no inference can be made as to the infective dose with exposure that may occur through natural feeding.

Although transmission of HPAI via artificial insemination is strongly suspected in turkeys, data on dose response to such exposure are lacking.

8.7.2 Dose Response in Chickens

Most experimental studies in chickens used intranasal inoculation as an entry point. For the intranasal route, in one study, the 50% chicken infectious dose (CID_{50}) for 11 H5 and H7 HPAI strains (of chicken and turkey origin) varied between $10^{1.2}$ and $10^{4.7}$ EID_{50} with a geometric mean of $10^{2.9}$ $EID_{50.}$[92] All but one strain (A/chicken/Rostock/1934 HPAI H7N1, which was endemic in Europe in the early 1900s) in this study had a mean CID_{50} above 10^{2} EID_{50} with

strains less adapted to chickens having the higher CID_{50} values. Other studies have also found similar estimates for the CID_{50} through the intranasal route, with higher CID_{50} values indicating a lack of adaptation for infection in chickens.[51,92,93] The initial cases in wild birds in the U.S. with Eurasian HPAI H5N8 (A/GF/WA/14) and reassortant H5N2 (A/NP/WA/14) viruses had high CID_{50} values (i.e., near or above $10^{4.7}$) and thus were likely poorly adapted to chickens, possibly explaining why poultry outbreaks were limited in the Pacific flyway during the 2014-2015 outbreak.[51]

Single-hit dose-response models (e.g., exponential) have been used for HPAI virus in chickens and mammals.[94,95] These models assume that each virion has the capacity to independently act and cause infection in the host. Dose-response models enable us to estimate the probability of infection when a bird is exposed to a dose different from the 50% infectious dose. For example, given a CID_{50} less than $10^{2.82}$ EID_{50}, a chicken exposed to 10 EID_{50} would have a 1% chance of infection according to the single-hit exponential dose-response model.

Given limited data, there is greater uncertainty regarding the infectious dose for other routes such as oral consumption of infected material. Kwon and Swayne (2010) found a substantially higher 50% infectious dose for HPAI H5N1 (A/Whooper Swan/Mongolia/244/) via oral consumption of chicken meat (10^{7} EID_{50}) or drinking of contaminated water ($10^{6.7}$ EID_{50}).[96] However, in this study, a group of 3 to 5 chickens were fed contaminated meat with a single virus concentration, and details regarding the uncertainty in the estimates were not provided. The study also found higher infectious doses for the intragastric inoculation route by gavage ($10^{6.2}$ EID_{50} for liquid and $10^{7.4}$ EID_{50} for meat) compared with the intranasal route.

In Swayne and Beck (2005), feeding of finely chopped meat from chickens infected with H5N1 HPAI viruses at higher doses ($10^{7.8}$ EID_{50}/bird) resulted in transmission of H5N1 HPAI (A/chicken/Korea/ES/2003) virus.[97] However, feeding of HPAI H5N2 (A/chicken/ Pennsylvania/1370/1983)-infected chicken breast or thigh meat to Specific Pathogen Free (SPF) chickens at lower doses ($10^{3.5–3.6}$ EID_{50}/bird) did not produce infection. The authors reasoned that lack of direct exposure of the respiratory tract (i.e., minced meat likely did not pass through the choanal cleft and contact nasal surfaces) could explain the lack of infection in H5N2 trials with lower doses. Moreover, a reference is made to a feeding trial by Purchase et al. (1931), in which 0.5 g of blood fed to chickens resulted in HPAI transmission, whereas feeding 5 g of meat did not, suggesting that transmission is more likely if a feedstuff is conducive to passage into the nasal cavity.[98] However, in the Purchase et al. study, the HPAI concentration in blood was not estimated, and it may have been sufficient to cause infection via the intragastric route.

Sergeev et al. (2013) found a CID_{50} for H5N1 HPAI (A/Chicken/Suzdalka/Nov-11/2005) virus of $10^{3.9}$ EID_{50} for oral inoculation and $10^{5.2}$ EID_{50} for intragastric inoculation via gavage tube.[99] The authors suggested contamination of the nasal mucosal membranes from the oral cavity via the choanal slit as a possible internal mechanism for transmission via the fecal-oral route.

There is considerable uncertainty regarding the infectious dose via the aerosol route. Direct aerosol data from Spekreijse et al. (2013) suggest very low transmission rates, even after 24 hours of exposure to H5N1 HPAI (A/turkey/Turkey/1/2005) virus in a concentration of more than 10^{3} EID_{50}/m^{3} in air coming from a room housing infectious chickens.[100] When we fit exponential and logistic dose-response models to data from Spekreijse et al. (2013), maximum likelihood estimation suggested a CID_{50} for the aerosol route between 5 and 6 log EID_{50}.[100] An

estimate of 5 to 6 log EID_{50} is more consistent with the lower transmission rates for AI observed between chickens housed in adjacent cages in most studies.[101]

Sergeev et al. (2013) found considerably lower CID_{50} estimates (approximately 1 log EID_{50}) for various HPAI H5N1 strains when susceptible chickens were exposed to 0.5- to 2-micrometer (µm) diameter aerosols generated from liquid contents of HPAI-infected embryonating eggs.[99] The results from this paper are not consistent with other studies that indicate lower aerosol transmission between infected and susceptible chickens housed in adjacent cages, and are also not consistent with data published in Spekreijse et al. (2013).[100] A possible explanation for the differences between this study and Spekreijse et al. (2013) is that the characteristics of 0.5- to 2-µm contaminated aerosols generated by nebulizing embryonating egg contents differ from naturally contaminated aerosols emanating from a chamber with infectious chickens. In addition, Spekreijse et al. (2013) allow that the viral titer determined by RT-qPCR includes inactivated virus, such that the titer of viable virus in the air sample was actually lower.[100]

8.7.3 Route of Entry and 50% Infectious Dose Estimate 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 susceptibility of different tissues to infection with HPAI virus (intranasal vs. intragastric) observed in laboratory inoculation and experimental feeding trials, there is considerable uncertainty as to the infectious dose that is appropriate for natural exposure via feeding of contaminated materials. The route of entry impacts the dose-response parameters in the exposure assessment.

We had obtained expert opinion regarding the appropriate route of entry and associated infectious dose (intranasal or intragastric) that best represents oral exposure in chickens, given the limited data on this aspect.[102] 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 as well as in chickens.

8.8 Latently Infected and Infectious Periods

In individual birds, the incubation period is dependent on the dose, route of exposure, 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.[103] For trade purposes, the OIE defines the flock incubation period as 21 days.

8.8.1 Latently Infected and Infectious Periods in Turkeys

The latently infected and infectious periods may vary considerably with HPAI strain and turkey breed. Saenz et al. (2012) estimated the mean infectious period for HPAI H7N1 (A/ostrich/Italy/984/00) in turkeys to be 1.47 days (95% CI [confidence interval], 1.3 to 1.7)

from experimental transmission studies.[17,104] The data from this study also suggested that the latent period for HPAI H7N1 in turkeys is likely less than 16 hours.[104]

Aldous (2010) evaluated the virus shedding patterns and mortality in turkeys inoculated with various doses of HPAI H5N1 (A/turkey/Turkey/1/05) virus.[89] Analysis of these data indicated a mean latent period of 1.27 days (SD, 0.40 days) and a mean infectious period of 1.28 days (SD, 1.17 days).[89,105] Further details on the estimation of these parameters are provided in Appendix 2 of the Turkey Hatching Eggs Risk Assessment.[106]

8.8.2 Latently Infected and Infectious Periods in Chickens

Latent and infectious periods have been documented for multiple HPAI virus strains, and periods may vary depending on virus strain and chicken type used in experimental conditions. Using 6-week-old SPF white Leghorn chickens, Van der Goot et al (2005) determined an infectious period of 6.3 days (95% CI 3.9-8.7 days) when birds were inoculated with HPAI H7N7 (A/Chicken/Netherlands/621557/03).[107] In another experiment also using 6-week-old SPF white Leghorn chickens, this time inoculated with HPAI H5N2 (A/Chicken/Pennsylvania/1370/83), the mean infectious period was 6.8 days (95% CI 4.91-8.69 days) and a modeled latent period was 1-2 days.[108]

Mean time to death (which includes both latent and infectious periods) was observed in 2- to 4-week-old SPF white Leghorn chickens using multiple strains of HPAI H5N1. Death was observed in 100% of birds in less than 36 hours when inoculated with one of the four following strains: DK/Vietnam/201/05, DK/Vietnam/206/05, DK/Vietnam/207/05, Muscovy DK/Vietnam/213/05. Mean time to death was estimated at less than 48 hours for DK/Vietnam/218/05 and at 48 hours for DK/Vietnam/203/05.[109]

In a study using 4-week-old SPF chickens of a layer breed inoculated with HPAI H5N1 (A/Chicken/Legok/2003), researchers reported a mean latent period of 0.24 days (95% CI 0.099-0.48 days) and a mean infectious period of 1.6 days (95% CI 0.90-2.5 days).[110]

In 5- and 8-week-old broiler chickens inoculated with 2015 EA/AM HPAI H5N2 (Tk/MN/2015), mean times to death of 4.8 and 3.2 days were observed, respectively.[111]

8.9 Clinical Signs

The presence and severity of clinical signs of HPAI infection depend on the virus strain and bird species affected.[31] Infected wild and domestic ducks may be asymptomatic, whereas clinical signs in gallinaceous poultry are usually severe, resulting in high mortality.[112] In chickens and turkeys, the clinical signs associated with HPAI infection include marked lethargy with ruffled feathers, lack of appetite, excessive thirst, neurological signs (e.g., tremors, torticollis, opisthotonos, etc.), decreased egg production, soft-shelled or misshapen eggs, respiratory signs (coughing and sneezing), watery diarrhea, or sudden, unexpected death.[78,112] Mature chickens frequently have swollen, cyanotic combs and wattles, and edema surrounding the eyes.[112] In turkeys, a cessation in flock vocalization ("cathedral syndrome") often accompanies infection.[79] 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.[103,105,113]

The mortality rate in an infected flock can reach 100%.[114] 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; and petechial hemorrhages which cover the abdominal fat, serosal surfaces, peritoneum, and surface under the keel.[78,112] In layers, the ovary may be hemorrhagic or degenerated and necrotic.[115] Ruptured ova have been reported in layers and broiler and turkey breeders; the peritoneal cavity may be filled with yolk from ruptured ova, causing severe peritonitis in birds that survive long enough.[112] In addition, most HPAI viruses can cause necrosis of the pancreas[78]; all species of birds affected in the 1999-2001 H7N1 HPAI outbreak in Italy had lesions at necropsy of pancreatitis, but this was most pronounced in turkeys and chickens.[116]

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 deaths. While a confirmed diagnosis depends on the isolation and identification of the virus, it is typically advantageous (for rapid control and eradication) to respond to a presumptive positive H5 or H7 result by PCR in accordance with any case definition.[117] In the United States, confirmation of an HPAI outbreak is made by the National Veterinary Services Laboratories in Ames, Iowa (NVSL). After 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).[117]

The reference standard for diagnosis of viable AI virus is virus isolation—an accurate method of confirming the presence of a virus that could infect other birds.[118] 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 its HA and NA subtype.[13]

Molecular methods for detection of viral nucleic acid and genetic 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 through-put, high sensitivity, and high specificity.[13]

Antigen detection immunoassay kits have also been used in prior outbreaks and have advantages of speed (15-20 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.[13]

8.11 Differential Diagnosis

HPAI can resemble several other avian diseases, including velogenic viscerotropic Newcastle disease, infectious bronchitis, infectious laryngotracheitis, mycoplasmosis, infectious coryza, fowl cholera, aspergillosis, and *Escherichia coli* infection. It also must be differentiated from heat exhaustion, toxicities, and severe water deprivation.

9 Risk Evaluation

9.1 Pathways for a Turkey Flock Becoming Infected with HPAI 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 proximity to infected farms. The implementation of a Control Area (e.g., 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 the majority of 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 insects, aerosols, and wild birds in this chapter.

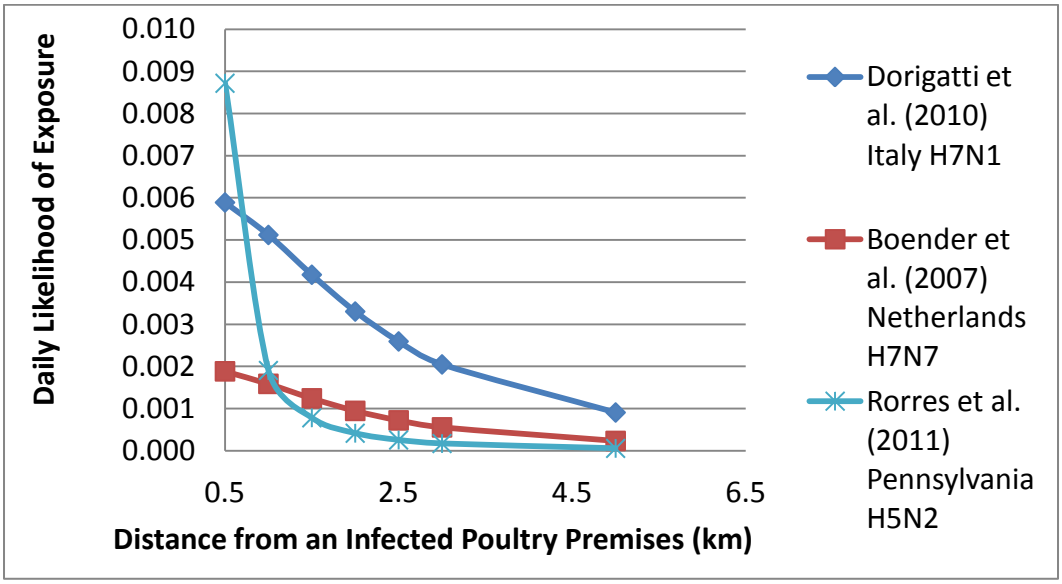


Figure 4. Relationship between the daily likelihood of exposure and distance from infected premises estimated from past HPAI outbreak data (also called a "transmission kernel"). Note that all these transmission kernels are not "mechanism-specific" and, hence, include movement of people, vehicles, and equipment between farms as possible transmission mechanisms.[119-121]

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.[122]

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 distance in all curves. The specific mechanisms by which the transmission likelihood increases with proximity is ambiguous based on these studies (see Appendix 2**:** 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 (an 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.[123] There are several instances where AI did not spread to other houses even on the same premises (see Appendix 2: 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 Insects in the Transmission of HPAI Virus

Insect or fly transmission of AI virus has been suspected in previous HPAI outbreaks based on anecdotal reports.[124,125] However, there are no quantitative epidemiological studies establishing transmission via flies. Houseflies (*Muscidae*) and blow flies (*Calliphoridae*) are reservoirs and vectors of a wide variety of pathogenic organisms affecting poultry.[126] The housefly is usually the most abundant and pestiferous fly species in poultry houses.[126] Most blowflies result from improper disposal of dead birds in a poultry operation, with very few associated with manure.[126] Some biosecurity plans and guidelines for AI control recommend fly control to minimize the spread of AI because of the uncertainty about fly transmission of HPAI.[127,128] A majority of turkey integrators specify some sort of fly control program within their biosecurity plans (TWG, personal communication, August 2016). Additionally, the STS Plan specifies that biosecurity measures following an outbreak of HPAI must include keeping vehicle windows rolled up at all times while on poultry premises in order to prevent flies from getting into vehicles and spraying insecticide inside trucks as needed to eliminate the transportation of flies from farm to farm during the warm months of the year.[10]

Below is a summary of the literature from previous outbreaks implicating insects in transmission of HPAI, survivability of AI viruses in flies, dispersion likelihood, and transmission of HPAI to a poultry flock in the 2 weeks prior to marketing. For a more in-depth discussion on transmission via flies, please see the Turkey Day Old Poults Risk Assessment.[129]

9.1.2.1 Literature Review

- Blow-flies were considered as a potential transmission route in the 2004 HPAI H5N1 outbreak in Japan.[130,131] In this outbreak, the prevalence of H5 virus genes was highest in blowflies collected 600 to 700 meters from the infected farm (20 to 30%), and HPAI virus gene-positive flies (10%) could be detected up to 2 km from the infected premises. The authors estimated the prevalence of viable virus at 5% in flies around the epidemic area.[131]
- Experimental studies indicate that flies can ingest AI virus and that there is a steady decrease in the viable virus titer over time.[132-134] Sawabe et al. (2009)[134] evaluated the survivability of H5N1 virus in blowflies 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 the 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.

- Wanaratana et al. (2013)[135] evaluated the potential of the housefly to serve as a mechanical vector of the H5N1 virus. H5N1 virus could survive within the body of the house fly and remain infective for up to 72 hours post-exposure. The viral titers in housefly 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. 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 1.90 log ELD_{50}/ml (the concentration at time 0 was 4.70 log ELD_{50}/ml), whereas virus could not be recovered by 48 hours post-exposure.
- While there are no known transmission studies in turkeys, Wanaratana et al. (2013), demonstrated transmission to chickens administered fly homogenate via oral drop with a pipette 1 day after exposure to $10^{8.5}$ $ELD_{50.}$[135] Based on the timing of virus shedding,[a] between 1 and 3 chickens out of 10 appeared to have been directly infected from the fly homogenate in this study.[135]
- Tsuda et al. (2009)[136] proposed a mechanism of transmission whereby poultry directly feed on HPAI-infected blowflies. It has been shown that a chicken can eat 31 blowflies placed in its cage in just 7 minutes.[134] However, feeding dead flies (*C. nigribarbis*) contaminated with H5N1 virus did not result in transmission (unpublished data).[137] The frozen dead flies were not attractive to chickens, and only small numbers of flies were consumed by the chickens in this experiment.[137]
- Fly dispersal behavior varies by species and environmental conditions. Houseflies tend to remain close to their breeding site as long as they find suitable food, breeding sites, and shelter. Also of note, the dispersal rate of flies decreases at temperatures below 53°F and increases during premises cleanout or spreading of litter.[138] A summary of dispersal rates appears in **Table 2**.

Table 2. 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	James & Harwood[139]
Housefly	Generally range less than 2 miles (3.2 km); range in a radius of 328-1,640 feet from breeding site if suitable food available; only 8-30% disperse beyond a poultry facility	Stafford[138]
Blowfly	Estimated 1.25-1.79 km/day on average	Tsuda et al.[136]
Blowfly	2-3 km in 24 hours	Sawabe et al.[130]

[a] Only 3 birds out 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.

- Beetles have also been implicated as a possible vector for transmitting AI viruses in a few studies.[140-142] However, there are minimal data on the experimental transmission of AI via beetles. In the 1983 HPAI H5N2 outbreak in Pennsylvania, testing of 144 pools of beetles (*Coleoptera*) yielded only two positive pools. One of the positive pools consisted of darkling beetles, and the second of hide beetles.[125]
    - Given that 10 to 60 insects were pooled together in each sample, Bayesian analysis indicates that the actual prevalence among beetles would be between 0.01% and 0.15%, which is quite small (see Appendix 3: Estimating an Approximate Posterior Distribution for the Prevalence Among Insects).

9.1.2.2 Expert Opinion

We obtained expert opinion on insects as a risk factor from veterinarians in the broiler and turkey sector working groups who had field experience in managing AI outbreaks. Overall, eight experts, including industry and regulatory veterinarians, rated this risk factor on a categorical scale ranging from negligible to extremely high (see Appendix 4: Expert Polling on Insect Transmission Routes, for details of the questionnaire). A majority of experts (six out of eight) rated the likelihood of insect transmission from known infected premises as low or negligible at 1.5 km, and negligible at 2 km or farther **(Figure 5).** The ratings for the likelihood of insect transmission from infected but undetected premises were similar; however, slightly more concern was noted at 1.5 km, where three of eight experts rated risk of infection as moderate or high (**Figure 6**).

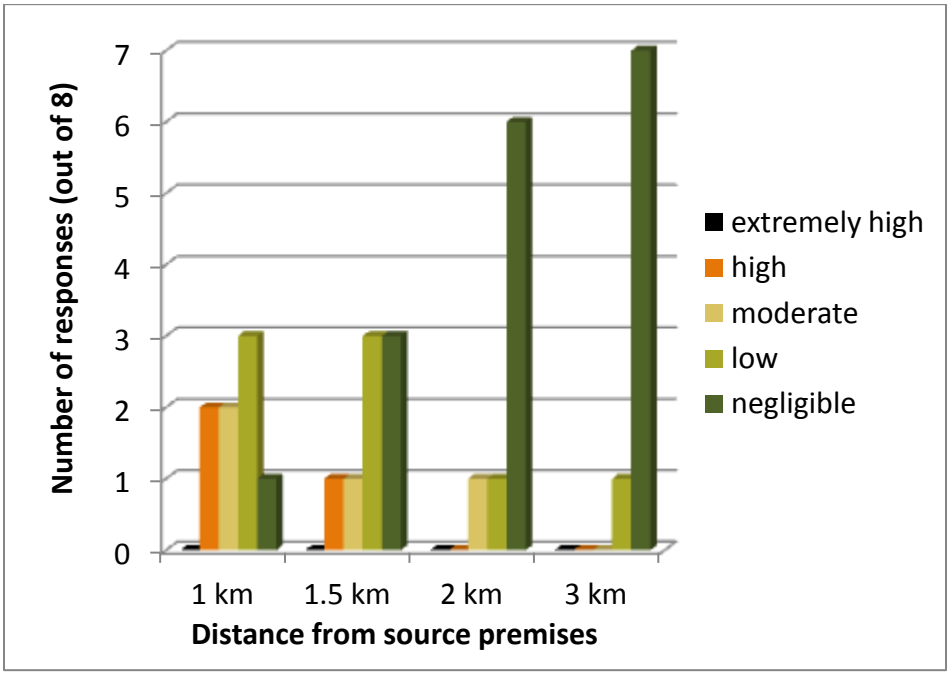


Figure 5. Ratings for likelihood of insect transmission **from a known HPAI-infected premises** by veterinarians with experience managing AI outbreaks

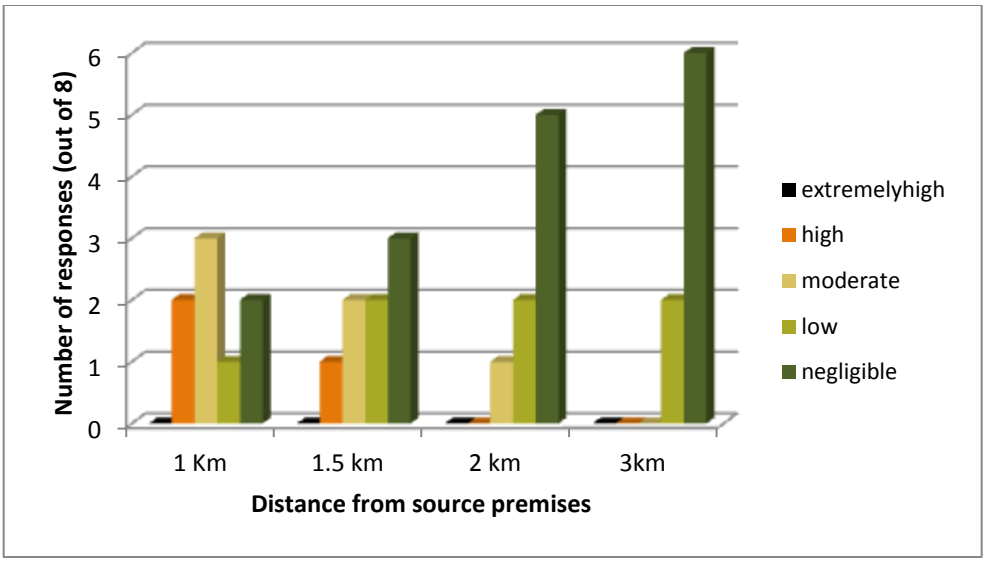


Figure 6. Ratings for likelihood of insect transmission from an **HPAI-infected but undetected premises** by veterinarians with experience managing AI outbreaks.

9.1.2.3 Qualitative Analysis

We considered the following factors in evaluating this pathway:

- In the period leading up to load-out, the inside of a turkey house likely contains a large amount of manure and other environmental conditions that may attract flies. Winpisinger et al. found the number of houseflies was significantly higher near (within 3.2 km) large (> 2 million birds) caged layer operations, compared with background fly levels in rural areas.[143] 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% of the mean value at layer farm) and 1.6 km (2 to 8% of the mean value at layer farm) was much lower than the number of flies trapped at the layer facilities.
- While fly transmission has been proposed as a possible mechanism for spread of HPAI, there has not been any epidemiological analysis evaluating flies as a risk factor for spread. Furthermore, local area spread components (mechanisms other than those 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.)
- Chickens have been shown to ingest live and actively flying houseflies,[134] and turkeys could likely show similar behaviors, but there has been no experimental evidence of chickens or turkeys 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.[135] 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 (i.e., virus ingested by a fly), the most likely inoculation route to the turkey is intragastric. As turkeys do not grind or

masticate their food within the oral cavity, the likelihood that fly gut contents would contact the choanal cleft during ingestion is decreased. The intragastric infectious dose is not well documented in turkeys, and an estimate (based on results from a study using esophageal inoculation) is very high at more than 20 times $10^{3.6}$ EID_{50} (see Hazard ID section).[49] Estimates for chicken infectious dose via the intragastric route also are quite high at $10^{5.2}EID_{50}$ to $10^{6.2}EID_{50}$ based on two studies.[96,99]

    - Wanaratana et al.(2013) have found a considerable decrease in the external HPAI virus concentration on an exposed fly within 24 hours.[135] While HPAI virus is inactivated at a slower rate in fly gut content, the likelihood of poultry infection (via fly ingestion) due to the virus encapsulated in the fly gut would be reduced because of the higher infectious dose needed for the intragastric route in poultry.

- 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 9.1.2.1 Literature Review). The relatively rapid inactivation of virus present in and on flies would result in reduced likelihood of transmission at greater distances.
- The number of flies around turkey premises typically increases seasonally (i.e., in warmer, damp weather) and if turkey houses are open (i.e., open-sided turkey houses have more flies than tunnel-ventilated houses) (TWG, personal communication, August 2016). The proportion of flies around an infected premises that could contain viable virus is likely low. Literature estimates report between 2 and 5% of flies may contain virus. Dispersal behavior may vary depending on environmental conditions and fly species, and dispersal is hypothesized to increase during outbreak activities such as premises depopulation.

9.1.2.4 Likelihood Rating and Conclusion

We concluded that the likelihood of a turkey finishing premises becoming infected with HPAI virus via insect transmission varies with distance as described in **Table 3**. Of note, at premises located closer than 1.5 km to an infected flock, there are too many variables to accurately assess the risk of infection with HPAI via insect transmission.

Table 3. Likelihood of a turkey finishing premises becoming infected with HPAI virus via insect transmission based on qualitative analysis and expert opinion

	Composite likelihood rating			
	Distance from source (km)			
Source premises type	1.5	2	3	5
Known infected premises	*Negligible to moderate*	*Negligible to low*	*Negligible*	*Negligible*
Infected but undetected premises	*Negligible to low*	*Negligible to low*	*Negligible*	*Negligible*

9.1.3 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 played a limited role.[68,144] 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.

9.1.3.1 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 as well. Aerosol spread has been implicated in very few HPAI outbreaks.
    - 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.[59] 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.[123] Appendix 2: 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%.[69] 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.
    - 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 close to and downwind of other infected flocks. 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.[145]
    - 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.[26]
    - A plume analysis model of infected farms in the 2014-2015 HPAI outbreak in Minnesota found that farms located 7 to15 km from an infected farm were at low to moderate risk of infection via aerosol transmission; however, wind speed and direction may affect the distance at which transmission can occur. Farms located

within 5 km of an infected premises were at increased risk regardless of wind conditions.[26]

- Activities that can generate AI virus-contaminated dust or aerosols very close to susceptible poultry have been implicated as a transmission mechanism.
    - Trucking of live birds actively infected with AI virus within 200 meters of a susceptible flock can pose a risk for aerosol transmission (D. Halvorson, personal communication, July 2016).[124]
    - Depopulation activities up to 400 yards (366 meters) upwind from a susceptible flock can present a risk for aerosol transmission.[26] 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 to 1.25 miles (~1600 to 2000 meters).[146] As noted above, 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.[145]
    - Spreading of non-composted contaminated litter on adjacent fields was suspected as a transmission mechanism during the 1983 HPAI H5N2 AI outbreak (D. Halvorson, personal communication, March 2016).[124] Spread of non-composted manure from infected farms approximately 1.25 miles from susceptible poultry was suspected to have resulted in transmission in one instance during an LPAI H7N2 outbreak in Pennsylvania.[146]
    - 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.[26]
- Only a couple of 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 2004 HPAI H7N7 outbreak in Canada.[147] Inside the barn, a viral titer of 292 $TCID_{50}/m^3$ was detected in air samples.[b] 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.
    - 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 8 farms was virus-positive; the positive sample was taken 45 meters downwind.[124]
    - The 2015 USDA epidemiology investigation report describes the results of 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) of affected barns, and at extended distances ranging from approximately 70 to

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

1,000 meters downwind from the barns. Five of the six flocks had at least one air sample test positive.[148]

9.1.3.2 Experimental Studies of Aerosol Transmission of AI Virus

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 studies, aerosol transmission of AI was not observed between groups of inoculated and susceptible chickens housed in adjacent cages or chambers with direct airflow.[14,44,149,150]
- A few studies have shown inefficient or low transmission of AI between groups of inoculated and susceptible birds housed in adjacent cages or chambers with direct airflow.
    - LPAI (Turkey/Wis/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.[151]
    - Two out of six strains of LPAI H9N2 were transmitted via aerosol from a cage with four infected chickens to chickens in an adjacent cage 100 cm away.[152]
    - For chickens housed in cages 10 cm apart, airborne transmission of HPAI H5N1 occurred inefficiently when 1 to 2 chickens were infected, but efficiently when 4 to 8 chickens were infected.[153] 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).[101]
    - For H5N1 (A/turkey/Turkey/1/2005), Spekreijse et al.[100,154] estimated a transmission rate of 0.10 new infections per infectious bird per day for chickens housed 1 meter away.
- Experimental studies indicate that variability between influenza virus strains can impact transmissibility via aerosols. For example, Zhong et al. (2014) found different strains of LPAI H9N2 to have markedly different aerosol transmissibility between chickens.[155] The study proposed that the influenza virus genes HA and PA are important in determining aerosol transmissibility.
- Several studies have indicated efficient transmission of HPAI H5N1 and LPAI H9N2 virus to chickens by aerosols that were mechanically generated by nebulizing virus containing stock fluid to very small particle sizes (2-5 μm).[99,156]
- Several studies have found that influenza A viruses show decreased survivability in aerosols at higher temperature and higher relative humidity.[157,158]

AERMOD plume models (see Appendix 5: Live Turkey Movement Aerosol Modeling for model parameters and scenarios) were utilized in the context of this risk assessment. The measure of interest was HPAI virus concentration:

- The dispersion model scenarios estimated the risk of transmission to a house of 14,000 turkey hens assumed to weigh 15.53 lb.
- 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 increased 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, see Appendix 5: Live Turkey Movement Aerosol Modeling).
    - 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.
    - 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.
- 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, see Appendix 5: Live Turkey Movement Aerosol Modeling).
- 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.

9.1.3.3 Expert Opinion

- We obtained expert opinion on aerosol spread as a risk factor from veterinarians in the turkey and broiler sector working groups with field experience managing AI outbreaks. Overall, eight experts, made up of industry and regulatory veterinarians, rated this risk factor on a categorical scale ranging from negligible to extremely high (see Appendix 6: Expert Polling on Aerosol Transmission Route for details of the questionnaire). In a scenario in which depopulation activities were not taking place, a majority of experts (7 out of 8) rated the likelihood of aerosol transmission from known infected premises as low or negligible at 1.5 km and negligible at 2 km or farther. The ratings for the likelihood of aerosol transmission from infected premises where depopulation activities were taking place were similar, but more concern was noted at 1.5 km, where 5 of 8 experts rated risk of infection as moderate or high (see Appendix Figures 1-2**,** Appendix 6: Expert Polling on Aerosol Transmission Route).

9.1.3.4 Qualitative analysis

We considered the following factors in evaluating this pathway:

- The housing and ventilation systems utilized in commercial turkey operations likely represent at least a partial barrier to local area spread when compared with alternative housing systems (free range or pasture-raised), which are not within the scope of this 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.[157-159]
    - 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.

9.1.3.5 Likelihood Rating and Conclusion

9.1.3.5.1 Likelihood of HPAI Spread to a Turkey Flock in a Control Area via Aerosol Transmission from a Known HPAI-Infected Flock

Given the higher predicted prevalence of infectious birds in known infected flocks, both the expert opinion ratings and exploratory dispersion modeling results indicated higher potential risk for this category (compared to spread from an infected but undetected flock). Given the uncertainties involved with parameters needed for modeling calculations, more weight was given to epidemiologic data from previous outbreaks and expert opinion than to modeling calculations for determination of risk associated with this pathway. Literature review and most previous outbreak reports indicated that local area spread and aerosol transmission were 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.

We provided the following likelihood ratings, considering the above factors.

- *High* if the turkey flock is located 0.5 km from a known infected poultry farm.
- *Moderate* if the turkey flock is located 1 km from a known infected poultry farm.
- *Low* if the turkey flock is located 1.5 km from a known infected poultry farm.

9.1.3.5.2 Likelihood of HPAI Spread to a Turkey Flock in a Control Area via Aerosol Transmission from an Infected but Undetected Flock

In this case, the expert opinion ratings and dispersion modeling results indicated lower risks (compared to spread from a known infected flock). We rated the likelihood of turkeys becoming infected with HPAI via aerosols from an infected but undetected poultry flock at a specific distance from the infected premises as follows:

- *Low* to *moderate* if the turkey flock is located 0.5 km from an infected but undetected poultry farm.
- *Low* if the turkey flock is located 1 km from an infected but undetected poultry farm.
- *Negligible* to *low* if the turkey flock is located 1.5 km from an infected but undetected poultry farm.

9.1.3.5.3 Conclusion

The likelihood of a turkey flock becoming infected with HPAI virus via bio-aerosols ranges from *negligible* to *high*, depending on the distance from, and prevalence of virus in, the source flock. The assessed risk is highest for flocks located within 0.5 km from a known infected poultry farm. We estimate the likelihood of HPAI spread to a turkey flock to be *negligible* to *low* if the premises is located 1.5 km from an infected but undetected poultry farm, and *low* if the premises is located 1.5 km from a known infected poultry farm.

9.1.4 Role of HPAI Spread to a Turkey Flock in a Control Area via Wild Aquatic Birds in Farm Vicinity

Wild aquatic birds are the main reservoir of influenza A viruses in nature. They harbor all 16 (H1-H16) HA and all 9 (N1-N9) NA subtypes of AI in their population. Most of the isolates from aquatic birds have been LPAI, which generally does not cause disease in the wild population. It is understood that the virus circulates continuously in the wild population, but often at low levels.[160]

Various 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 reared in semi-intensive or extensive (free-range) conditions.[161] Wild ducks have been found to carry a higher prevalence of virus during their southern migration in the fall (22.2%) than during their spring northerly migration (0.3%). This difference may be due to the increased number of susceptible young birds during the fall migration.[160]
- Snow geese are known to have died from EA/AM H5N2 during the 2014-2015 HPAI outbreak in the U.S.[27] Anecdotally, snow geese were observed near poultry houses that later became infected with H5N2.

- Shorebirds have also been found to carry influenza viruses in a higher percentage than that in ducks during the spring migration.[160]
- Gulls are susceptible to HPAI viruses[162] and are a known reservoir for AIVs.[163] Gulls are suspected to have been the source of a 2002 outbreak in the Chilean poultry industry. In this instance, the HPAI virus likely mutated from an LPAI strain.[164] 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 the virus, but transmission from gulls to other species is less clear.[162] Because they are opportunists, gulls 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 AIVs (more in-depth analysis of the role of scavengers can be found in section 9.2.4.2 Likelihood of Turkey Flock Becoming Infected with HPAI via On-farm Dead Bird Disposal and Scavengers during PMIP).

Influenza viruses have been shown to affect all types of domestic birds, and the primary infection depends on the degree of contact with wild birds. 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.[165] Potential pathways of HPAI virus transmission through wild aquatic birds in the farm vicinity are illustrated below.

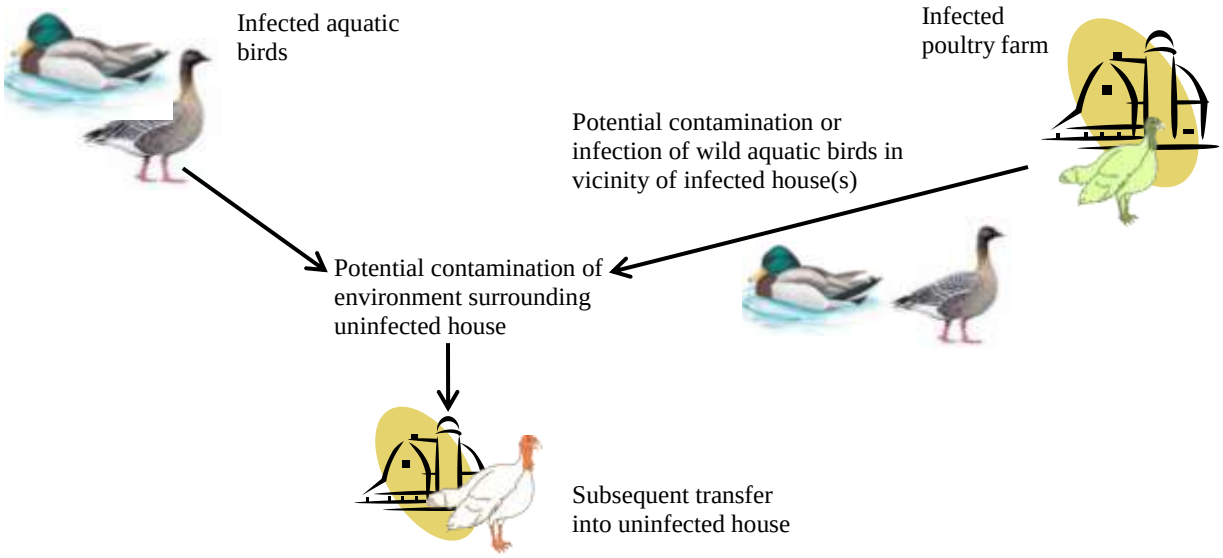


Figure 7. Pathway of HPAI virus transmission through wild aquatic birds

9.1.4.1 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[166] or from aquatic bird population surveillance sampling.[160]

Recent studies have shown that HPAI viruses, in particular Eurasian H5N1 and H5N8, 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.[167]
- 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.[168]
- 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.[19]
- 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 subsequently isolated in North America show 99% similarity to the Korean H5 strains.[169,170]
- 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.[171]
- 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 (**Table 4)**.[27]

Table 4. H5N2 cases in U.S. aquatic birds, December 2014 to June 2015[26]

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 2015 H5N2 outbreak in the midwestern U.S., sampling of wildlife took place on five infected and five uninfected farms. Out of 419 individual birds sampled, killdeers were the only aquatic birds 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.[26]

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 highly pathogenic H5 viruses in poultry in the U.S. and Canada dating back 43 years prior to the 2014-2015 outbreak.[172] 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.[172]

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 5** summarizes the results of several studies on HPAI virus in wild and domesticated aquatic birds.

Table 5. Summary of experimental studies of HPAI virus in wild and domesticated aquatic birds

HPAI virus	Bird species	Inoculation	Findings	Reference
H7N3 (A/chicken/Chile/184240-1/02)	Chiloe 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 chiloe wigeons at day 2 post inoculation. Virus efficiently transmitted to cinnamon teal contacts, not to chiloe wigeon contacts.	Sá e Silva et al., 2011 [173]
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.	Alexander et al., 1986 [174]
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.	Alexander et al., 1986 [174]
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.	van der Goot, 2005 [107]

HPAI virus, cont.	Bird species	Inoculation	Findings	Reference
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.	Wood et al., 1985 [175]
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 of the inoculated gulls. In both species virus was isolated from internal organs. Viral titers were higher in oropharyngeal swabs than in cloacal swabs.	Brown et al., 2006 [162]
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	Mary Pantin-Jackwood, personal communica-tion, August 2016

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.[176] These findings are summarized in **Table 6**.

Table 6. Shedding and transmission results of experimental infection of mallard ducks with H5 and H7 HPAI virus at10^6 EID_{50} intranasally[176]

Virus strain	Shedding (days)	OP vs. CL	Trans. to contacts	> Chicken BID_{50}
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 sighting.[177] 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, mallard, ring-billed gull, glaucous-winged gull, mew gull, killdeer, and trumpeter swan—were seen in the immediate barn area at least twice.[178] They were most frequently observed near feed silos. No wild aquatic birds were observed entering the poultry houses.

Some recent events have provided 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 was detected in 11 commercial broiler breeder, table egg layer, and turkey farms in British Columbia by December 17, 2014.[179] In addition, the Canadian Food Inspection Agency confirmed HPAI H5N1 on a noncommercial poultry farm on February 7, 2015.[179] Influenza viruses had been previously isolated from wild and domestic ducks in British Columbia.[180]

- 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.[181,182] Various positive aquatic birds were found during the outbreak, as shown in **Table 7**, cementing the possibility of introduction from wild aquatic birds.

Table 7. 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 Bevins et al[182]

Species	n	HPAI virus icA positive
Mallard, *Anas platyrhynchos*	1410	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.[183] On February 12, 2015, Eurasian H5N8 was also confirmed in a commercial chicken flock in Kings County, California.[181]
- Between March and June of 2015, an outbreak of H5N2 was observed in the Midwest; turkey premises were the most impacted in Minnesota, and chicken layer premises were

more involved in Iowa.[26] Although 3,139 waterfowl fecal samples were tested during this outbreak, HPAI virus was not isolated from any aquatic bird fecal sample.[184]

9.1.4.2 Qualitative Analysis

We considered the following factors in evaluating this pathway:

- Generally, total confinement and biosecurity measures practiced on commercial poultry operations effectively prevent wild aquatic birds from entering turkey barns.
    - The spread of the viruses via migratory waterfowl routes is far less likely to occur in poultry farms with bird-proof confinement.[185]
    - Feed bins are maintained so that wild birds do not frequent or access turkey premises, and spilled feed should be promptly cleaned up to avoid attracting wild birds.[10,186-188]
- Experimental studies suggest that the possibility of HPAI infection in wild aquatic birds varies considerably, and their ability to transmit viruses depends on the combination of virus strain and host.
    - A study in 1986 by Alexander et al. challenged ducks with eight different HPAI and LPAI viruses via three different routes (intranasal, intramuscular, and contact with inoculated ducks).[165] Ducks became infected with only four strains of viruses, and the infection rate for each strain varied with the route of infection. In addition, tracheal and cloacal viral shedding was inconsistent.
    - As illustrated in **Table 6**, mallard ducks experimentally infected intranasally with four strains of H5 HPAI viruses at10^6 EID_{50} responded differently from those infected with strains of H7 HPAI viruses at the same EID_{50}.[176]
- Despite the possibility of wild aquatic birds introducing HPAI to susceptible farmed poultry, there is no known evidence of secondary spread from total confinement farms within control zones via wild birds. 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 fall outside the scope of this assessment. 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.[185] Secondary spread caused by wild birds between poultry premises should be considered possible in rare instances.
    - Wood et al. (1985) demonstrated little to no fecal shedding of HPAI H5N2 in wild ring-billed gulls (and domestic Pekin ducks), suggesting these birds were unlikely to transmit virus from farm to farm in the 1983 Pennsylvania outbreak.[175]
    - None of the HPAI-infected wild ducks (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.[26,169,170,189]
    - 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.[183]

9.1.4.3 Likelihood Rating and Conclusion

While wild aquatic birds are natural reservoirs for influenza A viruses (including several strains of HPAI virus) and could potentially cause a spillover of disease to domestic poultry, primary infection in turkeys or other domestic poultry depends upon the degree of contact with wild birds. In addition, there is no historical evidence of secondary spread from total confinement barns within Control Areas via wild aquatic birds. Modern commercial poultry management systems, in combination with stringent biosecurity measures, make the contact between wild and domestic birds—and resulting secondary spread of HPAI virus among domestic poultry via wild aquatic birds—unlikely within a Control Area. Therefore, we conclude that the likelihood of HPAI infection in poultry via wild aquatic birds in the farm vicinity is *low*.

9.1.5 Role of HPAI Virus Spread to a Turkey 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.[190] A compilation of more recent surveys of wild birds describes an overall AI virus prevalence of 15.2% in Anseriformes (waterfowl), 2.9% in Passeriformes (perching birds), and 2.2% in Charadriiformes (waders, gulls, and auks).[185] Influenza viruses are primarily spread from wild birds to domestic poultry through the mechanical transfer of infective feces, usually via human activity.[185] For a thorough review of pathways associated specifically with aquatic bird species, please see section 9.1.4, Role of HPAI Spread to Turkey Flock in a Control Area via Wild Aquatic Birds in Farm Vicinity.

9.1.5.1 Likelihood of Infection via Passerine or Columbiforme Birds in Farm Vicinity

Since its appearance, HPAI H5N1 has demonstrated the unique ability among HPAI viruses to infect a wide variety of species, including wild birds. 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.[191] 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.[192] The potential pathways for HPAI transmission via passerine or Columbiforme birds include infection or contamination of the wild bird on an infected poultry farm or premises contaminated with infected wild bird feces, with subsequent primary or secondary transmission into an uninfected turkey house. The flying distances that some wild bird species travel depend on food supply and weather. For example, starlings and blackbirds disperse as far as 15 to 25 miles on average, with some individuals traveling up to 50 miles daily from their roost to their feeding grounds.[193] Any of these movements provide an individual bird the opportunity to contact and disseminate AI viruses.

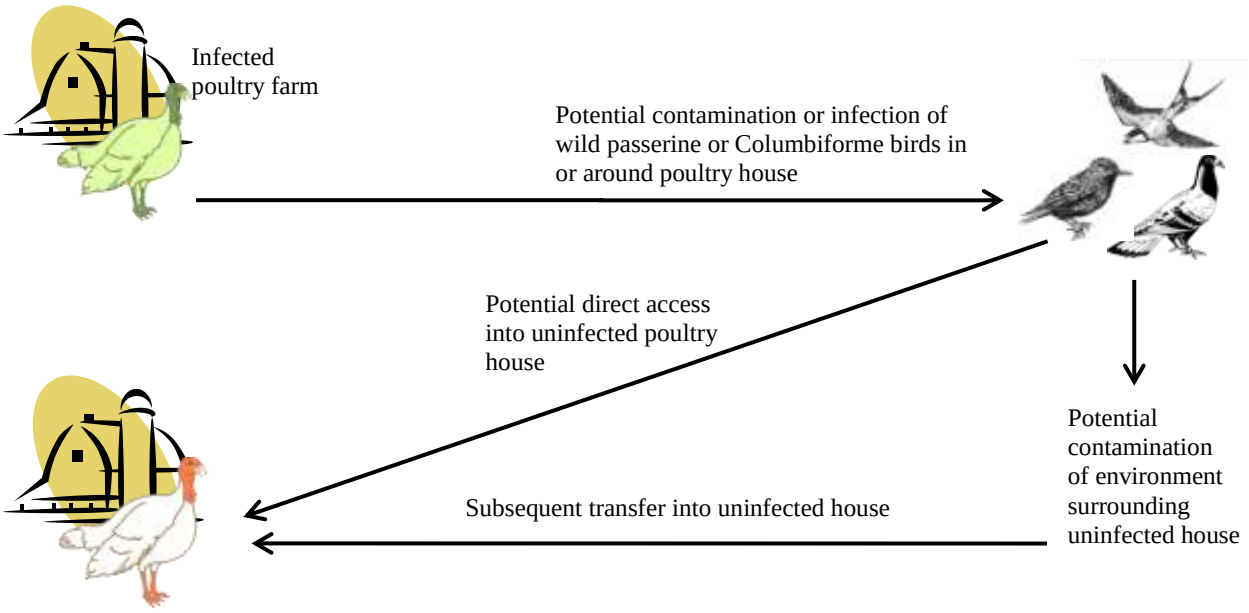


Figure 8. Pathway for exposure of a turkey farm via wild passerine or columbiforme birds

9.1.5.1.1 Literature Review

Small species of wild birds have been observed entering poultry barns.

