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Center for Animal Disease
Information and Analysis

2150 Centre Avenue
Building B, Mail Stop 2W4
Fort Collins, CO 80526

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

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

Safeguarding Animal Health

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1. Abbreviations and Definitions

AHPA	Animal Health Protection Act
AMS	Agricultural Marketing Service
APHIS	Animal and Plant Health Inspection Service (USDA)
AI	Avian influenza
CEAH	Centers for Epidemiology and Animal Health (USDA: APHIS: VS)
CFR	Code of Federal Regulations
EPA	Environmental Protection Agency
GMP	Good Manufacturing Practice
FDA	Food and Drug Administration
FSIS	Food Safety Inspection Service
HA	Hemagglutinin
HACCP	Hazard Analysis and Critical Control Point
HPAI	Highly Pathogenic Avian Influenza
HPNAI	Highly Pathogenic Notifiable Avian Influenza
LE	Liquid Egg
LPAI	Low Pathogenic Avian Influenza
NA	Neuraminidase
NAHEMS	National Animal Health Emergency Management System
NPLE	Nonpasteurized Liquid Egg
OIE	World Organization for Animal Health (Office International des Epizooties)
PLE	Pasteurized Liquid Egg
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)

Buffer surveillance zone

The zone immediately surrounding the infected zone. The buffer surveillance zone and the infected zone comprise the control area.

Check

Check means an egg that has a broken shell or crack in the shell but has its shell membranes intact and contents not leaking.

Continuous inspection

Continuous inspection requires that the FSIS inspector be on the premises of the egg products processing facility whenever pasteurization equipment is operating.

Control area

A control area, consisting of an infected zone and a buffer surveillance zone, will be established to ensure the rapid and effective containment of the disease. 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

Dirty egg or "Dirties" means an egg(s) that has an unbroken shell with adhering dirt or foreign material.

Egg

The shell egg of the domesticated chicken. Shell eggs of turkeys, ducks, geese, and guineas are outside the scope of this assessment.

Infected zone

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

- Preventing products from birds and other susceptible animals from leaving the zone unless a risk assessment determines that such movement can be permitted.
- Preventing movement of vehicles, equipment, and nonsusceptible animals out of the zone unless appropriate biosecurity procedures (as determined by a risk assessment) are followed.

Low risk

For this risk analysis, the term "low risk" means it is highly unlikely that moving shell eggs will cause infection in another poultry production premises. The specific magnitude cannot be determined, as there is no evidence that these products have ever served as a transmission pathway. The determination of "low risk" suggests that although not a strict requirement, additional resources to further evaluate or mitigate this risk may be considered (depending on circumstances).

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

Negligible risk

For this risk analysis, the term "negligible risk" means there is a very low likelihood that moving shell eggs will cause infection on another premises. The specific magnitude cannot be determined, as there is no evidence that shell eggs have ever served as a transmission pathway. In quantitative terms, "negligible risk" is the likelihood that moving shell eggs will result in infection on another premises is less than 1/1,000,000. This particular likelihood is used as it is consistent with other common meanings for the term, as discussed in Appendix 12. The determination of "negligible risk" suggests that allocating additional resources to mitigate this risk may not be a cost-effective use of resources (depending on circumstances).

Nest run egg

Eggs that have been packed as they come from the production facilities without having been washed, sized, and/or candled for quality, with the exception that some checks, dirties or obvious undergrades may have been removed.

Nonpasteurized liquid egg

Shell eggs that have been washed, sanitized and broken and converted to liquid egg which has not been subjected to pasteurization.

Restricted egg

Restricted egg means any check, dirty egg, incubator reject, inedible, leaker, or loss.

Shell egg

For this assessment, we define shell eggs as washed and sanitized eggs.

2. Executive Summary

This document assesses the risk that the movement of shell eggs during a highly pathogenic avian influenza (HPAI) outbreak in the poultry egg industry in the United States will result in HPAI infection on another poultry premises. This assessment includes egg packing facilities that wash and sanitize eggs under Federal regulations, State regulations, or industry Good Manufacturing Practices (GMPs) that are equivalent to 7CFR56.76 (f) parts (3) and (11),[a] which specify the temperature of the water used for washing and sanitizing, and mandate the concentration of sanitizer (chlorine or equivalent) to be not less than 100 ppm or more than 200 ppm. It is assumed that the layer farms included in this risk assessment will be participants in the active surveillance protocol to be implemented by industry in conjunction with APHIS during an outbreak. This program details active surveillance of flocks for clinical signs of illness, changes in feed and water intake, a drop in egg production, an increase in daily mortality above an established threshold, and submission of a pooled sample of swabs from 5 randomly chosen birds among the daily mortality for real-time reverse transcription polymerase chain reaction (RRT-PCR) testing each day.

This risk assessment is a joint effort between the Egg Products Industry working group, the University of Minnesota's Center for Animal Health and Food Safety, and the United States Department of Agriculture (USDA)[b] to support permits for moving shell eggs safely and in a timely fashion during an outbreak.

This risk assessment is intended to identify pathways of HPAI transmission associated with the movement of shell eggs, and assess their corresponding likelihoods of carrying the virus off of an infected premises and causing infection on another poultry premises despite implementation of all standard preventive measures as well as outbreak-specific measures. This risk assessment will ultimately provide the framework necessary for decision makers to:

a) Quickly assess the effectiveness of preventive measures as they pertain specifically to the washing, sanitizing, and movement of shell eggs.
b) Implement a permit system to allow uninfected shell egg packing facilities that process eggs under Federal regulations, State regulations, or industry Good Manufacturing Practices (GMPs) that are equivalent to 7CFR56.76(f) parts (3) and (11) to move shell eggs into, within, and outside of the control area during an HPAI outbreak.

To address these objectives, we first estimated the proportion of externally and internally contaminated eggs from an infected but undetected layer flock. We then estimated the degree of HPAI virus inactivation on the shell surface of contaminated eggs via washing and sanitizing. Finally, we evaluated the risks associated with post-sanitizing handling and transportation of shell eggs. In particular, this document assesses:

[a] Parts of 7CFR56.76 are included as Appendix 1.
[b] Specifically, the Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) Centers for Epidemiology and Animal Health (CEAH) within the USDA.

a) Likelihood of HPAI virus contamination of the contents of eggs from an infected but undetected flock.

b) Likelihood of HPAI virus contamination of the shell surfaces of eggs from an infected but undetected flock after washing and sanitizing.

c) Risk of recontamination of the shell surface of eggs after sanitizing via aerosols leading to infection of a susceptible flock.

d) Risk of HPAI virus from washed and sanitized eggs moved from an infected but undetected flock resulting in infection of a susceptible flock.

e) Risk of the vehicle or driver transporting shell eggs resulting in HPAI infection of a susceptible flock.

This document is an evolving product-specific risk assessment that will be reviewed and updated as necessary before and during an outbreak to incorporate the latest scientific information and preventive measures. If the Incident Command System is activated in response to an HPAI outbreak, APHIS (and Incident Command staff) will review this risk assessment with respect to the situation in order to assess industry requests for movement of washed and sanitized shell eggs.

Overall Finding and Conclusion

The risk that movement of shell eggs into, within, and outside of a control area during a highly pathogenic avian influenza outbreak would cause an HPAI outbreak on another poultry production premises in the United States is *negligible* when shell eggs are transported to destinations without poultry on the premises.

If there are poultry on the destination premises, it is concluded that the overall risk of moving washed and sanitized shell eggs into, within, and outside of a control area during an HPAI outbreak is *low*.

3. Introduction

In the event of a highly pathogenic avian influenza (HPAI) outbreak in the U.S. poultry industry, local, State and Federal authorities will implement a foreign animal disease emergency response. This response consists of a control and eradication strategy that will utilize depopulation, quarantine and movement control measures to prevent further spread of HPAI virus.[1] In addition to compliance with such measures, 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 associated with the movement of the product in question is acceptable.[c]

Completing these types of risk assessments in a timely manner during an outbreak can be challenging. Risk assessments can take more time to conduct than the shelf-life of some of the perishable ingredients or products that need to be moved. In addition, the available storage capacity might be inadequate for holding the product while the risk assessment is being completed and may result in disposal of product. For these products, the risk may be evaluated before an outbreak occurs. Shell eggs are one such product.

The purpose of this document is to determine the risk of disease spread due to the movement of shell eggs produced from an undetected, notifiable, HPAI-positive flock in a control zone. Shell eggs from a known (i.e., detected) positive flock are not considered, as it is assumed that they will be restricted from movement.

This document assesses the risk associated with the movement of shell eggs into, within, and outside of a control area during an HPAI outbreak. The facilities covered in this document are only those under Federal regulations, State regulations, or industry-generated protocols that are equivalent to 7CFR56.76(f) parts (3) and (11), which specify the temperature of the water used for washing and sanitizing, and mandate the concentration of sanitizer (chlorine or equivalent) to be not less than 100 ppm or more than 200 ppm.[d] This assessment takes into consideration all applicable regulations including preventive measures already in place as well as additional preventive measures that will be implemented during an outbreak.[e]

This risk assessment does not guarantee that movement will be permitted during an HPAI outbreak. However, this document provides the framework necessary for decision makers to quickly assess the effectiveness of the preventive measures as they pertain specifically to transport of shell eggs. This risk assessment will also allow decision makers to consider implementation of additional control measures which would allow shell egg

[c] During an outbreak, APHIS conducts numerous product-specific risk assessments, taking into consideration all permit requirements and preventive measures currently in place.
[d] See Appendix 1.
[e] Normal day-to-day operations and preventive measures are in place via Good Manufacturing Practices (GMP), State regulations and Federal regulations as required by FSIS, FDA, and APHIS.

movement for further processing into, within and outside of the control area during an HPAI outbreak.

4. Scope

This risk assessment applies to all eggs processed under Federal regulations, State regulations, or industry-generated protocols that are equivalent to 7CFR56.76(f) parts (3) and (11),[f] which specify the temperature of the water used for washing and sanitizing, and mandate the concentration of sanitizer (chlorine or equivalent) to be not less than 100 ppm or more than 200 ppm. In addition, it is assumed that the layer farms included in this risk assessment will be participants in the active surveillance protocol to be implemented by industry in conjunction with APHIS during an outbreak. This program details active surveillance of flocks for clinical signs of illness, changes in feed and water intake, drop in egg production, an increase in daily mortality above an established threshold, and submission of a pooled sample of swabs for RRT-PCR testing from 5 randomly chosen birds among the daily mortality.

This risk assessment focuses on the risk that movement of washed and sanitized shell eggs will result in HPAI spread to other susceptible poultry. Although the risks to humans or wildlife associated with the production or movement of shell 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.

This assessment is not intended to be a regulatory review of the effectiveness of Federal or State level shell egg regulations. We did not directly evaluate their utility, but did review the portions of the various regulations that are common practice in the shell egg industry and their relevance to risk reduction in the event of an HPAI outbreak. We also reviewed information concerning past HPAI outbreaks and any potential connections to shell eggs.

Scope Justification:

Regulation of shell eggs is shared among various Federal agencies and State governments, or shell eggs may be processed according to industry-generated protocols. Although eggs destined for breaking are washed and sanitized according to mandatory Federal Food Safety Inspection Service (FSIS) regulations (9CFR590.515-516), shell eggs destined for retail do not fall under any standardized regulatory policies. After careful review and assessment of the common practices used to process shell eggs in the United States, we elected to base this risk assessment on the assumption that the majority of shell egg operations in the United States have adopted shell egg washing and sanitizing procedures similar to those outlined in 7CFR56.76(f) (Agricultural Marketing Service Regulations Governing the Voluntary Grading of Shell Eggs), although they may be following State level regulations or industry protocols rather than enrolling in this

[f] See Appendix 1.

voluntary Federal program. Shell egg operations not that do not currently implement 7CFR56.76(f) or similar egg washing and sanitizing procedures may do so as an additional preventive measure during an outbreak. We specified the parts of 7CFR56.76(f) that we consider to be: a) common practice in most shell egg operations, and b) relevant to risk reduction in movement of shell eggs in the event of an HPAI outbreak.

5. 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 are making some assumptions to establish context and applicability. These assumptions are:

a) An HPAI outbreak has been detected, APHIS is implementing the HPAI Response Plan, and some degree of planning has taken place at other levels. The APHIS HPAI Response Plan is intended to complement regional, State, and industry plans and APHIS recommends their continued development.

b) Shell egg production facilities may have HPAI infection in their laying flocks but it has not yet been detected. If there was absolute certainty that HPAI infection was absent there would be no risk. If HPAI infection has been detected, however, it is assumed that Incident Command will shut down the production premises, movement of shell eggs will not be allowed, and any associated laying facilities will be depopulated. This situation also does not pose a risk associated with movement of shell eggs as the premises would cease production and be quarantined, depopulated, cleaned and disinfected before resuming production.

c) This assessment is applicable to most (*but not all*) of the situations that may arise during an outbreak. As discussed in the movement section, permits to move shell eggs may be issued for movement to slaughter/processing or for movement under conditions described on a movement permit. These conditions depend on the circumstances and cannot be known in advance, thus this assessment can only provide information and not generate recommendations.

6. HPAI Overview

6.1 Definition of Highly Pathogenic Notifiable Avian Influenza

HPAI is defined[g] 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 (1) of this definition, but has an amino acid sequence at the hemagglutinin cleavage site that is compatible with highly pathogenic avian influenza 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.[2 4-7]There are three antigenically distinct types of influenza viruses within the *Orthomyxoviridae* family: types A, B, and C. Types B and C are typically found only in humans. Influenza A viruses include all AI viruses and can infect a wide variety of animals including birds, pigs, horses, marine mammals, and humans.

The two surface glycoproteins of the influenza 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.[3,4,6-8] There are sixteen different subtypes of HA (H1 to H16), nine different subtypes of NA (N1 to N9) and 144 different HA:NA combinations.[9]

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.[3,4 10 11 12]While all bird species are thought to be susceptible to AI, some are more susceptible than others. Most AI viral infections in birds are subclinical or induce mild disease syndromes, consisting primarily of respiratory disease.[4 11,12]These viruses are designated as low pathogenic avian influenza (LPAI) viruses. A few isolates of H5 and H7 subtypes are very virulent and induce severe disease with morbidity and mortality rates reaching 100 percent in affected flocks.[3,11 8] These viruses are termed HPAI.

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 significant morbidity and production losses, they are not considered to be reportable diseases.

