United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Science, Technology, and Analysis Services

Center for Epidemiology and Animal Health

2150 Centre Avenue Building B
Fort Collins, CO
80526

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

August 2014 FIRST REVIEW

January 2015 FINAL REVIEW AND CLEARANCE

Collaboration between the Egg Sector Working Group, the University of Minnesota's Center for Animal Health and Food Safety, and USDA:APHIS:VS:STAS:CEAH.

Veterinary Services
Safeguarding Animal Health

University of Minnesota
Center for Animal Health and Food Safety

Suggested bibliographic citation for this report:

Timothy Goldsmith, Carie Alexander, David Halvorson, Sasidhar Malladi, Timothy Snider, Shauna Voss, Todd Weaver, Jamie Umber. **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.** Collaborative agreement between USDA:APHIS:VS:CEAH and University of Minnesota Center for Animal Health and Food Safety, Fort Collins, CO. January 2015. 142 pgs. Retrieved from the University of Minnesota Digital Conservancy: https://hdl.handle.net/11299/176192

This document was developed through the Continuity of Business / Secure Food Supply Plans / Secure Poultry Supply project initiative. Related documents can be found at: https://www.aphis.usda.gov - search for "Continuity of Business"

Contributors
Timothy Goldsmith, Assistant Professor, University of Minnesota (UMN)
Carie Alexander, Veterinary Public Health and Preventive Medicine Resident, UMN
David Halvorson, Professor Emeritus, UMN
Sasidhar Malladi, Risk Analyst, UMN
Timothy Snider, Veterinary Public Health and Preventive Medicine Resident, UMN
Shauna Voss, Veterinary Public Health and Preventive Medicine Resident, UMN
Todd Weaver, Epidemiologist, USDA:APHIS:VS:CEAH
Jamie Umber, Risk Analyst, UMN

Acknowledgement:

We thank the Turkey Sector Working Group for their support during the development of this risk assessment.

Keywords: avian influenza, continuity of business, HPAI, market, outbreak, risk, turkey, poultry

Questions or comments on data analysis, contact: Timothy Goldsmith, DVM, MPH, University of Minnesota College of Veterinary Medicine, Secure Food Systems, 612-625-0883, Email: gold0188@umn.edu

Table of Contents

1. Abbreviations and Definitions

APHIS	Animal and Plant Health Inspection Service (USDA)
AI	Avian influenza
CEAH	Centers for Epidemiology and Animal Health (USDA: APHIS: VS)
CFR	U.S. Code of Federal Regulations
C&D	Cleaning and Disinfection
EPA	U.S. Environmental Protection Agency
GMP	Good Manufacturing Practice
HA	Hemagglutinin
HPAI	Highly pathogenic avian influenza
HPNAI	Highly pathogenic notifiable avian influenza
LPAI	Low pathogenic avian influenza
NA	Neuraminidase
NAHEMS	National Animal Health Emergency Management System (USDA)
NPIP	National Poultry Improvement Plan
OIE	World Organization for Animal Health (formerly Office International des Epizooties)
P.I.	Probability Interval
PPE	Personal protective equipment
RRT-PCR	Real-time reverse transcription polymerase chain reaction
U.S.	United States of America
USDA	United States Department of Agriculture
VS	Veterinary Services (USDA: APHIS)

Batching

Filling setters with eggs that are close to the same maternal age, egg size and storage time in order to keep each batch with similar characteristics. This minimizes the variability in the hatch window, which is the amount of time from the hatching of the first to the last chick in a batch.

Biosecurity

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

Breeder farm

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

Buffer zone

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

Control Area

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

Dirty egg

A dirty egg or dirties are egg(s) that have an unbroken shell with adhering dirt or foreign material.

Egg

The hatching egg of domesticated breeder turkeys. Hatching eggs of chickens, ducks, geese, and guineas are outside the scope of this assessment.

Egg handling materials

Handling materials used in the transport and storage of hatching eggs such as plastic flats, pallets, buggies, setter trays, etc.

EID_{50}

50 percent embryo infectious dose, or dose at which 50 percent of inoculated embryos become infected.

Hatchery

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

Hatching egg

A fertilized egg produced by breeding birds. Day old poults hatched from hatching eggs may be used for commercial turkey production or to supply primary or multiplier breeding flocks.

Incident Command System

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.

Incident Command

An on-scene management function that supports incident response.

Infected zone

In an outbreak of HPAI, an infected zone will be established that 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 nonsusceptible animals out of the zone unless appropriate biosecurity procedures (as determined by a risk assessment) are followed.

Active surveillance for HPAI of presumptive, confirmed infected, and contact premises.

Infectious Period

The period of time that an individual bird is infectious (i.e. shedding HPAI virus at sufficient levels that could result in transmission 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.

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 hatching eggs from a premises or a geographic area described in a quarantine order.

National Poultry Improvement Plan (NPIP)

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.

Poult

A young turkey.

Permit Team

A team within the Incident Command that grants permits for the authorized movement of products or livestock from premises under quarantine within or out of the Control Area.

Secure Turkey Supply Plan

Contains science based outbreak measures developed by the Turkey Sector Working Group to mitigate the risk of HPAI spread.

Setter

An incubator used for hatching eggs.

Turkey Sector Working Group

A stakeholder work group consisting of turkey industry veterinarians, university extension veterinarians with expertise in the turkey industry, state and APHIS regulatory veterinarians, and representatives from NPIP and others involved in business continuity in the turkey industry.

Mention of companies or commercial products does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned. USDA neither guarantees nor warrants the standard of any product mentioned. Product names are mentioned solely to report factually on available data and to provide specific information.

2. Executive Summary

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. 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 turkey hatching eggs, during an HPAI outbreak, from a breeder farm, located within the Control Area, will result in HPAI virus spread to day old poults in a turkey hatchery or to other susceptible turkey breeder flocks.

This risk assessment is a joint effort between the Turkey Sector Working Group, the University of Minnesota's Center for Animal Health and Food Safety, and the United States Department of Agriculture (USDA) to support permits for moving turkey hatching eggs and associated handling materials during an HPAI outbreak. This assessment is applicable to commercial turkey hatcheries that participate in the USDA: APHIS National Poultry Improvement Plan (NPIP) and follow the Secure Turkey Supply Plan (STS Plan) in the event of an HPAI outbreak. The STS Plan contains science based outbreak measures to mitigate the risk of HPAI spread associated with the movement of hatching eggs. Measures included in the STS Plan were initially developed with input from members of the Turkey Sector Working Group (TSWG). The final list of measures is updated based on the risk evaluation for movement of hatching eggs. This risk assessment also considers applicable current industry practices and biosecurity measures (NPIP) as well as outbreak specific measures from the STS Plan. The main categories of outbreak specific measures from the STS Plan considered include:

- Active surveillance of turkey breeder hen and tom flocks by RRT-PCR testing, detection of abnormally high mortality, or a significant drop in egg production rate, before hatching eggs will be allowed to move from a breeder farm to a hatchery, in combination with a 2-day on-farm holding period.
- The STS Plan requires that turkey breeder hens and toms producing fertile hatching eggs must test negative for avian influenza matrix genes by RRT-PCR before hatching eggs will be allowed to move. One 5-bird pooled sample must be tested and found to be negative from every house on the premises for two consecutive days prior to movement of turkey hatching eggs. If there are fewer than 5 dead turkeys in the house, the remainder of the samples should be taken from sick turkeys.
- On farm biosecurity measures such as washing and sanitization of hatching eggs and biosecurity measures for farm personnel to include hand washing and special footwear.
- Reduction in the frequency of semen movement from twice weekly to once weekly.
- Modifications to workflow practices at the hatchery during an outbreak.

- Cleaning and disinfection of vehicles and biosecurity measures for the vehicle driver delivering hatching eggs and egg-handling materials.

The entry assessment section evaluates the likelihood of HPAI virus being transmitted onto a virus free turkey hatchery premises through pathways associated with the movement of turkey hatching eggs from breeder hen flocks located in the Control Area. For the entry assessment, we considered scenarios where either breeder hen or breeder tom flocks first become infected prior to the movement of hatching eggs, for two different HPAI virus strains (Asian H5N1 HPAI and a strain with a longer infectious period). Based on simulation model results for the two scenarios where hen flocks are first infected, the predicted mean number of internally contaminated hatching eggs moved from a HPAI infected but undetected flock was estimated to be very low. Multiple factors such as fewer birds per breeder house than in meat-type turkey houses and correspondingly lower normal house mortality; earlier detection with active surveillance RRT-PCR testing; and the 2-day holding time before the movement of eggs contributed towards the low predicted number of potentially contaminated eggs moved.

There is a possibility of transmission to breeder hen flocks via semen movement, if a breeder tom flock becomes infected with HPAI virus. In this case, multiple breeder hens could be simultaneously exposed through contaminated semen or the insemination crew, resulting in significantly different disease spread dynamics. In the scenarios where breeder tom flocks were first infected, HPAI disease is likely detected either in the breeder tom or hen flocks by the time contaminated hatching eggs may be moved from the hen flock. The model predicted number of internally or externally contaminated hatching eggs moved from a turkey breeder house given a 2-day holding time after production was very low in these scenarios as well.

The degree of external contamination of eggs moved from an infected breeder house is expected to be low on nest clean eggs under all the scenarios considered, due to sanitizing with an EPA registered disinfectant or chlorine rinse with the concentration equal to or greater than 200 ppm. The predicted number of eggs that were externally contaminated prior to sanitizing per movement was low under most scenarios.

The likelihood and degree of contamination of egg-handling materials would be reduced due to early detection with active surveillance and the correspondingly lower proportion of infectious birds before HPAI is detected. In addition, other on-farm measures from the STS Plan such as disinfection of the egg buggy wheels and change of footwear before entering the egg storage room floor further reduce the chance of movement of contaminated handling materials. The likelihood of setter (incubator) trays and buggies, moved from the premises being contaminated with HPAI virus was rated to be *negligible* t*o low*, provided that the outbreak measures from the STS Plan are strictly followed. The likelihood of entry of HPAI virus into the hatchery, via the vehicle or driver transporting hatching eggs was estimated to be *negligible* to *low.*

The exposure assessment evaluated the risk of susceptible poultry becoming infected with HPAI virus associated with the movement of hatching eggs from breeder flocks in the Control Area. The risk of day-old poults becoming infected with HPAI virus from hatching eggs and egg-handling materials via movements of equipment or personnel at the hatchery was estimated to be *negligible* to *low*, provided that the preventive measures

from the STS Plan are strictly implemented. Finally, we evaluated the risk of the movement of egg-handling materials from the hatchery resulting in HPAI spread to a susceptible breeder flock. The primary risk pathway for this risk event is through cross-contamination of vehicles, personnel, or egg-handling materials destined to breeder flocks by incoming vehicles, personnel or egg-handling materials—originating from HPAI infected turkey breeder farms. Provided the preventive measures specified in the STS Plan are strictly followed, we concluded:

- The risk of a susceptible breeder flock becoming infected with HPAI virus from the contamination on the exterior of the vehicle or driver transporting cleaned and disinfected egg-handling materials from the hatchery to a breeder farm is *negligible*.
- The risk of a susceptible breeder flock becoming infected with HPAI virus due to cross contamination of cleaned and disinfected egg handling materials from the hatchery to a breeder farm is *negligible* to *low*.

This assessment aids, but does not replace, the judgment of on-scene officials. This 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 turkey hatching eggs.

Overall Finding and Conclusion

The risk that movement of turkey hatching eggs into, within, and out of a control area during an HPAI outbreak results in the infection of susceptible poultry is *negligible* to *low*, provided that applicable preventive measures from NPIP regulations 9CFR145 and 9CFR147 and the STS Plan are strictly followed.

3. Introduction

In the event of a 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 that will utilize depopulation, quarantine and movement control measures to prevent further spread of HPAI virus. State and/or Federal authorities will also issue official permits to allow movement of birds and their products from premises identified in a quarantine order during an outbreak. 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.

Completing these types of risk assessments in a timely manner during an outbreak can be challenging. Turkey hatcheries have limited holding capacity for hatching eggs and extended movement restrictions may result in the loss of the value of hatching eggs. 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.

The purpose of this assessment is to: (1) identify plausible risk pathways for the spread of HPAI infection through the movements of turkey hatching eggs and associated handling materials; and (2) to assess the corresponding likelihoods of spread of HPAI onto another poultry premises (e.g. turkey breeder operation), given all current and future preventive measures that will be in place during an outbreak.

There are two types of movements between turkey breeder hen flocks and turkey hatcheries that were considered in this assessment:

- Movement of turkey hatching eggs from a breeder hen flock to a commercial hatchery.
- Movement of hatching egg handling materials from a hatchery to a breeder hen flock.

The production of hatching eggs is dependent on the movement of semen from breeder tom flocks. The risk of movement of semen is not addressed directly but only in the context of exposure of hens to contaminated semen and moving potentially contaminated hatching eggs.

Current industry practices and biosecurity measures as well as outbreak specific measures from the STS Plan that are applicable to the movement of hatching eggs were considered in the overall risk evaluation. The current biosecurity measures considered include guidelines followed by producers and hatcheries participating in the NPIP (9CFR145 and 9CFR147). Categories of outbreak specific measures from the STS Plan considered here include:

- Active surveillance of turkey breeder hen and tom flocks using RRT-PCR testing; detection of abnormally high mortality, or a significant drop in egg production rate, in combination with a 2-day on-farm holding period before hatching eggs will be allowed to move from a breeder farm to a hatchery.

- On farm biosecurity measures such as washing and sanitization of hatching eggs and biosecurity measures for farm personnel to include hand washing and footwear protocols.
- Reduction in the frequency of semen movement from breeder tom flocks from twice weekly to once weekly.
- Modifications to workflow practices at the hatchery.
- Cleaning and disinfection of vehicles and biosecurity measures for the vehicle driver delivering hatching eggs and egg-handling materials.

The risk evaluation was performed in two parts: an entry assessment and an exposure assessment. The entry assessment section evaluates the likelihood of HPAI virus being transmitted onto a virus free turkey hatchery premises through the movement of contaminated turkey hatching eggs, egg-handling equipment, or the delivery truck or driver from a turkey breeder hen flock located in the Control Area. The entry assessment considers current industry practices as well as outbreak specific preventive measures described in the STS Plan. Stochastic simulation models of within-flock HPAI disease spread, in conjunction with models of the active surveillance protocols from the STS Plan, were used to estimate the likelihood of moving potentially contaminated hatching eggs from a breeder premises prior to disease detection. Sanitization of hatching eggs and other farm personnel biosecurity steps were also considered in the evaluation of the likelihood of moving externally contaminated hatching eggs and egg handling materials.

Outbreak scenarios where turkey breeder tom flocks become infected with HPAI virus prior to the collection and movement of turkey semen were also evaluated in the analysis. The movement of turkey semen from tom to hen flocks has been implicated in the spread of avian influenza in past outbreaks. The management of tom flocks and collection, handling, and movement of semen are considered to be component steps in the production of fertile turkey hatching eggs. We evaluated the impact of active surveillance measures included in the STS Plan in both tom and hen flocks in reducing the risk of movement of potentially contaminated hatching eggs to the hatchery.

The impact of HPAI virus strain variation was also considered by performing the analysis for two different virus strains. Compared with Asian H5N1 HPAI virus, strains of HPAI virus with longer infectious periods can potentially lengthen the time to disease detection within a flock, increasing the number of infectious birds present before movement. Multiple detection mechanisms in addition to active surveillance testing were also considered in the analysis, such as an increase in flock mortality above a detection threshold in both hen and tom flocks, as well as a drop in the egg production rate over two consecutive days.

The exposure assessment evaluates the risk of susceptible poultry becoming infected with HPAI virus associated with the movement of hatching eggs. Specifically, we evaluated the following pathways for exposure of susceptible poultry:

- Exposure of day old poults via movements of equipment or personnel at the hatchery.

- Exposure of susceptible breeder flocks via the vehicle, driver or egg-handling materials moved from the hatchery.

The results from the entry assessment, hatchery workflow and biosecurity practices as well as outbreak measures from the STS Plan were considered in the exposure assessment.

This assessment aids, but does not replace, the judgment of on-scene officials. This 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 turkey hatching eggs.

4. Background & Industry Characterization: Turkey Hatching Egg Production

4.1 Definition of Hatching Eggs and Hatcheries

A hatching egg is a fertilized egg produced by breeding birds. Unique to turkey egg production, hens are artificially inseminated to produce fertile eggs. Semen from breeder tom flocks is collected and supplied to breeder hen flocks on the same day the hens are inseminated. Breeder tom and breeder hen flocks may be located on the same or different sites. Hatching eggs are produced and stored at the breeder hen farm and transported off the premises to a hatchery one or more times per week (**Figure 1**).

A hatchery is an establishment that is dedicated to the hatching of eggs for the production of poults. Commercial hatcheries receive hatching eggs from off-site breeder farms; incubate and hatch the eggs; process the poults; and then ship the day-old poults to turkey brooder farms (**Figure 1**). 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 production.

This risk assessment focuses specifically on the production and movement of hatching eggs intended to supply the turkey industry with breeder and commercial turkeys.

4.2 Overview of Turkey Hatching Egg Production in the United States

4.2.1 Breeder Farms that Produce Hatching Eggs

Turkey hatching eggs are produced at breeder farms and then moved to a hatchery for incubation and hatching. Flocks maintained at breeder farms consist of either primary breeder flocks or multiplier breeder flocks (**Figure 2**).

(i) Primary Breeder Flocks

Primary breeder flocks are the genetic stock for the industry. They are high value foundation flocks that are maintained under much higher biosecurity than multiplier farms for the purpose of establishing, continuing, or improving parent lines. Such flocks are designated pedigree great-grandparent (GGP), and grandparent (GP) stock that ultimately generate eggs that will hatch into multiplier parent breeding flocks (P). There are two primary turkey breeder companies in North America; one is in Ontario, Canada, and the other in West Virginia, United States.

(ii) Multiplier Breeder Flocks

Multiplier breeder flocks produce hatching eggs used for producing commercial turkeys. 337 million turkey eggs were set in incubators in 2011. States reporting turkey hatcheries are: Arkansas, California, Florida, Idaho, Indiana, Iowa, Massachusetts, Michigan, Minnesota, Missouri, North Carolina, Ohio, Pennsylvania, Texas, Virginia, West Virginia, and Wisconsin.(1)

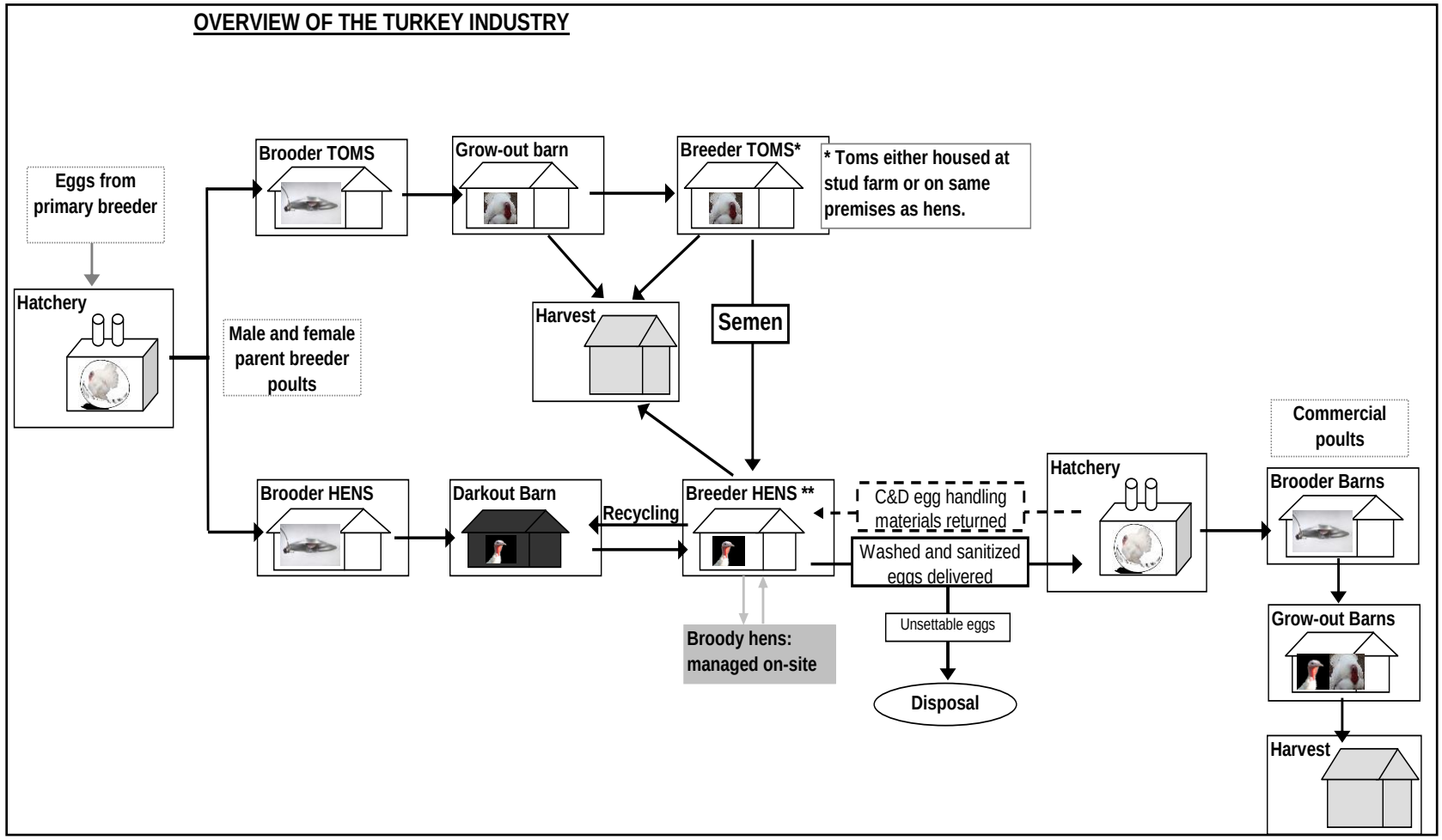


Figure 1.Turkey industry diagram.

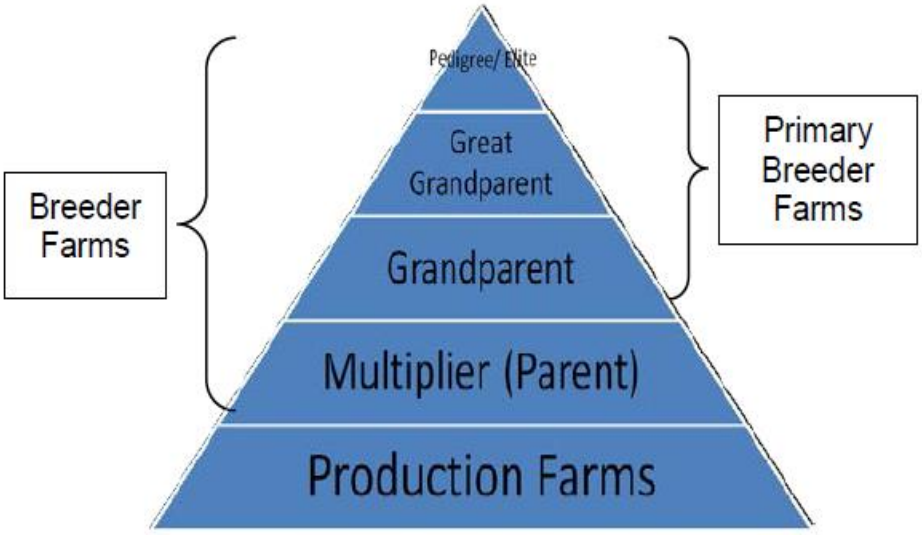


Figure 2 Structure of the turkey industry.

4.2.2 Hatchery Operations

Commercial hatcheries are typically located less than 100 miles distance from the breeder farm supplying hatching eggs. Commercial hatcheries may receive eggs from company-owned farms or may obtain eggs from breeder farms owned by a different company through contractual agreements. As of 2012 there were 49 turkey hatcheries in the United States with an approximate incubator capacity of 40 million hatching eggs. In 2011, 285 million poults were hatched in turkey hatcheries. The majority of turkey companies in the United States own hatcheries.(1) In some cases, day-old poults may be supplied to a different company's turkey farms.

4.2.3 Hatching Egg Distribution and Logistics

4.2.3.1 Hatching Egg Supply Chain Members

The supply chain members involved in the hatching egg process include breeder tom farms, breeder hen farms, and hatcheries as shown in **Figure 1**. During normal operations, hatching eggs at the breeder hen farms are inspected during the collection process to ensure they are clean and appropriate for hatching. Settable quality[a] eggs are cleaned and disinfected, stored on-farm for 1 to 2 days, and then transported to a hatchery. Dirty or defective (un-settable) eggs are disposed of on-farm. On occasion, excess hatching eggs may be sold and shipped to another hatchery.

[a] A settable quality egg is one that is not dirty, cracked, misshapen, checked, white shelled or sandy shelled.

4.2.3.2 Movement of Hatching Eggs and Egg-Handling Materials

Eggs are gathered in baskets or on trays and moved to an egg room on the same farm for sanitizing. Eggs are placed on plastic setter trays, or incubator racks, and then stored on metal buggies or carts in a cooler on the farm. At the time of shipping, the egg-laden metal buggies are loaded into a truck and delivered to the hatchery. During routine operations, the hatching egg transport vehicle may pick up eggs from multiple breeder farms on a single trip. Vehicles transporting hatching eggs to the hatchery also transport egg-handling materials back to the breeder farms. Most hatcheries, under normal operations, will transfer reusable, C&D egg-handling materials such as trays, flats, racks, dollies, or buggies to breeder premises within the same company without regard to farm of origin.

Typically, hatching egg handling materials are reusable and are returned to the breeder farm after cleaning and disinfection (C&D) at the hatchery. If eggs are sold externally to another commercial hatchery, they are placed on disposable paper or fiber flats, packed on single-use, new paper or fiber materials, packed in cases, and placed on pallets for storage and shipping. There is no circulation, or reuse, of materials used for these outside shipments.

4.3 Major Steps in the Production and Processing of Hatching Eggs During Routine Operations

4.3.1 On-Farm Operations

The main steps in hatching egg production, including transport of eggs to a hatchery, are as follows:

1. Semen collection, transport and delivery, and insemination
2. Maintenance of hen flocks
3. Egg production
4. Egg collection
5. Egg cleaning and on-farm storage
6. Egg pick-up and transportation to a hatchery

1. Semen collection, transport and delivery, and insemination

Breeder tom flocks are raised in separate houses, or barns, from breeder hens. Typically, there are 800 to1200 toms per house. Breeder tom houses may be located on the same site as breeder hens, or off-site on stud farms. If the tom flock is located on the same site, the same workers both collect semen and inseminate hens. If on different sites, there are separate teams of workers performing these tasks.

Semen is manually collected from a single breeder tom once or twice per week. Semen collection is scheduled to coincide with the hen insemination schedule, so that semen from different birds within a tom flock may be collected on different days. In turkeys, the semen volume averages 0.35 to 0.5 mL per tom, with a sperm count of 6 to 8 billion/ml.(2) Turkey semen is usually diluted with an extender and pooled together along with semen from several toms. The quality of the turkey semen may deteriorate

considerably beyond 6 hours of storage and transportation time. Turkey semen may be frozen but reduced fertility of frozen semen limits usage to special breeding projects (i.e. it is generally not used for commercial breeding).(2)

Semen is either delivered from off-site stud farms to the farm gate of the breeder hen flock or obtained from on-site breeder tom flocks. Hens are manually inseminated once per week. On the same farm, insemination may be undertaken in different barns each day.

2. Maintenance of hen flocks

Turkey breeder hens are transferred from a dark-out house to a breeder house at 28 to30 weeks of age when they begin producing eggs. According to industry experts, the number of breeder hens per house (flock) varies, depending on the company size and external sales requirements. The number of birds per house ranges from 2,000 to 5,000. Most breeder farms have all birds of the same age (all-in, all-out operations). Breeder hens are of single age within a breeder house. Breeder hens are raised in open floor houses with automatic watering and feeding systems

3. Egg production

Turkey breeder hens begin to produce eggs after light stimulation at 28 to 30 weeks of age and typically lay for 6 months. A single hen lays an average of 0.45 to 0.7 eggs per day, or approximately 75 to 117 eggs per laying cycle. In some instances, hens may be molted and go through another laying cycle.

4. Egg collection

Turkey hatching eggs are usually collected 3 to 4 times per day every day of the week. Frequent collection of hatching eggs is recommended in the National Poultry Improvement Plan (NPIP) regulations to reduce the incidence of floor-eggs and cracks, and resulting in cleaner eggs. On average, floor-eggs have significantly higher levels of bacterial contamination compared to nest-laid eggs.

Hatching eggs may be hand-collected using baskets or automatically collected using a mechanical nesting and conveyer belt system. Even with mechanical nests, some eggs may be laid on the breeder house floor (floor-eggs). These floor-eggs are gathered by hand and placed either in a collection basket or on the conveyer belt.

Eggs are manually transferred via cart or buggy several times per day from individual houses to the on-farm egg cleaning and storage facility. However, buggies or pallets used to transport eggs to the hatchery are not brought into the hen house.

5. Egg cleaning and on-farm storage

Most breeder hen sites sanitize hatching eggs following collection and prior to on-site storage and subsequent delivery to the hatchery. Some operations may have an egg washing step prior to sanitizing. For farms with multiple breeder flocks and barns in production, there is typically one common egg washing and storage facility on the premises.

Expert opinion elicited from Turkey Sector Working Group participants indicates most turkey breeder hen premises sanitize hatching eggs with a rinse, with some premises

washing the eggs with water (without detergent) before sanitizing. Eggs may be washed either mechanically or by hand. In mechanical systems, a conveyer moves eggs through a pressurized spray system that removes organic material. Some systems may also include a mechanized brush system to aid cleaning. Based on a summary of responses, common sanitizing agents included hypochlorite, quaternary ammonium compounds and hydrogen peroxide. After sanitizing, eggs are allowed to air dry.

In routine operations, carts used to carry setter trays into the hen house remain on the farm (i.e. they are separate from buggies used to transport setter trays to the hatchery). Depending on the type of egg washer/sanitizer used, some operations gather eggs onto setter trays so that trays and eggs are washed together. Other operations manually transfer eggs from baskets or flats to a belt washer, and then transfer washed eggs to setter trays. In this setup, setter trays would have been washed at the hatchery and distributed to the breeder farm.

For storage, the plastic setter trays and eggs are typically stacked on buggies or carts. Paper flats or fiberboard cases and pallets may be used in some instances when eggs are sold to external hatcheries. Most turkey breeder farms ship hatching eggs 3 or 4 times per week, depending on demand, storage capacity, and flock production levels. In most cases, egg storage buggies are parked in a climate controlled room adjacent to the loading area and are not moved until pick-up for delivery. Handling and movement is kept to a minimum in order to avoid damage and maximize quality. The eggs are stored at 65 to 70°F (18 to 21°C).

6. Egg pick-up and transportation to a hatchery

Hatching eggs are transported in specifically designed egg trucks with adequate ventilation to maintain appropriate temperature. The vehicles have equipment to hold egg buggies in place during transport. Prior to on-farm pick-up, it is common for egg trucks to undergo C&D (i.e. at the farm gate or off-site). C&D may include external (i.e. focus on undercarriage and wheels) and internal (i.e. trailer area and cab floor mats, etc.) disinfection. Some companies further require drivers to use personal protective equipment (PPE) on their premises. At the time of egg pick up, freshly C&D egg-handling materials, such as replacement carts and setter trays, are delivered from the hatchery to the farms.

At the time of pick-up, farm personnel typically move the egg-laden buggies, or carts, to the loading dock area. Prior to loading, some operations disinfect cart wheels by running the cart, or buggy, through a wheel bath. Typically, egg truck drivers will not enter the on-farm, egg storage area. In some instances, farm personnel may enter the trailer to assist the truck driver during loading. Depending on the design of the on-farm loading dock, carts may be either lifted into the truck with the use of a hydraulic lift, or rolled directly into the truck if the loading dock is elevated.

Once at the hatchery, eggs are unloaded and stored in the egg receiving room.

4.3.2 Processing of Hatching Eggs at Hatcheries

The steps in hatching egg processing from arrival at the hatchery to shipment of day-old poults are as follows (see **Appendix 8** for diagrams of hatchery layouts):

1. Egg arrival and storage at the hatchery
2. Incubation/Setting
3. Hatching
4. Poult processing
5. Poult storage and transportation

1. Arrival and storage at the hatchery

Turkey hatching eggs are typically 1 to 4 days old when they arrive at the hatchery. Prior to incubation, the eggs are held in the "egg room," which is maintained at 65 to 70°F (18 to 21°C) with a relative humidity of 75 to 80 percent. Eggs may be incubated directly after arrival or held in the egg room for up to seven to ten days before transferring to the setter room.

2. Incubation

Eggs are moved from the egg room to the incubation (setter)[b] room, where they are kept in a setter at a temperature of 98.6 to 100°F (37 to 39°C) and 55 to 70 percent relative humidity for 25 days. Eggs placed together in a setter or hatcher do not necessarily have the same history. Factors such as egg size, flock age, and storage time all have an effect on incubation time. Eggs are batched in setters, meaning that the setters are filled with eggs that are close to the same maternal age, egg size, and storage time to keep each batch with similar characteristics. Eggs of different ages may be present in different batches within a same setter. Hatchers however, are operated on an all-in, all-out basis in that eggs are placed in at the same time in a hatcher and removed at the same time after hatching. During this time the eggs may be visually inspected. Rarely, eggs may "explode" during incubation due to bacterial contamination. Exploding eggs are usually encountered a week or more into incubation. No embryo growth is expected in a contaminated egg before the embryo dies so there would be no amplification of HPAI virus in an egg before it explodes. In general, exploders are removed when the eggs are transferred from the setter to the hatcher, in order to prevent bacterial contamination of eggs before pipping. Setter trays are then cleaned and sanitized. Exploded eggs are discarded in hatchery waste. Setters take in air from the setter room and exhaust used air directly to the environment outside the hatchery.

3. Hatching

After 25 days of incubation, the eggs are moved to the hatcher room, transferred to hatcher trays and placed into hatchers. The hatchers are specialized incubating chambers which control temperature (95 to 99°F (35 to 37 °C)) and humidity (60 to 70 percent relative humidity) to facilitate hatching. After hatching, the poults remain in the hatcher up to 24 hours to dry and are subsequently transferred to processing. The air from the hatchers is typically exhausted directly to the outside of the building. The hatcher room is managed as an all-in all-out facility.

Infertile eggs, eggs that contain dead embryos, and eggshells that remain after pulling poults constitute hatchery waste.(3) Unhatched eggs from the hatcher tray are usually

[b] The term setter is commonly used for incubators within the hatching egg industry. Setter and incubator are used interchangeably in this document.

macerated to destroy unhatched embryos, and pipped eggs and cull chicks are either macerated or destroyed using carbon dioxide gas. Macerated debris is augured into a bin or removed by vacuum into a storage hopper. In the United States, most hatchery waste is disposed of in landfills, and the remainder is rendered or composted.(4)

4. Poult processing

After hatching and drying, poults are removed from the hatcher trays and moved to the poult take-off area[c], or room, to begin processing. The first step in the processing operation is called "pulling the hatch". In this initial step, poults are separated from hatch debris and transferred to processing conveyer belts or lines. Unhatched eggs are removed and macerated and hatchery debris is removed by vacuum. The poults are checked for quality, with any defective poults removed. Processing operations such as vaccination, beak treatment, toe treatment and gender separation or "sexing" may then be performed. These processes may be automated or performed manually.

5. Poult storage

Processed poults are packed in poult boxes and stacked on dollies or carts for shipment to turkey brooder facilities. The poults may be held in a poult holding room or area until shipped the same or next day. The optimal poult holding room conditions are 77°F to 84.2°F (25° to 29°C) and 50 to 70 percent relative humidity. The poult holding room is separated from the egg rooms, incubators and hatchers.

4.3.3 Sanitary Efforts in the Production of Hatching Eggs

The key sanitation goals in production of turkey hatching eggs are; 1) to produce a nest-clean egg that has undergone C&D prior to arrival at the hatchery; and 2) to structure hatchery operations such that egg contamination and cross-contamination of eggs and handling materials is minimized.

Production of a nest-clean egg

NPIP regulation 9CFR147.22 requires that visibly dirty eggs not be used for hatching purposes. Culled eggs are disposed of on the farm. As mentioned previously, non-visibly dirty eggs would be sanitized immediately after collection at the breeder farm.

Hatchery operations minimizing egg contamination

In accordance with NPIP requirements, the flow of hatchery products, personnel, air and waste is controlled to minimize contamination of eggs in the hatchery. In most hatcheries, these flows are designed to prevent the spread of contamination from dirty to clean areas. Clean areas include the egg room and setters and dirty areas include the hatcher, poult processing and wash areas. While this specified product flow is primarily designed to minimize contamination of sanitized hatching eggs from pathogens in hatch debris that reduce hatchability, some aspects of hatchery work flow also prevent the exposure of poults from eggs or materials potentially contaminated with HPAI virus and these practices are considered in the risk evaluation. Hatchery flow is described below.

[c] The poult "take off" room is used to separate day old poults from eggshells and other debris and transfer the poults to a processing conveyer line.

1) *Product flow*

There is no direct contact between eggs and poults in a hatchery. Eggs arrive at the egg room, and are then moved to setter incubators for a 25 day incubation period. The eggs are subsequently moved to the hatching room and transferred from setter trays to hatching trays before being placed in the hatcher incubator. After hatching, poults are moved to the poult room, processed, and loaded onto a truck for delivery.