- European starlings and house sparrows are frequently located near poultry houses.[194] During a field survey to estimate the incidence of bacterial pathogens in passerines near broiler houses, starlings were seen trying to gain entrance to all chicken houses on one farm, and a nest with young starlings was seen in the eaves of one house.[194] Numerous droppings on the sides of the houses on another farm indicated that sparrows and starlings were attracted to the house and possibly trying to gain entrance.
- In a survey of table-egg layer operations in California regarding pest management practices, producers ranked wild birds (passerines) as being somewhat more pestiferous on southern ranches[195] than on northern ones[191] when asked to rank pests in order of perceived importance.[196]
- Craven et al. note that starlings have the ability to peck through plastic wire mesh on the sides of chicken houses.[194] Burns et al. counted wild birds in the vicinity of poultry farms in Ontario and British Columbia[178] and found:
    - Barn swallows (*Hirundo rustica*), rock doves (*Columba livia*), and European starlings (*Sturnus vulgaris*) were all observed entering poultry barns, which included broiler, broiler breeder, layer, and turkey production.
    - Rock doves were observed entering barns the most frequently.
- In a survey of infected turkey farms during the 2014-2015 outbreak in Iowa, Minnesota, North Dakota, South Dakota, and Wisconsin (n=81), 33.7% of farms reported seeing wild birds (unspecified species) in poultry barns daily to occasionally; most reported that small perching birds are present on farm year-round.[26,197]
- Some species or populations of passerines could be termed synanthropic, as they occupy a distinct ecological niche in and around human agricultural activities. The behavioral

characteristics of passerines that may contribute to their ability to play a role in the transmission of AI to domestic poultry are summarized in **Table 8**.

Table 8. Behavioral characteristics of several members of the order Passeriformes that impact the potential role of transmission of HPAI virus in environments on the farm around poultry houses

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 domesticu*s)	Resident	Closely associated with people and their buildings	Prefers 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 (grain sorghum), etc.

Table from USDA-APHIS Poultry Feed Risk Assessment.[198]

Wild birds may also be attracted to poultry feed. For more information on specific risks of feed contamination if passerines breach biosecurity at feed mills or on farms, see USDA-APHIS Poultry Feed Risk Assessment.[198]

While passerine birds have not been directly implicated in the spread of HPAI in previous outbreaks, such birds have tested positive for AI in the vicinity of infected poultry premises.

- 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 as well.[199]
- 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) with an H5N2 serotype was reported.[200] 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 Pakistan in 2007, four wild crows were found to be H5N1-positive following outbreaks in backyard poultry and zoo birds.[201]
- In Hong Kong in 2009, among 22 birds found dead, including chickens, one large-billed crow (*Corvus macrorhynchus*) was infected with H5N1.[201]

- 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.[202]
- A chickadee 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 (Minnesota Department of Natural Resources 2015).[203] 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.
- As part of a case-control study of layer flocks in northwest Iowa in 2015, wild birds and mammals around the flocks were sampled.[26]
    - 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 from lung tissue of a juvenile European starling was positive for Eurasian H5 (icA).
    - Additional serologic evidence for icA H5 was found in a sparrow (*Passer domesticus*), another European starling (*Sternus vulgaris*), and two American robins (*Turdus migratorious*) sampled around the same positive farm.

With one exception (from a study in Slovakia), surveillance of passerines for AI virus has demonstrated a zero to low prevalence in the wild population.

- 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 birds.[26,204]
- No influenza virus was isolated from 83 cloacal swabs collected from four adult and 79 juvenile reed warblers (*Acrocephalus scirpaceus*) in 1993, despite proximity to aquatic habitats of known AI reservoir species.[161]
- In a survey of passerine birds in the U.S. state of Georgia from 1999 to 2009, zero of 234 birds of 25 different species tested positive for AI antibodies.[205]
- 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.[206]
- In China, from 2004 to 2007, RT-PCR on 7,320 cloacal, tissue, or fecal samples from 33 Passeriforme species identified 0.36% to be H5N1-positive; 1.09% of tree sparrows were positive.[167]
- During active surveillance of Passeriformes for HPAI H5N1 in Mongolia from 2005 to 2011, zero of 80 live-bird, fecal, and sick-bird samples were positive.[168]
    - No passerine birds were sampled during five H5N1 wild bird outbreak investigations.
- In 2006, out of 8,961 Passeriformes sampled via RT-PCR in Europe, 1 (0.01%) was H5N1-positive and 8 (0.09%) were LPAI-positive.[191]

- From a total of 670 cloacal swabs from 37 different species of migratory passerine birds in Slovenia from 2004 to 2006, there was 1 positive rRT-PCR in the only common starling (*Sturnus vulgaris*) tested, but virus isolation was unsuccessful.[207]
- In a 2007 study in Slovakia, 30% of 155 passerine birds of 12 species were AI virus positive via RT-PCR on cloacal and/or oropharyngeal samples, including three of six swallows (*Hirundo rustica*).[208] 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.
- Rectal samples from 1,300 tree sparrows (*Passer montanus*) in China in 2011 yielded no AI virus, while 94 of 800 were serologically positive for H5N1, and zero of 800 were seropositive for H7.[209]

Experimental susceptibility of passerine birds to HPAI H5N1 depends on the species of bird and strain of virus.

- In sparrows inoculated with four different H5N1strains, mortality was 66-100%, oropharyngeal and cloacal titers were as high as 4.7 and 4.1 $\log_{10}$ EID_{50}/ml, respectively, at 4 days post-inoculation (DPI), and there was no same-species contact transmission.[210] Mortality was 0% in European starlings, maximum cloacal titer was 3.8 $\log_{10}$ EID_{50}/ml at 2 DPI, and there was only one, unduplicated, instance of contact transmission. Oropharyngeal and cloacal titers were very low in pigeons (*Columba* spp.), and their mortality was 0%.
    - The authors deduce that sparrows may act as intermediate hosts for transmission to both poultry and mammals, but the lack of contact transmission and high mortality preclude them from serving as a reservoir species for H5N1.
    - While starlings may also act as intermediate hosts, the authors conclude the low contact transmission rate likely indicates they could not serve as an H5N1 reservoir.
    - Pigeons were determined to be likely to play a minor role in the ecology of H5N1.
- Brown et al. (2009) found similar mortality rates (60-100% at 10^2 to 10^6 EID_{50} inoculum/bird) and maximum oropharyngeal titers (4.2 $\log_{10}$ $TCID_{50}$/ml) in house sparrows (*Passer domesticus*) inoculated with A/whooper swan/Mongolia/244/05 HPAI H5N1, but maximum cloacal titers were significantly (P=0.002) lower than oropharyngeal. While 40% of pigeons (*Columba livia*) inoculated with the highest dose of H5N1 died, they and the survivors shed virus only briefly and at low titers. All pigeons in the low- and medium-dose groups survived and remained AI virus-free.[211]
    - These authors conclude that sparrows could play a role in AI virus transmission in an outbreak, though more likely via contamination of the environment and feed, due to their predominantly oropharyngeal shedding, or via turkeys scavenging infected sparrow carcasses.
- Two studies with the HPAI H5N1 strain A/chicken/Hong Kong/220/97 resulted in no mortality and infrequent histopathology lesions in house sparrows and European starlings.[212,213] While mortality among house finches (*Carpodacus mexicanus*) averaged 44%, histopathology 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.

- In another study, house sparrows experimentally infected with A/duck/Laos/25/06 H5N1shed virus for several days and rapidly contaminated their drinking water.[214] On the other hand, inoculated chickens shed undetectable levels of virus into their water troughs, despite high oropharyngeal and cloacal shedding; the authors surmise that this was due to rapid disease progression in the chickens (i.e., the chickens stop drinking water).
    - These authors concluded that sparrows may be unlikely to become infected by chickens under normal field conditions in an H5N1 outbreak.
    - They also inferred that the behavior of infected sparrows may be a determining factor in their potential as intermediate H5N1 hosts via viral shedding.
- In tree sparrows inoculated with A/chicken/Miyazaki/K11/2007 and A/chicken/Shimane/1/2010 H5N1, mortality was 100% 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.[215] While there was no intraspecies transmission among sparrows, 10 of 16 (62.5%) contact chickens died when housed with infected sparrows.
    - Due to the prolonged viral shedding observed here, the authors concluded that tree sparrows have the potential to serve as biological vectors of H5N1.
- Nestorowicz et al. infected house sparrows and starlings 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.[199] Uninfected birds were placed in contact with infected birds of the same species. Transmission to starlings was observed. More details from the experiment are provided in **Table 9** below.

Table 9. Summary of the experimental transmission of H7N7 HPAI virus in house sparrows and starlings by Nestorowicz et al. (1987)[199]

Common name	Mortality	Virus isolation	Transmission
Starlings	100%; all inoculated birds died within 48 hr. post-inoculation	Not reported	Contact birds died within 4 days of being placed with infected birds
Sparrows	30%	Isolated from all tissues from birds that died within 2 days post inoculation	Not transmitted to uninfected contact birds

- 23 of 23 stonechats (*Saxicola torquata*) inoculated with A/Cygnus cygnus/Germany/R65/2006 H5N1 died within 3 to 7 days, most with no clinical signs.[195] Oropharyngeal shedding peaked at 10^3 to 10^4 $TCID_{50}$/ml on 3 to 6 DPI.

9.1.5.1.2 Qualitative Analysis

We considered the following qualitative factors for evaluating this pathway:

- A majority of the studies cited above examine strains of HPAI H5N1.
  - To date, HPAI H5N1 has proven to be unique in its ability to infect a variety of species, and more ubiquitous than any other HPAI virus.
- The risk of AI transmission is much lower from a single infected bird than from a population of birds in which infection is established.
  - As noted in the literature review, surveys of passerine birds have demonstrated a low prevalence of AI virus, including the more pervasive H5N1.
  - Several experimental studies have resulted in no intraspecies transmission in passerine species.[195,210,215]
- Given the preponderance of passerine birds, 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.
- The estimated 50% aerosol infectious dose for turkeys is close to or less than 3 to 4 log EID_{50} , with significantly higher doses deemed necessary for infection via the oral route (see section 8.7.1 Dose Response in Turkeys).
  - The studies cited above demonstrate variability in oropharyngeal and cloacal HPAI virus titers in passerines, depending on the bird species and the H5N1 strain, but when shed titers were measured, most studies indicate they would be adequate to infect turkeys via the aerosol route.
- Biosecurity guidelines dictate measures to prevent wild bird access to turkey barns and maintenance of feed bins such that wild birds are neither frequenting nor accessing turkey premises (see section 7.3.3.5 Animal, Pest, Insect Control).[10,186,188]
  - While it is industry standard to discourage wild birds from accessing poultry barns as part of a company biosecurity plan, assuring that no birds are entering turkey barns is not considered feasible (TWG, personal communication, August 2016).
  - A case series describing 81 turkey farms in Iowa, Minnesota, North Dakota, South Dakota, and Wisconsin with HPAI infections during 2015 found that 33.7% of the farms observed wild birds inside barns; respondents reported seeing wild birds (most commonly starlings and sparrows) inside barns from daily to occasionally.[26,197]
  - Even with proactive industry management, wild birds are known to enter poultry barns. Burns et al. 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.[178]

- For more information on potential for contamination of finished feed products by passerine birds, see USDA-APHIS Poultry Feed Risk Assessment.[198]

- Secondary transmission of HPAI from a passerine bird outside the turkey house is unlikely.
  - As potential biological vectors, passerine birds shed lower cloacal viral titers, and their fecal volume is small.
  - 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[c] on five farms infected with HPAI H5N2 virus and five uninfected farms in Iowa indicates that mechanical transmission through contamination of the external surface 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).[26] As potential mechanical vectors, Passeriformes, due to their small size, can transfer a very small volume of contaminated feces from an infected turkey premises.

9.1.5.1.3 Likelihood Rating and Conclusion

While passerine and columbiforme birds 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, and they have demonstrated the ability to enter secure turkey houses. Thus, the likelihood of HPAI infection via passerine birds in the farm vicinity is *low to moderate.*

9.1.5.2 Likelihood of Infection via Other Non-Aquatic Bird Species in Farm Vicinity

Other non-aquatic avian species such as birds of prey or scavenger species vary greatly in number and behavior around poultry farms. Unlike passerines or columbiforme species, these birds are unlikely to be able to enter poultry barns, resulting in only secondary transmission pathways. Several studies have clearly shown that flying birds transport viruses such as HPAI H5N1.[32] These birds might have contact with manure stored outside the poultry house or manure that is land-applied. Although the quantity of manure wild birds can carry is unknown, as well as the host adaptability of other HPAI virus strains to different wild bird species, 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 an infected prey item, or potentially pick up and move that prey item to a location closer to a turkey premises.

Common predator and scavenging wild birds undertake 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.[32] Scavenger species may be attracted to premises with improperly secured daily mortality carcasses. Species known to scavenge avian carcasses in the U.S. considered in this assessment include vultures, some hawks and eagles, crows, ravens, and magpies. Some gull species known to scavenge are covered separately in section 9.1.4, Role of HPAI Spread to Turkey Flock in a Control Area via Wild Aquatic Birds in Farm Vicinity.

[c]220 individual birds across 18 species on infected farms, 199 individual birds across 16 species on uninfected farms

Finally, wild galliformes also may be attracted to poultry operations.

- In a survey of infected turkey farms during the 2015 outbreak in Iowa, Minnesota, North Dakota, South Dakota, and Wisconsin (n=81), 26% reported seeing wild turkeys, pheasants, and quail around their poultry barns.[26]

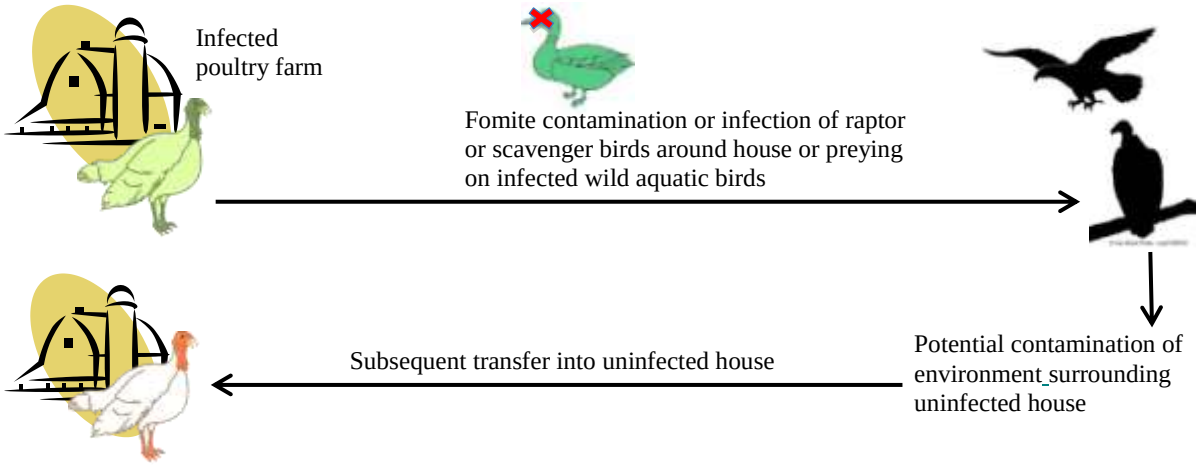


Figure 9. Pathway for exposure of a turkey farm via scavenging birds or raptor species. A similar process could be demonstrated for wild gallinaceous birds.

9.1.5.2.1 Literature Review

Non-passerine non-aquatic birds have not been directly implicated in the spread of HPAI in previous outbreaks, and few such birds have tested positive for AI in the vicinity of outbreaks in poultry or wild waterfowl.

- 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 and 22 black vultures from the quarantine zones were tested for H5N2 and none were positive.[216]
- 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 the quarantine area did not support this.[175]
- Raptors found dead during an H5N1 outbreak in wild water birds in Germany in 2006 revealed evidence of H5N1 infection in common buzzards and peregrine falcons.[217] The authors hypothesize that in this H5N1 outbreak in wild water birds, raptor exposure and mortality likely occurred more often in species that hunted or scavenged 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 is encephalitis.
- No infection was found in other species tested (Eurasian sparrow hawk, common kestrel, white-tailed sea eagle, undetermined species buzzard, undetermined species raptor, red kite, rough-legged buzzard, western marsh harrier, and goshawk).

- Other birds of prey in the order Accipitriformes, such as the common buzzard (*Buteo buteo*), have become infected in previous HPAI H5N1 outbreaks.
  - 10.5% of wild birds testing positive during the 2006 HPAI H5N1 outbreak in Germany were birds of prey, including common buzzards, peregrine falcons, kestrels, and European eagle owls.[218]
    - The buzzards reportedly displayed severe central nervous system infection without systemic virus distribution (unpublished data).
  - An HPAI H5N1-positive common buzzard carcass found in Bulgaria in 2010 contained no gross pathological lesions, suggesting the bird died shortly after infection and would not have served as a reservoir of infection.[219]
- 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.[27,148] Out of 100 positive birds, only seven were of non-passerine non-aquatic species (**Table 10**).

Table 10. HPAI H5-positive samples from non-passerine non-aquatic species collected from December 2014 to June 2015 in the U.S.[27]

Date	Species	Lineage	Sampling type (location)
4/14/15	Cooper's hawk	EA/AM H5N2	Mortality (MN)
4/13/15	Snowy owl	EA/AM H5N2	Mortality (WI)
1/20/15	Bald eagle	EA H5N8	Mortality (ID)
1/9/15	Red-tailed hawk	EA/AM H5N2	Mortality (WA)
12/31/14	Red-tailed hawk	EA/AM H5N2	Mortality (WA)
12/29/14	Cooper's hawk	EA/AM H5N2	Mortality (WA)
12/29/14	Peregrine falcon	EA H5N8	Mortality (WA)

- There also were cases of HPAI confirmed in captive wild birds (**Table 11**).[220]

Table 11. HPAI-positive samples from captive wild birds in the U.S.[220]

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

- Similarly, cases of HPAI in captive falconry birds in Dubai suggest that these raptors were likely infected through consumption of infected farmed or wild prey items.[221]
- 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 gyrfalcons and hybrid gyr/peregrine falcons.[221]

Surveillance of non-passerine non-aquatic birds for AI virus has demonstrated zero to low prevalence in the wild.

- A 2010 survey of antibodies to AI in wild birds revealed zero positives out of 184 black vultures sampled in Mississippi.[89] The authors note that nearly all species of terrestrial birds tested were negative for AI antibodies.
- A survey of antibodies to influenza A in 616 raptors admitted to two U.S. wildlife rehabilitation centers, and 472 peregrine falcons caught at a migratory banding station, found relatively low prevalence of antibodies (subtype not described) in a variety of species. Results of the survey are summarized in the table below (**Table 12**).[222]
    - Antibodies to influenza A (subtyping not possible due to low HI ratio in sera) were found in bald eagles, peregrine falcons, great horned owls, and Cooper's hawks.
    - No influenza A antibodies were found in turkey vultures or black vultures.

Table 12. Serologic evidence of influenza A in raptors admitted to two U.S. wildlife rehabilitation centers[222]

Species	Number tested	Number positive	Percent positive
Bald eagle	406	22	5.1
Peregrine falcon	472	1	0.2
Great horned owl	81	1	1.2
Cooper's hawk	100	1	1.0
Turkey vulture	21	0	0
Black vulture	8	0	0

- Gunnarson et al. (2010) sampled nestling white-tailed sea eagles (n=181) and peregrine falcons (n=168) in Sweden for influenza A infection.[223]
    - No evidence of infection or antibodies was detected in any samples.
    - Authors acknowledge that maternal antibodies last less than 3 weeks in nestlings, and that sampling older nestlings that haven't fledged may be a less sensitive screening population than adult birds.
- Peterson et al. (2002) found a 0% prevalence of AI virus in 70 wild turkeys (*Melleagris gallopavo*) in a survey in Texas.[224]
- A study of wild captured or hunter-harvested wild bobwhite quail (*Colinus virginianus*) in Texas found prevalence of 1.4% using rRT-PCR; however, no virus could be isolated from culture.[225]

Experimental susceptibility of non-passerine birds to HPAI is relatively unstudied.

- Experimentally infected American kestrels (*Falco sparverius*) with H5N1 (A/whooperswan/Mongolia/244/05) demonstrated 100% mortality within 7 days of inoculation.[226]
    - The American kestrels demonstrated oral viral RNA shedding and infectious virus and, to a lesser extent, cloacal shedding. Infectious viral particles as detected by embryonated egg inoculation were not detected in cloacal samples.
    - Seroconversion occurred by 4 to 5 DPI.
    - The most consistent histopathological lesions occurred in brain and pancreas; all infected birds had some evidence of meningitis and encephalitis.
- In commercial Japanese quail, Chukar partridges, ring-necked pheasants, and Guinea fowl experimentally infected with H5N8 (A/GF/WA/14) and H5N2 (A/NP/WA/14), there was 100% mortality, with a mean time to death of 2.5 to 3 days (M. Pantin-Jackwood, personal communication, November 2015).
    - Clinical signs included listlessness and ruffled feathers.
    - Histopathological signs included necrotic pancreas, mottled spleen, petechial hemorrhages on the myocardium, and pulmonary hemorrhage.
    - Conspecific birds (i.e., birds belonging to the same species) placed in contact with infected partridges or pheasants also showed high mortality from both H5N8 and H5N2 infections.

9.1.5.2.2 Qualitative Analysis

We considered the following qualitative factors in evaluating this pathway:

- To date, HPAI H5N1 has proven to be unique in its ability to infect a variety of species, and more ubiquitous than any other HPAI virus.
- 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, low circulating antibodies, solitary living patterns, and apparent rapid mortality in raptors make risk of spread within these predatory species less likely.

- As noted in the literature review, surveys of birds of prey and scavenging birds have demonstrated a low prevalence of AI virus, including the more pervasive H5N1.

- Given that some scavenger and other non-passerine species may have relatively large home ranges,[227-229] 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 in the dead bird disposal section (see section 9.2.4, Likelihood of Turkey Flock Becoming Infected with HPAI 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 turkey barns, manage dead bird disposal, and maintain feed bins such that wild birds are neither frequenting nor accessing turkey premises (section 7.3.3.5, Animal, Pest, Insect Control).[10,186-188]
  - Given proper biosecure disposal of dead birds on turkey premises and the fact that non-passerine and non-columbiforme species have not been observed inside turkey houses, direct contact with live poultry is not common given standard biosecurity measures in place in the turkey industry (TWG, personal communication, August 2016). However, turkey carcasses may remain uncovered outside turkey houses or storage containers for hours prior to biosecure disposal (TWG, personal communication, August 2016). For evaluation of the risk associated with potential wild bird contact with turkey carcasses, see section 9.2.4, Likelihood of a Turkey Flock Becoming Infected with HPAI via Dead Bird Disposal.

9.1.5.2.3 Likelihood Rating and Conclusion

Other (not passerine or columbiforme) non-aquatic bird species have the potential to contract HPAI virus and have home ranges of adequate size to contain adjacent turkey farms where they potentially may access contaminated carcasses, manure, or other material at an infected turkey premises. However, they are unlikely to have direct contact with poultry flocks if standard biosecurity measures are in place, and their ability to shed virus has not been studied in many species. For the above reasons, the likelihood of HPAI infection via non-passerine non-aquatic birds in the farm vicinity was rated as *low*.

9.1.6 Role of HPAI Virus Spread to Turkey Grow-Out Premises near Poultry Live-Haul Routes via Feathers, Feces, and Other Fomites

The evaluation of the risk of HPAI virus transmission to turkey grow-out premises in a Control Area near poultry live-haul routes assumes the release of potentially HPAI-contaminated material from live-haul trailers along roadways and transportation routes in close proximity to a turkey grow-out premises. The birds in transit may originate from premises inside or outside the Control Area. This evaluation is written specifically for turkey grow-out premises. However, since multiple poultry commodity operations involve live-bird movements, including turkeys to market, broilers to market, spent layer hens to market, layer pullets to egg production, turkeys

from brood to grow-out, and breeder movements, the concepts here can be translated across these other live-bird movements.

As a requirement of the Secure Poultry Supply Plan, the Pre-Movement Isolation Period (PMIP) decreases the likelihood of permitted movement of infected but undetected flocks within a Control Area. Additionally, turkey grow-out premises in a Control Area that wish to request permitted movement must 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 7: Cross-Commodity Pre-Movement Isolation Period).

9.1.6.1 Risk of HPAI Virus Transmission to Turkey Grow-out Premises near Poultry Live-Haul Routes

The transport of an infected but undetected flock near a turkey grow-out facility represents a potential pathway for local area spread. HPAI-virus transfer to premises near the live-haul route could occur via HPAI-contaminated feathers, feces, and other fomites, which may contaminate turkey premises close to the route and may subsequently be tracked into turkey barns. The two specific pathways identified are: (1) HPAI-contaminated fomites from a live-haul truck blow into, or are tracked into, turkey premises and introduce virus to a turkey flock, and (2) a contaminated live-haul road results in a vehicle bringing virus into turkey premises and subsequent virus transfer into a barn. **Figure 10** diagrams the exposure pathway.

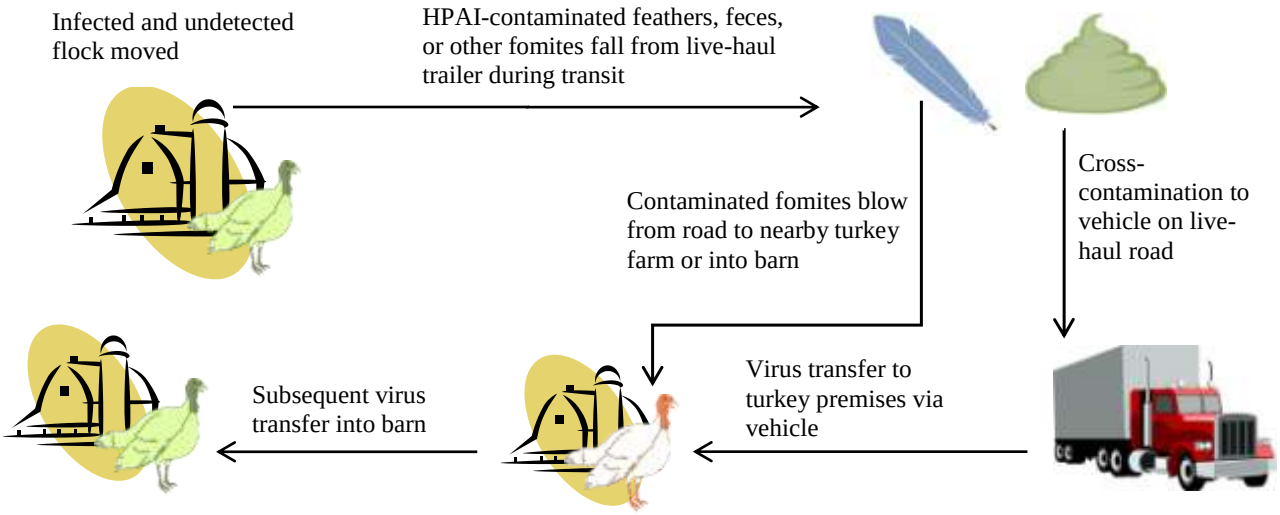


Figure 10. Pathway for exposure of a turkey grow-out premises via fomites originating from nearby live-haul route

- If infected poultry are transported to processing, the extent of virus contamination available to infect a flock near the live-haul route is affected by virus shedding, virus persistence, and quantity of virus transferred between 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 mL or per gram of tested substrate, although higher concentrations have been observed in some cases. Various units of measure are used.

- Immature feathers: In chicken feathers, the median viral titers for three HPAI H5N1 virus strains tested (A/duck/Sleman/BBVW-1003-34368/2007, A/duck/Sleman/BBVW-598-32226/2007, and A/Muscovy duck/Vietnam/453/2004) were ~10^5, ~10^6, and ~$10^{5.7}$ $TCID_{50}$/0.1mL for immature pectorosternal feathers, immature flight feathers, and immature tail feathers, respectively, in broilers inoculated with feather samples that had been ground with a mortar and pestle.[230] From chicks inoculated with an HPAI H7N1 strain (A/Chicken/Italy/5093/99) at 15 days old, viral RNA load was higher in feather pulp than in oropharyngeal and cloacal swabs for most days tested post-inoculation.[231] Feather pulp was obtained by squeezing the calamus (i.e., the feather quill).[231] 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 (39.2°F) for the 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 inoculated (Ck/Miya/K 11/07, Ws/Akita/1/08) 7- and 8-week-old chickens.[233] In ducks, 3.8% of mature pectorosternal feather samples were positive post-challenge, and, of the virus-positive feathers, viral titers ranged from ~$10^{0.6}$ to ~$10^{4.5}$ $TCID_{50}$/0.1 mL.[230] From inoculated (A/duck/Nigeria/1071-23/2007) 24-week-old Pekin ducks, 31.25% of breast and tail feather calami and 37.5% of wing feather calami were positive by rRT-PCR at 3 days post-inoculation.[234]
- Feces: In turkey feces, viral titers were estimated to be between 10^3 and 10^5 EID_{50}/mL with 2015 HPAI H5N2 viruses (A/turkey/MN/12528/2015 and A/chicken/IA/13388/2015) on the basis of cloacal swab data (E. Spackman, personal communication, May 2016).[235] 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).[65]
- Blood: In turkey blood, HPAI viral titers ranged from 10^1 to $10^{5.8}$ EID_{50}/0.1 mL at 1-3 days post-inoculation when turkeys were inoculated with 10^6 EID_{50} of an H7N1 virus strain (A/chicken/Italy/1067/1999).[49]

- Once virus is shed, 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 1: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates.
- This transmission pathway is likely multi-step. The available literature suggests virus concentration decreases when transferred between surfaces. In an experimental setting, mechanical transmission of an enveloped virus after multiple contact steps has occurred.[236]

- Virus transfer between surfaces for non-AI viruses ranges from undetectable to 46% transferred.[237]
- Mechanical transmission via a multiple-step pathway was documented using porcine reproductive and respiratory syndrome virus (PRRSV) in 1 of 10 replicates by virus isolation and in 8 of 10 replicates by RT-PCR at less than 0°C (32°F) in a swine industry-like setting.[236]
    - 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 kinds (cardboard, Styrofoam, metal, and plastic).*
    - PRRSV RNA was detected by PCR in 8 of 10 replicates on three container surfaces (Styrofoam, metal, and plastic) and 7 of 10 replicates on a cardboard container after the final transmission step at less than 0°C (32°F).[236]
    - At 10-16°C (50-60.8°F), infectious PRRSV RNA was detected by PCR in 2 of 10 replicates on the farm anteroom floor.[238]

- Findings from previous disease outbreaks suggest virus transmission to a poultry premises near a live-haul route is possible.
    - In a review of infectious laryngotracheitis (ILT) outbreaks on U.S. broiler operations, some experts have implicated live-haul trucks transporting infectious birds as a probable means of indirect spread to nearby susceptible flocks along the route.[239,240]
    - In the 2002-2003 outbreak of ILT on Mississippi broiler farms, the mean distance of the nearest live-haul road to case farms was 0.40 miles, while the distance of the nearest live-haul road to control farms was 1 mile (distance to nearest live-haul road [miles]: Odds Ratio=0.54; P-value=0.0392; univariate analysis).[241]
    - In the 1995 outbreak of LPAI H9N2 in Minnesota, spatial observations suggested exposure to the live-haul route used to transport a known infected turkey flock to slaughter was a risk for premises infection (eight of nine premises within 250 meters of the 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.[120,122,242] 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 2: Literature Review on the Role of Local Area Spread in Previous Outbreaks.

- If virus is transferred into a barn, the likelihood of infection is dependent on infectious dose. Mean infectious dose varies with poultry species and virus strain.[92]

- In turkeys, the infectious dose for various avian influenza virus strains was measured at $10^{0.8}$ BID_{50} with an LPAI H7N2 strain (A/turkey/Virginia/15851/02), $10^{2.2}$ BID_{50} with an HPAI H7N1 strain (A/ostrich/Italy/984/00), 10^{4} EID_{50} with an LPAI H7N3 strain (A/turkey/Italy/8000/02), and 10^{5} EID_{50} with an HPAI H5N2 strain (A/Northern Pintail/Washington/40964/2014).[89,235,243,244] With 2015 H5N2 poultry isolates (A/turkey/MN/12582/2015, A/chicken/IA/13388/2015), infectious dose in turkeys was 10^{5} EID_{50} and 10^{3} EID_{50}, respectively.[235]

9.1.6.2 Qualitative Analysis

We considered the following factors in evaluating this pathway:

- While this risk assessment is limited to evaluating risk of HPAI infection on premises located within the Control Area, poultry transport on routes passing through the Control Area may include flocks originating inside or outside the Control Area, which have different movement requirements.
  - Permitted terminal and transfer movements of live poultry originating from within a Control Area likely will require movement from a Monitored Premises, 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% probability or greater of detection in flocks exposed to HPAI virus before the PMIP begins, given a 100% effective PMIP (see Appendix 7: Cross-Commodity Pre-Movement Isolation Period). As an example of movement originating from inside the Control Area, **Table 13** shows simulation results of the detection probability for turkeys with SPS pre-movement testing and PMIP. This modeling assumed a 100% effective PMIP, which prevents flock exposure to virus during the PMIP. For modeling with a PMIP that is not 100% effective, see Appendix 10: Supplementary Testing Protocols.
  - Premises located outside a Control Area may not be subject to permitted movement. There may be variation in pre-movement testing as states or Incident Command may require testing for poultry movements from premises in the Free Area,[117] 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 of 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 consists of rRT-PCR testing of 2 pools of 11 swabs and a mortality trigger of 3 birds per 1000. Simulation results for turkeys are shown in **Table 13**. When detection by a mortality trigger is obtained depends on transmission parameters and virus characteristics.[245] In the models for movement originating outside a Control Area, 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 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).

Table 13. Detection probabilities for turkeys using three biosecurity and surveillance protocol scenarios[a]

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

[a]Probabilities estimated from 6,000 simulation iterations using MN HPAI H5N2 strain characteristics and considering virus exposure within 10 days of movement.

- If infected poultry are transported to processing, the initial contamination for this pathway depends on HPAI-contaminated material falling from the live-haul trailer. Feathers, feces, and other potential fomites fall from live-haul trailers because they are not enclosed (D. Halvorson, personal communication, July 2016), as shown in **Figures 11, 12,** and **13**. Day-old chicks and poults are transferred in different vehicles and are totally enclosed.
    - For permitted movement from premises in an HPAI Control Area, the STS Plan permit guidance for the movement of turkeys states that drivers and trucks must be biosecure.[10] There are no specific biosecurity measures to prevent virus-laden fomites from falling from live-haul trailers.
    - Netting systems to contain feathers in live-haul trailers typically are not used because they are ineffective and create an additional biosecurity problem as nets are difficult to clean. Thus, nets were not used on live-haul trucks during the 2015 or 2016 U.S. HPAI outbreaks (TWG, personal communication, July 2016).

Figures 11-13 show the openings in crates used for live-haul in the turkey and broiler industry.

Figure 11. Live-haul trailer of turkeys after load-out (Photo: anonymous).

Figure 12. Live-haul trailer of turkeys (Photo courtesy of Jill Nezworski).

Figure 13. Crates filled with broilers to be loaded onto a live-haul truck (Photo courtesy of GNP Company).

- The likelihood of this contamination reaching a premises and infecting a flock may depend on the distance of the premises from the live-haul road, weather conditions, natural barriers/landscape, and virus transfer steps.
    - Close proximity to a road seems quite likely for the majority of poultry premises, regardless of species.
        - Among turkey premises, a case-control study following the 2016 H7N8 outbreak in Indiana reported a majority of farms were located within 499 yards of the nearest public gravel or dirt road (55% of case farms were within 499 yards [0.28 miles] of a public road; 60% of control farms were within 499 yards of a public road).[246]
        - In a survey of operators of broiler and breeder-layer chicken premises in Georgia, similar distances from poultry houses to the nearest public road were identified (68% of growers were within 440 yards [0.25 miles] of a public road).[247]
    - During disease outbreaks, the distance between the live-haul road and poultry premises may be efficiently maximized by routing when possible, or based on company requests. According to members of the STS Working Group, poultry live-haul routes are determined by individual companies based on transit time and safety of the birds, both the birds on trucks and birds on premises in the area (TWG, personal communication, July 2016).
        - 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 (Broiler Working Group, personal communication, August 2016; TWG, personal communication, July 2016).
        - In geographic areas with many poultry production premises, routing may take on increased importance due to the density of susceptible birds near a route.
        - During recent HPAI outbreaks in the U.S., live-haul routing requirements were not needed for approval of permitted movement in 2015 in Minnesota (Minnesota Board of Animal Health, personal communication, October 2016) and were not mandated by Incident Command in 2016 in Indiana (TWG, personal communication, July 2016).
            - However, for permitted movement from premises in an HPAI Control Area, both the STS Plan and the Secure Broiler Supply (SBS) Plan recommend live-haul route approval from the Incident Command team or routes selected in consultation with a poultry veterinarian or production manager.[10,248]
        - 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.[240]
    - The transmission steps of this pathway could be affected by weather conditions, natural barriers/landscape, and cleaning and disinfection.

        - Since feathers are lightweight and turkey grow-out barns are often not fully closed (e.g., many are curtain-sided with screen openings), transmission into barns via feathers over short distances is a possibility. Weather conditions, such as wind and precipitation, as well as natural barriers/landscape between the live-haul route and turkey premises may affect whether virus arrives on farm.
    - Virus transmission from a live-haul trailer to a premises close to the road is likely a multi-step transmission pathway. With each virus transfer step, virus concentration is likely to decrease. Among the potential pathways identified, HPAI-contaminated fomites that blow to a turkey premises from a live-haul trailer directly into the barn involves fewer transfer steps compared to fomites blowing onto a premises (not into the barn) or a vehicle bringing virus to a turkey premises from a contaminated live-haul road, followed by subsequent transfer into the barn.

- Biosecurity measures at turkey grow-out premises reduce the likelihood of this contamination reaching the premises and infecting the flock. These biosecurity measures are NPIP Biosecurity Principles; Level 2 Biosecurity, as recommended by the STS Plan following an outbreak of HPAI; and the greatly intensified biosecurity of the PMIP for premises in a Control Area that wish to request live bird movement.
    - Standardized biosecurity in the poultry industry, such as rules about entering the perimeter buffer area (PBA), crossing the line of separation (LOS), and managing vehicle access, are intended to prevent flock exposure to diseases.[1]
    - STS Plan Level 2 Biosecurity measures (such as visitor restrictions; vehicle and equipment inspection and C&D; turkey barn entry restrictions; and turkey barn entry PPE and C&D) are intended to provide a high level of biosecurity.[10] Additional biosecurity recommendations are detailed for premises located in the Infected Zone, such as the use of a designated entry and exit checkpoint at the perimeter of the Infected Zone.[10]
    - For turkey grow-out premises in a Control Area that wish to request permitted movement of live birds, the enhanced biosecurity of the PMIP minimizes the chances of a flock being exposed to HPAI for the 8-day period prior to movement. The PMIP reduces the likelihood of a vehicle contaminated from a live-haul road bringing virus to a turkey operation, as all vehicles will be cleaned and disinfected before entering the premises. A requirement to use barn-specific footwear to enter the poultry house during the PMIP minimizes introduction of virus via tracking into the barn 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
        - Replacing all non-critical operational and routine visits with telephone communication or scheduling such visits outside the PMIP (see Appendix 7: Cross-Commodity Pre-Movement Isolation Period).
    - Vehicles and equipment arriving on a turkey premises may be difficult to disinfect thoroughly, especially during harsh winter conditions. Thus, virus may remain on vehicles contaminated from the live-haul route, despite C&D steps.

- Previously, ten experienced poultry veterinarians evaluated the risk of infecting susceptible poultry flocks via the microbial load from two truckloads of turkeys shedding a generic pathogen at varying distances (results shown in **Table 14**).[249]

Table 14. 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[249]

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 available for transmission, initial titer of the pathogen, age of contaminant/half-life of virus, and distance to susceptible poultry.[249]

9.1.6.3 Risk Rating and Conclusion

9.1.6.3.1 Risk of HPAI Transmission to Turkey Grow-Out Premises in a Control Area near Route of Live-Haul Trailers

Literature review and expert opinion indicate a potential for increased risk when a poultry premises is located close to live-haul routes for transporting infectious birds. The requirements for permitted movement of live birds in the SPS Plan, specifically implementing an effective PMIP, increase the likelihood of detection prior to scheduled movements that originate in a Control Area. Vehicles transporting live poultry from a Monitored Premises that has met the requirements of the SPS Plan (PMIP, PCR testing) are less likely to represent an infected but undetected movement than if the PMIP and testing were not in place. As presented in Appendix 10: Supplementary Testing Protocols, it is also unlikely that flocks moved after a PMIP and testing would contain large numbers of clinically infected birds.

During the 2015 HPAI outbreak in the U.S., 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, biosecurity and pre-movement testing practices in Free Areas may differ from those in Control Areas. 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.

9.1.6.3.2 Conclusion

The risk of HPAI virus spread to turkey grow-out premises near poultry live-haul routes via feathers, feces, and other fomites depends on both distance and source flock. Considering the above factors, assuming that the preventive measures specified in the SPS Plan are strictly followed when moving live poultry, and given that live-haul vehicles passing a premises within the Control Area may originate from within or outside the 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 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 a Turkey Flock Becoming Infected with HPAI via Movements of People, Vehicles, or Equipment

9.2.1 Likelihood of a Turkey Flock Becoming Infected with HPAI via Critical Operational Visits during PMIP

Routine operational visits to a farm include feed delivery, gas delivery, veterinarians, shavings suppliers, meter readers, repairmen, service personnel, managers, vaccination crews, clean-out services, de-caking services, and other visitors. The Secure Poultry Supply Plan Cross-Commodity PMIP requires most of the operational visits to occur outside of the PMIP before moving turkeys to market. However, some critical operational visits, such as feed delivery, emergency repair of equipment, and service visits for bird health, would need to continue during the PMIP with required and specific biosecurity measures in place.[1]

An average of 7 feed delivery visits (range 1-17 visits) was reported over 14 days for 80% (8/10) of case premises with this visitor type in the 2016 Indiana AI outbreak.[30] During this time period in the same outbreak, no repair or maintenance visits occurred, and the frequency of these visits in the broiler industry varied between 2 and 5 times a year.[30,250,251]

9.2.1.1 Likelihood of Infection via Feed

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 and contaminated with wild bird feces raised concerns 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.[198]

Adherence to biosecurity measures in the STS Plan (Level 1), recommended prior to an HPAI outbreak, reduces potential attraction of and contamination from wild birds and rodents by securing feed bins and promptly cleaning up spilled feed.[10] Turkey industry feedback indicated the practices in response to a feed spill may depend on the size of the spill during normal operations, but during an HPAI outbreak, spilled feed would likely be discarded (TWG, personal communication, August 2016).

On most turkey operations, feed programs consist of many different feeds (typically 5 to 7 or more) fed to turkeys in intervals of 2 to 4 weeks each, with variations depending on the growth and development of turkeys.[4] After 2 to 4 weeks of age, commercial turkeys are typically fed pelleted feed and, as turkeys grow, larger pellets are used.[4,198] The Feed Risk Assessment assessed the risk of HPAI transmission to poultry fed contaminated feed in a variety of scenarios listed in **Table 15**. Further information can be found in the Feed Risk Assessment.[198]

Table 15. Assessed risk of HPAI transmission to poultry fed contaminated feed in listed scenarios (pathways). Adapted from the Feed Risk Assessment[198]

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

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

During outbreak situations, feed trucks used on turkey premises are commonly shared with and used to service other farms, as reported from 2015 and 2016 HPAI outbreaks.[26,30] A quantitative estimate of turkey premises connectivity has not been documented to our knowledge, but in the broiler industry, under normal operations, Dorea et al. estimated feed trucks may visit a range of 0 to 5 premises daily.[251]

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 turkey finishing 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
- Emergency repair of critical mechanical equipment
- Service visits to address changes in bird health.

It has been suggested that company feed delivery should involve dedicated trucks for use in the Infected Zone and that feed delivery routes should avoid infected farms. Additionally, trucks within the Control Area should be limited to a single premises delivery per load rather than delivering feed to several farms on a route.[4] In addition to feed delivery, other critical operations visits (emergency maintenance and bird health service visits) are assumed to offer a similar potential pathway to that of feed trucks.