There are many examples of H5 and H7 virus isolates that are not pathogenic, so antigenic configuration alone does not determine pathogenicity.[8] Migratory waterfowl

[g] While there are other avian influenza types that are notifiable to OIE, this definition is used to maintain consistency with the APHIS HPAI Response Plan.

have yielded more influenza viruses than any other group, while turkeys and chickens have experienced the most substantial disease problems.[6,8]

6.3 Geographic Distribution of H5N1 HPAI

Since 2003, over 60 countries have reported H5N1 HPAI in domestic poultry and wildlife. The current list of all confirmed affected countries is maintained by the OIE.[14]

6.4 Susceptibility to Chemical and Physical Agents

AI viruses are easily inactivated by physical agents such as heat, extremes of pH, nonisotonic conditions, and dryness; however, their infectivity can be maintained for several weeks under moist, low temperature conditions. Infective virus can be retained in fecal matter for up to 82 days at 4 ºC[15-18] and about 32 days at 15-22 ºC[15,19] with one report claiming survival of infectious material up to 70 days.[18]

Due to their lipid envelope, AI viruses are relatively sensitive to disinfection agents and inactivation by lipid solvents such as detergents.[8,20] The Environmental Protection Agency (EPA) maintains a list of disinfectants with label claims for avian influenza viruses.[21] These products include halogens, aldehydes, quaternary ammoniums, phenols, alcohols, peroxides and some detergents. These label claims are for use on hard, non-porous surfaces, and in some instances removal of organic material is required for efficacious disinfection. Formalin and beta-propiolactone can be used to eliminate the infectivity of the viruses while preserving hemagglutinating and neuraminidase activity.

6.5 Transmission

Circumstantial evidence suggests that contact with migratory waterfowl, sea birds, or shore birds is a risk factor for introduction of virus into domestic poultry populations.[8,12,22-26] Since 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.[4,5,23] Once introduced into a flock, virus can be 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.[23]

6.6 Incubation Period

The incubation period can range from less than 1 day to 7 days depending on the virus strain, dose of inoculums, species, and age of the bird.[26,27]

6.7 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 terrestrial birds are usually severe, resulting in high mortality.[28]

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.[26] Mature chickens frequently have swollen, cyanotic combs and wattles, and edema surrounding the eyes. The mortality rate in an infected flock can reach 100 percent.

6.8 Gross Lesions

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.[26] In layers, the ovary may be hemorrhagic or degenerated and necrotic. The peritoneal cavity is frequently filled with yolk from ruptured ova, causing severe airsacculitis and peritonitis in birds that survive longer than 7 days.

6.9 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 followed by sudden deaths; however, a conclusive diagnosis is dependent on the isolation and identification of the virus.[26] In the laboratory, 9- to 11-day-old embryonated chicken eggs are inoculated with swab or tissue specimens. If HPAI virus is the causative agent, the embryo will die within 48–72 hours. If the virus isolated is identified as influenza type A, its serologic identity (HA and NA type) is determined using a RRT-PCR test.

6.10 Laboratory Specimens

AI viruses can be isolated from tracheal, oronasal, or cloacal swabs.[26] If large numbers of birds are to be sampled, swabs from up to 5 birds can be pooled in the same tube of brain and heart infusion broth.[29] These specimens are then taken to USDA-approved laboratories where an RRT-PCR test is run.

6.11 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.[30] It also must be differentiated from heat exhaustion and severe water deprivation.

6.12 Control and Eradication

The overall goal for response to a highly contagious disease such as HPAI is to detect, control, and eradicate the agent as quickly as possible to return individual farms to

normal production and regain disease-free status for the United States.[1] Control and eradication will rely on three basic principles:

a) Prevent contact between susceptible flocks and disease agents.

b) Stop the production of the agent by infected or exposed flocks (i.e. quarantine, depopulation and disinfection measures).

c) Increase the disease resistance of susceptible flocks.

7. Background Information on Shell Egg Operations

7.1 Purpose

This portion of the risk assessment will describe the major processes in shell egg operations and the current status of regulatory policies concerning shell egg operations in the United States.

7.2 Background: Description of Shell Egg Operations

7.2.1 Production and Distribution of Shell Eggs

In the United States, eggs are produced on several "small" or niche market as well as "large" or production oriented farms. Niche market-oriented operations may have as few as several hundred to several thousand hens and in some cases up to 50 or 75 thousand hens. Over 85 percent of the eggs in the U.S. are produced by "large" production oriented farms containing one million hens or more. These production facilities tend to be highly automated. The large production oriented facilities comprise egg production farms that do not process eggs onsite known as "offline" farms, and those that combine the washing and sanitizing operations on the production facility known as "inline" farms.

Offline egg farms may house 75,000 to over one million hens and typically gather eggs mechanically. Eggs produced at an offline farm are usually packed "as is" without having been washed, sized, and/or candled for quality. These unwashed eggs are referred to as "nest run" eggs. These eggs are eventually transported to a processing facility for washing and may at that point be combined with eggs from several offline farms.

Inline shell eggs are produced in facilities that move eggs on belts or conveyors directly to processing (thus, the term "inline") where they are processed on a daily basis. Inline operations generally involve large farm complexes that may have up to 6 million hens.

Some inline operations will also have equipment arranged so that shell eggs from other (offline) locations can be merged into the egg stream prior to the washing step. This practice is called "side loading." The inline operations that produce washed, sanitized and size-sorted shell eggs for retail or food service customers can divert undersized, oversized, and under-grade eggs for breaking either onsite or they can be transported to another site for breaking.

Figure 1 shows the various pathways for shell egg production, processing and distribution in the United States. In this risk assessment, we focus on the risks associated with movement of shell eggs from stand-alone processing facilities and inline processing facilities to destinations such as retail, food service or further processing. The risks associated with movement of eggs from offline farms to the processing facilities are addressed in a separate assessment for the movement of nest run eggs.

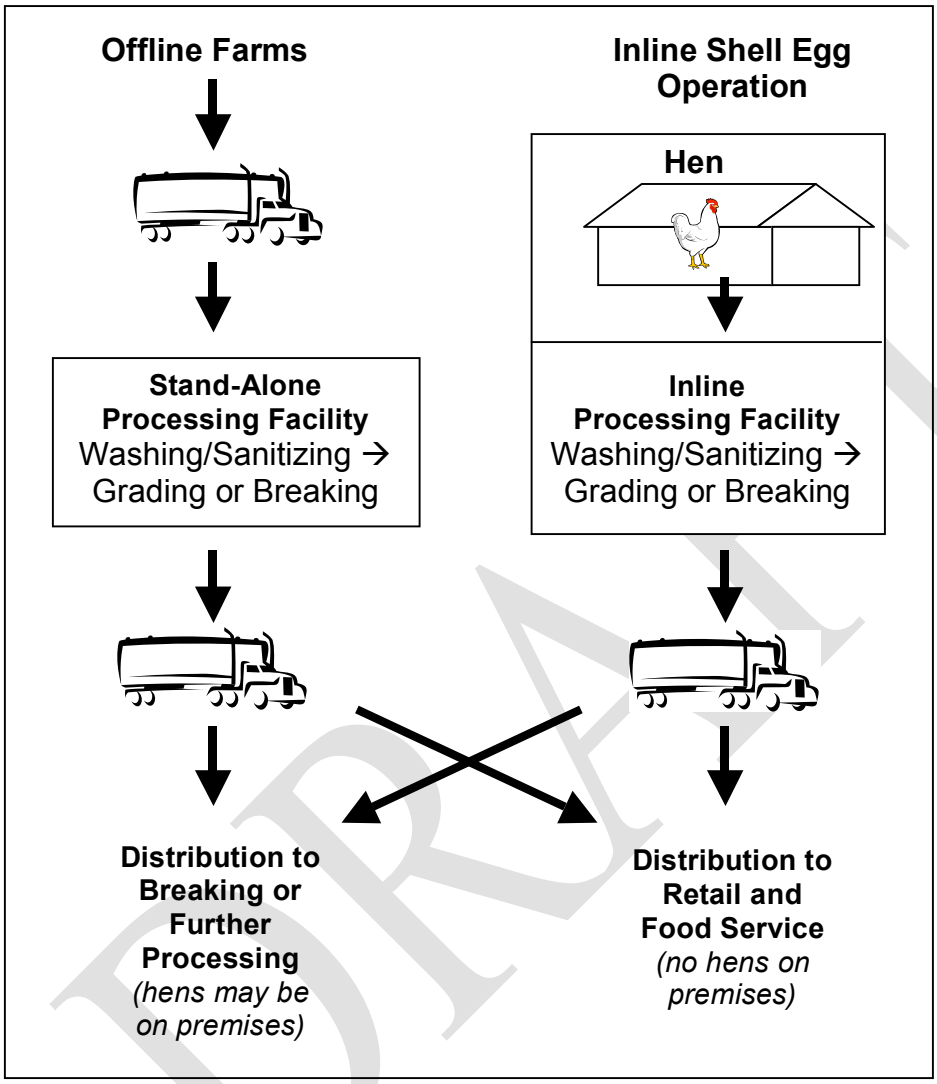

Figure 1. Pathways of shell egg production, processing and distribution

7.2.2 Regulation of Shell Egg Operations

The regulation of shell eggs is shared among various Federal agencies and State governments, or shell eggs may be processed according to industry-generated protocols. We reviewed current Federal shell egg regulations as well as State level regulations in the top five egg producing states in detail and included this information as Appendix 7. In brief, at the Federal level, the USDA Agricultural Marketing Service (AMS), the USDA food safety Inspection Service (FSIS), and the U.S. Food and Drug Administration (FDA) all regulate shell eggs in some manner. While there are no mandatory Federal regulations that detail specific requirements for washing and sanitizing shell eggs prior to sale as table eggs, the AMS has a voluntary shell egg grading program (7CFR56) which includes specific egg washing and sanitizing requirements. AMS also oversees a shell egg surveillance program that places limitations on restricted eggs (7CFR57). FSIS mandates specific washing and sanitizing conditions for eggs prior to breaking (9CFR590). Concerning shell egg storage, both the FSIS (9CFR590) and FDA (21CFR101) have identical temperature requirements; however FSIS regulations are directed at egg handlers[h] and FDA requirements are directed toward retail establishments. In addition, both agencies have labeling requirements; FSIS requires a label that indicates refrigeration is required and the FDA label includes safe handling instructions.

Shell egg operations that do not fall under 9CFR590 and choose not to participate in the voluntary AMS shell egg grading program typically adhere to State level regulations or follow industry-generated protocols. State level regulations vary, and many States rely on industry protocols rather than regulating shell operations. Shell egg operations not that do not currently implement 7CFR56.76(f) or similar egg washing and sanitizing procedures may do so as an additional preventive measure during an outbreak.

A general overview of the common processing steps used in shell egg operations in the United States is provided in the next section.

7.2.3 Major Processes in Shell Egg Operations

The primary shell egg operations are:

1. Shell egg washing
2. Sanitizing of shell egg surfaces
3. Candling/inspection
4. Weighing, grading and packaging
5. Storage and transport

1. Shell egg washing

[h] As defined in the 7CFR: Egg handler means any person, excluding the ultimate consumer, who engages in any business in commerce that involves buying or selling any eggs (as a poultry producer or otherwise), or processing any egg products, or otherwise using any eggs in the preparation of human food.

The initial phase of pre-wetting and washing shell eggs occurs as inline eggs are received via conveyor belts and accumulated on egg washer conveyors for entry into the washing machines, or when the offline shell eggs are removed from their bulk packaging (pallets, racks, and flats) and introduced via vacuum lifts to the egg washer conveyor. The pre-wetting of shell eggs is optional, and not all egg washing operations use this procedure.

The eggs are then moved into the egg washing machine where they are washed with an EPA approved detergent. AMS regulations 7CFR56.76(f) requires the temperature of the wash water to be 90°F or higher, and at least 20°F warmer than the temperature of the eggs.
The AMS regulations also stipulate requirements for sanitation of the washing equipment and condition of the wash water.

2. Sanitizing of shell egg surfaces

Immediately following the detergent wash, operations following 7CFR56.76(f) part (11) or equivalent sanitize shell eggs with a potable water rinse with a chlorine concentration of 100 (min) - 200 (max) ppm. The temperature of the sanitizing rinse water is maintained at 90°F or higher, and is at least 20°F warmer than the temperature of the eggs. All surfaces of the eggshell come into contact with the sanitizing rinse. After sanitizing and drying, the surface of shell eggs may be coated, as an optional process, with a food grade mineral oil to maximize conservation of quality.

3. Candling/inspection of shell eggs

Candling is a process where eggs are passed over an intense light that allows internal and external defects to be detected and removed. This includes removing eggs with residual specks of manure present on the surface.

4. Weighing, grading and packaging shell eggs

Individual egg weighing is done by specialized machines. The eggs are indexed into separate packing lanes based on individual egg weights. Eggs may be placed on cardboard, foam or plastic flats or in cartons in 12, 18 or 30 egg increments. Final packaging may be in a variety of case sizes.

5. Storage and transport

Most washed and sanitized shell eggs are destined to end customers such as retail and food service operations. A small proportion of washed and sanitized shell eggs may be transported for breaking or further processing. Egg cases can be stored on-site or transported to a distribution point or to the customer. Cooler rooms must be refrigerated and capable of maintaining an ambient temperature no greater that 45°F (7.2°C). Accurate thermometers must be provided for monitoring cooler room temperatures. Humidity control should be used in the coolers to prevent shrinkage (see Agriculture Marketing Service Egg Grading manual, Agricultural Handbook number 75).[31]

7.3 Analysis of Risk of HPAI Virus Spread through Movement of Shell Eggs

As shown in Figure 1, washed and sanitized eggs may be destined for retail distribution, foodservice operations, or for further processing. When washed and sanitized eggs are transported to further processing operations with susceptible poultry on the premises, there may be potential pathways for HPAI spread through contaminated eggs, trucks or personnel.

The likelihood that eggs from an infected but undetected flock are internally contaminated with HPAI virus is a major factor in evaluating the HPAI disease spread risk associated with the movement of shell eggs. In chapter 8, we address this question with an assessment of the nature of HPAI virus spread through a flock of poultry and the relationship between the time of disease detection and the number of internally contaminated eggs.

The external surfaces of shell eggs can experience a different degree of HPAI virus contamination than the internal contents due to different contamination pathways and egg washing and sanitizing procedures which reduce external viral load. In chapter 9, we estimate the likelihood of external virus contamination of eggs from an infected but undetected flock. In addition, we evaluate the degree of HPAI virus inactivation on the eggshell with the washing and sanitizing procedures.

In chapter 10, we evaluate the risk that the surfaces of shell eggs are recontaminated with HPAI virus via aerosols during post-sanitizing handling procedures.