2) *Personnel flow*

Generally, there is limited movement of personnel from the hatching area or poult room back to the egg storage area. There is little contact of personnel with eggs in the setters. Nonessential visitors to the hatchery are discouraged.

3) *Airflow*

Rooms considered less contaminated with pathogens are described as "clean," while "dirty" rooms may have a higher degree of contamination. "Clean" rooms are generally kept at positive air pressure relative to "dirty" rooms so that air flows from "clean" to "dirty" rooms. Examples of "clean" rooms that may have a positive air pressure include the vaccine room, egg storage room, setter room, and hatching room. Conversely, the poult take-off room where hatch debris is separated and equipment washrooms are considered "dirty" and are thus maintained at negative air pressure. Additionally, to minimize circulation of exhaust air and spread of contamination between different areas of the hatchery, the major hatchery areas such as egg room, setter room, hatching room and poult processing areas have separate air intake and exhausts.(5, 6) In particular, setters and hatchers usually have a separate air exhaust to the outside, either directly or through a plenum that accumulates air from different setters. The exhaust is not filtered.

4) *Waste flow*

There are separate floor drains for the egg room, setter room, hatching room, poult room and washing room. Hatchery waste, including hatch debris (broken eggshells) and culled poults, is transferred via a closed waste removal system to a waste storage bin outside the main hatchery rooms.

4.4 Current Disease Prevention and Containment Measures in Breeder-Hatchery Operations during Normal and Outbreak Situations

The NPIP is a cooperative Industry-State-Federal program focused on disease prevention in poultry and safety of poultry products throughout the country. Participation in NPIP provides breeders and hatcheries with standardized guidelines for poultry and egg management, as well as biosecurity practices. NPIP provisions 9CFR145 and 9CFR147 are pertinent to hatchery and breeder facilities and contain various C&D and biosecurity measures for the production and transportation of hatching eggs. Most commercial turkey breeders and hatcheries participate in the NPIP program. Hatcheries often implement additional biosecurity measures beyond the NPIP requirements which are considered to be minimum industry standards. Examples of the typical preventive biosecurity measures practiced in the hatching egg industry currently include 1) monitoring the health status of

flocks, 2) cleaning and disinfection of reusable materials, and 3) segregation of setting, hatching, and poult processing operations.

1. Monitoring the health status of flocks

As described previously, performance parameters for breeder flocks (egg production and mortality) are recorded on a daily basis and used to monitor flock health.

2. Cleaning and disinfection of reusable materials

Stainless aluminum racks and plastic setter trays are C&D in each work cycle. The level of C&D is much higher in the hatching egg system as compared to table-egg production systems due to the high value of hatching eggs. The effectiveness of the C&D process is monitored by testing swabs from trays, racks and other environmental surfaces on a regular basis for microbial contamination.

3. Segregation of setting, hatching and poult processing operations

The incubation and hatching, poult-processing operations are performed in separate rooms, thereby reducing the potential for cross contamination. Strict sanitary measures are in place for personnel working in the hatching and poult-processing areas. For example, personnel typically do not handle stored hatching eggs and poults on the same day.

5. Scope

This section describes the scope of the assessment with respect to the types of movements addressed and the facilities covered.

5.1 Facilities Covered Under this Risk Assessment

This risk assessment is applicable to multiplier turkey hen flocks and off-site commercial hatcheries producing turkey day-old poults that meet all of the criteria listed below[d]:

- Participate in the USDA-APHIS National Poultry Improvement Plan (NPIP) as stated in 9CFR145 and 9CFR147 (Error! Reference source not found.)
- Implement the STS Plan in the event of an HPAI outbreak.

This risk assessment also includes turkey breeder tom flocks for the purpose of evaluating the potential HPAI transmission to breeder hen flocks via semen movement and the resulting impact on the risk associated with hatching egg movement provided that they:

- Participate in the USDA-APHIS National Poultry Improvement Plan (NPIP) as stated in 9CFR145 and 9CFR147 (Error! Reference source not found.)
- Implement the STS Plan in the event of an HPAI outbreak.

5.2 Types of Movements Addressed Under this Risk Assessment

This risk assessment addresses the following types of movements into, within, and out of the Control Area during an HPAI outbreak:

- Movement of hatching eggs from a multiplier turkey breeder hen flock directly to a multiplier commercial turkey hatchery.
- Movement of cleaned and disinfected hatching-egg handling materials from the hatchery to a turkey breeder hen flock.
- The movement of semen from turkey breeder tom flocks was considered due to the potential transmission of HPAI virus to hen flocks via the insemination process and the resulting impact on the risks associated with hatching egg movement from the breeder hen flock.
- If the hatchery is located within the Control Area, it is possible that breeder flocks located outside the Control Area may need to move eggs to the hatchery. Because this movement originates from the Free Area, risk of movement into the Control Area from the Free Area is assumed to be negligible and is not evaluated. However, handling materials would have to be returned to the breeder farm from

[d] Although the scope of this assessment is limited to multiplier turkey breeder hen farms and hatcheries producing turkey day old poults, based on practices in this industry sector, the results may be applicable to primary turkey hatcheries that have more stringent biosecurity requirements. However, primary turkey breeder industry stakeholders were not part of the turkey sector working group, and practices in this sector of the industry were not considered in risk evaluation.

within the Control Area and there is a potential for cross contamination of these materials at the hatchery. This pathway was not evaluated explicitly. Control measures applied to materials returned to breeder farms within the Control Area should be sufficient for this pathway.

Although the production of hatching eggs is dependent on the movement of semen from tom flocks, the risk of movement of semen is not evaluated directly. However, exposure of breeder hens to contaminated semen is considered to be a possibility. Also, some hatcheries sell excess eggs to other hatcheries. The movement of hatching eggs between hatcheries is also outside the scope of this assessment.

5.3 Hatching Egg Process Steps Addressed in this Assessment

Two key risks were identified associated with the movement of hatching eggs and hatching egg-handling materials during an HPAI outbreak.

- The risk that movement of hatching eggs from an infected but undetected multiplier turkey breeder hen premises results in the infection of day-old poults at the hatchery.
- The risk of the movement of hatching egg-handling materials from a hatchery resulting in HPAI infection of susceptible turkey breeder hen flocks.

To estimate these risks, we evaluate all of the steps in the hatching egg process from the production of eggs at the turkey hen flock to the loading of day old poults into the transport vehicle at the hatchery. The risks associated with movement of day-old poults to brooder farms are addressed in a separate risk assessment.

6. Overview of Data Analysis Approaches

6.1 Risk Assessment Overview

The assessment follows the general qualitative risk assessment principles recommended by the OIE import risk analysis guidelines. However, the risk assessment organization has been modified from that proposed in the OIE import risk analysis handbook as appropriate for movement of turkey hatching eggs.(7) The risk assessment is comprised of hazard identification and two evaluation steps: (1) entry assessment (entry of HPAI virus onto a susceptible poultry premises through the movement of a commodity); and (2) exposure assessment (exposure of susceptible animals). If the entry assessment demonstrates a *negligible* likelihood of the commodity, associated vehicle, driver or handling materials being contaminated with HPAI virus, the risk assessment may be concluded at that point. However, if the likelihood is estimated to be greater than *negligible*, the next step in the risk assessment is the exposure assessment, which would assess the likelihood that susceptible poultry will be infected by HPAI virus through the pathogen-commodity pair in question. In this assessment, the risk ratings were determined on the basis of the likelihood of HPAI spread to the destination premises. The adverse consequences of HPAI virus spread to another poultry premises were assumed to be very high and a consequence assessment was not included.

In the current document, the entry assessment evaluates the likelihood of HPAI virus being released into a commercial hatchery through the movement of turkey hatching eggs. The exposure assessment then evaluates the risk of day old poults at the hatchery or other susceptible turkey breeder hen flocks becoming infected with HPAI virus associated with the movement of hatching eggs and egg-handling materials.

The assessment utilizes a qualitative evaluation approach where the likelihoods of individual events in the pathway were rated according to a qualitative scale (see **Table 1**). The qualitative ratings were based on multiple data sources and evaluation approaches, such as literature review, expert opinion, quantitative simulation model predictions, and past outbreak experiences. The quantitative simulation model results from previously completed proactive risk assessments were used to estimate the number of HPAI contaminated eggs produced from infected, but undetected turkey breeder hen flocks (a single house) supplying eggs to a commercial hatchery. The likelihood for main steps in each pathway was assessed and categorized using the descriptive scale described in **Table 1**.

6.2 Likelihood and Risk Ratings

The likelihood for main steps in each pathway was assessed and categorized using the descriptive scale in **Table 1**.

Table 1. Descriptive scale used 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 risk estimate for each pathway in the exposure assessment was determined by combining the likelihoods of the individual events. For determining the overall risk rating for pathways involving a sequential chain of events which all have to occur for the pathway to be completed, relatively more weight was given to events with the lowest likelihood in the chain. The risk rating scale used in this assessment is provided below.

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

Low risk: The spread of HPAI infection to susceptible poultry through the risk pathway is very unlikely.

Moderate Risk: The spread of HPAI infection to susceptible poultry through the risk pathway is unlikely to but does occur.

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

Extremely High Risk: The spread of HPAI infection to susceptible poultry through the risk pathway is almost certain to occur.

6.3 Uncertainty Estimation

The uncertainty of the likelihood/risk estimation was assessed by using a range defined by the terms in the descriptive rating scale provided in **Table 1**. For example, a risk estimate of *negligible* to *low* encloses the true risk, which is not deterministically known, where the interval between the two ratings represents the uncertainty in the analysis.

7. 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 make a number of assumptions to establish context and applicability. These assumptions are:

- That a HPAI outbreak has been detected and APHIS is implementing the HPAI Response Plan.(8) The APHIS HPAI Response Plan is intended to complement regional, State, and industry plans. APHIS recommends their continued development.
- Turkey Breeder farms may have HPAI infection in their flocks, but it has not yet been detected. If there were absolute certainty HPAI infection was absent, there would be no risk of HPAI spread from virus at the breeder farm. On the other hand, if HPAI infection has been detected on the premises, it is assumed Incident Command would quarantine the premises. As the movement of hatching eggs and handling materials would not be allowed, and the facility would be depopulated, cleaned and disinfected before resuming production. This situation does not pose a risk associated with movement of hatching eggs.
- All relevant preventive measures from the STS Plan are strictly followed. The assessment does not evaluate the risk that the preventive measures in the STS Plan are incorrectly implemented, either intentionally or unintentionally.
- The assessment focuses on the risk that movement of hatching eggs will result in the spread of HPAI to other susceptible poultry. Although the risks to humans or wildlife associated with the production or movement of hatching eggs are critical concerns that should be addressed, they are outside the scope of this assessment. The draft *National Highly Pathogenic Avian Influenza Response Plan* has personnel safety measures designed to mitigate the risk to humans.(8)
- We conservatively assumed that HPAI virus would be present in sufficient concentration in semen from infected turkey toms to be infectious to turkey hens. Data on the concentration of HPAI viruses in turkey semen and the dose response relationship for the insemination route is unavailable. However, field experiences in previous LPAI outbreaks where semen movement was implicated for AI spread suggest that transmission via semen may be possible.(9, 10)
- Because the adverse consequences of HPAI virus spread are assumed to be very high, the risk rating was determined based on the likelihood of HPAI spread to the destination premises 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.
- Given routine hatchery workflow practices, it is assumed that buggies and setter trays coming from a breeder flock are not taken into any hatchery room in which day old poults are present.
- It is assumed that turkey hatching eggs shall not move until diagnostic steps have ruled out HPAI in a breeder hen flock, if HPAI is detected in a breeder tom flock supplying semen to it.
- 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.

8. Hazard Identification

Hazard identification consists of listing the pathogenic agent(s) associated with the species from which a commodity is derived and whether the agent(s) can be classified as hazards (i.e. they have a potential to cause harm) for further consideration in the risk assessment.(7) For movement of turkey hatching eggs, the pathogenic agent of concern in this assessment is HPAI virus. This section includes background information from published literature and expert opinion to provide the most current knowledge about the epidemiology of HPAI viruses associated with trade in turkey products.

Properties of HPAI viruses, including environmental persistence, transmission characteristics, and physical and chemical inactivation, have been extensively reviewed in comprehensive texts.(11) The following is a brief summary of key properties of HPAI viruses, with emphasis on the variability between HPAI strains and transmission characteristics in turkeys.

8.1 Agent and Host Range

Avian influenza 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 and humans.(11, 12)

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. There are sixteen different subtypes of HA (H1 to H16), nine different subtypes of NA (N1 to N9) and 144 different HA:NA combinations.(11) 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 as:

a. Any influenza virus that kills at least 75 percent of eight, 4- to 6-week-old susceptible chickens, within ten days following intravenous inoculation with 0.2 ml of a 1:10 dilution of a bacteria-free, infectious allantoic fluid.

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

c. Any influenza virus that is not a H5 or H7 subtype and that kills one to five chickens and grows in cell culture in the absence of trypsin.

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

All H5 or H7 isolates of both low and high pathogenicity and all HPAI isolates regardless of subtype are reportable to State and national veterinary authorities and to the OIE.(13) Although other 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 low pathogenic avian influenza (LPAI) viruses, but most highly pathogenic avian influenza (HPAI) viruses responsible for high mortality in domestic birds do not have recognized wild bird reservoirs.(14) The phrase 'highly pathogenic for chickens' does not indicate or imply that the AI virus strain is highly pathogenic (HP) for other bird species, especially wild ducks or geese (Anseriformes). However, if a virus is highly pathogenic for chickens, the virus will usually be HP for other birds within the order Galliformes, family Phasianidae, such as turkeys and Japanese quail. To date, most HPAI viruses for chickens are generally non-pathogenic for ducks and geese in experimental studies.(12) However, lethality of HPAI viruses in ducks has changed with 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 ducks.(14)

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.(15) Recent identification of a H5N2 virus with a 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 a potential high pathogenicity for poultry could be maintained in a wild waterfowl community prior to introduction.(14)

Host adaptation is a key determinant in the ability to maintain transmission of a HPAI virus within domestic poultry. Once adapted to gallinaceous poultry, HPAI viruses are unlikely to return back to circulate among wild birds because they are adapted to poultry.(16) However, the emergence of Asian HPAI H5N1 strains have led to increased uncertainty regarding the role of wild birds as reservoirs in the maintenance of HPAI viruses in nature.(17) Prior to the outbreak of HPAI H5N1 virus in Europe, Asia, and Africa, HPAI viruses had only rarely been isolated from wild birds, usually associated with outbreaks in domestic poultry, with one exception.(18) An outbreak of HPAI H5N3 in South Africa in 1961 was observed in a population of terns. Asian HPAI H5N1 strains, however, have been isolated from multiple species of wild birds.(19) Both these H5N3

and H5N1 HPAI viruses were isolated in sick, moribund or dead wild birds. Despite extensive global wildlife surveillance efforts, infection with H5N1 HPAI viruses was not detected in healthy wild birds, except for a few isolated cases. Therefore, the significance of wild birds as a source of infection and their influence on the epidemiology of H5N1 HPAI viruses is yet to be fully established.(14)

8.2 Geographic Distribution of H5N1 HPAI

- The *Report of the Global Programme for the Prevention and Control of Highly Pathogenic Avian Influenza* contains information on incidence of H5N1 HPAI in animals as well as a global overview and worldwide situation report. The 2012 report can be viewed at http://www.fao.org/avianflu/en/strategydocs.html.
- 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/animal-health-in-the-world/update-on-avian-influenza/2014/.

8.3 Virus Shedding

HPAI viruses have been isolated from respiratory secretions, feces, and feathers, as well as the eggshell surface, albumen, yolk and meat from infected poultry. Estimates of HPAI virus fecal concentrations in chicken feces mostly ranged between 10^3 to10^7 EID_{50}/gram although concentrations as high as 10^9 EID_{50}/gram have been observed in some cases.(20-22)

H5N2 HPAI viruses have been isolated from the eggshell surface, yolk and albumen of eggs laid by experimentally inoculated chicken hens.(23) In 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 a HPAI virus strain isolated from chickens.(24) Italian HPAI H7N1 viruses have also been isolated from eggs laid by infected hens.(25)

In an experimental study, the concentration of H5N2 HPAI virus ranged from 0.97 to $10^{5.9}$ EID_{50}/eggshell and 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.(23)

As compared to chickens, avian influenza viruses in turkeys demonstrate a relatively high degree of affinity for oviduct tissue, compared to respiratory and digestive tissue.(26) A predilection for replication within these tissues may explain the precipitous drops in egg production reported in turkey breeder hen flocks during natural outbreaks.(27-30) Narayan et al. recovered HPAI virus from egg yolks from each of 3 eggs laid by 30 week old turkey hens that were infected through contact with a hen experimentally infected with a HPAI virus.(31) In turkey breeder hens experimentally inoculated with swine

origin LPAI H3N2, virus was recovered from eggshells and egg contents.(26) 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. HPAI virus concentration data from eggs laid by infected chickens were used in the risk evaluation, as data from eggs laid by HPAI infected turkey hens were not available.

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 Environmental Protection Agency (EPA) maintains a list of disinfectants with label claims for avian influenza viruses. These products include halogens, aldehydes, quaternary ammoniums, phenols, alcohols, peroxides and some detergents.(32-34) 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 inactivation.

8.5 Persistence of HPAI in Manure and Other Media

Persistence of AI viruses in the environment in different media is summarized in **Table 2**. 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 HPAI viruses have remained viable in liquid poultry manure for 105 days in winter under freezing conditions and 35 days at 4º C.(21, 35) H5N1 HPAI virus was viable for four days at 25 to 32 ºC when kept out of direct sunlight.(36)

Table 3 summarizes the literature on inactivation of AI viruses at 35 to 37 °C which approximates the range of temperatures in the incubator (37 to 39 º C). Most of these data suggest more than a 6.8 log EID_{50} inactivation of AI virus within 15 days at incubation temperature. Muhmmad et al. (2001) reported that HPAI H7N3 virus retained infectivity in allantoic fluid for a period of 35 days at 37 ºC although the hemagglutination titers were reduced to undetectable levels, suggesting very low virus concentrations.(37)

Table 2. Persistence of avian influenza viruses in the environment in different media under various environmental conditions.

Virus strain	Media	Conditions	Survival duration and temperature	Reference
Duck influenza viruses (H7N2 and H3N6)	Untreated Mississippi river water	Un- chlorinated water	4 days 22 ºC; 20 days 0 ºC	Webster et al. (1978)(38)
	Duck feces	Relatively high humidity (sealed in a vial)	30 days 4 ºC; 7 days 20 ºC	
Pennsylvania HPAI H5N2	Wet manure	In a barn (winter under freezing conditions)	105 days after depopulation	Fichtner et al. (2003)(35)
Pennsylvania HPAI H5N2	Wet feces	Closed vial	35 days > 4 ºC Between 2 to 3 days at 25 °C	Beard et al. (1984)(21)
	Wet feces	Open vial	Between 9 to 14 days 4 ºC Between 1 to 2 days at 25 °C	Beard et al. (1984)(21)
2 Clades of HPAI H5N1	Duck feathers	Relatively high humidity (sealed in a vial)	160 days 4 ºC; 15 days 20 ºC; Titers of $10^{4.3}$ EID_{50}/ml for 120 days at 4 °C	Yamamoto et al. (2010)(39)
	Drinking water (commercial mineral water)	Collected at 3 days post infection and stored at 4 C or 20 ºC	Inconsistently isolated from water stored at 4 ºC over a 30 day period; no virus isolated from drinking water at 20 ºC after 3 days	
H7N2 LPAI	10^{7-8} EID_{50} mixed with chicken manure from 3 sources	2 sources were chickens housed in BSL2 facilities; 1 source commercial layers	Inactivated in commercial layer manure after 6 days at 15 to 20 ºC; 2 days at 28 to 30 ºC	Lu et al. (2003)(40)

Virus strain	Media	Conditions	Survival duration and temperature	Reference
8 wild type LPAI viruses, H5 and H7 subtypes; and 2 HPAI H5N1 subtypes	Water 0, 15, and 30 parts per thousand salinity;	Simulated winter and summer coastal marshland temperatures in LA, 17 and 28 °C	H5N1 had shorter environmental survival times compared to wild-type LPAI viruses; 2 clades persisted for 94 to 158 days at 17 ºC	Brown et al. (2007)(41)
HPAI H5N1 from chickens in central Thailand	0.2ml $10^{6.3}$ EID_{50}/ml in allantoic fluid, feces, water, one cubic inch of meat or eggs	Virus added to allantoic fluid or feces	In *shade* (25-32 ºC): survived for 10 days in allantoic fluid; for 4 days in feces; for 3 days in water from a rice field In *sunshine* (32-35 ºC): killed within 30 minutes after placing the sample in sunlight	Songserm et al. (2005)(36)
		Virus added to meat or eggs	Killed if cooked for > 3 minutes at 70 ºC	
3 isolates from hunter killed ducks from various waterfowl habitats in Louisiana (H6N2, H4N6, H10N7)	Distilled water adjusted to pH (6.2, 7.2, 8.2); (0, 20 ppt); (17 ºC and 28 ºC); and surface water from a rice field and two marshes	Salinity for fresh and brackish sea water; mean winter and summer temps for coastal Louisiana	Survival in surface water ranged from 9 to 55 days; persistence in simulated water samples ranged from 9 to 100 days	Stallknecht et al. (1990)(42)
5 HA subtypes from hunter killed ducks in Louisiana (H3N8, H4N6, H6N2, H12N5, H10N7)	Distilled water at 17 and 28 ºC	Mean winter and summer temps for coastal Louisiana	Survival for 207 days at 17 ºC; and 102 days at 28 ºC depending on subtype	Stallknecht et al. (1990)(43)

Virus strain	Media	Conditions	Survival duration and temperature	Reference
H5N1 HPAI	Virus added to normal chicken manure; 2.38 x $10^{5.25}$ ELD_{50}	pH 9.23; 13.7% moisture (dry manure)*	No virus recovered after 24 hrs. at 25 ºC; or 15 minutes at 40 ºC. Virus recovered after 4 hrs of UV exposure at room temperature (25 ºC)	Chumpolbanchorn et al. (2006)(44)
3 LPAI viruses (H4N6, H5N1, H6N8)	3 different water types: Starting titers ranged from $10^{4.14}$/ml – $10^{5.14}$/l	Distilled water (pH 7.8); Normal saline 0.9% (pH 7.2); Surface Water) from Lake Constance, Europe. Incubated at -10, 0, 10, 20, and 30 ºC.	Viruses remained infective the longest in DW, followed by SW. Detectable in SW at all temps: H4N6 182 days; H5N1 182 days; H6N8 224 days; persistence inversely proportional to water temperature	Nazir et al. (2010)(45)
3 LPAI viruses (H4N6, H5N1, H6N8), and H1N1	Lake sediment, duck feces, and duck meat	$10^{6.25}$ $TCID_{50}$/ml virus loaded onto germ carriers incubated at 30, 20, and 0 ºC	Persistence highest in lake sediment (5 to 394 days), feces (1 to 75 days), meat (1 to 81 days)	Nazir et al. (2011)(46)
H7N1 LPAI; H7N1 HPAI	HPAI: Breast and thigh meat from chickens, turkeys and ducks infected oro-nasally, collected 3 days post infection	Homogenized meat samples were held at 4 ºC	Infectivity in meat at 4 ºC: 135 days in chicken meat; 90 days in turkey meat; 75 days in duck meat	Beato et al. (2009)(47)
	HPAI and LPAI: virus inoculated into allantoic fluid	Infectivity assayed after holding allantoic fluid samples at 4 ºC and 20 ºC	Infectivity in allantoic fluid: HPAI up to 210 days at 4 ºC; LPAI up to 270 days at 4 ºC; HPAI not detectable at 60 days at 20 ºC, LPAI 2.9 log EID_{50} at 60 days at 20 ºC.	

Virus strain	Media	Conditions	Survival duration and temperature	Reference
		Infectivity assayed after holding allantoic fluid samples at 4 ºC and 20 ºC with pH adjusted to 5 and 7	Persistence time higher for viruses at pH 7 than for pH 5; HPAI more persistent at pH 7; LPAI more persistent at pH 5	
H13N7 LPAI	Steel, wood, tile, tire, gumboot, feather, egg shell, egg tray (cardboard), plastic, latex, cotton fabrics, polyester fabric; 10 µl of 6.3 X 10^6 $TCID_{50}$/ml	Placed in sealed tubes and stored in a drawer at room temperature	Survival up to 72 hrs. on most surfaces; 24 hrs. on cotton; 6 days on latex; 6 days on feathers; 2 days on wood; 1 day on egg tray; 3 days on truck tires. Survival appeared to be less on porous vs. non-porous surfaces	Tiwari et al. (2006)(48)
H6N2	Treatments: Virus in allantoic fluid mixed with chicken manure, used litter, and feed; homogenized embryonated chicken egg in corn silage. 3.4 x 10^8 EID_{50}	Specimens: held in mesh bags buried in compost; vials of allantoic fluid buried in compost; Controls: held in sealed vials at ambient temperature (23-26 ºC)	Treatments: Virus in all mesh bag specimens inactivated at 40-50 ºC by day 3 except for one manure sample at 40 ºC; Viable virus from allantoic fluid in vials on day 3 (46- 43 ºC); day 7 (55.5 ºC); and day 10 (62.2 ºC) Controls: Viable virus at 21 days (22.7 – 25.7 ºC)	Guan et al. (2009)(49)

*Chicken fecal moisture may be as high as 60%

Table 3. Summary of literature on thermal inactivation of AI virus in wet media when incubated at 35 to 37 ºC.

Study	Virus and Media	Temperature and Time	Inactivation Log (EID_{50})

Terregino (2009) (50)	HPAI H7N1 A/turkey/Italy/1387/00 (Allantoic fluid)	37 ºC for 15 days	>6.5
Terregino (2009) (50)	HPAI H7N1 A/turkey/Italy/4580/99 (Allantoic fluid)	37 ºC for 15 days	>6.6
Terregino (2009) (50)	LPAI H7N1 A/turkey/Italy/3675/99 (Allantoic fluid)	37 ºC for 15 days	5.5
Terregino (2009) (50)	LPAI H7N1 A/turkey/Italy/4608/03 (Allantoic fluid)	37 ºC for 15 days	6.1
Shortridge et al., (1998)(22)	HPAI H5N1 (Wet feces)	35 ºC for 2 days	~4
Davidson et al., (2010)(51)	LPAI H9N2 (Allantoic fluid)	37 ºC for 4 days	>6.8
Davidson et al., (2010)(51)	LPAI H9N2 (Allantoic fluid)	37 ºC for 2 days	~5
Negovetich and Webster (2010) (52)	LPAI H2N3 (Allantoic fluid mixed with various liquid media)	37 ºC for 10 days	>6.8
Homme and Easterday (1970) (53)	LPAI A/Turkey/ Wisconsin/1966 (Allantoic fluid)	37 ºC for 4 days	8

8.6 Transmission

Contact with migratory waterfowl, sea birds, or shore birds is a risk factor for introduction of AI virus into domestic poultry populations.(54) 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.(11) Once introduced into a flock, AI virus can spread from flock to flock by direct movement of infected birds and indirect movement of contaminated equipment, egg flats, feed trucks, and service crews, or other means. Windborne transmission may occur when farms are closely situated and appropriate air movement exists.(55, 56) Wild animals such as raccoons and foxes have also been implicated in local area spread.(57)

As compared to chickens, there is an additional risk in turkeys of viral transmission via the artificial insemination process. 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 in natural outbreaks.(9, 58) Although semen was implicated in the spread of AI in field outbreak investigations, isolation of AI virus from tom semen was not reported in these studies. Avian influenza virus has previously been isolated from tom turkey semen, but titer levels were not reported.(59) It was unclear whether this virus came from the semen *per se,* or from the cloaca contaminated by fecal material (Halvorson, D, personal communication).(60) It is not anatomically or practically possible to collect semen without the collection device touching the cloaca. HPAI virus antigen has been observed in testes, suggesting that virus could be present in semen (Swayne, D.E., personal communication)(61).

In a recent study, tom turkeys were inoculated intranasally with 10^6 $TCID_{50}$/0.5ml of TR H3N2 swine influenza virus (SIV) A/Turkey/OH/313053/2004.(62) 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, the authors conclude that there is potential for venereal transmission of SIV in turkeys, based on the presence of viral RNA in the reproductive tract and semen, along with another recent study's conclusion that insemination was a significant risk factor in a seasonal human H1N1 outbreak in turkey hens.(63) In that outbreak, human to bird transmission was proposed, either directly via saliva during manual insemination or indirectly through contaminated semen.

Other studies have demonstrated AI viruses can be transmitted to turkey breeder hens through artificial insemination with semen experimentally contaminated with AI virus on the day of collection.(64) Pantin-Jackwood et al. (2010) transmitted pandemic H1N1 virus to hens by intracloacal or intrauterine inoculation, demonstrating that transmission is possible through contamination of these mucosal surfaces by semen or fomites.(65)

Evidence of vertical transmission of avian influenza from infected hens to day old poults or chicks has been lacking thus far, as most strains are lethal to chicken embryos.(47, 66-68) Groups of turkey hens in egg production, with no clinical evidence of influenza A infection, were inoculated intravenously or intratracheally or were inseminated with semen contaminated with 2 LPAI influenza viruses, and virus was not recovered from poults hatched from eggs laid by exposed turkey hens.(69) 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 avian influenza experts have stated that although there have been several LPAI outbreaks in the United States, vertical transmission or hatchery transmission has not been observed to-date. Expert opinion elicitation was conducted by the University of Minnesota to gather field data on the absence of evidence for vertical transmission of viral influenza during past outbreaks, in particular from breeder turkey hens to their hatching eggs and day old poults. Turkey industry representatives provided reports of 26 flocks that had undergone AI infection and where eggs from the flocks were set for hatching and not removed after the detection of AI virus. Most of the outbreaks reported were due to swine origin influenza A viruses (H1N1 or H3N2). In addition, a couple of outbreaks each were identified as due to Pandemic H1N1 and other LPAI viruses (H6N2 and H7). There was

no evidence of horizontal or vertical transmission of AI within the hatchery to day old poults in any of these instances (**Appendix 9**).

Chicks hatched from eggs produced by two HPAI H7N3 infected broiler breeder flocks tested negative for AI during an outbreak in British Columbia in 2004. The outbreak report of the Canadian food inspection Agency states, "Because AI does not survive long at incubator temperatures, day-old chicks are not a likely source of infection for broiler growers."(70) In the 1983 Pennsylvania HPAI H5N2 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.(21) Also the 214 chicks hatched from these eggs showed no sign of AI disease and had not developed AI antibodies to H5N2.

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.(71) In this study, turkeys were inoculated with H5N1 and H7N1 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} for H5N1 and $10^{2.2}$ EID_{50} for H7N1. Turkeys were found to be more susceptible than chickens by over 200-fold for H5N1 and over 100-fold for H7N1.

In another study, turkeys were inoculated with different doses of A/ostrich/Italy/984/2000 H7N1 HPAI by a combined intranasal/intraocular route.(72) 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), while there was greater than 50% (4/6) 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} at both 96 and 120 hours PI.

In their studies using a highly poultry-adapted LPAI strain, Pillai et al., (2010) demonstrated a 50% lower ID_{50} for turkeys ($10^{1.4}$ EID_{50}) than for chickens ($10^{2.6}EID_{50}$).(26) 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.(71) 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 to 4 log EID_{50}. Transmission of LPAI to turkeys has been demonstrated via an estimated aerosol dose between 3 to 5 log EID_{50}.(73) Data from this experimental study suggests that the 50% aerosol infectious dose is close to or less than 3 to 5 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 directly into the esophagus with 2 grams of

$10^{3.6}$ EID_{50}/0.1g HPAI H7N1 (for a total dose of $10^{4.9}$ EID_{50}) infective meat homogenate.(74) Tracheal and cloacal swabs collected out to day 7 remained negative, as did serum samples out 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 process.

Although transmission of HPAI via semen is strongly suspected in turkeys, data on dose response to such exposure are lacking. However, field experiences in previous LPAI outbreaks where semen movement was implicated for AI spread suggest that transmission via semen may be possible.(9, 10)

8.7.2 Dose Response in Chickens

Most experimental studies in chickens used intranasal inoculation as an entry point. For the intranasal route, the 50 percent chicken infectious dose (CID_{50}) for 10 HPAI strains varied between $10^{1.2}$ to $10^{4.7}$ EID_{50} with a geometric mean of $10^{2.82}$ EID_{50}.(75) Most strains in this study had a mean CID_{50} above 10^2 EID_{50} except for the HPAI H7N1. Other studies have also found similar estimates for the CID_{50} through the intranasal route.(76)

Single hit dose response models (e.g., exponential) have been used for HPAI virus in chickens and mammals.(77, 78) 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 a 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 via oral consumption of chicken meat (10^7 EID_{50}) or drinking of contaminated water$10^{6.7}$ EID_{50}.(79) 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 intra-gastric inoculation route by gavage ($10^{6.2}$ EID_{50} for liquid and $10^{7.4}$ for meat EID_{50}) compared to 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 virus.(80) However feeding of HPAI H5N2 infected chicken breast or thigh meat to SPF chickens ($10^{3.5–3.6}$ EID_{50}/bird) did not produce infection. The authors reasoned that lack of direct exposure of respiratory tract receptors (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), where 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.(81) However, in this

study, the HPAI concentration in blood was not estimated and it may have been sufficient to cause infection via intra-gastric route.

Sargeev et al., (2013) found a CID_{50} of $10^{3.9}$ EID_{50} and $10^{5.2}$ EID_{50} for oral inoculation and intra-gastric inoculation via gavage tube respectively.(82) The authors suggested contamination of the nasal mucosal membranes from the oral cavity via choanal slit as a possible internal mechanism for transmission via the fecal oral route.

8.7.3 Route of Entry and 50 Percent Infectious Dose Response Used in this Assessment

In turkeys, 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 the process of 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 for infection with HPAI virus (intranasal vs. intragastric) observed in laboratory inoculation and experimental feeding trials, there is considerable uncertainty as to the infectious dose that is appropriate for natural exposure via feeding of contaminated materials. The route of entry used 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 (83). 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 Infected 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 in turkeys to be 1.47 days (95% C.I., 1.3 to 1.7) from experimental transmission studies.(84) The data from this study also suggested that the latent period for HPAI H7N1 in turkeys is likely less than 16 hours.(84)

Aldous (2010) evaluated the virus shedding patterns and mortality in turkeys inoculated with various doses of HPAI H5N1 virus. Analysis of these data indicated a mean latent period of 1.27 days (std.dev. 0.40 days) and a mean infectious period of 1.28 days (std.dev. 1.17 days). (71, 85) Further details on the estimation of these parameters are provided in **Appendix 2.**

8.9 Clinical Signs

The presence and severity of clinical signs of HPAI infection depends on the type of bird species affected. Infected wild and domestic ducks may be asymptomatic, whereas

clinical signs in gallinaceous poultry are usually severe, resulting in high mortality. In poultry (chickens and turkeys), the clinical signs associated with HPAI infection include marked depression with ruffled feathers, lack of appetite, excessive thirst, decreased egg production, soft-shelled or misshapen eggs, respiratory signs (coughing and sneezing), watery diarrhea or sudden, unexpected death. In turkeys, a cessation in flock vocalization (Cathedral Syndrome) often accompanies infection. Depression, decreased feed consumption, diarrhea, ruffled feathers, 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.

The mortality rate in an infected flock can reach 100 percent. 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. In layers, the ovary may be hemorrhagic or degenerated and necrotic. The peritoneal cavity is frequently filled with yolk from ruptured ova, causing severe air sacculitis and peritonitis in birds that survive longer than 7 days. In addition, necropsy of birds affected in the 1999-2001 H7N1 HPAI outbreak in Italy revealed pancreatitis in all species of birds; this was most pronounced in turkeys and chickens.(86)

8.10 Diagnosis

HPAI is a differential diagnosis to be considered in any flock in which marked depression, inappetence, and/or a drastic decline in egg production are associated with sudden deaths; however, a conclusive diagnosis is dependent on the isolation and identification of the virus.

The reference standard for diagnosis of AI virus is virus isolation. In the laboratory, 9- to 11-day-old embryonated chicken eggs are inoculated with suspension from swab or tissue specimens. Additional tests on fluids from the egg are required to confirm the presence of AI virus and determine its serologic identity (HA and NA type).(11)

The application of molecular methods for detection of viral nucleic acid has become an important tool in the recent years. The real time reverse transcription polymerase chain reaction (RRT-PCR) has advantages for outbreak surveillance such as speed, scalability for high through put, high sensitivity and specificity.(11)

Antigen detection immunoassays kits have also been used in prior outbreaks and have advantages of speed (15-20 minutes) and good specificity. While the low analytical sensitivity (greater than 10^4 EID_{50}) is a limiting factor, birds presenting from clinical disease or that died due to AI infection generally shed adequate virus titers 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.(11)

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 and severe water deprivation.

9. Entry Assessment

An entry assessment determines the likelihood of a commodity (in this case, turkey hatching eggs and associated handling materials) being contaminated with a hazard (e.g. HPAI virus) and describes the biological pathways necessary for that hazard to be introduced into a particular environment with susceptible poultry. It includes an estimation of the likelihood (i.e., qualitative or quantitative) of each of the pathways occurring.(7)

The entry assessment of this risk assessment evaluates the likelihood of HPAI virus being transmitted onto a commercial turkey hatchery premises via movement of contaminated hatching eggs; associated handling materials; or the vehicle or driver, from a breeder premises in the Control Area.