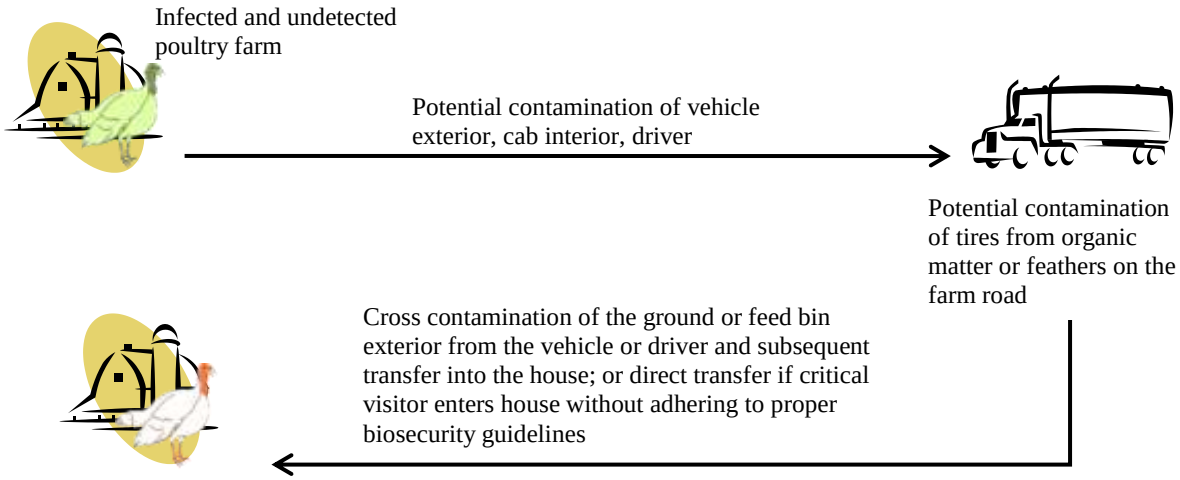


Figure 14. Pathway for exposure of a turkey farm via feed delivery or another critical operations visit

9.2.1.2.1 Literature Review

- Past AI outbreak data indicate a high frequency of feed delivery visits. Feed delivery was the most commonly reported visitor type at case turkey premises in the 2015 HPAI outbreak, occurring at 83% (n=41) of premises over an 8-day period.[26] A similar frequency was reported at case-control turkey and chicken premises in the 2002 LPAI H7N2 outbreak in Virginia.[70]
    - At case turkey premises in the 2016 Indiana H7N8 AI (LPAI and HPAI) outbreak with at least one feed visit, the average number of feed delivery visits was 7 visits in 14 days.[30]
    - Maintenance visits and service visits for bird health have been reported to occur less frequently than feed visits. Construction, repair, and maintenance workers did not visit any case turkey premises (n=10) in the Indiana 2016 AI outbreak over a 14-day period, and only 1-2 case premises had a visit from a veterinarian (or extension agent) during this time. [30]
- Between-farm transmission models and previous poultry disease outbreak epidemiology have assessed feed delivery risks.
    - In a model by Leibler et al., feed delivery accounted for 74% of total point estimates of risk for broiler farms using the same integrator as index farm, based in part on reports from surveyed broiler growers that feed was delivered once every 2 days (range 1 to 5 days).[250] 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 among Georgia broiler farms of HPAI virus by visitors was most frequently associated with feed trucks and company personnel.[251]

- On a per-contact basis, incorporating outbreak data from the HPAI H7N7 Netherlands outbreak, Ssematimba et al. estimated a probability of infection of 0.0414 (95% CI, 0.0043 to 0.085) for each feed delivery contact and attributed 2.63% of infections in the outbreak to this contact type. [252]
- 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).[253]
  - However, in the 2002 LPAI H7N2 outbreak in Virginia on turkey and chicken premises, feed truck visits occurred at a significantly higher frequency in a 2-week period at case premises than control premises (p=0.05; univariate analysis). But this question was excluded from multivariate model consideration due to limited data. [70]
- During the second series of outbreaks in the Italy AI (LPAI and HPAI) epidemic of 1999-2000, movement of feed vehicles was linked to virus spread.[254] Capua et al. noted that the semi-vertical integration system of the Italian poultry industry results in feed trucks often visiting multiple farms daily.[255]
- In a model of risk for ILT infection during an outbreak on broiler farms in Mississippi, farms with more visits per month by feed trucks were associated with higher risk for ILT (OR=1.18; P=0.0099; univariate analysis).[241]

- Transmission models have also estimated the risks of potential contacts for other critical operational visits.
  - Based on visitor type parameter estimates of frequency, probability of contamination, and number of farms visited, a stochastic model estimated that servicemen contribute ~26% or ~8% to HPAI spread between Georgia broiler farms, depending on farm density.[251]
    - In the LPAI H6N2 outbreak in California from 2000 to 2002, virus likely spread between premises in part through the use of common service crews.[128]
  - In contrast, a model by Leibler et al. estimated a 0% contribution of contacts via maintenance visits to total exposure risk at broiler premises in a U.S. poultry-dense region.[250]

9.2.1.2.2 Qualitative Analysis

We considered the following factors in evaluating this pathway:

- The cross-commodity PMIP outlines biosecurity practices for critical operational visits during the PMIP.[1]
  - People who have contact with other poultry must shower and change clothes before entering the premises and also wear necessary protective clothing and footwear as described in appropriate biosecurity protocols.
  - All vehicles and equipment will be cleaned and disinfected prior to entering premises.
- Feed truck visits and feed delivery are still likely to occur at a high percentage of turkey operations during the PMIP.

- While feed truck visits will be the most frequent type of contact during a PMIP, they are subject to specific biosecurity guidelines outlined in the STS plan, in addition to those stipulated by the cross-commodity PMIP (see STS Plan for a full list of biosecurity requirements relevant to feed trucks and drivers).[10]
    - Under normal operations, the STS Plan (Level 1) recommends that feed delivery drivers should be prohibited from entering turkey houses and that feed delivery vehicles should be routed in consultation with a poultry veterinarian.
    - During an HPAI outbreak in the region, recommendations of the STS Plan (Level 2) focus on truck and driver biosecurity to reduce the risk from potentially contaminated feed delivery moving between premises. This includes conducting hand hygiene, disinfecting the vehicle interior, disinfecting bottoms of feet, wearing boots on-farm, and inhibiting potential fly transmission aided by vehicle transport.
    - The STS Plan (Level 2) recommends heightened biosecurity within the Infected Zone in an HPAI outbreak to control or restrict movements of people and vehicles, and calls for prohibiting split-load feed deliveries.
- Relative to feed deliveries, however, other critical operational visits (e.g., emergency repair of critical mechanical equipment and service visits to address changes in bird health) likely happen less frequently, and visitors may or may not have visited poultry farms recently.
    - The STS Plan (Level 2) recommends biosecurity measures for some potential contact types of critical operational visits, with additional cross-commodity PMIP requirements if the visit were to occur during a PMIP.[10]
        - Service technicians, service crews, and veterinarians, according to the STS Plan (Level 2), in an outbreak should avoid exposure to equipment from other farms that has not been washed and disinfected, and should conduct hand hygiene after contact with several potentially infectious or contaminated sources.
- Critical visitors other than those associated with feed delivery may be required to enter a poultry house to complete their necessary tasks. Visitors who enter poultry houses during a PMIP may contact birds directly, thus decreasing the number of steps in the potential pathway to infection diagrammed above.
    - To enter poultry houses during PMIP, the cross-commodity guidelines require farm-specific clothing and barn-specific footwear.
    - Additionally, the STS Plan (Level 2) recommends farm-specific head coverings for service technicians, service crews, and veterinarians and the use of disinfection stations for visitors and contract laborers.[10]

9.2.1.2.3 Likelihood Rating and Conclusion

Critical operations visits will be limited during PMIP; however, delivery of feed during this period is likely, and the potential for emergency maintenance visits and service visits to address bird health also exists. Assuming all requirements for biosecurity during PMIP are followed, the likelihood of introducing HPAI virus to a turkey flock by feed, feed delivery, and other critical operations visits during PMIP is as follows:

Pathway	Risk
Contaminated feed	*Negligible*
Feed delivery (driver and/or vehicle)	*Low*
Other critical operations visitor (personnel and/or vehicle)	*Low to Moderate*

9.2.2 Likelihood of a Turkey Flock Becoming Infected with HPAI via People and Their Vehicles Entering the Premises

During a PMIP, all non-critical visitors are prohibited from entering turkey premises, and thus, vehicle and visitor traffic is likely to include only growers, employees, and critical visitors.[1] In this evaluation of people (namely growers and employees), the risk of people entering the poultry house and people not entering the poultry house was assessed separately. In previous outbreaks, off-site movements of poultry growers, their families, and their employees have been implicated as risk factors for disease transmission.[70,241]

Following an outbreak of HPAI, the STS Plan (Level 2) recommends heightened biosecurity for growers and employees who are in contact with the turkeys or the turkey premises.[10] During the PMIP, visiting other poultry farms is prohibited for people who work on poultry farms.[1] Off-site social contacts with other people may still occur.

For a discussion on critical operations visitors and their vehicles that may continue during PMIP, see section 9.2.1 Likelihood of a Turkey Flock Becoming Infected with HPAI via Critical Operational Visits during PMIP. For a discussion of any critical tools or equipment brought onto poultry premises during these visits, see section 9.2.3 Likelihood of a Turkey Flock Becoming Infected with HPAI via Machinery or Equipment Shared between Multiple Premises.

9.2.2.1 Likelihood of Infection via Movement of People Who Enter Poultry Houses

Social contact data reported among growers from other sectors of the poultry industry conservatively indicate the potential for cross-contamination via off-site movement of people in the turkey industry.[247,250] Variation in frequency of off-site contact among people is expected regionally.

The possible pathways for transmission via social contacts between people involve contamination of the person's clothes, shoes, hands, or vehicle at a meeting place with another person who came from an infected but undetected farm, and subsequent cross-contamination of a virus-free poultry premises. These pathways are shown below in **Figure 15**.

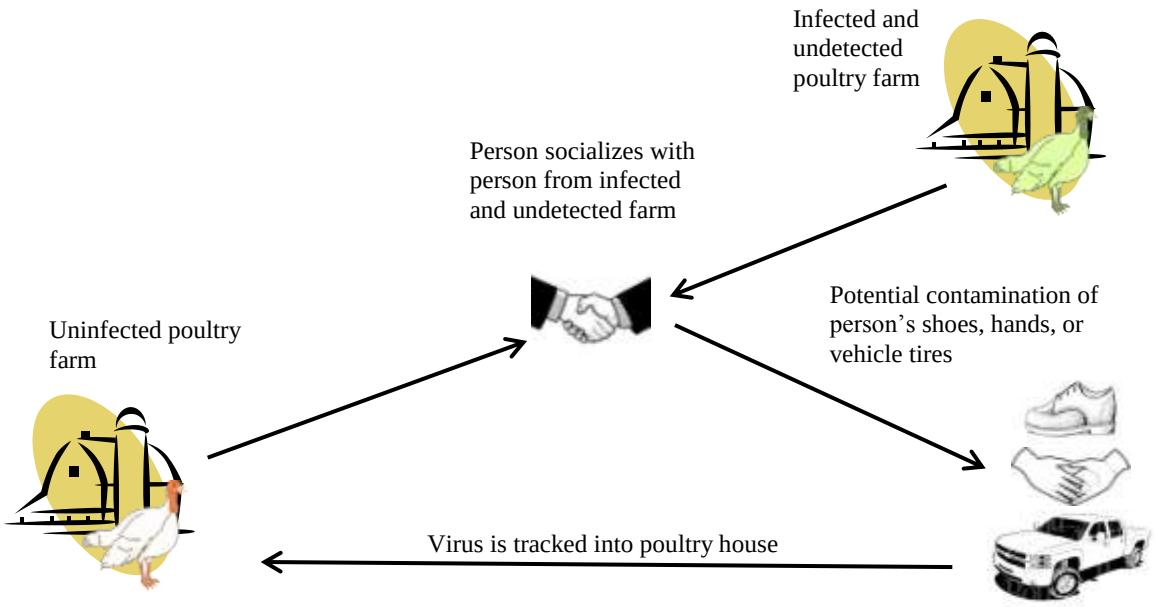


Figure 15. Pathway for exposure of a poultry premises due to virus introduction by people

9.2.2.1.1 Literature Review

- Social contacts between people have been evaluated as a risk for disease transmission in the poultry industry. Analytical studies specific to the turkey industry, including on the frequency of such contacts, are lacking.
    - In a study combining a national survey of broiler poultry growers and stochastic disease modeling, Leibler et al. (2010) determined that broiler grower social contacts contribute less than 1% to the AI transmission risk attributable to contacts between farms, based on an estimated frequency of social contacts of once monthly.[250]
        - The range in frequency of social visits among broiler growers reported by Leibler et al. was 10 times a month to no visits. [250]
        - However, a similar survey of broiler and breeder-layer growers in Georgia reported a higher frequency of personal interactions among growers, which demonstrates the likely variability of this parameter. In the two Georgia counties surveyed by Vieira et al., 49% (25/51) of broiler respondents reported personal interactions with another grower in the previous week, and 9.8% (5/51) reported 5 or more personal interactions with another grower in the previous week.[247]
        - Thus, the risk estimate by Leibler et al. may be too low. Nonetheless, the social contacts in Leibler et al. (2010) were "at a given visitor-receiving farm," and the PMIP measures prohibit such on-farm visits.[250]
    - In addition to other factors, Dunn et al. (2003) noted that there were significant social ties between growers in the 2001-2002 H7N2 LPAI outbreak in Pennsylvania in broiler chickens. Five infected flocks had growers who all belonged to the same church group, two flocks were managed by brothers, and two others were managed by people with close social and business ties.[256]

- Specifically, people accessing the poultry house have been identified as a risk factor for AI infection. In their study of risk factors in the spread of LPAI H7N2 in the 2002 Virginia outbreak on turkey and chicken farms, McQuiston et al. (2005) found the likelihood of infection to be significantly higher among farms using nonfamily caretakers to work in the poultry houses, with an odds ratio of 2.1 in the multivariate analysis (case farms: 45.7%; control farms: 30.4%).[70]

- HPAI virus has the potential to be transmitted via feces-caked 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 1: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates.
  - At low ambient temperatures of 4.0-6.7°C (39-44°F) and low to moderate relative humidity (15.2 to 46.3%), HPAI H5N1 (A/Vietnam/1203/2004) in chicken feces remained viable until day 13.[257] Data on AI virus survival in turkey feces is not in the published literature to our knowledge.
    - However, at temperatures closer to summer conditions in the United States (72.3-74.6°F and 89.1-91.2% relative humidity), the same HPAI H5N1 virus strain in chicken feces was inactivated at day 4.[257]
  - On two rubber surfaces (gumboot and tire) at an unspecified room temperature, LPAI H13N7 was below the detectable limit at day 6.[258]
- 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 the house on another farm (without cleaning and disinfecting shoes) to be P= 0.039 to 0.15 per transfer event.[259]
    - The model was based on small-scale broiler farms in Indonesia, and model parameters were estimated from survey data, literature review, and expert opinion.
    - Variables affecting the risk estimation included 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.
  - 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.
- Respiratory viruses can be transmitted via human hands, though studies with HPAI virus are lacking.
  - As detailed in Appendix 5 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.[237] Depending on the virus, percentage transferred via fingerpads ranged from undetectable to 23%.
  - Ansari et al. (1991) demonstrated that 20 minutes after deposition on donor fingertips, 0.7% of human rhinovirus transferred to recipient fingertips.[260] On the other hand,

transfer of human parainfluenza virus was undetectable at 20 minutes post-deposition. Both parainfluenza and rhinovirus are single-stranded RNA viruses similar to influenza.

- Assuming a virus transmission efficiency of 0-20%, and based on data extrapolation from other viruses (including the above study), modeling by Glanville et al. (2010) demonstrated an average 5% chance of a bird being infected with HPAI H5N1 virus via hand contact with someone who had *directly* handled an infected bird at another farm.[259] This estimate applies only to the first bird handled and incorporates the effect of estimated travel time—specific to the study locale in Indonesia—on virus decay.

9.2.2.1.2 Qualitative Analysis

We considered the following qualitative factors for evaluating this pathway:

- There is the potential for growers, farm employees, or members of their households to have regular social or other contacts with other growers, especially in poultry-dense regions. During the PMIP, these contacts will likely occur off the poultry premises.
- The potential level of contamination on the people when they are meeting may vary, and depends in part on the level of on-farm biosecurity at their respective premises.
  - People from turkey, broiler, and layer premises operating under heightened PMIP biosecurity may represent a lower risk. During PMIP, activities with a risk for lateral transmission of HPAI virus are prohibited, and enhanced biosecurity for people must be in place.[1]
  - In a region near an HPAI outbreak, people from commercial turkey premises are recommended to take measures to remove any potential virus contamination before departing the premises, such as disposing of PPE and practicing hand hygiene, in conjunction with the high level of biosecurity (Level 2) on the premises.[10]
  - There are fewer C&D stipulations for people in other sectors of the poultry industry in an outbreak when a PMIP is not being implemented. While it is reasonable to assume that biosecurity may be heightened in the face of an HPAI outbreak, the practices utilized on individual commercial or non-commercial poultry premises will likely vary.
- People who may potentially become contaminated via social contacts should change clothes and shoes before coming into contact with birds on their premises.
  - In a region near an HPAI outbreak, the STS Plan (Level 2) recommends farm-specific clothing, head covering, and footwear for growers and employees who enter turkey houses.[10]
  - During a PMIP, cross-commodity PMIP measures state people must wear clothing dedicated to the farm and shoes dedicated to the barn before entering poultry houses. See Appendix 7: Cross-Commodity Pre-Movement Isolation Period.
- Potential contamination to people's vehicles may be mitigated by biosecurity measures required during normal operations, such as vehicle access and traffic pattern guidelines at NPIP-participating turkey grow-out premises, and the STS Plan (Level 1) recommendation on vehicles parking away from turkey houses.[1,10]

- Truck and driver mitigations are intensified during an outbreak, as recommended by the STS Plan (Level 2), to address contamination of the vehicle cab, hands of the driver, and shoes of the driver and passenger.[10]
- However, during an HPAI outbreak, there is no requirement for C&D of the exterior of vehicles used near poultry barns, unless the premises is located in the Infected Zone.[10]
    - As outlined above, virus may survive days to weeks depending on weather conditions and type of contaminated surface.

- 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%) on a donor surface is transferred to the recipient surface in each direct contact event.[261]
    - As an example, viral contamination on the exterior of a vehicle on an infected and undetected farm, already reduced by dilution outside the house, would undergo multiple transfer steps *(e.g., vehicle tires→ travel to social meeting place→ ground→ tires of vehicle from uninfected farm→ travel to uninfected farm→ ground→ grower's boots→ uninfected barn)* and subsequent viral load reduction.
    - If, however, the social contact resulted in direct contamination and the newly contaminated person later entered the poultry house, fewer contact steps would be needed.
    - In the period before the PMIP begins, growers and employees may visit other poultry premises, thus potentially decreasing the number of transfer steps needed to bring virus onto the premises, where it may be tracked into the barn during PMIP.

- Biosecurity measures for people, such as PPE (coveralls and barn-dedicated footwear), showering, and hand hygiene, have been effective in reducing the likelihood of virus transmission in a previous infectious disease outbreak and experimental studies.[241,262,263]
    - Appendix 6 of the Broiler Hatching Eggs Risk Assessment details the effectiveness of PPE, showering, changing clothes, and hand hygiene in mitigating the transmission of infectious diseases.[261]
    - Post-outbreak questionnaire data from case turkey premises (n=81) in the 2015 outbreak in the Upper Midwest showed that 25.2% of surveyed premises had a changing area where poultry workers took a shower; at 71.8% of surveyed premises, poultry workers wore dedicated laundered coveralls before entering each house; and at 98.1% of surveyed premises, poultry workers wore rubber boots or boot covers in poultry houses.[197]

9.2.2.2 Likelihood of Infection via People Who Do Not Enter Poultry Houses

Movement of people among premises represents potential pathways of virus transmission. However, the risk posed by employees who work exclusively on one poultry premises and have no contact with other commercial or non-commercial flocks is likely lower. Employee data on

the turkey industry from 2015 and 2016 HPAI epidemiological investigations reported 0% (0/81) and 10% (1/10) of surveyed case premises employed workers who are also employed by other poultry operations.[26,30] On 40% (4/10) of surveyed case premises in Indiana in 2016, employees reported working on more than one company farm.[30] The results from these post-outbreak questionnaires highlight other potential risks and cross-contamination sites, such as employee family members working for another poultry operation, employees owning their own poultry, and premises sharing a common break area.[26,30]

Prior to an outbreak of HPAI, the STS Plan (Level 1) recommends that employees of poultry premises should not own other birds, should receive biosecurity training emphasizing the importance of avoiding contact with hunter-killed birds or other birds not owned by their employer, and must comply with 24-hour downtime between bird contact and returning to work.[10] Described below is the potential pathway for a person who has had contact with other birds and then has contact with the poultry on the premises.

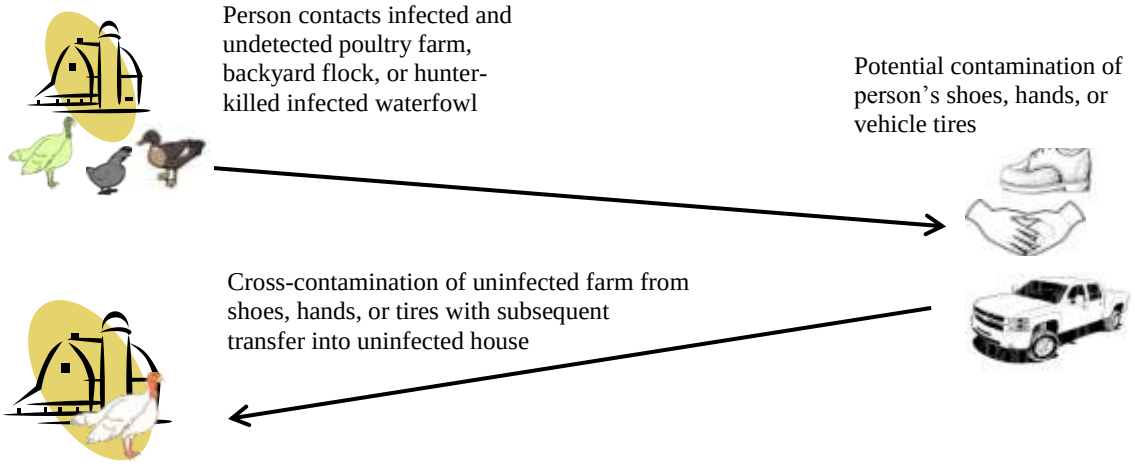


Figure 16. Potential pathway for exposure of a poultry premises due to virus introduction by a person who contacted birds outside the premises of interest

9.2.2.2.1 Literature Review

- 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.
    - In the epidemiological questionnaires and interviews conducted during the 2015 HPAI H5N2 outbreak on pullet and layer premises in Iowa and Nebraska, nine producers suggested potential virus spread via the movement of supervisors or employees who visited many company premises.[177]
    - In the 1999-2000 H7N1 outbreaks in Italy, which included LPAI and HPAI outbreaks in turkeys, broilers, layers, and other poultry types, it has been suggested that temporary staff on larger farms may have contributed to the identification of larger farm size as a risk factor for infection.[122]

- In the 1979 LPAI H7N3 outbreaks in four Texas turkey flocks, Glass et al. reported potential transmission from the index premises to another case farm by shared personnel (i.e., an insemination crew).[264]
- 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).[241]
- 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.[33]

- Previous modeling results indicate potential variation of virus spread in the poultry industry via hired help and day laborers, depending on farm density and virus characteristics.
  - In a stochastic disease model of HPAI spread among Georgia broiler farms, Dorea et al. (2010) estimated that hired help contributed between ~22% (low farm density) and ~37% (high farm density) to the overall spread of HPAI virus via visitors from one infected farm in 1 day.[251]
  - Stochastic disease modeling combined with national survey data on broiler poultry growers estimated that the relative risk of exposure to avian influenza for broiler farms employing day laborers ranges from 3.8 (2-day viral survival on a vehicle, within the same integrator as index farm) to 25.8 (7-day vehicle viral survival, across different integrator group than index farm).[250]
    - As detailed in Appendix 1: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates, HPAI virus has the potential to survive for prolonged periods in cool, moist conditions.

9.2.2.2.2 Qualitative Analysis

In addition to the factors outlined above for growers and employees accessing turkey houses, we considered the following qualitative factors for evaluating this pathway:

- The STS Plan's recommended mitigation measures aim to reduce employee contact with potentially contaminated or infectious sources, such as other birds and equipment.
  - During normal operations, employee biosecurity training should very clearly emphasize the importance of avoiding bird contact, and farm policy should restrict employee ownership of other birds.[10]
    - Biosecurity guidelines stipulate measures to use after employee-bird contact, including 24 hours of downtime before returning to work and procedures to re-enter the PBA.[1,10]
    - A detailed assessment of the risks of HPAI contamination or infection due to wild birds can be found in section 9.1.4 Role of HPAI Spread to Turkey Flock in a Control Area via Wild Aquatic Birds in the Farm Vicinity and section 9.1.5 Role of HPAI Virus Spread to Turkey Flock via Wild Non-aquatic Birds in Farm Vicinity.

- Exposure to potentially contaminated equipment, specifically equipment from other farms that has not been washed and disinfected, is prohibited.[10]
- After contact with several potentially contaminated or infectious sources or after removing gloves, hand hygiene is mandatory.[10]

- The PMIP measures further reduce the risk of HPAI virus introduction into the poultry house from contamination by people.
  - The pathway of people who do not access the poultry house requires an additional viral transfer step for virus to be tracked into the poultry house. An example pathway is: *contaminated hands/shoes/tires of a person (for example, a part-time employee)→ travel from infected and undetected farm A to uninfected farm B→ ground/surface contamination at uninfected farm B outside of poultry house→ hands/shoes of another person (for example, a full-time employee) at farm B→ poultry house.*
    - If the full-time employee became cross-contaminated via contact with the part-time employee outside of the poultry house, farm-specific clothing required during PMIP might still allow virus to enter the barn; however, barn-specific footwear may decrease the amount of contamination on boots or shoes worn into the poultry house.[1]
- According to the STS Plan, biosecurity training provided by turkey production companies should stress the importance of avoiding contact with other birds, including birds that may be commonly hunted, such as ducks, geese, waterfowl, and other game birds (e.g., quail, pheasants, etc). Farm policy should also bar employees from owning other birds. In the event that contact is made with other birds, employees agree that they will comply with a 24-hour waiting period before returning to work.[10]

9.2.2.3 Likelihood Rating and Conclusion

Although some contact may be unavoidable, it is recommended that growers minimize unnecessary contact with other growers during the PMIP and limit travel to other poultry premises during the entire grow-out period. Still, social and other non-business contacts have been documented between growers and members of their families. Similarly, while task-specific crews (such as vaccination or turkey insemination) and day laborers have been documented risks in the literature, many companies suggest that employees should limit contact with other commercial or non-commercial poultry flocks outside their job duties.

During the PMIP, vehicle and visitor traffic to a poultry premises is likely to be decreased to include only critical visitors, employees, and growers. The prevention of HPAI virus transmission by people during the PMIP depends on close adherence to the biosecurity measures outlined in the PMIP.

Provided the STS PMIP measures for people are strictly followed, we rate the likelihood of HPAI transmission via people (namely growers, employees, and critical operational visits) during the PMIP as follows:

Person type	Likelihood Rating
Critical operations visitors and vehicles	See section 9.2.1 Likelihood of a Turkey Flock Becoming Infected with HPAI via Critical Operational Visits during PMIP
People entering poultry barns during PMIP	*Low*
People not entering barns during PMIP	*Very low*

9.2.3 Likelihood of a Turkey Flock Becoming Infected with HPAI Virus via Machinery or Equipment Shared between Multiple Premises

Many types of equipment are used to maintain a turkey flock and premises, including some specialized types of equipment that may be shared between premises for activities such as house maintenance, removal of manure, and preparing an empty house for the next flock of birds. Shared equipment may be owned and managed by an integrator, a grower, or a visitor who utilizes an item on multiple poultry operations. For a more thorough assessment of equipment utilized for load-out, see section 9.3 Likelihood of Turkey Flock Becoming Infected with HPAI Virus via Load-Out Operations.

The STS Plan biosecurity measures state that sharing equipment between turkey farms is not recommended and that if equipment must be shared, effective C&D must take place between uses (Level 1 biosecurity).[10] Following an outbreak of HPAI (Level 2 biosecurity), mitigation measures further recommend prohibiting exposure [of growers, employees, service technicians, service crews, and veterinarians] to equipment from other farms that has not been washed and disinfected and that equipment should be inspected [for cleanliness] prior to entry onto a farm.[10] During PMIP, no equipment may be brought onto the premises except tools or items required to complete a critical operations visit, and such equipment must be cleaned and disinfected prior to entering the premises.

9.2.3.1 Likelihood of Infection via Shared Machinery or Equipment

During the final grow-out period prior to implementation of an 8-day PMIP, sharing equipment is not recommended, but may occur by equipment being returned to its owner (if grower-owned) or staged on a turkey premises (if owned by an integrator or a third party). While birds may not directly contact this equipment, it may arrive contaminated with HPAI virus, and if it is not cleaned and disinfected effectively, there is potential for cross-contamination to the ground around a barn and/or personnel who may then track virus into a turkey barn. Additionally, some shared equipment may enter a poultry barn in the final grow-out period during routine tasks, disease investigation, or building maintenance. **Figure 17** illustrates the transmission pathway.

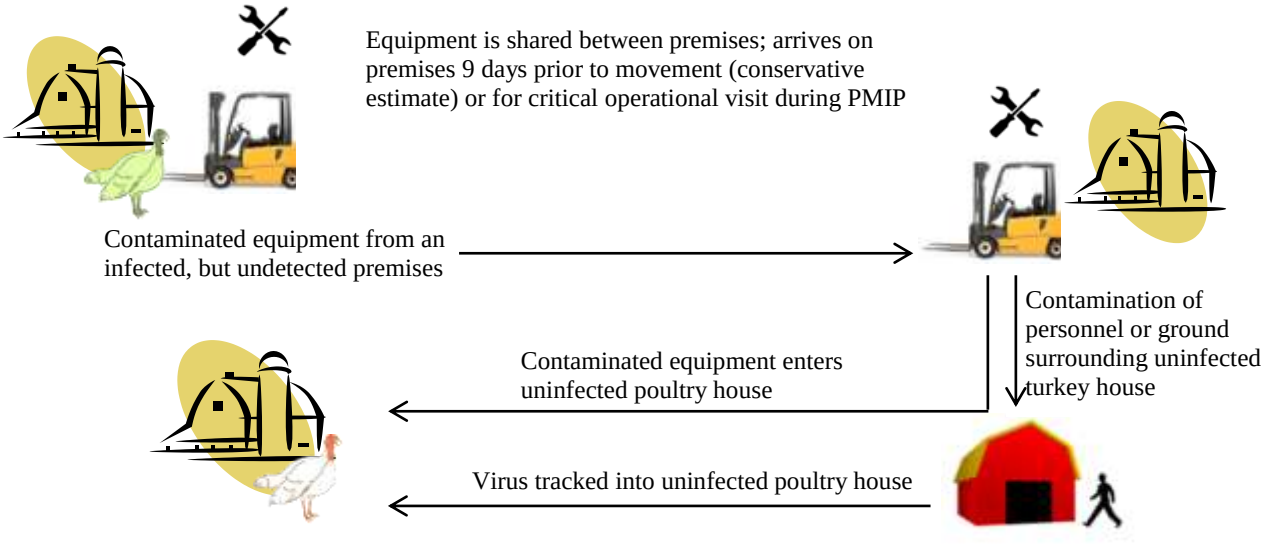


Figure 17. Pathway for exposure of a turkey flock via shared machinery or equipment during final grow-out period

9.2.3.2 Literature review

- Movement of contaminated equipment and, more specifically, shared equipment has been implicated as a potential transmission pathway in previous AI outbreaks in the U.S. poultry industry.
    - In the 2015 U.S. HPAI outbreak on turkey premises in the Upper Midwest (IA, MN, ND, SD, and WI), a descriptive analysis of epidemiologic findings based on narrative responses from infected turkey farms identified highly likely transmission routes for some of them. Of those routes, two involved sharing equipment, with the onset of clinical signs occurring 10 to 11 days after potential exposure to the shared equipment.[26]
    - On layer premises in the 2015 HPAI outbreak, preliminary univariate analysis of a case-control study considered sharing of egg racks or pallets and egg flats as potential infection risk factors for entry into the farm-level multivariate model (egg racks or pallets shared in previous 14 days: 29% of case farms, 11% of control farms, P=0.08; egg flats shared in previous 14 days: 30% of case farms, 14% of control farms, P=0.17). Because of model instability due to sparse data, equipment sharing was not included in the multivariate model, but the authors concluded that this could be a risk factor for infection.[177]
    - In the 2002 LPAI H7N2 outbreak in Virginia, the pattern of virus spread indicated likely movement by fomites, people, and equipment contaminated with virus.[59] However, in a case-control study of infected premises during the same outbreak, there was no statistically significant difference in the occurrence of equipment sharing on infected premises versus non-infected premises (farm equipment borrowed or loaned: case farms=17.3%, control farms=16.7%; P=0.88).[70]
    - In the 1996-1998 LPAI H7N2 outbreak in Pennsylvania, the source of virus exposure for 1 of 25 positive premises was suspected to be equipment contact.[146]

- Virus spread was attributed in part to equipment in the 1983-1984 HPAI H5N2 outbreak in Pennsylvania and in the 2000-2002 LPAI H6N2 outbreak in California.[253]
  - During the 1983-1984 HPAI H5N2 outbreak in Pennsylvania, Utterback identified movement of products, materials, equipment, tools, and supplies that may contact birds as a potential mode of AI transmission between flocks. In the analysis, the potential high-risk equipment included egg flats; equipment used for manure, dead bird disposal, and feed; and equipment moved from farm to farm.[265]
- Sharing of equipment has been associated with disease spread in outbreaks of other viral pathogens of poultry.
  - In a multivariate model with matched controls, removing litter using shared equipment increased the odds of infectious laryngotracheitis (ILT) infection on Mississippi broiler farms in a 2002-2003 outbreak (caked and/or total litter removed from house and/or farm using shared equipment: OR=5.39; P=0.0378).[241]
  - Analysis of the Newcastle disease epidemic mainly on layer and pullet premises in southern California from 1971-1973 showed evidence of mechanical spread of the disease from infected premises by fomites, such as egg flats.[266]

- Based on the data in the literature, equipment sharing occurs in the poultry industry during both outbreak and non-outbreak situations. The types and frequency of equipment sharing vary between operations, sectors, and regions.
  - The extent of equipment sharing during normal operations in the turkey industry has not been published, to our knowledge.
  - Regional biosecurity surveys of Georgia broiler and breeder-layer growers reported that 8-25.8% of respondents share equipment.[247,267] Findings by Dorea et al. indicate that shared equipment is most commonly used by one other grower, but this ranged from 1-20 growers.[267]
- During outbreaks (HPAI/LPAI H7N8 in Indiana in 2016 and LPAI H7N2 in Virginia in 2002), equipment was shared at 11% (1/9) and 17.3% (26/150) of surveyed case premises, respectively.[30,70] Among turkey case premises in Indiana in 2016, the only equipment reported to be shared was a live-haul loader.[30]
  - Data on sharing of specific types of equipment, as published in a case series of infected turkey flocks in the Upper Midwest and a case-control study of infected Iowa layer flocks during the 2015 HPAI outbreak, are shown in **Table 16**.

Table 16. Percentage of turkey and layer premises reporting equipment sharing (i.e., not farm-specific equipment) during the 2015 HPAI outbreak[26,268]

	Layer premises, 2015 HPAI outbreak		Turkey premises, 2015 HPAI outbreak[a]
	Case farms (n=26)	Control farms (n=33)	Case farms (n=67-80)[b]
Equipment	*Percent sharing equipment*		
Gates/panels	--[c]	--	9%
Lawn mowers	20%	28%	37%
Pressure sprayer/washer	8%	9%	43%
Skid-steer loader	12%	0%	39%
Tillers	--	--	13%
Manure handling	12%	3%	--

[a]Investigation questionnaire to turkey premises in 2015 case series asked if equipment used on the premises was farm-specific. In this analysis, non-farm-specific equipment was interpreted to be equivalent to shared equipment.

[b]The number of respondents varied for each equipment type listed in the survey.

[c]Dashes indicate data on that specific type of equipment was not reported.

- Historically, disinfecting shared equipment is a described mitigation measure in the event of an infectious disease outbreak in poultry. Little is documented, however, on the specific procedures for C&D during an outbreak.
    - For case turkey premises in the Upper Midwest 2015 HPAI outbreak, the majority of farms responded that pre-loaders were cleaned and disinfected by first power washing (to remove organic material, manure, and feathers) and then applying disinfectant.[26]
    - All layer case and control premises (n=59) surveyed in a case-control study in the 2015 HPAI outbreak reported that shared pressure sprayers/washers, skid-steer loaders, and manure-handling equipment were disinfected. Other shared equipment was disinfected at variable rates (lawn mowers, egg flats, racks, or pallets). Shared lawn mowers were disinfected by 65% of surveyed farms.[268]
    - During an outbreak of ILT in northern Georgia, the most frequently reported cleaning procedures for equipment on broiler premises were power washing (64.4%), water washing only (33.8%), disinfection (20.0%), and disassembly and cleaning of parts (3.8%); 3.5% of equipment was not cleaned.
        - On broiler premises in southern Georgia, where there was no ongoing ILT outbreak, the methods used to clean equipment were not statistically different; however, relatively fewer farms utilized a disinfectant in this region (9.4% versus 20% of farms, P= 0.056).[267]

9.2.3.3 Qualitative Analysis

We considered the following qualitative factors in evaluating this pathway:

- Sharing equipment between poultry operations represents a known risk to biosecurity and an opportunity for disease introduction. Although the standardized poultry industry biosecurity guidelines and STS Plan biosecurity measures prior to an outbreak of HPAI (Level 1) recommend not sharing equipment when possible,[1,10] this may not be economically or logistically feasible for all types of equipment and scenarios.[26]
    - Equipment sharing between poultry premises is documented in the literature, and per communication with industry representatives of the STS Working Group, many equipment types have been reported to be shared (TWG, personal communication, February 2016).
        - Equipment is more likely to be shared if it is company-owned or used among premises with common ownership or family ties (TWG, personal communication, February 2016).
        - Specific equipment types reported to be shared in the turkey industry include loaders, pre-loaders, panels, skid loaders, load-out boards, tractors, air compressors, hand tools, tillers, litter clean-out equipment, feed haul equipment, and cleaning equipment. The extent of equipment sharing reported by industry representatives varied (TWG, personal communication, February 2016).
            - For an evaluation of the risk of HPAI infection spread to a turkey flock during load-out, see Section 9.3 Likelihood of Turkey Flock Becoming Infected with HPAI Virus via Load-Out Operations.
        - During an HPAI outbreak, turkey grow-out premises may reduce non-essential activities requiring equipment, such as tilling litter, to minimize equipment sharing (TWG, personal communication, February 2016).
    - During a PMIP, sharing of equipment is prohibited. The exception is for equipment needed to complete a critical operational visit, such as emergency repair of critical mechanical equipment or service visits to address changes in bird health (see Appendix 7: Cross-Commodity Pre-Movement Isolation Period).
    - However, any equipment brought to the premises during the final grow-out period prior to implementation of a PMIP may continue to be used during a PMIP.
        - Recent HPAI outbreak experience suggests this scenario may still present a risk of infection as there were epidemiological links during the 2015 HPAI outbreak for turkey premises that used shared equipment 10 to 11 days prior to the onset of clinical signs.[26]
        - Arrival of contaminated shared equipment well in advance of movement to processing could provide more opportunities for personnel or equipment to track virus into the barn, especially under certain environmental conditions (e.g., cool temperatures and high humidity) in which viable virus could persist for days to months (see Appendix 1: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates).

- On shared equipment, the potential level of virus contamination depends on the infectious material, the contaminated substrate, and the survival characteristics of the virus.
    - Machinery or equipment that enters a poultry barn, has poultry contact, or comes in contact with poultry feces is at high risk for contamination at an infected but undetected premises. Such equipment (excluding load-out equipment) includes de-caking or tilling equipment, skid steers/front loaders, hand tools, and poultry monitoring equipment.
    - Estimates of HPAI virus concentrations in poultry carcasses, feces, and feathers from infected poultry generally range between 10^3 and 10^7 EID_{50} per gram or per milliliter of tested substrate, although higher concentrations have been observed in some cases.
        - For a detailed summary of the literature on virus titers in feces, feathers, blood, and poultry carcasses from infected poultry, see Section 9.2.4, Likelihood of Turkey Flock Becoming Infected with HPAI via Dead Bird Disposal, and on virus titers in immature and mature feathers from infected poultry and ducks, see section 9.1.6, Risk of HPAI Virus Spread to Turkey Grow-Out Premises Near Poultry Live-Haul Routes via Feathers, Feces, and Other Fomites.
    - Virus survival is generally longer in cooler temperatures and moist conditions. AI virus survival has not been tested in turkey feces, to our knowledge. In chicken feces, virus can remain infectious between 2 and 7 weeks at cooler temperatures (39 to 46°F), similar to winter conditions in many regions of the U.S.[257,269,270] Virus survival in chicken feces is reduced to less than 5 days in warmer temperatures (71 to 77°F).[257,269]
        - For virus survival data on feathers, feces, and equipment surfaces, see Appendix 1: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates.
- In a non-outbreak setting, premises participating in the NPIP will follow the site-specific biosecurity guidelines outlined in 9-CFR, which include C&D procedures for equipment when applicable.[1] Similarly, STS Plan biosecurity measures prior to an outbreak of HPAI (Level 1 biosecurity) recommend effective cleaning and disinfection of equipment when shared.[10]
    - As described in the literature above, disinfecting shared equipment is the best practice in non-outbreak situations, and many turkey industry company biosecurity plans require C&D of shared equipment between premises (TWG, personal communication, February 2016).
        - While C&D protocols should be described in most company biosecurity plans, strict compliance with plans cannot be assumed, especially when growers or third-party contractors are not directly observed. During an HPAI outbreak, compliance with C&D protocols is believed to be high (TWG, personal communication, February 2016).
    - There may be limitations to C&D of some equipment because of environmental concerns (e.g., excessive waste water from cleaning large equipment) or concerns about damaging the functionality of mechanical or electrical equipment that cannot be

heated or wet (i.e., laptops, cell phones, or other monitoring devices). Weather conditions, such as harsh winter weather, may make thorough C&D of equipment more difficult.

- Following an outbreak of HPAI, the STS Plan biosecurity recommendations (Level 2) include equipment inspection (for proper C&D) prior to entry on the premises and prohibiting exposure (of people who may contact birds, specifically growers, farm employees, service technicians, service crews, and veterinarians) to shared equipment that has not been washed and disinfected.[10]
- During a PMIP, equipment required for a critical operational visit must be cleaned and disinfected prior to entering the premises (see Appendix 7: Cross-Commodity Pre-Movement Isolation Period).

- For shared equipment that is not used inside barns during the final grow-out period, the pathway to infect a turkey flock requires multiple steps to introduce HPAI virus into a barn.
  - A potential pathway in this scenario involves four contact steps: *shared contaminated equipment→ ground area on uninfected turkey premise→ farm personnel's boots or clothing→ turkey house*. However, if personnel handle the equipment directly and then enter a turkey barn (e.g., to move equipment to a new location on the premises before working with the flock), fewer steps are needed in this pathway: *shared contaminated equipment→ farm personnel's boots, clothing, or hands→ turkey house*.
  - With each transfer step, there would likely be a reduction in the virus concentration transferred to the recipient surface. This is because only a fraction of the virus (6 to 27%) on the donor surface is transferred to the recipient surface in each direct contact.[261] However, depending on the initial viral load and infectious dose in turkeys for that strain, the potential level of virus concentration tracked into the barn may still be infectious.
  - If the equipment remains outside the PBA, there is a decreased likelihood of cross-contamination to the ground near the poultry house.
    - NPIP stipulations state that all biosecurity plans for poultry premises utilize a PBA.[1]
  - During a PMIP, individuals who have direct contact with poultry must wear premises-specific clothing and barn-specific footwear (see Appendix 7: Cross-Commodity Pre-Movement Isolation Period).
    - For more information on risks and mitigation measures related to farm personnel introducing virus into a barn, see section 9.2.2, Likelihood of a Turkey Flock Becoming Infected with HPAI via People and their Vehicles Entering the Premises.
  - Ssematimba et al. assessed the risk of people and/or equipment that access only the poultry premises or storage rooms on the premises on broiler and layer farms in the Netherlands. They proposed the exposure risk classification for a majority of these contacts to be medium. In the analysis, the risks identified for such contacts are the

potential of serving as fomites, expanding the farm network, and lack of or non-adherence to biosecurity protocols, such as non-thorough C&D.[73]

- Equipment that enters the poultry house may be a greater risk due to closer proximity to or potential direct contact with poultry.
    - For equipment utilized inside the poultry house, site-specific biosecurity plans should have provisions to cross the LOS, as required by the NPIP standardized biosecurity principles.
        - According to industry representatives of the STS Working Group, many companies require C&D of shared equipment between premises, but not between poultry houses on the same premises (TWG, personal communication, February 2016).
        - Some companies require C&D of tires to enter any poultry house on the same premises (TWG, personal communication, February 2016).
    - During the PMIP, equipment used in the house may include shared equipment that arrived before the onset of the PMIP and equipment used for a critical operational visit during a PMIP.
    - Critical visits during the PMIP, such as emergency repair of mechanical equipment or service visits to address changes in bird health, are assumed to occur at a situation-specific frequency. The available information from studies conducted in non-outbreak scenarios suggests a maintenance visit may be required during an 8-day PMIP.
        - According to surveyed poultry growers in Georgia, visiting repairpersons entered the chicken house at approximately 18% of broiler farms in the low-density region and 0% in the high-density region during a 7-day period.[247]
        - Among eight premises observed for 4 days in Ontario, Canada, there was one maintenance visit to service barn ventilation equipment, and this contact had visited three premises over a 3-day period.[271]
    - During a PMIP, it is plausible that required repair or service persons performing critical maintenance or evaluating changes in bird health may use equipment that has been used on multiple poultry premises.
        - For further information on the risks associated with the person or vehicle making a critical operational visit during PMIP, see section 9.2.1, Likelihood of a Turkey Flock Becoming Infected with HPAI via Critical Operational Visits During PMIP.
    - In a comparable evaluation of broiler and layer premises in the Netherlands, the exposure risk classification was proposed to be high for people and/or equipment that access the poultry house largely because of the frequency of the human contacts. Such contacts (e.g., veterinarians) may visit up to 100 farms per year, and their equipment may not always be thoroughly cleaned and disinfected between farm visits.[73] The same evaluation proposed an overall exposure-risk classification of medium for people and equipment that did not access the poultry house. [73]

9.2.3.4 Likelihood Rating and Conclusion

Previous poultry disease outbreaks demonstrate a known risk for virus spread as a result of movement of contaminated and shared equipment. Equipment is brought onto a poultry premises may contaminate the ground or personnel who work with poultry, or if contaminated equipment is used inside a barn, live poultry may directly contact it. In the U.S., C&D of shared equipment is recommended in the STS Plan and should be addressed in the biosecurity protocols for all NPIP-approved grower premises. However, adherence to protocols may be limited by feasibility, consistency, and logistics. In the absence of a PMIP, we rate the likelihood of a turkey flock becoming infected with HPAI virus via shared machinery or equipment to be *moderate.*

During the PMIP, in addition to standard biosecurity measures, no off-site equipment will be pre-staged, and only critical operational visits may continue, such as emergency repair of critical mechanical systems or service visits to address changes in bird health. Equipment for these critical operational visits must be cleaned and disinfected. Provided the SPS Plans, including the greatly intensified biosecurity of the PMIP, are strictly followed, we rate the likelihood of a turkey flock becoming infected with HPAI virus via shared machinery or equipment during a PMIP as *low.*

9.2.4 Likelihood of Turkey Flock Becoming Infected with HPAI via Dead Bird Disposal

The process of dead bird disposal in the Control Area addressed in this risk evaluation relates to normal mortality on turkey premises, as opposed to mortality from known infected premises, including depopulation. Processes described are recommended per the HPAI STS Plan 2015 and cross-commodity pre-movement isolation period (PMIP) document (see Appendix 7: Cross-Commodity Pre-Movement Isolation Period).

Dead turkeys must be collected and removed in a biosecure manner from houses each day and moved to an on-site location that is as far away from the barns as possible; containers (dumpsters) for dead turkeys should never leave the farm. Multiple poultry farms should not share initial dead bird collection containers or disposal sites located on poultry premises. For farms within the Control Area, no movement of dead birds off a farm is allowed without a permit issued by the Incident Commander.[10]

In normal operations, turkey premises may employ a variety of methods to dispose of daily mortality, both on- and off-site. With regard to carcass disposal, PMIP measures restrict off-site transportation of carcasses for the duration of the PMIP in the days leading up to movement of live birds to processing.

9.2.4.1 Dead Bird Disposal During PMIP

Due to the potential spread of HPAI via carcass disposal, the PMIP measures restrict off-site transportation of carcasses for disposal for the duration of the PMIP. Dead bird disposal is limited to secure on-site storage or disposal during the PMIP as outlined in the STS Plan.[10] Secure on-site storage or disposal options include composting, incineration, pit burial, individual burial, carcass fermentation, and refrigerator/freezer storage. As individual burial and carcass fermentation are not widely practiced in the turkey industry, this risk evaluation will focus on the more common on-site practices of composting, incineration, pit burial, and refrigerator/freezer storage.