Next, in chapter 11, we proceed with an evaluation of the risk of shell eggs infecting susceptible poultry on other premises. We considered: 1) the number of contaminated eggs expected from an infected but undetected flock (from chapter 8 analyses), 2) the degree of contamination given the processes and pathways discussed in chapters 8-10), and 3) the likelihood of exposure of shell eggs to susceptible poultry on another premises. This risk pathway only exists in situations where shell eggs are transported to breaking facilities with poultry on the premises.

Finally, in chapter 12, we evaluate the risk that vehicles or personnel moving shell eggs could indirectly transmit HPAI virus to susceptible poultry.

8. Likelihood of HPAI Virus Contamination of the Contents of Shell Eggs Moved from an Infected but Undetected Flock

This portion of the risk assessment describes the likelihood that shell eggs moved from premises containing an infected but undetected flock are internally contaminated with HPAI virus.

Likelihood of HPAI Virus Contamination of the Contents of Shell Eggs Moved from an Infected but Undetected Flock

- **Risk Factors:** Internal egg contents contaminated with HPAI virus; late detection of HPAI infection in a flock.
- **Current Preventive Measures:** None
- **Additional Preventive Measures** (to be implemented by industry in conjunction with APHIS during an outbreak): Active surveillance of flocks for clinical signs of illness, changes in feed and water intake, a drop in egg production, an increase in daily mortality above an established threshold, and submission of a pooled sample of swabs from 5 randomly chosen birds among the daily mortality for RRT-PCR testing.
- **Conclusions:**
  The expected number of internally contaminated eggs moved per day from an infected but undetected 100,000 bird layer house is 11 (90 percent probability interval, 0-44).

8.1 Background Information

Natural outbreak and experimental studies have found HPAI H5N2 virus in eggs laid by infected chickens.[22] To evaluate the disease spread risks associated with virus from the egg contents, we first need to estimate the proportion of eggs from an infected but undetected flock that would be contaminated.

The proportion of contaminated eggs from an infected but undetected flock would depend on the HPAI prevalence at various time periods before infection is detected. A characteristic feature of HPAI infection in a flock is the exponential increase in prevalence and mortality over time. Consequently, the proportion of contaminated eggs expected from an infected but undetected farm during an outbreak would depend on the time taken to detect infection given the surveillance protocol followed during the outbreak. As an extreme example, in the scenario of perfect surveillance, the number of contaminated eggs produced before detection would be zero.

The United Egg Producers/United Egg Association - USDA APHIS Veterinary Services Highly Pathogenic Avian Influenza Movement Control Model Plan (UEP/UEA – USDA APHIS VS Movement Control Model Plan) for use in an HPAI outbreak specifies active surveillance based on RRT-PCR testing (Appendix 8). We utilized a stochastic disease transmission model to simulate HPAI spread within the flock. The transmission model results were then used in conjunction with a simulation model of the active surveillance protocol to estimate the number of contaminated eggs that might be moved before infection is detected in the flock. We also estimated the viral titer and infectivity in the internal contents of the eggs. These results are used in subsequent chapters to evaluate the risk that susceptible poultry are infected via HPAI virus in the internal contents of eggs moved from an infected but undetected flock.

8.2 Evaluation

We estimated the likelihood that the internal contents of shell eggs moved from an HPAI infected but undetected flock are contaminated with HPAI virus in three parts:

a) The likelihood that the internal contents of eggs laid by an HPAI infected hen are contaminated.

b) The HPAI infection prevalence and the number of internally contaminated eggs at various time periods post infection of the flock.

c) The time to detect HPAI infection in the flock and the maximum daily number of contaminated eggs before detection when following the active surveillance protocol.

8.2.1 Likelihood that the Internal Contents of Eggs Laid by an Infected Hen are Contaminated

In this section, we use results from laboratory studies to estimate the likelihood that eggs laid by an HPAI infected hen are internally contaminated with HPAI virus. We also summarize published data concerning HPAI viral titers in the contents of contaminated eggs.

8.2.1.1 Likelihood that Internal Contents of Eggs Laid by an HPAI Infected Hen are Contaminated

Although HPAI H5N1 virus was isolated from eggs laid by infected Japanese quail,[32] to date, the HPAI H5N1 virus has not been isolated from eggs laid by infected chickens. The HPAI H5N2 virus, however, has been recovered from the internal contents of eggs laid by experimentally infected hens.[33] Data from experimental infection of hens with the HPAI H5N2 virus indicate that the likelihood of contamination of eggs from an HPAI infected hen depends on the duration of infection. In addition, these data indicate a drop in egg production rate in the period during which an infected hen may lay contaminated eggs.

(i) Time necessary to produce an egg

Egg yolk (ovum) development begins when the pullet reaches sexual maturity in response to a sequence of hormonal changes.[34] Approximately 10 days are required for an individual egg yolk to mature, and 5 to 10 egg yolks are in the growth process at any one time. The egg follicle is suspended on a highly vascular pedicle until the ova reaches maturity and ovulation occurs.

Upon release from the ovary, the yolk transverses the oviduct where the remaining portions of the egg are secreted. The yolk passes briefly through the infundibulum (15 minutes)[35] and successively through the magnum (2 to 3 hours) where the thick albumen is secreted, and the isthmus (75 minutes) where inner and outer shell membranes are formed which act as a barrier to the penetration of bacterial organisms. The egg then enters the tubular shell gland where water and electrolytes enter the albumen (approximately 5 hours) and then into the shell gland pouch (uterus) where it remains for the longest time during development (15 hours).[35] The total time necessary for an egg to transverse the oviduct varies from 23 to 26 hours. The timing of the formation of the shell membrane barrier and deposition of the shell egg matrix may prevent viral contamination of the internal contents of an egg during the final stages of egg shell development.

(ii) When eggs become contaminated in HPAI infected hens

In order to estimate the fraction of time an infected hen may lay a contaminated egg prior to death, we first consider the physiological and biological plausibility of the event. Given that a developing egg may be effectively protected from exposure in the shell gland (uterus) during the later stages of egg shell formation (about 15 hours), and considering the minimum latent period of at least 6 hours[27], we posit that eggs laid prior to 21 hrs post infection (PI) would not be contaminated and that eggs laid after 21 hrs PI could be contaminated.

This reasoning is supported by experimental studies and data collected from outbreak investigations (Table 1). In Swayne *et al*., adult laying hens were challenged by intranasal inoculation with HPAI H5N2 (Pennsylvania 1983).[33] No virus contamination was recovered from eggs laid for the first day PI, but 17 of 22 eggs laid on days 2 and 3 were contaminated. All birds either died or ceased egg production by day 4. Using these data, the fraction of time post inoculation that hens produced contaminated eggs prior to death is 0.67 (excluding day 4 where no egg production occurred).

In Beard *et al*.,[36] the first mortality observed occurred on day 4 PI and birds stopped laying eggs by day 5 PI. Eggs laid through day 2 were not contaminated, but eggs laid on days 3 and 4 were contaminated. Using these data, the fraction of time post inoculation that hens produced contaminated eggs until they stopped laying eggs is 0.5.

In our model, we assumed that eggs laid during the first 19 hours after infection are not contaminated and eggs laid after the first 19 hours are all contaminated. This threshold distribution implies that an infected bird would produce contaminated eggs in 0.56 fraction of the time lived. This estimate is comparable to that in the draft FSIS-FDA-

APHIS interagency risk assessment but is less than that observed from the experimental data (0.67) by Swayne *et al*. [33]

Table 1. Summary of data on the proportion of internally contaminated eggs from HPAI infected layers.

Study	*Percent contaminated eggs*	*Time of detection*	*HPAI strain*	*Source*
1	8% (3/37)	Last egg	Lab study (H5N2)	Bean *et al.* (1985)[23]
2	35% (15/42)	From 3rd day post infection onwards	Lab study (H5N2)	Beard *et al.* (1984)[17]
3	7-57% for different flocks[i]	Not shown	(H5N2) Natural Outbreak	Cappucci *et al.* (1985)[22]
4	45%	Day 2 and day 3 post infection	Lab study (H5N2)	Swayne and Suarez (2008)[33]

According to Beard *et al.* and Swayne *et al.,* there is no statistically significant difference in virus recovery frequencies between egg yolk and albumen.[17] For this assessment, we assumed that the virus recovery frequencies are the same for egg contents regardless of whether we are referring to egg yolk or albumen.

(iii) The decrease in egg production rate

A decrease in egg production is a frequently reported sequela of avian influenza infection in domestic poultry. Empirical evidence for a decrease in egg production in birds infected with Asian HPAI H5N1 is not yet available. A decrease in egg production was attributed to the isolation of Asian HPAI H5N1 from affected flocks both in Sudan[37] and Nigeria.[38] In this report, the precise rate of laying pre and post infection was not reported for the flock, thus the magnitude of the drop in egg production is not known.

Data concerning decreases in egg production are available from other HPAI H5N2 studies (Table 2). The shaded cells in Table 2 represent egg production on the days on which at least one contaminated egg was laid. In Beard *et al*. a total of 17 eggs were produced on days 3 and 4 PI.[36] Based on the egg production on day 2 (14 eggs), we projected that a total of 28 eggs would have been produced on day 3 and day 4 combined if there were no drop in egg production rate due to HPAI infection. We estimated a 39 (17/28) percent decrease in egg production rate in an infected layer hen compared to the normal rate of lay from Beard *et al.*[36] Similarly, we estimated a 29 percent drop in egg production rate from Swayne *et al.* data as shown in Table 2.[33] Based on the above data,

[i] This natural outbreak data may not be directly applicable for estimating the probability of contaminated eggs from an HPAI infected hen as the results are subject to variations in the HPAI infection prevalence within the flock and potential cross-contamination of eggs dependent on the layer management practices during the outbreak.

we assumed that the egg production rate in HPAI infected hens is 29 percent lower than the normal egg production rate of 0.7 eggs/hen/day.

Table 2. Estimate of fractional drop in egg production rate using the actual number of eggs laid after infection of hens with H5N2 HPAI and the projected number of eggs laid by healthy hens.

Study	Day 1	Day 2	Day 3	Day 4	Total eggs laid on the days when contaminated eggs were laid		Percent drop in egg production
					Actual	Projected (with no drop in egg production)	
Beard *et al*.[j,36]	-	14	14	3	17	28	39
Swayne *et al*. [k,33]	15	16	6	0	22	31	29

8.2.1.2 HPAI Titer and Infectivity in Egg Contents

Studies on HPAI virus titers in egg contents are summarized in Table 3. We did not model HPAI viral titers in egg yolk and albumen separately as the data on differences between their viral titers are limited. Bean *et al*[23] showed a 2 log difference between albumen and yolk, but only 3 eggs were analyzed. Swayne *et al*. [33] showed a one log difference between the albumen and yolk HPAI viral titers on average, but the difference may not be statistically significant. [33]

Based on a weighted average of the data summarized in Table 3, we used $10^{4.5}$ EID_{50}/ml as the viral titer in the internal contents of contaminated eggs. Here, EID_{50} refers to the 50 percent chicken embryo infectious dose. This viral titer represents a relatively high level of infectivity to chickens. We developed a dose response model for estimating the probability of infection in a chicken orally exposed to various doses of HPAI virus (details are provided in Appendix 4). The model predicts that the mean probability of infection in a chicken orally exposed to 0.1 ml of contaminated egg contents is 0.88 (90 percent probability interval 0.47-0.999).

Table 3. Summary of data on the HPAI viral titer within egg contents.

Source	*Sample*	*Viral titer (EID_{50}/ml)*	*HPAI Strain*
Swayne and Beck (2004)[l],[39]	egg contents	$10^{4.9}$	H5N2
Bean et al (1985)[23]	yolk	$10^{3.6}$	H5N2
Bean et al (1985)[23]	albumen	$10^{5.6}$	H5N2
Beard et al (1984)[17]	egg contents	$>10^{4.0}$	H5N2

[j] 25 leghorn hens, IN or IO with "SEPRL-PA" HPAI H5N2 Isolate
[k] 24 leghorn hens, A/chicken/Pennsylvania/1370/83 HPAI H5N2, cessation of egg production by day 4
[l] The reported virus titer is from unpublished data (M. Brugh) cited within this reference.

Swayne and Suarez (2007)[40]; Swayne et al (2008)[33]	yolk	$10^{3.0}$	H5N2
Swayne and Suarez (2007)[40]; Swayne et al (2008)[33]	albumen	$10^{3.6}$	H5N2

8.2.2 HPAI Infection Prevalence and the Number of Internally Contaminated Eggs at Various Time Periods Post Infection of the Flock

We used an infectious disease transmission model to estimate the prevalence of HPAI infection that might occur in a typical 100,000-bird layer house over time and the prevalence of infection in pools of daily mortality over time. The deterministic version of the transmission model was developed as part of a draft interagency risk assessment conducted by FSIS in collaboration with FDA and APHIS.[41] The disease transmission model is an extension of the Reed-Frost Susceptible-Latent-Infectious-Died (or Removed) model to include more infectious states. The model estimates the number of susceptible, infected, and dead birds and the number of contaminated eggs every 6 hours post-infection.

The inherent variability in the time course of HPAI spread within a flock can have a significant impact on the time to detection of infection. For instance, if the mortality due to HPAI infection is low on a particular day, then the likelihood of detecting infection when following a surveillance protocol based on daily mortality sampling is reduced. Data from the H7N7 outbreak in the Netherlands showed a relatively high variance in daily mortality among various infected flocks.[42] We developed a stochastic version of the transmission model to incorporate the effects of variability in spread of HPAI in a flock. Details of the disease transmission model are in Appendix 3.

We estimated the latently infected and infectious periods for the transmission model based on experimental studies of HPAI inoculated hens.[27] In the transmission model, the expected latently infected period is 13.8 hours and the expected infectious period is 29.6 hours. An important parameter for the transmission model is the effective contact rate, defined as the number of birds an infectious chicken comes into contact with that is sufficient to transmit infection per unit time. The effective contact rate has been estimated using a variety of approaches such as experimental studies, HPAI outbreak data, and simulation studies in the literature.[43-47] We used an effective contact rate of 2 chickens/6-hour time period considering the contact rate estimates from the literature for caged layers. We discuss the impact of the uncertainty in the effective contact rate estimate and perform a sensitivity analysis for our estimate in section 8.3. Given a contact rate of 2 chickens/6 hours, the basic reproductive number (R_0) for our model is 9.9. Finally, we used the data presented in section 8.2.1.1 to estimate the number of contaminated eggs from an infected flock. The main assumptions of the disease transmission model are summarized below. Details concerning our estimates are included in Appendix 3.