Outbreak specific preventive measures (i.e. control measures to be implemented in the event of a HPAI outbreak) described in the STS Plan were considered when making risk estimates. The active surveillance protocol described in the STS Plan is an important measure for reducing the likelihood of moving contaminated hatching eggs and egg-handling materials.

9.1 Likelihood of Hatching Eggs Moved from an HPAI Infected but Undetected Breeder Premises Being Contaminated with HPAI

Risk Factors: HPAI virus contamination of eggs; late detection of HPAI infection in a flock; movement of contaminated turkey semen.

Current Preventive Measures: Frequent collection of eggs to produce nest clean eggs; sanitizing hatching eggs.

Outbreak Specific Measures (to be implemented by industry during an outbreak): Active surveillance RRT-PCR testing of flocks; reduction in frequency of semen movement from twice weekly to once weekly; 2-day holding period after production (egg collection) before movement of eggs off the farm.

9.1.1 Introduction

Experimental studies have found HPAI virus in the internal contents and shell surfaces of eggs laid by infected chickens. HPAI virus has also been recovered from the yolk of eggs laid by infected turkey hens (**Section 8.3**).

The likelihood of moving contaminated hatching eggs from a breeder premises before detection is dependent on the HPAI disease spread dynamics within a breeder henhouse and the time taken to detect infection via active surveillance. We utilize simulation models of disease spread within an infected breeder tom and hen house and models of the active surveillance protocols from the STS Plan in our evaluation.

We consider the sanitization of hatching eggs and other on-farm personnel biosecurity steps from the STS Plan for evaluating the likelihood of moving externally contaminated hatching eggs.

9.1.2 Preventive Measures

9.1.2.1 Current Preventive Measures

High levels of microbial contamination can adversely impact hatchability of eggs. Consequently, most turkey breeder farms have incorporated measures to increase the proportion of nest-clean eggs (eggs without excessive amounts of adhering organic matter when they are collected). NPIP regulations 9CFR147 recommend that only clean eggs are used for hatching purposes (Error! Reference source not found.).

Practitioners participating in the Turkey Sector Workgroup who offered expert opinion (i.e. not a random sample) suggested that most turkey breeder hen operations sanitize hatching eggs, with some operations washing the eggs with water (without detergent) before sanitizing. Common sanitizing agents reported included hypochlorite and quaternary ammonium compounds. Hydrogen peroxide based agents were used in a few cases. Some operations gather eggs onto setter trays first and then sanitize the trays and eggs together while others may manually transfer eggs from baskets or flats to a belt for sanitizing and then transfer sanitized eggs onto setter trays. In this setup, setter trays would have been cleaned and disinfected at the hatchery before being distributed to the breeder farm.

In routine operations, carts used to carry setter trays into the henhouse remain on the farm (i.e. remain separate from carts used to transport setter trays to the hatchery).

9.1.2.2 Outbreak Specific Preventive Measures

The outbreak specific measures considered in this evaluation are from the STS Plan.

a. The STS Plan requires that turkey breeder hens and toms producing fertile hatching eggs must test negative for avian influenza matrix genes by RRT-PCR before hatching eggs will be allowed to move.
    - One 5-bird pooled sample must be tested and found to be negative from every house on the premises for two consecutive days prior to movement of turkey hatching eggs.
    - If there are less than 5 dead turkeys in the house, the remainder of the samples should be taken from sick turkeys.

b. If daily mortality is abnormally high (more than 2/1,000 birds in a flock) immediately prior to a scheduled movement, turkey hatching eggs shall not move until diagnostic steps have ruled out HPAI as the cause of elevated mortality.

c. If a significant drop in egg production rate is detected by the farm manager immediately prior to a scheduled movement, turkey hatching eggs shall not move until diagnostic steps have ruled out HPAI as the cause of the drop in egg production. Here, a total decrease in egg production rate of 15 percent or more occurring over a two day period is considered as a quantitative trigger for a drop in egg production.

For example, if the egg production rate in a 3000 hen turkey house was 1800 eggs on Monday and 1350 or fewer eggs were produced on Wednesday, then the trigger for drop in egg production rate would activate further diagnostic steps.

d. Hatching eggs must be washed and sanitized with a chlorine rinse with a concentration equal to or greater than 200 ppm or with an approved disinfectant for avian influenza virus according to the manufacturer's label directions for application on hatching eggs. Eggs are washed and sanitized while on flats in cases where flats are taken into the hen house, or are transferred to clean flats after being washed or sanitized. Employees who manually transfer eggs must wash their hands with soap and water or use a hand sanitizer before handling eggs.

e. Eggs are held in the egg storage room for 2-days after production (collection) before hatching eggs are moved from the premises. As an example, given a 2-day hold, eggs produced on Monday can be moved on Wednesday after receiving 2 negative RRT-PCR tests on Tuesday and Wednesday. The 2-day interval before the movement of eggs in conjunction with the active surveillance protocol reduces the likelihood of moving contaminated eggs before HPAI infection is detected.

f. One 5-bird pooled sample must be tested by RRT-PCR per turkey breeder tom house on 2 consecutive days before the day of semen movement to the breeder hen flocks. For example, negative RRT-PCR test results from samples collected on Monday and Tuesday should be required in order to move semen collected on Wednesday under this protocol.

g. Reduction in the frequency of semen movement from twice weekly to once weekly.

h. Farm personnel should wear gloves and disposable or cleaned and disinfected boots before entering egg storage coolers.

9.1.3 Predicting the Number of Internally Contaminated Eggs Moved from an Infected but Undetected Turkey Breeder Hen Flock

The key factors determining the likelihood of moving HPAI contaminated hatching eggs from an infected but undetected breeder flock (house) include:

- Characteristics of HPAI disease in infected hens such as the length of latently infected and infectious periods and the likelihood that eggs laid by HPAI infected turkeys are contaminated with virus.

- The HPAI disease mortality and number of infectious turkeys at different points over time given the dynamics of HPAI spread within the flock.

- The variability in detecting HPAI infection with the active surveillance protocol, given the prevalence of HPAI disease in the pools of daily mortality.

We used stochastic simulation models to predict the number of HPAI contaminated eggs moved from an infected but undetected house, given the above factors and under different outbreak scenarios. The stochastic disease transmission model estimates the HPAI prevalence, the disease mortality, and the proportion of eggs produced at various time

points post infection of the flock (house) that are internally contaminated. The active surveillance protocol model then uses the transmission model output to estimate the number of contaminated eggs that may be moved before HPAI infection is detected. In the following sections, we first describe the simulation models of disease transmission and active surveillance and then discuss the results under alternate outbreak scenarios.

9.1.3.1 Stochastic Disease Transmission Model

Overview of the disease transmission model

We developed a stochastic SLIR model to simulate disease spread within an infected turkey house. Disease states included in the model are susceptible (S), latently infected (L), infectious (I), and removed (R). The model updates the number of hens in disease states at 0.1 day time steps. The uncertainties in input variables and the inherent variability associated with HPAI infection among different hens within a flock were incorporated into the model.

The model was programed with Visual Basic.NET, and data were output into a spreadsheet for analysis (Excel®, Microsoft Corporation, Redmond, WA). The statistical packages R(87) and statconnDCOM (88) were used to fit statistical distributions to data and model random variables. Further technical description of the disease transmission and active surveillance models is provided in **Appendix 2**.

Modeling of disease state transitions

The number of susceptible hens that become newly infected with HPAI virus in each time step in the model is dependent on the adequate contact rate and the proportion of infectious birds in the house at that time step. Here, β is the adequate contact rate (also called the transmission parameter) and is defined as the mean number of birds each bird comes into contact with per unit time such that the contact is adequate to transmit infection. The other disease transitions, such as transitions from latently infected to infectious states and from infectious to removed disease states, are based on estimated distributions for duration of latently infected or infectious periods in individual hens from experimental studies.

Modeling of egg contamination: timing and drop in egg production rate

In this model, eggs produced before a certain lag period τ_{egg} (hours) after the infection of a hen would not be internally contaminated. Infectious hens were assumed to produce contaminated eggs after τ_{egg} hours post infection onwards. This modeling assumption is supported by experimental data from HPAI infected chicken hens[e] where eggs laid on the first day post inoculation were not contaminated but a significant proportion of eggs laid on day 2 or 3 post infection were contaminated.(23, 31, 89) A possible rationale for this observed data is that a developing egg could be protected from exposure to HPAI virus by membranes during the later stages of egg shell formation when the egg is in the uterus.

[e] Although Narayan et al. recovered HPAI virus from egg yolks from each of 3 eggs laid by 30 week old turkey hens, experimental data on the number of internally or externally contaminated eggs produce at various time points after infection for HPAI infected turkey hens are unavailable.

As described in the hazard identification (**Section 8.9**), a drop in the egg production rate is a frequently observed clinical sign of AI infection and may be particularly pronounced in turkey flocks. We modelled that an infected hen would have decreased egg production rate in the time period post infection when it may produce contaminated eggs. The egg production rates for individual hens were then integrated to estimate the impact on the flock level egg production rate.

Estimation of baseline parameters

The probability distributions for the infectious and latent times are key inputs impacting the disease spread dynamics in an infected flock. In scenarios (A & C), we estimated these parameters from experimental data from HPAI H5N1 inoculated and contact turkeys as shown in **Table 4**.(71, 85) However, there is a considerable variation in these parameters between different HPAI strains (e.g., the infectious period is longer for the 1983 Pennsylvania HPAI H5N2 strain). We considered the impact of a HPAI strain having a longer infectious time in scenarios (B & D).

There is considerable uncertainty regarding the adequate contact rate for turkey flocks in the United States, as the outbreak data required for this type of analysis is limited. The estimates of contact rates in chickens thus far have been based on various approximate methods with estimates ranging from 0.76 to 33 birds per day(90-93). A recent study has evaluated the spread of HPAI H7N1 in 3 sets of experiments. (84) In each of these three experiments, one inoculated bird was introduced to a group of 10, 20, or 40 contact turkeys. Using a simplifying assumption of zero latent time, this study estimated an adequate contact rate of 2.1 (range 1 to 7.1 per day from different experiments). Because a latently infected period at a bird level (defined as a period when a bird is infected but is not shedding detectable virus via RRT PCR) has been observed in most experimental studies, we believe that contact rate obtained from estimation procedures using a non-zero latent period is more applicable for within flock models of HPAI transmission. For the current risk analysis, we chose to reanalyze the data in Saenz et al. (2012) in order to specifically take into account a latent period and estimated β distribution with a mean of 4.35 per day (95% credibility interval of 2.67 to 7.05) per day. We also performed sensitivity analysis with a contact rate of 1.5 per day and 12 per day to evaluate the impact of uncertainty in this parameter as detailed in **Appendix 2**.

Quantitative data on the extent of a decrease in egg production in turkey hens infected with HPAI virus is unavailable. Drops in egg production rates of more than 80 percent have been observed in several LPAI infected turkey breeder houses over a few days.(86, 94) A drop in egg production in turkey breeder hens from 57 percent to 13 percent was observed during the 1983 H5N2 HPAI outbreak in Virginia with 19 percent mortality.(95) Turkey industry experts have stated that similar drops in egg production of over 80 percent would be expected in turkey breeder flocks with HPAI infections as well.

In the 2003 HPAI H7N7 outbreak in Netherlands, a drop in egg production rate of more than 90 percent was observed in table-egg layers and breeders over several days.(96)

Table 4. Baseline parameter estimates for the HPAI transmission model in turkey tom and hen breeder flocks.

Parameter name	Parameter description	Distribution/Value
Effective 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 distribution: mean 4.35; std.dev. 1.13; contacts per day
Latent period distribution	length of the latent period	Scenario A and C: Gamma distributed: mean 1.27 days; std.dev. 0.40 days (71). Scenario B and D Gamma distributed: mean 0.40 days; std.dev. 0.14 days.(84, 92)
Infectious period distribution	length of the infectious period	Scenario A and C: Weibull distributed: mean 1.28 days; std.dev. 1.17 days (71). Scenario B and D Weibull distributed: mean 3.75 days; std.dev. 2 days. (23, 92)
Normal egg production rate	Egg production rate in hens that are not infected with HPAI disease	Uniform (0.45, 0.7) eggs/hen/day (STS Workgroup)
HPAI Infected egg production rate	Estimated egg production rate for an individual HPAI infected hen when it may lay contaminated eggs	75% lower than normal egg production rate
Number of turkey hens/house	Distribution of the number of hens per house	Gamma (7.88, 426) distribution truncated at 1200 and 5500. Mean 3314 birds, std.dev. 1092 birds per house. Estimated from industry data.
Number of turkey toms/house	Average number of toms per house	Uniform (800, 1200) distribution. Mean 1000 birds (STS Workgroup).
Contaminated egg lag period(τ_{egg})	Infectious hens were assumed to produce contaminated eggs after τ_{egg} hours post exposure	19 hours (21, 23, 97)

Data from HPAI H5N2 inoculated chicken hens showed a 29 to 39 percent drop in the egg production rate in infected chicken hens.(21, 23). We used a 75 percent drop in the egg production rate in infected turkey hens on an individual basis in the baseline scenario (i.e. when an individual hen becomes infected, it produces 75 percent fewer eggs than the normal rate of lay). The 75 percent drop in egg production rate was chosen based on data from LPAI and HPAI infected turkey flocks as well as expert opinion from turkey industry veterinarians.

9.1.3.2 Active Surveillance Model

The active surveillance model predicts the potential number of contaminated eggs in a shipment of hatching eggs moved from a turkey hen house by simulating disease detection via diagnostic testing, observation of increased mortality, decrease in egg production rate or where applicable via detection in turkey breeder tom houses supplying semen to the hen flock. Some of the factors considered in the surveillance model may vary under different model scenarios (details provided in **Appendix 2**).

The active surveillance model first simulates the number of diseased birds in pooled samples tested via RRT-PCR, given the number of normal and diseased birds in the daily mortality pool, on each day of RRT-PCR testing prior to movement of hatching eggs. The normal mortality distributions for turkey tom and hen breeder houses were estimated from industry data. The number of diseased dead birds was obtained from the transmission model results.

In the next step, the model simulates detection of HPAI via RRT-PCR testing (matrix gene) of the pooled sample, or by observation of increased mortality or by observing decreased egg production. The sensitivity of the RRT-PCR test is estimated to be 86.5 percent, so there is a 13.5 percent chance infection will not be detected even when the pooled sample contains an HPAI-positive swab.(98, 99) We note that AI Experts commented that this is a conservative estimate of the diagnostic sensitivity given recent enhancement to test protocols.(100-102)

Normal daily mortality data for 26 turkey breeder hen houses were provided by industry representatives. Overall there were 7364 points in this dataset. The daily mortality percentage ranged from 0.0 (5^{th} percentile) to 0.13 percent (95^{th} percentile) with a mean of 0.057 percent.

9.1.3.3 Simulation Model Scenarios

We considered scenarios where either breeder hen or breeder tom flocks first become infected prior to the movement of hatching eggs. Because breeder tom flocks have relatively more stringent biosecurity requirements compared with breeder hen flocks, industry representatives considered HPAI infection of breeder hens to be more likely to occur, so direct infection of breeder hens was considered in the main risk assessment scenarios. As mentioned in **Section 9.1.3.1**, virus strain transmission characteristics can impact time to disease detection in a flock and the disease prevalence at the time of detection. Given the variability in strain characteristics and its impact on the surveillance

outcomes, we evaluated HPAI virus strains with 2 different infectious periods (short and medium length).

9.1.3.3.1 Baseline Scenarios A and B (Breeder Hen Flock is Assumed to Have Been Infected First)

Scenarios A and B consider that HPAI infection was first introduced into a turkey breeder henhouse starting with one exposed bird.

- *Scenario A*: Latent and infectious (1.28 days) period distributions representative of Asian HPAI H5N1 strains characteristics were used.
- *Scenario B*: A longer infectious period of 3.75 days was used in this scenario compared with scenario A.

9.1.3.3.2 Alternate Scenarios C and D. (Breeder tom flock is assumed to have been infected first)

Supposing breeder toms were to become infected prior to movement of turkey semen, there is a possibility that multiple breeder hens could be simultaneously exposed through contaminated semen or through the insemination crew, resulting in significantly different disease spread dynamics (1 vial of semen may be used to inseminate as many as 200 hens). In scenarios C and D, we assumed that HPAI virus is present in semen from infectious turkey toms in sufficient titers to be infectious, and can result in simultaneous exposure of multiple hens via insemination.

- *Scenario C*: Latent and infectious (1.28 days) period distributions representative of Asian HPAI H5N1 strain characteristics were used.
- *Scenario D*: A longer infectious period of 3.75 days was used in this scenario compared to scenario C.

9.1.3.4 Simulation Results on the Likelihood of Moving HPAI Contaminated Hatching Eggs from an Infected but Undetected Turkey Breeder Hen House under Various Scenarios

Turkey Breeder Hens First Infected (Results for Scenarios A and B)

Figure 3 and **Figure 4** show the mean number of latent, infectious and dead birds on progressive days post infection of a barn under scenarios A and B. The key transmission model results under scenarios A and B, based on 8000 simulation iterations, are summarized in **Table 5**. The predicted mean number of internally contaminated eggs moved from a HPAI infected but undetected turkey house was very low based on simulation results, 0.008 (90 percent P.I., 0-0) or 0.007 (90 percent P.I., 0-1) eggs/house-moved, depending on the HPAI virus strain. Multiple factors such as the smaller flock size and correspondingly lower normal house mortality resulting in earlier detection with RRT-PCR testing, and the 2-day holding time after production before the movement of eggs, contribute toward reducing the likelihood of moving a large number of internally contaminated eggs before infection is detected.

Figure 5 illustrates the interaction between various factors impacting the number of internally contaminated hatching eggs moved before infection is detected. The box plots depict the predicted number of internally contaminated eggs produced from an infected turkey hen house 2 days before movement day. The overall probability of detection via diagnostic testing and observation of flock mortality increases with each progressive day post infection of the flock.

There is considerable variability in the rate of decrease in egg production in HPAI infected breeder flocks. For example, the variability in the drop in egg production for four simulation model iterations is shown in **Figure 6**. This variability is derived from the variability in the course of HPAI disease spread within the flock. In this assessment, a total decrease in egg production rate of 15 percent or more occurring over a two day period was considered as a quantitative detection trigger to detect a drop in egg production. In the active surveillance simulation model, HPAI detection in a breeder flock occurs when a simulated drop in egg production reaches or exceeds this quantitative trigger.

The simulation results from **Table 5** for the predicted number of infectious birds, in iterations where HPAI infection was not detected by movement day, are used in evaluating the likelihood of hatching egg handling materials being contaminated.

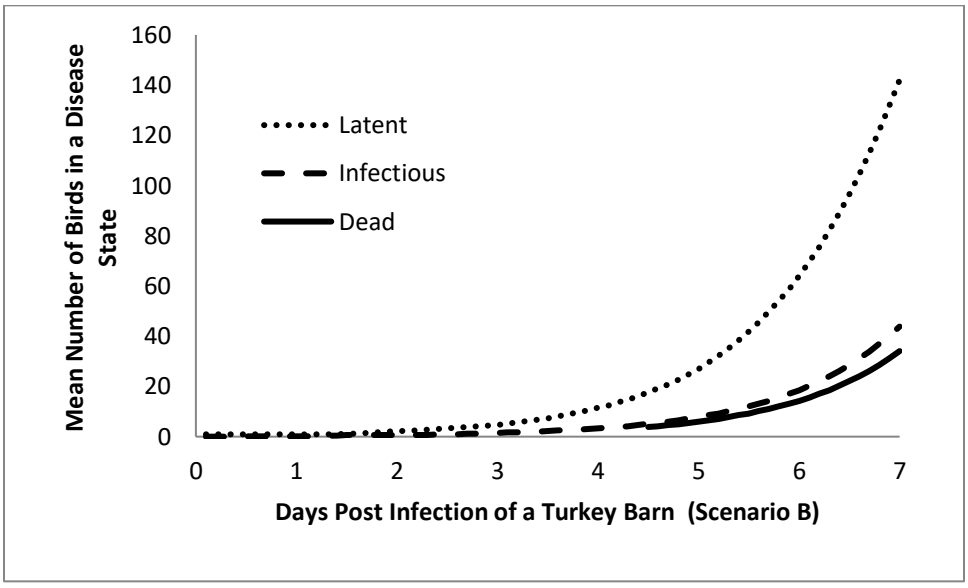


Figure 3. Average number of latent, infectious, and dead birds from days 0 to 7 post flock infection under scenario A.

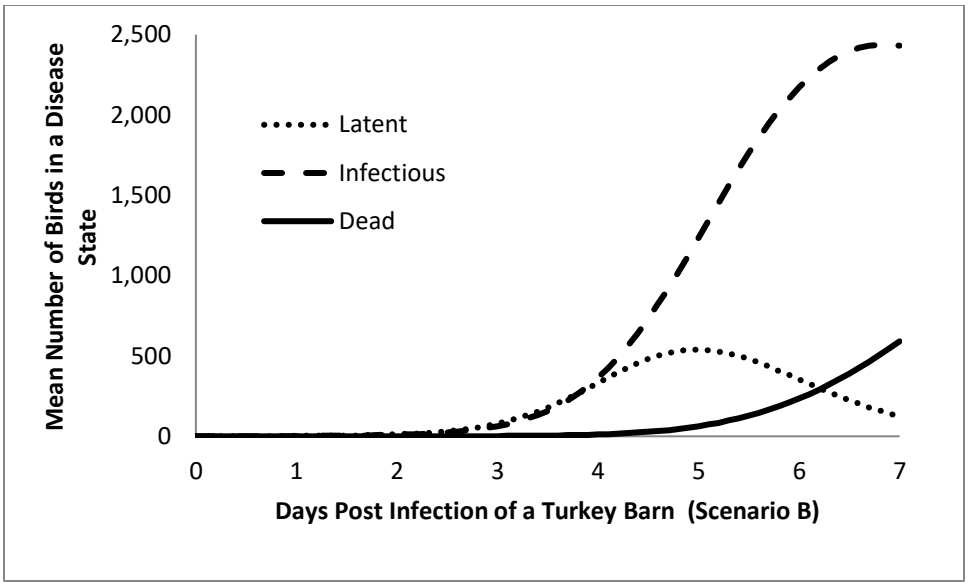


Figure 4. Average number of latent, infectious, and dead birds from days 0 to 7 post flock infection under scenario B.

Table 5. Results from simulation models of disease transmission and active surveillance for scenarios where breeder hen flocks are first infected (Scenarios A and B).

Output parameter	**Scenario A (Infectious period 1.28 days)**	**Scenario B (Infectious period 3.75 days)**
Model predicted number of internally contaminated hatching eggs moved from a turkey breeder hen house given a 2-day holding time after production (eggs per movement)	0.008 (0-0)*	0.007 (0-0)
Approximate probability of detecting HPAI in breeder hen flocks by hatching egg movement day that is *Y* days post infection of the flock (averaged over all days *Y*)	74 % (*Y*: 2-7 days)	81% (*Y*: 2-7 days)
Predicted number of infectious birds at movement in iterations where infection was not detected by movement day	1.2 (0-4)	21 (2-78)
Predicted number of infectious birds 2-days before movement (latest possible packing time for eggs moved with a 2-day hold) in iterations where infection was not detected by movement day	0.09(0-1)	0.48 (0-2)

*Approximate 90 percent probability interval

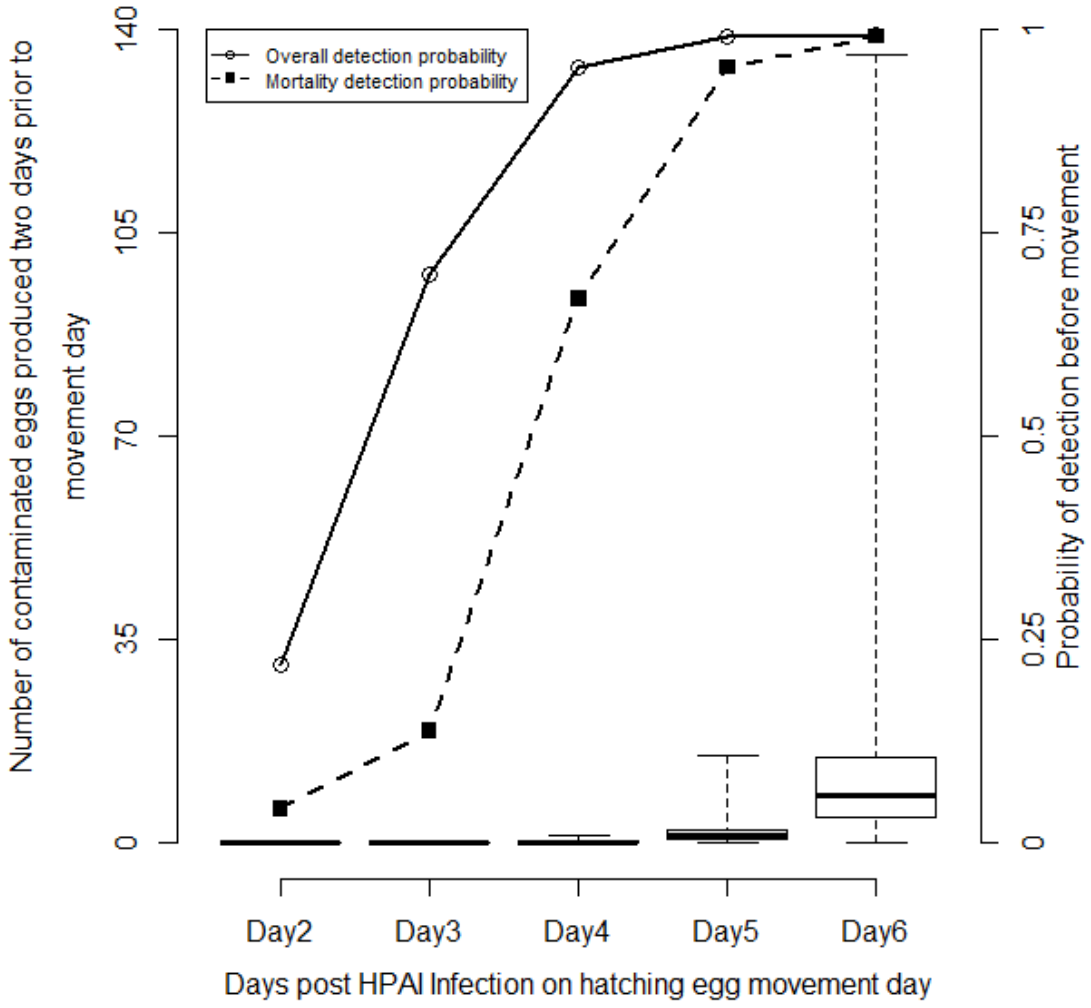


Figure 5. Probability of detecting HPAI infection in a turkey henhouse by the day of movement of hatching eggs. Movement day may occur on different days post infection of the house. The box plots indicate the number of contaminated eggs produced 2 days before hatching egg movement day. The mortality detection probability is for detection via observation of increased mortality alone without diagnostic testing, whereas the overall detection probability includes detection by the mortality trigger as well as active surveillance testing in hen flocks.

We conclude that the likelihood of moving internally contaminated hatching eggs from an infected but undetected breeder farm under scenarios A and B is *low*.

Turkey Toms First Infected (Results for Scenarios C and D)

We first briefly discuss results regarding the likelihood of detecting infection in tom flocks and then discuss the likelihood of moving contaminated eggs based on simulation of HPAI disease spread and detection in breeder hen flocks.

Detection in Tom Houses

Turkey breeder tom houses are smaller in capacity relative to henhouses and thus have a lower normal daily mortality count (typically 0 or 1 birds per day). In general, a lower normal mortality is expected to result in earlier detection of HPAI infection through observation of abnormally high mortality or through diagnostic testing of dead birds prior to semen movement. **Table 6** shows simulation results on the likelihood of detection of HPAI in a breeder tom flock by semen movement day.

Table 6 Mean detection likelihoods and the number of infectious birds present on semen movement day in tom flocks under Scenarios C and D (once weekly movement of semen).

Output	**Scenario C (Baseline infectious period 1.28 days)**	**Scenario D (Longer infectious period 3.75 days)**
Mean detection likelihood by semen movement day [a]	79	75
Infectious turkey toms present on semen movement day if HPAI is undetected by the day of semen movement	2.49 (0-10) [d]	11 (1-49)
Mean detection likelihood in tom flocks by two days after semen movement day [b]	96	95
Mean detection likelihood in tom flocks by three days after semen movement day [c]	99.6	99.3

[a] Probability of detection before contaminated semen movement.

[b] The earliest day that externally contaminated eggs could be moved with a 2-day hold.

[c] The earliest day that internally contaminated eggs could be moved with a 2-day hold.

[d] Approximate 90 percent probability interval from 8000 iterations.

The results indicate that HPAI infection is detected in a breeder tom flock by semen movement day in the majority of cases. However, there may be a moderate likelihood of HPAI being detected after semen movement day. A potential reason for not detecting HPAI before semen movement in some cases is that the tom flock was exposed to HPAI

virus within a short duration (1-2-days) of semen movement and disease mortality has not yet occurred or is very low.

Detection in Breeder Hen Houses or Breeder Tom Houses Before Potential Movement of Internally Contaminated Hatching Eggs

In this case, there is a high probability of HPAI disease detection in either turkey breeder tom or hen flocks by the time internally contaminated hatching eggs may be moved from the hen flock after exposure via insemination. Multiple detection mechanisms are applicable for this scenario as detection may occur either in breeder tom or hen flocks. In the following, we briefly review the relevant detection mechanisms and then present the overall simulation results.

We use the example timeline shown in **Figure 7** to explain the analysis in this section. In this hypothetical timeline, a breeder hen flock is exposed to HPAI virus via insemination on Monday. The earliest day that internally contaminated eggs could be laid would be Tuesday. Given a 2-day hold as described in **Section 9.1.2.2**, the soonest those eggs could be moved would be Thursday. By Thursday, there is a high probability of detection either in tom or hen flocks via multiple mechanisms.

The overall probability of detection (i.e. considering all of the HPAI detection mechanisms) by the earliest estimated day when internally contaminated eggs may be moved from a breeder hen flock exposed 3 days after insemination was estimated to be 99.9 under scenarios C and D (**Table 7**). The model predicted number of internally contaminated hatching eggs moved from a turkey breeder house given a 2-day hold time after production (eggs per movement) was 0 (95 percent P.I., 0-0) under these scenarios.

- Detection via RRT-PCR (Matrix Gene) testing in turkey breeder henhouses: Simulation results indicate approximately 99.9 percent detection via RRT-PCR tests in scenarios C and D, before movement of internally contaminated eggs. In these scenarios, given that multiple hens are expected to have been simultaneously exposed via insemination, the disease mortality is predicted to be relatively high (i.e. meaning that multiple pools of 5 swabs are tested due to the high numbers of dead birds), increasing the likelihood of detection via diagnostic testing of dead birds.
- Detection via increased mortality in turkey breeder henhouses: There was 99.9 percent detection via the mortality trigger before the day internally contaminated eggs may have been moved after exposure of hen flocks via insemination in Scenarios C and D.
- Detection via drop in egg production in turkey breeder henhouses: There was 2.7 percent detection via a drop in egg production in Scenario C and a 98 percent detection in Scenario D before the day internally contaminated eggs may have been moved after exposure of hen flocks via insemination. As proposed in Weaver et al. (2012), the infectious period and latent period of the virus strain may have a considerable impact on the detection by drop in egg production.(103)

- Detection in breeder tom flocks: We estimated a 91.3 and 97.5 percent likelihood of detection in breeder tom flocks in Scenarios C and D respectively before internally contaminated eggs may be moved from a breeder hen flock that became infected via semen movement after a 2-day hold. These likelihood estimates were made only for outbreaks where infection was missed in the tom flock.

We conclude that the likelihood of moving internally contaminated hatching eggs from an infected but undetected breeder farm under scenarios C and D is *negligible* to *low*.

Table 7 Results from simulation models of HPAI disease transmission and active surveillance for scenarios where the breeder tom house is first infected and the hen flock is subsequently infected via insemination (Scenarios C and D). The predicted detection probability is the overall detection probability for all detection mechanisms in both the hen and tom flocks.

Output parameter	Scenario C (Infectious period 1.28 days)	Scenario D (Infectious period 3.75 days)
Model predicted number of internally contaminated hatching eggs moved from a turkey breeder house given a 2-day hold after production (eggs per movement).	0.00 (0-0)*	0.00 (0-0)*
Predicted detection probability by 2-days after exposure via insemination.	99.9%	99.9%
Predicted detection probability by 3 days after exposure via insemination.	99.9 %	99.9 %

*Approximate 90 percent probability interval from 8000 iterations.

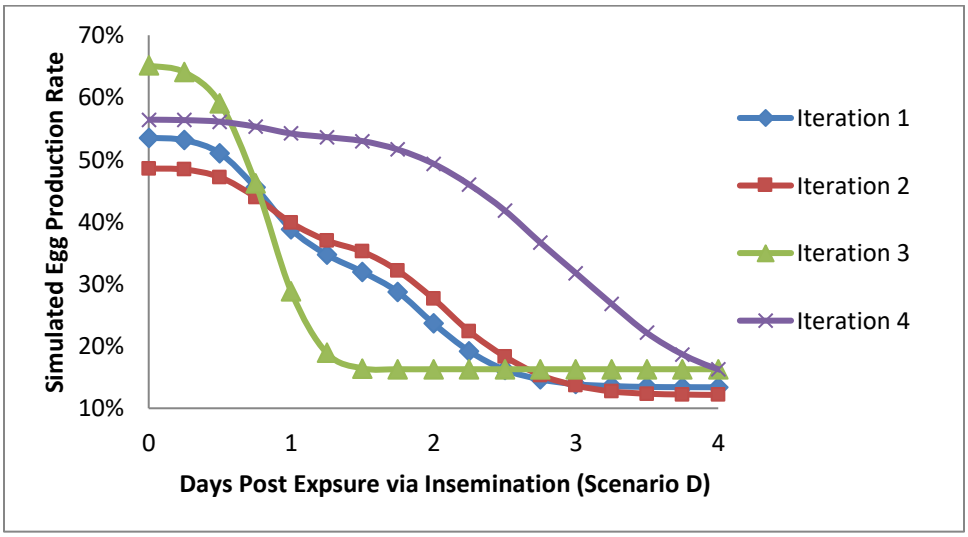
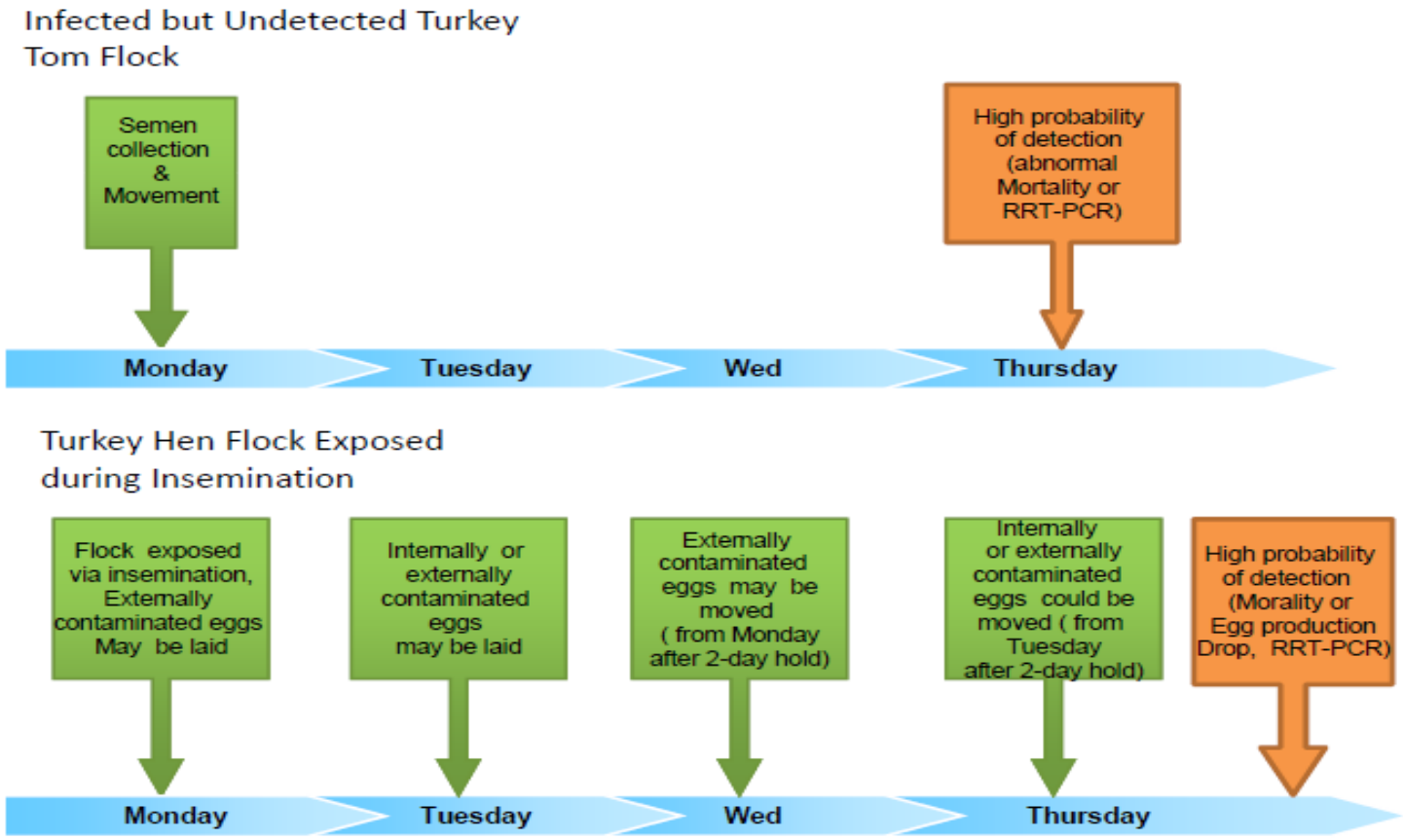


Figure 6. Examples of simulated egg production over progressive days post infection of a breeder hen flock via insemination under Scenario D for four iterations. The variability in

the egg production rate in these simulation iterations is derived from variability in the course of HPAI disease spread within the flock.