Composting

Composting, or controlled decomposition under thermophilic and aerobic conditions,[272] is the most widely used method of on-site carcass disposal in the turkey industry. Under conditions of routine mortality, carcasses are composted together in piles or bins to which a supplemental carbon source, such as litter or sawdust, has been added (see **Figure 18**). According to the STS Plan,[10] following an HPAI outbreak, composting must be managed to ensure carcasses are covered to prevent exposure to wild animals and maintain adequate temperatures. 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 (130-150 ^{o}F) achieved. Composted turkey carcasses may be used as fertilizer, soil amendment, or as a source of organic material for composting additional material.[273]

Poultry composters are typically constructed on a concrete slab to prevent nutrient leaching and vermin entrance. They typically are three-sided and have an overhead roof.[272] If mass depopulation is needed, in-house composting may be used. Multiple peridomestic species have been shown to access poultry carcass compost piles (**Figure 19**), including, but not necessarily limited to, raccoon (*Procyon lotor*), opossum (*Didelphis virginiana*), striped skunk (*Mephitis mephitis*), and domestic cats (*Felis catus*).[274]

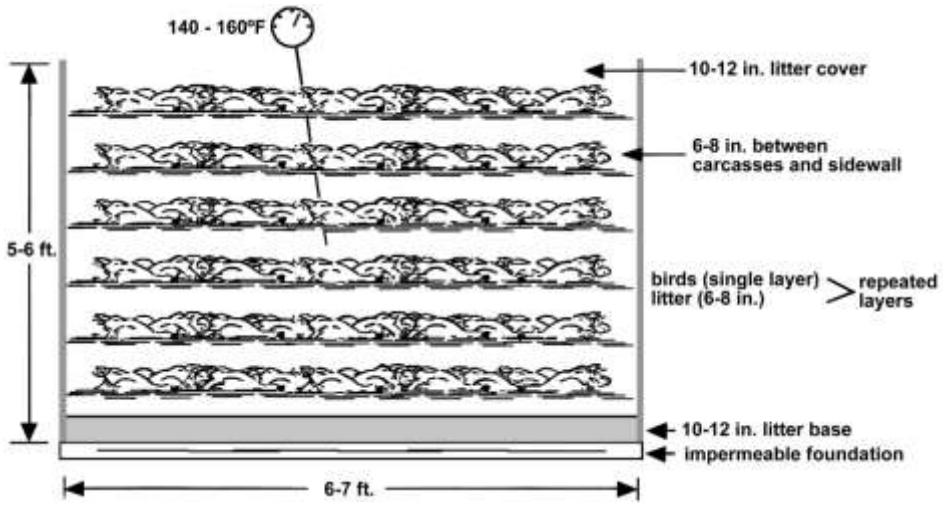


Figure 18. Mortality composter profile[272]

Figure 19. Wild mammals accessing poultry mortality compost piles. Photos courtesy of USGS

Incineration

Incineration is one of the most biosecure methods for carcass disposal and is a commonly used method for chicken carcass disposal; however, due to the size of turkeys and relative expense, incineration is less commonly used in the turkey industry.[4] If on-farm incineration is used in the Control Area, carcasses must be protected from exposure to wild animals.[10] Complete carcass combustion occurs within the incinerator unit and the resultant residue does not attract animal or insect pests.[275]

Pit Burial

Burial of carcasses in disposal pits can be a less costly method of disposal, but it is restricted in many areas and, while widely used in the chicken industry, it is not widely used in the turkey industry due to scavenger and water contamination concerns.[4] Nonporous soil and a deep water table are the most amenable to pit burial, while sandy soil requires reinforcement of the sides of the pit. To prevent access by animal and insect pests, the top of the pit must be solid and have a tight-fitting lid. Due to groundwater concerns, the placement of burial pits is usually closely regulated by state departments of agriculture.[276] Disposal via burial of hundreds of dead turkeys near market age can present problems and may require taking carcasses to a landfill that will accept them.[4]

Refrigeration/Freezer storage

Carcasses may be stored in a vermin-proof refrigerator or freezer until off-site carcass movement for rendering or another disposal method is permitted. In large turkey operations, daily mortality usually requires the use of a high-capacity unit. Freezers typically contain leak-proof carcass storage boxes, which are also used to transport the carcasses to the rendering plant[277] or other destination.

In areas where refrigeration/freezing is not commonly practiced during normal operations, short-term on-site refrigeration/freezing of carcasses may provide a viable choice for dead bird management during the PMIP (e.g., using refrigerated trucks or refrigerated shipping containers) until other on-site or off-site disposal can be coordinated or permitted.

9.2.4.2 Likelihood of Turkey Flock Becoming Infected with HPAI via On-farm Dead Bird Disposal and Scavengers During PMIP

Carcass disposal on the farm presents an opportunity for vermin and scavengers to access

infected wildlife or poultry carcasses and transmit the HPAI virus to a neighboring uninfected turkey house or mortality disposal site, either mechanically or via virus shedding. The virus could subsequently be transmitted into a turkey house via farm personnel or other mechanisms.

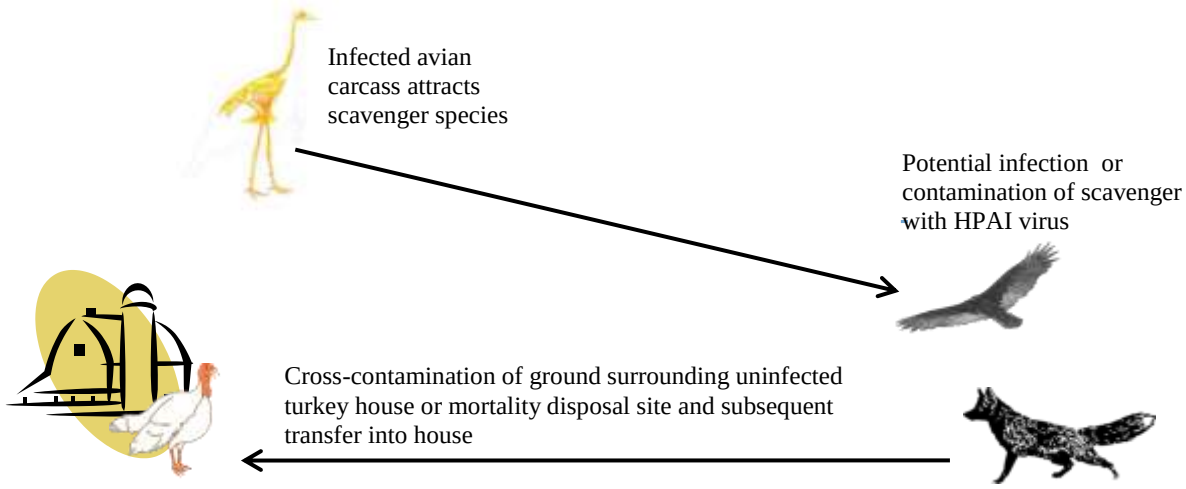


Figure 20. Pathway for exposure of a turkey farm via dead bird disposal on-site

9.2.4.2.1 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.[273]
    - 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 at 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.[278] 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.

    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. [279] 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 60°C (140°F).
- 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 61.5°C (143°F) at the top and 50.3°C (123°F) at the bottom of the bin.[280] 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.[281] 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 37.8°C (100°F) for 3 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.

- The observation of scavengers near poultry houses has been identified as a risk factor for AI transmission.
  - In the 2002 LPAI H7N2 outbreak in Virginia, multivariate analysis determined that the presence of foxes, raccoons, and opossums posed an approximately twofold increase in risk of infection.[70]
- Multiple studies have demonstrated the susceptibility of mammals, including scavenger species, to HPAI.
  - Ferrets and foxes 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.[282,283] Lipatov et al. (2009) measured presence of viral antigen in ferret tissue, not actual viral shedding. Reperant (2008), however, demonstrated pharyngeal shedding in foxes 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 days post-inoculation (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.
  - Following experimental gastrointestinal HPAI H5N1 infection, cats became systemically infected and viral shedding was detected (via RT-PCR) in pharyngeal and rectal swabs.[284] Pharyngeal shedding occurred in both cats with gastrointestinal exposure, beginning 2 DPI. Rectal shedding was observed in only one of these cats, and only 2 DPI.

- Songserm et al. (2006) describe a fatal HPAI H5N1 infection in a dog following ingestion of infected duck carcasses.[285]
- When they were experimentally fed carcasses of LPAI H4N6-inoculated mallards or H4N6-spiked and coated chicken eggs, raccoons failed to subsequently shed AI virus RNA. While this study does not support scavengers as a source of LPAI biological transmission, the authors propose that HPAI virus may be more likely to be shed by scavengers because of its ability to cause more disseminated infection.[286]
  - More recently, experimentally infected striped skunks (as well as cottontail rabbits) have been shown capable of transmitting LPAI H4N6 to birds (mallards) through indirect contact with shared resources (i.e., through contaminating the environment).[40]
- Both striped skunks and raccoons have been shown to shed LPAI H4N8 and H4N6, respectively, following experimental nasal inoculation with those strains.[38,287] For most of the skunks, nasal shedding of H4N8 peaked at 8 DPI at an average $10^{5.65}$ PCR EID_{50} equivalents/ml,[d] and oral shedding at7 DPI at an average $10^{4.82}$ PCR EID_{50} equivalents/ml. Nasal shedding of H4N6 in the raccoons 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) 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.[288]

- 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 avian influenza 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.[219]
  - For a detailed assessment of susceptibility and pathogenicity in avian scavenger species, please see section 9.1.5, Risk of HPAI Virus Spread to Turkey Flock via Wild Non-aquatic Birds in Farm Vicinity**.**

9.2.4.2.2 Qualitative Analysis

We considered the following qualitative factors for evaluating this pathway:

- The pathway may involve one or more virus transfer steps between contact surfaces. For example, if a wild animal is acting as a mechanical vector, the pathway *infected undetected carcass>scavenger>ground area on uninfected premises>farm personnel's boots>turkey house* involves four contact steps. 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, the virus concentration transferred would likely decrease substantially with each contact step. This is because only a fraction of the virus (6 to 27%) on a donor surface is transferred to the recipient surface in each direct contact .[237] The ground traveled by the scavenger between the carcass and the

[d] 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

uninfected turkey premises would further lessen the amount of virus present on the scavenger for transmission once at the premises.

- If a wild animal were contaminated by an infected carcass, we would expect virus may be transferred via feces, bodily fluids, or feathers. One gram of organic matter from a poultry carcass may contain 10^6 EID_{50}/g.[237]
- For perspective, using a mid-range viral transfer concentration, if 15% of virus is transferred at each contact step described above, enough virus particles still remain after four steps to infect five turkeys (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.

- If, however, the scavenger becomes infected with and subsequently sheds HPAI virus on the grounds outside the uninfected turkey house, there are only two contact steps: from the contaminated grounds to the personnel's boots, and from the boots to the turkey house floor. The likelihood of a scavenger actively shedding HPAI virus following ingestion of an infected carcass is, thus, a critical consideration.
  - The studies cited above demonstrate that mammalian and avian scavengers can become infected with HPAI virus following ingestion of infected poultry, both naturally and experimentally.
    - In the studies in which rectal shedding following consumption of HPAI-infected meat was studied, it was short-lived and occurred inconsistently.[283,284]
  - Additionally, HPAI H5N1 strains that replicate mostly in the lower respiratory tract may not be readily excreted via the upper respiratory system of mammals.[289] The role of other excretory systems, such as the gastrointestinal and urinary tracts, as portals of viral exit is unknown at this time.
- Other plausible pathways where fewer contact steps are involved include those where the grower or other poultry farm personnel directly contact an infected or contaminated scavenger species:
  - An infected or contaminated scavenger species is trapped and killed or dies on an uninfected farm. The grower or employee disposes of the scavenger, then enters a turkey house, introducing virus to the flock.
  - Using calculation like that above (with an infectious dose of 10^2 EID_{50}), but assuming only one transfer step from the scavenger to the person, enough virus particles could be on the person to infect 1,500 turkeys.
  - A domesticated scavenger (e.g., dog or cat) is infected or contaminated on an infected neighboring farm. Grower or employee contacts the animal, then enters a turkey house, introducing virus into the flock.
    - In a study of commercial poultry farms in Virginia, over half of all farms had cats on the premises, and over two-thirds of farms had dogs on premises.[70]
    - The number was slightly lower in a case-control study of turkey flocks affected by the 2016 HPAI outbreak in Indiana; between 30 and 44% of flocks had dogs and/or cats on the premises.[246]

        - In December 2016 a highly pathogenic strain of AI (H5N6) was discovered in two dead cats in South Korea, 2 km from a chicken farm where HPAI H5N6 was first reported in November 2016.[290]
        - Among HPAI-positive turkey farms in the 2015 outbreak in the northern Midwest, 25 to 30% had dogs and/or cats.[26]

- The distance between poultry farms (i.e., the distance a scavenger must travel between a carcass and an uninfected farm) also impacts the likelihood of HPAI transmission by the scavenger. The infected carcass and the uninfected farm must be within the likely range of the scavenger for transmission to potentially occur and, in view of known scavenger ranges, this is very likely.
    - The home range of red foxes (*Vulpes vulpes*) is generally up to 8 km (5 miles) in diameter, being largest in the winter.[291]
    - Raccoons (*Procyon lotor*) normally have a home range diameter of 1.8 to 3 km (1.1 to 1.95 miles).[292]
    - The diameter of the opossum's (*Didelphis virginianis*) home range is between 1.3 and 2 km (0.8 to 1.2 miles).[293]
    - The striped skunk (*Mephitis mephitis*) has a home range 2.2 to 2.5 km (1.4 to 1.6 miles) in diameter.[294]
    - Turkey vultures can travel up to 225 km (140 miles) per day.[295]
- The enhanced biosecurity required during the PMIP applies only to farms located in a Control Area that wish to move birds off the premises. While the STS Plan states that no movement of dead birds off a farm in the Control Area is allowed without a permit issued by the Incident Commander[10] and it is assumed that biosecurity practices may be elevated across all poultry farms in an outbreak situation, other poultry farms in the Control Area are not subject to any particular stipulations on dead bird disposal or other movements on and off the farm. It is assumed that there may be marked variation in the practices on farms within the Control Area that are not currently adhering to a PMIP.
- In a case-control study of commercial poultry farms in Virginia in 2002, a 1.9-fold increased risk of LPAI H7N2 infection was associated with the sighting of wildlife near poultry houses.[70] Scavengers must, however, gain access to the infected carcass at the source farm in order to contact and transmit HPAI virus.
    - As described above, it may be unlikely for scavengers to access carcasses in burial pits, incinerators, or storage freezers, as all are designed to prevent animal entrance.
        - However, we assume industry variation in frequency of mortality collection and type of storage container used to gather carcasses from the time they are removed from the poultry house to the point when they are placed in the pit, incinerator, or storage freezer.
        - While ideally these intermediate transport or storage containers also should prevent access by scavengers on premises observing PMIP,[10,248] turkey industry representatives have indicated that turkey carcasses may remain uncovered,

sometimes on the ground, outside turkey houses or storage containers for hours (TWG, personal communication, 2016).

- 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 of a depth (10 to 12 inches) designed to prevent odor production that would attract scavengers.[272]
  - When the 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.[296]
  - Reports vary on the prevalence of vermin and scavengers with a properly managed composter; however, such animals have been shown to access poultry compost barns.[272,274]
  - In their univariate analysis, McQuiston et al. (2005) found uninfected farms were significantly more likely to dispose of dead birds via composting than infected farms (77.9% versus 63.9%, P=0.008).[70]

9.2.4.2.3 Likelihood Rating and Conclusion

The risks for HPAI introduction associated with off-site disposal methods such as rendering are well-documented in the literature (see section 9.2.4.3, Likelihood of Turkey Flock Becoming Infected with HPAI via Dead Bird Disposal that Takes Place before PMIP), and off-site disposal of mortality must be discontinued during PMIP. Best practices for on-site carcass disposal, and STS Plan biosecurity measures, should decrease the likelihood of attracting scavenger species to poultry mortality on a turkey farm in the days leading up to marketing. Other poultry farms in the Control Area including an infected farm, however, may not be subject to the intensified biosecurity practices required by PMIP, and turkey carcasses may remain accessible to scavenger species for hours before biosecure disposal, particularly when dumped outside the house to be deposited at the disposal site later. Mammalian and avian scavengers have the potential to biologically or mechanically carry HPAI virus. Given that many scavenger species have home ranges of adequate size to contain adjacent poultry farms, we thus rate the likelihood of a turkey flock becoming infected with HPAI during the PMIP via scavengers within the Control Area to be *moderate*.

9.2.4.3 Likelihood of Turkey Flock Becoming Infected with HPAI via Dead Bird Disposal that Takes Place before PMIP

Turkey operations are free to utilize their preferred disposal method for daily mortality in the days leading up to the PMIP (although off-site disposal in the Control Area is expected to require a permit from the Incident Commander per the STS Plan). For facilities which exclusively use an on-farm disposal method listed above, please refer to protocols and procedures listed in section 9.2.4.2, Likelihood of Turkey Flock Becoming Infected with HPAI via On-farm Dead Bird Disposal and Scavengers during PMIP, for an evaluation of risk.

Off-site disposal methods include rendering, landfill (garbage), or sourcing animal byproduct as feed for other farmed carnivorous species (such as fox and mink for fur production). When dead birds are moved off a farm, trucks should be covered (to prevent dissemination of potentially contaminated feathers) and should follow a designated approved route. Trucks that carry dead

birds must be cleaned and disinfected, using an appropriate protocol, after delivery of the carcasses before returning to the turkey farm.[10]

Rendering

The typical turkey mortality collection process for rendering was described in personal communications (D. Halvorson and M. Smeltzer, personal communication, June 2016). During non-outbreak operations, dead birds are collected by hand inside each house throughout the day and deposited in one or more designated areas (sometimes a bin, sometimes the ground) at the ends of the house as well as on the sides of longer houses, either inside or outside. Once or twice per day, an employee collects the carcasses or the bin containing the carcasses from each house and loads them into a pickup truck, utility vehicle, or small front-loader (such as a skid-steer or mini-track loader). The carcasses are transported to a common collection bin at some distance from the houses. The distance between the houses and the collection bin varies from farm to farm. The bins typically are dumpster-type containers and may or may not be covered. On large farms, roll-off containers may be used.

Several times per week a company-owned or contracted rendering truck collects the contents of the collection bins: lift arms on the truck engage the dumpster and raise it over the bed of the truck, dumping the carcasses into the truck. While the truck bed is sealed against leakage, it may or may not be covered. However, per the STS Plan, following an HPAI outbreak trucks moving dead birds *should* be covered to prevent the dissemination of potentially contaminated feathers. Depending on the company ownership, the rendering truck may or may not visit other premises on the same day for carcass pick-up, although following an HPAI outbreak (again, according to the STS Plan), the rendering truck *should* follow a designated approved route.

Ideally, provisions should be in place to limit contact with dead birds from other farms. This may include prohibiting growers or farm employees from using company or personal vehicles to transport carcasses to a rendering site, utilizing a neutral off-site area for pickup by rendering company, and ensuring all containers used in transporting mortality from the poultry house to the final destination are secure against wildlife, leaks, and spills.[297]

The transfer of dead birds via on-site equipment to collection bins from which carcasses are then collected by a rendering truck presents an opportunity for truck and ground contamination with HPAI virus and subsequent virus transfer to other premises and other houses on the same premises. The rendering truck may directly enter other premises, and/or transfer virus to other premises along the route if the truck is uncovered. The virus could subsequently be transmitted inside other poultry houses via farm personnel. For further analysis of infection of premises near the route, please see section 9.1.6, Risk of HPAI Virus Spread to Turkey Grow-out Premises near Poultry Live-Haul Routes via Feathers, Feces, and Other Fomites.

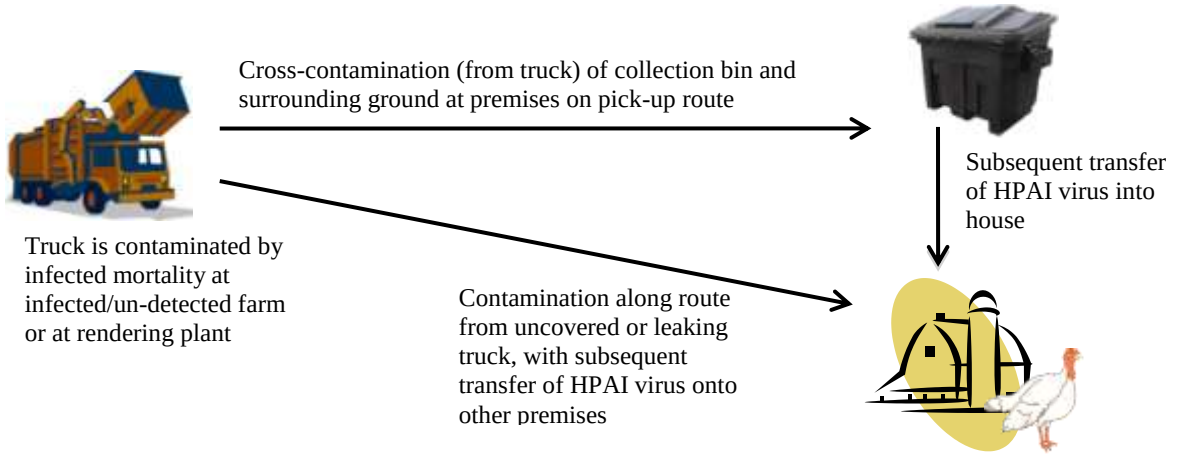


Figure 21. Pathway for exposure of a turkey farm via rendering. Other off-site disposal methods are assumed to share a similar pathway.

Other off-site dead bird disposal

- *Landfill* – Less commonly, poultry farm mortality may be transported to a landfill with other garbage products from the operation. A survey of poultry industry representatives revealed that 1 out of 15 turkey industry respondents knew of growers who routinely dispose of turkey carcasses in their trash, and 9 out of 37 respondents from the layer industry reported disposing of pullet or layer mortality in the trash (2 out of 8 broiler industry respondents knew of growers who would "maybe" dispose of broiler carcasses in their trash in the event of an HPAI outbreak)(see Appendix 9: Poultry Industry Survey on Garbage Management Practices). Further information about this pathway can be found in section 9.2.5, Likelihood of a Broiler Flock Becoming Infected with HPAI Virus due to Garbage Management.
- *Transportation for use as feed for other carnivore-raising operations* – Use of poultry carcasses and byproducts from the poultry industry has been described domestically and internationally in the feeding practices for fur-bearing animals (such as mink and fox),[289,298,299] other exotic species (e.g., alligator farms,[300] captive wildlife and zoos[301]) and anecdotally in some commercial dog breeding operations. Carcasses may be transported directly to feeding operations locally (personal communication, Jill Nezworski, July 2016),[299] or may be consolidated at regional collection centers for distribution.[302]

9.2.4.3.1 Literature Review

- Rendering has been implicated in the spread of avian influenza virus in previous outbreaks.
    - Following the 2015 HPAI H5N2 outbreak in Minnesota, a case-control study of 43 case and 40 control turkey farms found that the rendering of dead birds was a risk factor for H5N2 infection, with an odds ratio of 9.8 (i.e., farms that used rendering were 9.8 times more likely to be infected than farms that did not use rendering).[303]

- Also in the 2015 HPAI H5N2 outbreak, a case-control study of Iowa layer farms and barns found that 39% of case farms versus 13% of control farms reported that the renderer came onto the farm. Rendering trucks came near the barns in 29% of case farms, compared with 3% of control farms.
  - The adjusted odds ratio (OR) for rendering trucks coming near the barns was 22.3 ($P< 0.001$).[177]
- In a case series of 81 infected turkey farms in Iowa, Minnesota, North Dakota, South Dakota, and Wisconsin in the 2015 HPAI H5N2 outbreak , 47% of case farms used off-site carcass disposal (renderer, landfill, other) in the 14 days prior to disease detection.[26]
- Data from the 2003 HPAI H7N7 outbreak in the Netherlands were used to estimate H7N7 transmission probabilities to susceptible farms by individual contact types (e.g., feed delivery, egg transport, etc).[68]
  - The analysis determined that, per contact, rendering visits posed a 25% chance of transmission.
- A case-control study of the 2002 LPAI H7N2 outbreak in Virginia found dead bird disposal by rendering to be the most significant risk factor for avian influenza infection in turkey and chicken farms.
  - The odds ratio was 7.3 ($P < 0.001$) in a multivariate analysis.[70]
- Rendering pick-ups of dead birds likely played a part in the spread of avian influenza virus in the California LPAI H6N2 outbreak from 2000 to 2002.[128,253]

- Among carnivorous species that may be fed poultry carcasses or byproducts from the poultry industry, HPAI infection has been documented in a variety of species that may be used in the commercial or exotic pet trade, in zoos, or in the fur industry.
  - In reviews of HPAI H5N1 infection in carnivorous species, tigers, leopards, other exotic felids, domestic cats, domestic dogs, civets, and ferrets were identified as potential host species. Within-population transmission was documented in various cat species and in ferrets.[289,304]
    - It was hypothesized in all cases, except that of infection in a colony of civets, that carnivores were infected by consuming or scavenging infected bird carcasses.[289]
  - In captive large felids, onset of clinical signs has been correlated with feeding poultry carcasses in areas with ongoing HPAI outbreaks.[301]
- In captive mink, clinical signs when inoculated with H9N2 (A/Chicken/Hebei/4/2008) were relatively mild, including lethargy and dry nose. No mortality was observed, but pulmonary edema and inflammatory infiltrates were noted on histopathology of lung tissue. Upper respiratory shedding of virus was evident up to 15 days post inoculation.[298]
- In non-mammalian carnivores, there is limited evidence for AI virus infection. One study noted antibodies against an unknown subtype of AI virus in captive crocodilians of three species in Florida, and PCR testing revealed over 99% identity with the NS1 gene of duck AI virus isolates in four crocodilian species tested.[305]

9.2.4.3.2 Qualitative Analysis

The following qualitative factors were considered for evaluating off-site dead bird disposal:

- Collection dumpsters for dead birds 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), this presents the opportunity for mechanical or biological transfer of HPAI virus via scavengers from infected and undetected carcasses onto the surrounding grounds. This could potentially result in cross-contamination of the rendering truck or other mortality transport truck tires and personnel boots, with subsequent contamination of other premises and other turkey houses.
- The transfer of infected and undetected poultry carcasses from the collection dumpster into the rendering truck or mortality transport truck 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 on another premises, the lift arms could contaminate the dumpster there, and the truck tires could contaminate the ground near the dumpster.
    - Many studies have demonstrated high titers and the persistence of HPAI virus in various poultry tissues and fluids:
        - When turkeys were experimentally infected oro-nasally with 100 µl of $10^6 EID_{50}$ of HPAI H7N1, virus persisted for > 1 day at > $10^4 EID_{50}$/g of muscle tissue at 4°C (39°F).[46]
        - Toffan et al. (2008) experimentally infected turkeys with A/turkey/Italy HPAI H7N1 and measured titers up to $10^{6.8}$ EID_{50}/ml in their blood.[49]
        - In experimental infections of chickens with EA/AM HPAI H5N2, viral titers were 10^7 EID_{50}/g in spleen and lung samples.[51]
        - Chicken thigh meat contained up to $10^{7.5}$ EID_{50}/g of HPAI H5N1 virus at 24 hours after experimental infection.[50]
        - EA/AM HPAI H5N2 viral titers of $10^{3 \text{ to}}$ 10^5 EID_{50}/mL of turkey feces were interpolated from cloacal swab data (E. Spackman, personal communication, May 2016).
        - Experimental infections with the 1983 Pennsylvania HPAI H5N2 strain resulted in ~10^9 EID_{50}/g of chicken feces.[65]
        - Turkey feather tip pools from experimentally infected birds contained $10^{4.168}$ to $10^{5.79}$ EID_{50}/ml of HPAI H5N1 virus (M. Slomka, personal communication, January 2014).
        - Indiana HPAI H7N8 viral titers were$10^{5.9}$ EID_{50}/ml in feather root samples (M. Pantin-Jackwood and E. Spackman, personal communication, May 2016).
    - A 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 8.7.2 (Hazard ID), only 1.5 ounces (~44 ml) of carcass fluid contains enough viral particles to infect approximately 4400 birds. If a higher amount of virus was present to begin with (e.g., 10^7 EID_{50}) then a single milligram could contain enough virus to infect 100 birds.
  - In a study of 1-week-old chicks that had been infected intratracheally with 2.5×10^4 $TCID_{50}$ of HPAI virus (H5N1), homogenates of liver, lung, kidney, and brain from those infected chicks contained $10^{6.3}$ to $>10^{9.3}$ $TCID_{50}$/g tissue. On the basis of the relative weight of the lungs, liver, kidneys, and brain of 1-week-old chicks weighing 50 to 55 g, the volume of two chick carcasses represents a minimum of 10^{10} $TCID_{50}$.[283]

- If the rendering truck or other mortality transport truck bed is not covered, 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 along the route.
  - Transport trucks may be owned by the integrator or by a third-party contractor. Trucks operated by contractors may or may not be covered, and it may be difficult to require use of covered trucks in these situations (TWG, personal communication, 2016). Additionally, even if a truck is covered, feathers and other material may still escape at driving speeds.
  - In addition to literature cited above, another study found that feathers harvested from ducks experimentally infected with HPAI H5N1 maintained viral titers of $10^{5.5}$ EID_{50}/ml for 3 to 6 days at 4°C (39°F) and $10^{4.0}$$EID_{50}$/ml for 3 to 6 days at 20°C (68°F).[232]
    - The authors point out that, while fecal material containing high viral loads may be quickly diluted in the environment, contaminated feathers may persist as solid materials in the field.
  - In the 1983 to1984 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.[253]

- Some companies require rendering trucks to be cleaned and disinfected (C&D) between farms. The STS Plan requires <u>all vehicles</u> that have been to a rendering plant to be C&D before returning to a turkey farm.[10]
  - This is more likely to be effective at eliminating virus contamination in warmer and drier conditions than in colder and wetter conditions.
    - Section 9.1.4 of the Turkey Hatching Eggs risk assessment[8] and Appendices 4, 5, and 6 of the Nest Run Eggs risk assessment[306] discuss the effectiveness of disinfectants and C&D under different environmental conditions.
  - If C&D is inadequate to remove viral contamination or is not performed between farms, HPAI virus survival is not likely to be affected by the relatively short time period between farms, especially in the protected, moist environment provided by poultry carcasses.

    - Appendix 1: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates details HPAI virus survival on various substrates under different environmental conditions.

- Similar to rendering, collection of mortality from turkey grower operations for use in the feeding of captive carnivores or fur-industry commercial mink and fox operations represents a risk of premises contamination.
    - Growers may individually contract with a local mink operation, or may source mortality to a centralized feeding operation that collects byproducts from many agricultural industries, including egg, dairy, swine, beef, fish, and slaughter facilities.[302]
    - Protocols for truck and driver biosecurity of such operations, in addition to C&D of equipment used to store and transport mortality, are not known and are suspected to vary by premises.
    - Feed for many mink and some farmed fox is prepared on-site and may include ground raw poultry carcasses combined with other agricultural products that the grower may source and store on-site. This practice occurs both in the United States and in other major fur-producing countries such as Canada and China.[298,307,308]
- Ranches and farms in the U.S. that raise fur-bearing animals are geographically limited; they are mostly located in states with cooler winter climates, and the number of registered premises in the U.S. is likely under 400.[309]
- Both mink and foxes have been shown experimentally to be susceptible to multiple HPAI and LPAI viruses,[283,310] and mink have been shown to transmit avian H3N8, H11N4, H7N7, H5N3, and H9N2 by contact with other mink.[298,310]
    - In a Chinese study of farmed mink, there was serological evidence of exposure to H5N1 strains (RE-5 and RE-7) and H9N2 strains (A/Chicken/Hebei/4/2008 and A/chicken/Shanghai/10/01) in both juvenile and breeding adult mink.[298] Of note, researchers could not find a mink farm where poultry carcasses or byproducts were not fed to mink as part of their regular ration.
    - This may represent a means of perpetuating virus and a potential source of contamination for turkey premises that contract with mink farms for mortality disposal.
- During PMIP, entering a poultry house is prohibited unless the person is wearing clothing dedicated to the farm and footwear dedicated to the house (see Appendix 7: Cross-Commodity Pre-Movement Isolation Period).
    - If virus from off-site dead bird disposal had contaminated the ground before the onset of PMIP, premises-specific clothing and footwear could still become contaminated before entering the poultry house.

9.2.4.3.3 Likelihood Rating and Conclusion

While off-site disposal methods are prohibited during the PMIP leading up to load-out because of the associated high risk of virus transmission, there remains the potential for contamination of the ground around a poultry house that could lead to virus being tracked into the house on

personnel boots or equipment. The PMIP is designed to increase the probability of detection if virus were introduced to a flock 9 or more days before load-out (since PMIP duration for turkeys to market is 8 days), but virus could be tracked into the barn any time during PMIP, potentially allowing an infected but undetected flock to move to market. Given that off-site dead bird disposal occurs in the Control Area before a PMIP, we thus rate the likelihood of a turkey flock becoming infected as a result of HPAI virus introduction to the flock (before or during the PMIP) via off-site dead bird disposal that takes place prior to the PMIP to be *moderate*, provided that best on-site carcass disposal practices and the cross-commodity and STS Plan PMIP measures are followed.

9.2.5 Likelihood of a Turkey Flock Becoming Infected with HPAI Virus due to Garbage Management

Garbage is typically removed from poultry premises by contracted garbage management services (see Appendix 9: Poultry Industry Survey on Garbage Management Practices). Garbage trucks coming near the barns were a significant risk factor in a case-control study of egg layer flocks in the 2015 U.S. HPAI outbreak.[177] This evaluation considers the possible ways a turkey flock could become infected with HPAI virus by garbage management before movement to processing.

9.2.5.1 Likelihood of HPAI Virus Infection via Garbage Management

Garbage management represents a potential pathway for HPAI virus infection of a turkey flock, as multiple poultry premises may share a common disposal site (e.g., landfill), trash collection service provider, or trash collection site (i.e., shared dumpster for multiple premises). HPAI virus may enter a turkey premises via contaminated garbage trucks or drivers. **Figure 22** diagrams the transmission pathway.

Potential sources of HPAI virus contamination at landfill
Mortality
Processing plant offal
Eggs from layer premises
Infected, undetected premises
Infected, detected premises

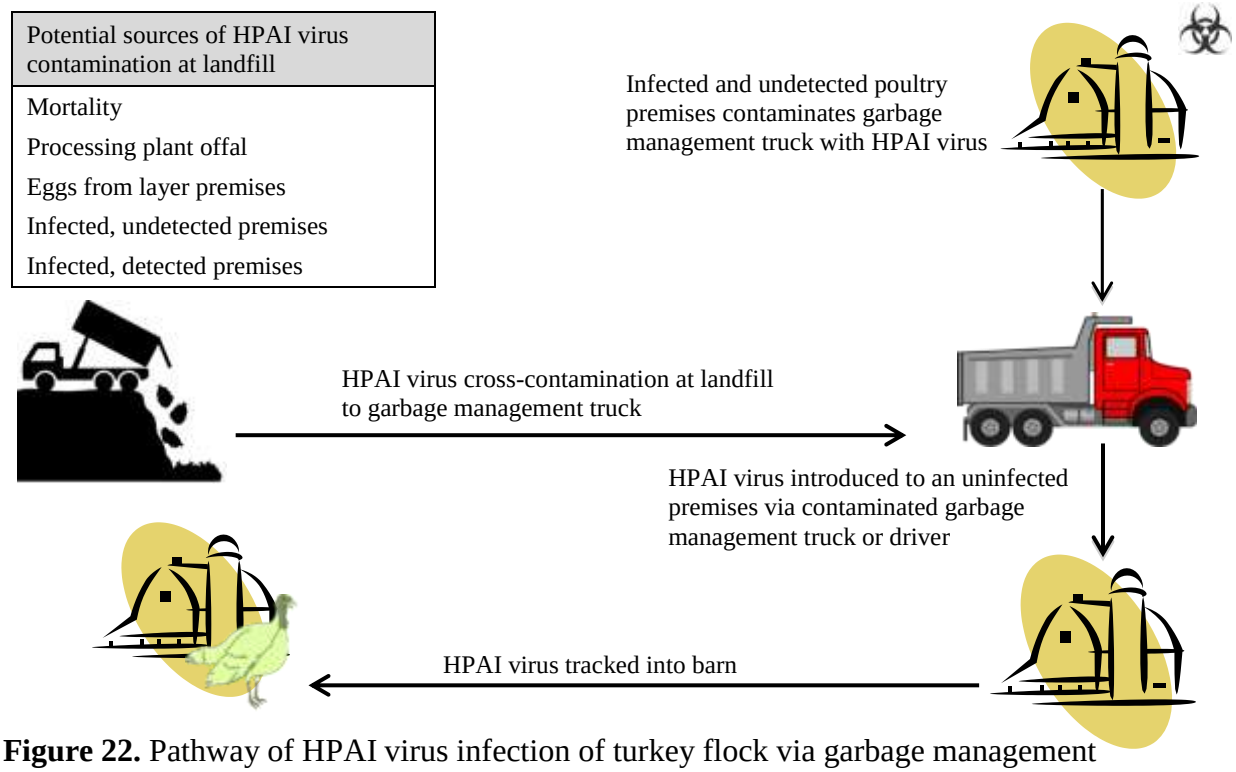


Figure 22. Pathway of HPAI virus infection of turkey flock via garbage management

9.2.5.2 Literature Review

- In the 2015 HPAI outbreak, garbage management was identified as a novel risk factor for disease spread.[177]
    - In the 2015 outbreak of HPAI H5N2 in the U.S., 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 <.001). This practice occurred at 61% of case farms and 23% of control farms.[177]
        - The univariate analyses (of factors considered for the farm-level multivariable model) showed that 39% of control farms did not allow garbage trucks beyond the perimeter of the premises; this did not occur at case farms (i.e., garbage trucks either entered the farm or did not come to the farm at all) (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%; control farms, 26%).[177]
    - Of note, 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 and may visit or contract with multiple poultry premises in an area.
    - 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).[241]
        - 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%) to off-farm transmission.[251]
        - The models estimated the percent contribution to off-farm transmission. Visitor activities in the high-poultry-density region (1.45 farms/5 miles^2) and low-poultry-density region (0.49 farms/5 miles^2) were calculated separately.
- In many areas, non-commercial poultry operations (i.e., live poultry markets and backyard flocks) may utilize the same garbage management contractors as commercial poultry farms. On non-commercial 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% CI, 1.8-3.4).[311]

- In an analysis of risk factors associated with H5N1 in backyard poultry in Egypt from 2010-2012, disposing of mortality and poultry feces in garbage piles outside was significantly correlated in the regression model (F=15.7; P<0.0001).[312]

- Landfills may serve as a potential site for cross-contamination as garbage management services for 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.[59,256]
  - In the 2002 LPAI H7N2 outbreak in Virginia, disposal of depopulated flocks mainly occurred at "mega-landfills" by sealed, leak-proof trucks that were cleaned and disinfected on-farm and at the landfill.[59]
  - During the 2001-2002 Pennsylvania H7N2 LPAI outbreak, some euthanized case flocks were disposed of at landfills after being transported in closed containers.[256]

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. 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 broiler, turkey, and layer sectors, as listed in **Table 17**.
  - The survey results referenced throughout this qualitative analysis were obtained from a small convenience sample of individuals with knowledge of garbage industry practices in various poultry sectors and with a low response rate. Statistical analyses were not conducted for these data. Absence of an affirmative response cannot be assumed to indicate a high-risk activity is not occurring, and this has been taken into account in the assessment. Despite these limitations, the data are informative for the purpose of the risk assessment. For a summary of the survey results, see Appendix 9: Poultry Industry Survey on Garbage Management Practices.

Table 17. Survey results 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)
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)

Item	Broiler sector (n=8 respondents)	Turkey sector (n=15 respondents)	Layer sector (n=39 respondents)
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)
Lunch room and restroom garbage[c]	--	--	Yes (37/39)

[a]Yes indicates 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.

  - Additional items reported to be disposed of in the garbage on turkey premises were empty med containers, poult box papers, and supply containers. On broiler premises, additional items included boxes, buckets, jugs from disinfectants, litter treatments, and disposable chick feeder lids.

- Of potential HPAI-contaminated or infectious material reported to be disposed of in the garbage on poultry premises (i.e., dead wildlife, poultry carcasses, egg shells, and potentially contaminated materials that have contacted poultry), the hypothetical expected virus concentration on each type of item varies.
  - HPAI virus has been recovered in many tissues of poultry carcasses, such as muscle, liver, kidney, brain, spleen, and blood. For detailed information on virus concentration in various tissues, feces, and feathers, see section 9.2.4, Likelihood of Turkey Flock Becoming Infected with HPAI via Dead Bird Disposal.
    - If garbage is contaminated with infectious poultry carcasses, the risk of infection via garbage management is likely similar to the risk via off-site dead bird disposal. Off-site dead bird disposal has been implicated in previous AI outbreaks. For a detailed literature review, see section 9.2.4.2.1, Literature Review.
  - 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 9.2.4, Likelihood of Turkey Flock Becoming Infected with HPAI via Dead Bird Disposal.
        - 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 9.1.4, Risk of HPAI Spread to Turkey Flock in a Control Area 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 9.1.5, Risk of HPAI Virus Spread to Turkey Flock via Wild Non-Aquatic Birds in Farm Vicinity.
    - Eggs from infected hens have tested positive for HPAI virus, including egg shells, albumen, and yolk. Unusable eggs from commercial and breeder flocks may be disposed of in garbage. Measured concentrations have varied. See the Secure Egg Supply Egg Shell Risk Assessment for more details.[102]

- 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, egg shells, egg trays, wood, steel, glass, and PPE, see Appendix 1: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates.
- In a survey sent to representatives of the broiler, turkey, and layer sectors, it was reported that garbage is likely to be transported to a landfill by a contracted service provider for the majority of commercial poultry operations.
    - 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 at landfills.[313] 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.[313]
    - 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 arrives at a turkey 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.
            - In the Netherlands, poor management practices pertaining to liquid waste (e.g., waste water) and solid waste have been identified as potentially

increasing the risk of avian influenza transmission in the neighborhood of the infected farms (A. Ssematimba, personal communication, August 2016).[73]

- A shared dumpster or common trash collection point for multiple poultry premises, while not commonly used in the poultry industry, represents an additional site of potential cross-contamination between commercial poultry operations.

- Garbage trucks and drivers typically do not contact live poultry while completing contracted duties on a poultry premises. Biosecurity recommendations and site-specific biosecurity plans may not stipulate specific biosecurity measures for garbage management drivers; however, it is recommended that visitors follow procedures to cross the PBA and LOS.[1]
  - In a qualitative evaluation of potential AI transmission pathways on broiler and layer premises in the Netherlands, Ssematimba et al. 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.[73] The analysis considered contact frequency, biosecurity practices, and risk category.
- Virus introduction into poultry houses via garbage management may involve one or more virus transfer steps. Although there would likely be reduction in the virus concentration (6 to 27%) between a donor surface and recipient surface in each direct contact,[261] the virus concentration potentially tracked into the barn may still be above the infectious dose. This depends on the initial viral load and infectious dose of that virus strain in turkeys.
  - It is assumed that the distance traveled by the vehicle between the time of contact with infected garbage and the uninfected turkey premises may lessen the amount of virus present on the tires for transmission once at the premises. However, mechanical transmission of a similar type virus (PRRSV) has been demonstrated experimentally in a swine industry-like setting.[236]
  - Alternatively, if a contaminated load of garbage is in the truck at the time of arrival on an uninfected turkey premises, fewer transfer steps are required.
  - The enhanced biosecurity required during a PMIP applies only to farms located in a Control Area that wish to move birds off the premises. It is assumed that there may be marked variation in the biosecurity and garbage practices on farms within the Control Area 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, there is a decreased likelihood of cross-contamination between contaminated garbage management and personnel, equipment, or other potential fomites that may access the poultry house.
    - Based on survey responses from representatives of the turkey industry, it is common practice for the dumpster or trash collection point to be located at the entrance or perimeter of the farm. This equates to a distance of 100 to 250 feet

from the nearest poultry barn for the majority of respondents, but this distance varies.

- As is true with other third-party contractors, poultry growers or integrators may find it difficult to control or influence certain practices by contract garbage haulers, including C&D of garbage trucks, pickup routing, and landfill practices.

- On turkey premises, the frequency of garbage pickup is most often weekly, based on survey responses from representatives of the turkey industry.

9.2.5.4 Likelihood Rating and Conclusion

9.2.5.4.1 Likelihood of a Turkey Flock Becoming Infected with HPAI Virus due to Garbage Management

Garbage management was identified as a novel risk factor for HPAI virus introduction in the 2015 outbreak in the U.S. 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. Given that there is potential for HPAI virus associated with garbage management to be tracked into the poultry house, the likelihood of a turkey flock becoming infected with HPAI virus due to garbage management without or prior to a PMIP is *moderate to high.*

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

During the PMIP, garbage will not be removed from the premises, given the risk it presents, and the producer is responsible for managing the risks associated with any on-site garbage movement that must occur. The greatly intensified biosecurity measures of the PMIP, such as using footwear specific to each poultry house, should decrease the likelihood that virus is tracked into barns during the final days before load-out (see Appendix 7: Cross-Commodity Pre-Movement Isolation Period). Provided the on-farm biosecurity measures are strictly followed during a PMIP, the likelihood of a turkey flock becoming infected with HPAI virus due to garbage management during PMIP is *low*.

9.3 Pathways for a Turkey Flock Becoming Infected with HPAI Virus 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., load-out crews, which may load out more than one flock within 12 hours, have been associated with the spread of AI.[314] 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 removal.[253,315] During the 1986 LPAI H5N2 outbreak in Pennsylvania, restricting farm access to only sanitized load-out trucks and crates interrupted infection transmission.[316] Currently, a majority of commercial turkey grower premises practice an “all-in, all-out” management system, in which a single-age flock is placed on a premises and then the farm is completely depopulated at load-out; however, approximately 40 to 45% of commercial turkey grower premises are multi-age farms

where younger birds will remain on the premises (sometimes for multiple weeks depending upon their life stage) after older, market-age birds are loaded out for processing.[4,7,26]

In this chapter we are assessing the likelihood that a turkey flock will become infected during the load-out process (either birds that will be loaded out or younger birds on a multi-age premises that will not be loaded out), resulting in movement of infected but undetected birds to market. Pathways considered include contaminated load-out equipment, vehicles, and/or crews from an infected and undetected farm, followed by introduction of HPAI virus into a flock at the next load-out premises.[e]

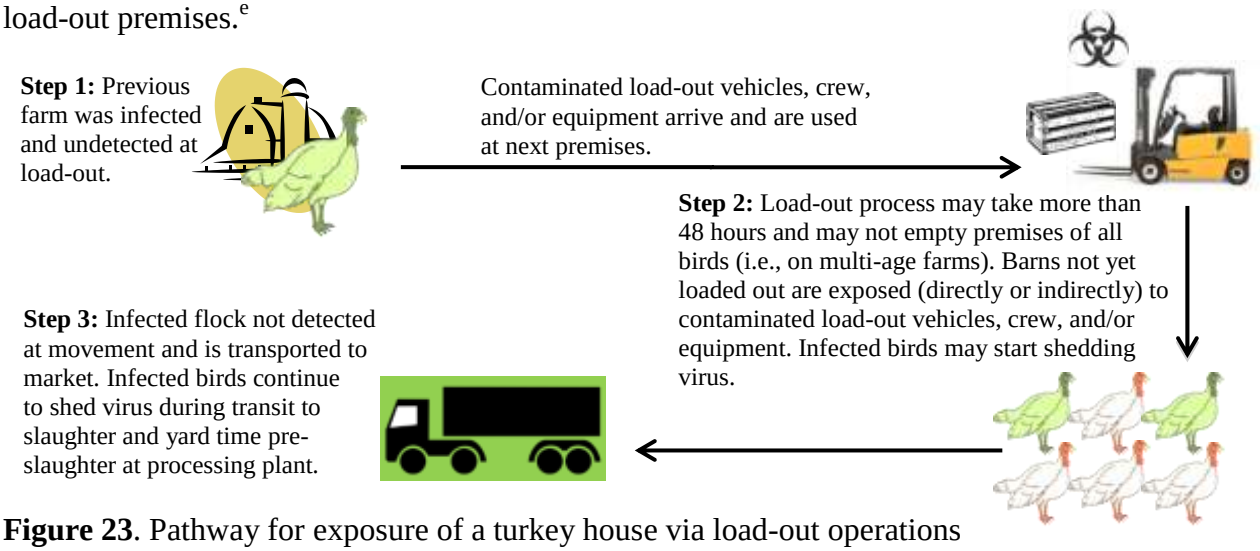


Figure 23. Pathway for exposure of a turkey house via load-out operations

9.3.1 PMIP Measures for Moving Turkeys to Slaughter

For premises within a Control Area that wish to move turkeys to slaughter, a Pre-Movement Isolation Period (PMIP) is defined that limits non-critical visits and personnel on the farm, while biosecurity and flock disease surveillance are increased (see Appendix 7: Cross-Commodity Pre-Movement Isolation Period).[248] 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 virus in the final days before load-out. 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 Likelihood of Detecting HPAI in an Infected Turkey House.