(i) Assumptions

- *The effective contact rate for disease transmission estimated from HPAI outbreak data from the Netherlands and Thailand (2 birds/6 hours) is applicable to layer flocks (i.e. a flock of birds contained in a single layer house) in the U.S.* Differences in the layer management practices between the United States and other countries and the characteristics of the HPAI strain causing the outbreak may result in a different contact rate than that used in this assessment. Sensitivity analysis with respect to the contact rate (presented in section 8.3) indicates that our risk estimates are conservative with respect to the range of contact rates estimated in the literature for HPAI infection in caged layers.
- *The mean infectious period and latent period for the HPAI strain are similar to those for Asian H5N1 strains*. We estimated a latent period of 13.8 hours and an infectious period of 29.6 hours using experimental data from HPAI H5N1 inoculated hens. The transmission model output may be sensitive to the infectious period of the HPAI strain causing the outbreak and may vary considerably for strains with a longer infectious period (e.g., Netherlands HPAI H7N7).
- *A flock size of 100,000 is typical in the industry.* A greater flock size would likely result in higher normal mortality and a decreased likelihood of detecting HPAI infection within a given time based on the active surveillance protocol. We consider the flock size of 100,000 as a conservative estimate given that the USDA APHIS Layer 1999 data indicates that the mean and median flock sizes are less than 83,000 hens (see Appendix 3 for further details).[48]

(ii) Simulation Results

The above model was coded in Excel using Visual Basic for Applications and @RISK software.[m] An effective contact rate of 2 chickens/6-hour time period and a flock size of 100,000 layers (contained within a single layer house) were used as input parameter values. We conducted simulations for 12000 iterations with Latin Hypercube sampling. The daily mortality results from the transmission model are presented in Table 4. Table 5 shows the estimated number of contaminated eggs from the output of the disease transmission model.

[m] @RISK version 4.5.3 Copyright © 2004, Palisade Corporation.

Table 4. Daily mortality predicted by the transmission model in a 100,000 bird layer house starting with one infected bird.

	Daily Mortality					
Parameter type	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Deterministic	0	1	4	31	235	1757
Stochastic mean	0	0.67	3.8	31	235	1761
Stochastic 90% probability interval	0	1	1-8	11-59	82-442	617-3,228

Table 5. Number of internally contaminated eggs predicted by the transmission model in a 100,000 bird layer house starting with one infected bird.

	Estimated Daily Number of Contaminated Eggs					
Parameter type	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Stochastic mean	0.122	1.2	9	67.9	511	3590
Stochastic 90% probability interval	0-1	0-2	3-17	23-127	177-959	1,316-6443

8.2.3 The Time to Detect Infection and the Maximum Daily Number of Contaminated Eggs under the Active Surveillance Protocol

In the targeted active surveillance protocol described in the UEP/UEA – USDA APHIS VS Movement Control Model Plan, swabs from 5 randomly selected birds among the daily mortality sample are pooled together and tested via RRT-PCR each day. The number of days to detect infection under this protocol depends on the variability in the normal mortality independent of HPAI and the variability in the mortality due to HPAI. We used outputs from the transmission model in conjunction with a simulation model of the active surveillance protocol to estimate the number of days to detect infection in a flock.

According to the UEP/UEA – USDA APHIS VS Movement Control Model Plan, eggs or egg products from flocks within the control area will be allowed to move with a permit only after the flock tests negative with RRT-PCR testing as described above.[n] This

[n] Such holding time for eggs or egg products awaiting negative RRT –PCR results may increase the risk that storage capacity is exceeded leading to disposal of product.

protocol is explicit in that if infection from a flock is detected on a particular day by RRT-PCR testing, then the eggs or egg products from the flock on that day will not be moved from the premises. Therefore, for this analysis we assumed that eggs produced on the day on which infection is detected are not relevant for risks associated with movement of shell eggs. Given this assumption, we defined the maximum daily proportion of contaminated eggs as the highest daily proportion of contaminated eggs among all the days starting from the day the flock is infected to one day before the infection in the flock is detected.

The simulation model of the active surveillance protocol can best be explained by using a scenario tree (Figure 2). The number of diseased birds present in the 5 randomly chosen birds from the daily mortality group follows a hypergeometric distribution (hypergeometric (*M, n, D*)) where *M* is the total mortality, *D* is the mortality due to HPAI and $n = 5$ is the sample size.[o] Given this protocol, there is a 95 percent chance of including at least one diseased bird in the pooled sample if the HPAI prevalence among dead birds is greater than 39 percent.[p] The pooled sample of 5 swabs from the selected birds is tested via RRT-PCR. The sensitivity of this test is estimated to be 86.5 percent, so there is a 13.5 percent chance that infection will not be detected even when the pooled sample contains an HPAI-positive bird.[29] We modeled the process of RRT-PCR testing as a simple Bernoulli trial. We also considered that HPAI virus would be detected due to increased mortality if there is very high disease mortality. Specifically, we assumed that HPAI infection would be detected due to increased mortality if the daily mortality *M* is greater than 0.5 percent of the flock. Details for the estimation of this threshold are provided in appendix 10.

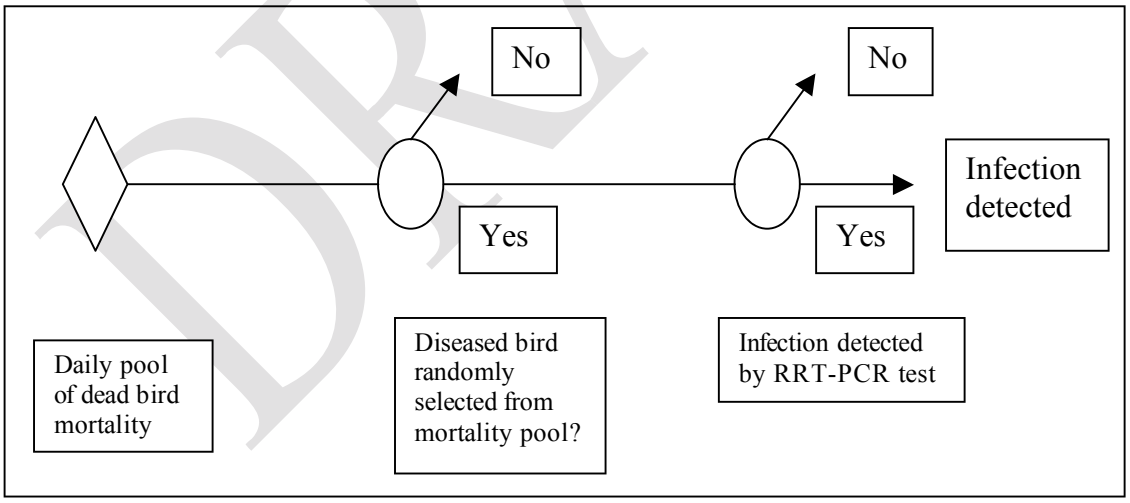

Figure 2. A scenario tree analysis illustrating the probability of reporting at least one test-positive bird via RRT-PCR.

[o] Although some dead birds might be missed (not detected) while counting mortality each day, this will not impact the probability of detecting HPAI infection under the active surveillance protocol provided that all dead birds are equally likely to be missed regardless of whether they have HPAI or not.

[p] The confidence limit was based upon on simulation of the hypergeometric distribution with @RISK for 20,000 iterations. The daily mortality pool size M was estimated from Agristats data as shown in table 6.

(i) Assumptions:

- Apart from active surveillance of mortality via RRT-PCR, other clinical indicators of HPAI infection such as a drop in egg production and decreased feed intake are not considered towards detecting infection.
- The sampling of daily mortality is random; i.e., swabs from dead birds with clinical signs and those without clinical signs are equally likely to be included in the pooled sample for RRT-PCR testing.
- Weekly mortality data was used to estimate the normal daily mortality.
- The active surveillance protocol with daily RRT-PCR testing of a pooled sample from the flock is implemented before the flock becomes infected with HPAI. The assumption is well justified after the initial few days of RRT-PCR testing during the HPAI outbreak. However, differences in model results without this assumption are not significant (as discussed in appendix 11).

(ii) Model Inputs:

The main input parameters for this model are summarized in Table 6.

Table 6. Key input parameters for the simulation model to estimate the number of days to detect infection in a flock when the active surveillance protocol is followed.

Input Parameter	*Value*	*Unit*	*Source*
Flock size	100,000	Birds	Average flock size (FSIS risk assessment)[49]
Normal daily mortality independent of HPAI infection	Empirical distribution mean 28 (95 percent CI 26.4-29.6), std. dev. 33	Birds/ day	Unpublished Data from 27 layer flocks for the entire production cycle, Agri Stats, Inc.[50] (See Appendix 3 for details)
RRT-PCR test sensitivity	86.5%	--	Elvinger *et al*.(2007)[29]; Dr Erika Spackman, pers. comm., 2007

(iii) Model Results:

Number of days to detect HPAI after the first chicken is infected:

The distribution of the number of days to detect HPAI infection from simulated output is shown in Figure 3. The mean time to detection was 3.3 days. However, as shown in Figure 3, there is a 7.4 percent chance that ≥ 5 days are required to detect infection. One reason for the late detection of HPAI (≥ 4 days) is a high normal mortality rate. A high normal mortality rate implies that a swab from a bird that dies due to HPAI is less likely to be included in the random sample for the pooled specimens sent for RRT-PCR testing. Another factor leading to late detection is the low sensitivity (86.5 percent) of the RRT-PCR test.

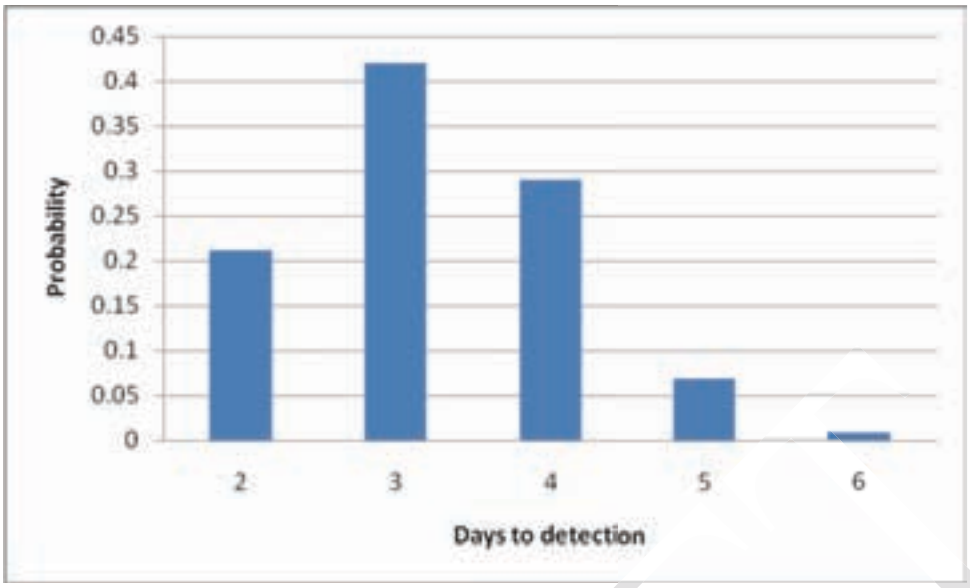

Figure 3. Simulated number of days to detect HPAI in a flock of 100,000 layers with active surveillance via RRT-PCR testing.

Maximum number of contaminated eggs per day from an infected but undetected flock:

The expected maximum number of contaminated eggs produced per day from a 100,000 layer flock prior to detection was 11 contaminated eggs/day (90 percent probability interval 0-44). In 5 percent of the simulation iterations, however, 44-1018 contaminated eggs were produced prior to detection. Typically, the cases in the simulation results exhibiting a greater number of contaminated eggs produced prior to detection were associated with late detection of infection.

8.3 Sensitivity Analysis for the Effective Contact Rate

We conducted a sensitivity analysis on the effective contact rate because there is less certainty associated with its estimation compared with other transmission parameters. An increase in the effective contact rate would have two competing effects that would impact the number of contaminated eggs produced from a flock before detection. The first effect is that a higher contact rate would result in a faster rate of disease spread and a higher mortality. It follows that the higher mortality rate would likely result in a reduced time to detect infection in the flock. The second effect is that the greater number of infected chickens would likely result in a higher number of HPAI virus contaminated eggs being produced over a given time.

For the sensitivity analysis, we performed 6000 simulation iterations of the disease transmission and the active surveillance models for the effective contact rates of 1, 2 and 4 birds/6 hours. The contact rate of 4 birds/6 hours can be considered an upper bound estimate for caged layers as predicted by the simulation model of Savill *et al*.[47] As detailed in Appendix 3, the contact rates estimated from true HPAI outbreak data were mostly less than 2-birds/6 hours. From our simulation results, the expected maximum daily number of contaminated eggs moved before infection is detected with contact rates

1, 2 and 4 birds/6 hours was 4 (90 percent probability interval 0-15), 11 (90 percent probability interval 0-44) and 24 (90 percent probability interval 0-49), respectively.

The above sensitivity analysis indicates that the number of contaminated eggs from an infected but undetected flock of 100,000 birds (within a single layer house) following the active surveillance protocol increases with the effective contact rate. The effective contact rate of 2-birds/6 hours used in our assessment is higher than the estimates from HPAI outbreak data in the literature and is therefore considered a conservative estimate.

8.4 Conclusion

In this chapter, we utilized a stochastic disease transmission model to estimate the proportion of HPAI virus contaminated eggs from an infected but undetected layer flock following the active surveillance protocol described in the UEP/UEA – USDA APHIS VS Movement Control Model Plan. The key inferences from this chapter are as follows:

a) The number of contaminated eggs from an infected but undetected flock varies with the time of disease detection (i.e. the level of surveillance effort).

b) Our simulation models predict that the expected value of the maximum daily number of internally contaminated eggs moved from an infected but undetected flock following the active surveillance protocol is 11 (90 percent probability interval 0-44).

c) Based on the estimated viral titer in the contents of contaminated eggs and dose response analysis, a chicken orally exposed to 0.1 ml of contaminated egg contents has a high probability of becoming infected.

The above results were used in subsequent chapters to evaluate the risk of HPAI spread to susceptible poultry due to virus in the contents of washed and sanitized eggs.

In our analysis, we considered RRT-PCR as the sole means for detecting infection. However, in practice, clinical signs such as increased mortality, decreased egg production and supplemental PCR testing may lead to earlier detection. An important caveat is that some deviation from modeling results is to be expected depending on the characteristics of the HPAI strain causing the outbreak such as length of latently infected and infectious periods, variation in the flock size, and layer management practices.