Figure 7. Example timeline and considerations for scenarios C and D.



9.1.4 Likelihood and Degree of HPAI Virus Contamination on the Eggshell

Eggs may be externally contaminated directly at oviposition by fecal material within the cloaca, or may become cross-contaminated after they are laid through contact with organic material in the environment such as feces from infected hens or contact with other eggs on the egg-belt or floor.

Studies of HPAI H5N2 infected chickens have shown the frequency of contamination on the eggshell to be similar to, or lower than, the frequency of internal content contamination.(23, 89, 97, 104) Specifically, in these studies, eggs laid on the first day post-inoculation were not contaminated; however, by the time of death (3 to 6 days), 35 to 45 percent of the eggs laid by infected hens overall were contaminated internally and externally.

Available data from chicken flocks naturally infected with avian influenza viruses also indicate the frequency of contamination of the eggshell is less than or equal to the frequency of internal content contamination. In a natural outbreak study of HPAI H5N2 infected commercial layer hens, virus was isolated from the albumen in 11 out of 24 eggs and from the shell surface in 2 out of 22 eggs.(95) Assuming these data were obtained through random sampling, there is a 99 percent chance the true proportion of externally contaminated eggs in this flock was lower than the true proportion of internally contaminated eggs.[f] On LPAI H7N2 infected broiler breeder farms, virus was not found on any eggshells sampled, although virus could be isolated from 90 percent of chickens within the flock (tracheal or cloacal swabs) and 50 percent of dust and manure swabs. (105)

In turkey breeder hens experimentally infected with swine origin LPAI H3N2, virus was detected on the shell surfaces of eggs at 3 days post infection, and virus was isolated from all eggs laid on and after day 4 post infection.(26) In this study, the frequency of external contamination of turkey eggs with LPAI virus was estimated to be statistically higher (34/69 eggs or 49 percent) compared with albumen (20/73 eggs or 27 percent).

In recent unpublished data, the average viral titer on contaminated eggshells from HPAI H5N2 infected chickens on days when contaminated eggs were laid was between $10^{3.4}$ to $10^{3.6}$ EID_{50}/eggshell from 16 contaminated eggs.(97, 104) A 2011 study, showed maximum virus titer on individual eggs from HPAI H5N2 infected chicken hens can be as high as $10^{5.9}$ EID_{50}/eggshell.(23) In this assessment, we considered that the viral titer on a contaminated eggshell, before disinfection, ranges from $10^{3.4}$ EID_{50} to $10^{5.9}$ EID_{50} /eggshell.

[f]The uncertainty associated with the true proportion of externally contaminated eggs was modeled as a Beta (12, 14) probability distribution. The uncertainty associated with the true proportion of externally contaminated eggs for this flock was modeled as Beta (3, 21) distribution.

Turkey Breeder Hens First Infected (Results for Scenarios A and B)

Given observations from field studies and laboratory experiments, we used the results of our model for the daily number of internally contaminated eggs to estimate the daily number of externally contaminated eggs, regardless of the source of external contamination. We estimate that the number of externally contaminated eggs per movement of hatching eggs from infected but undetected premises would be 0.008 (90 percent P.I., 0-0) or 0.007 (90 percent P.I., 0-0) eggs/house without disinfection, depending on the HPAI virus strain.

Turkey Breeder Toms First Infected (Results for Scenarios C and D)

It is theoretically possible that semen contaminated with HPAI virus, deposited in the uterus during artificial insemination, could contaminate the eggshell surface of eggs in utero. We are uncertain as to whether or not semen is contaminated systemically or by virus replication in male reproductive and accessory sex organs, as well as to the level of virus titer that might be present in semen from birds infected with HPAI virus. In addition, breeder toms in the later stages of HPAI infection (24 to 30 hours post infection), when viral titers reach high levels (10^7 EID_{50}) systemically, may not be capable of producing semen.(106) However, HPAI virus antigen was detected in testes tissue in experimentally inoculated toms.(107) To address this uncertainty, we assumed that semen could become cross contaminated with feces in the cloaca during collection and that titers would be similar to those in feces (10^3 to 10^7 EID_{50}/ml) during the period of viral shedding in the gut before the bird enters an advanced disease state. Because one vial of extended semen (1:20 dilution) is used to inseminate as many as 200 turkey breeder hens, several eggs may become externally contaminated via contact with HPAI contaminated semen in these scenarios. In addition, at a dilution of 1:20 (1.3 log reduction), there would not be a significant reduction in viral titer within the semen.

In Scenarios C and D, there is a high probability of detection (99.9 percent based on simulation results) before any externally contaminated eggs may be moved from the breeder house. Multiple detection mechanisms such as RRT-PCR testing, drop in egg production, or by increased mortality in tom or hen flocks are applicable for these scenarios. Given the two day holding time after production before movement of eggs, the simulation models indicate a *very low* likelihood that eggs externally contaminated with HPAI virus from semen would be moved off the breeder premises even in the absence of washing and sanitizing in these scenarios.

Impact of sanitizing

In this section, we estimate the degree of HPAI viral inactivation on hatching eggs with the application of an EPA registered disinfectant active against avian influenza virus and used according to manufacturer label directions OR using a chlorine based sanitizer with a 200 ppm or more concentration.

For EPA registered disinfectants, the label claims against influenza viruses must be supported by efficacy data that demonstrate a 3-log reduction of avian influenza viral titers on applicable surfaces. Additionally, the data must be generated in GLP (Good Laboratory Practices, 40CFR160) certified labs utilizing agency accepted protocols. Details concerning the nature of the various EPA registered virucides, registration

requirements, efficacy testing and samples of virucide testing tables are included in Appendices 4, 5 and 6 of the previously completed *Nest Run Eggs Risk Assessment*.(108) We expect at least a 3-log reduction in the viral titer on the egg shell surface (not including the pores)[g] of HPAI virus contaminated hatching eggs that are nest clean (do not have excessive adhering organic matter) when using an EPA registered disinfectant under operational conditions that match or exceed the requirements for virucidal label claims against avian influenza virus. A review of manufacturer labels indicated that there are disinfectants for which the recommended concentration for application on hatching eggs is within the concentration range required for virucidal uses against avian influenza virus.

A previous *Risk Assessment on the Movement of Washed and Sanitized Shell-eggs* estimated that the viral load on the eggshell would be reduced by a factor of at least 1,000 (a 3-log reduction) given a 1 to 8 second exposure time with a 200 ppm chlorine rinse.(109, 110) This was based on experimental data testing HPAI H5N1 virus inactivation in allantoic fluid at neutral sanitizer pH (7 to 8) and simulation of decrease in available chlorine due to organic load.

Efficacy of HPAI virus reduction with chlorine at higher pH has not been tested to our knowledge. Higher pH values may cause the less potent ionic form of chlorine (OCl^-) to predominate in solution, making virus inactivation less efficient. A 97 percent chance that a 1000 factor (3-log) inactivation of HPAI virus on eggshells is achieved was estimated in the *Washed and Sanitized Shell-egg Risk Assessment* at a sanitizer pH of 10, at 32 °C, and utilizing data on inactivation of other indicator viruses (Hepatitis B).(109)

Dirty eggs are typically not preferred for setting because they have lower hatchability and may contaminate other hatching eggs. NPIP regulation 9CFR147 requires participating hatcheries not to set dirty hatching eggs in setters. Given the higher organic load, inactivation with sanitizing may be less than 3-logs for hatching eggs that are dirty.

9.1.5 Conclusion

In this section, we evaluated the likelihood and degree of internal and external contamination of turkey hatching eggs from an infected but undetected breeder flock. In our evaluation, we considered potential mitigation steps to reduce external contamination, such as the sanitizing of eggs and biosecurity steps for farm personnel. Additionally, we considered the active surveillance protocol and the 2-day egg holding period specified in the STS Plan that would be implemented in the event of a HPAI outbreak.

Based on our reported assumptions and methodology, various scenarios of HPAI disease transmission in breeder hens that were directly exposed or exposed via the insemination process with HPAI virus from breeder tom flocks were simulated. Results for a turkey breeder flock that adheres to the active surveillance protocol and 2-day, on-farm, egg-holding period specified in the STS Plan are as follows:

[g] For the purpose of this assessment, HPAI virus that may be present within the eggshell pores is not considered when referring to eggshell surface contamination. Based on expert opinion (Dr. Syed Sattar and Dr. Dean Cliver), virus present within the pores (of porous materials in general) is less likely to be transferred on to contact surfaces.

- The predicted number of internally contaminated hatching eggs per movement from an infected breeder henhouse before detection was very low under all the scenarios considered (the mean varied between 0 to 0.008 eggs per movement under different scenarios). The results indicated a 95 percent chance of no internally contaminated eggs being moved from an infected house before detection for the model scenarios considered. (There are around 6700 eggs per movement from a house on average when eggs are moved twice weekly).

We concluded that the likelihood of moving internally contaminated hatching eggs from an infected but undetected breeder farm under scenarios A and B is *low*. We also concluded that the likelihood of moving internally contaminated hatching eggs from an infected but undetected breeder farm is *negligible* to *low* under scenarios C and D where the possibility of breeder hen farm becoming infected via semen movement from the breeder tom flock is considered. Therefore, the overall likelihood of moving internally contaminated eggs is estimated to be *low*, given the uncertainty in HPAI disease spread model parameters.

- The predicted number of eggs that were externally contaminated prior to sanitizing per movement was *low* under all the scenarios considered. Sanitizing with an EPA registered disinfectant or chlorine rinse with the concentrations equal to or greater than 200 ppm is predicted to cause at least a 3 log reduction in HPAI virus concentration on the eggshell. Considering the impact of sanitizing, the degree of external contamination is expected to be *low* in in the unlikely case where an externally contaminated egg is moved from an infected breeder house before detection.

9.2 Likelihood that Hatching Egg-Supporting Materials Loaded onto a Vehicle at an HPAI Infected but Undetected Breeder Premises are Contaminated

- **Risk Factors:** Late detection of HPAI infection in a flock; movement of personnel and equipment between the henhouse and egg packing or storage areas; cross contamination of egg handling material via personnel; activities associated with loading eggs on the truck.
- **Current Preventive Measures:** Sanitizing of setter trays along with eggs in some operations; dedicated hatchery buggies that are not taken inside the henhouse; use of baskets or trays that do not leave the farm for hand gathering eggs.
- **Outbreak Specific Measures:** (to be implemented by industry during an outbreak)**:** Active surveillance of flocks; 2-day holding period after production before movement of eggs off the farm; disinfection of hatching egg buggy wheels and the storage room floor prior to loading or disinfection of buggy wheels before moving them into the storage room; use of PPE by farm personnel before entering the hatching egg storage rooms.

9.2.1 Introduction

Egg handling materials have been implicated in the spread of AI between table-egg layer premises in previous outbreaks.(67, 111) Pearson et al. (1984) reported that several samples from the egg belt, egg room floor, and egg flats were positive for HPAI virus during the 1983 Pennsylvania outbreak.(112) Hence, contamination of setter trays from environmental surfaces must be considered a possibility, although quantitative data are unavailable. We performed a qualitative review of this pathway as follows.

In this chapter we estimate the likelihood and degree of contamination of turkey hatching egg handling materials moved from an infected breeder premises before detection considering the applicable current and outbreak specific measures from the STS Plan.

9.2.2 Preventive Measures

9.2.2.1 Current Preventive Measures

Some operations gather eggs onto setter trays first and then sanitize the trays and eggs together, while others may manually transfer eggs from baskets or flats to a belt for sanitizing and then transfer sanitized eggs onto setter trays. In this setup, setter trays would have been cleaned and disinfected at the hatchery before being distributed to the breeder farm.

In routine operations, carts used to carry setter trays into the hen house remain on the farm (i.e. remain separate from carts used to transport setter trays to the hatchery).

9.2.2.2 Outbreak Specific Preventive Measures

The outbreak specific measures considered in this document are from the STS Plan.

- Hatching eggs must be washed and sanitized with a chlorine rinse with concentrations equal to or greater than a 200 ppm or with an approved disinfectant for avian influenza virus according to the manufacturer's label directions for application on hatching eggs. Eggs are washed and sanitized while on flats in cases where flats are taken into the hen house, or are transferred to clean flats after being washed or sanitized. Employees who manually transfer eggs must wash their hands with soap and water or use a hand sanitizer.
- Farm personnel should disinfect the egg storage room floor and buggy wheels before the buggies are moved for loading; or soak the buggy wheels with disinfectant (being careful to cover the entire circumference of the wheel) prior to moving egg buggies into the egg storage room cooler.
- Farm personal should don gloves and disposable or cleaned and disinfected boots before entering egg storage coolers.

9.2.3 Plastic Setter Trays

The likelihood and degree of contamination of setter trays depends on whether the eggs are hand gathered or belt gathered. This is because the setter trays are often brought inside the henhouse when eggs are hand gathered, unlike in belt gathering. We evaluate these scenarios separately as follows.

9.2.3.1 For Farms Employing Belt Gathering

Some turkey breeder houses employ belt gathering of eggs. The key pathways for contamination of setter trays for this scenario are: 1) indirectly via personnel contacting contaminated egg belts, eggs, and other henhouse surfaces and subsequently contacting setter trays; 2) via contact with externally contaminated eggs; 3) via contact with contaminated environmental surfaces; and 4) via aerosolized dust or organic matter originating from the henhouse.

The likelihood and degree of contamination of trays during the packing process depends on the number of infectious birds present at the time of packing. In general, active surveillance reduces the time to detect HPAI infection in the flock. Shorter time to detection would also imply a lower proportion of infectious birds in the time interval before HPAI infection is detected. In addition, the 2- day holding time would further reduce the likelihood that externally contaminated eggs would be moved off the farm, as they are less likely to be packed at a time when the proportion of infectious birds is high. The likelihood and degree of contamination of setter trays moved from the premises after production and before eggs are moved off the farm, would be significantly reduced given the active surveillance protocol and the 2-day holding period as specified in the STS Plan.

Turkey Breeder Hens First Infected (Results for Scenarios A and B)

The predicted mean number of infectious birds on the day of packing from simulation results (48 hours before movement) for eggs moved from an infected but undetected premises was 0.09 (95 percent P.I., 0-1) to 0.48 (95 percent P.I., 0-2) infectious birds per turkey breeder hen house under scenarios A and B, respectively (**Table 5)**. Logically, a

lower proportion of infectious birds at the time of packing should also result in a lower likelihood and degree of contamination of the setter trays from environmental surfaces, such as the egg belt, via direct and indirect pathways.

A greater number of infectious birds are expected to be present in the flock on the day of movement of eggs to the hatchery (1.2 (90 percent P.I., 0-4) to 21 (90 percent P.I., 2-78) infectious birds (**Table 5**)), relative to the number of infectious birds on the day of packing. However, for setter trays containing eggs that are stored in the cooler, the likelihood of contamination is *low,* because there would be minimal opportunity for contact with contaminated surfaces or personnel. Once setter trays are packed with eggs and placed onto buggies or pallets, there is no specific need for farm personnel to contact them directly. In addition, there would be no direct airflow from the henhouse to the egg storage room on the breeder farm. While aerosol contamination through recirculation of henhouse aerosols from outside is a possibility in theory, we estimate that the degree of contamination through this pathway would be insignificant. In a natural outbreak study, there was a 100 factor (2-log $TCID_{50}$) reduction in HPAI viral titer from the inside of an infected barn (with a high proportion of infectious birds) to the outside of the barn (at a titer of 12 $TCID_{50}/m^3$ outside the barn).(113) The HPAI viral concentration in air from an undetected farm under active surveillance would be lower compared to the estimates from the above study, due to the lower number of infectious birds.

The likelihood of setter trays contaminated by HPAI virus present on the eggshells being moved from the premises would be *low,* considering the low estimated number of externally contaminated eggs moved before HPAI infection is detected (0.008 (90 percent P.I., 0-0) or 0.007 (90 percent P.I., 0-0) eggs/house without disinfection, depending on the HPAI virus strain). Efforts to produce nest clean eggs reduce the likelihood that floor-eggs or visibly dirty eggs are placed onto setter trays.

The pathway for contamination of setter trays from eggs via the hands of farm personnel involves two virus transfer steps via direct contact: 1) contaminated egg to hands; and 2) hands to setter trays. Data from expert opinion and experimental studies with indicator viruses suggest 6 to 27 percent of virus may be transferred onto a recipient surface in each transfer step. Considering the two transfer steps, the viral titer on setter trays contaminated through this pathway would be 1 to 2 logs lower than the titer on eggshell surface (approximate range 0 to 2 log EID_{50}/cm^2).

Outbreak specific measures proposed by the STS Plan require that turkey hatching eggs be washed and sanitized with an EPA registered disinfectant against avian influenza or sanitized with a chlorine rinse of equal to or greater than 200 ppm. Turkey hatching eggs may be washed while on the setter trays, or eggs and setter trays may be sprayed with a disinfectant after packing. Turkey hatching eggs are not typically brushed during washing to remove organic debris, and a detergent soap is typically not used in the wash water. Using both washing and sanitizing steps would have a greater impact in reducing viral load as compared to operations that only use sanitization. Considering the above factors, we rated the likelihood of setter trays from an infected but undetected breeder premises where eggs are belt gathered being contaminated with HPAI virus at the time they are loaded onto the vehicle as *negligible* to *low*, depending on the method of disinfection used.

Turkey Breeder Toms First Infected (Results for Scenarios C and D)

For scenarios where the turkey breeder tom flock is first infected, there is a high likelihood of detection by 3 days after insemination day (**Table 8** and **Table 9**). For eggs moved within two days post insemination, the predicted number of infectious birds on the day of packing (48 hours before movement) was close to 0 in 8000 iterations (**Table 8**).[h]

Table 8. Simulation model estimates of the overall probability of HPAI detection and the number of infectious birds at the time of hatching egg packing and movement for Scenario C.

	Day of movement in relation to insemination day (ID)		
	ID +1 day	**ID + 2-days**	**ID + 3 days**
Detection percent by movement	99.3%	99.9%	99.9%
Infectious birds at movement if not detected	50 (41-61) *	—	—
Infectious birds at packing (2-days prior to movement) if not detected	0	—	—

* Interval is approximate based on 5th and 95th percentiles in 8000 simulation iterations.

—HPAI infection was detected in all of the observed simulation iterations and hence there were no simulation results to estimate this parameter.

Table 9. Simulation model estimates of the overall probability of HPAI detection and the number of infectious birds at the time of hatching egg packing and movement for Scenario D.

	Day of movement in relation to insemination day (ID)		
	ID +1 day	**ID + 2-days**	**ID + 3 days**
Detection percent by movement day	99.2 %[#]	99.9%	99.9%
Infectious birds at movement if not detected	371 (266-647)*	—	—
Infectious birds at packing (2-days prior to	0	—	—

[h] Insemination day (semen movement day) was assumed to be independent of hatching egg movement day. Hatching eggs could be moved at any time after insemination.

movement) if not detected

* Interval is approximate based on 5th and 95th percentiles in 3000 simulation iterations

In this scenario, detection only occurs in tom flocks as no mortality has occurred in hen flocks.

— Indicates insignificant

We rated the likelihood of setter trays from an infected but undetected breeder premises where eggs are belt gathered being contaminated with HPAI virus at the time they are loaded onto the vehicle to be *negligible* to *low*, depending on the method of disinfection used. This likelihood estimate considers the small proportion of outcomes (0.8%) where infectious birds may be present in the flock if the movement day occurred on the day after insemination (50 (90 percent P.I., 41-61) and 371 (90 percent P.I., 266-647) infectious birds/hen flock for Scenarios C and D respectively.)

9.2.3.2 For Farms Employing Hand Gathering

In this case, the setter trays may be brought into the henhouse on farm carts or similar equipment to pack hand gathered eggs. There would be a relatively greater likelihood of the hands of farm personnel becoming contaminated due to contact with nests and other henhouse surfaces. The very low estimated number of infectious birds on the day of packing (48 hours before movement) for eggs moved from an infected but undetected premises, as discussed previously, would significantly reduce the likelihood of contamination through this pathway (**Table 5**, **Table 7**, and **Table 8**).

The likelihood of contamination of setter trays via aerosolized dust within the henhouse at the time of packing would also be *low*, given the small number of infectious birds at the time of packing. A virus titer of 292 $TCID_{50}/m^3$ was estimated inside an infected layer house where birds experienced a high mortality during the HPAI H7N7 outbreak in Canada (or approximately 2920 EID_{50}/m^3)(114). The number of infectious birds in an undetected flock under active surveillance at the time of packing (two days before movement) in our scenarios is likely significantly lower than number of infectious chicks in the barn in this experiment (**Table 9**). The relatively lower number of infectious birds and the correspondingly lower aerosol virus concentrations is unlikely to cause any significant contamination of setter trays.

As described in **Section 9.2.2.2**, eggs are washed and sanitized while on flats in cases, where flats are taken into the henhouse, or are transferred to clean setter trays after being washed or sanitized. Outbreak specific measures from the STS Plan require that hatching eggs must be washed and sanitized with a chlorine rinse equal to or greater than a 200 ppm concentration or with an EPA registered disinfectant for avian influenza virus according to the manufacturer label directions for application on hatching eggs. Egg trays used to handle hatching eggs are designed in such a way that there is minimal contact between the egg and the support on the tray, such that washing should still expose the majority of the egg surface to cleaning and disinfection (i.e. the eggs do not sit in an enclosed cup on the tray as in Table egg trays). Employees who transfer washed and sanitized eggs onto cleaned and sanitized setter trays are required to wash their hands or

use hand sanitizer before handling sanitized eggs to prevent cross contamination. We rated the likelihood of setter trays from an infected but undetected breeder premises where eggs are hand gathered being contaminated with HPAI virus at the time they are loaded onto the vehicle as *low*.

9.2.4 Buggies or Pallets

Buggies (farm carts) used to collect eggs in the henhouse generally remain on the farm. However, there is a possibility that cleaned and sanitized buggies or pallets delivered to the farm from the hatchery could become contaminated. The main pathways for buggies or pallets to become contaminated with HPAI virus include:

- Through contact with egg packing room or egg storage room floors which were contaminated via movement of personnel or equipment (farm carts or buggies) from the henhouse.
- Through personnel contacting infected birds or contaminated surfaces within the henhouse and subsequently handling buggies or pallets.

The likelihood and degree of contamination through these pathways would depend on various factors. These factors include the proportion of infectious turkeys; the frequency of personnel movements between the henhouse and the packing and storage areas; the extent of mixing of organic matter within the henhouse; and the amount of contaminated material that adheres to shoes and is transferred to the packing area floor with each movement.

9.2.4.1 Likelihood of Contamination of Buggies at the Time of Packing Hatching Eggs

The active surveillance protocol reduces the likelihood that an infected flock with a high proportion of infectious birds will not be detected. The 2-day holding period after collection further reduces the likelihood that eggs packed at a time when the proportion of infectious birds is high will be subsequently moved off the farm. For Scenarios A and B, simulation results indicate that 2-days prior to the day eggs are moved from an infected but undetected flock, the average number of infectious birds would be 0.09 (95 percent P.I., 0-1) or 0.48 (95 percent P.I., 0-2) birds for a flock of turkey breeder hens, depending on HPAI virus strain (**Table 5**).

For Scenarios C and D, the number of infectious birds would be insignificant at the time of packing of eggs that may be moved from an undetected flock after holding for two days (**Table 8** and **Table 9**). For eggs produced on the day of insemination, a very low number of infectious birds would be expected, considering the latent period.

The likelihood of contamination of buggies also depends on factors such as the amount of virus shed per infectious bird and the degree of mixing of organic matter on the henhouse floor from infectious and uninfected birds. The HPAI viral titer in fresh chicken manure may range from $10^{3.5}$ to 10^{7} EID_{50}/gram, depending on the strain of the virus **(Section 8.3)** . However, there is considerable uncertainty associated with factors such as the degree of mixing of organic material within a henhouse and the quantity of organic material transferred to the egg packing area via personnel traffic.

For buggies or pallets moved from infected but undetected premises 2-days after packing, our overall conclusion is that the likelihood of HPAI virus contamination of these materials from the floor at the time of packing is *low,* considering the small estimated number of infectious birds at the time of packing (**Table 5, Table 8**, and **Table 9**).

9.2.4.2 Likelihood of Contamination of Buggies during Storage or at the Time of Loading Hatching Eggs Onto a Truck

The 2-day holding period has less of an impact on the likelihood of contamination of the buggies from the storage room floor. For scenarios A and B, the estimated number of infectious birds in an undetected flock during the time interval when the packed buggies may be present in the egg storage room is 1.2 (90 percent P I., 0-4) or 21 (90 percent P.I., 2-78) birds per hen house, depending on the HPAI virus strain scenario.

In scenarios C and D, the predicted number of infectious birds while hatching eggs are present in the egg storage room and before detection of HPAI can be relatively high (estimated means of 50 and 371 breeder hens under different movement scenarios). However, the predicted probability of detection was very high implying that buggies loaded with contaminated hatching eggs would not be moved off the farm in most cases. The on-farm outbreak specific measures considered in the STS Plan play a greater role in reducing the likelihood of contamination of buggies during storage and loadings in these scenarios.

The frequency of personnel movements from the henhouse to the egg storage room is likely lower compared with movements to the egg-packing area. Contamination of buggies from the egg-storage room floor may occur during the holding period, before eggs are moved from the premises. For example, this event may occur as buggies are rearranged in the storage room when new eggs are brought into the storage room, or while loading buggies into the transportation vehicle. However, based on input from industry representatives, egg buggies are not frequently rearranged while in storage, although it is performed in a few cases. The lower frequency of rearrangement reduces the likelihood of contamination between the buggies and storage room floor by the hands of farm personnel.

Furthermore, the outbreak measures considered in this risk assessment (**Section 9.2.2.2**) require breeder farm personnel to use disposable gloves and disposable or clean boots before entering the egg storage area. The use of PPE would reduce the likelihood of contamination of the egg storage area.

As mentioned in **Section 9.2.2.2**, one of the options in the STS Plan is the disinfection of buggy wheels and the setter room floor prior to loading eggs. Poultry industry experts have stated that the organic load on the buggy wheels is not high and is not expected to impact performance of the disinfectant significantly. These measures would likely cause more than a 3-log inactivation of AI viruses, given their susceptibility to most disinfectants when applied according to manufacturer’s directions.(32, 33) There are several EPA registered disinfectants for AI viruses with data showing at least a 3-log inactivation of virus under conditions similar to intended use.(115) In the alternate option from the STS plan, where the buggies wheels would be disinfected prior to moving into

the storage room, the likelihood of contamination of the storage room floor would be reduced.

Based on the above factors and considering the variable number of infectious birds that might be present in the hen flock under alternate scenarios, we conclude that the likelihood of buggies or pallets being contaminated with HPAI virus at the time they are loaded onto a vehicle for movement from an HPAI infected but undetected flock is *negligible* to *low*.

9.2.4.3 Degree of Contamination

We evaluated the degree of contamination of egg-handling materials through various pathways using exploratory scenario analysis. Factors considered included: 1) the proportion of infectious birds; 2) the frequency of personnel movements between the henhouse and the packing and storage areas; 3) the extent of mixing of organic matter within the henhouse; and 4) the amount of contaminated material that adheres to shoes and is transferred to the packing area floor with each movement. Experimental data on the adherence of soil to shoes and the transfer rates of various viruses between inanimate surfaces and finger pads, and expert opinion regarding the viral transfer rates between relevant surfaces encountered in the poultry environment, were utilized in this analysis (details provided in Nest Run Eggs Risk Assessment(108).(116)

The estimated HPAI viral titer on egg-handling materials contaminated from the packing room floor was 2.27 EID_{50}/cm^2 in the baseline scenario. In a conservative scenario using very cautious values for the input parameters, the estimated viral titer on the materials was $10^{2.8}$ EID_{50}/cm^2. The estimated viral titers on egg-handling materials contaminated from the egg-storage room were 4.4 EID_{50}/cm^2 in the baseline scenario and $10^{3.3}$ EID_{50}/cm^2 in the conservative scenario. The viral titers estimated in the scenarios referenced above are approximate given the lack of direct data for viral transfer rates on surfaces encountered in the poultry environment. Validation of this exploratory modeling through empirical data is an important area for further research.

Several breeder farms require the use of footbaths before and after entering the henhouse. We do not consider this biosecurity measure in our evaluation, as it is ambiguous whether it is followed across all breeder farms. In addition, there may be considerable variation in the effectiveness of the footbaths, depending on operational factors such as the frequency of replacing the disinfectant.

9.2.5 Conclusion

In this section, we evaluated the likelihood and degree of contamination of egg handling materials at an infected but undetected turkey breeder hen operation. We considered on farm measures to reduce the likelihood of contamination of the egg handling materials such as the sanitization of eggs, C&D of the egg storage room floor and buggy wheels, and other biosecurity steps for farm personnel from the STS Plan. Additionally, we considered the active surveillance protocol and the 2-day egg holding period specified in the STS Plan that would be implemented in the event of an HPAI outbreak.

The likelihood of egg handling materials such as setter (incubator) trays and buggies moved from the premises being contaminated with HPAI virus was rated to be *negligible* t*o low*, provided that the outbreak measures from the STS Plan are strictly followed.

9.3 Likelihood of the Vehicle or Driver Moving Hatching Eggs from an HPAI infected but Undetected Breeder Premises Being Contaminated

Risk Factors: Contamination of the breeder farm egg storage room floor and passageways to the loading dock; high proportion of infectious birds in an undetected breeder flock.

Current Preventive Measures: Recommended measures from NPIP program (9CFR147) such as C&D of the delivery vehicle and protective clothing for the driver.

Outbreak Specific Measures (to be implemented by industry during an outbreak)**:** Required protective clothing for the driver at the breeder farm; disinfecting the egg storage room floor and buggy wheels concurrently before moving egg buggies onto the loading dock or soaking buggy wheels with disinfectant just before moving egg buggies into the egg storage room; footwear protocols for farm personnel before entering the egg storage room; C&D of delivery vehicle.

Conclusions: The risk of release of HPAI virus via the vehicle or driver transporting hatching eggs from the breeder premises is *negligible* to *low,* provided the proposed preventive measures from the STS Plan are strictly followed.

9.3.1 Introduction

In this section, we first evaluate the likelihood that a vehicle or driver leaving an infected but undetected breeder farm is contaminated with HPAI virus, with consideration of current on farm mitigation measures from NPIP and the STS Plan. While vehicles moving hatching eggs have not been implicated in spread of AI virus to day-old chicks or poults, movement of vehicles (rendering or manure hauling) in general has been implicated in farm-to-farm spread in previous outbreaks.(11, 54, 111)

9.3.2 Preventive Measures

9.3.2.1 Current Preventive Measures

The NPIP program includes several biosecurity practices for hatching egg delivery truck drivers and helpers transporting eggs from breeder farms to hatcheries. Recommended driver and vehicle biosecurity measures from NPIP 9CFR147.24 are listed below:

a. Spray truck tires thoroughly with disinfectant before leaving the main road and entering the breeder farm driveway.

b. Put on sturdy disposable plastic boots or clean rubber boots before getting out of the truck cab. Put on a clean smock or coveralls and a hairnet before entering the poultry house.

c. After loading eggs, remove the dirty smock or coveralls and place into plastic garbage bag before re-entering the truck.

d. Reenter the cab of the truck and remove boots before placing feet onto floorboards. Remove hairnet and leave with disposable boots on farm.

e. Sanitize hands using appropriate hand sanitizer.

9.3.2.2 Outbreak Specific Measures

Biosecurity measures for the truck driver and farm personnel that have been proposed for inclusion in the STS Plan are listed below:

9.3.2.2.1 Truck Driver

a. All drivers and passengers must wear boots (rubber or disposable) before getting out of the vehicle. Boots must be worn the whole time on the farm. When exiting the farm, put disposable boots in an appropriate disposal container prior to exiting the farm. Then spray shoes with disinfectant before entering your vehicle. Rubber boots and any tools used on the farm must be cleaned and disinfected prior to being removed from the turkey premises (draft STS Plan version 20).

b. Must move directly and only to a hatchery (draft STS Plan version 20).

c. Use a hand sanitizer before leaving and after re-entering the cab (draft STS Plan version 20).

d. Vehicle windows should be rolled up at all times while on the poultry farm in order to prevent flies from getting into the vehicle (draft STS Plan version 20).

e. Spray insecticide inside trucks as needed to eliminate the transporting of flies from farm to farm during warm months of the year (draft STS Plan version 20).

f. Spray the floors, pedals, and bottoms of feet with disinfectant after every stop (draft STS Plan version 20).

9.3.2.2.2 Farm Personnel

g. Farm personnel should disinfect the egg storage room floor and buggy wheels before the buggies are moved for loading; or soak the buggy wheels with disinfectant (being careful to cover the entire circumference of the wheel) prior to moving egg buggies into the egg storage room cooler.

9.3.2.2.3 Cleaning and Disinfection of the Vehicle

h. The outside of all vehicles will be C&D under supervision of regulatory personnel with an approved disinfectant at a C&D station at or near the turkey premises within the Infected Zone. If C&D cannot be completed at the turkey premises, the vehicles must be accompanied by a permit issued by the Incident Commander to travel to a C&D station within the Infected Zone (Draft STS Plan version 20).

9.3.3 Evaluation of Risk

The potential risks associated with vehicles and personnel leaving the breeder farm are:

- Risk of release of HPAI virus via the vehicle transporting hatching eggs from an infected but undetected breeder premises, and
- Risk of release of HPAI virus via the driver transporting hatching eggs from an infected but undetected breeder premises.

9.3.3.1 Risk of Release of HPAI Virus via the Vehicle Transporting Hatching Eggs from an Infected but Undetected Breeder Premises

We evaluate this risk pathway in two parts:

- The likelihood and degree of HPAI virus contamination of the interior surfaces (truck and cab interior) of the vehicle while loading hatching eggs at breeder premises.
- The likelihood and degree of HPAI virus contamination of the exterior of the vehicle transporting hatching eggs

9.3.3.1.1 The Likelihood and Degree of HPAI Virus Contamination of the Interior Surfaces of the Vehicle While Loading Hatching Eggs at the Breeder Premises

9.3.3.1.1.1 Truck Cargo Interior

During normal operations, the truck driver enters the egg storage room to load hatchery buggies into the hatchery truck. In situations where specific on-farm mitigation measures are not implemented, the interior of vehicles moving hatching eggs may potentially become contaminated with HPAI virus from environmental surfaces or materials via successive virus transfers between contact surfaces (e.g., breeder house → shoes of farm personnel or egg buggy wheels → egg storage room floor → driver's shoes → trailer interior). The NPIP (9CFR145 and 9CFR147) and STS Plan have several measures that mitigate such risk pathways. We considered the following factors in our evaluation.

- In general, active surveillance would reduce the likelihood that HPAI is not detected when a high proportion of the breeder hen flock is in an infectious state, reducing the degree of contamination on environmental surfaces in and around the egg storage room and loading dock. For scenarios A and B, the estimated number of infectious birds in an infected, undetected flock during the time interval when the packed buggies may be moved from the egg storage room was estimated to be 1.2 (90 percent P I., 1-4) or 21 (90 percent P.I., 2-78) birds per hen house, depending on the HPAI virus strain scenario. In scenarios C and D, there is a *low* to *moderate* likelihood that a high number of infectious birds are present at the time of loading. The predicted mean number of infectious birds when hatching eggs are moved 1-day post insemination ranged from 50 (Scenario C) to 371 (Scenario D) hens under different movement scenarios (**Table 8** and **Table 9**). However, as discussed in the previous section, there is a

high likelihood of HPAI infection being detected by active surveillance or clinical signs by 2 days post insemination under scenarios C & D.

- Farm personnel are required to wear shoe covers or boots that have been disinfected before entering the egg storage room cooler, reducing the degree of contamination of the egg storage room floor through this pathway. The reduced contamination of the egg storage room would also reduce the likelihood and degree of contamination of the vehicle trailer via the driver's shoes while loading.
- The likelihood and degree of contamination of the egg storage room floor at the time of loading of hatching eggs would be *low*. The outbreak measures described in **Section 9.2.2.2** include a couple of options for mitigating this pathway. In the first option, the disinfection of the storage room floor and disinfection of buggies wheels would prevent the cross contamination of trailer interior at loading. In the second option, the likelihood of the contamination of storage room floor would be reduced due to C&D of buggy wheels prior to moving them into storage and due to the footwear measure described above. The susceptibility of AI to appropriate disinfectants would reduce the likelihood of contamination of the cargo interior through this pathway.(32)
- There may be a low degree of contamination of the farm loading dock through settling of aerosols generated from an infected but undetected henhouse, depending on the number of infectious hens at the time of loading. The degree of contamination transferred via the shoes of the driver or farm personnel during loading would be further reduced due to 2 virus transfer steps involved.(20, 117, 118)

Given these factors, we estimated the likelihood of contamination of the cargo interior of the hatching egg truck through the movement and loading of hatching eggs from the egg storage room to be *low*.