9.3.1.1 Additional Load-out Mitigation Measures for Permitted Movement of Poultry to Market from a Control Area

Load-out begins as the first crew, vehicle, or equipment arrives on the premises and ends when the last load of birds departs the premises. As noted, on multi-age premises there still may be

[e] Premises contamination with HPAI virus by load-out crews or processes may also represent a pathway by which day-old poults could become infected upon placement of the next flock in the brooder house of the same poultry premises if virus were not inactivated during downtime. These pathways leading to infection of day-old poults are outside the scope of this risk assessment (see Turkey Day-Old Poults Risk Assessment).

turkeys on the premises after a load-out is completed. Pre-staging of equipment during a PMIP is prohibited (see Appendix 7: Cross-Commodity Pre-Movement Isolation Period).

The biosecurity and sampling stipulations pertinent to load-out of turkeys become more stringent as the duration of load-out increases. If birds are infected because of contaminated load-out equipment entering the premises, they have the potential to shed virus up until the time of slaughter. Viral contamination may be tracked into occupied poultry houses which are still awaiting load-out, or, on multi-age premises, into occupied poultry houses which will not be loaded out for several more weeks. This extends the period for HPAI virus to replicate and spread through the flock, and includes any time remaining in the poultry house until load-out, in addition to transit time, and any hold time at the plant before slaughter. Load-outs of longer duration pose an increased risk of transporting infected but undetected birds to market.

To meet the permit guidance criteria for movement from a Control Area, all turkey premises (regardless of load-out time) should adhere to mitigation measures after completion of load-out, such as load-out crew stipulations and live-haul routing requirements, and sanitation procedures for live-haul trucks and equipment.[10]

A greater emphasis is placed on decreasing the likelihood of HPAI-contaminated load-out equipment being used for permitted movement within a Control Area, and on diligent biosecurity between barns to minimize spread between poultry houses in the event of a virus introduction during load-out. Specifically, turkey loading equipment and live-haul trucks and equipment must be cleaned and disinfected under an appropriate protocol[317] after a turkey house is depopulated and before entering another premises[10] so that they remain free or nearly free of contamination. Additionally, loading crews are prohibited from entering other turkey houses on the same farm.[10] These suggested measures become more stringent as the amount of time needed for a load-out increases. For turkey premises where load-out takes more than 48 hours, daily PCR testing is required of barns that will be moved after the initial 48 hours of load-out. In addition to the PCR testing, some PMIP biosecurity measures must continue throughout the multi-day load-out (see Appendix 11: Load-out Mitigation Measures). Results of modeling simulations to support the increased biosecurity and sampling requirements for premises with longer load-out times can be found in Appendix 10: Supplementary Testing Protocols.

9.3.2 Literature Review

In past LPAI outbreaks in the U.S. poultry industry, load-out equipment and crews have been implicated as a means of virus spread between farms, specifically involving partial flock removal and movement of load-out crews between premises.

- In the 1995-1996 LPAI H9N2 outbreak in Minnesota, likely transmission pathways between commercial turkey premises included exposure from contaminated processing trucks during partial flock load-outs and contaminated load-out personnel and equipment from an infected flock.[315]
- In a 1978 outbreak of LPAI (H6N1, H4N8, H6N2) in Minnesota turkey flocks, the management practice of marketing turkey hens while leaving growing toms on the farm allowed potentially contaminated load-out equipment and crews to contact birds (toms) that would remain on the farm.[253]

- In an overview of the Minnesota Cooperative Control Program, Poss et al. identified orderly marketing as a procedure to prevent AI virus spread, as there is potential for heavy contamination to personnel and equipment involved in the transport of an infected flock. Previously, load-out crews, which may load out more than one flock within 12 hours, have been associated with the spread of AI.[314]
- The use of clean load-out vehicles and equipment has been protective in a past AI outbreak. During the 1986 LPAI H5N2 outbreak in Pennsylvania, which likely spread in part through movement of contaminated crates, transmission was interrupted when premises access was restricted to sanitary crates and clean trucks.[316]

During previous poultry disease outbreaks (LPAI, HPAI, and ILT), movement of contaminated transport vehicles, transport equipment, and infected poultry likely contributed to virus spread between farms.

- Shared equipment likely played a role in the 2014-2015 HPAI outbreak in the U.S. Load-out equipment was among the most commonly shared equipment found in a case series of 81 infected turkey farms in Iowa, Minnesota, North Dakota, South Dakota, and Wisconsin (including 63 meat production farms). The case series showed that 85% of farms shared pre-loaders; 92% of farms shared live-haul loaders; and 69% of farms shared poultry trailers.[26] For more information on other shared equipment, see section 9.2.3, Likelihood of a Turkey Flock Becoming Infected with HPAI virus via Machinery or Equipment Shared between Multiple Premises.
- A 2001-2002 outbreak of LPAI H6N2 in Northern California is suspected to have spread from flock to flock in part through moving birds to slaughter.[128,318]
- In the 1983-1984 H5N2 LPAI and HPAI Pennsylvania outbreak, contaminated transport trucks and coops and movement of live birds were among the factors that contributed to spread of infection.[253]
- 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 the route in past outbreaks, see section 9.1.6 Risk of HPAI Virus Spread to Turkey Grow-Out Premises near Poultry Live-Haul Routes via Feathers, Feces, and Other Fomites.

The load-out process inherently places crews, vehicles, and equipment in close contact with live poultry, poultry feces, and poultry feathers.

- Estimates of HPAI virus concentrations in feathers, feces, and blood from infected poultry generally range between 10^3 and10^7 EID_{50} per gram or per milliliter of tested substrate, although higher concentrations have been observed in some cases.[44,50,51]
- While turkey load-out equipment is typically C&D between farms, not all items are easy to disinfect, especially if all organic matter is not removed in the cleaning step (typically power-washing); power-washing practices may vary in thoroughness and effectiveness in practice.[26]
- For further information on viral load on substrates related to live-bird movement, see section 9.1.6, Risk of HPAI Virus Spread to Turkey Grow-Out Premises near Poultry

Live-Haul Routes via Feathers, Feces, and Other Fomites and section 9.2.4, Likelihood of Turkey Flock Becoming Infected with HPAI via Dead Bird Disposal.

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 U.S.

- Kurmi et al., Beard et al., and Wood et al. reported that HPAI virus strains were inactivated in chicken feces in less than 5 days in warm temperatures (71 to 77^{o}F) and nearly 2 to 8 weeks in cooler temperatures (39.2 to 46.04^{o}F).[77,257,270] In these experimental studies, when temperature was constant, time to virus inactivation in feces usually increased as moisture level increased.[77,257] On substrates that may be found on vehicles or poultry transport crates, 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.[258] In cool temperatures (39.2-46.0 ^{o}F), an HPAI H5N1 strain (A/Vietnam/1203/2004 [H5N1 clade 1]) on glass and galvanized metal persisted longer in low relative humidity than in high humidity, whereas the opposite was true for the same virus in feces. (On glass and metal, virus was recovered at day 13 in low relative humidity and at 4-9 days in high relative humidity; in feces, virus was recovered at day 8 in low relative humidity and at day 13 in high relative humidity in feces).[257]
- For further data on viral persistence on different substrates and in varying environments, see Appendix 1: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates.

9.3.3 Qualitative Analysis

We considered the following qualitative factors for evaluating this pathway:

- The time required to load out a turkey premises depends on the size and type of operation, crew and equipment logistics, and capacity of the slaughter facility.
    - Industry representatives report that some premises can complete load-out in one night; but load-out may take up to 5 days for multi-age premises or up to 10 days for all-in, all-out farms with up to 280,000 birds (TWG, personal communication, January 2016).
    - Transportation time from the farm to a processing plant ranges from 10 minutes to 6 hours. This timeline is optimized to minimize transit mortality and protect carcass value and likely cannot be shortened further (TWG, personal communication, January 2017).
    - Turkey farm size and slaughter plant capacity vary with geography and logistics. Industry representatives from the TWG have indicated that load-out crew and processing plant schedules may be adjusted slightly during an outbreak situation, if possible (e.g., depending upon crew availability), in an attempt to expedite the load-out process (TWG, personal communication, January 2016).
- As discussed in section 9.4, Likelihood of Detecting HPAI in an Infected Turkey House, the likelihood of a turkey house 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 a PMIP.

- The enhanced biosecurity measures in place during a PMIP are not required to continue into the load-out period (see Appendix 7: Cross-Commodity Pre-Movement Isolation Period); thus it is possible that contaminated equipment or crews brought to the premises for load-out could lead to cross-contamination of houses that have yet to be loaded out via people or equipment entering occupied poultry houses.
    - In the event of a single point-source infection, **Table 18** shows the estimated number of birds in a given barn which may be infectious, depending on duration of time between infection and slaughter (i.e., load-out, transit time, and yard time at plant).

Table 18. Estimated number of infectious birds in a single poultry house if exposed at the time of load-out from a single-point source infection[a]

Initial number of infected birds	Mean number of infectious birds post-exposure to HPAI									
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days
1 bird	0	2	6	19	64	211	621	1,542	3,125	5,187

[a]Transmission model estimations are from 6,000 iterations using EA/AM HPAI H5N2 strain characteristics from an isolate from a turkey infected in Minnesota during the 2015 outbreak and a mean flock size of 15,188 birds.

    - If, however, equipment were heavily contaminated (which may be less likely following proper C&D outlined in the STS Plan) or virus strain characteristics caused initial infection in multiple birds, the mean number of infectious birds in a barn at a given time-point is much higher (**Table 19**).

Table 19. Estimated number of infectious birds in a single poultry house if exposed at the time of load-out from an initial infection in multiple birds[a]

Initial number of infected birds	Mean number of infectious birds post-exposure to HPAI									
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days
10 birds	5	17	55	182	572	1,574	3,522	6,196	8,638	9,822
100 birds	48	164	516	1,447	3,424	6,344	9,095	10,406	9,854[b]	7,936

[a]Transmission model estimations are from 6,000 iterations using EA/AM HPAI H5N2 strain characteristics from an isolate from a turkey infected in Minnesota during the 2015 outbreak and a mean flock size of 15,188 birds.

[b]In the model, a large proportion of birds died before 9 days, leading to smaller mean numbers of infectious birds at 9 and 10 days post-exposure.

- If birds are infected by load-out equipment, they have the potential to shed virus up until the time of slaughter. This includes load-out, transit time, and any hold time at the plant before slaughter. Longer load-outs thus pose an increased risk of transporting a considerable number of infected but undetected birds to market.

- During non-outbreak operations, turkey live-haul trucks and load-out equipment are routinely C&D prior to entry onto turkey premises. Following an outbreak of HPAI, live-haul trucks and equipment must be C&D at the processing plant before going to turkey farms, and turkey-loading equipment must be C&D after a turkey house is depopulated, both using National Animal Health Emergency Management System (NAHEMS) guidelines. Despite such C&D recommendations, some feces, feathers, and other contaminants may remain on surfaces that will contact a subsequent flock (TWG, personal communication, January 2016). The STS Plan describes further recommended mitigation measures for load-out following an outbreak of HPAI:
  - Turkey loading crews are prohibited from entering other turkey houses on the same farm, and load-outs within the Control Area will be limited to one farm per night per crew. Crews must shower and change to clean clothes and footwear between farms.[10]
  - Turkeys from farms in an Infected Zone should be loaded on a day at the end of a week to maximize opportunity for equipment C&D and downtime.[10] The plan does not stipulate any specific downtime requirements for crews or their personal vehicles.
- While sharing load-out and live-haul equipment between farms is not recommended, industry representatives have indicated that, in practice, such equipment is frequently shared (e.g., loaders, pre-loaders, load-out boards, live-haul trucks, etc) (TWG, personal communication, January 2016).[26] It is currently unrealistic to prohibit sharing of load-out and live-haul equipment, as it is typically owned by the processor.
  - Ideally, shared load-out equipment is adequately C&D before moving between farms, but load-out equipment that remains on the same premises is not generally C&D between houses (TWG, personal communication, January 2016).
- As pre-staging of load-out crews or equipment is not allowed during a PMIP, potential viral introduction via contaminated crews or load-out equipment would occur after the conclusion of flock sampling for HPAI required for permitted movement of turkeys to market.
  - The latent period for an individual turkey varies with virus strain and infectious dose; it has been estimated to range from less than 1 day to 1.41 days (Erica Spackman, personal communication, December 2015).[89,104,105] Thus, considering both latent period and contact rate in the event of exposure to HPAI virus, the number of infectious birds shedding virus in a flock at the end of a 48-hour load-out period would be low (**Table 18** above).
    - Greater variation exists in infectious period and mean time to death for turkeys infected with AI. Experimental transmission studies with H7N1 and H5N1 strains have estimated the mean infectious period to be between 1 and 1.5 days, while inoculation with 2015 EA/AM HPAI H5N2 (Tk/MN/2015) resulted in a mean time to death of 5.61 days.[89,104,105]

        - For a more detailed review of experimental studies of latency period, infectious period, and mean time to death from AI infections in turkeys, see section 8, Hazard Identification – HPAI Overview and Section 9.4, Likelihood of Detecting HPAI in an Infected Turkey House.
    - Large turkey flocks may require more than 3 to 4 days to load out. In the transmission model shown in **Table 18**, extending load-out to 5 or 6 days results in more than a 10-fold increase in the number of infectious birds compared with load-outs of 3 to 4 days or less.
        - In a scenario in which contaminated load-out crews or equipment infect more than one bird initially, the potential number of infectious birds at the point of slaughter could be much higher much sooner. For example, if there are 10 birds initially infected, the model estimates close to 200 infectious birds by the fourth day after exposure to HPAI (**Table 19**).
    - Transport time from farm to slaughter plant represents additional time for potential viral shedding. As noted earlier, the transportation time for commercial turkey systems in the U.S. is generally less than 1 hour up to 6 hours (TWG, personal communication, January 2017).
- More stringent load-out biosecurity is required for premises with extended (multiple-day) load-outs. These measures should decrease the likelihood of contaminated people or equipment bringing HPAI virus to a premises or tracking virus between barns during the load-out process.
    - In addition to STS Plan biosecurity (level 2) recommendations, for premises with load-outs longer than 48 hours, two key components of the more stringent PMIP biosecurity measures should continue throughout the load-out: (1) pre-staging of equipment in a barn before beginning load-out in that barn is not allowed, and (2) all persons entering a barn must utilize barn-specific footwear and farm-specific clothing.
    - For additional details on mitigation measures for extended load-outs, see Appendix 11: Load-out Mitigation Measures.
- Most companies stipulate, per the STS Plan, that load-out crews enter only the barn where they are currently working. However, growers and employees may still need to enter multiple barns on the premises in the course of caring for birds (TWG, personal communication, January 2016).
- Flocks which are infected via load-out equipment may not be detected by clinical signs or mortality trigger alone.
    - PCR testing of barns during load-out on a premises should increase the probability of detecting infections that occurred because of the load-out process, especially as the duration of load-out increases.
    - For further information on load-out testing and surveillance protocols and sensitivity analysis of such protocols, see Appendix 10: Supplementary Testing Protocols.

- On multi-age premises, younger birds remaining on the farm after load-out are at increased risk of coming into contact with HPAI virus that is brought to the premises via contaminated load-out equipment, crews, or vehicles.
    - Once load-out is complete, heightened biosecurity and surveillance measures stipulated for PMIP and load-out are no longer required. If the practice of using barn-specific footwear is not voluntarily continued, the potential to track HPAI virus contamination into occupied barns remains realistic.
- Premises outside the Control Area may be less likely to be infected, as the role of local area spread diminishes with distance. However, if HPAI-contaminated load-out crews, vehicles, or equipment were used outside the Control Area, there are fewer safeguards in place to decrease the likelihood of heavy contamination of a poultry flock during load-out that could lead to movement of an infected but undetected flock.
    - Premises outside the Control Area may not be subject to surveillance and pre-movement testing requirements beyond routine NPIP surveillance for LPAI.
    - Premises outside the Control Area have no limitations on load-out duration or pre-staging of load-out equipment.
    - Load-out vehicles and crews outside the Control Area may have less stringent biosecurity requirements.
- Load-out crews have the potential to carry virus off the premises on clothing, boots, and vehicles, and may pose additional risks in transit to and from job sites, by living in homes with backyard poultry or shared with individuals who work on other poultry operations.
    - STS Plan measures require a change of clothes and shower for load-out crews before going on to work with other poultry.[10]
    - Interaction between load-out crews and other poultry industry employees or other poultry is addressed in Section 9.2, Likelihood of Turkey Flock Becoming Infected with HPAI via Movements of People, Vehicles, or Equipment.

9.3.4 Risk Rating and Conclusion

Previous outbreaks have implicated contaminated load-out crews and equipment in the spread of AI. In the U.S. commercial turkey industry, C&D of vehicles and equipment associated with terminal movements is routine practice. If a flock were infected via contaminated load-out crews or equipment, shortening the time from load-out to slaughter for the complete premises limits how long the virus may spread within the flock. However, the time required to load out and completely depopulate a turkey premises depends on the size and type of operation (i.e., multi-age premises may not be completely depopulated), crew and equipment logistics, and capacity of the slaughter facility.

Given that PMIP enhanced biosecurity and testing measures are utilized, and that additional load-out mitigation measures are in place for multiple-day load-outs, we estimate the likelihood of a turkey flock becoming infected with HPAI virus via load-out operations and resulting in an infected but undetected movement to market to be *low to moderate.*

Multi-age premises, which house two or more distinct age groups of turkeys for grow-out, represent a unique deviation from the assumptions used for single-age (i.e., all-in, all-out)

management plans, as movement of birds to market does not necessarily result in complete depopulation of a premises. Turkeys remaining on a premises represent a susceptible host population at increased risk of exposure to HPAI-contaminated load-out equipment, vehicles, or crews due to proximity. Given that load-out mitigation measures are in place, specifically a prohibition of load-out crews entering other turkey houses on the same farm, the risk of the remaining (younger) turkeys on a multi-age premises becoming infected with HPAI virus via load-out operations on that premises is estimated to be *moderate* to *high*.

9.4 Likelihood of Detecting HPAI in an Infected Turkey House Prior to Movement

9.4.1 HPAI Surveillance Measures

9.4.1.1 Current Measures

Current routine influenza surveillance measures involve testing of turkey flocks for H5/H7 subtypes of AI for birds processed at slaughter plants participating in the U.S. H5/H7 avian influenza monitored program of the NPIP (see 9CFR part 146.33 for further information).

9.4.1.2 Outbreak Measures

Active Surveillance by rRT-PCR Testing

The active surveillance protocol option outlined in the STS Plan and evaluated in this risk assessment document involves testing two pooled samples via rRT-PCR (influenza matrix gene real-time reverse transcriptase polymerase chain reaction) at National Animal Health Laboratory Network (NAHLN) labs. One pooled sample with swabs from 11 dead birds must be tested by rRT-PCR for every 50 dead birds from each house on the premises for 2 consecutive days prior to the start of load-out. The sample for the second (later) rRT-PCR test must be collected within 24 hours before the start of load-out.[10]

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, in which case earlier sample collection times for rRT-PCR tests may be needed on a case-by-case basis. An active surveillance protocol involving one pooled sample of 11 swabs on two consecutive days in which the second (later) test is collected within 48 hours prior to the start of load-out is evaluated in Appendix 10 (Supplementary Testing Protocols) for those premises that anticipate the turnaround time for rRT-PCR results to take longer than 12 hours. Collecting rRT-PCR samples earlier reduces the likelihood of detecting HPAI prior to the load-out start; thus, it is important to note that this alternate testing protocol is outside the scope of preferred testing protocols as outlined in the SPS plan.

Detection through Trigger for High Mortality

If daily mortality exceeds 2/1000 birds (excluding culls) in a house immediately prior to a scheduled movement, turkeys shall not move until diagnostic steps have been initiated and HPAI has been ruled out as the cause of elevated mortality.[10]

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

The likelihood of detecting HPAI in a turkey house 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 house, 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. (2016).[319] Some of the input parameters of the simulation models from Weaver et al. (2016) have been updated according to recent research on the 2015 EA/AM HPAI H5N2 outbreak in the U.S.[319] A summary of the input parameters is given in **Table 20** and details on their estimation is given in Appendix 8: 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 in a house, which impact the rate of rise in mortality and morbidity over time. The HPAI spread dynamics depend on parameters such as the length of latently infected and infectious periods in individual birds and the "contact rate" between infectious and susceptible turkeys.
- 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. The chances of detecting a virus positive sample depend on the diagnostic sensitivity of the test.

The HPAI spread dynamics in a house 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 turkeys in each disease state is updated at 0.1-day time intervals. Transitions from the latent to the infectious state and the infectious to removed state are determined by latent and infectious period distributions estimated for a given HPAI strain based on data from experimental studies. Once a bird is in the removed state, it is considered to be 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 transmission contacts (i.e., contact is adequate to transmit infection) each bird has per unit time. Higher adequate contact rates result in a higher likelihood of infection. Similarly, as the number of infectious birds increases, the likelihood of infection increases.

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 mortality and disease mortality available on the test day. The normal mortality is simulated based on industry-provided daily and weekly 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 one of the days prior to the start of load-out.

9.4.2.2 Model Scenarios

The likelihood of detecting HPAI in a turkey house prior to movement is evaluated using data from two different isolates of the EA/AM HPAI H5N2 strain. Latent and infectious period distributions were estimated for each of the isolates from turkey inoculation data (E. Spackman, personal communication, December 2015). One isolate, from a turkey infected in Minnesota during the 2015 outbreak, was estimated to have a mean latent period of 1.41 days and mean infectious period of 4.20 days for a total mean time to death (combined length of the latent and infectious periods) of 5.61 days. Similarly, the other isolate, from a chicken infected in Iowa during the 2015 outbreak, was estimated to have a mean latent period of 0.73 days and mean infectious period of 4.82 days for a total mean time to death of 5.55 days.

The latent and infectious period distributions can impact the time to detection. HPAI strains with a long mean time to death, for example, will generally take longer to detect via active surveillance due to the slower rise in mortality. As the latent and infectious periods are strain-specific and can vary considerably, the probability of detection depends on the characteristics of the given strain. The HPAI strains evaluated in Weaver *et al*. (2015) for example, Pennsylvania HPAI H5N2 and Asian HPAI H5N1, had estimated mean times to death in turkeys of 4.15 and 2.55 days, respectively.[319] These HPAI strains are not included in the risk assessment since the mean times to death are less than that of either EA/AM HPAI H5N2 isolate. As the EA/AM HPAI H5N2 isolates are expected, therefore, to have relatively lower detection probabilities, the two scenarios are deemed sufficient for assessing the effectiveness of the active surveillance protocols and risk management strategies, since high probabilities of detection under the EA/AM HPAI H5N2 scenarios imply high probabilities of detecting strains with lower mean times to death. The two isolates are evaluated for turkey toms only, because toms, with generally higher non-disease mortality, which makes HPAI harder to detect, represent a more conservative approach. Thus, if detection probabilities achieve acceptable levels for toms, they will also be sufficient for hens.

Table 20. Parameter estimates for the HPAI transmission model for turkey tom houses

Parameter name	Parameter description	Distribution/Value
Contact rate (transmission parameter)	The number of direct or indirect contacts a bird has that is sufficient to transmit infection per unit time	Inverse gamma distributed (shape=16.775, scale=68.581, mean 4.35 birds per day) truncated at 0.25 and 100
Latent period distribution	Length of the latent period	EA/AM HPAI H5N2 Turkey isolate: Gamma distributed (shape=1.7733, scale=0.7976; mean 1.41 days) EA/AM HPAI H5N2 Chicken isolate: Gamma distributed (shape=4.0307, scale=0.1809; mean 0.73 days) Truncated at 3 days
Infectious period distribution	Length of the infectious period	EA/AM HPAI H5N2 Turkey isolate: Gamma distributed (shape=7.7624, scale=0.5405; mean 4.20 days) EA/AM HPAI H5N2 Chicken isolate: Gamma distributed (shape=2.9655, scale=1.6248; mean 4.82 days) Truncated at 10 days
Number of toms/house	Distribution of the number of toms per house	Exponential distribution truncated at 2000 and 40000. Mean 15188 birds per house. Estimated from industry data.

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 house becoming exposed to HPAI close to the start of load-out. **Table 21** gives the detection probabilities for a house from 1 to 11 days following exposure to HPAI under the active surveillance protocol of one rRT-PCR test of 11 swabs taken the day of and day before load-out begins on the premises.

Under the turkey isolate scenario, if a house were exposed to HPAI 5 days prior to the start of load-out, the estimated probability of detection is 59%. Given that the house was exposed for five days prior to load-out, testing would occur on the fourth and fifth day of HPAI being present in the house. In this example, the probability of detection improves as the number of days post exposure increases. The probability of detection increases due to the exponential growth in mortality that occurs as HPAI moves through the house, which increases the likelihood of including at least one bird that is dead due to HPAI in the pooled sample taken for diagnostic testing or observing total mortality above 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 house.

Table 21 can be used to inform the length of the PMIP under an assumption that the PMIP is 100% effective in preventing exposure to the pathogen. In these scenarios, it is conservatively assumed that the house is infected immediately before implementation of the heightened biosecurity of PMIP. For example, under a 4-day PMIP, a house is assumed to have been infected four days before the start of load-out, just prior to the start of the PMIP. The detection probability in this case, considering the turkey isolate scenario, is estimated to be 37%. Similarly, the scenario under a 5-day PMIP is estimated to result in a 59% likelihood of detection.

The length of the PMIP decided upon by the STS Working group is 8 days, which results in a high probability of detection under both scenarios. The detection probabilities are lower under the turkey isolate scenario primarily because it has a longer average latent period, which delays spread of the infection through the flock due to the increased time required for a bird to become infectious. The overall longer mean time to death for the turkey isolate relative to the chicken isolate would also be expected to cause lower probabilities of detection. As discussed previously, the strains considered in Weaver et al. (2015), Pennsylvania HPAI H5N2 and HPAI H5N1, both have lower mean times to death than the isolates given in **Table 21**.[319] Therefore, an 8-day PMIP is almost certainly sufficient for the Weaver et al. (2015) strains as well.[319] Thus, there is evidence that an 8-day PMIP is adequately robust for the characteristics exhibited by different strains and is long enough to consistently achieve high probabilities of detection.

Table 21. Simulation model results showing the predicted probability of HPAI detection for a turkey tom house infected some given number of days prior to the start of load-out on the premises[a]

Scenario	Number of days prior to movement when exposure to HPAI occurs										
	1	2	3	4	5	6	7	8	9	10	11
	Predicted probability of HPAI detection										
EA/AM HPAI H5N2: Turkey isolate	0.09	0.15	0.22	0.37	0.59	0.78	0.91	0.97	0.99	1.00	1.00
EA/AM HPAI H5N2: Chicken isolate	0.09	0.18	0.37	0.67	0.90	0.97	1.00	1.00	1.00	1.00	1.00

[a]The detection probabilities are estimated from 6000 simulation iterations. The active surveillance protocol consists of 1 sample of 11 swabs taken for rRT-PCR testing both the day of and the day before the start of load-out.

Table 22 compares the probability of detection under three different active surveillance and PMIP strategies. Under the scenarios with no PMIP, exposure is assumed to occur sometime between 1 and 12 days prior to the start of load-out. Under the scenario with an 8-day, 100% effective PMIP, meaning the PMIP guarantees the house 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 (i.e., more) than the twelve days prior to load-out are not considered since the infection is almost certain to be detected via diagnostic testing

and monitoring of mortality, so the risk of moving infected but undetected turkeys would be minimal in such cases.

The results in **Table 22** indicate that performing active surveillance using only a mortality trigger without implementing a PMIP is insufficient for detecting HPAI in a turkey house. Including diagnostic testing consisting of one pooled sample of 11 swabs taken for rRT-PCR testing both the day of and the day before load-out begins on the premises substantially improves the likelihood of detecting HPAI in the house prior to movement. However, when a PMIP is not implemented, exposures occurring within twelve days of load-out still fail to result in high levels of detection. The results in **Table 22** suggest that HPAI could go undetected in roughly 1/4 to 1/3 of these cases. When exposure close to the time of movement is prevented through an 8-day PMIP, on the other hand, HPAI is estimated to be detected in the house with a high degree of confidence.

Also included in **Table 22** is the mean number of infectious birds at the start of load-out in the houses that go undetected, along with the 5^{th} and 95^{th} percentiles. The mean number of infectious birds at the start of load-out in houses where HPAI goes undetected is highest in the turkey isolate scenario under the surveillance protocol of diagnostic testing with an eight-day PMIP. This is because the infection is present in the house for at least 8 days, which leads to more birds becoming infected. The diagnostic testing with no PMIP protocol and mortality-trigger-only protocol, on the other hand, allow for infections to occur within 8 days of the start of load-out, which provides less time for a large number of infectious birds to accumulate. As the mortality due to HPAI will also be lower at the time of load-out following exposure within 8 days, the probability of detection will be lower. Thus, the exposures close to the time of load-out represent a greater proportion of the outcomes where HPAI is not detected in the house, leading to the lower mean number of infectious but undetected turkeys. Despite the high number of infectious but undetected turkeys under the protocol of diagnostic testing with PMIP active surveillance, the likelihood of detection is so high that the other two surveillance protocols pose a greater risk for HPAI spread. The mortality-trigger-only protocol in particular represents an extremely risky practice due to the relatively high number of infected but undetected birds paired with a low detection probability.

In the chicken isolate scenario, diagnostic testing with a PMIP protocol clearly produced the most favorable result for the mean number of infected but undetected turkeys, as HPAI was estimated to have been detected in every house exposed within twelve days of load-out, leading to no undetected birds. The mortality-trigger-only protocol resulted in the highest number of infectious but undetected birds, which is further evidence this protocol is not adequate. The mean number of infectious but undetected birds under the protocol of diagnostic testing with no PMIP is moderately few, though the probability of detection is far from the desired 95% threshold. Based on the results for both isolates, the optimal active surveillance protocol is diagnostic testing with a PMIP, which is estimated to achieve low levels of risk for HPAI spread considering the probability of detection and mean number of infectious but undetected turkeys at the start of load-out.

Table 22. Likelihood of detecting HPAI in a turkey tom house prior to the start of load-out on the premises followed by the mean number of infectious turkeys in undetected houses at the time of movement

Strain scenario	Active surveillance and PMIP scenario varying by status and effectiveness[a]		
	Mortality trigger only, no PMIP	rRT-PCR testing and mortality trigger, no PMIP[b]	rRT-PCR testing and mortality trigger, 100% effective 8-day PMIP[c]
	Likelihood of detection Mean number of infectious turkeys		
EA/AM HPAI H5N2: Turkey isolate	0.53 137 (0, 723)	0.68 22 (0, 96)	0.99 258 (25, 627)
EA/AM HPAI H5N2: Chicken isolate	0.65 126 (1, 694)	0.77 20 (1, 96)	1.00 0 (0, 0)

[a]Parentheses indicate the 5th and 95th percentiles estimated from 6000 iterations.

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

[c]Houses are 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 a Turkey Tom House Prior to the Start of Load-out on the Premises

The overall probability of not detecting HPAI in an infected turkey tom house by the start of load-out considers two events: the probability a susceptible house becomes infected provided it is some given distance from an infectious premises, and the probability that the infection is not detected in the house 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 in Section 9.4.2.4.1, Estimation of the Probability of Infection via a Spatial Transmission Kernel. The probability that infectious undetected birds are not detected by the start of load-out, given that the house 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. (2015) that considers the 12 days prior to the start of load-out.[319]

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 some given distance from a susceptible premises. The spatial transmission kernel theoretically averages the risk over all transmission pathways at the given inter-premises distance, thereby providing a summary view of outbreak spread. The current analysis considers two different spatial transmission kernels: a transmission kernel estimated from the 2003 HPAI H7N7 outbreak in the Netherlands by Boender et al. (2007) and a transmission kernel estimated from the 2015 HPAI H5N2 outbreak in Minnesota.[120] 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.[120] 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*.[120]

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 in the estimation of the spatial transmission kernel for Minnesota, giving the following expression[26]:

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

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 8: 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 24** 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. The Netherlands HPAI H7N7 transmission kernel suggests that local transmission pathways to infectious premises, such as wild animals, aerosols, or equipment sharing, were the primary forces behind outbreak spread, while the Minnesota HPAI H5N2 transmission kernel suggests that transmission pathways involving moderate distances, such as garbage or rendering truck visits, played a significant role in outbreak spread in addition to the local pathways.

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. Because the overall probability of not detecting HPAI in a house prior to the start of load-out is derived using the transmission kernel-based probability of infection, it is expected to exhibit similar behavior.

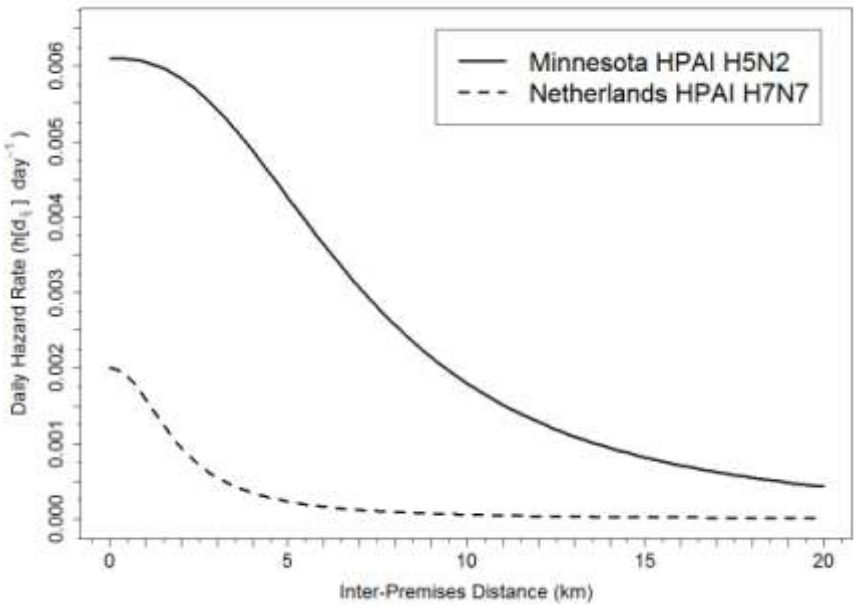


Figure 24. Spatial transmission kernels estimated from the 2003 HPAI H7N7 outbreak in the Netherlands by Boender et al. (2007) and the 2015 HPAI H5N2 outbreak in Minnesota[120]

9.4.2.4.2 Estimated Overall Likelihood of Not Detecting HPAI in a Turkey Tom House Prior to the Start of Load-out

Estimates for the overall likelihood of not detecting HPAI in a turkey tom house prior to the start of load-out are given in **Table 23**. The overall likelihood is the combined probability of a house first being exposed to HPAI and then HPAI not being detected in the house 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 and Netherlands HPAI H7N7 spatial transmission kernels. The overall likelihood under the Netherlands transmission kernel is given by **Table 23** in parentheses. The probability that the infection goes undetected in the house is estimated using the active surveillance simulation model under a diagnostic testing protocol of one pooled sample of 11 swabs taken for rRT-PCR both the day of and the day before the start of load-out. The overall likelihood is estimated under the EA/AM HPAI H5N2 turkey isolate scenario in order to obtain conservative estimates.

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 Minnesota or Netherlands HPAI outbreaks. 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 23** 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% effective at preventing exposure, which means the daily probability of infection during the PMIP is reduced to 1/5 of the probability prior to the PMIP. The last scenario considers a 100% effective PMIP, which means the daily probability of exposure during the PMIP is zero.

The estimates given in **Table 23** 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 kernels. In addition, the higher mean hazard rate estimated from the Minnesota HPAI H5N2 outbreak leads to the higher estimates for the overall likelihood.

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 house prior to the start of load-out. This risk can be further reduced by implementing a sound active surveillance protocol. **Table 23** indicates that the heightened biosecurity during the PMIP combined with an active surveillance protocol of one pooled sample of 11 swabs taken for rRT-PCR testing both the day of and the day before the start of load-out is a viable strategy to reduce the overall likelihood, resulting in low likelihoods of moving infected but undetected birds even at relatively close distances to infectious premises and under the higher hazard rates of the Minnesota transmission kernel.

Table 23. Predicted percent likelihood of a turkey tom house being: (1) exposed to HPAI from an infected premises at a specific distance and (2) undetected prior to the start of load-out following exposure, under three PMIP scenarios varying by biosecurity effectiveness[a] [120]

Distance from an infected premises (km)	Scenario for the daily likelihood of exposure during 8 days before load-out[b]		
	Baseline-no PMIP	80% effective PMIP	100% effective PMIP
	Predicted likelihood (%)		
1.5	2.33(0.48)	0.49(0.10)	0.022(0.004)
2	2.28(0.37)	0.48(0.08)	0.022(0.003)
3	2.14(0.22)	0.45(0.05)	0.020(0.002)
5	1.72(0.09)	0.36(0.02)	0.016(0.001)

[a]First estimate in each cell is based on the 2015 Minnesota EA/AM HPAI H5N2 outbreak, while the estimates in parenthesis are based on the 2003 Netherlands HPAI H7N7.

[b]In all scenarios, an active surveillance protocol of one pooled sample of 11 swabs taken for rRT-PCR testing both on the day of and the day before the start of load-out on the premises was implemented under the EA/AM HPAI H5N2 turkey isolate strain.

9.4.3 Likelihood of Moving Infectious but Undetected Turkeys Following Exposure During Load-out

Contaminated load-out crews and equipment entering a poultry premises pose an infection risk that is especially relevant during extended (i.e., multiple-day) load-outs. As discussed in Section 9.3, Likelihood of Turkey Flock Becoming Infected with HPAI Virus via Load-out Operations, the number of infectious birds can increase rapidly in houses infected early in the load-out process, which could cause potentially 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 of infectious birds following exposure to HPAI during the load-out process. The estimated likelihood of detection for a single house 2 to 10 days following exposure to HPAI under an active surveillance protocol of one rRT-PCR sample of 11 swabs taken daily is given in **Table 24.**

The protocol is evaluated under three scenarios varying by the number of birds assumed to be initially infected, which is a proxy for increasing levels of contamination on the load-out crews and equipment. The EA/AM HPAI H5N2 turkey isolate is used in order to obtain conservative estimates. The testing protocol for multiple-day load-outs decided upon by the STS Working group involves daily rRT-PCR testing of 11 swabs for all houses scheduled to be loaded out, beginning 48 hours after the arrival of load-out equipment onto the premises. On multi-age premises, houses that are not scheduled to be loaded out are not included in the daily testing requirement and similarly are not included in the calculations to predict the probability of detection prior to transportation as those birds will not be moved. On large operations, it is possible that multiple poultry houses will be awaiting load-out at the 48-hour mark after premise-wide load-out has begun. Since more than one house could be tested, the premises-wide likelihood of detection would be at least as high as and generally higher than the estimates given in **Table 24**, which are for surveillance in a single house only.

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 house. The results suggest that the infection in a house exposed early in an 8-, 9- or 10-day load-out would likely be detected regardless of the level of contamination. When the initial number of infected birds is 10, the probability of infection detection exceeds the 95% threshold 5 days post exposure. When the initial number of infected birds is 100, the 95% threshold is estimated to be exceeded 4 days post exposure. The low detection probabilities for those houses exposed close to the time of movement can be improved through the use of supplementary antigen capture (AC) testing. The likelihood of detection with supplementary AC testing is evaluated in Appendix 10: Supplementary Testing Protocols.

Table 24. The likelihood of detecting HPAI in a house prior to the transportation of turkey toms to processing for different numbers of days post exposure and different numbers of initially infected birds, meant to represent the contamination level of the load-out crew and equipment[a]

	Days post exposure								
Initial no. of birds infected	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	Predicted detection probability[a]								
1	0.15	0.23	0.37	0.59	0.80	0.92	0.98	1.00	1.00
10	0.17	0.46	0.85	0.98	1.00	1.00	1.00	1.00	1.00
100	0.37	0.92	1.00	1.00	1.00	1.00	1.00	1.00	1.00

[a]The active surveillance protocol consists of a daily rRT-PCR sample of 11 swabs. The likelihood of detection was estimated from 6000 simulation iterations based on the EA/AM HPAI H5N2 turkey isolate strain.

As infections occurring even 6 to 7 days prior to movement can have a low likelihood of detection, the exposure mitigation and biosecurity measures implemented during a multiple-day load-out are especially important in reducing the risk of transporting infectious but undetected birds to processing. Despite the low probabilities of detection, the likelihood of sending large numbers of infectious but undetected turkeys to processing is expected to be quite low. **Table 25** shows the predicted percent probability of not detecting HPAI in a house where the number of infectious but undetected turkeys exceeds 300, given exposure occurred during load-out, some number of days prior to movement. The percent probabilities are estimated from the EA/AM HPAI H5N2 turkey isolate strain under the active surveillance protocol of daily samples (of houses to be loaded out) of 11 swabs taken for rRT-PCR testing. Similar results were determined for numbers of infectious but undetected turkeys exceeding 100, 500, and 1000 birds at the time of movement and are given in Appendix 10: Supplementary Testing Protocols.

The results in **Table 25** suggest the risk of sending infectious but undetected turkeys to processing in numbers of at least 300 is generally quite low. However, an 8.3% probability of moving at least 300 infectious but undetected turkeys where 100 birds were initially infected 3 days prior to movement is substantial. The nontrivial likelihood of such an event underscores the importance of using relevant biosecurity to prevent heavy contamination of load-out equipment and contamination of other barns yet to be loaded out on a premises.

Current recommended practices for all turkey load-outs (including multiple-day load-outs) include cleaning and disinfecting the load-out equipment before use on the premises. Cleaning and disinfecting load-out equipment should prevent the equipment from being highly contaminated, making the scenario with one initially infected bird more likely than scenarios with many initially infected birds. In addition, for multiple-day load-outs, heightened barn-to-barn biosecurity, such as barn-specific footwear, is recommended, which limits the likelihood of HPAI entering a populated barn before load-out begins in that barn. This may prevent HPAI virus from being present in a house for multiple days. Considering these recommended exposure mitigation measures for multiple-day load-outs, the likelihood of sending at least 300 infectious but undetected turkeys to processing is expected to be low.

Table 25. The estimated percent probability of not detecting HPAI in a house following exposure during load-out where the number of infectious but undetected turkey toms at the time of movement exceeds 300 birds

Initial number of birds infected[a]	Days post Exposure								
	2	3	4	5	6	7	8	9	10
	Predicted percent probability of at least 300 infectious but undetected turkey toms at the time of movement[b] (%)								
1	0.00	0.00	0.02	0.43	0.82	0.68	0.40	0.12	0.02
10	0.00	0.02	2.23	1.78	0.20	0.00	0.00	0.00	0.00
100	2.38	8.33	0.32	0.00	0.00	0.00	0.00	0.00	0.00

[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]Percent probabilities are estimated from 6000 simulations based on the EA/AM HPAI H5N2 turkey isolate strain and an active surveillance protocol of 1 sample of 11 swabs taken daily for rRT-PCR testing.

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 house. This leads to higher levels of disease mortality, increasing 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 is estimated to be sufficient for the differing latent and infectious periods of various HPAI strains, consistently achieving high probabilities of detection. Exposure of a turkey house to HPAI during a multiple-day load-out may be difficult to detect since the infection occurs close to the time of movement. However, given the load-out biosecurity and active surveillance measures in place, if an infected but undetected movement were to take place, it would be unlikely to contain large numbers of infectious birds.

Given that an effective PMIP is implemented, and that both mechanisms for active surveillance outlined in the STS Plan (trigger for elevated mortality and rRT-PCR mortality testing) are utilized as described, and that load-out biosecurity measures are implemented, the likelihood of HPAI in an infected turkey house going undetected is rated as follows:

- The overall likelihood of HPAI-infected but undetected turkeys in a house at the conclusion of PMIP and prior to the start of load-out on the premises is estimated to be *low* at a distance of 1.5 km or more from an infected premises.
- The likelihood of a movement with large numbers (≥ 300) of HPAI-infected but undetected turkeys being sent to slaughter following infection during load-out is estimated to be *low*.

10 Overall Conclusion

The objective of this assessment was to estimate the risk that the movement of market-age turkeys to processing (i.e., turkeys to market), from a premises located within a Control Area during an HPAI outbreak in the poultry industry in the U.S., would result in the introduction of HPAI infection to another poultry population (e.g., another poultry farm or birds remaining on a multi-age premises).

The assessment considered relevant current industry practices and current biosecurity measures as well as outbreak-specific measures from the STS Plan, in particular the PMIP. The assessment focused on the risk pathways for HPAI infection of market-age turkeys on grow-out premises located within an HPAI Control Area 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, but rather relate to the likelihood of infection of live birds that will move and potential 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 turkeys to market **(Figure 25)**.

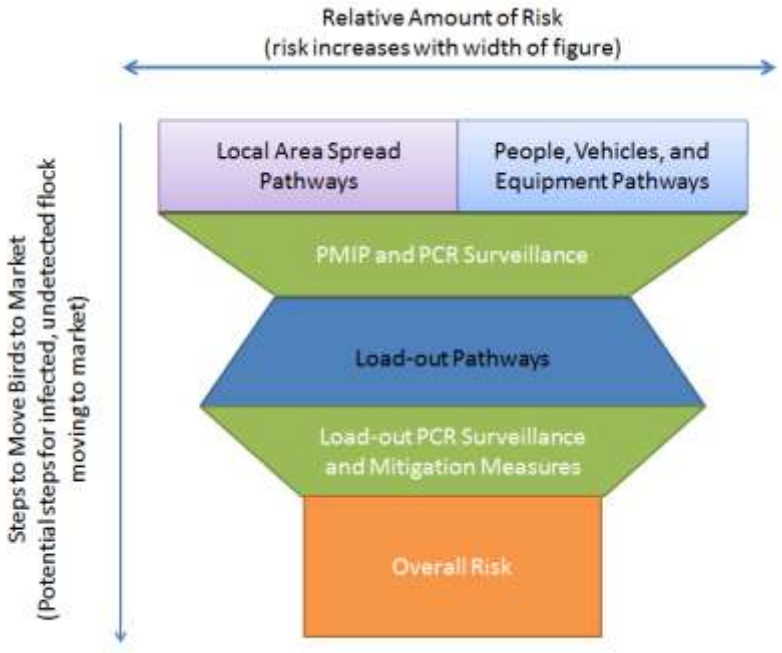


Figure 25. Diagrammatic representation of the overall assessed risk. The overall risk assessment is based on consideration of the steps needed to move live birds to market and the pathways that could lead to infection of a flock, the subsequent likelihood of detection of the infected flock, and potential movement of an infected but undetected flock.