9. Likelihood of HPAI Virus Contamination of the Shell Surfaces of Washed and Sanitized Eggs

This portion of the risk assessment describes the likelihood that the shell surfaces of eggs from an infected but undetected flock are contaminated with HPAI virus after washing and sanitizing.

Likelihood of HPAI Virus Contamination of the Shell Surfaces of Washed and Sanitized Eggs from an Infected but Undetected Flock

- **Risk Factors:** Shell egg surface contaminated with HPAI virus; egg washing, inspection and sanitizing steps do not remove the HPAI virus.
- **Current Preventive Measures:** Egg washing, sanitizing and inspection procedures as specified in 7CFR56, 9CFR590, State regulations or industry generated protocols.
- **Additional Preventive Measures:** Egg washing and sanitizing with sanitizing rinse temperature and concentration as specified in 7CFR56.76(f) parts (3) and (11)[a]. Specifically, the concentration of sanitizer (chlorine or equivalent) should be between 100 to 200 ppm.
- **Conclusions:** The egg washing and sanitizing procedures as specified in 7CFR56.76(f) parts (3) and (11)[a] would inactivate HPAI virus on the shell surface by a factor of 1,000 (a 3-log reduction).

9.1 Background Information

This section of the risk assessment evaluates the risk of HPAI virus on a shell egg surface surviving the washing and sanitizing processes. As described in Chapter 8, HPAI (H5N2) virus has been isolated from both the shell and contents of eggs laid by infected chickens.[22 17,40,51] Current egg washing and sanitizing procedures as outlined in section 7.2.3 are aimed at reducing shell egg surface contamination with microbial agents such as *Salmonella*. Washing and sanitizing likely results in some inactivation of HPAI virus on the eggshell as well, given that the avian influenza virus is an enveloped virus that exhibits marked sensitivity to disinfectants.[52] The degree of inactivation is dependent on specific operational conditions including the pH of the wash water and sanitizing rinse, contact times of the wash and rinse steps, and organic load. These conditions are not standardized in the egg industry and may result in varying degrees of virus inactivation.

In this section, we estimate the degree of viral inactivation from egg washing and sanitizing processes using data from inactivation studies of HPAI viruses and other viruses.

9.2 Current Preventive Measures

Preventive measures considered in this risk assessment are those specified in section 7.2.3 regarding shell egg washing, inspection and sanitizing prior to transport.

As described in section 7.2.3, the washing operation utilizes a combination of heat, pH, detergent action, contact time and mechanical agitation to accomplish the removal of soil from the shell egg surface. Detergents used in the washing process must be USDA approved[q] and labeled for such use by the EPA. In addition, the wash temperatures are assumed to reach a minimum of 90°F. Unclean eggs are disposed of or rewashed.

In this risk assessment, it is assumed that all shell eggs are sanitized prior to packaging for transport. A spray rinse containing a sanitizer of 100 - 200 ppm available chlorine or its equivalent is applied to all surfaces of the egg. After sanitizing, shell eggs are inspected for interior and exterior defects. This process is performed for removal of any defects such as unclean eggs, eggs with interior defects, misshapen shells (an indicator of disease), and broken shells.

9.3 Evaluation

We address the likelihood of HPAI virus remaining on washed and sanitized shell egg surfaces in three parts:

a) The likelihood of HPAI being present on the eggshell.

b) The likelihood of HPAI on the eggshell not being removed during the wash step.

c) The likelihood of HPAI virus on the eggshell not being deactivated or removed during the shell sanitizing step.

9.3.1 Likelihood of HPAI being Present on the Eggshell

Laboratory studies with HPAI H5N2 infected hens have found the frequency of contamination of the eggshell to be similar to the frequency of internal content contamination.[33] Specifically, in this study, eggs laid on the first day post-inoculation weren't contaminated, but by time of death 30-43 percent of eggs laid by infected hens overall were contaminated.[17,40] Based on this finding, we assumed that the frequency of contamination on the shell surface of eggs from infected hens is similar to that of internal content contamination.

We used the experimental data presented in section 8.2.1.2 in the disease transmission model to estimate the number of eggs with HPAI virus on the shell surface from an infected but undetected flock. The simulation results indicate that the number of eggs with a contaminated shell surface from an infected but undetected flock of 100,000 layers is 11(90 percent probability interval 0-44).

[q] The current approval process for cleaners and sanitizers is included as a letter from the National Supervisor of Shell Eggs (Appendix 9).

To our knowledge, there are no published data on the HPAI viral titer on the shell surface of contaminated eggs. In recent unpublished data[33] the viral titer on contaminated eggshells from HPAI (H5N2) infected hens was between $10^{3.4}$ - $10^{3.6}$ EID_{50}/eggshell from 16 contaminated eggs. We assumed that the viral titer on a contaminated eggshell is $10^{3.6}$ EID_{50}/eggshell for this assessment.

In addition to the eggs that are contaminated at oviposition, eggs may also be cross-contaminated with organic material (such as feces) from infected hens. Data on the cross-contamination of eggs is limited. A study of LPAI H7N2 infected broiler breeder farms did not find any virus from 120 eggshell swabs, although virus could be isolated from 90 percent of chicken (tracheal or cloacal swabs) and 50 percent of dust and manure swabs. This suggests that virus transfer from the cloaca, manure or dust to the eggshell surface is inefficient. Among eggs from an HPAI infected flock studied in Cappucci *et al.*,[22] virus could be recovered from only 10 percent of shell surface swabs, however, 40 percent of albumen samples were positive for the virus. These studies indicate that the fraction of eggs from layer farms that are cross-contaminated on the surface is low, regardless of the mode of contamination. While we do not consider cross-contamination of the shell surface in our model, the expected proportion of eggs laid by an HPAI infected hen that are contaminated in our model (47 percent) is higher than the 30-43 percent observed in experimental studies and is thus conservative.

9.3.2 The Risk of HPAI on the Eggshell not being Removed during the Wash Step

In a typical commercial egg washer, eggs are passed through conveyer rollers while being cleaned with brushes and sprayed with recycled wash water containing an approved cleaning agent.[r] Regulations concerning egg washing operations (9CFR590.515, Appendix 2) require the wash water temperature to be above 32.2°C and be replaced once every 4 hours. During the washing process, egg contents, manure, dirt and microbes in accumulate in the recycled water. A higher wash water pH is preferable for reducing *Salmonella* contamination.[53] Typically, the wash water pH is in the range of 10-11.5 and the total dissolved solids are greater than 2 grams/L.[54] Although avian influenza virus might be inactivated at high pH with sufficient contact time,[55] it is unclear whether the pH of 10-11.5 in the egg washing process would cause any inactivation of HPAI virus with a short contact time (typically less than one minute). Lu *et al.*[15] found no inactivation of LPAI H7N2 virus at pH 10 and 12 with contact times of 5, 10, and 15 minutes.

HPAI virus can be inactivated by the detergents and alkalis in the approved egg washing compounds.[20] Detergents act on the lipid components of enveloped viruses via their surfactant property.[56] A recent article reported a 2 to 3 log factor reduction in the surface viral titer of LPAI H7N7 virus after ten minutes of treatment with a laundry detergent (2-6 grams/L) and peroxide.[57] Shahid *et al.* found that HPAI H5N1 virus in suspension form was inactivated after exposure to 0.1 percent soap solution for 5 minutes.[55] Grayson *et al.*

[r] See Appendix 9.

found that washing with soap and water was very effective in inactivating human influenza A H1N1 virus on volunteers' hands.[58] Alkalis can inactivate influenza virus by denaturing proteins. Abe *et al.*[59] found greater than a 3-log reduction of avian influenza virus with an alkali after a contact time of 30 minutes. The effectiveness of alkalis can decrease in the presence of organic matter, however.

Direct data on virus inactivation under the typical operational conditions encountered in the egg washing process (pH, organic load, detergent concentration, etc.) is not available. A recent study indicates that commercial washing procedures are successful in removing a significant proportion of the *Enterobacteriaceae* (higher than 50 percent).[60] Knape *et al*.[54] found a 10 to100 factor reduction of aerobic plate counts with egg washing. Musgrove *et al.*[61] found that *Salmonella* was recovered more frequently from unwashed eggs (in 15.8 percent of eggs compared with 8.3 percent in washed eggs). Given the disinfectant activity of detergents on enveloped viruses such as HPAI and the empirical evidence of the reduction of bacterial contamination in commercial egg washing operations, it is reasonable to postulate that a 0.5 to 1 log inactivation of HPAI virus is achieved through egg washing.

9.3.3 The Risk of HPAI Virus on the Eggshell not being Deactivated or Removed during the Shell Sanitizing Step

9.3.3.1 Egg Sanitizing using Chlorine Sanitizers

AMS regulations outlined in 7CFR56 require shell egg operations participating in their voluntary grading program to spray-rinse the shell eggs with potable water containing an approved sanitizer with a chlorine concentration between 100-200 ppm or its equivalent. In addition, the sanitizing rinse is maintained at a temperature greater than 90°F. The sanitizing activity of chlorine is dependent upon operational conditions such as chlorine concentration, organic load, temperature, pH, and contact time. Among these conditions, only the chlorine concentration range and sanitizing spray temperature are specified in most regulations. The variability in the other conditions such as pH and contact time may result in varying degrees of inactivation.

We developed an @RISK simulation model to incorporate the uncertainties of these conditions into our estimate of HPAI reduction on shell egg surfaces due to chlorine sanitizer spray. The model is presented below with additional details on the model design included as Appendix 5.

(i) Definitions:

Exposure time (t) - The time for which the eggshell surface is exposed to the sanitizing rinse.

Ct values (Ct) - The chlorine concentration (*C*) multiplied by exposure time *(t)*.[s] *Ct* is a frequently used measure of exposure to disinfecting agents.

[s] *Ct* values indicate the chlorine concentration (in mg/L, or ppm) and time (in minutes).[137] *Ct* values can reflect various combinations of chlorine concentration and time and can be considered equivalent. For

Computed Ct values - The output *Ct* values from our simulation of the sanitizing process.

Chlorine decay rate (k) - The exponential decay rate constant for chlorine concentration. We consider that the sanitizing rinse chlorine concentration decays exponentially due to the organic matter on the eggshell surface.

(ii) Assumptions:

- Eggs have been washed and cleaned prior to sanitizing, leaving no soil or feces on the shell egg surface.
- HPAI virus is present on the shell egg surface.[t]
- Eggs are in contact with the sanitizing spray for 1 to 8 seconds.[u]
- Hepatitis A virus is less sensitive to inactivation by chlorine than HPAI virus. As Hepatitis A is a non-enveloped virus, it is likely less sensitive to chlorine inactivation than the enveloped HPAI virus. Hepatitis A virus is relatively resistant to inactivation and has been used as an indicator virus for disinfection of water by the EPA.
- The *Ct* values and chlorine decay rates are reasonable values to use for egg processing operations.

(iii) Simulation Model and Parameters:

In the simulation model, we first estimated a probability distribution for *Ct*, using exposure times provided by industry experts and chlorine concentration specified in regulations. We then compared *Ct* values from the simulation output with values required to achieve a 1000 factor inactivation (referred to as a 3-log reduction) as reported in various sources.[62] The values used for the key parameters of the simulation model are provided below. Details of the simulation model are provided in Appendix 5.

Parameter	*Uniform distribution range*	*Source*
Initial chlorine concentration (C_o)	100 – 200 ppm	Assumed
Exposure time (t)	1 – 8 seconds	Expert opinion[63]
Chlorine decay (k)	1.24 – 2.33 min^{-1}	Rice *et al.*, 2007[62]

(iv) Simulation Results:

example, a *Ct* value of 10 could indicate an exposure of 10 minutes to a 1-ppm concentration or an exposure of 1 minute to a 10-ppm concentration.

[t] The amount of virus present was not specified in the model, as there has been no research on this issue. However, HPAI has been detected on shell eggs.[22]

[u] The 8-second value is considered by an industry expert to be a minimum value.[63]

1) The chlorine concentration time *Ct*:

The mean *Ct* value from the simulation was 11.9 mg-min/L, falling between 4.2 and 19.9 mg-min/L with 90 percent probability (Figure 4).

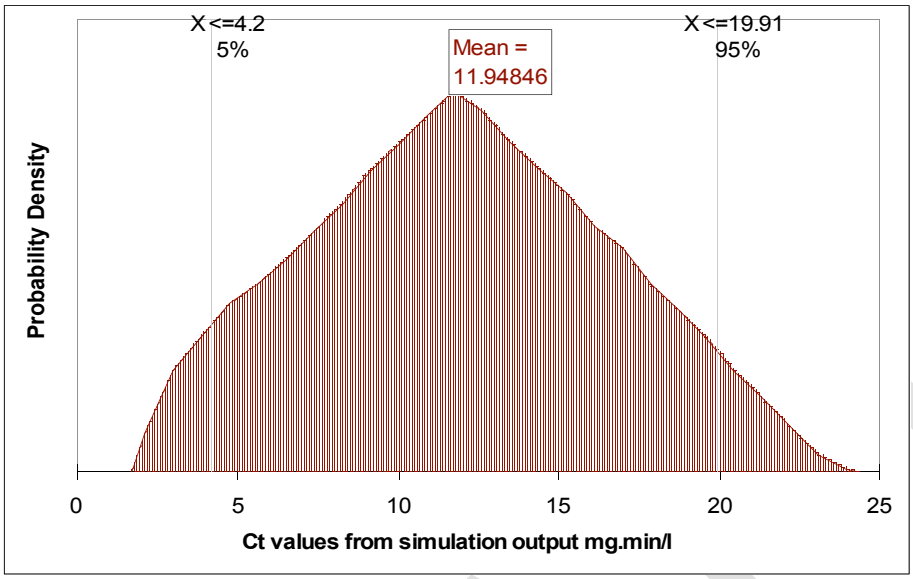

Figure 4. Distribution of computed *Ct* values from simulation output.

2) Degree of HPAI virus inactivation with computed *Ct* values:

The chlorine activity for a given *Ct* is dependent on the pH and temperature. Higher pH values cause the less potent ionic form of chlorine (OCl^-) to predominate in solution, making virus inactivation less efficient (*Ct* value increases). Conversely, higher temperatures increase virus inactivation efficiency, decreasing the *Ct* value. As previously mentioned, the sanitizing rinse temperature is required to be above 32°C.

During shell egg sanitizing, the effective shell egg surface pH would be between the pH of wash water (typically higher than 10) and the pH of the sanitizer spray. We model the scenarios where the shell egg surface is at neutral pH or high pH separately as follows.