There is also a possibility that the trailer interior could become contaminated via leakage of internal egg contents during transport. However, given the low estimated number of internally contaminated eggs moved and the low rate of leakage of turkey hatching eggs (**Appendix 3**), the likelihood of contamination of trailer interior through this pathway would be *negligible* to *low*.

Overall, we estimated the likelihood of contamination of the cargo interior of the hatching egg truck through the movement of hatching eggs to be *low*.

9.3.3.1.1.2 Truck Cab Interior

The primary risk pathway for the contamination of the cab interior is through the driver becoming contaminated via the egg storage room floor and farm loading dock, surrounding ground areas, or by handling hatching egg-handling materials. We considered the following factors in our evaluation.

- The low likelihood and low degree of contamination of the egg room storage floor at time of loading. This would reduce the potential for transmitting HPAI virus via the driver's shoes.
- Aerosol contamination of surrounding ground areas of the breeder farm is a possibility. However, the degree of contamination through this pathway would be low, considering the relatively small number of infectious birds in an undetected flock under active surveillance for scenarios A and B (1.20 (90 percent P I., 1-4) or 21 (90 percent P.I., 2-78) birds per hen house) and dispersion of potentially contaminated aerosols generated from within the henhouse. The degree of contamination for scenarios C and D (mean ranged from 50 to 371 birds per hen house) would be higher.
- The use of PPE such as disposable or clean rubber boots and coveralls while at the breeder farm—and following specific protocols for removing and isolating them before entering the cab—would further reduce the likelihood of contamination of the cab interior.
- In addition, proposed measures in the STS Plan require the cab interior to be cleaned and disinfected if the driver steps outside the cab. Specifically, the driver is required to spray the floors, pedals, and bottoms of feet with disinfectant after every stop and use a hand sanitizer before leaving and after re-entering the cab. The use of hand sanitizer would likely inactivate any HPAI virus contamination of the driver's hands from removing PPE.(119, 120)

We rated the likelihood of the cab interior of the vehicle leaving an infected but undetected premises being contaminated with HPAI virus to be *low*, provided the applicable preventive measures from NPIP 9CFR147 and the STS Plan are strictly followed.

9.3.3.1.2 The Likelihood and Degree of HPAI Virus Contamination of the Exterior of the Vehicle Transporting Hatching Eggs

Although there are no direct data on the frequency or degree of HPAI contamination of tires of vehicles leaving an infected farm, the movement of vehicles has been implicated for spreading HPAI in previous outbreaks, and the C&D of truck tires is frequently recommended in response plans.(11, 121, 122) There is a possibility that truck tires may become contaminated with HPAI virus from the surrounding ground areas of the farm, which were previously contaminated through deposition of aerosols and movement of equipment or personnel. However, the degree of contamination through this pathway would be low under scenarios A, B and C, considering the lower proportion of infectious birds in an undetected flock under active surveillance and considering the dispersion of aerosols generated from within the henhouse. A relatively higher proportion of infectious birds and likelihood of contamination would be expected in scenario D (**Table 9**). In a natural outbreak study there was a 100 factor (2-log $TCID_{50}$) reduction in HPAI viral titer from the inside of an infected barn (with a high proportion of infectious birds) to the outside of the barn (at a titer of 12 $TCID_{50}/m^3$ outside the barn).(113) The HPAI viral concentration in air from an undetected farm under active surveillance would be lower

compared to the estimates from the above study due to the lower number of infectious birds.

As described in **Section 9.3.2.2**, the exterior of the vehicle should be C&D after leaving the breeder farm. These plans are similar to those developed to control the 2002 Exotic Newcastle Disease (END) outbreak in California and were found to be effective in that situation. Similarly, other relevant guidelines such as the National Animal Health Emergency Management System (NAHEMS, USDA) guidelines address the C&D of vehicles in detail.(123) These C&D procedures would effectively inactivate HPAI virus on the vehicle exterior, given the sensitivity of HPAI virus to most detergents and disinfectants (see **Section 8.4**).(32, 124) We conclude that the risk of HPAI virus remaining on the exterior of a vehicle that has been cleaned and disinfected as specified in the STS Plan is *negligible*.

9.3.3.2 Risk of Release of HPAI Virus via the Driver Transporting Hatching Eggs from an Infected but Undetected Breeder Premises.

There is a possibility that the vehicle driver's hands, clothing and shoes could become contaminated with HPAI virus while loading hatching eggs at the breeder farm. We consider the on farm C&D and driver biosecurity measures from the STS Plan in evaluating this risk. We rated the risk of the hands, clothing and shoes of the driver transporting hatching eggs from an infected, undetected breeder premises being contaminated with HPAI virus when leaving the farm to be *low,* considering the impact of PPE and on farm measures.

9.3.4 Conclusion

The likelihood of entry of HPAI virus via the vehicle or driver transporting hatching eggs from the breeder premises is *low,* provided the proposed preventive measures from the STS Plan are strictly followed.

10. Exposure Assessment

In the exposure assessment, we estimate the risk of susceptible poultry becoming infected with HPAI virus released from an infected but undetected breeder flock through the movement of potentially contaminated hatching eggs, associated handling materials, or the vehicle or driver transporting hatching eggs. By susceptible poultry, we are referring to day old poults at a hatchery orsusceptible breeder flocks within or outside the control zone that may receive egg handling materials from the hatchery.

10.1 Risk of Day Old Poults Becoming Infected with HPAI Virus from Hatching Eggs or Egg Handling Materials via Movements of Equipment or Personnel at the Hatchery

Risk Factors: Egg-handling materials originating from a breeder flock in a Control Area being contaminated; dissemination of HPAI virus via movements of equipment or personnel within the hatchery.

Current Preventive Measures: Separation of hatchery operations in different rooms; hatchery work flow and C&D measures from NPIP.

Outbreak Specific Measures (to be implemented by industry during an outbreak)**:** Hatchery employees must wash hands before entering/leaving rooms and wear disposable shoe covers or utilize foot baths; egg pick-up drivers will not enter poult processing areas or conduct poult deliveries; C&D of loading docks, passages, receiving storage areas after receiving hatching eggs; poult processing occurs before eggs are received.

Conclusions: The risk of day-old poults becoming infected via movements of contaminated equipment or personnel at the hatchery is *negligible* to *low* provided the preventive measures from the STS Plan are strictly implemented.

10.1.1 Introduction

In this section, we evaluate the risk of day-old poults becoming infected with HPAI virus from eggs or materials originating from infected but undetected breeder flocks via movements of equipment or personnel within the hatchery.

Although chicken and turkey breeder flocks have been infected in previous avian influenza outbreaks, there are currently no published reports of transmission of HPAI infection to day-old chicks or poults through hatchery operations. However, given the uniformly negative test results in hatching eggs and chicks from infected broiler breeder flocks during the 2004 HPAI outbreak in British Columbia, and considering the high temperature inside the setters, the Canadian Food Inspection Agency (CFIA) concluded that the movement of chicks and hatching eggs does not pose a risk for HPAI transmission.(70) Considering the similarities in hatchery design and workflow, it stands to reason that CFIA's conclusion is applicable for turkey hatcheries as well.

There is a potential risk pathway for movement of equipment or personnel at the hatchery to transmit HPAI virus from hatching eggs or materials originating from breeder flocks to day-old poults. However, as evaluated in **Section 9.1** the likelihood of incoming hatching eggs—or materials from an infected but undetected breeder farm (**Section 9.2**)—being contaminated with HPAI virus is *negligible* to *low,* given the mitigation measures from STS Plan. We consider these results as well as current hatchery sanitary measures from the NPIP program in evaluating this risk.

10.1.2 Preventive Measures

10.1.2.1 Current Preventive Measures at the Hatchery

10.1.2.1.1 NPIP provisions (9CFR parts 145 and 147)

The National Poultry Improvement Plan (NPIP) has specific provisions for participating hatcheries. Complete details can be found in 9CFR parts 145 and 147. Hatcheries must be kept in sanitary condition. All cleaning and disinfection procedures should be as outlined in § 147.24.

- Egg rooms[i] should be cleaned and disinfected at least two times per week.
- Incubator rooms should be cleaned and disinfected after each set or transfer.
- Incubator rooms should not be used for storage.
- Egg trays and buggies should be cleaned and disinfected after each transfer.
- After each hatch, hatcher walls, ceilings, floors, doors, fans, vents, and ducts should be cleaned and disinfected. The hatcher room should not be used for storage.
- Chick/poult processing equipment and rooms should be thoroughly cleaned and disinfected after each hatch.

10.1.2.1.2 General Work Flow Design in a Hatchery

- The hatchery building should be arranged so that separate rooms are provided for each of the four operations: 1) egg receiving; 2) incubation and hatching; 3) chick/poult processing; and 4) egg tray and hatching basket washing (9CFR147.23 b).
- The poult area is separate from the hatching egg-storage room (egg cooler) and setter rooms (see **Appendix 8** for an example hatchery layout). There is limited movement of people between the poult area and the egg-storage room (egg cooler) during poult processing. In addition, there is no common equipment used for egg handling and poult processing.(125)

[i] "room" includes floor, ceilings, walls, drains, air filters and humidifiers

10.1.2.2 Outbreak Specific Measures at the Hatchery

10.1.2.2.1 Cleaning and Disinfection and Biosecurity Measures

- Hatchery personnel must wash their hands with soap and water and/or apply a hand sanitizer before entering or leaving the hatcher room or poult processing rooms (Appendix 8).
- Egg pick-up drivers will not enter poult processing areas, conduct poult deliveries or handle poults on the same day that they have delivered eggs to the hatchery.
- Egg contents leaked onto hatchery floors or equipment must be C&D according to the hatchery SOP as soon as possible.
- Employees must take precautions to prevent the potential transfer of contamination into the poult processing room via shoes by utilizing a foot bath or clean disposable shoe covers.

10.1.2.2.2 Outbreak Work Flow in a Hatchery

- If the hatchery has a loading dock that is used in common with eggs, the poults will move first before the eggs are received on the same day. The loading docks must be cleaned and disinfected at the end of the day.
- Poult processing will occur and be completed prior to any egg-room work or egg movement to the setter room. As an alternate option, dedicated personnel will be used for poult processing and egg movement at the hatchery.

10.1.2.2.3 Low Chance of Leakage

During the egg grading process at the breeder premises, any cracked, misshapen, or leaking eggs are removed. At the hatchery, handling of turkey hatching eggs before incubating is less likely to result in eggs cracking or leaking, as compared with the washing and handling of table-eggs. This is due to the stronger precautions taken to prevent damage when handling hatching eggs (turkey hatching eggs have higher economic value) as well as the thicker shell of turkey eggs compared to chicken eggs. In current hatchery operations, any leakage of hatching eggs would result in C&D of the floors as soon as possible after completing the current work task.

10.1.2.2.4 Handling and C&D of Buggies in a Hatchery

Buggies originating from the breeder farm are held in the egg storage room until the hatching eggs are suitable for placement in the incubator (up to 5 days). After setter trays are removed, the buggies and racks are C&D in the washroom. In general, hatchery buggy sanitation requirements exceed the requirements for handling material sanitation in other sectors of the industry. Visual inspection and routine microbiological monitoring (e.g., weekly intervals) is performed to ensure the effectiveness of the C&D process. Buggies may be returned to breeder farms immediately after sanitation.

10.1.3 Evaluation of Risk

We considered two pathways for evaluating this risk. The major steps in these pathways are illustrated in **Figure 8** and **Figure 9**.

1. Risk that day-old poults contract HPAI from shoes or equipment cross-contaminated with HPAI virus from hatchery floors.
2. Risk that day-old poults contract HPAI from virus transferred to the hands of hatchery personnel via contact with contaminated equipment or surfaces.

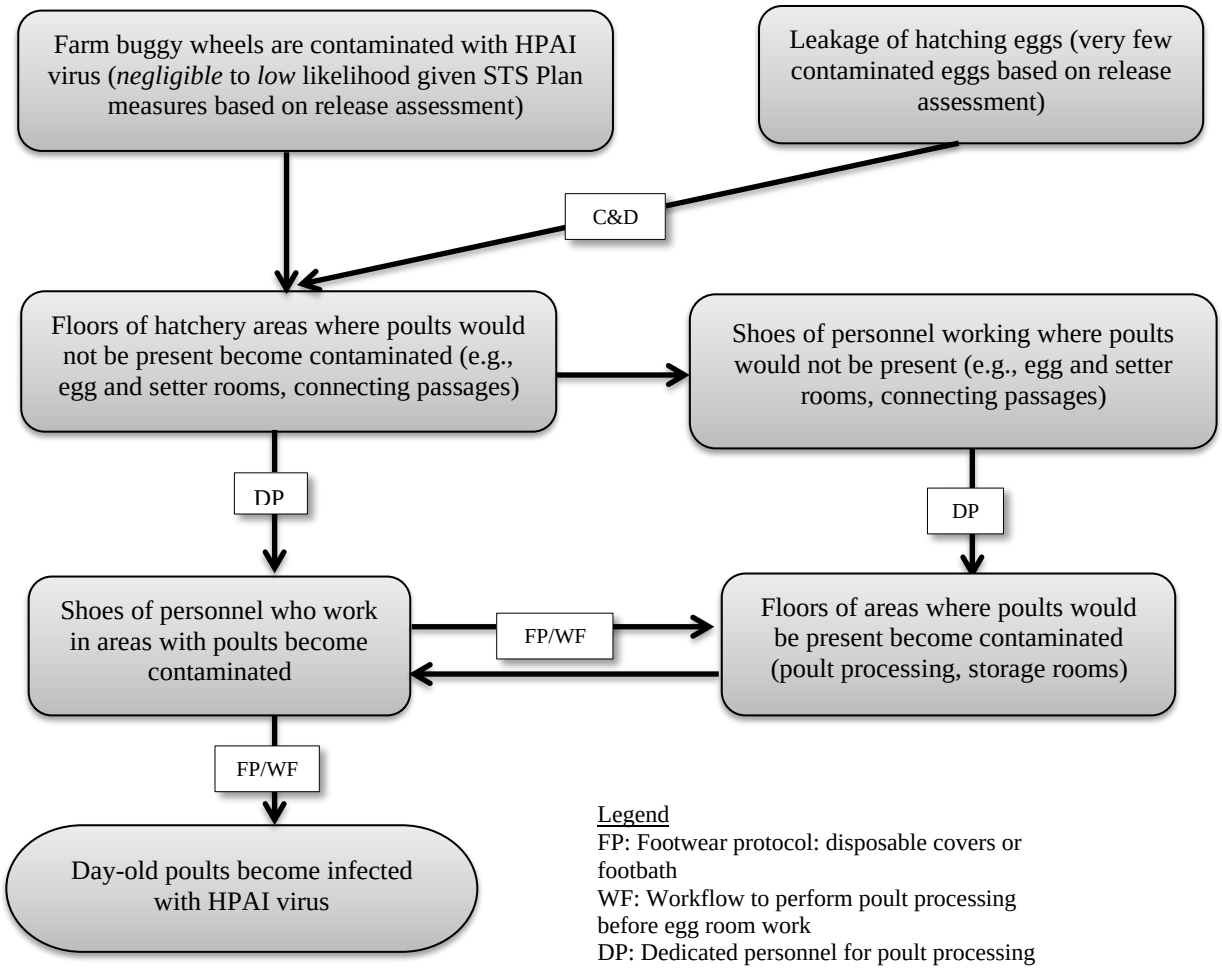


Figure 8. Risk pathway for day-old poults becoming infected with HPAI virus via transmission through shoes or the wheels of equipment at the hatchery and risk mitigation measures evaluated towards reducing the risk for each step in the pathway.

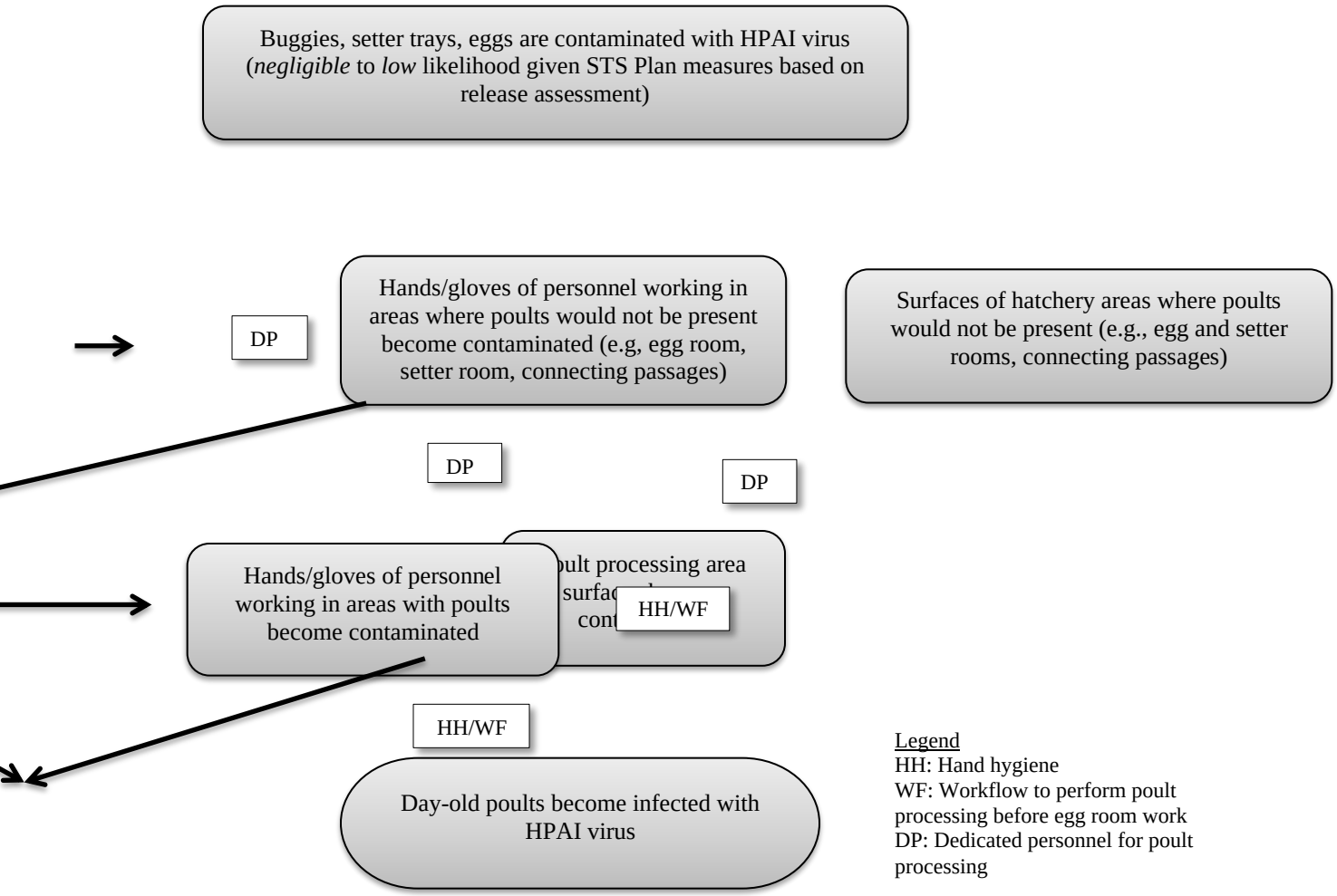


Figure 9. Risk pathway for day-old poults becoming infected with HPAI virus via transmission through contaminated hands of hatchery personnel and risk mitigation measures evaluated towards reducing the risk for each step in the pathway.

10.1.3.1 Risk that Day-old Poults are Infected with HPAI Virus from Hatchery Floors

10.1.3.1.1 Risk that Day-old Poults Contract HPAI via Contaminated Hatching Eggs that Leak onto Hatchery Floors

The likelihood that a contaminated egg would leak due to handling or transport is *very low*. This is due to the low predicted number of internally contaminated hatching eggs moved from an infected but undetected breeder farm as estimated in **Section 9.1**, as well as the *low* likelihood of leakage of turkey hatching eggs.

The estimated mean number of leaking internally contaminated hatching eggs in quantitative simulation scenarios (**Appendix 3**) varied between 0 to 0.000021 eggs per shipment of eggs from the breeder farm. A mean leakage rate for normal virus free turkey hatching eggs of 0.05 percent was used in these scenarios (based on a couple of point estimates from industry representatives). Also, HPAI contaminated eggs without visible defects were considered to be 3.7 times more likely to leak than uncontaminated eggs based on expert opinion.

In the rare instances where contents from a contaminated hatching egg leaks onto floors, the current practice in most hatchery guidelines is to clean and disinfect the floors immediately. Overall, considering the low likelihood of leakage of HPAI virus-contaminated eggs and the procedure for C&D of hatchery floors in case of leakage, we conclude that the risk of day-old poults becoming infected with HPAI virus from the contents of contaminated hatching eggs that leak onto hatchery floors is *negligible.*

10.1.3.1.2 Risk that Day-old Poults Contract HPAI via Virus Transferred to Hatchery Floors from Contaminated Materials or Shoes

Floors of the egg room, connecting passages, and the setter room at the hatchery could potentially become contaminated from the movements of HPAI contaminated materials (buggies or pallets). Subsequent movements of equipment or personnel across the contaminated floors could result in cross-contamination and dissemination of HPAI virus to the day-old poult processing areas via successive viral transfer between contact surfaces (**Figure 8**).

The following factors were considered in our evaluation:

a. From **Section 9.2.5**, the likelihood of egg handling materials such as setter (incubator) trays and buggies moved from the premises being contaminated with HPAI virus at the time of movement was estimated to be *negligible* to *low* while considering various on-farm mitigation measures from STS Plan.

b. Biosecurity precautions to reduce the potential for transferring contamination between different rooms of the hatchery are implemented in most hatcheries. The egg processing, setter, hatcher, and poult processing areas are typically well separated. The typical workflow for farm buggies in the hatchery is as follows: storage in egg room → movement from egg room to setter room → movement of empty buggies to washroom → and storage in a clean room before shipping back to farms. The farm buggies are not brought into hatcher rooms or poult processing rooms. In addition, as per 9CFR145.6, the setter room floors are C&D after each transfer and the setter room is not used for storage of buggies. This will reduce the likelihood for dissemination of HPAI virus through the setter room floors.

c. Poult processing will occur and be completed prior to any egg-room work or egg movement to setter room on the same day, according to the STS Plan. As an alternate option, dedicated personnel will be used for poult processing and egg movement at the hatchery. Given these protocols, there would be limited movement of personnel from potentially contaminated areas of the hatchery, such as the egg room or wash room, into the hatching or poult take-off rooms while day old poults are being processed. Any dissemination of HPAI virus from

buggies to connecting passages or the setter room floor would be further inactivated by the next day when the next batch of poults are processed, due to the temperature of these rooms. The buggies themselves would have been moved to wash room after setting of the eggs.

d. The STS Plan contains measures to prevent the exposure of poults via cross contamination of loading docks or connecting passages in situations where a shared dock is used for loading of poults. The C&D of loading docks and connecting passages after receipt of each truckload of hatching eggs from a Control Area is expected to effectively inactivate any HPAI virus transferred to the dock, given the effectiveness of most disinfectants to inactivate AI viruses (**Section 8.4**). Alternatively, restricting the unloading of eggs to be conducted after the shipment of poults on the same day may also reduce the likelihood of cross contamination, due to some inactivation of virus resulting from the additional time between the two movements.

e. Risk pathways for transmission of HPAI virus via hatchery floors would involve multiple transfer steps through direct contact between surfaces. The final surface concentration of HPAI virus transferred through such contact steps would be lowered by the multiple steps. This is because only a fraction of the virus (6 to 27 percent) on a donor surface is transferred to the recipient surface in each direct contact event. (20, 117, 118, 126, 127)

f. The temperature in most rooms (except for the egg-storage room) is typically 77°F (25°C) or higher. Experimental studies showed that HPAI H5N1virus is inactivated at 77°F (25°C) within a day on steel or glass surfaces and within 1 to 2-days in dry manure (**Appendix 4**).(22, 128, 129) Given the hatchery temperatures, hatchery C&D protocols, and the time interval between egg setting and poult processing, any HPAI virus potentially transferred to environmental surfaces during setting operations would likely be inactivated before the commencement of poult processing operations on the following day.

g. The STS Plan (**Section 10.1.2.2**) recommends that employees take precautions to prevent transfer of microbial contamination into the poult processing room via shoes (e.g., change shoes; use disposable boot covers). In addition, most hatcheries place disinfectant foam-baths or foot baths in the entrances or exits between various rooms of the hatchery.

In summary, given a *negligible* to *low* likelihood of the buggies being contaminated (**Section 9.2.4.2**), physical inactivation of virus due to high hatchery temperatures, hatchery sanitary practices, and hatchery workflow designs, we conclude that the risk of day-old poults being infected by HPAI virus due to equipment or the shoes of hatchery personnel contaminated through the hatchery floors is *negligible* to *very low*, provided the preventive measures discussed above are followed.

10.1.3.1.3 Risk that Day-old Poults Contract HPAI via Contaminated Hands of Hatchery Personnel

Personnel working in the poult processing operations at the hatchery may directly contact day-old poults. Hence, there is a high likelihood of day-old poults becoming infected if

the processing personnel's hands are contaminated with HPAI virus. The following factors were considered in our evaluation:

a. The likelihood of buggies being contaminated with HPAI virus at the time of being loaded onto the egg delivery vehicle was estimated to be *negligible* to *low* in **Section 9.2.4.2**. The likelihood of setter trays being contaminated with HPAI virus at the time of being loaded onto the egg delivery vehicle was estimated to be *negligible* to *low* for belt gathered eggs (**Section 9.2.3.1**) and *low* for hand gathered eggs (**Section 9.2.3.2**).

b. The transfer of hatching eggs from the egg room into setters is performed at the end of the day after poults are processed. Even if the hands of hatchery personnel were contaminated during handling (before setting) and setting of hatching eggs, there is little opportunity for transmitting virus to day old poults the following day.

c. Any HPAI virus associated with the setter trays would likely be inactivated due to incubation temperature (99 to 102°F, 37 to 39°C) and humidity (55 to 70 percent relative humidity) within 1 or 2-days (**Appendix 4**). The likelihood of HPAI virus on the setter trays being viable after 25 days of incubation is *negligible.*

d. The inactivation of HPAI virus on hatchery surfaces (apart from surfaces of eggs in the egg storage room cooler) within 1 to 2 days due to the relatively high temperatures and the impact of setter room sanitary measures would reduce the likelihood of this pathway as well.

e. Personnel handling day old poults are required to take sanitary measures, such as washing their hands with a detergent after each absence from the workstation. The STS Plan (**Section 10.1.2.2**) recommends employees wash their hands with soap or apply a hand sanitizer before entering the hatcher room or poult take-off rooms. Given the relative susceptibility of avian influenza virus to most detergents, this measure is expected to be effective in inactivating HPAI virus on hands.(119, 130)

We conclude that the risk of day-old poults being infected from HPAI virus via contact with the contaminated hands of hatchery personnel is *negligible* to *low* if the preventive measures in the STS Plan are strictly implemented.

10.1.4 Validation from Previous Outbreak Experiences

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 avian influenza experts have stated that, although there have been several LPAI outbreaks in the United States, vertical transmission or hatchery transmission has not been observed to-date. Poss et al. (2003) state "There has been no evidence of egg transmission from infected breeder flocks in Minnesota over the years. Poults hatched during the 1978 outbreak in Minnesota from an acutely ill Avian Influenza infected flock just as egg production was subsiding were monitored and no viral shed could be detected." (131) Expert opinion was elicited from turkey practitioners participating in the STS Workgroup by analysts at the University of Minnesota, and

reports of 26 flocks which had undergone Avian Influenza infection and where eggs from the flocks were set and not removed were provided. Most of the outbreaks reported were due to swine origin influenza A viruses (H1N1 or H3N2). In addition, a couple of outbreaks each were identified as due to Pandemic H1N1 and other LPAI viruses (H6N2 and H7). There was no evidence of horizontal or vertical transmission of AI within the hatchery to day old poults in any of these instances (**Appendix 9**).

The above observations are also consistent with outbreak experiences in broiler hatcheries. Given the uniformly negative test results in hatching eggs from infected broiler breeder flocks during the 2004 HPAI outbreak in British Columbia—and considering the high temperature inside the setters—the Canadian Food Inspection Agency concluded that the movement of day old chicks and hatching eggs does not pose a risk for HPAI transmission.(70) In the 2002 HPAI outbreak in Queretaro, Mexico, eggs already present in the hatchery at the time of infection in broiler breeder flocks were allowed to hatch following normal procedures. The broilers hatched from these eggs were not infected until 3 weeks old, suggesting that dissemination of virus in the hatchery did not occur. These outbreak experiences validate and support the risk assessment conclusions that the spread of HPAI virus to day-old poults via hatchery dissemination is unlikely.

10.1.5 Conclusion

We conclude that the risk of day-old poults becoming infected with HPAI virus from hatching eggs and materials via movements of equipment or personnel at the hatchery is *negligible* to *very low*, provided that the preventive measures from the STS Plan are strictly implemented. We also conclude the following with respect to specific risk pathways if the preventive measures in the STS Plan are implemented:

- The risk that day-old poults are infected with HPAI virus from the contents of contaminated hatching eggs that leak onto hatchery floors is *negligible.*
- The risk of day-old poults being infected by HPAI virus due to contamination of equipment or shoes of hatchery personnel through the hatchery floors is *negligible to very low*.
- The risk of day-old poults being infected from HPAI virus via contact with the contaminated hands of hatchery personnel is *negligible to very low*.

10.2 Risk that Day-old Poults are Infected with HPAI Virus Associated with the Vehicle or Driver Delivering Hatching Eggs

Risk Factors: Shoes of the hatching egg delivery driver, cross contamination of hatchery loading docks while unloading hatching eggs.

Current Preventive Measures: NPIP recommended measures: vehicle C&D, protective clothing for the driver at the breeder farm; C&D of egg-handling materials at the hatchery; hatchery workflow.

Outbreak Specific Measures (to be implemented by industry during an outbreak)**:** STS Measures for C&D of loading docks, hatchery work flow and segregation of duties between those working in egg room and with day old poults.

Conclusions: We conclude that the risk that HPAI virus from the interior of the vehicle or driver delivering hatching eggs results in the infection of day old poults is *negligible* to *low* provided that the preventive measures from the STS Plan are strictly followed.

10.2.1 Introduction

This section evaluates the risk of day-old poults at the hatchery becoming infected with HPAI virus from vehicles or drivers moving hatching eggs from a breeder farm in the Control Area. Spread of AI virus to day old poults at the hatchery via movement of hatching eggs has not been demonstrated in previous outbreaks, although it has been considered a theoretical possibility.(70, 132, 133) While hatching egg delivery vehicles have not been implicated for AI transmission to day-old poults, other vehicles (rendering or manure hauling) have been implicated in previous outbreaks.(11, 54, 111)

The emphasis of this section is for pathways for HPAI dissemination of virus from the vehicle or driver onto hatchery floors and environmental surfaces that may result in exposure of day old poults. We consider the qualitative rating of *negligible* to *low* for the likelihood of entry of HPAI virus from breeder flocks in Control Area via vehicle surfaces or drivers in our evaluation (**Section 9.3**). We also take into account the hatchery biosecurity steps in an outbreak from the STS Plan.

10.2.2 Preventive Measures

10.2.2.1 Current Preventive Measures

The current preventive measures include biosecurity measures for hatching egg vehicles and drivers at the breeder farm from the NPIP program and current industry practices as detailed in **Section 9.3.2.1**.

10.2.2.2 Outbreak Specific Measures

The on-farm biosecurity measures and vehicle C&D measures described in **Section 9.3.2.2** are important measures for this risk pathway overall. Relevant outbreak measures at the hatchery from the STS Plan include the following.

- Egg pick-up drivers will not enter poult processing areas, conduct poult deliveries or handle poults on the same day that they have delivered eggs to the hatchery.
- If the hatchery has a loading dock that is used in common with eggs, the poults will move first before the eggs are received on the same day. The loading docks must be cleaned and disinfected with a disinfectant approved by the Incident Command at the end of the day.
- Employees must wash their hands with soap or apply a hand sanitizer before entering the hatcher room or poult processing rooms. Employees must take precautions to prevent the transfer of microbial contamination into the poult processing room via shoes by utilizing a foot bath or clean disposable shoe covers.

10.2.3 Evaluation of Risk

In **Section 9.3**, we concluded that the risk of HPAI virus remaining on the exterior of a vehicle that has been cleaned and disinfected as specified in the STS Plan is *negligible*, so no further evaluation of exposure of day-old poults at the hatchery through this pathway is required However, we estimated the likelihood of contamination of the cargo interior, the truck cab interior and driver to be *low*.

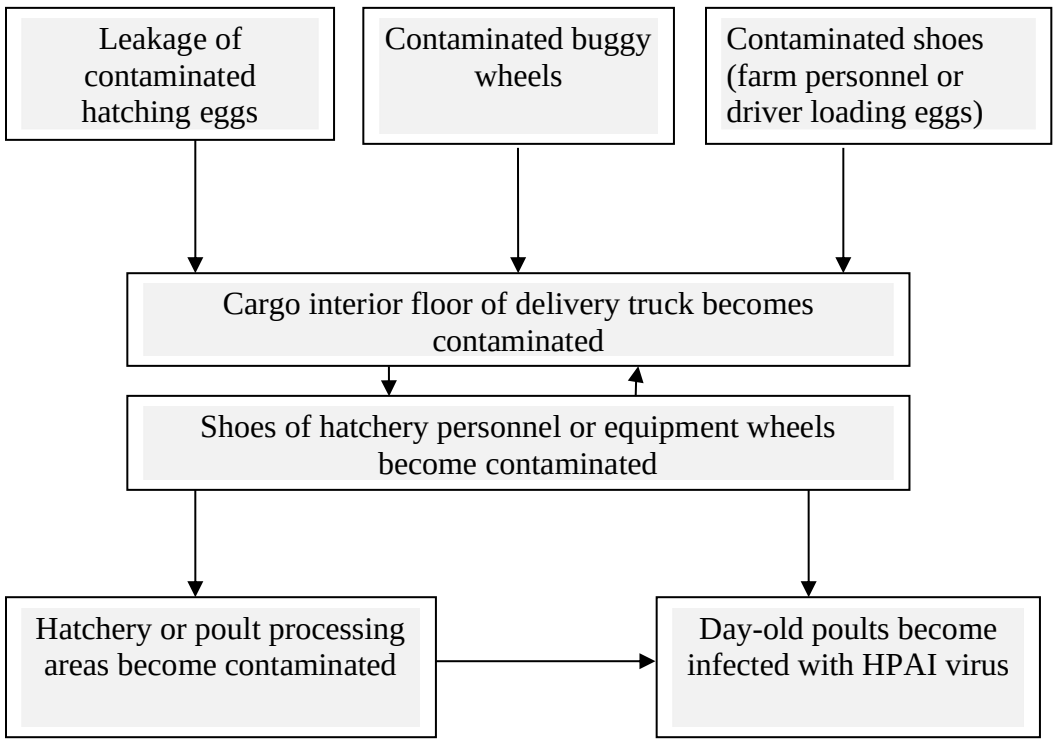


Figure 10. Potential risk pathways for day-old poults becoming infected with HPAI virus at the hatchery via transmission through shoes or equipment wheels contaminated via the egg delivery vehicle interior.

Pathways for this risk involve the transmission of HPAI virus from the contaminated vehicle interior or egg buggy surfaces to day-old poults via successive virus transfer steps between contact surfaces. The main steps in the pathway for transmission of HPAI virus from the cab interior or the cargo interior are as follows (**Figure 10**):

- The cargo or truck cab interior of the vehicle becomes contaminated while loading hatching eggs at the breeder premises (*Low* likelihood from **Section 9.3**).
- The loading docks, egg storage room floor, or connecting passages at the hatchery become contaminated from personnel or equipment that contact the cab or cargo interior of the truck.
- HPAI virus is subsequently transmitted to day-old chicks via movements of personnel or equipment.

We consider the following measures when evaluating this risk pathway.

- From the STS Plan (**Section 10.2.2.2**), egg pick-up drivers are prohibited from entering poults processing areas on the same day that they have delivered eggs to the hatchery. This measure reduces the likelihood of transfer of any HPAI virus from the driver's shoes onto passageways in the hatchery where day old poults may be taken across in the same day. Note that virus transferred to other hatchery surfaces would have a relatively lower likelihood of being transmitted to day old poults due to the greater number of steps in the pathway.
- Another important preventive measure for this risk is either; 1) the temporal separation of the poult processing operations and the transfer of hatching eggs from the egg storage room to the setters; or 2) the separation of hatchery personnel performing these duties. This measure reduces the likelihood of transmitting any HPAI virus from the hatchery floors to day old poults by increasing the number of virus transfer steps required. The measure would also increase the chances of HPAI virus becoming inactivated before infecting day old poults.
- The STS Plan contains measures to prevent the exposure of poults via cross contamination of loading docks or connecting passages in situations where a shared dock is used for loading of poults. Restricting the unloading of eggs to be conducted after the shipment of poults on the same day in combination with C&D of loading docks at the end of the day, mitigates the risk of exposure of poults via cross contamination of loading docks.
- There is a possibility that the truck cargo interior could become contaminated via leakage of internal egg contents during transport. In **Section 10.1.3.1.1**, we estimated the likelihood that a contaminated egg would leak due to handling or transport to be *very low*. We conclude that the likelihood of the trailer interior becoming contaminated with HPAI virus from hatching eggs from an infected but undetected farm that leak during transportation is *negligible*.
- Measures evaluated in **Sections 10.1.3.1.2** and **10.1.3.1.3** that reduce the risk of exposure of day-old poults through potential dissemination of HPAI virus from

the egg room to day old poults processing areas through the movement of personnel or equipment would be effective for this risk pathway as well.