The evaluation of the major risk pathways identified resulted in the following conclusions:

Local Area Spread Pathways

- **Insects.** The likelihood of a turkey premises becoming infected with HPAI virus via insect transmission varies with distance and with source premises infection status. The

estimated likelihood ratings range from *negligible to moderate*, with a higher likelihood of infection closer to a known infected premises. For premises located closer than 1.5km to an infected flock, there are too many variables to accurately assess the risk of becoming infected with HPAI via insect transmission.

- **Aerosols.** The likelihood of a turkey 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 a turkey premises becoming infected via bioaerosol transmission is rated as follows:
    - *Low to moderate* if <1.5 km from an infected but undetected premises.
    - *Moderate to high* if <1.5 km from a known infected premises.
- **Wild Birds.** The likelihood of HPAI virus spread to a turkey grow-out premises via wild birds depends upon the type of wild birds and exposure to the wild birds. With an effective PMIP, the likelihood of HPAI infection via wild aquatic birds and via non-passerine non-aquatic birds is *low* as these birds and their waste are unlikely to access or be tracked into a turkey grow-out barn. Given that passerine birds may access the inside of turkey grow-out barns (even during a PMIP) and have been shown to be capable of shedding the virus, the likelihood of HPAI infection via passerine birds in the farm vicinity was assessed as *low to moderate.*

People, Vehicles, and Equipment Pathways

- **Live-haul Routes.** The risk of HPAI virus spread to turkey grow-out 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 risk is estimated to be *negligible to low* no matter the distance. In contrast, for trucks hauling birds that had no PMIP and no diagnostic tests (e.g., from premises outside the Control Area), the risk ranges from *low to high,* with premises within 100 meters of the live-haul route at highest risk.
- **Feed and Critical Operational Visits.** Critical operations visits will be limited during PMIP; however, delivery of feed during this period is likely and the potential for emergency maintenance visits also exists. The likelihood of a turkey flock becoming infected with HPAI via critical operational visits during PMIP was assessed as *negligible to moderate,* as follows:
    - *Negligible* via feed.
    - *Low* via feed delivery (i.e., driver and or vehicle).
    - *Low to moderate* via other critical operational visits (i.e., personnel or vehicle).
- **People and their Vehicles.** Provided PMIP measures for people are strictly followed and people wear farm-specific clothing and barn-specific footwear, we rate the likelihood of a turkey flock becoming infected with HPAI via people and their vehicles entering the

premises during the PMIP as *low* for people entering the poultry barns and *very low* to *low* for people who do not enter the poultry barns.

- **Shared Equipment (other than load-out).** Previous outbreaks have demonstrated that shared equipment poses a disease transmission risk; however, during the PMIP, no off-site equipment will be pre-staged and only critical operational visits may continue. Thus, we rated the likelihood of a turkey flock becoming infected with HPAI virus via shared equipment as *low* during PMIP, and *moderate* prior to PMIP.
- **Dead Bird Disposal.** The risks of HPAI introduction associated with off-site dead bird disposal methods, such as rendering, are well documented, and off-site disposal of mortality must be discontinued during PMIP. However, the risky practice of off-site dead bird disposal may still occur outside of a PMIP, and daily mortality on turkey grow-out premises may remain uncovered outside turkey houses for hours before being moved to a biosecure mortality collection area on-site.
    - For on-farm dead bird disposal, given that many scavenger species can biologically or mechanically carry HPAI virus and have home ranges large enough to contain adjacent poultry farms, we assessed the likelihood of HPAI introduction to a turkey farm during the PMIP as *moderate.*
    - Off-site dead bird disposal methods prior to a PMIP may possibly result in premises contamination. However, the implementation of a PMIP does reduce the likelihood that such contamination will be tracked inside a grow-out barn during the PMIP. We thus assessed the likelihood of a turkey flock becoming infected as a result of HPAI virus introduction to the flock via off-site dead bird disposal that takes place prior to the PMIP as *moderate.*
- **Garbage Management.** There is potential for HPAI virus associated with garbage management to be tracked into a poultry house, and thus we assessed the likelihood of a turkey flock becoming infected with HPAI virus due to garbage management without a PMIP to be *moderate to high.* During a PMIP, no off-site movement of garbage is allowed, and thus we assessed the likelihood of a turkey flock becoming infected with HPAI virus due to garbage management during a PMIP as *low*.

Load-out Pathways

- **Birds moving to market.** Assuming that 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 load-out process, the risk that a turkey 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 *low to moderate*.
- **Multi-age premises**. Premises that house two or more distinct age groups of turkeys for grow-out represent a unique deviation from the assumptions used for single-age (i.e., all-in, all-out) management plans, as movement of birds to market does not necessarily result in complete depopulation of a premises. Turkeys remaining on premises represent a susceptible host population at increased risk of exposure to HPAI-contaminated load-out equipment, vehicles, or crews due to proximity. Given that load-out mitigation measures are in place, specifically a prohibition of load-out crews entering other turkey houses on

the same farm, the risk of the remaining (younger) turkeys on a multi-age premises becoming infected with HPAI virus via load-out operations on that premises is estimated to be *moderate to high*.

Overall Risk

It is concluded that the overall risk of HPAI spread to susceptible poultry associated with the movement of turkeys to market into, within, and outside of a Control Area from a single-age (e.g., all-in, all-out management) premises is *moderate*, provided that all applicable preventive measures from the STS Plan, in particular the PMIP, are strictly followed.

On multi-age turkey grow-out premises, susceptible hosts may remain on the premises after load-out of market-age birds, and the remaining (younger) birds are at significant risk for infection due to proximity and potential for contamination directly into barns. It is concluded that the overall risk of HPAI spread to susceptible poultry (on the same premises) associated with the movement of turkeys to market into, within, and outside of a Control Area from a multi-age premises is *moderate to high*, provided that all applicable preventive measures from the STS Plan, in particular the PMIP, are strictly followed.

In using the results from this risk assessment, it should be remembered that:

- This assessment is based on current (February 2017) information 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 in question.

Appendix 1: AI Virus Survival at Various Humidity Levels, at Various Temperatures, and on Various Substrates.

Appendix 1 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 general trend in persistence and survival time in the environment for AI viruses appears to be decreased survival in conditions of lower moisture and higher temperature. Virus survival and persistence in the environment has also been reported to be longer near neutral pH, in lower salinity, and without UV exposure.[66,257,320-322]

These tables are compiled to describe virus survival and persistence across a range of conditions. Of note, there are multiple methodologies 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 on HPAI virus were given preference over LPAI studies. Where information on AI virus was not available, data on other influenza 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 OIE Terrestrial Animal Health Code (Article 10.4.25),[323] and inactivation times at high temperatures have been summarized by USDA documents on parameters to inactivate HPAI virus using heat treatment.[324]

Appendix 1 Table 1. Summary of experimental studies on survival of AI viruses in feces and manure 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
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}[f] value of 75 days	Nazir et al., 2011[325]
Wet Chicken feces	4°C (39.2°F)	Closed vial	HPAI H5N2	#1370 isolate	Viable virus through 35 days (last time point tested)	-	Beard et al., 1984[77]

[f] T_{90} value: time required for 90% loss of virus infectivity

Substrate, *cont.*	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
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	-	Lu et al., 2003[326]
Wet chicken feces	4°C (39.2°F)	Capped vials	HPAI H5N1	A\Ck\Sikkim\151466\2008	-	0% infectivity at week 7	Kurmi et al., 2013[270]
Dry chicken feces	4°C (39.2°F)	Capped vials	HPAI H5N1	A\Ck\Sikkim\151466\2008	-	0% infectivity at week 8	Kurmi et al., 2013[270]
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	Wood et al., 2010[257]
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)	-	Wood et al., 2010[257]
Duck feces	10°C (50°F)	Moist germ carrier; feces in closed 50-ml plastic tubes	LPAI H6N8	A/Mute Swan/Germany/R29 27/07	-	T_{90} value of 14 days	Nazir et al., 2011[325]
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	-	Lu et al., 2003[326]
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	Nazir et al., 2011[325]
Fecal material	22°C (71.6°F)	Capped glass vials	LPAI H3N6	A/Duck/Memphis/ 546/74	-	Infectious virus not detected at day 13	Webster et al., 1978[327]
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	Wood et al., 2010[257]
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	Wood et al., 2010[257]

Substrate, *cont.*	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
Wet chicken feces	25°C (77°F)	Closed vial	HPAI H5N2	#1370 isolate	-	No viable virus at day 3	Beard et al., 1984[65]
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	Lu et al., 2003[326]
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	Nazir et al., 2011[325]
Dry chicken feces	37°C (98.6°F)	Capped vials	HPAI H5N1	A\Ck\Sikkim\151466\2008	-	0% infectivity at hour 30	Kurmi et al., 2013[270]
Wet chicken feces	37°C (98.6°F)	Capped vials	HPAI H5N1	A\Ck\Sikkim\151466\2008	-	0% infectivity at hour 30	Kurmi et al., 2013[270]
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	Lu et al., 2003[326]
Dry chicken feces	42°C (107.6°F)	Capped vials	HPAI H5N1	A\Ck\Sikkim\151466\2008	-	0% infectivity at hour 24	Kurmi et al., 2013[270]
Wet chicken feces	42°C (107.6°F)	Capped vials	HPAI H5N1	A\Ck\Sikkim\151466\2008	-	0% infectivity at hour 24	Kurmi et al., 2013[270]
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	Lu et al., 2003[326]

Appendix 1 Table 2. Summary of experimental studies on survival of AI viruses in compost 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
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)	Guan et al., 2009[280]
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)	Guan et al., 2009[280]
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	Guan et al., 2009[280]
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	Guan et al., 2009[280]

Appendix 1 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	Nazir et al., 2010[328]
Surface water (Lake Constance)	0°C (32°F)	-	LPAI H5N1	A/teal/Germany/Wv 632/05	-	T_{90} value of 208 days	Nazir et al., 2010[328]
Contaminated fecal material in river water	4°C (39.2°F)	-	LPAI H3N6	A/Duck/Memphis/5 46/74	Viable for all contact times (32 days)		Webster et al., 1978[327]
Sea water (Black Sea)	5-6°C (41-42.8°F)	-	LPAI H6N2	Not specified	-	No infective virus detected at day 7	Zarkov, 2006[329]
Sea water (Black Sea)	5-6°C (41-42.8°F)	-	LPAI H11N6	A/duck/England/ 56	-	No infective virus detected at day 9	Zarkov, 2006[329]
Surface water (Koprinka dam)	5-6°C (41-42.8°F)	-	LPAI H6N2	Not specified	-	No infective virus detected at day 16	Zarkov, 2006[329]
Surface water (Koprinka dam)	5-6°C (41-42.8°F)	-	LPAI H11N6	A/duck/England/ 56	-	No infective virus detected at day 18	Zarkov, 2006[329]
Surface water (Lake Constance)	10°C (50°F)	-	LPAI H4N6	A/mallard/Germany/ Wv1732-34/03	-	T_{90} value of 85 days	Nazir et al., 2010[328]
Surface water (Ovcharitsa dam)	10-12°C (50-53.6°F)	-	LPAI H6N2	Not specified	-	No infective virus detected at day 1	Zarkov, 2006[329]
Surface water (Ovcharitsa dam)	10-12°C (50-53.6°F)	-	LPAI H11N6	A/duck/England/ 56	-	No infective virus detected at day 1	Zarkov, 2006[329]
Distilled water	17°C (62.6°F)	-	HPAI H5N1	A/WhooperSwan/ Mongolia/244/05	-	Predicted persistence of 158 days	Brown et al., 2007[322]
Surface water (Lake Constance)	20°C (68°F)	-	LPAI H4N6	A/mallard/Germany/ Wv1732-34/03	-	T_{90} value of 23 days	Nazir et al., 2010[328]

Substrate, *cont.*	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
Contaminated fecal material in river water	22°C (71.6°F)	-	LPAI H3N6	A/Duck/Memphis/546/74	-	Virus not detected at day 7	Webster et al., 1978[327]
Distilled water	28°C (82.4°F)	-	HPAI H5N1	A/DuckMeat/Anyang/01	-	Predicted persistence of 30 days	Brown et al., 2007[322]
Surface water (Lake Constance)	30°C (86°F)	-	LPAI H4N6	A/mallard/Germany/Wv1732-34/03	-	T_{90} value of 14 days	Nazir et al., 2010[328]

Appendix 1 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)	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 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	Yamamoto et al., 2010[232]
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)	-	Guan et al., 2009[280]
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	Guan et al., 2009[280]
Duck feathers	20°C (68°F)	Placed in incubator	HPAI H5N1	A/WhooperSwan/Akita/1/2008	-	Negative for virus isolation at day 20	Yamamoto et al., 2010[232]
Chicken meat	57.8°C (136.04°F)	PCR tubes in thermo-cycler heating block	HPAI H5N1	A/chicken/Korea/ES/2003	-	Predicted 11-log EID_{50} reduction at 39.6 minutes	Thomas et al., 2007[330]

Appendix 1 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)	-	Guan et al., 2009[280]
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)	-	Wanaratana et al., 2010[331]
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	Zou et al., 2013[332]
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	Wanaratana et al., 2010[331]

Appendix 1 Table 6. Summary of experimental studies on survival of influenza 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)	-	Wood et al., 2010[257]
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	Wood et al., 2010[257]
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	Wood et al., 2010[257]
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	Greatorex et al., 2011[333]
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	Greatorex et al., 2011[333]
Steel, tile, gumboot, tire, egg shell, 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	Tiwari et al., 2006[258]
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)	-	Tiwari et al., 2006[258]
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	Tiwari et al., 2006[258]
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	Tiwari et al., 2006[258]
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	Tiwari et al., 2006[258]

Substrate, *cont.*	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	22°C (71.6°F)	50-60% relative humidity	H1N1	A/PR/8/34	Viable virus at hour 24 (last time examined)	-	Noyce et al., 2007[334]
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	Wood et al., 2010[257]
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	Wood et al., 2010[257]
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	Wood et al., 2010[257]
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	Wood et al., 2010[257]
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)	-	Sakaguchi et al., 2010[335]
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)	-	Bean et al., 1982[43]
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	Bean et al., 1982[43]

Substrate, *cont.*	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	55°C (131°F)	50% relative humidity	H1N1	A/PR/8/34	Minute 60 (last time point tested)	-	McDevitt et al., 2010[336]
Stainless steel	60°C (140°F)	50% relative humidity	H1N1	A/PR/8/34	-	Virus below detectable limit at minute 30	McDevitt et al., 2010[336]
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)	McDevitt et al., 2010[336]

Appendix 2: Literature Review on the Role of Local Area Spread in Previous Outbreaks

Appendix 2 Table 1 below summarizes the results from studies (to include modeling) on the influence of local area spread in AI transmission during previous outbreaks.

Appendix 2 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 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 as: shorter distance to dead bird disposal and litter compost area, presence of wild mammals, and visitors entering barns.	USDA Epi Report Indiana, March 18, 2016[246]
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.	Garber et al., 2016[177]
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.	Nishi-guchi et al., 2007[242]
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]).	Pelzel et al., 2006[337]
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 $\leq$1 km from an infected premises were at least 8 times more likely to become infected than farms $\geq$5 km.	Boender et al., 2007[120]

AI strain (Location)	Year of outbreak (species involved)	Study approach	Key findings	Source
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.	Senne et al., 2005[338] McQuis-ton, et al 2005[70]
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.	Dunn et al., 2003[256]
HPAI H7N1 (Italy)	1999-2000 (turkeys [meat and breeder], chickens [breeders, layers, and broilers], geese, quail, ostriches, guinea fowl, pheasants)	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.	Mannelli et al., 2006[339]
		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.	Busani et al., 2009[122]
		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 ban on restocking were identified through simulation to reduce infection spread.	Dorigatti et al, 2010[119]
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.	Sharkey et al., 2008[123]; Irvine et al., 2007[113]

AI strain (Location)	Year of outbreak (species involved)	Study approach	Key findings	Source
LPAI H5N3 (California)	1984 (turkeys)	Outbreak observation	Spread associated with insemination at 5 breeder premises across 110 miles, linked to one company and insemination crew. No spread to 193 other turkey premises or >800 chicken premises in the state.	McCapes et al., 1986[79]
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.	Halvorson et al., 1980[340]
LPAI A/T/Minn./67 (Minnesota)	1967 (turkeys)	Outbreak observation	Spread between houses on same premises and between premises. Spread between premises appeared associated with insemination; some houses on severely infected premises were not infected.	Kleven et al., 1970[82]

Appendix 3: Estimating an Approximate Posterior Distribution for the Prevalence among Insects

A Bayesian approach with Monte Carlo simulation was used to estimate the posterior distribution of the prevalence among insects given the observed data from testing pools of insects.[125] In the observed data, the pool size varied between 10 and 60 insects, and 2 of the 144 pools tested were positive for AI. The steps in the simulation iteration were as follows.

For each of the 144 pooled samples, the pool size N_j was simulated as an integer Uniform (10, 60) distribution. The prevalence among individual insects P_r was simulated using a uniform (0,1) prior (uninformed). The probability of a pooled sample being positive P_{pool} (j) was calculated according to the equation below,

$$P_{pool}(j) = 1 - (1 - P_r)^{N_t}$$

An indicator variable for whether the pooled sample *j* is positive, X(j) was simulated as a Bernoulli trial with the probability equal to P_{pool} (j).

The prevalence P_r in an iteration was accepted if the sum $\sum_j Xj = 2$, as only 2 out of 144 pools were positive in the data. The simulation was run for 2,000,000 iterations to estimate the approximate posterior distribution of P_r. An approximate two-sided 95% credibility interval was (0.01%, 0.15%) based on 391 values.

Appendix 4: Expert Polling on Insect Transmission Routes

A panel of eight experts in the turkey and broiler industries with field experience managing AI was surveyed between November 2013 and January 2014 on risk of HPAI transmission via multiple routes of infection. Surveys were administered through the online polling service SurveyMonkey.[g] Experts were asked to provide their opinion, based on previous experience, 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 insects with associated expert responses shown in **Appendix 4 Tables 1-2** and **Appendix 4 Figures 1-2**.

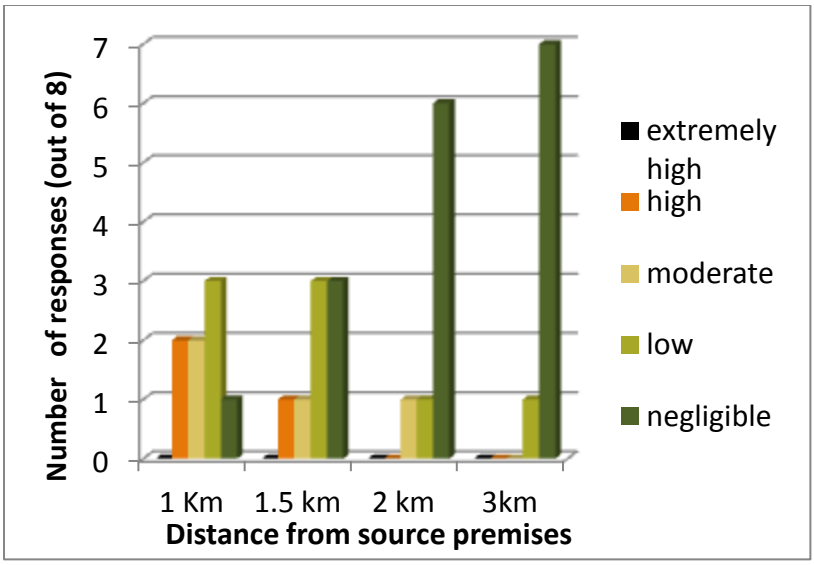
Q1. Please qualitatively rate the likelihood of AI transmission from a known infected flock to an uninfected turkey flock via insects located at distances specified in the table. Please complete the following table, selecting a risk rating for each scenario as negligible, low, moderate, high, or extremely high, based on your expert opinion.

Q2. Please qualitatively rate the likelihood of AI transmission from an infected but undetected flock (lower prevalence) to an uninfected turkey flock via insects located at distances specified in the table. 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=8) to the question of likelihood of AI transmission from a **known infected** flock to an uninfected turkey flock via insects at specified distances (Question 1)

Distance from source flock	Likelihood rating				
	Negligible	Low	Moderate	High	Extremely high
1 km	1	3	2	2	0
1.5 km	3	3	1	1	0
2 km	6	1	1	0	0
3 km	7	1	0	0	0

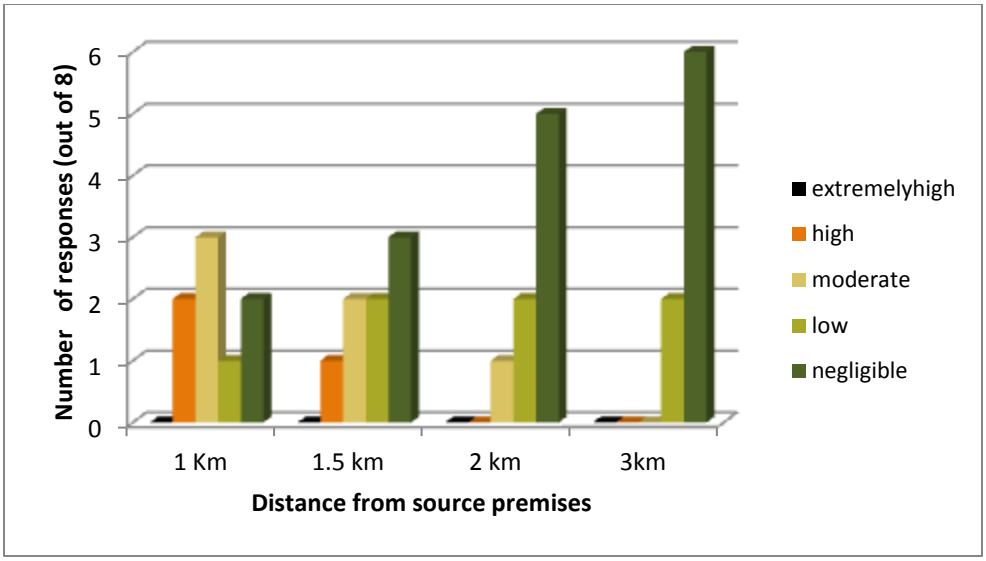
[g] SurveyMonkey, Inc., Palo Alto, CA, www.surveymonkey.com



Appendix 4 Figure 1. Expert responses (n=8) to the question of likelihood of AI transmission from a **known infected** flock to an uninfected turkey flock via insects at specified distances (Question 1)

Appendix 4 Table 2. Expert responses (n=8) to the question of likelihood of AI transmission from an **infected but undetected** (lower prevalence) flock to an uninfected turkey flock via insects at specified distances (Question 2)

Distance from source flock	Likelihood rating				
	Negligible	Low	Moderate	High	Extremely high
1 km	2	1	3	2	0
1.5 km	3	2	2	1	0
2 km	5	2	1	0	0
3 km	6	2	0	0	0



Appendix 4 Figure 2. Expert responses (n=8) to the question of likelihood of AI transmission from an **infected but undetected** (lower prevalence) flock to an uninfected turkey flock via insects at specified distances (Question 2)

Appendix 5: Live Turkey Movement Aerosol Modeling

Introduction

AERMOD[341,342] is a regulatory model used by, among others, the EPA for air quality assessment. The model inputs include information on source location and parameters, receptor locations, and meteorological variables (such as wind speed, wind direction, and turbulence parameters), and outputs include aerosol concentrations at various receptor locations over selected time periods. The model can output printed summaries of, for example, high values by receptor, overall maximum values for each averaging period, and tables of concurrent values summarized by receptor. These values can then be used to generate outputs such as contour plots that depict the concentrations at the various receptor locations. The model can also generate a file of all occurrences when a concentration value equals or exceeds a user-specified threshold.

Scenario A

Source and receiving flock size

The source farm in this scenario is a 25,000-bird HPAI-infected broiler house and the receiving flock is a 14,000-bird turkey house. In this scenario, the receiving turkeys were 14-week-old hens assumed to weigh 15.53 lb,[343] and the air intake per bird was 1.84 m^3/day, estimated using the equations presented in Lasiewski and Calder (1971).[344]

Meteorological Parameters

Meteorological data such as wind speed, relative humidity, temperature, etc. were obtained from Tupelo, Mississippi, for the year 2011.

Aerosol Source Emission Rate

The emission rate was directly estimated from the total suspended particle emission rate for a broiler house in the literature. Burns et al. (2008) estimated a mean particle emission rate of 2.78 ± 1.87 kg/day-house for a broiler house with average placement of 25,000 chickens.[345] Assuming that 50% of the suspended particles were contaminated at an HPAI virus concentration of 10^5 EID_{50}/g and a particle emission rate of 4.65 (2.78+1.87) kg/day-house, the aerosol source emission rate would be $10^{3.43}$ EID_{50}/second = 50%*4.65*1000 g/day/(24*3600 s/day)*$10^5$$EID_{50}$/g.

Particle Size Distribution

Particle pollution, also known as particulate matter or PM, is a mixture of very small particles and liquid droplets in the air. Generally, the smaller the particle, the more likely it could be inhaled and cause health problems. The EPA is most concerned with particles less than 10 µm in diameter, as these are generally considered small enough to pass through the nose and throat and potentially enter the lungs.[346] We considered that 41% of particles are PM_{10} and 27% of PM_{10} particles are $PM_{2.5}$. The particle size fraction in this scenario was chosen cautiously from the data points in **Appendix 5, Table 1** to have a greater proportion of small particles (conservative approach). For particles greater than 10 µm in size, a diameter of 25 µm was used based on the mean mass diameter estimate from Redwine et al.[347]

HPAI 50% Egg Infectious Dose

The H5N2 EID_{50} was estimated from two inoculation experiments involving the Eurasian/American HPAI H5N2 virus turkey field isolate whose data are presented in **Appendix 5 Table 2**. We used an exponential dose-response model parameterized from data to obtain the EID_{50} as 10^4 and $10^{3.2}$ for experiment 1 and 2 respectively. There could be considerable uncertainty regarding the parameters as well as the shape of the dose-response model for the aerosol route, given the limited data. However, as aerosol may represent a very low concentration exposure for a large number of birds, the risk estimate would be considerably lower if there existed a threshold dose below which the probability of an exposed bird becoming infected were zero. The exponential dose response model is a "single hit" model without a threshold dose and we take 10^4 EID_{50} in the analyses for Scenarios A and B.

Appendix 5 Table 1. Fraction of particles from poultry operations with size less than or equal to 10 µm (PM_{10}) and 2.5 µm ($PM_{2.5}$)

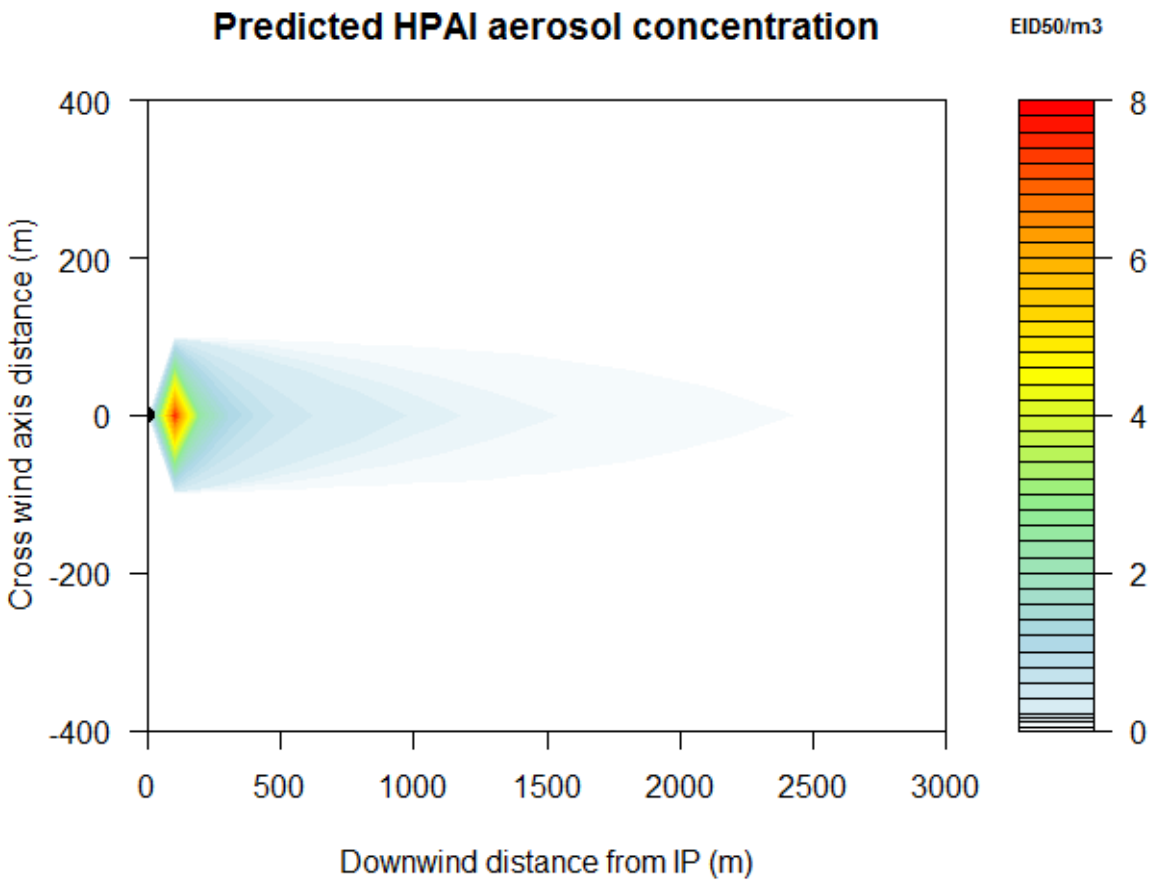
Study	PM_{10} fraction of total suspended particles (%)	$PM_{2.5}$ fraction of PM_{10} (%)
Burns et al. (2008)[345] (background PM controlled)	41	
Roumeliotis et al. (2010)[348] (background PM controlled)		15.6
Wathes et al. (1997)[349]	10	
Li et al. (2008),[350] turkey toms		11
Redwine et al. (2002)[347] (background PM not controlled)	5.9 (2.7-8.4)	
Takai et al. (1998),[351] inhalable vs. respirable dust (background PM not controlled)	13	
Li and Burns (2009),[352] layers (background PM not controlled)		10.5
Roumeliotis and Van Heyst (2007)[348] (background PM not controlled)	77	27
Unpublished industry data	50-70	50

Appendix 5 Table 2. Data points from the two inoculation experiments with Eurasian/American HPAI H5N2 virus turkey field isolate used to parameterize an exponential dose-response model through maximum likelihood methods

	Number Infected / Total Inoculated	
EID_{50}	Experiment 1	Experiment 2
10^2	0/5	0/10
10^4	5/5	5/10
10^6	20/20	40/40

Scenario A results:

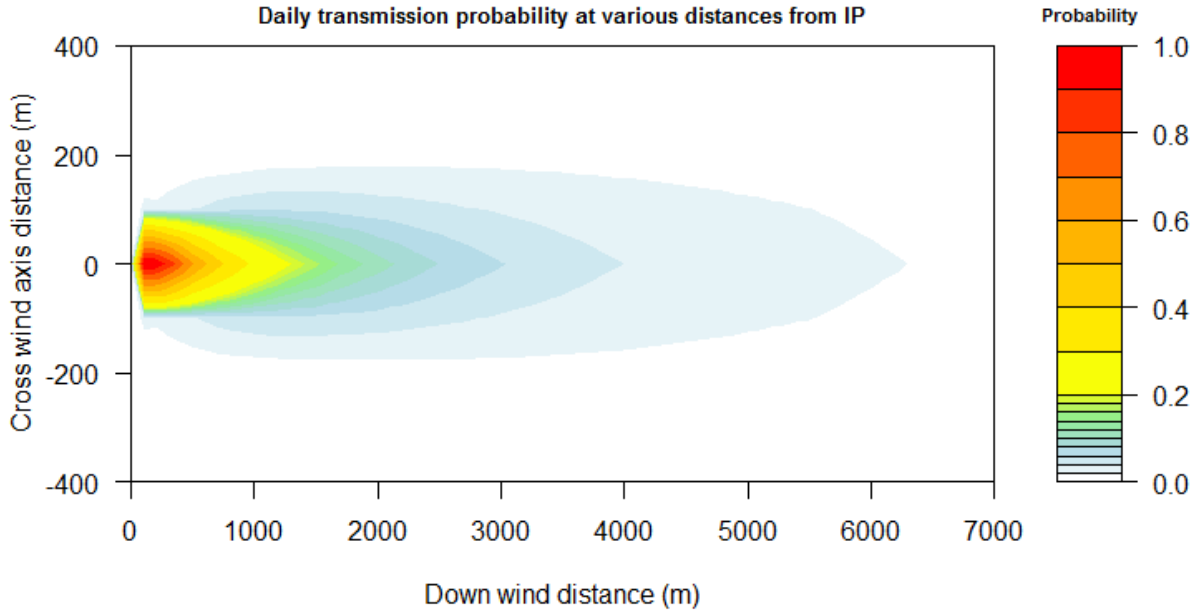
Scenario A results are shown in **Appendix 5 Figures 1-2** and **Appendix 5 Table 3.**



Appendix 5 Figure 1. AERMOD model-predicted HPAI virus concentrations at various points downwind of a source infected broiler premises (Scenario A)

Appendix 5 Table 3. AERMOD model-predicted HPAI virus concentrations and daily exposure probability at various points downwind of a source infected broiler premises (Scenario A)

	Distance from source (known infected broiler premises)				
Outcome Variable	***0.5 km***	***1 km***	***1.5 km***	***3 km***	***5 km***
Predicted HPAI concentration (EID_{50}/m^3)	0.54	0.19	0.1	0.04	0.02
Predicted probability of exposure in a day	0.62	0.28	0.16	0.06	0.03



Appendix 5 Figure 2. AERMOD model-predicted daily probability of infection for a susceptible turkey hen flock located at various points downwind of a source infected broiler premises (Scenario A)

Scenario B

Source and Receiving Flock Size

The source farm in this scenario is a 14,000-bird HPAI-infected turkey flock, and the receiving flock is a 14,000-bird turkey house. In this scenario, the receiving turkeys are 14-week-old hens assumed to weigh 15.53 lb[343] and the air intake per bird is 1.84 m^3/day, estimated using the equations presented in Lasiewski and Calder (1971).[344]

Meteorological Parameters

Meteorological data such as wind speed, relative humidity, and cloud cover, etc. were obtained from Olivia, Minnesota, for the year 2011.

Aerosol Source Emission Rate

In this scenario, the aerosol source emission rate was approximated using air sampling data from the 2015 HPAI outbreak. In these data, the cycle threshold (CT) values of three air samples from a liquid cyclone collector taken in a high HPAI prevalence turkey barn were 35.9, 34.2, and 33.9 for the EA/AM HPAI H5N2 strain. We used a standard curve for EA/AM HPAI H5N2 from the Southeast Poultry Research Laboratory (SEPRL) (SEPRL, personal communication, January 2016) to approximate the HPAI concentration in an infected turkey house. We note that the standard curve may vary depending on the virus strain and lab processes, and therefore the use of this standard curve is an approximation to inform qualitative ratings. Specifically based on the standard curve, the concentration in log EID_{50}/ml was calculated as CT *(-0.30+13.43). Using this approach, estimated virus concentration per m^3 of air was 2.67 log EID_{50}. We used a ventilation rate of 3 cubic feet per minute (CFM)/turkey[350] and estimated an overall emission rate of $10^{3.96}$ EID_{50}/s for a 14,000-bird turkey barn.

Distribution of HPAI Virus on Different Particle Sizes

The distribution of HPAI virus on different particle sizes was estimated using data on the number of RNA copies/m^3 in different particle size stages presented in Torremorell et al.[353] The first three columns of **Appendix 5 Table 4** below are directly based on data from Torremorell et al[353] (Table 4 results from Andersen Cascade Sampler). The fractions in the last column of **Appendix 5 Table 4** were calculated by dividing the estimated RNA copies per m^3 in a particle size stage by the sum of the RNA copies per m^3 across all the stages. The estimated distribution of airborne virus suggests that most of the virus is carried by large particles that tend to settle faster.

Scenario B results:

Scenario B results are shown in **Appendix 5 Tables 4-5** and **Appendix 5 Figure 3**.

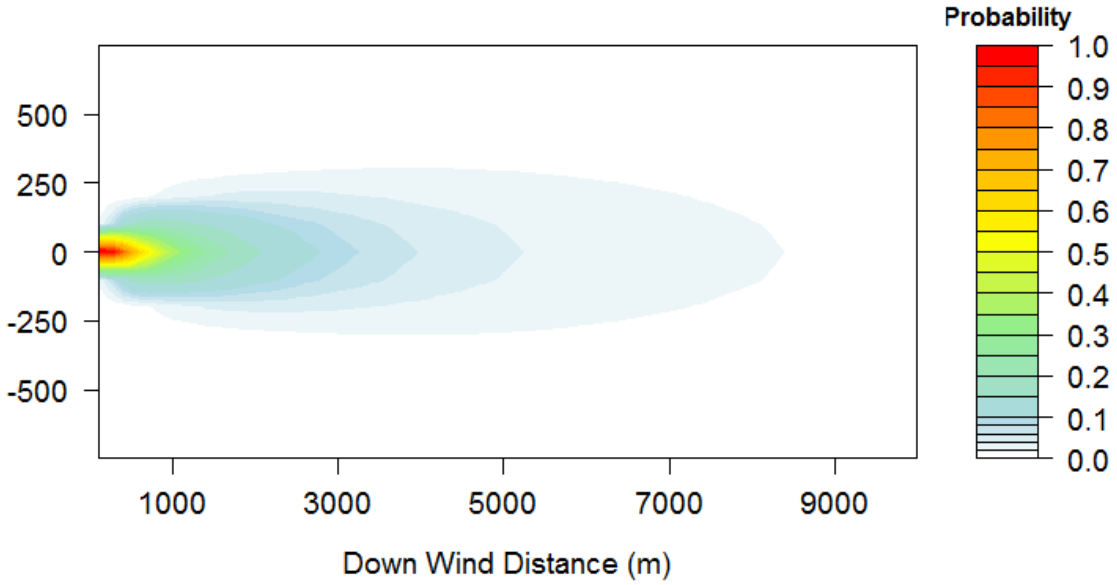
Appendix 5 Table 4. The concentration of virus on the different particle size stages and the proportion of virus RNA in a size fraction based on Torremorell et al. results[353]

Particle size stage lower limit (µm)	Particle size stage upper limit (µm)	Log RNA copies/m^3	Fraction of particles in this stage
0.01	0.4	2.939519253	0.00038
0.4	0.7	2.568201724	0.00016
0.7	1.1	2.62324929	0.00018
1.1	2.1	4.342422681	0.00951
2.1	3.3	5.041392685	0.04754
3.3	4.7	5.342422681	0.09509
4.7	5.8	5.653212514	0.19450
5.8	9	5.322219295	0.09077
9	-	6.113943352	0.56188

We assumed that 25% of suspended particles in the air from a turkey house are PM_{10} and 11% of PM_{10} particles are $PM_{2.5}$.[345,349,350] We assigned a diameter of 25 µm to particles larger than 10 µm, based on the mean mass diameter estimate from Redwine et al.[347] Diameters of 6.25 and 1.5 µm were assigned to PM_{10} and $PM_{2.5}$ size fractions respectively.

HPAI 50% Egg Infectious Dose

The infectious dose for this scenario is the same as in Scenario A.



Appendix 5 Figure 3. AERMOD model-predicted daily probability of infection for a susceptible turkey hen flock located at various points downwind of a source infected turkey premises (Scenario B)

Appendix 5 Table 5. AERMOD model-predicted HPAI virus concentrations and daily exposure probability at various points downwind of a source infected turkey premises (Scenario B)

	Distance from source (turkey premises)				
Outcome variable	**0.5 km**	**1 km**	**1.5 km**	**3 km**	**5 km**
Predicted HPAI concentration (EID_{50}/m^3)	0.76	0.27	0.15	0.05	0.02
Predicted probability of exposure in a day	0.74	0.38	0.23	0.09	0.04

Scenario C:

This scenario is aimed at showing the impact of the uncertainty in the aerosol infectious dose. The difference in infectivity for the different inoculation routes for H5N2 in turkeys is not yet clearly elucidated. In this scenario, the other 50% HPAI virus infectious dose of $10^{3.2}$ EID_{50} that was estimated from the second experiment dataset (**Appendix 5 Table 2**) was used instead of the 10^4 used in Scenario A. The rest of the inputs were not changed from Scenario A.

Scenario C results:

Scenario C results are shown in **Appendix 5 Table 6**. We observe that the predicted likelihood of aerosol transmission is much higher in Scenario C compared to Scenario A. These results indicate that the likelihood of aerosol transmission is very sensitive to the aerosol infectious dose for turkeys, and further studies to reduce the uncertainty in this parameter would be helpful.

Appendix 5 Table 6. AERMOD model-predicted HPAI virus concentrations and daily exposure probability at various points downwind of a source infected broiler premises (Scenario C)

	Distance from source (known infected broiler premises)				
Outcome variable	**0.5 km**	**1 km**	**1.5 km**	**3 km**	**5 km**
Predicted HPAI concentration (EID_{50}/m^3)	0.54	0.19	0.1	0.04	0.02
Predicted probability of exposure in a day	0.99	0.88	0.67	0.32	0.16

Appendix 6: Expert Polling on Aerosol Transmission Route

A panel of eight experts in the turkey and broiler industries with field experience managing AI was surveyed between November 2013 and January 2014 on risk of HPAI transmission via multiple routes of infection. Surveys were administered through the online polling service SurveyMonkey.[h] Experts were asked to provide their opinion, based on previous experience, 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 and responses that pertains 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 6 Tables 1-2** and **Appendix 6 Figures 1-2**.

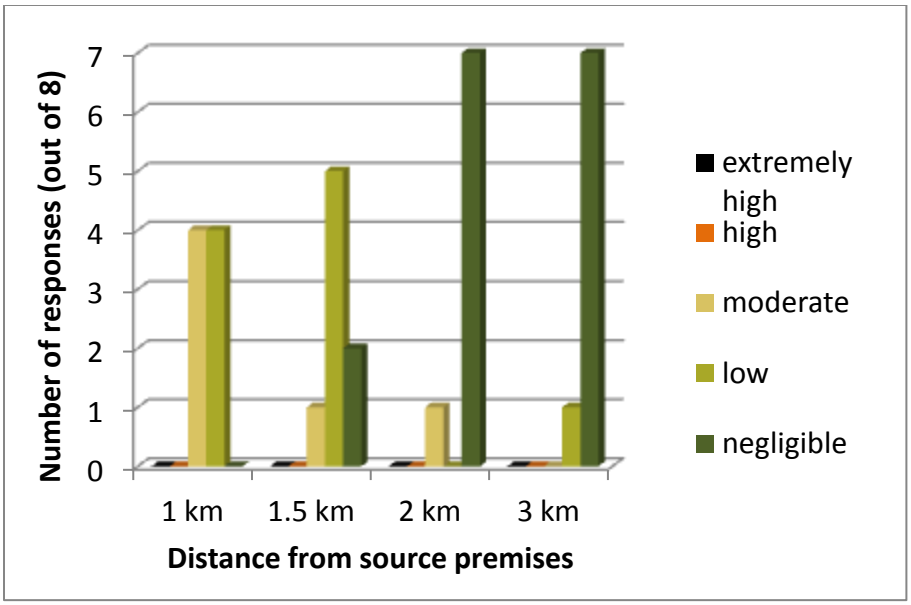
Q1: Please qualitatively rate the likelihood of AI transmission from a known infected flock to an uninfected turkey flock located at distances specified in the table. In this scenario, ***there are no*** *depopulation activities happening at source flock. Please complete the following table, selecting a risk rating for each scenario as negligible, low, moderate, high, or extremely high, based on your expert opinion.*

Q2: Please qualitatively rate the likelihood of AI transmission from a known infected flock to an uninfected turkey flock located at distances specified in the table. 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.*

Appendix 6 Table 1. Expert responses (n=8) to the question of likelihood of AI transmission from a known infected flock to an uninfected turkey flock at specified distances when no depopulation activities are happening at source flock (Question 1)

Distance from source flock	Likelihood rating				
	Negligible	Low	Moderate	High	Extremely high
1 km	0	4	4	0	0
1.5 km	2	5	1	0	0
2 km	7	0	1	0	0
3 km	7	1	0	0	0

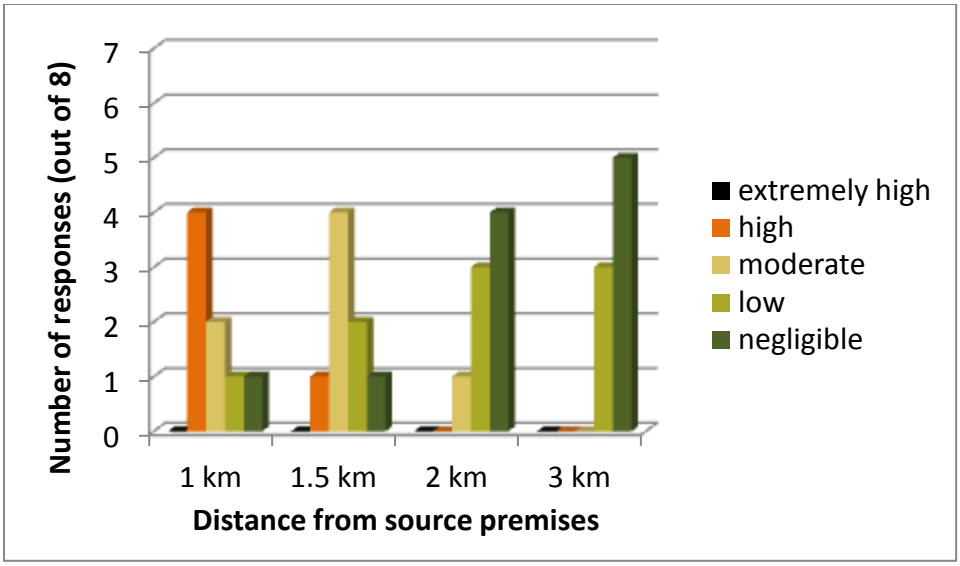
[h] Survey Monkey, Inc., Palo Alto, CA, www.surveymonkey.com



Appendix 6 Figure 1. Expert responses (n=8) to the question of likelihood of AI transmission from a known infected flock to an uninfected turkey flock at specified distances when no depopulation activities are happening at source flock (Question 1)

Appendix 6 Table 2. Expert responses (n=8) to the question of likelihood of AI transmission from a known infected flock to an uninfected turkey flock at specified distances where depopulation activities are happening at source flock (Question 2)

Distance from source flock	Likelihood rating				
	Negligible	Low	Moderate	High	Extremely high
1 km	1	1	2	4	0
1.5 km	1	2	4	1	0
2 km	4	3	1	0	0
3 km	5	3	0	0	0



Appendix 6 Figure 2. Expert responses (n=8) to the question of likelihood of AI transmission from a known infected flock to an uninfected turkey flock at specified distances where depopulation activities are happening at source flock (Question 2)

Appendix 7: Cross-Commodity Pre-Movement Isolation Period (PMIP)

TO MOVE BIRDS DURING AN HPAI OUTBREAK, PRODUCERS NEED TO AGREE TO A PMIP FOR A SET NUMBER OF DAYS PRIOR TO MOVEMENT. DURING THE PMIP:

1. **No live or dead poultry will be moved onto or off the premises.**
2. **Only critical operational visits to the premises will continue.**
3. **Manure, litter, and garbage will not be removed from the premises; the producer is responsible for managing the risks associated with any on-site movement that must occur.**
4. **Enhanced biosecurity for people and vehicles; no off-site equipment will be pre-staged.**

GOAL: for producers to actively and effectively implement enhanced biosecurity procedures in the critical time period before live poultry is moved, thus reducing the risk of lateral HPAI transmission.