SCENARIO A: Neutral pH, chlorine sanitizer

For a chlorine sanitizer at pH 7 to 8, all simulated *Ct* values exceeded previously reported *Ct* values for chlorinated sanitizers[62] that achieved a 3-log inactivation of HPAI virus at 5°C. On average, the *Ct* from simulation was greater than that required for 3-log inactivation by a factor of 24. The simulated *Ct* values were also higher than those reported for 4-log inactivation of other viruses such as Hepatitis A (a more chemical resistant, non-enveloped virus[64]) at pH 6 and 5°C. Furthermore, the required *Ct* values at the sanitizing rinse temperature of 32°C would likely be lower than those from the above experiments conducted at 5°C. These results indicate a 3-log inactivation of virus is achieved when the effective pH is less than 8.

SCENARIO B: High pH, chlorine sanitizer

The effectiveness of chlorine as a sanitizer can decrease significantly at pH greater than 10. To our knowledge, experimental data concerning chlorine inactivation of HPAI virus at pH ≥ 10 are not available. We used the following approaches to evaluate HPAI virus inactivation high pH: 1) Using correction factors for pH and temperature to extrapolate from the experimental data on HPAI virus at pH 7-8, and 2) Using data on Hepatitis A virus at high pH as a proxy for HPAI virus.

Effect of high pH on Ct value:
From studies of chlorine inactivation of other viruses,[64,65,66] the *Ct* value required for inactivation of viruses at a pH of 10 is 5 to 20 times higher than that required at a pH of 7 or 8.

Effect of high temperature on Ct value:
Experimental data concerning chlorine inactivation of HPAI virus at 32°C is also not available to our knowledge. However, the EPA guidance manual (1991)[95] suggests a 2-log decrease in *Ct* values for inactivation of other viruses for every 10°C increase in temperature. For example, a *Ct* value of 1 mg-min/L is sufficient to achieve a 4 log inactivation of Hepatitis A at 25°C and pH 10,[66] whereas a Ct value above 20 mg-min/L was required for a 3-log inactivation of Hepatitis A virus at 5°C and pH 10.[67]

Using the above correction factors and Hepatitis A virus as a proxy for HPAI virus, we conclude that the *Ct* value required for 3-log inactivation of HPAI virus at 25°C and a pH of 10 is between 1-5 mg-min/L. These *Ct* values are roughly 1 to 5 times greater than the reported *Ct* values for chlorine inactivation of HPAI virus in experiments performed at 5°C and pH 8.[62]

3) Simulated *Ct* values compared to required *Ct* values:

We calculated the ratio between the simulated *Ct* values (simulation results section 1) and the *Ct* values required for 3-log inactivation of HPAI virus at pH 10, 25°C (simulation results section 2), and simulated a distribution of the values (Figure 5). The simulation results show that there is a 97 percent chance that the simulated *Ct* value is higher than that required for 3-log inactivation of HPAI virus (i.e. the ratio is > 1), and on average the simulated *Ct* value is 5 times higher than the *Ct* value required for 3-log inactivation of HPAI virus in the high pH conditions reflective of industry conditions.

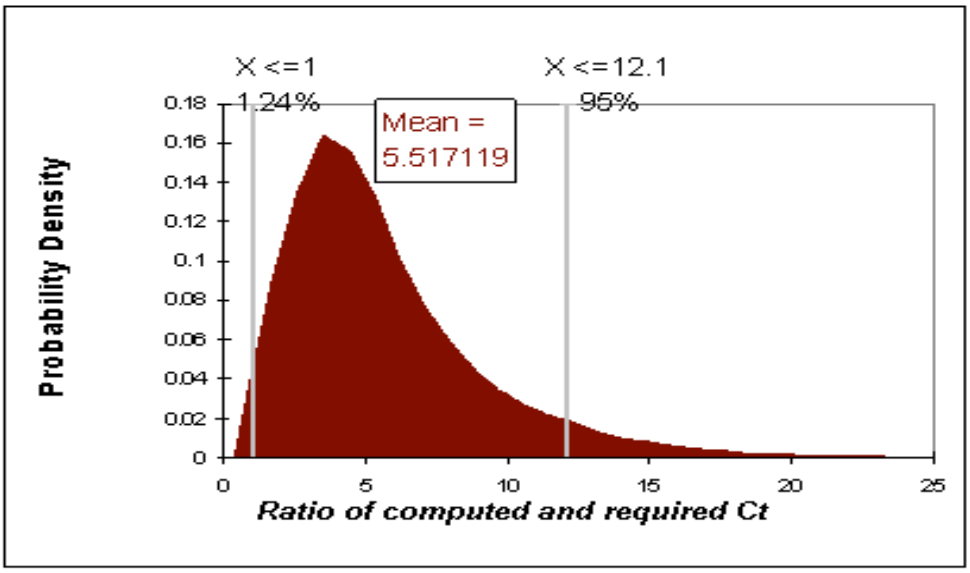

Figure 5. Ratio of computed and required Ct values.

9.3.3.2 Egg Sanitizing Using Non-Chlorine Sanitizers

As required under 9CFR590.516 (Appendix 2), all shell eggs that are used for breaking must be spray rinsed with potable water containing an approved sanitizer with a chlorine concentration between 100-200 ppm or its equivalent. Although chlorine compounds are most commonly used in industry, the FDA has also approved several non-chlorine compounds such as quaternary ammonium and iodine compounds. As of the writing of this risk assessment, FSIS does not review new compounds for use in official processing plants but rather requires that the compounds used be unaltered since their initial approval.[v] Non-chlorine sanitizers should thus be cleared through FSIS and deemed acceptable alternatives to 100-200 ppm chlorine. We do not model the use of non-chlorine sanitizers in this assessment.

9.3.3.3 Summary

Experimental data testing HPAI H5N1 virus inactivation in allantoic fluid at neutral sanitizer pH (7-8) shows the viral load on the eggshell to be reduced by a factor of at least 1,000 (a 3-log reduction).[62] Efficacy of HPAI virus reduction in conditions reflective of industry (pH 10 sanitizer, high temperature) has not been tested to our knowledge. At a sanitizer pH of 10, utilizing data on inactivation of other indicator viruses, we conclude that there is a 97 percent chance that a 1000 factor (3-log) inactivation of HPAI virus on eggshells is achieved.

9.4 Conclusion

For an infected but undetected flock following the active surveillance protocol described in the UEP/UEA – USDA APHIS VS Movement Control Model Plan, the number of

[v]The current approval process for cleaners and sanitizers is included as a letter from the National Supervisor of Shell Eggs (Appendix 13).

eggs moved per day that would have been externally contaminated with HPAI virus before washing and sanitizing is 11 (90 percent P.I. 0-44).

Based on our simulations of shell egg sanitizing, there is a 97 percent chance that the chlorine concentrations specified in 7CFR56 inactivate HPAI virus by a factor of 1,000 (a 3-log reduction).

Given our current knowledge of the viral titer on the surface of an egg from an infected hen ($10^{3.6}$ EID_{50}/eggshell; Swayne 2008)[33], we conclude that the viral load remaining on the eggshell surface after washing and sanitizing is a 3-log reduction from $10^{3.6}$ EID_{50}/eggshell, or approximately $10^{0.6}$ EID_{50}/eggshell. We examine the risk of susceptible poultry being infected via cross-contamination from the shell surface of washed and sanitized eggs in chapter 11.

10. Likelihood of Re-introduction of HPAI Virus to Shell Eggs during Post Sanitizing Handling

This portion of the risk assessment evaluates the likelihood that HPAI virus would be present on the surface of a shell egg after washing and sanitizing through cross-contamination.

Cross-contamination of Shell Eggs Post-Sanitizing

- **Risk Factors:** Cross-contamination of washed and sanitized shell eggs with HPAI virus via aerosols and bioaerosols.
- **Current Preventive Measures:** Mechanical ventilation systems that have airflow directed from the egg packing room into the henhouse (used in approximately 70 percent of henhouses).
- **Additional Preventive Measures:** Active surveillance protocol which includes RRT-PCR testing of swabs from 5 randomly selected birds among daily mortality.
- **Overall Risk**: *negligible*

10.1 Background Information

Following sanitizing, shell eggs are candled, inspected, weighed and packaged. During this time, it is possible that aerosolized HPAI virus from nearby henhouses could enter the egg packing room air space and contaminate shell eggs. This mode of contamination is unlikely if the henhouses are mechanically ventilated such that airflow is from the egg packing room to the henhouses. Although a 1999 survey found that more than 70 percent of henhouses are mechanically ventilated,[68] we deemed it necessary to determine whether a significant amount of infectivity would be transferred onto the eggshell via aerosols when henhouses are naturally ventilated.

This portion of the risk assessment will describe:

a) The density of aerosols and bioaerosols in poultry facilities.
b) The risk that HPAI virus in aerosols could contaminate washed and sanitized shell eggs and infect susceptible poultry via cross-contamination.

10.1.1 Characterization of Aerosols

Aerosols are small but variably sized particles that can remain suspended in the air for prolonged periods because of their low settling velocity.[69-70] Bioaerosols are a subset of aerosols that are comprised of particulate matter of biological origin (microbial, plant, or animal).[71]

Bioaerosols may be solid or liquid, with air serving as a mode of transport for dispersal.[72] The length of time bioaerosol particles remain suspended in air depends partly on particle size (Table 7) although these times are affected by air turbulence, shape and composition of the particles, animal activity, airflow patterns in facilities, and environmental conditions. During bioaerosol transport downwind, the concentration of particles and viable microorganisms decreases with time due to biological inactivation and gravitational settling. Bioaerosols generally move down thermal gradients from regions of warmer temperature to cooler regions.[73-76]

Table 7. Settling times for a 3 meter fall for spherical particles in still air.[70]

Particle Size	Settling Time
100 µm	10 sec
20 µm	4 min
10 µm	17 min
5 µm	62 min
<3 µm	Do not settle

10.1.2 Aerosols in Egg Layer Operations

Maghirang *et al*.[77] examined air quality in a commercial layer house in Pennsylvania with 120,000 layers. They found particle concentrations to range from 25 to 103 particles/ml. Ninety-nine percent of airborne-particles were smaller than 10 µm and 97 percent were smaller than 5 µm in diameter.

In another study of aerosols in a commercial layer house in South Africa, airborne particle concentration in the packing/grading area (0.02-0.05)mg/m^3 was significantly lower than that in the henhouse (0.1-0.15)mg/m^3.[78] In this study, the total bacterial counts in the henhouse were in the ranged from10^4-10^5 CFU/m^3.

A study in pullet facility found airborne dust concentrations ranging from 0.5 to 1.95 mg/m^3.[79] The study also determined that skin squames accounted for the majority of the small particles, followed by a lesser amount of down, feather fragments, food and fecal debris. Total aerobic bacteria, mold/yeast, coliform and pseudomonas levels were measured in offline, inline and mixed commercial shell egg operations in the U.S. by Northcutt *et al*.[53] The study found highest microbial counts in the henhouse followed by the air near the egg washer and dryer. Egg storage coolers had the lowest microbial counts.

10.1.3 Aerosols and Avian Influenza Virus

Avian influenza (AI) viruses are released in the nasal secretions and feces of infected birds.[4,5,23] Studies generally agree that cool, moist conditions in the range of 20-50 percent humidity favor the survival of AI viruses in the environment (reviewed in Sattar and Ijaz, 1987).[69] Influenza aerosols generated by sneezing or coughing are most stable in

low relative humidity, and infection with these lipid-enveloped viruses occurs most frequently during the winter.[73]

10.1.3.1 Experimental avian influenza transmission studies

Several experimental studies indicate that airborne transmission of HPAI infection in chickens is possible but inefficient.

- HPAI H5N1 virus was transmitted via aerosol from geese to quail housed 0.3 m away, but not from geese to chickens.[80]
- HPAI H5N1 virus was not transmitted between chickens in adjacent cages; authors concluded that the fecal-oral route was the primary mode of transmission.[16]
- HPAI H5N2 virus from infected chickens was not transmitted to sentinel chickens in another corner of the room.[81]
- Airborne transmission of HPAI H5N1 occurred inefficiently when 1-2 chickens were infected, but efficiently when 4-8 chickens were infected.[82]
- Air-borne transmission of LPAI H5N2 virus was demonstrated experimentally.[83]
- Aerosolized LPAI H3N2 virus was efficiently transmitted to guinea pigs at 5°C and 20°C in relative humidity of 20-50 percent.[84]

10.1.3.2 Aerosol sampling in HPAI outbreaks

- High volume air sampling was conducted in and near an infected layer flock where birds experienced a high mortality during the HPAI H7N7 outbreak in Canada.[85] Inside the barn, air sampler detected a viral titer 292 $TCID_{50}/m^3$.[w] Air sampling at a command post outside the barn showed a much lower viral load of 12.5 $TCID/m^3$. One virus positive air sample was also collected 800 m downwind of the infected farm, although the virus concentration was very low.
- Five of 6 air samples taken 3-6 meters downwind of H5N2 affected flocks on 6 premises were virus positive; one of 12 air samples taken 45-85 meters downwind of affected flocks on 8 premises was virus positive (Brugh and others, unpublished data described in Brugh and Johnson, 1986).[86]

10.1.4 Relevant Eggshell Contamination Studies

DeReu *et al*. found that the aerobic flora and gram negative bacteria on eggshell surfaces were lower on eggs collected at the packing station compared with those collected at the candling area.[87] Similarly, Musgrove *et al.* found that microbial populations decreased with egg washing and remained low throughout packaging.[88] These studies suggest that there is no significant increase in the shell egg microbial counts after washing and sanitizing via aerosol or other pathways.

Lu *et al*.[89] studied LPAI H7N2 infections in two broiler breeder farms in Pennsylvania. In this study virus could be isolated from roughly 90 percent of chicken swabs and 50 percent of dust and manure swabs; however it was not isolated from 120 eggshell swabs.

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

These results suggest that cross-contamination of eggs via aerosols may not result in significant contamination of eggs.

10.2 Evaluation of Risk

a) Experimental transmission studies indicate that aerosol transmission between chickens housed in adjacent cages is possible but inefficient depending on the virus strain. In addition, the studies suggest that the fecal-oral route is the primary means of transmission for the HPAI H5N1 strains in chickens.

b) We derived a very conservative upper bound on the amount of infectivity that might be transferred onto an eggshell via aerosols in a contaminated packing room air as follows. We assumed that it takes less than one minute for eggs to be packed after completion of washing and sanitizing. Given studies reporting that that more than 97 percent of aerosol particles in a henhouse are smaller than 5 μm, it is reasonable to assume a settling velocity of less than 0.048 m/minute (see Table 7). If we assume that the particles settle over the entire surface area of an egg (0.07 m^2 , Rahn and Paganelli 1989)[90] then particles in a 0.00035 m^3 volume of air might settle on the egg. Even if we assume the packing room has a viral concentration found in an HPAI infected barn experiencing a high mortality (292 $TCID_{50}/m^3$), the expected concentration of virus settling on the eggshell via aerosols is only 0.1$TCID_{50}$/eggshell or approximately 1 EID_{50}/eggshell.[x] We note that this viral titer was estimated from air within an HPAI-infected layer house with birds dying at a rapid rate; the viral titer in the egg packing room would be significantly lower. Furthermore, given active surveillance with RRT-PCR testing, we would expect HPAI infection to be detected before a significant portion of the flock is infected.