Other preventive measures include current hatchery biosecurity practices, such as the placement of disinfectant footbaths or foam baths at the connecting passages between different regions of the hatchery, and restricting employees from moving from dirty areas to clean areas. We conclude that the risk that HPAI virus from the interior of the vehicle delivering hatching eggs results in the infection of day old poults is *negligible* to *low*, provided that the preventive measures from the STS Plan are strictly followed.

The likelihood of HPAI being released into the hatchery environment through the vehicle driver was rated to be *low* in the entry assessment. The STS Plan measures that do not to allow the egg delivery driver into the poult processing areas on the same day would further reduce the likelihood of the poults becoming infected. The factors considered above in reducing the likelihood of HPAI virus from the trailer interior being disseminated through the hatchery surfaces would also be applicable for any virus associated with the shoes of the driver. We conclude that the risk of HPAI virus from the driver delivering hatching eggs resulting in the infection of day old poults is *low* provided that the preventive measures from the STS Plan are strictly followed.

10.2.4 Conclusion

We conclude that the risk that HPAI virus from the cargo or cab interior of the vehicle or vehicle driver delivering hatching eggs results in the infection of day old poults is *negligible* to *low*, provided that the preventive measures from the STS Plan are strictly followed.

10.3 Risk of HPAI Spread to Other Breeder Premises via the Vehicle, Driver or Egg-handling Materials Moved from the Hatchery

Risk Factors: Cross-contamination of the delivery vehicle via loading docks or personnel; post C&D handling resulting in contamination of reusable hatching egg handling materials.

Current Preventive Measures: Recommended measures from NPIP program (9CFR147) such as C&D of the delivery vehicle and protective clothing for the driver and C&D of egg-handling materials.

Outbreak Specific Measures (to be implemented by industry during an outbreak)**:** Required protective clothing for the driver at the breeder farm; C&D of the vehicle before arriving at the hatchery; C&D of reusable materials at the hatchery; storage of C&D material in a segregated clean room; workflow practices to prevent C&D materials from being moved through potentially contaminated areas.

Conclusions:

- The risk of susceptible poultry being infected with HPAI virus from the vehicle transporting egg-handling materials from the hatchery to breeder farm is *negligible.*
- The risk of susceptible poultry being infected with HPAI virus via the vehicle driver transporting egg-handling materials from the hatchery to breeder farm is *negligible.*
- The risk of susceptible poultry being infected with HPAI virus via egg-handling materials moved from the hatchery to a breeder farm is *negligible* to *low.*

10.3.1 Introduction

In this section, we evaluate the risk that the vehicle or driver transporting egg-handling materials from the hatchery are contaminated, resulting in HPAI spread to a susceptible breeder flock. The primary risk pathway for this risk event is through cross-contamination of vehicles or personnel by vehicles, personnel or handling materials originating from HPAI infected turkey breeder farms.

Egg trays and reusable materials have been implicated as possible fomites for HPAI transmission between poultry farms in previous avian influenza outbreaks.(54, 134-136) For hatching eggs specifically, movement of egg racks has been implicated as a possible fomite for HPAI spread between breeder flocks in previous outbreaks. To our knowledge, outbreak data on the frequency or degree of contamination of egg handling materials from avian influenza infected farms is unavailable.

The STS Plan includes various movement specific biosecurity and C&D measures to address this risk. These measures reduce the likelihood that hatching eggs, egg-handling materials, the vehicle or driver originating from a breeder farm in the Control Area are contaminated with HPAI virus. We consider these measures in a qualitative evaluation of this risk.

10.3.2 Preventive Measures

10.3.2.1 Current Preventive Measures

General provisions from NPIP (Error! Reference source not found.) as well as NPIP biosecurity requirements for the vehicle and driver reviewed in **Section 10.2.2.1** apply to this risk pathway. Normal operations include washing and disinfecting reusable egg flats and buggies before they are returned to the breeder premises to prevent microbial contamination. Turkey hatchery buggy sanitation and the time that hatchery buggies are held in the egg room cooler were also considered in the risk evaluation (**Section 10.1.2.2.4**). Typically, hatcheries have automatic tray washers that use high pressure (up to 1000 psi) and temperature to ensure that the egg trays are completely clean. After setter trays are removed, the buggies and racks are C&D in the washroom. Visual inspection and routine microbiological monitoring (e.g., weekly intervals) is performed to ensure the effectiveness of the C&D process. Buggies may be returned to breeder farms immediately after sanitation.

10.3.2.2 Outbreak Specific Measures

Outbreak specific measures proposed in the STS Plan to address this risk include active surveillance of breeder flocks before moving eggs or equipment, C&D of the egg-buggy wheels and egg storage room floor before buggies are loaded into the delivery vehicle, C&D of the exterior of the vehicle before arriving at the hatchery, and proper use and disposal of PPE for the driver. Overall, these measures reduce the likelihood that hatching eggs, egg-handling materials, or the vehicle or driver originating from a breeder farm in the Control Area are contaminated with HPAI virus.

10.3.3 Risk Evaluation

10.3.3.1 Risk that Susceptible Poultry are Exposed to HPAI Virus via the Vehicle Transporting Egg-Handling Materials from the Hatchery

The potential risks associated with vehicles and personnel leaving the hatchery are:

- Risk that susceptible poultry are exposed to HPAI virus via the vehicle transporting egg-handling materials from the hatchery.
- Risk that susceptible poultry are exposed to HPAI virus via the vehicle driver transporting egg-handling materials from the hatchery.

10.3.3.1.1 Risk Associated with the Vehicle Exterior

In **Section 9.3**, we concluded that the risk of HPAI virus remaining on the exterior of a vehicle C&D before arriving at the hatchery as specified in the STS Plan is *negligible*. The risk of contamination of the hatching egg vehicle during transport would be reduced by selecting a route to avoid other poultry premises by a reasonable distance, once the C&D of the vehicle is completed. There are no plausible pathways for direct contamination of the vehicle exterior at the hatchery.

In **Section 9.3**, we concluded there is a *low* likelihood of the driver moving hatching eggs being contaminated. It is possible that the ground areas around the hatchery or the exterior surfaces of the vehicle delivering handling materials could become contaminated by the hatching egg delivery vehicle driver, with subsequent contamination of the exterior surfaces or tires of a vehicle destined to poultry premises. However, the STS Plan requires that the tires and wheel wells be C&D before delivering materials at the breeder premises. We conclude that the risk of susceptible poultry becoming infected with HPAI virus via contamination of the exterior of the vehicle transporting egg-handling materials from the hatchery is *negligible*.

10.3.3.1.2 Risk Associated with the Vehicle Interior

Depending on the hatchery design, a common shipping dock may be used for incoming hatching eggs and outgoing egg-handling materials. Given this practice, we evaluate the possibility that the cargo interior of the vehicle transporting clean handling materials becomes cross-contaminated during the loading process at the hatchery. The pathway for this risk involves contamination of the loading dock, or receiving storage areas, while unloading incoming hatching eggs, and subsequent contamination of the trailer interior of the outgoing vehicle via personnel or equipment.

- In the release assessment (**Section 9.3.3.1.1.1**), we estimated the likelihood of contamination of the cargo interior of the hatching egg truck through the movement and loading of hatching eggs from the egg storage room to be *low*.
- We estimated the likelihood of egg handling materials such as buggies moved from the breeder premises being contaminated with HPAI virus to be *negligible* to *low*, provided that the outbreak measures from the STS Plan are strictly followed. In particular, the buggy wheels would have been soaked with a disinfectant.

The pathway for contamination of outgoing materials would involve multiple transfer steps with direct contact between surfaces in order to transfer virus from the truck cargo interior to the hatchery loading dock. For example the overall pathway: loading dock at the breeder farm → driver boot covers → trailer interior floor → shoes of hatchery personnel → loading dock at hatchery → contamination of C&D'd materials going to another breeder farm would involve more than 4 steps. The final surface concentration of HPAI virus transferred through such contact steps would be diluted through the multiple steps. Only a fraction of the virus (6 to 27 percent) on a donor surface is transferred to the recipient surface in each direct contact event (Sayed Sattar and Susan Springthorpe, Personal communication, 2011).(20, 117, 118)

Because of the number of transfer steps involved in the release and entry pathways:

- We conclude that the likelihood of the hatchery loading dock becoming contaminated from the hatching egg delivery truck driver is *negligible* because the hatching egg driver uses PPE at the breeder farm, and STS Plan requires that the driver spray the floors, pedals, and bottoms of feet with disinfectant after every stop.
- We conclude that the likelihood of the hatchery loading dock becoming contaminated via wheels of incoming hatching egg buggies from a Control Area to be *negligible to low.*

Moreover, multiple transfer steps would also have to occur on the farm to transfer HPAI virus from the wheels of the incoming cross-contaminated buggies to susceptible poultry in the breeder hen-house. Based on the above qualitative evaluation, we conclude that the risk of susceptible poultry becoming infected with HPAI virus via the interior of the vehicle transporting egg-handling materials from the hatchery is *negligible*.

10.3.3.2 Risk of the Driver of the Vehicle Transporting Cleaned and Disinfected Handling Materials being Contaminated with HPAI Virus and Exposing Susceptible Poultry

In **Section 9.3**, we concluded that there is a *low* likelihood of the driver being contaminated on the breeder farm. Furthermore, the driver would be required to spray the floors and pedals of the delivery vehicle with disinfectant after entering the cab at the breeder farm. Finally, the driver delivering C&D'd materials would be required to wear PPE at the destination breeder farm. The likelihood of the transfer of contamination by the driver on either transfer step would be reduced by the use of PPE. As summarized in the *Broiler Hatching Egg* and *Table-egg Layer Hatching Egg* Risk Assessments, PPE such as disposable boots and gloves were found to be effective in preventing disease transmission in a majority of cases for other animal pathogen.(137, 138) We rate the likelihood of PPE not preventing transmission, resulting in HPAI spread to susceptible poultry, as *negligible.* We conclude that the risk of the driver transmitting HPAI virus to a susceptible breeder flock is *negligible* if the relevant PPE and biosecurity guidelines are strictly followed.

10.3.3.3 Risk that Susceptible Poultry are Exposed to HPAI Virus via Egg-Handling Materials Returned to a Breeder Farm

This risk evaluation is focused on the pathways involving cross-contamination of egg handling materials at the hatchery, before being distributed to breeder flocks. In **Section 9.2**, we concluded that the likelihood of buggies or pallets being contaminated with HPAI virus at the time they are loaded onto a transportation vehicle for movement from an HPAI infected but undetected flock is *low*. In **Section 9.3.3.1.1.1**, we also concluded that the likelihood of the truck cargo interior being contaminated is also low. Therefore, there is a possibility of buggies or pallets from uninfected premises becoming cross contaminated with HPAI virus from materials originating from infected but undetected premises through contamination of the hatchery loading dock and floors. The likelihood

of this event would largely depend on biosecurity procedures and the workflow practices at the hatchery.

- Any HPAI virus present on hatchery floors (other than the egg room) would likely be inactivated within 24 hours due to the daily C&D procedures at the hatchery as required by NPIP. In addition, the temperature of the setter and hatcher rooms (25°C or higher) is expected to result in more than 3-log EID_{50} inactivation of HPAI virus on dry surfaces within a day (see environmental persistence data in **Section 8**).

- Although hatchery personnel may contact buggies in the egg storage room and then handle C&D'd egg buggies, the degree of contamination transferred to buggy surfaces is estimated to be low.

We conclude that the likelihood of buggies, pallets or other materials being cross contaminated through the hatchery floors after C&D is *low* when the preventive measures discussed above are strictly followed. Similar to the evaluation of the risk of exposure of a susceptible breeder flock via the truck interior in Section10.3.3.1.2, multiple virus transfer steps would also have to occur on the farm to transfer HPAI virus from the wheels of the incoming cross-contaminated buggies to susceptible poultry in the breeder hen-house. Therefore, we estimated the risk of a susceptible breeder flock becoming infected with HPAI virus due to cross contamination of cleaned and disinfected egg handling materials from the hatchery to a breeder farm is *negligible* to *low*.

10.3.4 Conclusion

In this section, we evaluated the risk of a susceptible breeder flock being infected with HPAI virus from contamination on the vehicle, the vehicle interior, or driver transporting egg-handling materials, from a hatchery. Provided the preventive measures specified in the STS Plan are strictly followed, we conclude:

- The risk of a susceptible breeder flock becoming infected with HPAI virus from the interior or exterior of the vehicle transporting egg-handling materials from the hatchery to a breeder farm is *negligible*.

- The risk of a susceptible breeder flock becoming infected with HPAI virus via the vehicle driver transporting egg-handling materials from the hatchery to a breeder farm is *negligible.*

- We conclude that the risk of movement of setter trays resulting in HPAI infection of susceptible poultry is *negligible.*

- We conclude that the likelihood of buggies and pallets being cross contaminated through the hatchery floors after C&D is *low* when the preventive measures discussed above are strictly followed.

- Finally, we estimated the risk of a susceptible breeder flock becoming infected with HPAI virus due to cross contamination of cleaned and disinfected egg handling materials (buggies and pallets) from the hatchery to a breeder farm is *negligible* to *low*.

11. Overall Conclusion

In this assessment, we evaluated the risk that the movement of turkey hatching eggs and associated handling materials from breeder hen flocks located in a Control Area to commercial turkey hatcheries will result in HPAI spread to susceptible poultry (i.e. day old poults in the hatchery or other turkey breeder flocks). We evaluated scenarios where the breeder hen flock became infected through semen movement from infected breeder tom flocks or where it was directly infected from other pathways independent of the tom flocks. The risk assessment considered relevant current industry practices, current biosecurity measures (NPIP) as well as outbreak specific measures from the STS Plan.

Overall this assessment concludes that the risk of HPAI spread to susceptible poultry associated with the movement of turkey hatching eggs into, within, and outside of a Control Area during an outbreak is *negligible to low*, provided that the outbreak measures from the STS Plan are strictly followed.

The key results from the pathways evaluated in the Entry Assessment section are:

- The predicted mean number of internally and externally contaminated hatching eggs per movement from an infected breeder henhouse was very low under all the scenarios considered.
- The degree of external contamination of eggs moved from an infected breeder house is expected to be low for nest clean eggs under all the scenarios considered, due to egg sanitizing steps using an approved disinfectant or chlorine rinse with the concentration equal to or greater than 200 ppm.
- The likelihood of egg handling materials moved from the premises being contaminated with HPAI virus was rated to be *negligible* to *low.*

The key results from the pathways evaluated in the Exposure Assessment section are:

- The risk of day-old poults becoming infected with HPAI virus from hatching eggs or handling materials via movements of equipment or hatchery personnel is *negligible* to *low*.
- The risk of day-old poults becoming infected with HPAI virus from the vehicle or driver transporting hatching eggs or materials is *negligible* to *low*.
- The risk of a susceptible breeder flock becoming infected with HPAI virus from contamination on the exterior of the vehicle or driver transporting cleaned and disinfected egg-handling materials from the hatchery to a breeder farm is *negligible*.
- The risk of a susceptible breeder flock becoming infected with HPAI virus due to cross contamination of cleaned and disinfected egg handling materials from the hatchery to a breeder farm is *negligible* to *low*.
- Based on a review of published literature, and expert opinion provided by turkey industry practitioners participating in the STS working group, there is no documented evidence of horizontal or vertical transmission of AI in turkey hatcheries to day old poults to date.

The key quantitative results from the simulation models of HPAI disease spread within a turkey breeder flock and detection via active surveillance are as follows:

- In the scenarios where a breeder hen flock in the Control Area that was infected through mechanisms other than semen movement, the mean predicted number of internally contaminated hatching eggs varied between 0.007 to 0.008 eggs per movement.
- In the scenarios where a breeder hen flock in the Control Area was infected via HPAI virus associated with semen movement, no internally contaminated hatching eggs from an infected breeder henhouse were predicted to be moved before detection in the 8000 simulation model runs that were performed.
- The simulation results indicated a 95 percent chance that there are no internally contaminated eggs in movements of eggs from an infected hen house before detection under all the scenarios considered. There are approximately 6700 eggs per movement from a house on average in the scenarios considered here, when eggs are moved from a breeder flock twice weekly.

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

- This assessment is based on current (February 2014) 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. National Poultry Improvement Plan (Provision for Breeder-Hatchery Operations) 9CFR147.21-27

1. Measures at Breeder Premises.

- Cleaned and disinfected containers such as egg flats should be used in collecting the nest eggs for hatching. Egg handlers should thoroughly wash their hands with soap and water prior to and after egg collection. Clean outer garments should be worn.
- Dirty eggs should not be used for hatching purposes and should be collected in a separate container from the nest eggs. Slightly soiled nest eggs may be gently dry cleaned by hand.
- Hatching eggs should be stored in a designated egg room under conditions that will minimize egg sweating. The egg room walls, ceiling, floor, door, heater, and humidifier should be cleaned and disinfected after every egg pickup. C&D procedures should be as outlined in 9CFR147.24.
- The egg processing area should be cleaned and disinfected daily.
- Effective rodent and insect control programs should be implemented.

2. Measures at Hatchery

- The hatchery building should be arranged so that separate rooms are provided for each of the four operations: Egg receiving, incubation and hatching, chick/poult processing, and egg tray and hatching basket washing.
- Setter room walls, ceilings, floors, doors, fan grills, vents, and ducts should be cleaned and disinfected after each set or transfer. Setter rooms should not be used for storage. Plenums should be cleaned at least weekly.
- Egg trays and buggies should be cleaned and disinfected after each transfer. C&D procedures should be as outlined in 9CFR147.24.
- The entire hatchery should be kept in a neat, orderly condition and cleaned and disinfected after each hatch. The hatchery rooms, tables, racks, and other equipment in them should be thoroughly cleaned and disinfected frequently.
- Only clean eggs should be used for hatching purposes.
- Only new or cleaned and disinfected egg cases should be used for transportation of hatching eggs. Soiled egg case fillers should be destroyed.

3. Truck and Driver Biosecurity

The egg and chick/poult delivery truck drivers and helpers should use the following good biosecurity practices while picking up eggs or delivering chicks/poults:

- Spray truck tires thoroughly with disinfectant before leaving the main road and entering the farm driveway.

- Put on sturdy, disposable plastic boots or clean rubber boots before getting out of the truck cab. Put on a clean smock or coveralls and a hairnet before entering the poultry house.
- After loading eggs or unloading chicks/poults, remove the dirty smock/coveralls and place into plastic garbage bag before loading in the truck. Be sure to keep clean coveralls separate from dirty ones.
- Reenter the cab of the truck and remove boots before placing feet onto floorboards. Remove hairnet and leave with disposable boots on farm.
- Sanitize hands using appropriate hand sanitizer.
- Return to the hatchery or go to the next farm and repeat the process.
- Prevent indirect transmission from outside sources through contaminated equipment, footwear, clothing, vehicles, or other mechanical means.
- All vehicles used for transporting eggs or chicks/poults should be cleaned and disinfected after use. C&D procedures should be as outlined in 9CFR147.24

Appendix 2. Technical Details of Simulation Models of Disease Transmission and Active Surveillance

This appendix provides technical details and mathematical equations used for simulating the HPAI disease spread within a flock and detection via active surveillance.

Stochastic Disease Transmission Model

We developed a stochastic SLIR model to simulate disease spread within infected turkey breeder houses. Disease states included in the model are susceptible (S), latently infected (L), infectious (I), and removed (R). The model updates the number of hens in disease states at 0.1 day time steps. The uncertainties in input variables and the inherent variability associated with the HPAI infection among different hens within a flock were incorporated into the model.

The model was programed with Visual Basic.NET, and data were output into a spreadsheet for analysis (Excel®, Microsoft Corporation, Redmond, WA). The statistical packages R (87) and statconnDCOM (88) were used to fit statistical distributions to data and simulate random variables.

Notation

Appendix Table 1. Input variables and Indices used in the disease transmission model.

Variable	Description
β	Effective contact rate or the expected number of contacts that a bird has with other birds in a time period that are adequate to transmit HPAI infection.
N	The house size or the total number of birds in the house.
$N_i(t)$	The total number of infectious birds at the beginning of period t.
$N_s(t)$	The total number of susceptible birds at the beginning of period t.
$N_d(t)$	The total number of dead birds at the beginning of period t.
P_t	Probability that a susceptible hen at the beginning of period t becomes infected by beginning of period $t+1$.
$I_{t,t+1}^{new}$	Number of newly infected birds between the beginning of period t and the beginning of $t+1$.
i	Index of birds in the flock ($i \in 1, \dots, N$).
t	Index of time periods ($t \in 1, \dots, t_{max}$).
$\hat{S}(t)$	Set of indices of birds that are in a susceptible state in time period t.
$\hat{L}(t)$	Set of indices of birds that are in a latent state in time period t.
$\hat{I}(t)$	Set of indices of birds that are in an infectious state in time period t.
$\hat{R}(t)$	Set of indices of birds that are a removed state (dead) in time period t.
$\tau^L(i)$	Length of the latently infected period for a hen in a specific simulation iteration.

$\tau^I(i)$	Length of the infectious period for a hen in a specific simulation iteration.
$T^L(i)$	Simulation time at which hen i entered into the latently infected state.
$T^I(i)$	Simulation time at which hen i entered into the infectious state.
λ	Number of hours represented by each time period t of the simulation model.

Disease state transitions in the transmission model

The transmission equation estimates the number of susceptible birds that become newly infected with HPAI virus in each time period. The transmission equation is based on calculation of P_t the probability that a susceptible bird has an adequate contact with at least one infected bird in a time period. In general, a higher adequate contact rate (β) or higher proportion of infectious bird will lead to increased transmission. We use the transmission equation derived in Dietz and Schenzle (1985) as shown in Appendix Equation 1.(139) This transmission equation assumes that the number of effective contacts each bird has is Poisson distributed with a mean (β). A Poisson process indicates a continuous and constant opportunity for an event to occur. Appendix Equation 2 gives the number of newly infected birds in the next time period($I_{t,t+1}^{new}$), from a binomial distribution, where the outcome is dependent on the probability that a bird becomes infected (P_t) and the number of susceptible hens $N_s(t)$ in time period *t.* We note that Equations 1 and 2 have been utilized in several published studies of HPAI transmission in chickens.(93)

$$P_t = 1 - e^{-\frac{\beta(N_i(t))}{N-N_d(t)-1}}$$

Appendix Equation 1

$$I_{t,t+1}^{new} \sim Binomial(N_s(t), P_t)$$

Appendix Equation 2

The transmission equation provides the basis for calculating the number of birds transitioning from the susceptible to the latently infected state in one time period. The model updates the disease states in unit time steps (e.g., 0.1 days). The transitions from latently infected to infectious disease states and from infectious to removed (dead) disease states are accomplished by keeping track of the length of the individual hen's disease state and timing of when each hen transitioned into a disease state. For example, in the case of transitioning between the latent to infectious state, the model first calculates the length of the latent period for the hen (τ^L) based on the latent time distribution. The model also keeps track of the model time when a hen transitioned into the latently infected state (T^L). The model transitions the bird from the latently infected to the infectious state in the first time period *t* where $t^*\lambda \geq \tau^L + T^L$. Other disease state transitions are performed in a similar manner. The model can be run for a specified

number of time periods (60 6-hour intervals for this analysis) and provides estimates of number of birds in various disease states over time.

The main algorithmic steps associated with the various disease state transitions are described as follows.

Begin;
For t = 1 to t_{max} (
$\hat{R}(t+1) = \hat{R}(t)$;
$\hat{I}(t+1) = \hat{I}(t)$;
$\hat{L}(t+1) = \hat{L}(t)$;
$\hat{S}(t+1) = \hat{S}(t)$;
Transitions between infectious to removed state.
For each bird i ∈ $\hat{I}(t+1)$(
if t*λ ≥ $\tau^I(i) + T^I(i)$ then (
$\hat{R}(t+1) = \hat{R}(t+1)$ U {i};
$T^R(i) =$ t*λ;
$\hat{I}(t+1) = \hat{I}(t+1) -$ {i} ;
)
)
Transitions between latent to infectious state.
For each bird i ∈ $\hat{L}(t+1)$ (
if t*λ ≥ $\tau^L(i) + T^L(i)$ then (
$\hat{I}(t+1) = \hat{I}(t+1)$ U {i};
$T^I(i) =$ t*λ;
$\hat{L}(t+1) = \hat{L}(t+1) -$ {i} ;
)
)
Transitions between susceptible to latently infected state.
Appendix equations 1 and 2 were utilized to simulate and obtain the value for $I_{t,t+1}^{new}$
For each bird *i* among the first $I_{t,t+1}^{new}$ hens in $\hat{S}(t+1)$ (
$\hat{L}(t+1) = \hat{L}(t+1)$U {i},
$T^L(i) =$ t ∗ λ};
$\hat{S}(t+1) = \hat{S}(t+1) -$ {i}};)
)
End;

Estimation of Transmission Model Parameters

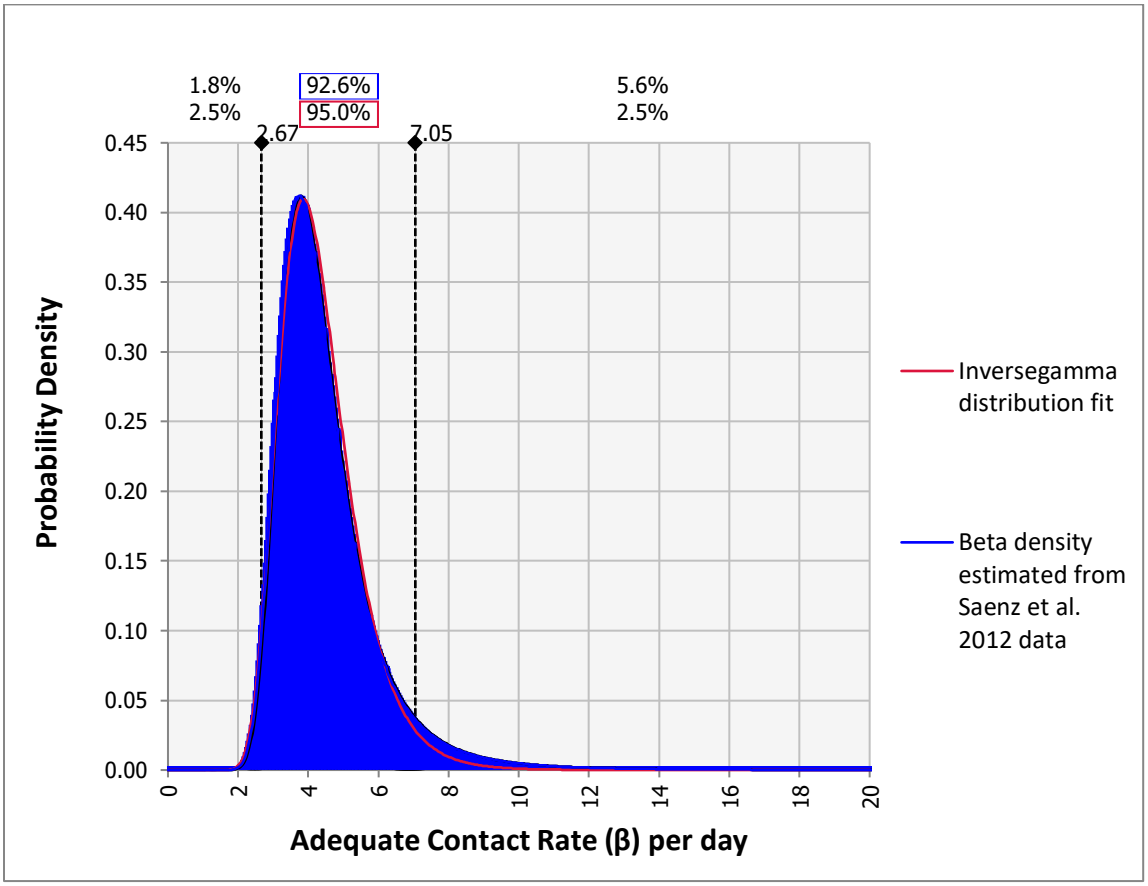
There is a considerable uncertainty regarding the adequate contact rate for turkey flocks in the United States, as the required outbreak data is limited. Moreover, most of the outbreak and experimental data is only partially observed where the timing of when birds have transitioned between various diseased states is not precisely known. Given these data limitations, various approximate methods and simplifying assumptions have been utilized in estimating the adequate contact rate in poultry flocks.

A recent experimental study has evaluated the spread of HPAI H7N1 in a set of three transmission experiments. In each of these three experiments, one inoculated bird was introduced to a group of 10, 20, or 40 contact turkeys.(89) The experiments ran for periods up to a week and all end in 100% mortality. Given that the timing of when a contact bird has become infected is not directly observable; a simplifying assumption of zero latent time was used. The study estimated an adequate contact rate of 2.1 (range 1 to 7.1 per day from different experiments). The authors mention that the simplifying assumption of zero latent period could result in a slight underestimation of the adequate contact rate. For the case of chickens, Bos et al. (2009) found that including a latent time could result in a considerable increase in the contact rate estimated from outbreak mortality data through back calculation.(93) Estimated contact rate increased from 4.5 per day to 8.94 per day when a latent time of 0/1 (mean 0.5) days was included in the estimation procedure.

Because a latently infected period at a bird level (defined as a period when a bird is infected but is not shedding detectable virus via RRT PCR) has been observed in most experimental studies, we believe that contact rate obtained from estimation procedures using a non-zero latent period is more applicable for within flock models of HPAI transmission. For the current risk analysis, we chose to reanalyze the data in Saenz et al. (2012) in order to specifically take into account a latent period. The parameters estimates were developed using Bayesian framework in order to evaluate the likelihood of a stochastic SEI model generating the experimental HPAI transmission data presented in Saenz et al. (2012) (84). Forward models were simulated with continuous-time Markov chains (CTMC) and Monte Carlo. Both methods resulted in similar results. The SEI model used for both approaches assumes a latent period governed by a gamma distribution with shape parameter *k* of 2. In practice, the latent period for the CTMC model was implemented using a two compartment latent period, according to the method of stages (e.g., Bos et al. 2007). (140) Both density-based and frequency-based transmission was considered, with similar results. Further, diagnostic sensitivities less than one were considered and explicitly modeled in order to allow for potential false negatives.

A grid-based approach was used to generate parameter values for the mean latent period (γL) and transmission rate parameters (β) for the three transmission experiments presented in Saenz et al. (2012).(84) Since the transmission experiments were conducted in a similar manner in a laboratory setting, it was possible to use the three generated likelihood surfaces in order to create a combined estimate for the relative likelihood of β and γL. An a priori estimate (using a likelihood approach based on a gamma distribution with shape parameter k=2) of the mean latent period was created using data from a separate inoculation experiment in Saenz, et al. (2012). (84) The a priori allowed using a Bayesian philosophy to update our estimates of the likelihood surfaces—additional information about γL provided additional information about β. Since the likelihood for the resulting distribution was reasonably concentrated, it was possible to integrate the likelihood surface with respect to γL to obtain a likelihood function solely parameterized by β (**Appendix Figure 1**). For use in simulation model, we found an InverseGamma (16.775, 0.0146) distribution to be the best fit for the β likelihood estimated above (**Appendix Figure 1**). We note that the estimated β distribution with a 95% credibility

interval of 2.67 to 7.05 per day is also comparable to other estimates in the literature (19)(141). Additionally, we performed sensitivity analysis with mean β as 1.5 per day and 12 per day to evaluate the impact of uncertainty in this parameter.



Appendix Figure 1. Posterior distribution of adequate contact rate (β) estimated from data presented in Saenz et al. (2012) and the Inverse Gamma distribution fit used in the simulation. (84)

In the scenarios (A and C), representing Asian HPAI H5N1 strains, we jointly estimated the infectious and latent period distributions using the experimental turkey data presented in Aldous et al. (2010) (71) A maximum likelihood approach that considers that the data is censored and birds were only sampled at daily intervals was utilized. A brief description of this is given below.

Given that we have censored data, the exact time point when a bird transitioned from the latent to the infectious state and the time point when a bird transitioned from infectious to the dead state is not known. Let sampling times *a* and *b* respectively be the time periods of the last negative and the first positive swab for an inoculated bird signifying that the latent period ended in the interval (a,b]. Let sampling times *c* and *d* represent the time of the last positive swab and either a negative swab or death signifying the end of infectious period in the interval (c,d].

Let event F be that the transition from latent to infectious period occurs in the interval (a,b]. Let event G be that the transition from the infectious period to death occurs in the

interval (c,d]. Given a gamma distributed latent period with shape k_1 and scale θ_1 and a and Weibull distributed infectious period with shape k_2 and scale θ_2, the likelihood of observing a specific data point is given by,

$$P(F \cap G) = \int_a^b f_{gamma(k_1,\theta_1)}(y)\left(\int_{c-y}^{d-y} f_{weibull(k_2,\theta_2)}(x)\,dx\right)dy$$

Appendix Equation 3

The combined likelihood for all the data points for HPAI H5N1 experiments from Aldous et al. (2010) was maximized using the Optim function in R Version 3.0.2 (A combination of simulated annealing and Nelder-Mead methods were utilized) to estimate $k_1, \theta_1, k_2, \theta_2$. The maximum likelihood estimates for k_1, θ_1, k_2 and θ_2, were 10.032, 0.126, 1.103 and 1.329 respectively.

Similar methods were utilized to estimate the parameters for scenarios B and D representing HPAI H5N2 strains. For the latent period, we estimated a Gamma (shape: 8.054, scale: 0.05) days distribution based on data from van der Goot et al. (2003) and Saenz et al. (2012).(84, 141) The infectious period was estimated to be Weibull (Shape: 1.965, Scale: 4.237) days distributed using HPAI H5N2 data from van der Goot et al. (2003) and Swayne et al. (2012). (23, 141)

Modeling of Active Surveillance

Notation

t is the index of disease days, $t = 1,\ldots,t^{max}$; where t=1 is the first day on which the flock is infected and t^{max} is the maximum number of days in the simulation. $t^{max} = 15$ in our simulations.

N^{dsp} is the number of days of eggs moved in a single shipment. N^{dsp} was set to 7 or 4 while modeling weekly once or twice movements respectively.

t^{mov} is the number of days post HPAI infection of the house on the day when hatching eggs are being moved in a particular simulation iteration $1 \leq t^{mov} \leq t^{max}$

t^{smov} is the number of days post HPAI infection of the breeder tom house on the day when turkey semen is moved in a particular simulation iteration $1 \leq t^{mov} \leq t^{max}$

t_{pcr}^{d} is the minimum detection day t via RRT-PCR testing in a single iteration.

t_{mor}^{d} is the minimum detection day t via observation of increased mortality in a single iteration.

t_{egg}^{d} is the minimum detection day t via observation of decreased egg production in a single iteration.

t^d_{tom} is the minimum detection day *t* that HPAI is detected in a turkey tom flock supplying semen to the breeder henhouse in question with the insemination day considered as day zero.

t^d_{all} is the minimum detection day *t* via all detection mechanisms in a single iteration.

t^d_{alltom} is the minimum detection day *t* via all detection mechanisms in a breeder tom house in a single iteration. The detection mechanisms applicable for a breeder tom house include through observation of unexpectedly high mortality or through RRT-PCR testing

$M^d(t)$ is mortality due to HPAI on day *t* (birds/house) estimated from HPAI disease transmission model.

$M^n(t)$ is normal mortality independent of HPAI on day *t* (birds/ house)

$M^t(t)$ is total mortality on day *t*, birds/house. $M^t(t) = M^d(t) + M^n(t)$

M_{lim} is threshold for total percentage mortality above which HPAI infection in the flock would be detected via observing unexpectedly high mortality regardless of diagnostic testing. M_{lim} was set 0.2 percent.

n_1 is number of swabs in the pooled sample submitted for RRT-PCR. $0 < n_1 \leq 5$

$\eta(t)$ is average egg production rate on day *t* post infection of the birds in the house (settable eggs/birds in the house) estimated from HPAI disease transmission model.

$X^{pool}(t)$ is number of swabs from HPAI infected birds present in the pooled sample on day *t* $0 \leq X^{pool}(t) \leq n_1$

$$X^{pcr}(t) = \begin{cases} 1 \text{ if } RRT-PCR \text{ test result for the pooled sample on day } t \text{ is positive} \\ 0 \text{ otherwise} \end{cases}$$

$$X^{mort}(t) = \begin{cases} 1 \text{ if HPAI infection is detected due to unexpectedly high mortality on day } t \\ 0 \text{ otherwise} \end{cases}$$

$$X^{egg}(t) = \begin{cases} 1 \text{ if HPAI infection is detected due to decreased egg production on day } t \\ 0 \text{ otherwise} \end{cases}$$

Se is diagnostic sensitivity of the RRT-PCR testing procedure.

$E(t)$ simulated number of HPAI internally contaminated eggs produced on day *t* post infection of the flock based on the disease transmission model, contaminated eggs/breeder houses.

E_{sum} is total number of contaminated eggs per movement depending on whether infection is detected.