What is PMIP?

- The Pre-Movement Isolation Period (PMIP) is a critical biosecurity component of the process to obtain a continuity of business permit that involves a defined period of greatly intensified biosecurity for an entire premises prior to permitted movement of live poultry.
- The PMIP is a component of the Secure Poultry Supply Plan, which provides guidelines for poultry premises that seek to move poultry products or live poultry within, into, or out of a regulatory Control Area during an HPAI outbreak.

What poultry movements require a PMIP?

- The PMIP is required for these live poultry movements:
  - **Terminal movements** (e.g., broilers to processing, turkeys to processing).
    - All out movements, and
    - Movements that do not remove all birds from a premises (e.g., multi-age premises).
  - **Transfer movements** (i.e., live bird movements between farms)
    - All out movements, and
    - Movements that do not remove all birds from a premises.
- These live poultry movements have a moderate to high risk of causing lateral disease transmission if infected but undetected poultry are moved.
- Movement of poultry that are infected but undetected will have subsequent epidemiologic, regulatory, and economic consequences.

What poultry movements do not require a PMIP?

- The PMIP does not apply to day-old chicks or poults (however, a post-move quarantine does apply to these birds).
- The PMIP does not apply to poultry by-products (except as specifically related to live bird movement).
- The PMIP does not apply to eggs or egg products.

When does the PMIP start and end?

- The PMIP starts the specified number of days prior to the scheduled movement date and ends when load-out begins (i.e., the hours or days of load-out are not considered part of the PMIP).
- The load-out period begins when the first crew, vehicle, or equipment arrives on the premises and ends when the last load of birds departs the premises. Pre-staging of equipment during the PMIP is prohibited.

How long is the PMIP?

- The PMIP takes place for a defined number of days immediately prior to the permitted movement of poultry. This period is as follows (determined by some combination of 95% probability of detection for the type of poultry, the type of housing [contact rate], and the characteristics of HPAI viruses [mean death time], as well as by the ultimate consequences of moving an infected, undetected specific type of flock):
    - For 8 days prior to movement of turkeys to processing
    - For 5 days prior to movement of broilers to processing
- Movements of other types of live poultry may not be advised or may require additional post-movement quarantine.

What critical operational visits to a premises may continue during the PMIP (when specific biosecurity measures are in place)? *Please defer to designated regulatory officials to determine what types of movements require a permit in the Control Area; while these critical operational visits need to continue during the PMIP, a permit may or may not be required.*

1. Feed delivery in a dedicated truck directly from a stand-alone feed mill. Trucks delivering individual feed ingredients that are stored on poultry premises will require a permit.
2. Emergency repair of critical mechanical equipment.
3. Service visits to address changes in bird health.

What is the specific biosecurity for these critical operational visits?

- People who have contact with other poultry must shower and change clothes before entering the premises and also wear necessary protective clothing and footwear as described in appropriate biosecurity protocols.
- All vehicles and equipment will be cleaned and disinfected prior to entering premises.

What activities are prohibited during PMIP?

Activities that pose a risk for lateral transmission of HPAI virus are prohibited. Specific prohibited activities are:

1. Off-farm disposal of mortality is prohibited. Risks associated with dead birds on-site must be managed.
2. Off-farm removal of manure or litter is prohibited. Risks associated with manure or litter movement on-site must be managed.
3. Off-farm garbage disposal is prohibited. Risks associated with garbage storage on-site must be managed.
4. Visiting other poultry farms is prohibited for people who work on poultry farms. People should have contact only with their assigned flock.
5. All non-critical visitors are prohibited from entering farms. All non-critical, routine, or operational visits must be replaced by telephone communication or must be scheduled outside of the PMIP. Non-critical visitors who work with or have contact with another commercial poultry operation (farm, hatchery, processing plant, etc.) or have contact with a noncommercial poultry flock (backyard birds, hobby farms with birds, or game birds) are prohibited from entering farms.
6. Entering a poultry house is prohibited unless the person is wearing clothing dedicated to the farm and footwear dedicated to the house.
7. Noncritical equipment from off-site is prohibited from being moved on-site.
8. Moving live poultry onto or off the premises is prohibited.

Why is the PMIP critical?

- The PMIP biosecurity requirements will minimize the likelihood of a flock being exposed to HPAI close to its scheduled movement date.
- The PMIP will increase the likelihood of detection of an infected flock prior to movement of birds.
- The PMIP will decrease the likelihood of moving infected but undetected poultry, thus reducing the risk of lateral transmission of HPAI virus from specific categories of live poultry.
- The PMIP assists regulatory officials evaluating movement permit requests, since producers seeking permits will actively document, for the record, that they have achieved specific biosecurity requirements.

How does the PMIP work?

- Signs of disease take time to develop following exposure to and infection with HPAI virus.
- If a flock is exposed to HPAI virus close to its scheduled movement date, signs of disease (or significant disease spread and mortality) within the flock may not be evident yet, and thus the probability of virus detection is relatively lower.

- If the flock is exposed to and becomes infected with HPAI virus prior to the implementation of the PMIP, disease signs of HPAI are likely to be detected by the day of scheduled movement (i.e., by the end of the PMIP); thus, movement can be halted and the flock is unlikely to pose a movement-associated risk.

What can be done for birds scheduled to move before a PMIP can be completed?

- When a Control Area is first established or is expanded, some flocks newly in that Control Area may already be scheduled to move before a full PMIP can be completed. Additionally, rapid marketing to reduce the susceptible poultry population in a Control Area may be beneficial for HPAI outbreak control, provided that birds can be moved in a biosecure manner. In such situations, PMIP biosecurity measures should be implemented as soon as possible, and additional information must be provided to the designated regulatory officials to request movement prior to a full PMIP.
- Additional information that must be provided to the designated regulatory officials includes: mortality and morbidity data, test results, destination premises location, farm visitor and farm activity records from the days prior to the Control Area designation, additional equipment requirements, route to be used for transport, defined load-out duration and situation, and defined biosecurity and downtime protocols for load-out crews.

Appendix 8: 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 Turkey House. 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).[319] The models from Weaver *et al*. (2016) were updated based on research on the 2015 EA/AM HPAI H5N2 outbreak in the U.S.[319] The derivation of the updated 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 before the start of load-out. The overall likelihood combines the probability of two events: First, the probability 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. 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).[319] Two different transmission kernels were used to estimate the overall likelihood: a transmission kernel derived from data on the 2003 HPAI H7N7 outbreak in the Netherlands by Boender et al. (2007) and a transmission kernel estimated from data on the 2015 HPAI H5N2 outbreak in Minnesota.[120] Details on the estimation of the Minnesota transmission kernel are given following an explanation of the estimation of the disease transmission and surveillance model parameters used in the simulation.

Estimation of Transmission Model Parameters

Adequate Contact Rate

The adequate contact rate distribution was estimated using the data and method from Saenz et al. (2012) assuming a non-zero latent period.[104] The experiment performed by Saenz et al. (2012) consisted of groups of 10, 20, and 40 contact turkeys that were each introduced to one turkey inoculated with HPAI H7N1 (for a total of three inoculated turkeys).[104] Saenz et al. (2012) estimated a contact rate distribution from the resulting data with an assumption of no latent period.[104] However, since non-zero latent periods have generally been observed in HPAI inoculation studies, the contact rate distribution used for this risk assessment is re-estimated from the Saenz et al. (2012) data assuming a non-zero latent period.[104] For more details and discussion see Weaver et al. (2016).[319]

Latent and Infectious Period Distributions

Latent and infectious period distributions were estimated for two EA/AM HPAI H5N2 isolates from experimental data using a method first derived in "An Assessment of the Risk Associated with the Movement of Turkey Hatching Eggs into, within, and out of a Control Area during a Highly Pathogenic Avian Influenza Outbreak."[8] The experimental data, provided by Dr. Erica Spackman, involved the inoculation of turkeys with an EA/AM HPAI H5N2 isolate from a

turkey infected in Minnesota and an EA/AM HPAI H5N2 isolate from a chicken infected in Iowa during the 2015 outbreak (E. Spackman, personal communication, December 2015). Turkeys were inoculated with one of the isolates in the morning of day 0. Inoculated turkeys were tested daily by rRT-PCR and checked every 12 hours for mortality. Birds showing severe symptoms in the evening were euthanized. Some birds showing severe symptoms were euthanized for necropsy, which could have been performed either in the morning or evening. Since birds are not continuously monitored and some are euthanized, the exact time of transition between infection statuses is unknown.

A maximum likelihood approach developed in "An Assessment of the Risk Associated with the Movement of Turkey Hatching Eggs into, within, and out of a Control Area during a Highly Pathogenic Avian Influenza Outbreak" that accounts for time-censored data is utilized to estimate the latent and infectious period distributions for the two EA/AM HPAI H5N2 isolates.[8] Briefly, the method consists of estimating parameters for gamma distributions integrated across the time in which a transition in infection status could have occurred. Inoculated birds that first tested positive for HPAI later than 4 days post inoculation were not included in the estimation of the latent and infectious period distributions since these birds could have been infected through contact rather than inoculation, making the length of the latent period uncertain.

Let event F be the transition from latently infected to infectious, and let event G be the transition from infectious to removed (death) state. Furthermore, let *a* be the time period of the last negative rRT-PCR test result and *b* be the time period of the first positive test result, so $F \in (a, b]$. The assumptions for the set containing event G depend on whether the turkey was observed dead, euthanized, or necropsied.

Let T_{obs} be the morning observation time of the day on which a turkey is observed as dead. The mortality could have been observed at time T_{obs} or 12 hours later in the evening at time $T_{obs} + \frac{1}{2}$. If the observation occurred at T_{obs}, the bird is assumed to have died within 8 hours of the observation since if the turkey had been very sick the evening before, 12 hours prior to T_{obs}, it would have been euthanized. Thus, the bird is assumed to have survived for at least 4 hours following the previous evening's inspection. If the observation occurred at time $T_{obs} + \frac{1}{2}$, the latest the bird could have died is at this time of the evening observation. Let $c_{obs} = T_{obs} - \frac{1}{3}$ and let $d_{obs} = T_{obs} + \frac{1}{2}$. Under the assumptions, $G \in [c_{obs}, d_{obs}]$.

Next, suppose the turkey is euthanized. Let T_{euth} be the morning observation time on the day of euthanasia. The earliest the turkey could have died naturally is the time of euthanasia, which occurs in the evening at time $T_{euth} + \frac{1}{2}$. Since only very sick birds are euthanized, the turkey is assumed to have died naturally no later than 8 hours after the evening euthanasia. Let $c_{euth} = T_{euth} + \frac{1}{2}$ and let $d_{euth} = T_{euth} + \frac{5}{6}$, so $G \in [c_{euth}, d_{euth}]$ in this case.

Finally, suppose the turkey is necropsied. Let T_{necro} be the morning observation time on the day of necropsy. The necropsied turkey could have been euthanized in the morning or evening observation period. If euthanized in the morning, at time T_{necro}, the earliest the turkey could have died naturally is T_{necro}. As only very sick turkeys were euthanized for necropsy, if euthanized in the evening, the turkey is assumed to have died within 8 hours post-evening. Let $c_{necro} = T_{necro}$ and let $d_{necro} = T_{necro} + \frac{5}{6}$. Thus, $G \in [c_{necro}, d_{necro}]$ under the assumptions.

Let N be the total number of inoculated turkeys and $I\{\cdot\}$ be an indicator function. Given a gamma-distributed latent period with shape k_1 and scale θ_1, and a gamma-distributed infectious period with shape k_2 and scale θ_2, the likelihood function is given by

$$L(x, y|k_1, \theta_1, k_2, \theta_2) = P(F \cap G)$$
$$= \prod_{i=1}^{N} \int_{a}^{b} f_{gamma}(y \mid k_1, \theta_1) \left(\int_{c_{obs}-y}^{d_{obs}-y} f_{gamma}(x \mid k_2, \theta_2)\, dx\, I\{turkey\ i\ observed\ dead\} \right.$$
$$+ \int_{c_{euth}-y}^{d_{euth}-y} f_{gamma}(x \mid k_2, \theta_2)\, dx\, I\{turkey\ i\ euthanized\}$$
$$\left. + \int_{c_{necro}-y}^{d_{necro}-y} f_{gamma}(x \mid k_2, \theta_2)\, dx\, I\{turkey\ i\ necropsied\} \right) dy$$

The likelihood function was maximized for each of the isolates using the "nlminb" algorithm, a quasi-Newton method in R's "optimx" function.[354-356] The maximum likelihood estimates for the Minnesota turkey isolate were $k_1 = 1.7733$, $\theta_1 = 0.7976$, $k_2 = 7.7624$, and $\theta_2 = 0.5405$. The maximum likelihood estimates for the Iowa chicken isolate were $k_1 = 4.0307$, $\theta_1 = 0.1809$, $k_2 = 2.9655$, and $\theta_2 = 1.6248$.

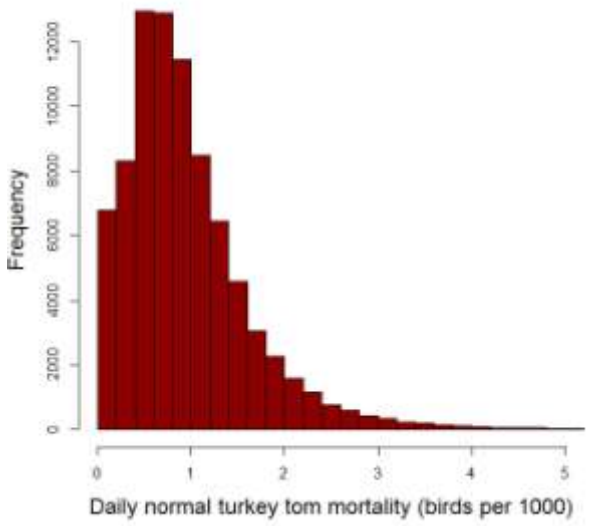
Number of Turkey Toms per House

The flock size is randomly generated from an exponential distribution with a mean of 15,188 birds truncated at 2,000 and 40,000 estimated from industry data.

Estimation of Active Surveillance Model Parameters

Daily Mortality

Daily normal (non-disease) mortality data on 32 turkey tom houses was provided by industry representatives. Normal mortality was simulated by first selecting 14 consecutive days of daily mortality from one of the 32 turkey tom houses. The end point for the 14 days of daily mortality is randomly selected to be 1, 2, or 3 days prior to the movement day. The daily mortality is then rescaled by a random number generated from a lognormal distribution with a mean of -5.0369 and standard deviation of 0.3330, which was estimated from additional industry-provided weekly mortality data. This rescaling of the daily mortality introduces variability into the simulation of normal mortality. A histogram of simulated turkey tom normal mortality in birds per 1,000 is given in **Appendix 8 Figure 1**. The simulated normal mortality has a mean of 1.08 birds per 1,000, 5th percentile of 0, and 95th percentile of 2.35. The disease mortality component of the total daily mortality is selected from the disease transmission simulation model output.



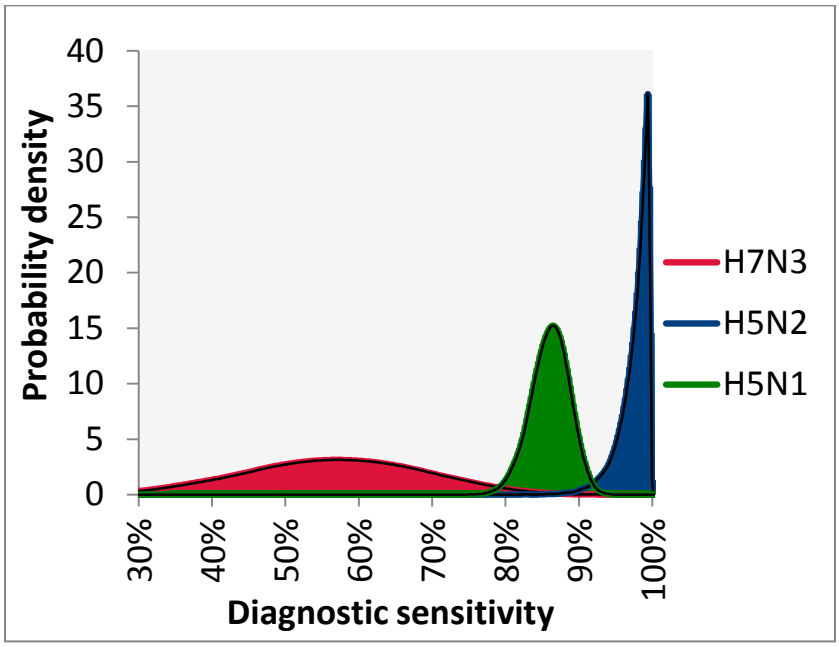
Appendix 8 Figure 1. Histogram of simulated daily mortality in a turkey tom house in the last 2 weeks prior to movement

Diagnostic Test Sensitivity

The sensitivity of the rRT-PCR test is estimated to be 86.5%, meaning there is a 13.5% chance the infection will not be detected even when the pooled sample contains an HPAI-positive swab.[357] AI experts noted this sensitivity estimate is conservative considering recent enhancements to test protocols.[8]

Supplementary antigen-capture (AC) immune assays using lateral flow devices are utilized in additional testing protocols evaluated in Appendix 10: Supplementary Active Surveillance Protocols. These tests require high virus concentrations to detect AI virus (detection limit is between 10^4 and 10^6 EID_{50}).[358,359] The diagnostic sensitivity of these tests therefore depends on the clinical status of the infectious birds, which affects the level of virus shedding. A study performed at the USDA Agricultural Research Service's Southeast Poultry Research Laboratory (SEPRL) was undertaken to provide data on 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 8 Figure 2**. The estimated means and 95% credibility intervals for the AC test sensitivities are 57% (33-80%) for the HPAI H7N3 Jalisco strain, 86% (80-91%) for the HPAI H5N1 strain, and 97.9% (92-99.9%) for the HPAI H5N2 strain.[360] 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 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%. Given the uncertainty and

variance surrounding the estimates for AC test sensitivity, a conservative estimate of 50% is chosen for this analysis.



Appendix 8 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 Comparison

Four candidate parameterizations of the spatial transmission kernel were assessed for best fit for the 2015 HPAI H5N2 Minnesota outbreak data using Akaike's Information Criterion (AIC).[361] The likelihood function used in the evaluation of AIC takes the form given in Boender et al. (2007).[120] The resulting AIC values were all within 2 of each other, meaning none of the parameterizations is a definitive best fit. The same parametrization as was used in Boender et al. (2007) was chosen for use in the Minnesota outbreak analysis in order to make the two spatial transmission kernels more comparable.[120] The spatial 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.

Due to phylogenetic evidence of primary introductions occurring concurrently with lateral spread, an additional parameter was added to the force of infection equation from Boender *et al*.

(2007).[120] 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.[120] The force of infection from Boender et al. (2007) is given below as a function of *t*:

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

This equation is modified 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:

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

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. However, as some of the risk from long-distance movements of people and equipment may be captured by *k*, a third force-of-infection equation was evaluated with a constant, distance-independent parameter, δ, that varies with the number of infectious premises:

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

Infection risk related to distance-independent movements of people and equipment and distance-independent environmental factors is therefore partitioned between δ and *k*, respectively. The three different force-of-infection equations were compared using AIC. Adding *k* to the force of infection significantly improved the model fit based on the resulting AIC values. The addition of δ, on the other hand, resulted in a larger AIC (AIC = 1393.393 with *k*, compared with 1395.332), which suggests that the inclusion of δ is trivial, and that long-distance movements of people and equipment provide only marginal contributions to the risk represented by *k*. Thus, the force of infection with *k* only was chosen for use in the analysis of the Minnesota outbreak.

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).[120] 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 8 days prior to the detection date. The infectious period is assumed to begin three days later, 5 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% confidence intervals under these assumptions regarding infection status are given in **Appendix 8 Table 1**, along with the parameters estimated from the Netherlands outbreak by Boender et al. (2007).[120] Infection risk in Minnesota—with its higher mean hazard rate, significantly higher r_0, which suggests higher infection risk persisted over significantly longer distances, and additional parameter *k* representing distance-independent environmental risk factors—would be expected to be considerably higher and less responsive to changes in distance, thereby posing an overall greater threat of HPAI spread.

Appendix 8 Table 1. Mean estimates and 95% confidence intervals of spatial transmission kernel model parameters estimated from HPAI outbreaks in Minnesota and the Netherlands

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)
Netherlands 2003 HPAI H7N7: Case premises are infected 6 days prior to the first rise in mortality; infectious period starts 2 days later and lasts until depopulation. Estimates from Boender et al. (2007) (#).	0.0020 (0.0012, 0.0039)	1.9 (1.1, 2.9)	2.1 (1.8, 2.4)	NA

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 from the Minnesota and Netherlands outbreaks are used to estimate the probability of infection applied in the estimation of the overall probability. Note that as the force of infection increases, the probability of infection increases. The uniformly higher mean hazard rates over distance along with the additional risk represented by *k* leads to a higher force of infection under the Minnesota outbreak. Thus, the probability of infection is higher for the Minnesota outbreak transmission kernel, which results in a higher estimated overall probability of not detecting HPAI in a flock prior to the start of load-out.

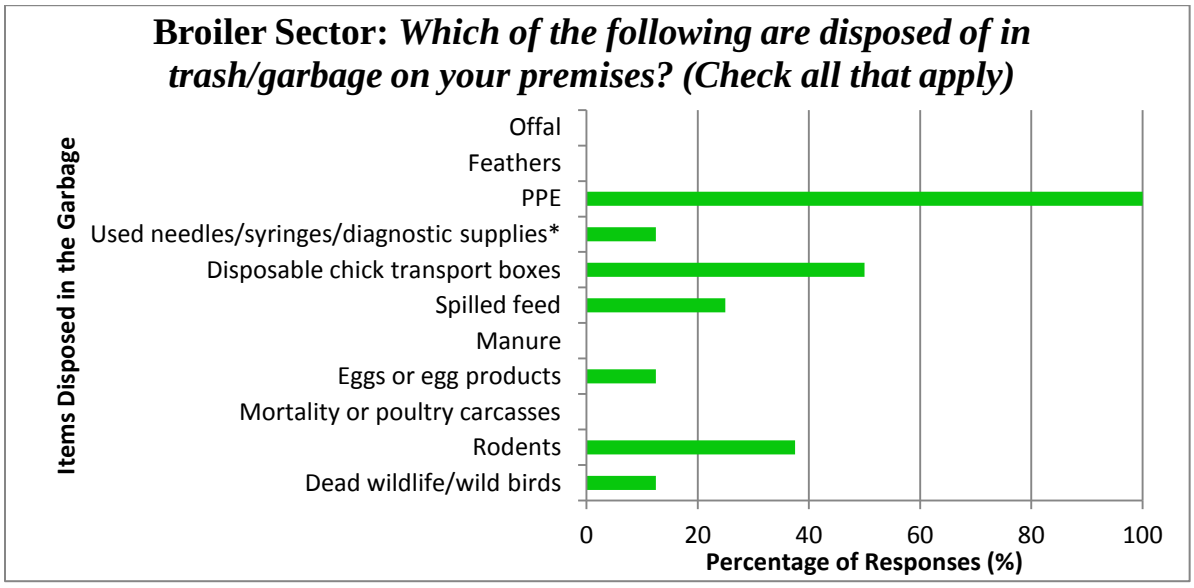
Appendix 9: Poultry Industry Survey on Garbage Management Practices

A convenience sample of veterinarians and other managers in the turkey (n=15), broiler (n=8), and layer (n=40) industries was surveyed between June and August 2016 on standard practices for garbage management on farms that they manage or supervise. Surveys were administered by the University of Minnesota HPAI Team using the online polling service Qualtrics.[i]

A convenience sample of participants with significant experience in the poultry industry was solicited; however, this survey was limited by small sample size. No additional analyses were conducted for these data beyond descriptive statistics. Still, the results are informative for the purpose of the risk assessment, and serve to illustrate the variations in industry practice and potential differences between poultry sectors that may operate in the same geographic area. As such, readers should note that absence of an affirmative response to a high-risk activity does not definitively indicate it is not occurring. The results of the survey are shown in **Appendix 9 Figures 1-19**. Of note, some survey questions and answer choices were modified to better match the industry to which the survey was distributed. Additionally, some participants declined to answer all questions within the survey.

Types of Items Disposed in the Garbage on Poultry Premises

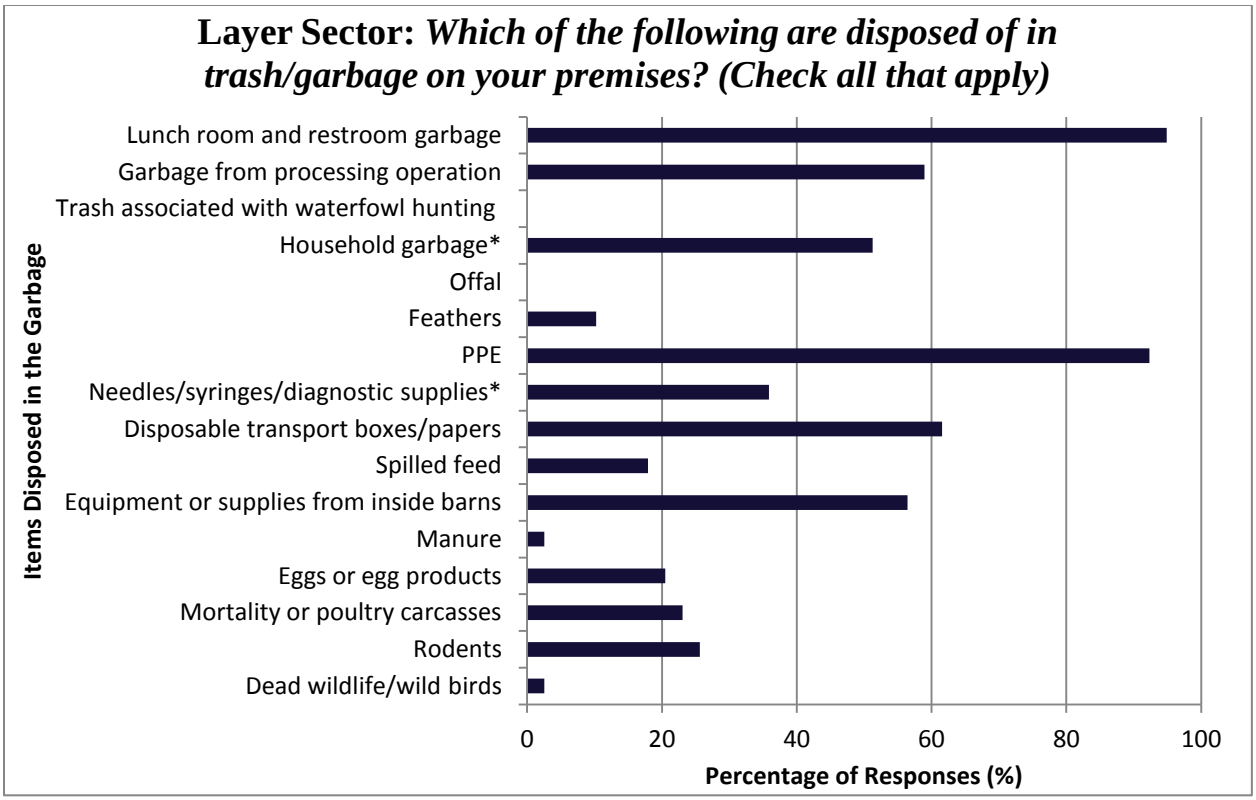
Appendix 9 Figures 1-3 show items disposed in garbage which may be potentially infectious or contaminated with HPAI in the event of an outbreak. Respondents answered the question: Which of the following are disposed of in trash/garbage on your premises (check all that apply)?[j]



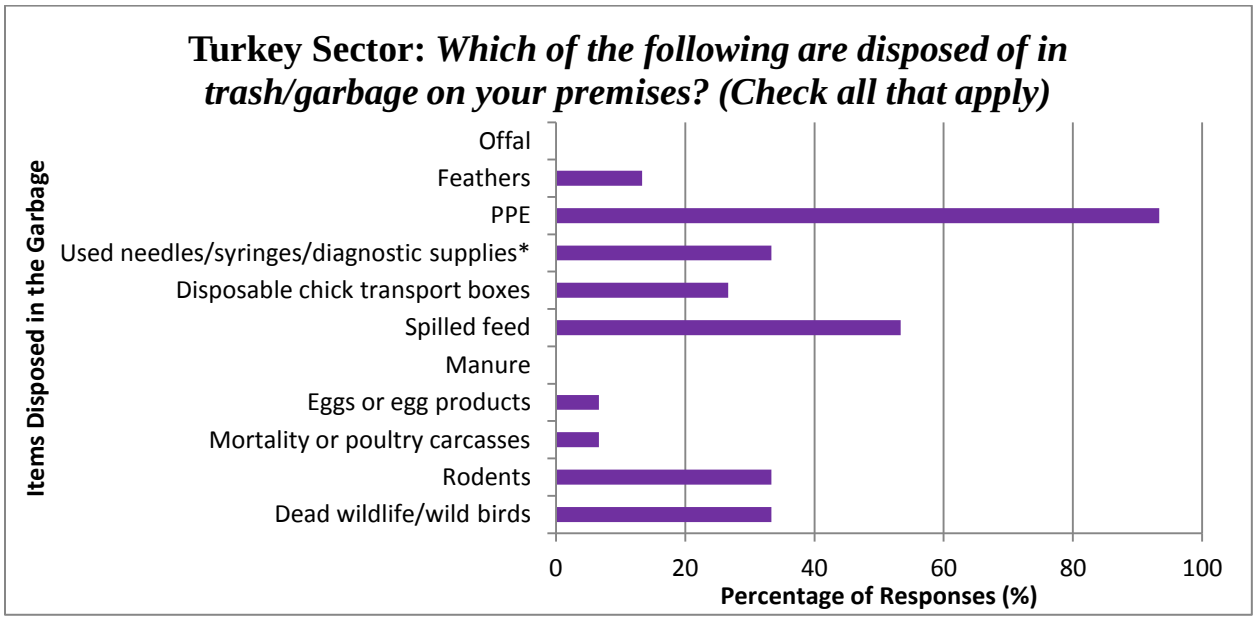
Appendix 9 Figure 1. Responses of broiler industry representatives (n=8) to types of items disposed in the garbage on broiler premises. Respondents in the broiler industry wrote in additional items such as boxes, buckets, jugs from disinfectants, litter treatments, disposable chick feeder lids, cans, and bottles.

[i] Qualtrics© 2015 Provo, UT, USA. http://www.qualtrics.com

[j] Item abbreviated for graphic display (*). Full text prompt as used in survey: "Used needles/syringes/diagnostic supplies that have contacted birds;" "Household garbage from farm manager residence or any other residence."



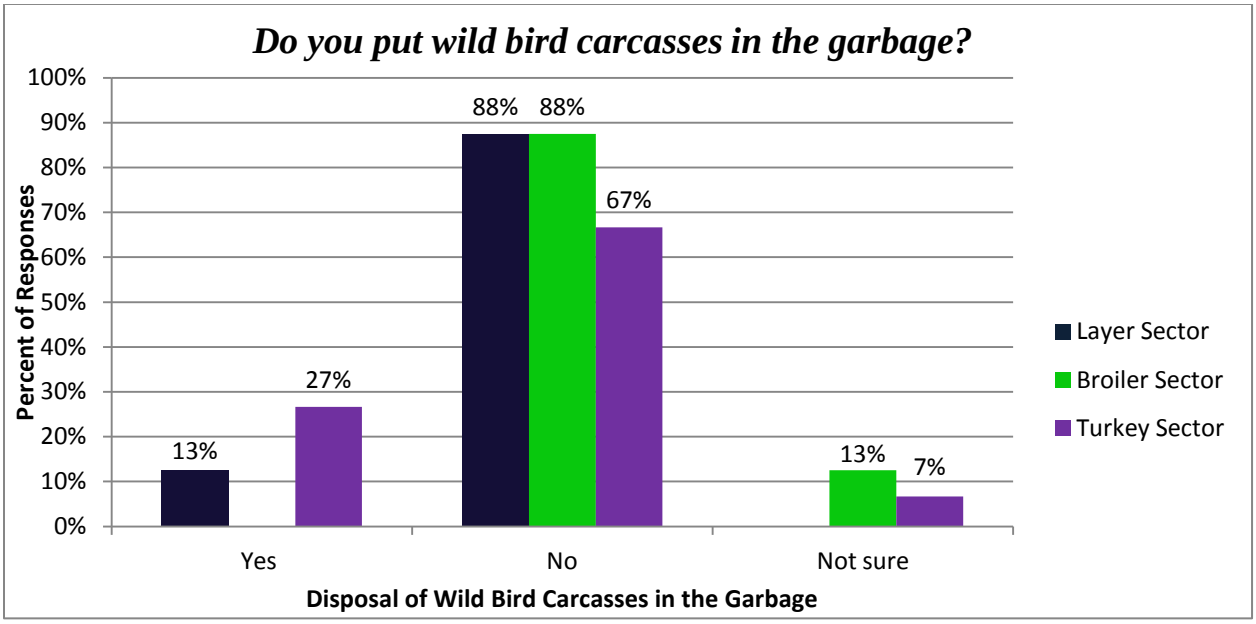
Appendix 9 Figure 2. Responses of layer industry representatives (n=39) to types of items disposed in the garbage on layer premises



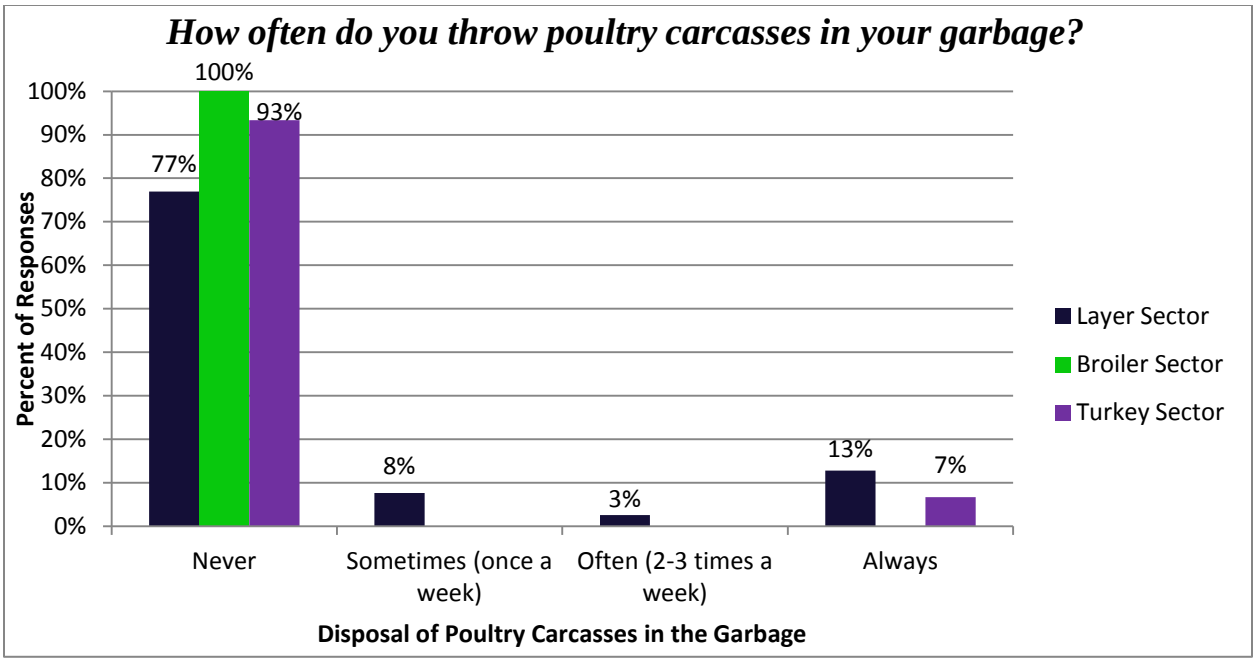
Appendix 9. Figure 3. Responses of turkey industry representatives (n=15) to types of items disposed in the garbage on turkey premises. Respondents in the turkey industry wrote in additional items such as trash from farm manager residence, empty medication containers, poult box papers, and supply containers.

Disposal of Potentially HPAI Infectious or Contaminated Items in the Garbage on Poultry Premises

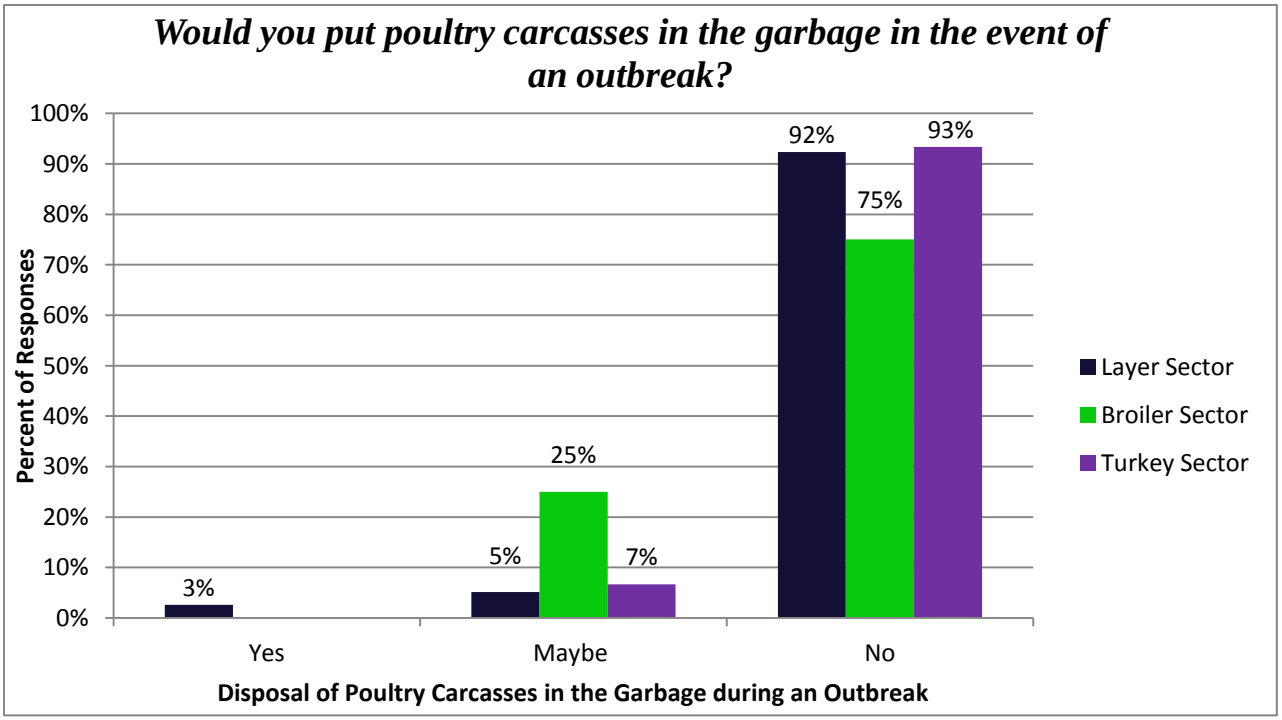
Appendix 9 Figures 4-7 show percentages of poultry industry respondents surveyed that reported disposing in the garbage potentially high-risk items, such as wild bird carcasses, poultry carcasses, and other items that may act as fomites.



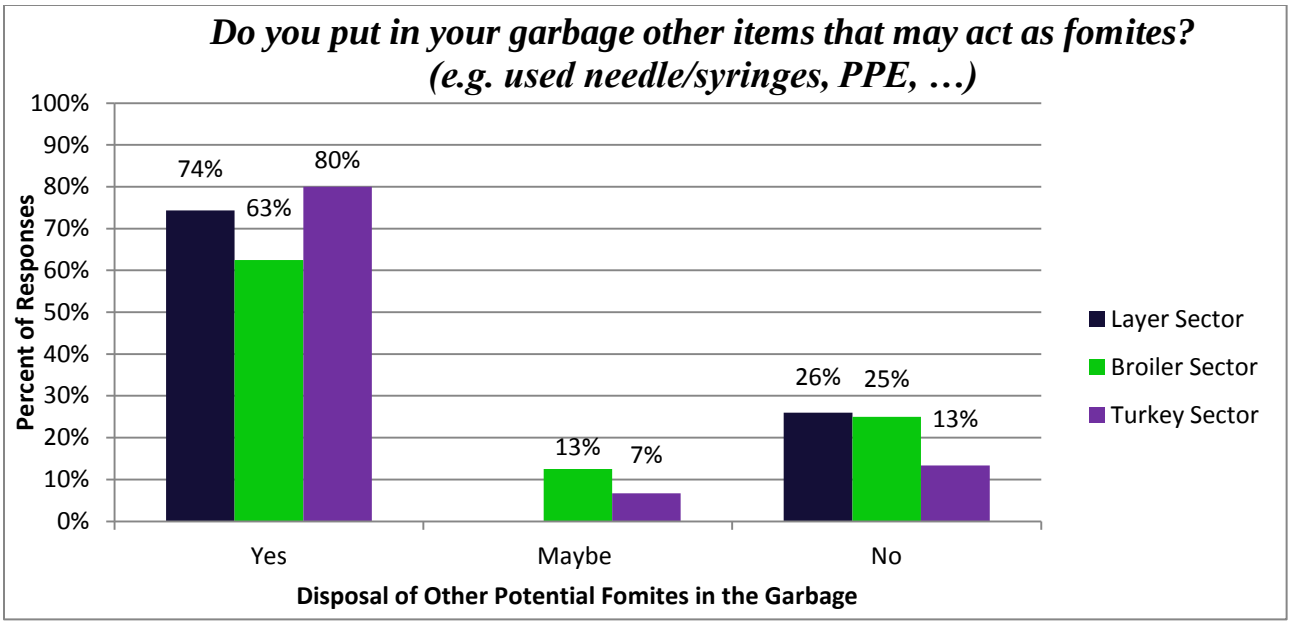
Appendix 9 Figure 4. Responses of poultry industry representatives to disposal of wild bird carcasses in the garbage (layer sector: n=40; broiler sector: n=8; turkey sector: n=15). The answer choice "not sure" was not available in the survey of layer industry representatives.



Appendix 9 Figure 5. Responses of poultry industry representatives regarding the frequency of disposing poultry carcasses in the garbage (layer sector: n=39; broiler sector: n=8; turkey sector: n=15). The carcass type (layer/pullet, broiler, or turkey) cited in the question matched the industry sector to which the survey was distributed.



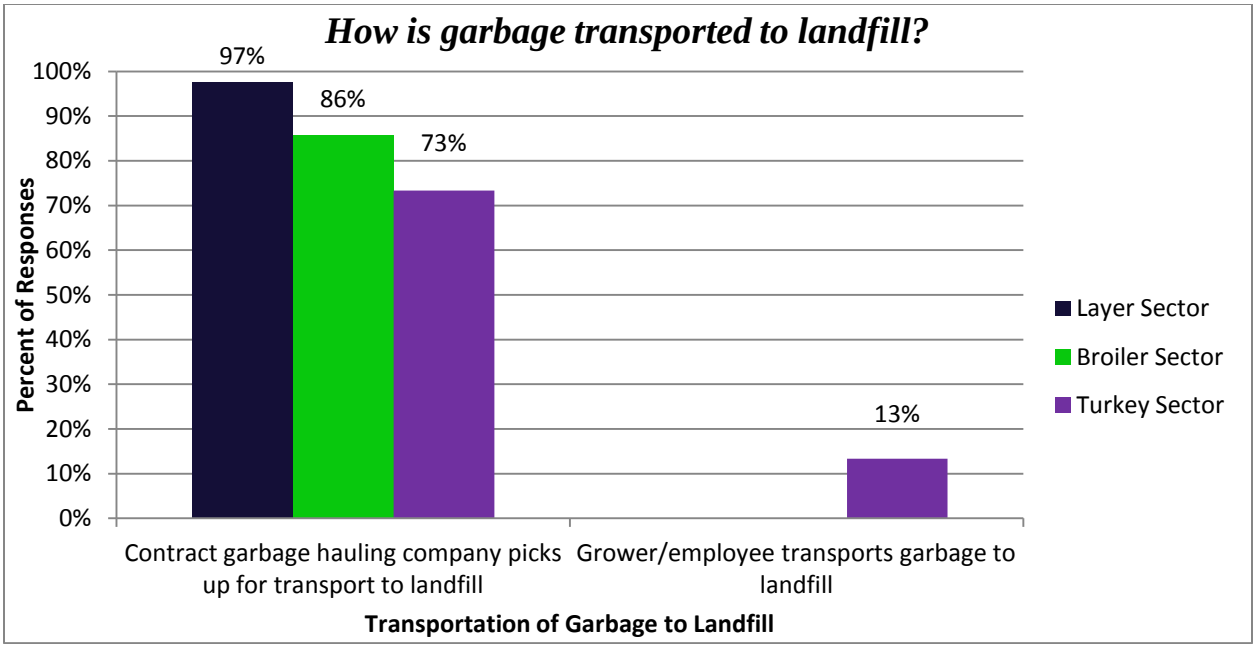
Appendix 9 Figure 6. Responses of poultry industry representatives regarding disposal of poultry carcasses in the garbage in the event of an outbreak (layer sector: n=39; broiler sector: n=8; turkey sector: n=15). The carcass type (layer/pullet, broiler, or turkey) cited in the question matched the industry sector to which the survey was distributed.



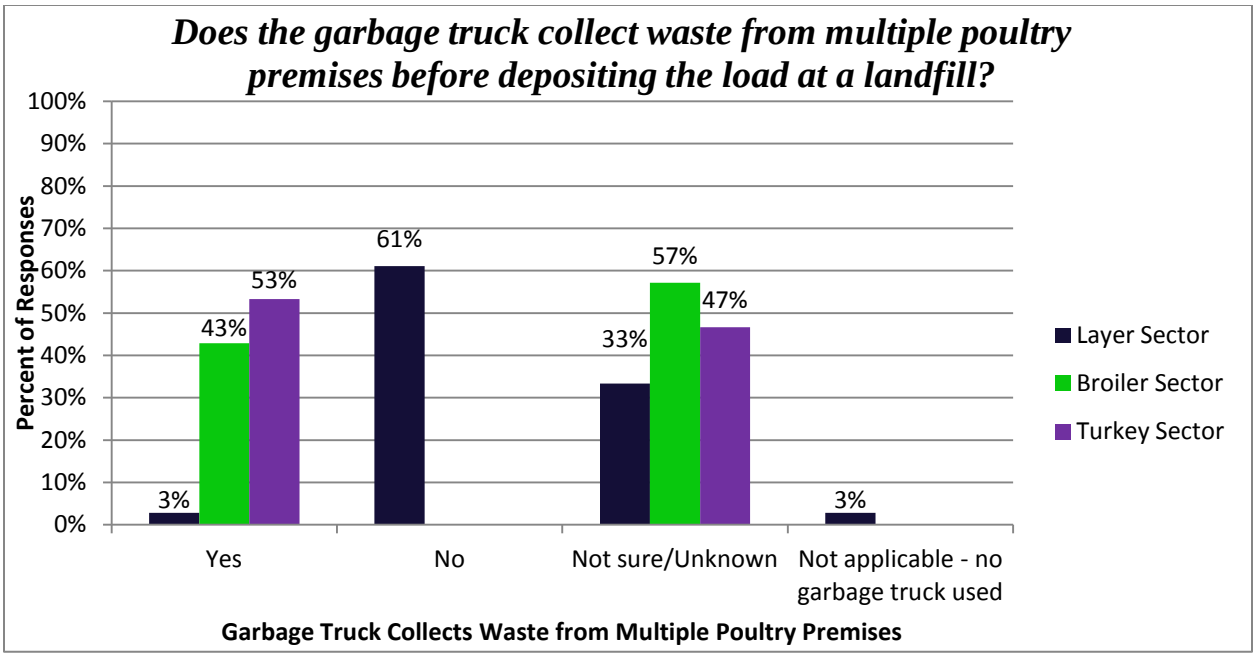
Appendix 9 Figure 7. Responses of poultry industry representatives regarding disposal of other items that may act as fomites (layer sector: n=39; broiler sector: n=8; turkey sector: n=15). The answer choice "maybe" was not available in the survey of layer industry representatives.