Considering the relatively inefficient transmission of H5N1 virus from birds housed in adjacent cages in experimental studies, the upper bound estimates on the amount of HPAI virus transferred onto eggshells via aerosol contamination after packing, and the studies showing no increase in bacterial counts on the eggshell from the candling to the packing station, we conclude that the magnitude of cross-contamination of eggshells after sanitizing via aerosols would be extremely small, and the risk that this contamination would lead to infection of susceptible poultry is negligible.

10.3 Conclusion

Cross-contamination of washed and sanitized eggs via aerosols is theoretically possible from the packing room into the henhouse in naturally ventilated henhouses without positive airflow. Based on conservative upper bounds derived from HPAI outbreak data and experimental studies of shell egg microbial quality at various points in egg

[x] For Canadian HPAI H7N3 a dose of 1 TCID is approximately equal to 10 $EID_{50,}$ personnel communication Dr Swayne.

processing, we conclude that the risk of recontamination of eggshells after sanitizing via aerosols leading to infection of susceptible poultry is negligible.

11. Risk that HPAI virus from Washed and Sanitized Eggs Infects Susceptible Poultry

This portion of the risk assessment describes the risk that washed and sanitized eggs transported from an infected but undetected premises infect susceptible poultry on another premises.

Risk that HPAI virus from Washed and Sanitized Eggs Infects Susceptible Poultry

• **Risk Factors:** Personnel contacting contaminated eggs or egg contents. Personnel traffic between henhouse and egg processing rooms. Spilling of liquid egg during handling. Pests entering the processing rooms.

• **Current Preventive measures:** FSIS requirements for egg processing facilities outlined in 9CFR590. FDA Good Manufacturing Practices (GMPs) outlined in 21CFR110.80. Industry practice of segregation of duties to have personnel dedicated to working in the henhouse or in egg processing areas.

• **Additional Preventive measures** (to be implemented by industry during an outbreak)**:** None needed

• **Overall Risk:** Negligible when washed and sanitized eggs are transported to locations without poultry on the premises. Low when washed and sanitized eggs are transported to locations with poultry on the premises.

11.1 Background Information

In this chapter, we evaluate the risk that susceptible poultry are infected with HPAI due to contaminated washed and sanitized eggs from an infected but undetected layer flock. For this risk to occur, contaminated shell eggs must first be transported to a facility where susceptible poultry are present.

Most shell eggs are distributed directly to locations without poultry on the premises such as retail and food service operations. In this case, there is no plausible pathway by which susceptible poultry are exposed to HPAI virus from the eggs. Industry experts estimate that less than 1 percent of washed and sanitized shell eggs are transported to premises with poultry for breaking or further processing.[91] The analysis in this chapter focuses on the small fraction of washed and sanitized eggs that are transported to premises with nearby poultry. On these premises, eggs must be washed and sanitized again before breaking, a common step in processing the washed and sanitized eggs.

Egg breaking and processing facilities come under FDA and FSIS regulatory measures designed to prevent microbial contamination of products. In this chapter, we evaluate whether these measures could also prevent HPAI virus associated with incoming washed and sanitized eggs from being transmitted to the henhouse.

11.1.1 Handling and Processing of Washed and Sanitized Eggs Transported to an Egg Breaking Facility

FSIS regulation 9CR590.516 requires eggs to be sanitized with 100-200 ppm chlorine or its equivalent immediately prior to breaking. Due to this requirement, incoming washed and sanitized eggs would be sanitized for a second time before breaking. The incoming egg flats would be loaded on to a conveyer system for sanitizing, then cracked or leaking eggs would either be removed for specialized personnel to break separately or handled as inedible eggs as specified in 9CFR590.510. The egg breaking machines are located in a separate room from the transfer/washing processes. After sanitizing, the eggs are transferred to the breaking room by a conveyer belt. Eggs are picked up by the breaking machine, the shell is broken, and the contents of each egg are secured in an individual "cup" for inspection. 9CFR590.522 requires that each egg's contents be evaluated for acceptance before being combined with other eggs for production of liquid egg. The liquid egg produced by the breaking process is screened and collected as streams of whole egg, whites, or yolk. The liquid egg is held in chilled and agitated storage tanks until transfer for further processing either at the breaking location or at an off-site facility. The risk of HPAI spread due to movement of nonpasteurized liquid egg has been addressed in a separate risk assessment.[92]

Given the above process, potential pathways for HPAI virus associated with contaminated eggs to be transmitted into an adjacent henhouse include cross-contamination by personnel or pests, or through aerosol spread. In the following section, we summarize regulatory and industry preventive measures that are relevant to these contamination pathways.

11.2 Preventive Measures

Current preventive measures include Federal regulations 9CFR590 and 21CFR110 and industry sanitary and biosecurity practices.

11.2.1 Regulatory Measures

Regulatory measures considered in this assessment are those specified in 9CFR590 (Egg products Inspection Act) and 21CFR110 (Current Good Manufacturing Practice in manufacturing, packing or holding human food). The USDA FSIS continually inspects egg products processing operations as specified in 9CFR590.24. Inspectors ensure that products are properly processed and that facilities are maintained and operated in a manner that prevents contamination (appendix 2). Daily verification of adherence to standards is documented by the inspector on Form PY203 or Form 159 (see appendices

of nonpasteurized liquid egg risk assessment).[92] Relevant measures from 9CFR590 and 21CFR110 are summarized as follows.

Plant requirements: The shell egg and egg product processing rooms should be designed and maintained in a clean and sanitary condition free from objectionable odors or vapors (9CFR590.500). The shell egg washing and breaking operations should be in separate rooms as stated in 9CFR590.515 (b). Liquid egg storage rooms, including surface coolers and holding tank rooms, should be kept clean and free from objectionable odors.

9CFR590 as well as 21CFR110 have multiple measures to prevent pests from entering the processing rooms. The processing rooms are required to be clean and free of flies, insects, rodents, refuse, odors and waste materials and from any conditions which may engender insects and rodents. Egg processing rooms are required to have a positive air pressure system relative to the henhouse,[y] and must have openings that prevent the entrance of flies or other insects (9CFR590.520(d)(1); 9CFR590.500). Doors and windows leading into rooms where edible product is processed are required to be of solid construction and fitted with self closing devices (9CFR590.500).

Equipment specifications: All utensils and food contact surfaces of equipment should be cleaned as frequently as necessary to protect against contamination of food (21CFR110.35). Tanks, vats and their covers must be of approved construction and operated under sanitary conditions (9CFR590.532). Surface coolers and liquid holding vats containing product must be kept covered while in use (9CFR590.530).

Personnel: 9CFR590 and 21CFR110 both require the personnel handling egg products or product contact surfaces to maintain their hands in a clean condition with thorough washing with soap after each absence from their workstation and whenever the hands become soiled or contaminated (21CFR110.10; 9CFR590.504; 9CFR590.522). Gloves, if used, should be maintained in a clean and sanitary condition (21CFR110.10)

Breaking Room Operations: 9CFR590 requires regular cleaning and sanitizing of all equipment and utensils used in the egg breaking process. Specifically, all non-mechanical breaking equipment such as trays and knives should be cleaned and sanitized every two and half hours. Belt type shell egg conveyors, mechanical egg breaking equipment and pumping system equipment (except for pipelines) should be cleaned and sanitized every 4 hours (9CFR590.522). The breaking room floor should be kept clean, dry and free of egg meat and shells (9CFR590.522).

The breaking room personnel should have easy access to hand washing facilities with supply of soap, clean towels. The hand washing facilities should be operated by other than hand operated controls 9CFR590.520. Liquid egg containers should not pass through the candling room.

[y] Federal regulations require this direction of airflow in blending and packaging rooms for pasteurized products as well as breaking rooms.

11.2.2 Industry Preventive Measures

These are preventive measures commonly practiced in the egg products industry in addition to the regulatory requirements.

Industry experts stated that most inline egg processing plants implement segregation of duties to have personnel dedicated to working in specific portions of the plant such as egg processing plants.[93]

The majority of egg processing operations also have additional sanitary measures and environmental testing. A survey found that more than 80 percent of egg processing plants perform mid-shift cleanups (with chlorine as the sanitizer), inspect nonproduct contact zones daily and conduct additional environmental microbial testing. These additional measures are intended to reduce microbial contamination by pathogens such as *E. coli*, *Salmonella enteritidis* and *Listeria monocytogenes*.[94]

11.3 Evaluation of Risk

We evaluated the risk that susceptible chickens are infected with HPAI virus from washed and sanitized eggs transported to an inline breaking facility in two parts.

a) Risk that pre-breaking handling of washed and sanitized eggs results in HPAI infection of susceptible chickens.

b) Risk that the breaking process and post-breaking handling of washed and sanitized eggs result in HPAI infection of susceptible chickens.

11.3.1 Risk that Pre-breaking Handling of Washed and Sanitized Eggs Results in HPAI Infection of Susceptible Chickens

For this risk to occur HPAI virus from the egg contents or the surface of washed and sanitized eggs must be transmitted into the henhouse via personnel or pests. We evaluated the risks associated with the HPAI virus on the shell surface or in the contents of eggs separately as follows.

11.3.1.1 Risk that Pre-breaking Handling of Eggs Results in Infection of Susceptible Chickens from HPAI Virus on the Eggshell

(i) Cross-contamination via personnel

The pre-breaking processing such as sanitizing, candling and transfer of eggs is usually mechanical. Consequently, the proportion of eggs that personnel contact directly is relatively small. Personnel might contact cracked or leaking eggs to separate them for manual breaking or processing as inedible eggs. Data on the leakage rate of HPAI virus contaminated eggs is unavailable to date. A recent expert opinion panel[z] indicated that HPAI virus contaminated eggs with no visual defects would probably be 2 to 5 times

[z] Details included as Appendix 6.

more likely to leak relative to virus-free eggs. Lederer *et al.*[95] reported a 0.002 probability that uninfected shell eggs leak during transportation. Considering the expert opinion, it is reasonable to assume a 0.01 as the probability of leakage for contaminated eggs. Given that we expect 11(90 percent probability interval 0-44) contaminated eggs from a flock of 100,000 layers, 0.11 (98 percent probability interval 0-1) contaminated eggs would leak. We conclude that the likelihood of personnel contacting the shell surface of contaminated eggs is low.

As described in section 11.2.2, inline operations usually implement segregation of duties; personnel are dedicated to working in specific portions of the plant. During an HPAI outbreak there will be increased awareness in the implementation of biosecurity measures for personnel. We conclude that the likelihood of personnel transmitting the HPAI virus into the henhouse is very low.

(ii) Cross-contamination via pests

As described in section 11.2.1, 9CFR590 as well as 21CFR110 codify multiple measures to prevent pests from entering the processing rooms. The egg processing rooms are to be clean and free of flies, insects, rodents, refuse, odors and waste materials and from any conditions which may engender insects and rodents. Furthermore, pests such as flies are less likely to enter egg cooler rooms due to the lower temperature. We conclude that the likelihood of pests transmitting HPAI virus into the henhouse is very low.

(iii) Amount of exposure and dose response.

The residual HPAI infectivity on a contaminated egg post-sanitizing is estimated to be small (0.6 log EID_{50}/eggshell). We assumed that half of the residual infectivity on a sanitized egg (0.3 log EID_{50}) is a conservative estimate of exposure to susceptible chickens through pathways such as cross-contamination via personnel or pests as discussed above. Based on an exponential dose response model (details provided in Appendix 4), the expected probability of infection in a chicken exposed through the oral route is 0.0035 (90 percent P.I. 0.0004 -.001) percent. Note that the incoming washed and sanitized eggs will be sanitized a second time immediately prior to breaking (9CFR590). This second sanitizing step at the breaking facility would reduce the viral titer on the eggshell to insignificant levels.

Overall, considering the low probabilities of personnel or pests contacting the contaminated eggs and transmitting the virus into the henhouse, and the low chance of infection in an exposed chicken, we conclude that the risk that susceptible chickens are infected with HPAI virus present on the eggshell surface due to pre-breaking handling is negligible.

11.3.1.2 Risk that Pre-breaking Handling of Eggs Results in Infection of Susceptible Chickens from HPAI Virus Contaminated Egg Contents

During pre-breaking handling, personnel may come in contact with contaminated egg contents while removing leaking eggs from the mechanical processing stream. From

section 11.3.1.1, the expected number of contaminated eggs that leak is 0.11(98 percent probability interval 0-1). Therefore, we conclude that the likelihood of personnel contacting the egg contents before breaking is low. Considering the segregation of duties discussed in section 11.2.2, the likelihood of personnel transmitting the HPAI virus into the henhouse is low.

The estimated mean HPAI viral titer within the contents of contaminated eggs ($10^{4.5}$ EID_{50}/ml) represents a relatively high degree of infectivity to chickens (section 8.2.1.2). Our dose response model predicts that the probability of infection in a chicken orally exposed to 0.1 ml of contaminated egg contents is 0.88 (90 percent probability interval 047-.999).

Although the chance of infection in a chicken exposed to HPAI virus contaminated egg contents is high, after considering the low probabilities of personnel contacting the contaminated eggs and transmitting the virus into the henhouse, we conclude that the risk that susceptible chickens are infected with HPAI virus in egg contents due to pre-breaking handling is low.

11.3.2 Risk that Susceptible Chickens are Infected with HPAI Virus from Washed and Sanitized Eggs due to Breaking and Post-breaking Handling Processes

We focus on the risks associated with HPAI virus within the egg contents in this section. As described in section 11.1.1, the risk associated with HPAI virus on the eggshell is negligible due to sanitizing for the second time before eggs are presented for breaking. The risk associated with the movement of nonpasteurized liquid egg was addressed in a separate assessment and found to be negligible.[92] The risks associated with the movement of byproducts of the breaking processes such as eggshells and inedible eggs will be addressed in forthcoming risk assessments.