$\hat{I}(t)$ is the number of infectious turkey breeder toms on day *t* in a specific simulation iteration from the disease transmission model.

I_{sem}^{eff} is the effective number of infectious turkey breeder toms on the semen movement days for calculating the number of hens infected via insemination. I_{sem}^{eff} is set to 0 when HPAI infection in the breeder tom flock is detected before the semen movement day in a simulation iteration.

$E_{int}^{scencd}(t)$ is the estimated number of internally contaminated eggs produced from the disease transmission model with modifications for scenarios C&D. In these scenarios, the possibility of contaminated eggs being laid on the first day post insemination was considered.

$E_{int}^{scencd}(t)$ is the estimated number of externally contaminated eggs from the disease transmission model with modifications for scenarios C&D. In these scenarios, the possibility of direct contamination of the eggshell with contaminated semen was considered.

E_{emov}^{int} is total number of internally contaminated eggs per movement on day 3 post insemination under scenarios C&D.

E_{emov}^{ext} is total number of externally contaminated eggs per movement on day 2 post insemination under scenarios C&D.

Surveillance Model for Breeder Hen Houses in the Baseline Scenarios (A and B)

These scenarios consider that the breeder henhouse has become directly infected (regardless of breeder tom flocks) starting with one infected hen. The first step of the active surveillance model is to choose a scenario for the number of days a flock had been infected on a day of hatching egg movement. In scenarios A and B, we considered that the house could have been infected between 2 to 7 days on the day of hatching egg movement. If the house were infected for more than 7 days, then infection would automatically be detected due to increased mortality. Conversely, if the house were only infected for one day, no contaminated eggs would be moved given the 2-day holding time.

For scenario A, t^{mov} was simulated as

$$t^{mov} \sim Uniform(2,7)$$

Appendix Equation 4

For scenario B, t^{mov} was simulated as

$$t^{mov} \sim Uniform(2,7)$$

Appendix Equation 5

The next step is to simulate the number of diseased birds in pooled samples tested via RRT-PCR, given the number of normal and diseased birds in the daily mortality pool. The normal mortality distributions for turkey tom and hen breeder houses were estimated from industry data. The number of diseased dead birds was obtained from the transmission model results. Given the relatively large sample size, we sampled directly from the data set to estimate the mean daily mortality percentage (empirical distribution).

The mean daily mortality rate for tom houses was estimated from the mean weekly mortality data for 25 houses provided by industry representatives (range 0.1 to 1.1 percent /week). The variation in the daily mortality was then simulated as a Poisson distribution, with the rate equal to the mean daily mortality estimated in the preceding step.

In the following, we provide the equations used for simulating detection of HPAI virus in the flock via RRT-PCR testing (matrix gene) of the pooled sample, by observation of increased mortality or by observing decreased egg production.

Detection via RRT-PCR

The number of birds included in the pooled sample on each day was calculated as follows:

$$n_1 = Min(5, M^t(t))$$

Appendix Equation 6

The number of infected dead birds included in the pooled samples on each day of testing ($t = t^{mov}$, and $t = t^{mov} - 1$) was modeled via the hyper geometric distribution as shown below.

$$X^{pool}(t) \sim HyperGeometric\left(M^t(t), n_1, M^d(t)\right) for\ t = t^{mov}, t^{mov} - 1$$

Appendix Equation 7

$$X^{pool}(t) = 0\ for\ t \notin \{t^{mov}, t^{mov} - 1\}$$

Appendix Equation 8

Depending on the sensitivity of the test, there is a chance that infection may not be detected even if a pooled sample contains a contaminated swab. We modeled the outcomes of RRT-PCR testing as a simple Bernoulli trial with probability *P* equal to the sensitivity of the test *Se*.

$$X^{pcr}(t) \sim Bernoulli(Se) Min(1, X^{pool}(t)) for\ t = t^{mov}, t^{mov} - 1$$

Appendix Equation 9

$$X^{pcr}(t) = 0\ for\ t \notin \{t^{mov}, t^{mov} - 1\}$$

Appendix Equation 10

$$t^{d}_{pcr} = \min(t \in \{1 \dots, t^{max}\} | X^{PCR}_{(t)} = 1)$$

Appendix Equation 11

<u>Detection via observation of increased mortality</u>,

$$X^{Mort}_{(t)} = \begin{Bmatrix} 1\ if\ M^{t}(t) > M_{\lim}\ * flock\ size \\ 0\ otherwise \end{Bmatrix} for\ 1 \le t \le t^{max}$$

Appendix Equation 12

$$t^{d}_{mor} = \min(t \in \{1 \dots, t^{max}\} | X^{Mort}_{(t)} = 1)$$

Appendix Equation 13

<u>Detection via observation of decreased egg production</u>

$$X^{egg}_{(t)} = \begin{Bmatrix} 1\ if\ \eta(\max(0, t-2)) - \eta(t) \ge 0.15 \\ 0\ otherwise \end{Bmatrix} for\ 1 \le t \le t^{max}$$

Appendix Equation 14

$$t^{d}_{egg} = \min(t \in \{1 \dots, t^{max}\} | X^{egg}_{(t)} = 1)$$

Appendix Equation 15

<u>Overall detection day</u>

$$t^{d}_{all} = \min(t^{d}_{egg}, t^{d}_{pcr}, t^{d}_{mort})$$

Appendix Equation 16

<u>Estimated Number of Internally Contaminated Eggs Moved before detection</u>

If HPAI virus is not detected by the movement day, then then contaminated eggs produced before the two day holding time (from transmission model) may be moved off the premises.

$$E_{sum} = \left\{ \begin{array}{c} 0 \; if t_{all}^{d} \leq t^{mov} \\ \sum_{t=\max(0, t^{mov}-2-N^{ndsp})}^{\max(0, t^{mov}-2)} E(t) \; if \; t_{all}^{d} > t^{mov} \end{array} \right\}$$

Appendix Equation 17

Surveillance Model Modifications for Scenarios C and D

Surveillance for breeder tom flocks

The surveillance model for breeder tom flocks estimates the likelihood that contaminated semen from an HPAI infected tom flock is moved off the premises for insemination. It also estimates the effective number of infectious toms in the breeder tom flock at the time of movement. This parameter is used to estimate the number of initially infected hens through the insemination process.

For modeling of surveillance in breeder tom houses, the RRT-PCR test days were changed to two consecutive days before (not including) the movement day (Appendix Equation 18 to Appendix Equation 21). These changes were made to accommodate the logistical constraints as holding semen on the movement day while awaiting negative RRT-PCR results may not be practicable.

$$X^{pool}(t) \sim HyperGeometric\Big(M^{t}(t), n_1, M^{d}(t)\Big) \; for \; t = t^{smov} - 1, t^{smov} - 2$$

Appendix Equation 18

$$X^{pool}(t) = 0 \;\; for \; t \notin \{t^{smov} - 1, t^{smov} - 2\}$$

Appendix Equation 19

$$X^{pcr}(t) \sim Bernoulli(Se) Min\Big(1, X^{pool}(t)\Big) for \; t = t^{smov} - 1, t^{smov} - 2$$

Appendix Equation 20

$$X^{pcr}(t) = 0 \; for \; t \notin \{t^{mov} - 1, t^{mov} - 2\}$$

Appendix Equation 21

For the case of semen movement, the output variables of interest are the overall minimum detection day and the effective number of infectious toms on the semen collection and movement day. These variables were calculated according to Appendix Equation 22 and Appendix Equation 23.

$$t_{alltom}^{d} = \min(t_{pcr}^{d}, t_{mort}^{d})$$

Appendix Equation 22

$$I_{sem}^{eff} = \begin{Bmatrix} 0 \; if \; t_{alltom}^{d} \leq t^{smov} \\ \hat{I}(t^{smov}) \; if \; t_{alltom}^{d} > t^{smov} \end{Bmatrix}$$

Appendix Equation 23

Surveillance for breeder hens infected via insemination process (Scenarios C and D)

Scenarios C& D consider the possibility of multiple breeder hens becoming simultaneously infected with HPAI virus associated with an infected but undetected tom flock via insemination process. There are additional model considerations and assumptions applicable for these scenarios.

The following additional assumptions are applicable for scenarios C & D

- We conservatively assumed that turkey hens infected through the insemination process may lay HPAI contaminated eggs by one day post infection. In most experimental studies it was observed HPAI contaminated eggs were not laid on the first day post inoculation via intranasal or intraocular route **(Section 9.1.3**). However, based on expert opinion, we considered the possibility that HPAI virus infection of the oviduct may occur relatively sooner when infected through insemination. Given this assumption and considering the two day hold before movement according to the STS plan, the earliest internally contaminated eggs could be moved from breeder hens infected through insemination is by 3 days after insemination.
- We conservatively assumed that turkey hens infected with HPAI through the insemination process may lay externally contaminated eggs immediately after insemination due to direct contact with semen. Given this assumption, we considered each turkey hen exposed via contaminated semen may lay an externally contaminated egg within the first day post insemination according to its normal egg production rate. Given this assumption, and considering the two day hold after production before movement, the earliest externally contaminated eggs could be moved from breeder hens infected through insemination is by 2 days after insemination.
- We assumed that if HPAI infection is detected in the turkey tom flock then the movement of eggs from the breeder hen flocks to which semen was supplied would be restricted until further diagnostic investigation. Hence, detection in turkey tom flocks was considered as an additional detection mechanism for turkey breeder hen flocks infected through insemination process.

In the following we describe changes to the surveillance model that are applicable for scenarios C and D. Given the large number of hens simultaneously predicted to be infected via insemination under these scenarios, a t^{mov} of 4 or greater would likely result in a trivial outcome of HPAI infection being detected and no contaminated eggs being moved. Therefore we set t^{mov} to be 3 for estimating this parameter. HPAI disease detection in turkey tom flocks was considered towards HPAI detection in the hen flocks.

In the next step, the earliest detection day in turkey tom flocks with respect to insemination day was calculated (insemination day is day zero under this time scale). Here the variables t^d_{alltom} t^{smov} are from the surveillance model simulation for the breeder tom house.

$$t^d_{tom} = t^d_{alltom} - t^{smov}$$

Appendix Equation 24

The overall detection day under these scenarios is the minimum detection day among the four detection mechanisms: unexpected high mortality, RRT-PCR testing, decreased egg production or detection in tom flocks.

$$t^d_{all} = \min(t^d_{egg}, t^d_{pcr}, t^d_{mort}, t^d_{tom})$$

Appendix Equation 25

The total number of internally contaminated eggs in a shipment of hatching eggs moved on day 3 post insemination is

$$E^{int}_{emov} = \begin{Bmatrix} 0 \; if t^d_{all} \leq 3 \\ E^{scencd}_{int}(1) \;) \; if \; t^d_{all} > 3 \end{Bmatrix}$$

Appendix Equation 26

The total number of externally contaminated eggs in a shipment of hatching eggs moved on day 2 post insemination day is

$$E^{ext}_{emov} = \begin{Bmatrix} 0 \; if t^d_{all} \leq 2 \\ E^{scencd}_{ext}(0) \; if \; t^d_{all} > 2 \end{Bmatrix}$$

Appendix Equation 27

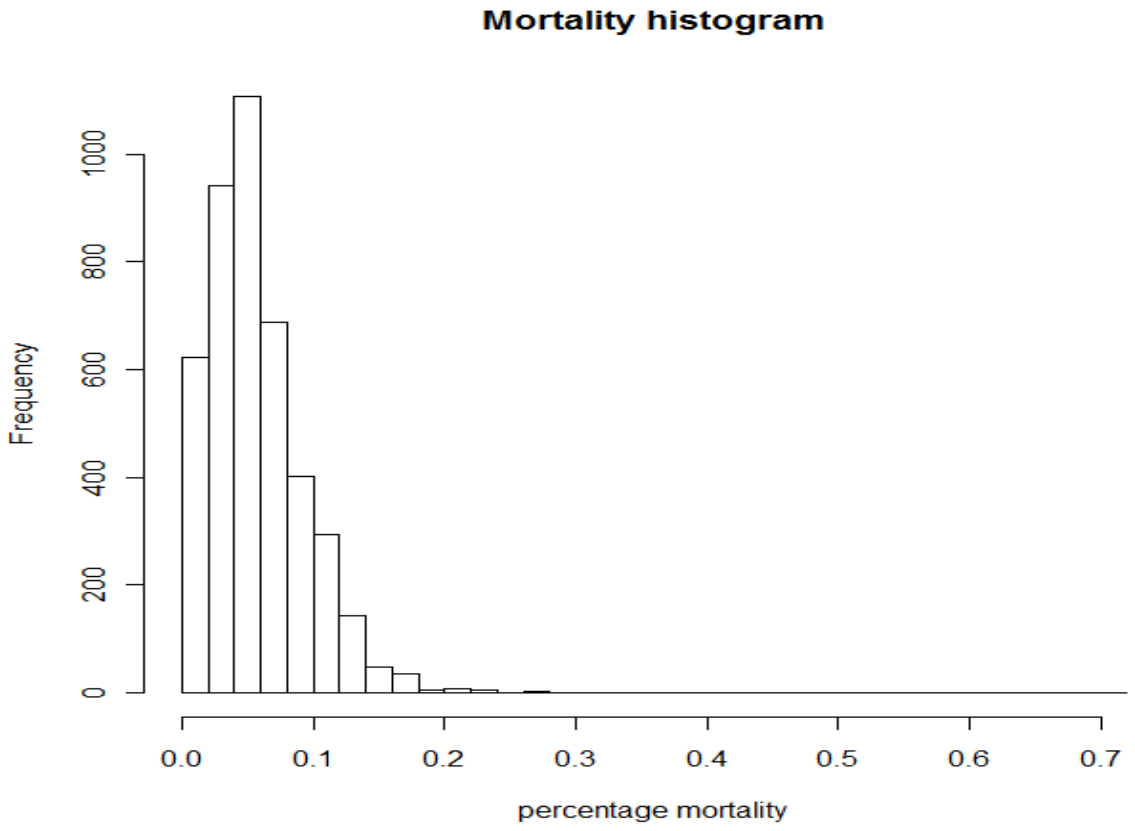
Parameters values Used in the Surveillance Model

The sensitivity of the RRT-PCR test was estimated to be 86.5 percent.(98) We note that AI Experts commented that this is a conservative estimate of the diagnostic sensitivity given recent enhancement to test protocols (Personal Communication, Jan Pederson, Erica Spackman, Marek Slomka).(100-102)

Normal daily mortality data for 26 turkey breeder hen houses were provided by industry representatives. Overall there were 7364 points in this dataset. The daily mortality percentage ranged from 0.0 (5th percentile) to 0.13 percent (95th percentile) with a mean of 0.057 percent (**Appendix Figure 2**). Given the relatively large sample size, we sampled directly from the data set to estimate the mean daily mortality percentage (empirical distribution). The mortality limit M_{lim} was set to 0.2 percent based on industry expert opinion. Note that this mortality is expected to result in a low false trigger

rate given the distribution of normal mortality (around 2 percent false trigger rate based on the industry data provided).

For turkey breeder tom mortality, we had 26 weekly flock mortality data points with a mean weekly mortality of 0.656 percent (range 0.1 to 1.1 percent). The daily mortality was simulated using a Poisson distribution with a mean mortality obtained by resampling of the weekly mortality data.



Appendix Figure 2. Histogram of daily mortality percentage from 26 turkey breeder hen flocks

Sensitivity Analysis for Adequate Contact Rate

We evaluated the impact of a slower or faster adequate contact rate compared to the baseline scenarios by simulating the disease transmission and active surveillance models with mean contact rates of 1.5 per day and 12 per day. The simulation results from **Appendix Table 2**, **Appendix Table 3**, **Appendix Table 4** and **Appendix Table 5** indicate that the predicted number of contaminated eggs moved from a turkey breeder hen house under active surveillance is robust to changes in the adequate contact rate within the range of values reported in the literature.

Appendix Table 2. Results from simulation models of disease transmission and active surveillance for scenarios where breeder hen flocks are first infected (Scenarios A and B) using a mean adequate contact rate of 1.5 contacts per day.

Output parameter	**Scenario A (Infectious period** 1.28 **days)**	**Scenario B (Infectious period** 3.75 **days)**
Model predicted number of internally contaminated hatching eggs moved from a turkey breeder hen house given a 2-day holding time after production (eggs per movement)	0.029 (0-0)*	0.033 (0-1)
Approximate detection probability by hatching egg movement day that is *Y* days post infection of the flock	58% (*Y*: 2-7 days)	68% (*Y*: 2-7 days)
Predicted number of infectious birds at movement in iterations where infection was not detected by movement day	0.68 (0-2)	6.6 (0-22)
Predicted number of infectious birds 2-days before movement day (at packing time for eggs moved with a 2-day hold) in iterations where infection was not detected by that day	0.12 (0-1)	0.91 (0-3)

*Approximate 90 percent probability interval

Appendix Table 3. Results from simulation models of HPAI disease transmission and active surveillance in Scenarios C and D using a mean adequate contact rate of 1.5 per day.

Output parameter	**Scenario C (Infectious period** 1.28 **days)**	**Scenario D (Infectious period** 3.75 **days)**
Model predicted number of internally contaminated hatching eggs moved from a turkey breeder house given a 2-day hold after production (eggs per movement).	0.00 (0-0)*	0.00 (0-0)*
Predicted detection probability by 2-days after exposure via insemination.	99.9%	99.9%
Predicted detection probability by 3 days after exposure via insemination.	99.9 %	99.9 %

*Approximate 95 percent probability interval.

Appendix Table 4. Results from simulation models of disease transmission and active surveillance for scenarios where breeder hen flocks are first infected (Scenarios A and B) using a mean adequate contact rate of 12 per day.

Output parameter	**Scenario A (Infectious period** 1.28 **days)**	**Scenario B (Infectious period** 3.75 **days)**
Model predicted number of internally contaminated hatching eggs moved from a turkey breeder hen house given a 2-day holding time after production (eggs per movement)	0.001(0-0)*	0.003 (0-0)
Approximate detection probability by hatching egg movement day that is *Y* days post infection of the flock	82% (*Y*: 2-7 days)	89% (*Y*: 2-7 days)
Predicted number of infectious birds at movement in iterations where infection was not detected by movement day	1.84 (0-8)	62 (11-155)
Predicted number of infectious birds 2-days before movement day (at packing time for eggs moved with a 2-day hold) in iterations where infection was not detected by that day	0.05 (0-1)	0.1 (0-1)

*Approximate 90 percent probability interval

Appendix Table 5. Results from simulation models of HPAI disease transmission and active surveillance in Scenarios C and D using a mean adequate contact rate of 12 per day.

Output parameter	**Scenario C (Infectious period** 1.28 **days)**	**Scenario D (Infectious period** 3.75 **days)**
Model predicted number of internally contaminated hatching eggs moved from a turkey breeder house given a 2-day hold after production (eggs per movement).	0.00 (0-0)*	0.00 (0-0)*
Predicted detection probability by 2-days after exposure via insemination.	99.9%	99.9%
Predicted detection probability by 3 days after exposure via insemination.	99.9 %	99.9 %

*Approximate 95 percent probability interval.

Appendix 3. Estimation of the Number of Contaminated Hatching Eggs that Leak During Transportation

We evaluated the likelihood that contaminated eggs leak during transportation or handling as follows:

- Based on industry data, the rate of leakage during transportation and handling combined varied between 0 out of 5000 eggs to 5 out 5000 (considered to be a high estimate) in a couple of observations. We used a uniform (beta(1,5001), beta(6,4996)) distribution to simulate the rate of leakage.
- HPAI virus contaminated eggs are more likely to be defective and thus may have a higher fraction of leakers compared to normal eggs.
- Based on the results of an expert opinion panel, we estimated that HPAI virus-contaminated eggs, with no visual defects, are 3.7 times more likely to leak than virus-free eggs (90 percent P.I. 2.2-7).(142)
- We estimated that 0.26 percent (90 percent P.I. 0.05-0.63 percent) of HPAI virus contaminated eggs may leak during transportation to the hatchery.

We estimated the likelihood of eggs leaking during transport or handling using the leakage probability estimated above in a simulation model. Based on the simulation results, the expected number of HPAI virus-contaminated eggs that leak during transportation to the hatchery or during handling is given in **Appendix Table 6**.

Appendix Table 6. Estimated mean number of internally contaminated hatching eggs per movement and the estimated mean number of leakers.

Scenario (Infectious period length)	**A (1.28 days)**	**B (3.75 days)**	**C (1.28 days)**	**D (3.75 days)**
Internally contaminated eggs	0.008 (0-0)*	0.007 (0-0)	0.00(0-0)*	0.00(0-0)*
Mean estimated number of contaminated leakers	0.000021	0.000018	0	0

*Approximate 90 percent probability interval

Hatching eggs are also less likely to leak during transport due to the mechanisms used for holding egg carts in place during transport. We conclude that the likelihood of the trailer interior becoming contaminated with HPAI virus from hatching eggs from an infected but undetected farm that leak during transportation is *negligible* to *low*.

Appendix 4. AI Virus Survival on Various Substrates

Appendix Table 7 and **Appendix Table 8** summarize the results of studies documenting survival on various substrates (poultry feces, glass, metal etc.). Based on these data, moisture content appears to be a major determinant of the survival time of avian influenza viruses in both feces and on plastic/metal surfaces. We conclude that, if sufficiently dried, AI virus within poultry feces is likely inactivated within 2-days and virus present on a metal or plastic surface (buggies, carts) would be inactivated within 1 day under room temperature and humidity conditions conducive to drying.

Appendix Table 7. Summary of experimental studies on survival of avian influenza virus on various substrates for HPAI inactivation studies in dried substances or under conditions that facilitate drying.*

Virus	Substrate	Survival	Humidity	Temperature	Reference
H5N1	Chicken feces	Not detected at 2-days	30-42% humidity	22-23°C	Wood et al., (2010) (129)
H5N2	Dried feces from AI-infected hens	Contained viable virus for 1 day	Stored in open vials	25°C	Beard et al., (1984)(21)
H5N1	Chicken manure	Lost infectivity at 24 hours	Not specified	25°C	Chumpolbanchorn et al., (2006) (44)
H5N1	Dried chicken feces	Nondetectable after 1 day	Not specified	25°C	Shortridge et al., (1998) (22)
H5N1	Glass, galvanized metal	No detection at 1 day	30-89% humidity (tested at both low and high relative humidity)	22-23°C	Wood et al., (2010) (129)
H1N1	Tyvek, surgical mask, wood desk, N95 respirator, gloves	No detection at one day except on gloves	55%	25°C	Sakaguchi (2010) (143)
H1N1 (pandemic)	Plastic, pine, steel, cloth	No detection of viable virus by one day	23-24%	17-21°C	Greatorex (2011) (144)

*Low moisture: Inoculated substrate was kept at low humidity (<70% RH), dried prior to testing, and/or stored in conditions conducive to the maintenance of low moisture content (e.g., storage in open vials)

Appendix Table 8. Summary of experimental studies on survival of avian influenza virus on various substrates for HPAI inactivation studies in moist substances or under conditions not conducive to drying. **

Virus	Substrate	Survival	Humidity	Temperature	Reference
H5N1	Chicken feces	Not detected at 4 days	91% humidity	22-23°C	Wood et al., (2010) (129)
H5N2	Feces from infected hens	Contained viable virus for 2-days	Stored in closed vials	25°C	Beard et al., (1984)(21)
H13N7	Steel, plastic	Inactivated by 6 days	stored in a cabinet	Room temperature	Tiwari et al. (2006) (48)
H7N1	Egg-shell, PVC, metal (tin)	Inactivated by 15 days	50-84% humidity	17-25°C	Vrtlak and Kapitancik, (1967) (145)
H7N1	Wood, burlap, grain, mixed feed	Inactivated by 8 days	50-84% humidity	17-25°C	Vrtlak and Kapitancik (1967)(145)

**High moisture: Inoculated substrate was kept at high humidity (>70% RH), not dried prior to testing, and/or stored in conditions conducive to the maintenance of higher moisture content (e.g., storage of moist feces in closed vials).

Appendix 5. The Definition of Non Negligible Risk Levels Used in this Assessment

Low Risk

For this risk analysis, the term “low risk” means it is very unlikely that moving hatching eggs and egg trays and buggies will cause infection in susceptible poultry. The determination of “low risk” suggests that, although not a requirement, additional resources to further evaluate or mitigate this risk may be considered (depending on circumstances).

Use in Risk Analysis

The term “low risk” has been frequently used in risk-rating systems for qualitative risk analysis. These risk-rating systems are often customized according to the specific objectives of the risk assessments. Consequently, there is considerable variation in the interpretation of the terms used to describe risk among various risk assessments. For example, in the USDA-APHIS Guidelines on Pathway-Initiated Pest Risk Assessments, the rating of *low* is interpreted as “the pest will typically not require specific mitigation measures”.(146) The FDA Guidance Document 152 states that “for a drug to be ranked as low risk overall, two of three major components (release, exposure and consequence) of the risk assessment should be ranked as low and the third component ranked as moderate”.(147) In a risk-rating system used in USDA APHIS for qualitative risk assessment for potential Federal noxious weeds, the overall pest risk potential is *low* as long as the likelihood of introduction of the weed is *low*, regardless of the consequences of introduction.(148) Overall, various definitions of “low risk” have been used as appropriate in different situations.

Negligible to Low Risk

When there is a considerable uncertainty in the risk estimate, we may not be able to ascertain whether the risk is *negligible* or *low*. This uncertainty can be expressed as a probability distribution for the risk in a fully quantitative risk assessment. For a qualitative risk assessment, there are no universally followed guidelines for expressing this uncertainty. Therefore, when there is uncertainty about whether the risk is *negligible* or *low*, we rate it as *negligible* to *low* risk. With *negligible* to *low* risk, depending on the circumstances, further evaluation to determine whether the risk is *negligible* or *low* may be conducted.

Definitions of Moderate, High and Extremely High Risk Levels Considered in the Risk Evaluation Process

These risk levels were defined on the basis of the likelihood of the spread of HPAI infection to susceptible poultry. The specific levels are defined as follows.

Moderate Risk: The spread of HPAI infection to susceptible poultry through the risk pathway is unlikely to but does occur.

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

Extremely High Risk: The spread of HPAI infection to susceptible poultry through the risk pathway is almost certain to occur

Appendix 6. The Use of "Negligible Risk" in this Assessment

Negligible Risk Defined for this Analysis

For this risk analysis, the term "negligible risk" means that the spread of HPAI infection to susceptible poultry through the risk pathway is insignificant or not worth considering. In quantitative terms, this is defined as a likelihood of less than 1/1,000,000 that the risk pathway will result in infection in other premises. This particular likelihood is used to be consistent with other common meanings for the term, as discussed below. The determination of "negligible risk" suggests that allocating additional resources to mitigate this risk pathway may not be a cost-effective use of resources (depending on circumstances).

Negligible Risk as Less Than 1/1,000,000

Origins

Use of the term "negligible risk" originated in efforts to regulate chemical exposures. While there is no formal definition, the term evolved in the human exposure risk assessment literature as a lifetime cancer risk of less than 1/1,000,000. This particular level was selected as it was thought to be a level of "essentially zero" risk.(149-152) While this level has not been normally defined in legislation, The House Committee on Commerce evaluated the use of this term by the Environmental Protection Agency, and agreed that the agency's interpretation of the term "negligible risk" to be approximately a one-in-a-million lifetime risk, as appropriate.(153)

Use in Agricultural Risk Analysis

The use of risk analysis for imports of agricultural products became mandatory with the adoption of the SPS Agreement[j] in 1995.(154) Specific recommendations and standards were to be established by the appropriate technical body. For animals and animal products, this is the Office International des Epizooties (OIE, or World Organisation for Animal Health). The OIE has published standards and guidance for conducting risk analysis, but has not formally defined "negligible" in a quantitative sense.(7) However, in a World Trade Organization trade dispute case, negligible risk was considered to be a risk whose probability is very low, or, as an expert consultant to the WTO Dispute Panel put it, "the standard scientific definition of "negligible" was a likelihood of between zero and one-in-one million."(155-158)

Policy Implications of a Quantitative Definition for Negligible Risk

While the 1/1,000,000 definition for negligible risk has substantial precedence (as shown above), there are difficulties with this approach. The 1/1,000,000 likelihood has been described as "folklore," vague, and inconsistent, and has been "used and (abused) in various policy contexts."(152, 159, 160) However, use of this figure is meant to be a very

[j] Formally known as the "Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) and Agreement on Technical Barriers to Trade (TBT)."

rough approximation and should not be given the same degree of certainty that may be applied when quantitative risk assessments can be used.

Negligible Risk as a Qualitative Measure for Agricultural Risk Analysis

The OIE has issued guidance that recommends using "negligible" to mean "not worth considering; insignificant."(7) The use of qualitative risk analysis methods by APHIS and the implied non-requirement for attaching a specific number to a level of risk has been challenged in the U.S. Court system and has been upheld as appropriate, if the analysis presents adequate scientific information.(161) When used in this manner, the courts have held that the determination of risk may be based on "the cumulative effects of the multiple, overlapping, safeguards." Furthermore, the courts have held that an "imposition of such a bright-line prohibition on qualitative standards was incorrect," and that the Animal Health Protection Act does not require a quantified permissible level of risk.(155) These opinions by the court system are also consistent with U.S. views expressed in WTO trade disputes.

Appendix 7. Qualitative Scales of Likelihood

This appendix defines the qualitative likelihood scale used to describe the probability of events in this risk assessment. Qualitative scales attach a specific narrative phrase which conveys a meaning to terms used to describe the likelihood of an event occurring. Generally, it is best to choose an expression where there is some evidence for a high degree of consensus for its interpreted meaning.(162) For example, use of the narrative phrase *"there is a high likelihood that the event will occur"* has been interpreted as a probability that ranges from 0.60 to 0.97 (60 to 97 percent chance of occurrence); and the expression *likely* has been interpreted to range from 0.63 to 0.77.(162, 163) To date, there is no one universally accepted or utilized likelihood scale, and the scales are customized as appropriate for specific assessments. The OIE handbook on qualitative risk analysis does not prescribe a specific likelihood scale although it provides examples for terms which might be used in likelihood scales such as *low, negligible, high* etc.(164) **Appendix Table 9** provides examples of qualitative scales used in risk assessments elsewhere and **Appendix Table 10** lists adjectives to describe likelihoods considered appropriate by the OIE. The likelihood scale used in this assessment is defined by **Appendix Table 11**.

Appendix Table 9. An example likelihood scale adapted from Standards Australia for qualitative risk assessment in fisheries management(165)

Category	Probability Range
Likely	It is expected to occur
Occasional	May occur sometimes
Possible	Some evidence to suggest this is possible here
Unlikely	Uncommon, but has been known to occur elsewhere
Rare	May occur in exceptional circumstances
Remote	Never heard of, but not impossible

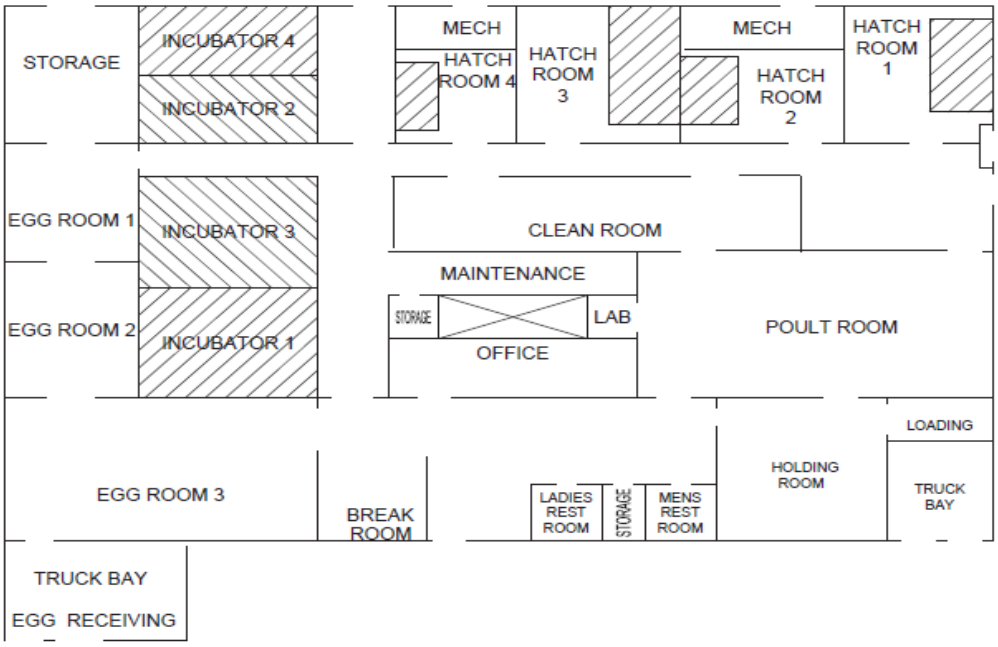
Appendix Table 10. Terms used as adjectives to qualify likelihood estimates considered appropriate by the OIE.(7)

Category	Descriptor
Extremely	Outermost, furthest from the center; situated at either end; utmost; the highest or most extreme degree of anything
High	Extending above the normal or average level
Highly	In a high degree
Significant	Noteworthy; important; consequential
Average	The usual amount, extent, rate
Low	Less than average; coming below the normal level
Remote	Slight, faint
Insignificant	Unimportant; trifling
Negligible	Not worth considering; insignificant

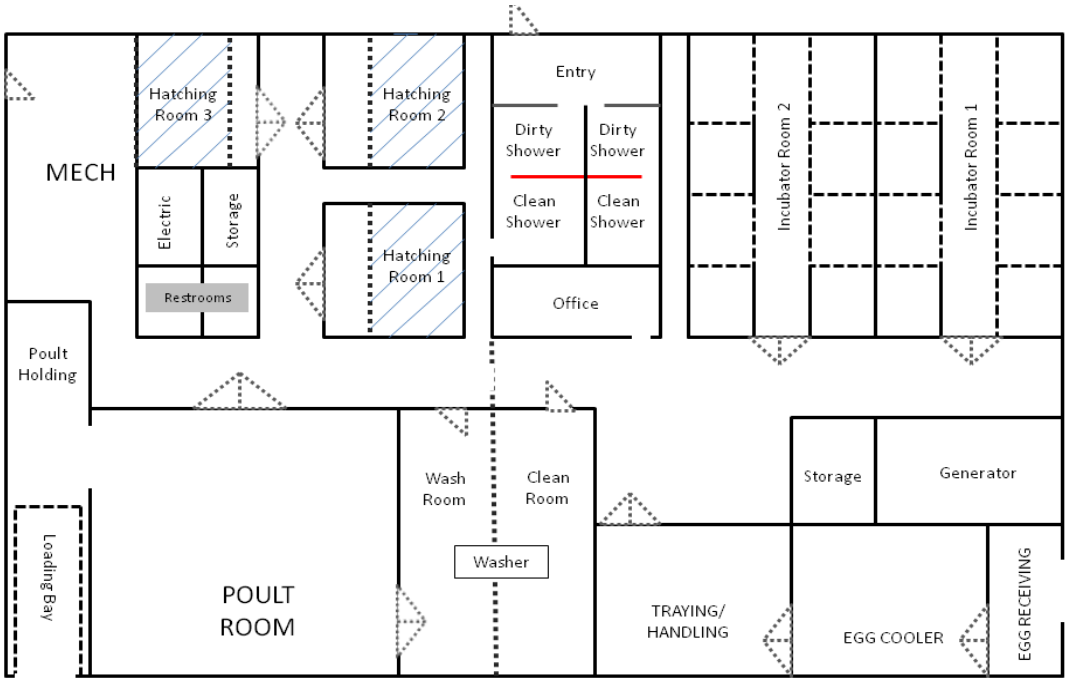
Appendix Table 11. Qualitative likelihood scale used in this assessment.

Category	Descriptor
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

Appendix 8. Examples of Hatchery Layouts



Appendix Figure 3. A hatchery that produces commercial poults from multiplier flocks.



Appendix Figure 4. A hatchery that produces poults from primary breeders (genetic stock) with shower-in facilities.

Appendix 9. Summary of Influenza Transmission Questionnaire

Expert opinion elicitation was conducted as part of an effort to assess the risk of HPAI infection of day-old poults with virus associated with hatching eggs or materials due to movements of personnel or equipment in the hatchery. The aim was to gather field data on the vertical transmission of viral influenza, in particular from breeder turkey hens to their hatching eggs. Members of the Association of Veterinarians in Turkey Production were queried for information on their experiences with HPAI in turkey egg production. Eight practitioners replied, and the following is a summary of their responses.

Four of the respondents had experienced outbreak(s) in breeders with one type of influenza, two practitioners had experienced outbreaks with two types of influenza, one had experienced three different types, and one four different types. Four of these types were characterized as Swine H1N1, one as H1N1, and two as Pandemic H1N1. Another four were characterized as Swine H3N2, one as Swine H1N2, one as H6N2, one as LPAI H7, and one was not characterized.

Practitioners reported a total of 162,200 hens involved in the influenza outbreaks, with the responses varying from no answer in 5 instances, and from 1,000 to 45,000 hens per influenza type in 10 outbreaks where the question was answered.

All respondents had set eggs from breeders experiencing influenza from the pre-clinical, clinical and post-clinical periods. One practitioner did not answer this question for one of the influenza types they had experienced.

In 12 instances, respondents stated that they did not recommend removing eggs from incubation due to current or previous influenza in the breeding hens. One practitioner reported recommending removal of some eggs from incubation in three cases, due to perception, but not due to concern over influenza transmission from the breeders to the eggs. None of the practitioners had observed influenza in poults hatched from infected breeders, nor had they suspected that such vertical transmission would occur.