Transportation of Garbage to Landfill

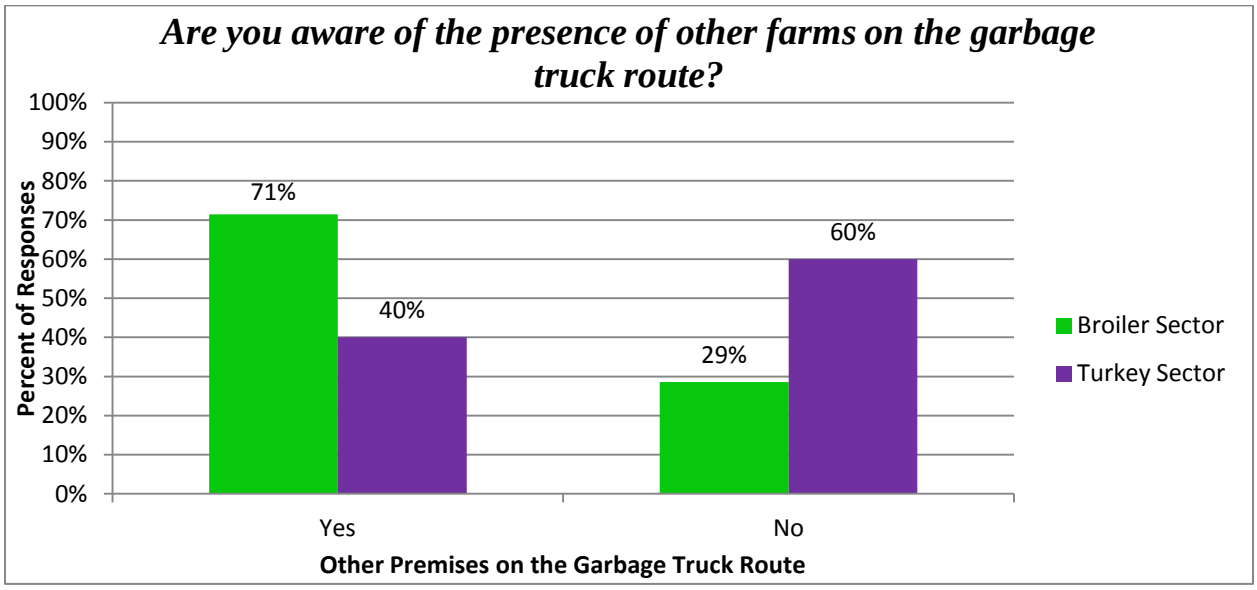
Industry representatives indicated that most commercial poultry operations utilize an off-site landfill for garbage disposal (BWG, TWG, personal communication, May 2016). **Appendix 9 Figures 8–10** show survey results related to garbage transportation to landfill, such as use of a contracted service and the garbage truck route.



Appendix 9 Figure 8. Responses of poultry industry representatives regarding methods of transporting garbage to landfill (layer sector: n=39; broiler sector: n=7; turkey sector: n=15). Respondents wrote in that some premises may use a combination of grower transport and contracted hauling services.



Appendix 9 Figure 9. Responses of poultry industry representatives regarding whether their garbage hauler collects waste from multiple poultry premises (layer sector: n=36; broiler sector: n=7; turkey sector: n=15).

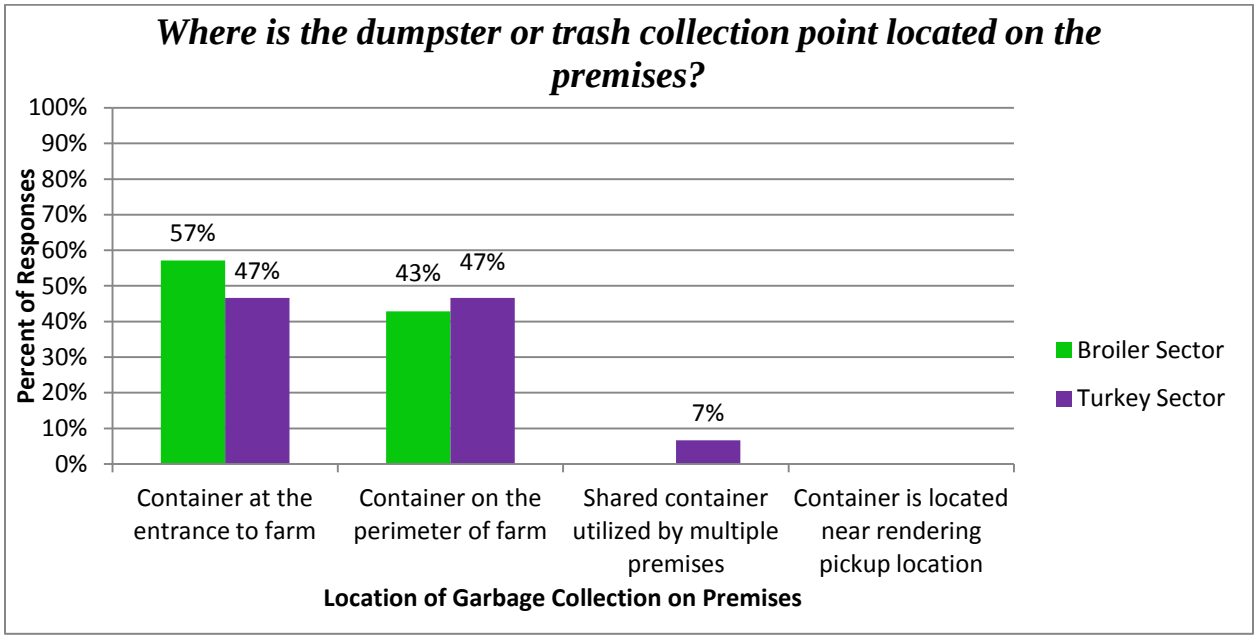


Appendix 9 Figure 10. Responses of broiler and turkey industry representatives concerning their awareness of other farms on the garbage truck route (broiler sector: n=7; turkey sector: n=15). This question was not asked of layer industry representatives.

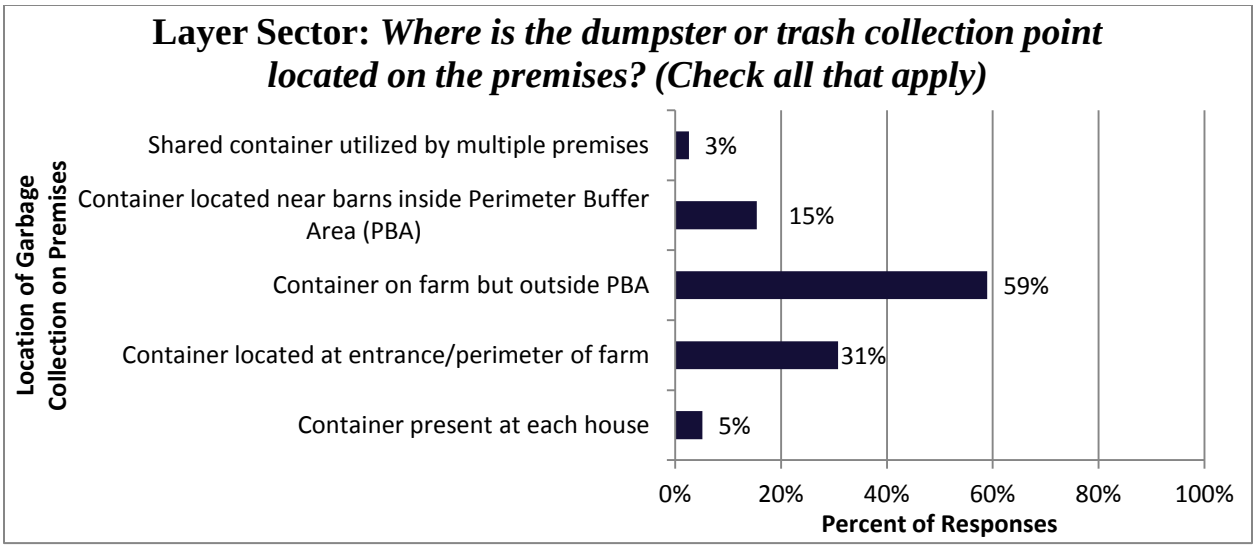
Location of Garbage Collection Area on Commercial Poultry Premises

Premises often have dumpsters or a designated location where trash is collected for transportation to a landfill. **Appendix 9 Figures 11–15** show survey results related to location of the garbage collection area on the premises relative to other features on the farm, such as poultry barns, other premises, and rendering collection point.

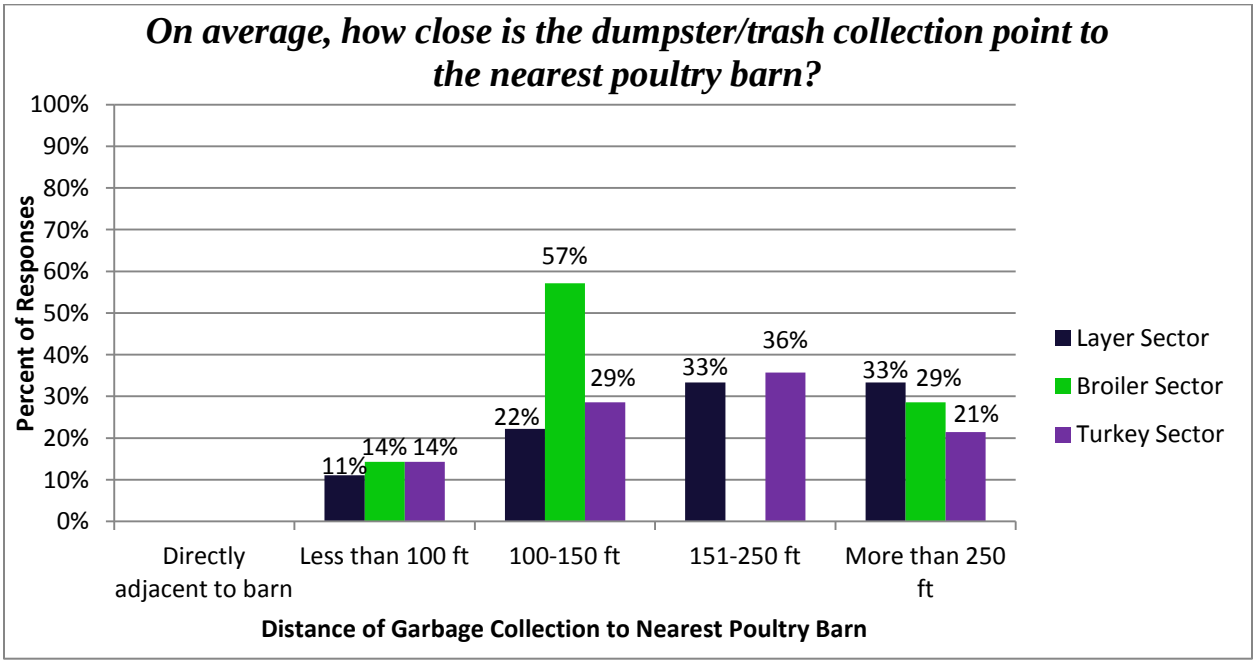
Note that in **Appendix 9 Figure 14**, the option "no rendering used" was available to respondents in the layer sector. The low number of responses from the broiler and turkey sectors may suggest that some individuals declined to answer this question because it was not applicable to their premises. An additional question about biosecurity practices at the dumpster site was posed only to layer industry representatives (**Appendix 9 Figure 15**).



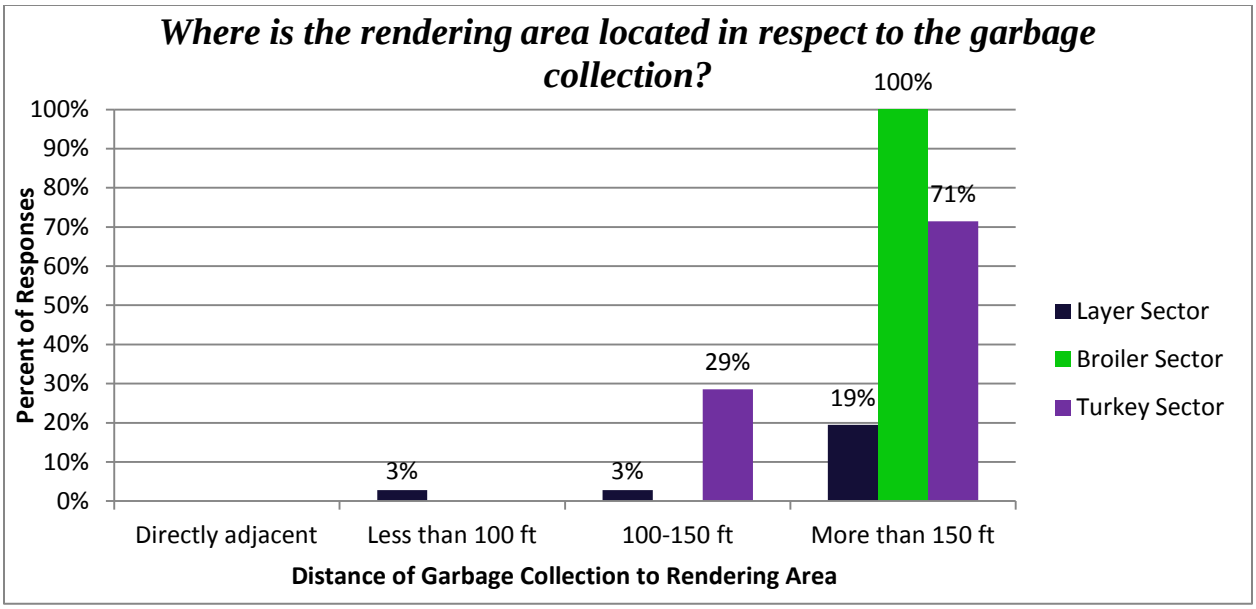
Appendix 9 Figure 11. Responses of broiler and turkey industry representatives regarding the location of the dumpster or trash collection point on their premises (broiler sector: n=7; turkey sector: n=15).



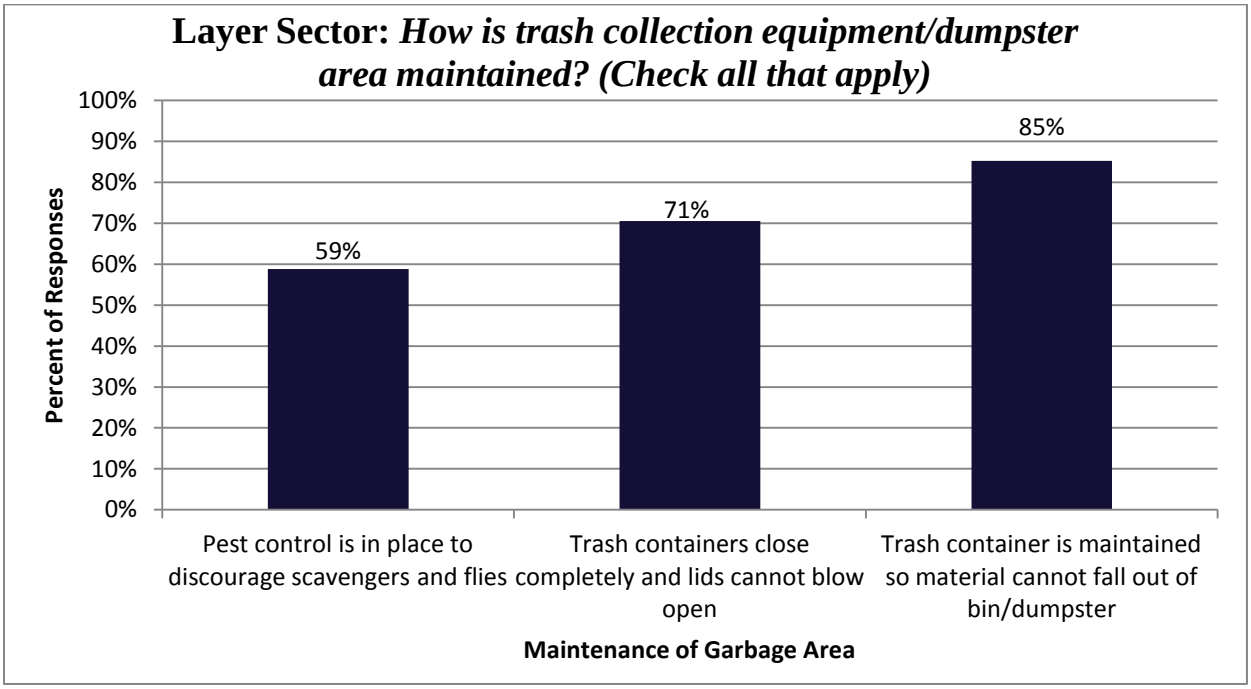
Appendix 9 Figure 12. Responses of layer industry representatives (n=39) regarding the location of the dumpster or trash collection point on the premises



Appendix 9 Figure 13. Responses of poultry industry representatives regarding the distance of the dumpster or trash collection point from the nearest poultry barn (layer sector: n=36; broiler sector: n=7; turkey sector: n=14). In the survey of layer industry representatives, it was specified that the nearest poultry barn may be on the same premises or neighboring premises.



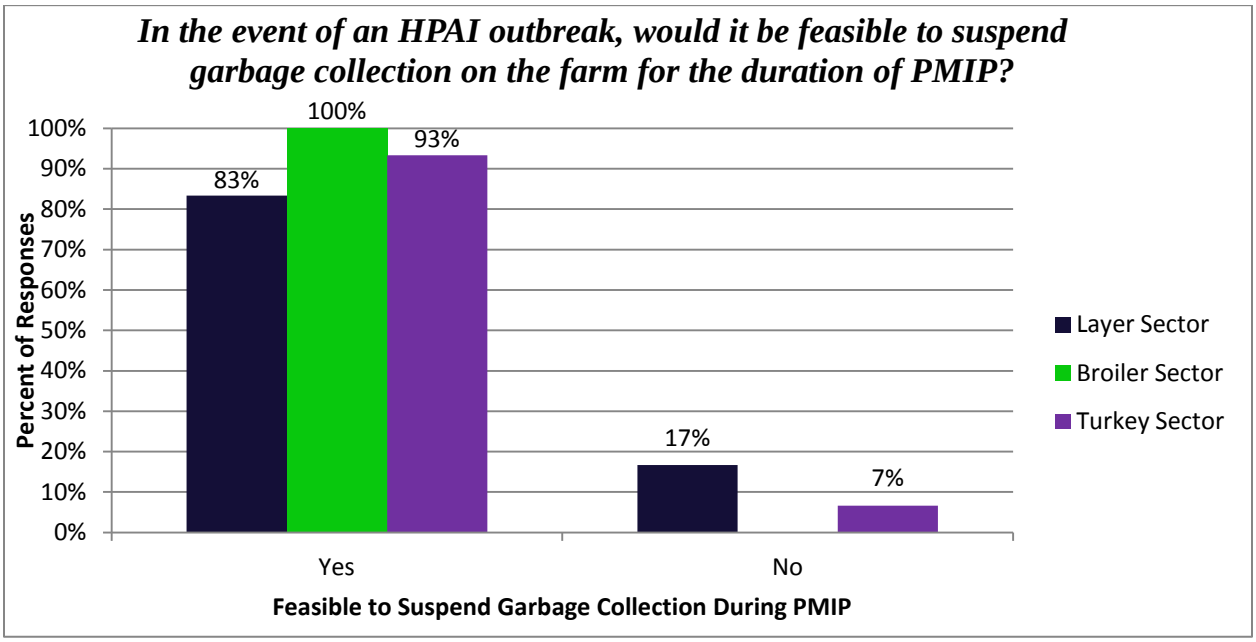
Appendix 9 Figure 14. Responses of poultry industry representatives regarding the distance of garbage collection from the rendering area (layer sector: n=36; broiler sector: n=3; turkey sector: n=7). A majority (75%) of layer industry respondents indicated no rendering is used (not shown).



Appendix 9 Figure 15. Responses of layer industry representatives (n=34) concerning maintenance of the trash collection equipment/dumpster area

Feasibility of Suspending Garbage Collection during PMIP

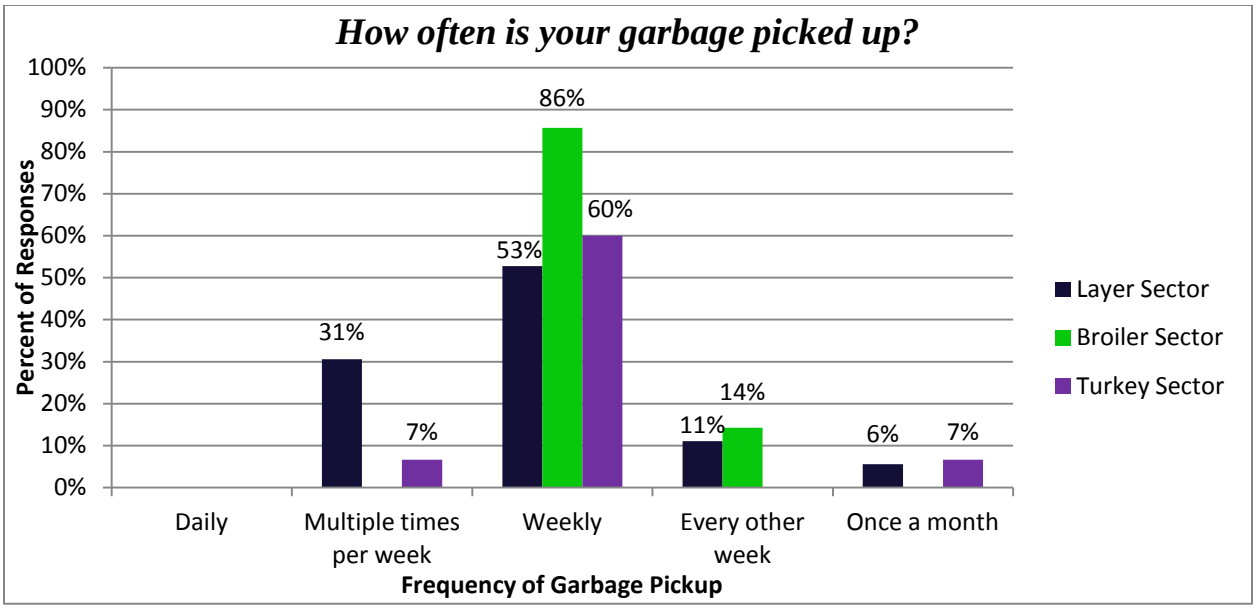
Premises that wish to move product out of a Control Area during an HPAI outbreak likely will need to observe a Pre-Movement Isolation Period (PMIP), during which no non-critical operations (including off-site disposal of poultry mortality and garbage) are allowed. **Appendix 9 Figure 16** shows survey results related to the feasibility of suspending garbage collection during PMIP. The duration of PMIP may vary by industry sector and type of movement requested. In addition to the results shown, respondents indicated their answers may depend on the size of the farm and the duration of PMIP. For suspension of garbage services to be feasible, some respondents noted they would need more on-site trash storage.



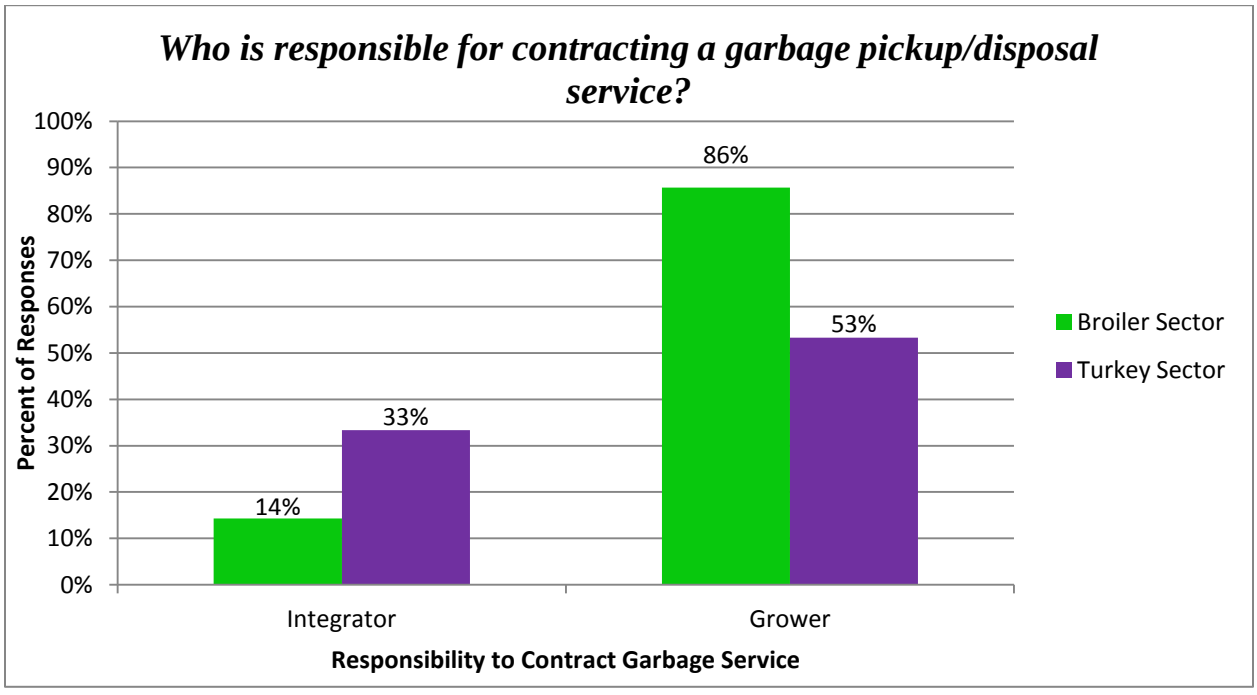
Appendix 9 Figure 16. Responses of poultry industry representatives concerning the feasibility of suspending garbage collection on the farm during PMIP (layer sector: n=36; broiler sector: n=7; turkey sector: n=15).

Frequency of Garbage Pickup and Responsibility of Contracting Garbage Service

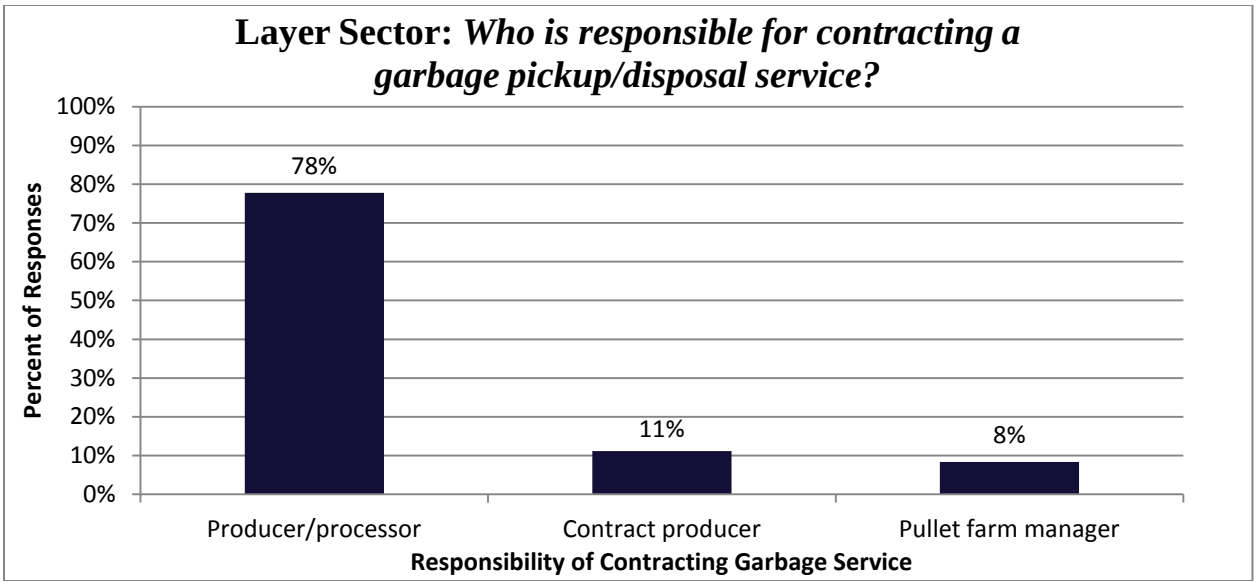
The person responsible for contracting third-party garbage hauling services and the frequency of garbage collection on a poultry premises may vary by farm size and type of operation and are not uniform across the poultry industry. **Appendix 9 Figures 17-19** show survey results related to frequency of garbage pickup and responsibility of contracting garbage service.



Appendix 9 Figure 17. Responses of poultry industry representatives regarding the frequency of garbage pickup (layer sector: n=36; broiler sector: n=7; turkey sector: n=15). Turkey industry respondents wrote in that frequency of garbage pickup may depend on the size of the farm and on some premises it is not pre-scheduled but occurs as needed.



Appendix 9 Figure 18. Responses of broiler and turkey industry representatives concerning the responsibility for contracting garbage pickup/disposal service (broiler sector: n=7; turkey sector: n=15).



Appendix 9 Figure 19. Responses of layer industry representatives regarding the responsibility of contracting garbage pickup/disposal service (layer sector: n=36). Answer choices presented in the layer sector survey were modified from those presented to broiler and turkey representatives to better align with industry practices.

Appendix 10: Supplementary Testing Protocols

Likelihood of Detecting HPAI in a Turkey Flock Prior to Movement: Protocols for Premises with rRT-PCR Test Result Turnarounds Greater than 12 Hours

The protocol evaluated in the main risk assessment document involving samples of 11 swabs taken for rRT-PCR the day of and day before the start of load-out assumes the turnaround time needed to obtain results from NAHLN labs is less than 12 hours. As a same-day turnaround is not always feasible, an additional protocol is evaluated in **Appendix 10 Table 1** consisting of one sample of 11 swabs taken for rRT-PCR testing 1 and 2 days prior to the start of load-out to allow more time for sending and receiving the test results. Earlier sampling for rRT-PCR generally results in lower detection probabilities because the infection has less time to spread through the flock. Supplementary AC testing performed immediately prior to load-out can be employed to offset the loss in detection probability from earlier rRT-PCR sampling.

To estimate the effect of AC testing on the detection probability, a third protocol was evaluated consisting of one sample of 11 swabs taken for rRT-PCR 1 and 2 days prior to movement with the addition of two samples of five swabs each taken for AC testing at the same time immediately prior to the start of load-out. The detection probabilities, and mean with the 5^{th} and 95^{th} percentiles of the number of infectious birds present in an undetected flock at the time of movement, are given in **Appendix 10 Table 1,** under the assumption that exposure occurred between 8 and 12 days prior to movement due to a 100% effective 8-day PMIP. The estimates are obtained from 6000 iterations of the simulation model.

As expected, the protocol with the earlier rRT-PCR sampling times is estimated to have lower detection probabilities than the baseline protocol of one rRT-PCR sample of 11 swabs taken the day of and day before movement. Furthermore, the mean number of infectious and undetected birds is lower under the baseline protocol, making it clearly preferable to the early rRT-PCR sampling protocol. The results show that supplementary AC testing in addition to the rRT-PCR samples taken 1 and 2 days prior to movement can counteract some of the drawbacks of earlier testing, because detection probabilities are at least as high as under the baseline active surveillance protocol for both isolates. Since the mean number of infectious and undetected birds is no lower than under the baseline active surveillance protocol and in the case of the turkey isolate scenario is higher, the baseline remains the optimal testing strategy. However, the earlier rRT-PCR sampling with supplementary AC testing represents an effective approach when the turnaround time for rRT-PCR results exceeds 12 hours.

Appendix 10 Table 1. Likelihood of AI detection and mean number of infectious undetected birds for two EA/AM HPAI H5N2 isolates and three different active surveillance protocols. A 100% effective 8-day PMIP is assumed to have been implemented.

Strain	Active surveillance protocol[a]		
	One sample rRT-PCR taken day of and day before movement	**One sample rRT-PCR taken 1 and 2 days before movement**	**One sample rRT-PCR taken 1 and 2 days before movement with supplementary AC testing[b]**
	Predicted detection probability[c] **Mean number of infected undetected birds (5th, 95th percentile)**		
EA/AM HPAI H5N2: Turkey isolate	0.99 258 (25, 627)	0.98 371 (40, 1192)	0.99 274 (24, 864)
EA/AM HPAI H5N2: Chicken isolate	1.00 0 (0, 0)	0.99 13 (0, 53)	1.00 0 (0, 0)

[a]Samples taken for rRT-PCR testing consist of 11 swabs.
bThe supplementary AC testing consists of two pools each with five swabs taken at the same time immediately prior to the start of load-out.
[c]Probabilities are estimated from 6,000 simulation iterations.

Likelihood of Moving Infectious and Undetected Turkeys Following Exposure during Load-out: Evaluating the Effect of Supplementary AC Testing

Exposures to HPAI occurring during the load-out process in houses to be loaded out are difficult to detect as they typically occur close to the time of movement. Supplementary AC testing can be used to improve the likelihood of detection since the samples can be taken immediately prior to movement, allowing greater time for HPAI to move through the flock prior to testing. **Appendix 10 Table 2** compares the detection probabilities for the baseline protocol of daily rRT-PCR testing of 11 swabs with a protocol consisting of two samples of five swabs taken for AC testing immediately prior to movement in addition to the daily rRT-PCR sampling. The probabilities are estimated for a single turkey tom house from 6,000 simulation iterations using the EA/AM HPAI H5N2 turkey isolate strain and considering different numbers of days post exposure to HPAI and levels of contamination on the load-out crew and equipment.

The results suggest supplementary AC testing can in some cases drastically increase the probability of detection. The largest gain of 33% is observed in the case of 100 birds initially infected 2 days prior to movement. Other gains of at least 10% are observed 3 days post-infection in the scenario with 10 birds initially infected and 5 days post-infection with one bird initially infected. In addition to increasing the likelihood of detection, the supplementary AC testing would be expected to decrease the mean number of infectious but undetected birds at the time of movement. Thus, additional AC testing can reduce the overall risk of HPAI spread resulting from exposure during load-out.

Appendix 10 Table 2. The likelihood of AI detection for 2 to 10 days following exposure using two different surveillance protocols

		Days post-exposure								
Initial number of birds infected[a]	**Protocol[b]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
		Predicted detection probability[c]								
1	rRT-PCR testing only	0.15	0.23	0.37	0.59	0.80	0.92	0.98	1.00	1.00
	rRT-PCR with supplementary AC testing	0.15	0.26	0.46	0.69	0.87	0.95	0.99	1.00	1.00
10	rRT-PCR testing only	0.17	0.46	0.85	0.98	1.00	1.00	1.00	1.00	1.00
	rRT-PCR with supplementary AC testing	0.26	0.66	0.93	0.99	1.00	1.00	1.00	1.00	1.00
100	rRT-PCR testing only	0.37	0.92	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	rRT-PCR with supplementary AC testing	0.70	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00

[a]The initial number of infected birds is meant to represent the level of contamination on the load-out personnel and equipment.
[b]The rRT-PCR testing consists of daily samples of 11 swabs. The AC testing consists of two pooled samples of five swabs taken immediately prior to movement.
[c]Probabilities were estimated from 6,000 simulation iterations using the EA/AM HPAI H5N2 turkey isolate strain.

Likelihood of at least 100, 500, or 1,000 Infectious but Undetected Turkeys in a Flock at the Time of Movement following Exposure during Load-out

The likelihood of at least 300 infectious but undetected turkeys in a house at the time of movement following exposure to HPAI during load-out was used in section 9.4.3: Likelihood of Moving Infectious but Undetected Turkeys Following Exposure during Load-out to demonstrate that despite lower probabilities of detection, the likelihood of releasing large numbers of infectious but undetected birds is predicted to be low. Similar probabilities are provided in **Appendix 10 Tables 3-5** for the cases where at least 100, 500, and 1000 infectious but undetected turkey toms are present in a flock at the time of movement. **Appendix 10 Table 5** provides evidence that the likelihood of releasing 1000 or more infectious turkeys is negligible. Similarly, **Appendix 10 Table 4** suggests the likelihood of releasing 500 or more infectious turkeys would generally be quite low, though there is estimated to be a fair amount of risk when crews and equipment are heavily contaminated, as indicated by the 6.20% probability of releasing 500 or more infectious turkeys if 100 birds were initially infected and exposure occurred 3 days before movement. The likelihood of moving at least 100 infectious but

undetected turkeys to processing, on the other hand, consistently reaches nontrivial levels, especially 2 days post-exposure under the scenario with 100 initially infected birds. These results in **Appendix 10 Table 3** reiterate the possible risk related to exposure during load-out and the importance of reducing contamination on the crews and equipment as well as ensuring the practice of the suggested biosecurity measures during the load-out period. Supplementary AC testing performed immediately prior to movement is an additional measure that could be employed to reduce the likelihood of releasing at least 100 infectious but undetected birds to processing.

Appendix 10 Table 3. The estimated percent probability of not detecting HPAI in a house following exposure during load-out where the number of infectious but undetected turkey toms at the time of movement exceeds 100 birds

Initial number of birds infected[a]	Days post-exposure								
	2	3	4	5	6	7	8	9	10
	Predicted percent probability of at least 100 infectious but undetected turkey toms at the time of movement[b]								
1	0.00%	0.00%	1.13%	4.40%	4.23%	2.85%	1.10%	0.23%	0.05%
10	0.02%	7.42%	12.6%	2.28%	0.20%	0.00%	0.00%	0.00%	0.00%
100	63.5%	8.33%	0.32%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

[a]The initial number of birds infected is a proxy for the level of contamination present on the load-out crew and equipment.
[b]Probabilities are estimated from 6,000 simulation using the EA/AM HPAI H5N2 turkey isolate strain and an active surveillance protocol of one sample of 11 swabs taken daily for rRT-PCR testing.

Appendix 10 Table 4. The estimated percent probability of not detecting HPAI in a house following exposure during load-out where the number of infectious but undetected turkey toms at the time of movement exceeds 500 birds

Initial number of birds infected[a]	Days post-exposure								
	2	3	4	5	6	7	8	9	10
	Predicted percent probability of at least 500 infectious but undetected turkey toms at the time of movement[b]								
1	0.00%	0.00%	0.00%	0.12%	0.28%	0.28%	0.17%	0.02%	0.00%
10	0.00%	0.00%	0.60%	1.02%	0.18%	0.00%	0.00%	0.00%	0.00%
100	0.05%	6.20%	0.32%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

[a]The initial number of birds infected is a proxy for the level of contamination present on the load-out crew and equipment.
[b]Probabilities are estimated from 6,000 simulation using the EA/AM HPAI H5N2 turkey isolate strain and an active surveillance protocol of one sample of 11 swabs taken daily for rRT-PCR testing.

Appendix 10 Table 5. The estimated percent probability of not detecting HPAI in a flock following exposure during load-out where the number of infectious but undetected turkey toms at the time of movement exceeds 1000 birds

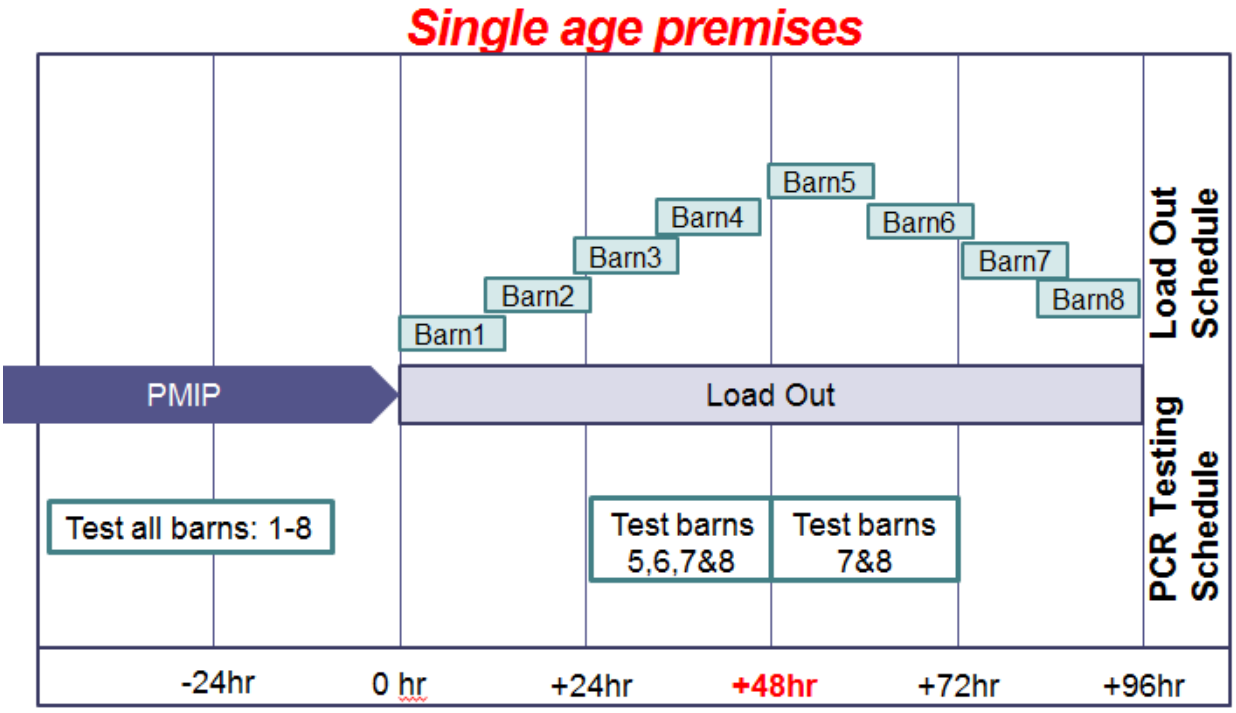
Initial number of birds infected[a]	Days post-exposure								
	2	3	4	5	6	7	8	9	10
	Predicted percent probability of at least 1000 infectious but undetected turkey toms at the time of movement[b]								
1	0.00%	0.00%	0.00%	0.02%	0.03%	0.07%	0.07%	0.02%	0.00%
10	0.00%	0.00%	0.08%	0.27%	0.07%	0.00%	0.00%	0.00%	0.00%
100	0.00%	0.63%	0.28%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

[a]The initial number of birds infected is a proxy for the level of contamination present on the load-out crew and equipment.
[b]Probabilities are estimated from 6,000 simulation using the EA/AM HPAI H5N2 turkey isolate strain and an active surveillance protocol of one sample of 11 swabs taken daily for rRT-PCR testing.

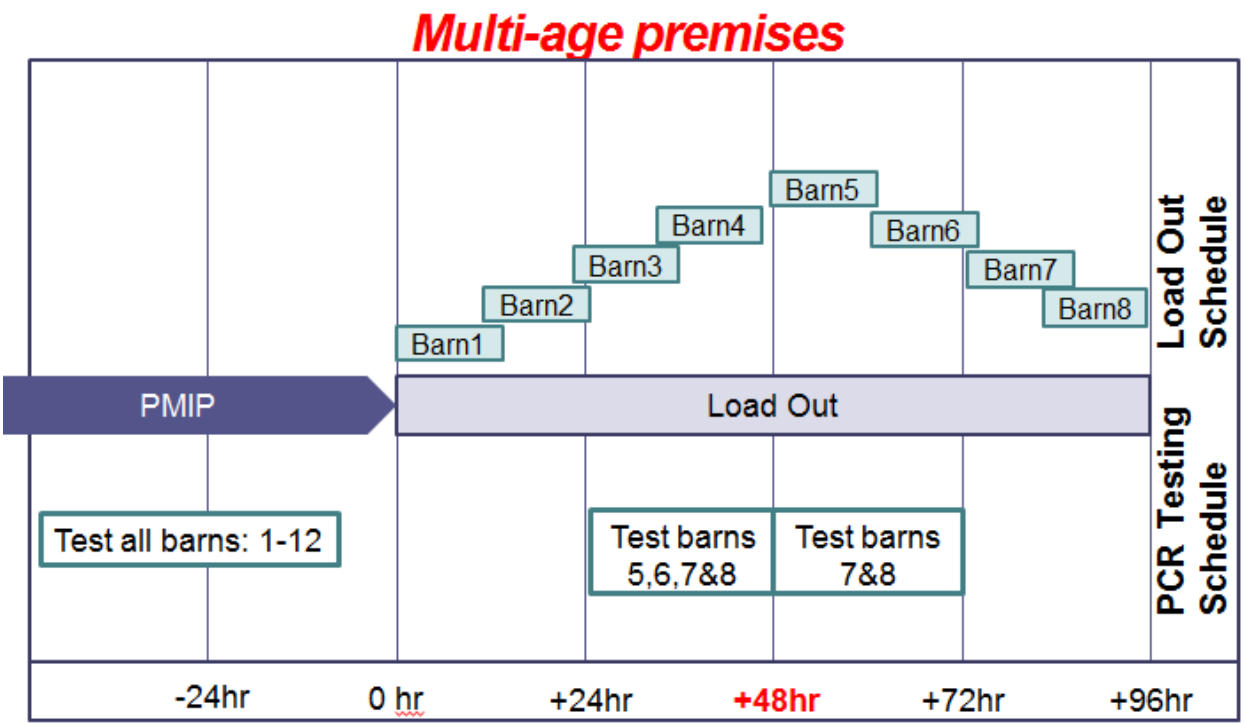
Appendix 11: Load-out Mitigation Measures

Due to the increased risk associated with extended load-out durations (demonstrated by simulation modeling), additional biosecurity measures are required for multiple-day load-outs at the premises level. Additional biosecurity and mitigation measures are summarized below:

1. Additional barn-to-barn biosecurity must be implemented. The following PMIP measures must be continued throughout the load-out process:
    - Pre-staging of equipment in a barn prior to beginning load-out in that barn is not allowed.
    - All persons entering a barn must use *barn-specific* footwear and *farm-specific* clothing.
2. For all barns loading out after the initial 48 hours of load-out on a premises, daily PCR testing is required.
    - Negative results of tests taken within 24 hours of scheduled movement (at barn level) must be documented before birds depart premises. (This is diagrammed in **Appendix 11 Figure 1** for single-age premises and **Appendix 11 Figure 2** for multi-age premises.)



Appendix 11 Figure 1. Daily testing is required of birds in all barns that are not loaded out within 48 hours of the start of premises-level load-out. In this example, there are eight barns on a single-age premises and complete depopulation of the premises takes 96 hours.



Appendix 11 Figure 2. Daily testing is required of birds in all barns that are not loaded out within 48 hours of the start of premises-level load-out. In this example, barns 1-8 are market-age turkeys, and barns 9-12 are 12-week-old turkeys. Depopulation of the market-age birds takes 96 hours.

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