Appendix Table 1. Expert elicitation response summary table.

Respondent	Subtype	No. hens	Were Eggs Set?	Were Eggs Removed?	Was AI Observed in Poults?	Was Transmission Suspected?
1	Swine H1N1	30,000	Yes	No	No	No
	Swine H3N2	20,000	Yes	No	No	No
2	Swine H1N2	NA*	Yes	No	No	No
3	Swine H1N1	45,000	Yes	No	No	No
4	Low Path H7	1,000	Yes	Yes/No**	No	No
	Swine H3N2	5,000		Yes/No**	No	No
	Swine H1N1	4,000	Yes	Yes/No**	No	No
	Pandemic H1N1	NA*	Yes	No	No	No
5	Swine H3N2	NA*	Yes	No	No	No
	Swine H1N1	NA*	Yes	No	No	No
	Swine H1N2	NA*	Yes	No	No	No
6	Unknown	15,000	Yes	No	No	No
7	Swine H3N2	5,000	Yes	No	No	No
8	LPAI H6N2	18,600	Yes	No	No	No
	Pandemic H1N1	18,600	Yes	No	No	No

*Not Available

**Some eggs were removed due to perception of a problem, not due to concern of egg transmission.

References

1. USDA:NASS. Hatchery Production 2011 Summary. 2012.
2. Bramwell, R. K. Overview of artificial insemination in poultry. Merck Veterinary Manual. 2012.
3. Cobb-Vantress, I. Hatchery Management Guide. 2014.
4. Das, K. C., M. Y. Minkara, N. D. Melear, and E. W. Tollner. Effect of poultry litter amendment on hatchery waste composting. The Journal of Applied Poultry Research 11:282-290. 2002.
5. Beckman, B. Personal communication.2010.
6. Wineland, M. Personal communication.Department of Poultry Science, North Carolina State University.2010.
7. World Organisation for Animal Health (OIE). Handbook on Import Risk Analysis for Animals and Animal Products, 2nd ed. The World Organisation for Animal Health (OIE), Paris, France. 2010.
8. USDA :APHIS :VS. Summary of the National Highly Pathogenic Avian Influenza Response Plan (Draft). 2007.
9. McCapes, R. H., R. Bankowski, and G. B. West. Avian Influenza in California: the nature of the clinical disease 1964-1985. Avian Diseases 47:118-132. 2003.
10. Kleven, S. H., R. C. Nelson, D. R. Deshmukh, J. I. Moulthrop, and B. S. Pomeroy. Epidemiologic and field observations on avian influenza in Minnesota turkeys. Avian Diseases:153-166. 1970.
11. Swayne, D. E., ed. Avian Influenza. Blackwell Publishing, Ames, Iowa. 2008.
12. Swayne, D. E., and D. L. Suarez. Highly Pathogenic Avian Influenza. Revue Scientifique et Technique de l'Office International des Epizooties 19:463-482. 2000.
13. Clifford, J. R. Veterinary Services Memorandum No. 565.14; Reporting Confirmed Findings of Low Pathogenic Notifiable Avian Influenza (LPNAI) (H5 and H7 Subtypes) to the World Organization for Animal Health (OIE) and to Trading Partners.Deputy Administrator, Chief Veterinary Officer.2006.
14. Gaidet, N., G. Cattoli, S. Hammoumi, S. H. Newman, W. Hagemeijer, J. Y. Takekawa, J. Cappelle, T. Dodman, T. Joannis, and P. Gil. Evidence of infection by H5N2 highly pathogenic avian influenza viruses in healthy wild waterfowl. PLoS pathogens 4:e1000127. 2008.
15. Senne, D. A., D. L. Suarez, D. E. Stallnecht, J. C. Pedersen, and B. Panigrahy. Ecology and epidemiology of avian influenza in North and South America. Dev Biol (Basel) 124:37-44. 2006.
16. Swayne, D. E. Understanding the complex pathobiology of high pathogenicity avian influenza viruses in birds. Avian Dis 51:242-249. 2007.
17. Boyce, W. M., C. Sandrock, C. Kreuder-Johnson, T. Kelly, and C. Cardona. Avian influenza viruses in wild birds: a moving target. Comp Immunol Microbiol Infect Dis 32:275-286. 2009.
18. Alexander, D. J. An overview of the epidemiology of avian influenza. Vaccine 25:5637-5644. 2007.
19. Stallknecht, D. E., and J. D. Brown. Wild birds and the epidemiology of avian influenza. Journal of Wildlife Diseases 43:S15-S20. 2007.

20. Bean, B., B. M. Moore, B. Sterner, L. R. Peterson, D. N. Gerding, and H. H. J. Balfour. Survival of influenza viruses on environmental surfaces. J Infect Dis 146:47-51. 1982.
21. Beard, C. W., and M. Brugh. Laboratory Studies on the Pennsylvania Isolates of Avian Influenza (H5N2) in Specific Pathogen-Free Chickens. Journal of the American Veterinary Medical Association 185:340. 1984.
22. Shortridge, K. F., N. N. Zhou, Y. Guan, P. Gao, T. Ito, Y. Kawaoka, S. Kodihalli, S. Krauss, D. Markwell, K. G. Murti, M. Norwood, D. Senne, L. Sims, A. Takada, and R. G. Webster. Characterization of Avian H5N1 Influenza Viruses From Poultry in Hong Kong. Virology 252:331-342. 1998.
23. Swayne, D. E., D. Eggert, and J. R. Beck. Reduction of high pathogenicity avian influenza virus in eggs from chickens once or twice vaccinated with an oil-emulsified inactivated H5 avian influenza vaccine. Vaccine 30:4964-4970. 2012.
24. Moses, H., C. Brandly, E. E. Jones, and E. Jungherr. The isolation and identification of fowl plague virus. Am. J. Vet. Res 9:314-328. 1948.
25. Starick, E., and O. Werner. Detection of H7 Avian Influenza Virus Directly From Poultry Specimens. Avian Diseases 47:1187-1189. 2003.
26. Pillai, S., Y. Saif, and C. Lee. Detection of influenza A viruses in eggs laid by infected turkeys. Avian Diseases 54:830-833. 2010.
27. Suarez, D., P. Woolcock, A. Bermudez, and D. Senne. Isolation from turkey breeder hens of a reassortant H1N2 influenza virus with swine, human, and avian lineage genes. Avian Diseases 46:111-121. 2002.
28. Mohan, R., Y. Saif, G. Erickson, G. Gustafson, and B. Easterday. Serologic and epidemiologic evidence of infection in turkeys with an agent related to the swine influenza virus. Avian Diseases 25:11-16. 1981.
29. Ficken, M., J. Guy, and E. Gonder. An outbreak of influenza (H1N1) in turkey breeder hens. Avian Diseases 33:370-374. 1989.
30. Akey, B. L. Low-pathogenicity H7N2 avian influenza outbreak in Virginia during 2002. Avian Diseases 47:1099-1103. 2003.
31. Narayan, O., G. Lang, and B. Rouse. A new influenza A virus infection in turkeys. Archives of Virology 26:149-165. 1969.
32. Birnbaum, N. G., and B. O'Brien. Methods for Inactivation of Avian Influenza Virus in the Environment. 1st ed. Wiley-Blackwell, Ames, Iowa. pp 391-405. 2008.
33. De Benedictis, P., M. S. Beato, and I. Capua. Inactivation of Avian Influenza Viruses by Chemical Agents and Physical Conditions: A Review. Zoonoses and Public Health 54:51-68. 2007.
34. Lombardi, M. E., B. S. Ladman, R. L. Alphin, and E. R. Benson. Inactivation of avian influenza virus using common detergents and chemicals. Avian Dis 52:118-123. 2008.
35. Fichtner, G. J. The Pennsylvania/Virginia experience in eradication of avian influenza (H5N2). Avian Diseases:33-38. 2003.
36. Songserm, T., R. Jam-on, N. Sae-Heng, and N. Meemak. Survival and Stability of HPAI H5N1 in Different Environments and Susceptibility to Disinfectants. In: Abstracts of the Office International des Epizooties/Food and Agricultural Organization International Conference on Avian Influenza. 2005.

37. Muhmmad, K., P. Das, T. Yaqoob, A. Riaz, and A. Manzoor. Effect of physico-chemical factors on survival of avian influenza virus (H7N3 type). Int. J. Agric. Biol 3:416-418. 2001.
38. Webster, R. G., M. Yakhno, V. S. Hinshaw, W. J. Bean, and K. G. Murti. Intestinal influenza: replication and characterization of influenza viruses in ducks. Virology 84:268-278. 1978.
39. Yamamoto, Y., K. Nakamura, M. Yamada, and M. Mase. Persistence of avian influenza virus (H5N1) in feathers detached from bodies of infected domestic ducks. Applied and Environmental Microbiology 76:5496-5499. 2010.
40. Lu, H., A. E. Castro, K. Pennick, J. Liu, Q. Yang, P. Dunn, D. Weinstock, and D. Henzler. Survival of Avian Influenza Virus H7N2 in SPF Chickens and Their Environments. Avian Diseases 47:1015-1021. 2003.
41. Brown, J. D., D. E. Swayne, R. J. Cooper, R. E. Burns, and D. E. Stallknecht. Persistence of H5 and H7 avian influenza viruses in water. Avian Dis 51:285-289. 2007.
42. Stallknecht, D. E., M. T. Kearney, S. M. Shane, and P. J. Zwank. Effects of pH, Temperature, and Salinity on Persistence of Avian Influenza-Viruses in Water. Avian Diseases 34:412-418. 1990.
43. Stallknecht, D. E., S. M. Shane, M. T. Kearney, and P. J. Zwank. Persistence of Avian Influenza-Viruses in Water. Avian Diseases 34:406-411. 1990.
44. Chumpolbanchorn, K., N. Suemanotham, N. Siripara, B. Puyati, and K. Chaichoune. The effect of temperature and UV light on infectivity of avian influenza virus (H5N1, Thai field strain) in chicken fecal manure. Southeast Asian J Trop Med Public Health 37:102-105. 2006.
45. Nazir, J., R. Haumacher, A. Ike, P. Stumpf, R. Böhm, and R. E. Marschang. Long-term study on tenacity of avian influenza viruses in water (distilled water, normal saline, and surface water) at different temperatures. Avian Diseases 54:720-724. 2010.
46. Nazir, J., R. Haumacher, A. C. Ike, and R. E. Marschang. Persistence of avian influenza viruses in lake sediment, duck feces, and duck meat. Applied and environmental microbiology 77:4981-4985. 2011.
47. Beato, M. S., I. Capua, and D. J. Alexander. Avian influenza viruses in poultry products: a review. Avian Pathology 38:193-200. 2009.
48. Tiwari, A., D. P. Patnayak, Y. Chander, M. Parsad, and S. M. Goyal. Survival of two avian respiratory viruses on porous and nonporous surfaces. Avian Dis 50:284-287. 2006.
49. Guan, J., M. Chan, C. Grenier, D. Wilkie, B. Brooks, and J. Spencer. Survival of avian influenza and Newcastle disease viruses in compost and at ambient temperatures based on virus isolation and real-time reverse transcriptase PCR. Avian Diseases 53:26-33. 2009.
50. Terregino, C., M. S. Beato, E. Bertoli, M. Mancin, and I. Capua. Unexpected heat resistance of Italian low-pathogenicity and high-pathogenicity avian influenza A viruses of H7 subtype to prolonged exposure at 37 C. Avian Pathology 38:519-522. 2009.
51. Davidson, I., S. Nagar, R. Haddas, M. Ben-Shabat, N. Golender, E. Lapin, A. Altory, L. Simanov, I. Ribshtein, and A. Panshin. Avian influenza virus H9N2 survival at different temperatures and pHs. Avian Diseases 54:725-728. 2010.
52. Negovetich, N. J., and R. G. Webster. Thermostability of subpopulations of H2N3 influenza virus isolates from mallard ducks. Journal of Virology 84:9369-9376. 2010.

53. Homme, P. J., and B. C. Easterday. Avian Influenza Virus Infections. I. Characteristics of Influenza A/Turkey/Wisconsin/1966 Virus. Avian Diseases 14:66-74. 1970.
54. Alexander, D. J. The Epidemiology and Control of Avian Influenza and Newcastle-Disease. Journal of Comparative Pathology 112:105-126. 1995.
55. Ssematimba, A., T. J. Hagenaars, and M. C. M. de Jong. Modelling the Wind-Borne Spread of Highly Pathogenic Avian Influenza Virus between Farms. PLoS ONE 7:e31114. 2012.
56. Ypma, R. J. F., M. Jonges, A. Bataille, A. Stegeman, G. Koch, M. van Boven, M. Koopmans, W. M. van Ballegooijen, and J. Wallinga. Genetic data provide evidence for wind-mediated transmission of highly pathogenic avian influenza. Journal of Infectious Diseases. 2012.
57. McQuiston, J. H., L. P. Garber, B. A. Porter-Spalding, J. W. Hahn, F. W. Pierson, S. H. Wainwright, D. A. Senne, T. J. Brignole, B. L. Akey, and T. J. Holt. Evaluation of Risk Factors for the Spread of Low Pathogenicity H7N2 Avian Influenza Virus Among Commercial Poultry Farms. JAVMA-Journal of the American Veterinary Medical Association 226:767-772. 2005.
58. Smithies, L., F. Emerson, S. Robertson, and D. Ruedy. Two different type A influenza virus infections in turkeys in Wisconsin II. 1968 outbreak. Avian Diseases 13:606-610. 1969.
59. Halvorson, D. A. The Control of H5 or H7 Mildly Pathogenic Avian Influenza: a Role for Inactivated Vaccine. Avian Pathology 31:5-12. 2002.
60. Halvorson, D. A. Personal Communication: AI virus isolation from turkey semen.University of Minnesota.2012.
61. Swayne, D. E. Personal Communication: AI virus isolation in turkey semen.Southeast Poultry Research Laboratory USDA/Agricultural Research Service.2012.
62. Ali, A., H. Yassine, O. O. Awe, M. Ibrahim, Y. M. Saif, and C.-W. Lee. Replication of Swine and Human Influenza Viruses in Juvenile and Layer Turkey Hens. Veterinary Microbiology 163:71-78. 2013.
63. Pedroni, E., X. Muñoz, V. Sotomayor, J. Muñoz, M. Arenas, R. Fasce, and A. Olea. Outbreak of human A (H1N1) influenza in turkeys of a commercial poultry farm, Valparaiso, Chile: August 2009. Revista chilena de infectologia: organo oficial de la Sociedad Chilena de Infectologia 29:420-426. 2012.
64. Samadieh, B., and R. Bankowski. Effect of avian influenza-A viruses upon egg production and fertility of turkeys. Avian Diseases:715-722. 1970.
65. Pantin-Jackwood, M., J. L. Wasilenko, E. Spackman, D. L. Suarez, and D. E. Swayne. Susceptibility of turkeys to pandemic-H1N1 virus by reproductive tract insemination. Virology Journal 7:27. 2010.
66. Kreager, K. Avian Influenza Control Philosophies in the Layer and Layer Breeder industries. Avian Diseases:344-348. 2003.
67. Ssematimba, A., T. Hagenaars, J. de Wit, F. Ruiterkamp, T. Fabri, J. Stegeman, and M. de Jong. Avian influenza transmission risks: Analysis of biosecurity measures and contact structure in Dutch poultry farming. Preventive Veterinary Medicine. 2012.

68. Stegeman, J. A., and A. Bouma. Epidemiology and Control of Avian Influenza. 11th International Conference of the Associations for Tropical Veterinary Medicine and 16th Veterinary Association Malaysia Congress. Petaling Jaya. pp 141-143. 2004.
69. Samadieh, B., and R. A. Bankowski. Transmissibility of Avian Influenza-A Viruses. American Journal of Veterinary Research 32:939-945. 1971.
70. Canadian Food Inspection Agency. Comprehensive Report on the 2004 Outbreak of High Pathogenicity Avian Influenza (H7N3) in the Fraser Valley of British Columbia, Canada. 2004.
71. Aldous, E. W., J. M. Seekings, A. McNally, H. Nili, C. M. Fuller, R. M. Irvine, D. J. Alexander, and I. H. Brown. Infection dynamics of highly pathogenic avian influenza and virulent avian paramyxovirus type 1 viruses in chickens, turkeys and ducks. Avian Pathology 39:265-273. 2010.
72. Nili, H., A. McNally, E. Aldous, A. Nunez, J. Banks, and I. Brown. Pathological changes in turkeys experimentally infected with different doses of A/ostrich/Italy/984/2000 H7N1 avian influenza virus. Iranian J Vet Res 9:330-335. 2008.
73. Homme, P. J., B. C. Easterday, and D. P. Anderson. Avian Influenza Virus Infections II. Experimental Epizootiology of Influenza A/Turkey/Wisconsin/1966 Virus in Turkeys. Avian Diseases 14:240-247. 1970.
74. Toffan, A., M. Serena Beato, R. De Nardi, E. Bertoli, A. Salviato, G. Cattoli, C. Terregino, and I. Capua. Conventional inactivated bivalent H5/H7 vaccine prevents viral localization in muscles of turkeys infected experimentally with low pathogenic avian influenza and highly pathogenic avian influenza H7N1 isolates. Avian Pathology 37:407-412. 2008.
75. Swayne, D. E., and R. D. Slemons. Using mean infectious dose of high- and low-pathogenicity avian influenza viruses originating from wild duck and poultry as one measure of infectivity and adaptation to poultry. Avian Dis 52:455-460. 2008.
76. Spekreijse, D., A. Bouma, J. A. Stegeman, G. Koch, and M. C. M. de Jong. The effect of inoculation dose of a highly pathogenic avian influenza virus strain H5N1 on the infectiousness of chickens. Veterinary Microbiology 147:59-66. 2011.
77. Kitajima, M., Y. Huang, T. Watanabe, H. Katayama, and C. Haas. Dose–response time modelling for highly pathogenic avian influenza A (H5N1) virus infection. Letters in applied microbiology 53:438-444. 2011.
78. Schijven, J. F., P. F. M. Teunis, and A. M. de Roda Husman. Quantitative Risk Assessment of Avian Influenza Virus Infection via Water. Rijksinstituut voor Volksgezondheid en Milieu RIVM. 2006.
79. Kwon, Y., and D. Swayne. Different routes of inoculation impact infectivity and pathogenesis of H5N1 high pathogenicity avian influenza virus infection in chickens and domestic ducks. Avian Diseases 54:1260-1269. 2010.
80. Swayne, D. E., and J. R. Beck. Experimental Study to Determine if Low-Pathogenicity and High-Pathogenicity Avian Influenza Viruses Can Be Present in Chicken Breast and Thigh Meat Following Intranasal Virus Inoculation. Avian Diseases 49:81-85. 2005.
81. Purchase, H. Experiments on the viability of the virus of fowl-plague under trade conditions. Vet. Rec 11:644-648. 1931.
82. Sergeev, A. A., O. Demina, O. Pyankov, O. Pyankova, A. Agafonov, S. Kiselev, I. Agranovski, A. A. Sergeev, A. Shikov, and L. Shishkina. Infection of Chickens Caused

by Avian Influenza Virus A/H5N1 Delivered by Aerosol and Other Routes. Transboundary and Emerging Diseases 60:159-165. 2013.
83. USDA: APHIS: VS: CEAH, University of Minnesota Center for Animal Health and Food Safety, Egg Sector Working Group. An Assessment of the Risk Associated with the Movement of Eggshells and Inedible Egg Product Into, Within, and Out of a Control Area During a Highly Pathogenic Avian Influenza Outbreak. Fort Collins, CO. 2013.
84. Saenz, R. A., S. C. Essen, S. M. Brookes, M. Iqbal, J. L. Wood, B. T. Grenfell, J. W. McCauley, I. H. Brown, and J. R. Gog. Quantifying Transmission of Highly Pathogenic and Low Pathogenicity H7N1 Avian Influenza in Turkeys. PLoS ONE 7:e45059. 2012.
85. Kilany, W. H., E. Abdelwhab, A. S. Arafa, A. Selim, M. Safwat, A. A. Nawar, A. M. Erfan, M. K. Hassan, M. M. Aly, and H. M. Hafez. Protective efficacy of H5 inactivated vaccines in meat turkey poults after challenge with Egyptian variant highly pathogenic avian influenza H5N1 virus. Veterinary Microbiology 150:28-34. 2011.
86. Mutinelli, E., I. Capua, C. Terregino, and G. Cattoli. Clinical, Gross, and Microscopic Findings in Different Avian Species Naturally Infected During the H7N1 Low- and High-Pathogenicity Avian Influenza Epidemics in Italy During 1999 and 2000. Avian Diseases 47:844-848. 2003.
87. Team, R. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. 2012.
88. Baier, T. R: Windows Component Services Integrating R and Excel on the COM Layer. 3rd International Workshop on Distributed Statistical Computing (DSC 2003) Vienna, Austria. K. Hornik, F. Leisch, A. Zeileis eds. pp 1-14. 2003.
89. Beard, C. W., M. Brugh, and D. C. Johnson. Laboratory studies with the Pennsylvania avian influenza viruses (H5N2). Fort Worth, TX, USA. pp 462-473. 1984.
90. Bouma, A., I. Claassen, K. Natih, D. Klinkenberg, C. A. Donnelly, G. Koch, and M. van Boven. Estimation of transmission parameters of H5N1 avian influenza virus in chickens. PLoS Pathog 5:e1000281. 2009.
91. van der Goot, J. A., G. Koch, M. C. M. De Jong, and M. van Boven. Quantification of the Effect of Vaccination on Transmission of Avian Influenza (H7N7) in Chickens. Proceedings of the National Academy of Sciences of the United States of America 102:18141-18146. 2005.
92. van der Goot, J. A., M. C. M. De Jong, G. Koch, and M. van Boven. Comparison of the Transmission Characteristics of Low and High Pathogenicity Avian Influenza A Virus (H5N2). Epidemiology and Infection 131:1003-1013. 2003.
93. Bos, M. E., M. Nielen, G. Koch, A. Bouma, M. C. De Jong, and A. Stegeman. Back-calculation method shows that within-flock transmission of highly pathogenic avian influenza (H7N7) virus in the Netherlands is not influenced by housing risk factors. Prev Vet Med 88:278-285. 2009.
94. Pillai, S. P. S., M. Pantin-Jackwood, S. J. Jadhao, D. L. Suarez, L. Wang, H. M. Yassine, Y. M. Saif, and C. W. Lee. Pathobiology of triple reassortant H3N2 influenza viruses in breeder turkeys and its potential implication for vaccine studies in turkeys. Vaccine 27:819-824. 2009.

95. Cappucci, D. T., D. C. Johnson, M. Brugh, T. M. Smith, C. F. Jackson, J. E. Pearson, and D. A. Senne. Isolation of Avian Influenza-Virus (Subtype-H5N2) From Chicken Eggs During a Natural Outbreak. Avian Diseases 29:1195-1200. 1985.
96. Elbers, A. R. W., G. Koch, and A. Bourna. Performance of Clinical Signs in Poultry for the Detection of Outbreaks During the Avian Influenza a (H7N7) Epidemic in the Netherlands in 2003. Avian Pathology 34:181-187. 2005.
97. Swayne, D. E., A. Lipatov, Y. K. Kwon, M. Jackwood, and J. Beck. Potential Role of Exposure to Poultry Products and By-products for Human H5N1 Infections. 2008.
98. Spackman, E., D. A. Senne, T. J. Myers, L. L. Bulaga, L. P. Garber, M. L. Perdue, K. Lohman, L. T. Daum, and D. L. Suarez. Development of a Real-Time Reverse Transcriptase PCR Assay for Type a Influenza Virus and the Avian H5 and H7 Hemagglutinin Subtypes. Journal of Clinical Microbiology 40:3256-3260. 2002.
99. Elvinger, F., B. L. Akey, D. A. Senne, F. W. Pierson, B. A. Porter-Spalding, E. Spackman, and D. L. Suarez. Characteristics of diagnostic tests used in the 2002 low-pathogenicity avian influenza H7N2 outbreak in Virginia. J Vet Diagn Invest 19:341-348. 2007.
100. Spackman, E. Personal Communication: QC testing for NIH funded labs for AIV detection.Southeast Poultry Research Laboratory USDA/Agricultural Research Service.2012.
101. Slomka, M. Personal Communication: Evaluation of lateral flow devices.Animal Health & Veterinary Laboratories Agency, United Kingdom.2012.
102. Pedersen, J. Personal Communication: Diagnostic sensitivity of matrix gene RRT-PCR test for Avian Influenza.Microbiologist, USDA:APHIS:VS.2012.
103. Weaver, J. T., S. Malladi, T. J. Goldsmith, W. Hueston, M. Hennessey, B. Lee, S. Voss, J. Funk, C. Der, and K. E. Bjork. Impact of Virus Strain Characteristics on Early Detection of Highly Pathogenic Avian Influenza Infection in Commercial Table-Egg Layer Flocks and Implications for Outbreak Control. Avian diseases 56:905-912. 2012.
104. Swayne, D. E., and D. L. Suarez. Current developments in avian influenza vaccines, including safety of vaccinated birds as food. Dev Biol (Basel) 130:123-133. 2007.
105. Lu, H. G., P. A. Dunn, E. A. Wallner-Pendleton, D. J. Hertzler, D. C. Kradel, J. B. Liu, D. P. Shaw, and P. Miller. Investigation of H7N2 Avian Influenza Outbreaks in Two Broiler Breeder Flocks in Pennsylvania, 2001-02. Avian Diseases 48:26-33. 2004.
106. Das, A., E. Spackman, C. Thomas, D. E. Swayne, and D. L. Suarez. Detection of H5N1 high-pathogenicity avian influenza virus in meat and tracheal samples from experimentally infected chickens. Avian Dis 52:40-48. 2008.
107. Pantin-Jackwood, M. Personal Communication: AI virus in turkey semen.Southeast Poultry Research Laboratory USDA/Agricultural Research Service.2012.
108. USDA: APHIS: VS: CEAH, University of Minnesota Center for Animal Health and Food Safety, Egg Sector Working Group. An Assessment of the Risk Associated with the Movement of Nest Run Eggs Into, Within, and Outside of a Control Area During a Highly Pathogenic Avian Influenza Outbreak. Fort Collins, CO. 2010.
109. USDA: APHIS: VS: CEAH, University of Minnesota Center for Animal Health and Food Safety, Egg Sector Working Group. An Assessment of the Risk Associated

with the Movement of Washed and Sanitized Shell Eggs Into, Within, and Outside of a Control Area during a Highly Pathogenic Avian Influenza Outbreak. Fort Collins, CO. 2009.
110. Rice, E. W., N. J. Adcock, M. Sivaganesan, J. D. Brown, D. E. Stallknecht, and D. E. Swayne. Chlorine inactivation of highly pathogenic avian influenza virus (H5N1). Emerg Infect Dis 13:1568-1570. 2007.
111. Halvorson, D. A. Prevention and management of avian influenza outbreaks: experiences from the United States of America. Rev Sci Tech 28:359-369. 2009.
112. Pearson, J. E., and D. A. Senne. Genesis of an Avian Influenza Outbreak. Journal of the American Veterinary Medical Association 185:330. 1984.
113. Schofield, L., J. Ho, B. Kournikakis, and T. Booth. Avian Influenza Aerosol Sampling Campaign in the British Columbia Fraser Valley, 9-19 April 2004. Defense Research and Development Canada. 2005.
114. Swayne, D. Personal Communication: TCID vs embryo infectious doses.Southeast Poultry Research Laboratory USDA/Agricultural Research Service.2009.
115. U.S. Environmental Protection Agency. Registered Antimicrobial Products with Label Claims for Avian Influenza (Bird) Flu Disinfectants. 2007.
116. Hunt, A., D. L. Johnson, and D. A. Griffith. Mass transfer of soil indoors by track-in on footwear. Sci Total Environ 370:360-371. 2006.
117. Ansari, S. A., S. A. Sattar, V. S. Springthorpe, G. A. Wells, and W. Tostowaryk. Rotavirus survival on human hands and transfer of infectious virus to animate and nonporous inanimate surfaces. J Clin Microbiol 26:1513-1518. 1988.
118. Bidawid, S., N. Malik, O. Adegbunrin, S. A. Sattar, and J. M. Farber. Norovirus cross-contamination during food handling and interruption of virus transfer by hand antisepsis: experiments with feline calicivirus as a surrogate. J Food Prot 67:103-109. 2004.
119. Grayson, M. L., S. Melvani, J. Druce, I. G. Barr, S. A. Ballard, P. D. Johnson, T. Mastorakos, and C. Birch. Efficacy of soap and water and alcohol-based hand-rub preparations against live H1N1 influenza virus on the hands of human volunteers. Clin Infect Dis 48:285-291. 2009.
120. Larson, E. L., B. Cohen, and K. A. Baxter. Analysis of alcohol-based hand sanitizer delivery systems: Efficacy of foam, gel, and wipes against influenza A (H1N1) virus on hands. American Journal of Infection Control 40:806-809. 2012.
121. Davison, S., R. J. Eckroade, and A. E. Ziegler. A Review of the 1996-98 Nonpathogenic H7N2 Avian Influenza Outbreak in Pennsylvania. Avian Diseases 47:823-827. 2003.
122. Kelly, T. R., M. G. Hawkins, C. E. Sandrock, and W. M. Boyce. A review of highly pathogenic avian influenza in birds, with an emphasis on Asian H5N1 and recommendations for prevention and control. J Avian Med Surg 22:1-16. 2008.
123. USDA: APHIS: VS. National Animal Health Emergency Management System Guidelines: Cleaning and Disinfection (Draft). pp 6-8. 2005.
124. Alphin, R. L., K. J. Johnson, B. S. Ladman, and E. R. Benson. Inactivation of avian influenza virus using four common chemicals and one detergent. Poult Sci 88:1181-1185. 2009.
125. Hatchery cleaning and disinfection. Technical advice sheet. Aviagen Turkeys. 2014.

126. Sattar, S. A. Personal Communication: Virus transfer between contact surfaces.Centre for Research on Environmental Microbiology (CREM) Faculty of Medicine, University of Ottawa.2010.
127. Springthorpe, S. Personal Communication: Virus transfer between contact surfaces.Centre for Research on Environmental Microbiology, University of Ottawa.2010.
128. Mauldin, J. M., MacKinnon, I. R. Hatchery ventilation and environmental control. Avian Biology Research 2:87-91. 2009.
129. Wood, J. P., Y. W. Choi, D. J. Chappie, J. V. Rogers, and J. Z. Kaye. Environmental persistence of a highly pathogenic avian influenza (H5N1) virus. Environmental Science & Technology 44:7515-7520. 2010.
130. Shahid, M. A., M. Abubakar, S. Hameed, and S. Hassan. Avian influenza virus (H5N1); effects of physico-chemical factors on its survival. Virol J 6:38. 2009.
131. Poss, P. E., K. A. Friendshuh, and L. T. Ausherman. The control of avian influenza. Avian Diseases 47:318-326. 2003.
132. Canadian Food Inspection Agency. Final Report Response to Findings of Highly Pathogenic Avian Influenza (H7N3) in Saskatchewan, September 2007. 2008.
133. Halvorson, D. Personal Communication: Hatchery transmission of AI viruses.University of Minnesota.2010.
134. Swayne, D. E. Transcript of the Question and Answer Sessions From the Fifth International Symposium on Avian Influenza. Avian Diseases 47:1219-1255. 2003.
135. Zanella, A., P. Dall'ara, and P. A. Martino. Avian Influenza Epidemic in Italy Due to Serovar H7N1. Avian Diseases 45:257-261. 2001.
136. Cardona, C. Low-pathogenicity avian influenza outbreaks in commercial poultry in California. The Threat of Pandemic Influenza. A. M. Stacey L Knobler, Adel Mahmoud, and Stanley M Lemon, ed. National Academies Press (U.S.). 2005.
137. USDA:APHIS:VS:CEAH, University of Minnesota Center for Animal Health and Food Safety, Broiler Sector Working Group. An Assessment of the Risk Associated with the Movement of Broiler Hatching Eggs Into, Within, and Out of a Control Area during a Highly Pathogenic Avian Influenza Outbreak. Fort Collins, CO. 2012.
138. USDA: APHIS: VS: CEAH, University of Minnesota Center for Animal Health and Food Safety, Egg Sector Working Group. An Assessment of the Risk Associated with the Movement of Hatching Eggs Into, Within and Outside of a Control Area during a Highly Pathogenic Avian Influenza Outbreak. Fort Collins, CO. 2010.
139. Dietz, K., and D. Schenzle. Proportionate mixing models for age-dependent infection transmission. Journal of Mathematical Biology 22:117-120. 1985.
140. Bos, M. E., M. Van Boven, M. Nielen, A. Bouma, A. R. Elbers, G. Nodelijk, G. Koch, A. Stegeman, and M. C. De Jong. Estimating the day of highly pathogenic avian influenza (H7N7) virus introduction into a poultry flock based on mortality data. Vet Res 38:493-504. 2007.
141. van der Goot, J. A., G. Koch, M. C. de Jong, and M. van Boven. Transmission dynamics of low- and high-pathogenicity A/Chicken/Pennsylvania/83 avian influenza viruses. Avian Dis 47:939-941. 2003.
142. USDA: APHIS: VS: CEAH, University of Minnesota Center for Animal Health and Food Safety, Egg Sector Working Group. An Assessment of the Risk Associated

with the Movement of Nest Run Eggs Into, Within, and Outside of a Control Area During a Highly Pathogenic Avian Influenza Outbreak. Fort Collins, CO. 2010.
143. Sakaguchi, H., K. Wada, J. Kajioka, M. Watanabe, R. Nakano, T. Hirose, H. Ohta, and Y. Aizawa. Maintenance of influenza virus infectivity on the surfaces of personal protective equipment and clothing used in healthcare settings. Environmental Health and Preventive Medicine 15:344-349. 2010.
144. Greatorex, J. S., P. Digard, M. D. Curran, R. Moynihan, H. Wensley, T. Wreghitt, H. Varsani, F. Garcia, J. Enstone, and J. S. Nguyen-Van-Tam. Survival of Influenza A (H1N1) on Materials Found in Households: Implications for Infection Control. PLoS ONE 6:e27932. 2011.
145. Vrtlak, O., and B. Kapitancik. Study of Fowl Plague Virus Resistance in Biological and Technical Material. Bull. Off. Int. Epiz. 67:969-988. 1967.
146. USDA: APHIS: PPQ. Guidelines for Pathway-Initiated Pest Risk Assessments. 2000.
147. US Department of Health and Human Services: Food and Drug Administration: Center for Veterinary Medicine. Guidance for Industry: Evaluating the Safety of Antimicrobial New Animal Drugs with Regards to Their Microbiological Effects on Bacteria of Human Health Concern. Document #152. 2003.
148. USDA: APHIS. Qualitative Risk Assessment for Potential Federal Noxious Weeds- Template. 2004.
149. Rodricks, J. V. Some attributes of risk influencing decision-making by public health and regulatory officials. Am J Epidemiol 154:S7-S12. 2001.
150. Fiori, J. M., and R. D. Meyerhoff. Extending the threshold of regulation concept: de minimis limits for carcinogens and mutagens. Regul Toxicol Pharmacol 35:210. 2002.
151. National Library of Medicine. IUPAC Glossary of Terms Used in Toxicology -R. 2009.
152. Peterson, M. What Is a de Minimis Risk? Risk Management: An International Journal 4:47-55. 2002.
153. U.S. House of Representatives, Committee on Commerce. Section 405. Tolerances And Exemptions For Pesticide Chemical Residues. 1996.
154. Zepeda, C., M. Salman, A. Thiermann, J. Kellar, H. Rojas, and P. Willeberg. The role of veterinary epidemiology and veterinary services in complying with the World Trade Organization SPS agreement. Preventive Veterinary Medicine 67:125-140. 2005.
155. United States Court of Appeals, N. C. Ranchers Cattleman Action Legal Fund United Stockgrowers of America v. U.S. Dept. of Agriculture Web. 2005.
156. USDA. WTO Resolution of the U.S.-Japan Apple Dispute. October 2005. 2005.
157. Gruszczynski, L. A. The Role of Science in Risk Regulation under the SPS Agreement. SSRN eLibrary. 2006.
158. World Trade Organization, D. S. B. Japan--Measures Affecting The Importation Of Apples: Report Of The Panel. World Trade Organization. 2003.
159. Hrudey, S., and D. Krewski. Is There a Safe level of Exposure to a Carcinogen? Environmental Science & Technology 29:370A- 375A. 1995.
160. Graham, J. D. Historical perspective on risk assessment in the federal government: Biological Mechanisms and Quantitative Risk Assessment. Toxicology 102:29-52. 1995.

161. Bryson, N. Citrus, Clementines and Mad Cows: Judicial Standards of Review for Sanitary and Phytosanitary Decisions of USDA's Animal and Plant Health Inspection Service (APHIS). International Conference on Sanitary & Phytosanitary (SPS) Risk Assessment Methodology. Washington, D.C. 2005.
162. Theil, M. The role of translations of verbal into numerical probability expressions in risk management: a meta-analysis. Journal of Risk Research 5:10. 2002.
163. Behn, R. D., and J. W. Vaupel. Quick analysis for busy decision makers. Basic Books, New York, New York, USA. 1982.
164. World Organisation for Animal Health (OIE). Guidelines for Import Risk Analysis. 2006.
165. Fletcher, W. The application of qualitative risk assessment methodology to prioritize issues for fisheries management. ICES Journal of Marine Science: Journal du Conseil 62:1576. 2005.