We considered the risk that HPAI virus within the egg contents is transmitted into the henhouse via personnel and pests. Although there is a hypothetical exposure pathway that HPAI virus is transmitted into the henhouse through aerosols generated in the breaking process, we do not evaluate this pathway. Experimental studies suggest that aerosol spread of HPAI H5N1 virus is inefficient even between chickens housed in adjacent cages (see chapter 10). Moreover, the breaking process generates a very small amount of aerosols (255 μg of egg protein/m^3 of air in plants processing roughly 75,000 eggs per hour).[96] The quantity of aerosols generated by our estimated 11 (90 percent probability interval 0-44) HPAI virus contaminated eggs would be insignificant.

11.3.2.1 Likelihood that Personnel Indirectly Transmit HPAI Virus from Contaminated Egg Contents to Susceptible Hens

The conveyance and breaking process of eggs is usually mechanical. The liquid egg produced in the breaking process is usually screened and transferred to agitated and chilled storage tanks via enclosed steel pipes. The likelihood of personnel contacting contaminated egg contents in this process is low. If a contaminated egg is cracked or

leaking,[aa] there is a possibility that personnel will contact the egg contents during manual breaking. The expected number of leaking contaminated eggs is 0.11 (98 percent probability interval 0-1).

As discussed in section 11.2.1, personnel working in the breaking room are required to maintain their hands in a clean condition with thorough washing. Furthermore, it is a plant requirement that breaking room personnel be provided easy access to hand washing facilities with odorless soap. As discussed in section 9.3.2 hand washing with soap has been found to be very effective in inactivating Influenza A viruses (3-log inactivation). We conclude that if the hand washing requirements in 21CFR110.10, 9CFR590.504 and 9CFR590.522 are strictly enforced, the likelihood of viable HPAI virus remaining on the hands of personnel is low.

Finally, due to the segregation of duties described in section 11.2.2 (personnel are dedicated to working in specific portions of the plant), we conclude that the likelihood of personnel transmitting HPAI virus from contaminated egg contents into the henhouse is low.

Considering the low likelihood of personnel contacting the contaminated egg contents, the hand washing requirements and the segregation of duties, the likelihood of personnel transmitting HPAI virus from contaminated egg contents into the henhouse is low.

11.3.2.2 Likelihood that Susceptible Chickens are Exposed to HPAI Virus from Contaminated Eggs via Pests

The FSIS regulations require egg breaking premises to be free of materials and conditions which constitute a source of odors or a harbor for insects, rodents, and other vermin. In addition, the doors and openings of the processing rooms are required to be of solid construction and fitted with self closing devices. 9CFR590 also requires cleaning and sanitizing of processing equipment every 4 hours. The breaking room floor is required to be free from egg contents and shells.

Further requirements in 21CFR110 contain several sanitary measures to avoid pests in any food processing facilities. In addition, given the continuous inspection of egg breaking operations, the likelihood of pests in the processing and storage areas is low. We conclude that the likelihood of pests transmitting the virus from contaminated egg contents in the processing room to the henhouse is negligible.

11. 4 Conclusion

Most shell eggs are destined for locations without poultry on the premises such as retail, food service or offline processing facilities. We conclude that the risk that a susceptible flock is infected via HPAI virus from washed and sanitized eggs that are moved to locations without poultry is negligible.

[aa] According to USDA FSIS, any egg with a portion of the shell or egg membranes missing is classified as a "leaker" regardless of whether the egg contents exude outside the shell.

Less than 1 percent of washed and sanitized eggs are estimated to be transported to breaking or further processing facilities. For the scenario where washed and sanitized eggs are transported to egg breaking facilities with adjacent poultry, assuming that the preventive measures in 9CFR590 and 21CFR110 are strictly enforced, we conclude the following:

- The risk of a susceptible flock being infected from HPAI virus on the shell surface of contaminated eggs is negligible.
- The risk of a susceptible flock being infected from HPAI virus originating from the contents of contaminated eggs is low.

The determination of "low risk" suggests that additional resources to further evaluate or mitigate this risk may be considered depending on circumstances, although this is not a strict requirement (see Appendix 13).

12. Risk of HPAI Spread to Another Poultry Premises via Vehicle or Driver Transporting Washed and Sanitized Shell Eggs

In this chapter, we evaluate the risk of HPAI spread to other poultry premises via cross-contamination from the vehicle or the truck driver transporting the shell eggs.

Risk of HPAI Spread to Another Poultry Premises via Vehicle or Driver Transporting Washed and Sanitized Shell Eggs

• **Risk Factors:** Cross-contamination of transport vehicle, inadequate cleaning and/or disinfection, and failure of biosecurity practices for personnel.

• **Current Preventive Measures:** 7CFR56 regulations applicable for operations participating in the AMS voluntary shell egg grading program; 9CFR590 regulations primarily applicable to eggs used for breaking or further processing; industry GMPs.

• **Additional Preventive Measures** (to be implemented by industry during an outbreak; see Appendix 14 for suggested protocols)**:** Cleaning and disinfection procedures for the truck exterior and interior and biosecurity requirements as described in UEP/UEA – USDA APHIS VS Movement Control Model Plan; active surveillance protocol which includes RRT PCR testing of swabs from 5 randomly selected birds among daily mortality;

• **Overall Risk:** Negligible if washed and sanitized shell eggs are transported to premises without poultry; Low if washed and sanitized shell eggs are transported to premises with poultry.

A review of the scientific literature and response planning documents indicate that movements of contaminated equipment, vehicles and personnel between poultry premises is the primary means of spreading HPAI.[97,98] Feed and rendering vehicles were associated with elvated risk of spread of AI virus due to their movement among poultry farms and congregation at common facilities;[98] however, there have been no reports of vehicles transporting shell eggs causing infection at another poultry premises.[24,99-102] Vehicles transporting eggs from processing facilities use different docking areas with a greater separation from the henhouse compared to the docks used by henhouse operations and related vehicles such as feed and rendering trucks.

Washed and sanitized shell eggs may be destined for foodservice, retail marketing, further processing or breaking. Most of these destinations do not have poultry on the premises. Industry experts estimate that less than 1 percent of shell eggs may be transported to locations with poultry on the premises (e.g., inline breaking plant).[58]

Nevertheless, the potential risk of cross-contamination when vehicles transport shell eggs to a facility with poultry on the premises needs to be evaluated. Existing preventive measures include Federal regulations for AMS regulated shell egg grading facilities or FSIS regulated breaking facilities, and industry GMPs. In the event of an HPAI outbreak, various movement and control measures will be implemented by various State and Federal authorities as well as by the egg industry.

In this chapter, we evaluate the existing and planned preventive measures for their ability to reduce the risk of HPAI virus spreading from the vehicle or driver to susceptible poultry.

12.2 Preventive Measures

12.2.1 Current Preventive Measures

Current preventive measures are based on regulatory requirements described in 9CFR590 (FSIS requirements for breaking facilities) and 7CFR56 (AMS requirements for participants in the voluntary shell egg grading program) as summarized below:

a) 7CFR56.76 requires outside premises to be free from refuse, rubbish, waste, unused equipment, and other materials and conditions which constitute a source of odors or a harbor for insects, rodents, and other vermin. Furthermore, the outside premises adjacent to grading, packing, cooler, and storage rooms must be properly graded and well drained to prevent conditions that may constitute a source of odors or propagate insects or rodents.

b) 9CFR590.50 requires that containers and packaging materials in which shell eggs are received into an official processing plant be free of odors.

c) Both 9CFR590.50 and 7CFR56 require shell eggs to be transported in refrigerated containers when shipping to the final customers.

12.2.2 Preventive Measures during an Outbreak

In the event of an HPAI outbreak, various movement control measures will be implemented by local, State and/or Federal authorities as part of the disease control effort.[97 103-105] Vehicle cleaning and disinfection procedures are included in these measures.

APHIS and the UEP recognize the potential risk from vehicles or conveyances and the importance of reducing this risk. The UEP/UEA – USDA APHIS VS Movement Control Model Plan thus instigates regulation of the movement of vehicles transporting eggs during an outbreak (Appendix 8).[106]

The UEP/UEA – USDA APHIS VS Movement Control Model Plan includes cleaning and disinfecting all vehicles transporting shell eggs that move into,[bb] within or outside of

[bb] Vehicles carrying egg products into a control area will be subject to cleaning and disinfection when they arrive at their delivery points.

a control area. The cargo interior and exterior of the movement vehicle must be cleaned before a permit is issued. In addition, tires and wheel wells of vehicles moving shell eggs must be cleaned and disinfected before leaving premises within a control area. The driver will not be allowed outside of the cab or the cab interior must also be cleaned and disinfected. Cleaning and disinfection requires the use of an EPA approved disinfectant[107] with efficacy against AI virus[cc] following a standard protocol that will require vehicle interior and exterior cleaning and disinfection. The movement control plan does not require vehicles to be cleaned and disinfected on the premises, but it does require that cleaning and disinfection be done before a movement permit is issued.

Additional protocols for cleaning and disinfection exist and new ones may need to be developed depending on the circumstances. For example, vehicle cleaning and disinfection guidelines are given in the November 2005 Draft National Animal Health Emergency Management System (NAHEMS) Cleaning and Disinfection Operational Guidelines from the USDA,[104] and general biosecurity guidelines are given in the April 2005 Draft National Animal Health Emergency Management System (NAHEMS) Biosecurity Operational Guidelines from the USDA.[105] Other cleaning and disinfection and biosecurity guidelines can be found in published literature.[108-110]

12.3 Evaluation of Risk

The potential risks associated with the transportation of shell eggs are:

a) Risk of the vehicle transporting shell eggs being contaminated with HPAI virus.

b) Risk of the driver being contaminated with HPAI virus.

c) Risk of cleaning and disinfection not inactivating the HPAI virus on the vehicle.

d) Risk that susceptible poultry are exposed to HPAI virus via contaminated vehicle or driver.

12.3.1 Risk of the Vehicle Transporting Shell Eggs being Contaminated with HPAI Virus

Potential pathways through which a vehicle may become contaminated include aerosol, flies, and through shoes or clothes of personnel while loading.

(i) Aerosol

Aerosol transmission is a possibility for inline shell egg operations with poultry on the premises. As discussed in chapter 10, aerosol transmission of HPAI virus is inefficient in chickens and becomes increasingly inefficient with distance from the infective source.[16,80,81] A study of air quality 3-6 m downwind of HPAI H5N2 infected flocks found that a majority of the samples taken with large volume air sampling (30,000 liters) were positive. Air sampling during an HPAI outbreak in Canada found a substantial

[cc] The EPA web page referenced in the text states the following: "Although there are no antimicrobial products registered specifically against the H5N1 subtype of **avian influenza A** viruses, EPA believes based on available scientific information that the currently registered **avian influenza A** products, when applied in strict accordance with the label directions, will be effective against the H5N1 strain."

reduction (about 2-log) in the virus concentration in the air at a command post outside the barn compared to air inside the barn where birds were dying rapidly. Overall, both these studies indicate that the frequency and concentration of HPAI virus recovery in the air decreases with distance from the henhouse.[86] Vehicles transporting eggs from processing facilities use different docking areas with greater separation from the henhouse compared to the docks used by henhouse operations related vehicles such as feed and rendering trucks, etc. Furthermore, the shell egg related docks are usually separated from the henhouse by multiple rooms such as the cooler, washing room, etc. Considering the greater separation from the henhouse and the relative inefficiency of aerosol transmission, we conclude that the amount of infectivity transferred onto trucks transporting shell eggs via aerosolized HPAI virus is low.

(ii) Flies

We have found no direct evidence showing that avian influenza can be transmitted via flies. Transmission through flies should be considered a possibility, however, as HPAI virus was isolated from flies near infected henhouses during the 1983-84 HPAI H5N2 outbreaks in Pennsylvania. Two studies isolated HPAI virus from approximately 1-5 percent of flies[dd] collected near severely infected henhouses.[86,111] In these studies, the amount of infectivity from virus-positive flies was not quantified.

The risk of HPAI spread via contaminated flies traveling in or on the shell egg transportation vehicle is pertinent to the small proportion of cases (less than 1 percent) where washed and sanitized shell eggs are transported from an inline facility with poultry to another inline processing facility. We believe that the risk is low for shell egg transportation as the washed and sanitized eggs are usually transported in enclosed refrigerated trailers which are unlikely to attract flies due to the colder temperature (typically 45°F). Additionally, our disease transmission and surveillance models predict that infection is likely detected before a significant proportion of the flock (90 percent probability interval 0.02-5.4 percent) is infected. Therefore, we expect the frequency of HPAI contamination of flies to be lower when the active surveillance protocol is followed compared to that observed near severely infected flocks. We conclude that the risk of HPAI spread via flies contaminating the interior of the vehicle transporting shell eggs is low.

(iii) Cross-contaminated from personnel and equipment while loading shell eggs

If personnel loading the eggs (driver or staff) walk across areas contaminated with HPAI virus they may carry virus on their shoes or clothes and cross-contaminate the interior of the truck. The pallet mover may be contaminated the same way. Inline operations usually have workers dedicated to working in specific portions of the plant such as the henhouse or the processing area, etc. For these operations, the likelihood that the floor of the cooler area is cross-contaminated with virus from the henhouse would be low. In offline operations, the loading dock and the cooler might get contaminated from egg packaging

[dd] The virus recovery frequency reported in Wilson et al 1984 is for pooled samples of 10-60 flies. Based on the HPAI virus recovery rate in pooled samples and the size of the insect pools, we estimated the virus recovery rate in individual flies to be approximately 1 percent.

materials while unloading incoming nest run eggs, depending on the level of biosecurity-practices at the offline farm. In the egg movement control protocol, nest run eggs are not allowed to move to an inline operation; contamination from outside eggs of an inline dock and cooler where washed and sanitized eggs are stored is thus prevented. We conclude that the amount of infectivity transferred to the truck interior via cross-contamination from personnel loading the truck is low.

The risk of contamination of the vehicle by the above pathways is lowered further when the active surveillance protocol is followed, as our surveillance models predict that infection would be detected before a significant proportion of the flock is infected (90 percent probability interval 0.04-5.4 percent).

12.3.2 Risk of the Driver of the Vehicle Transporting Shell Eggs being Contaminated with HPAI Virus

Drivers moving shell eggs generally do not have direct contact with live bird production and thus have limited opportunity to become contaminated with HPAI virus. As described in the previous subsection, there might be a low risk that the driver's shoes or hands are contaminated from the floor of the egg storage area if the driver loads the eggs. The Model Cleaning and Disinfection guidelines described in UEP/UEA –USDA APHIS VS Movement Control Model Plan require the driver to wear protective clothing and boots while outside the cab and remove them immediately before reentering the cab. The Incident Command and movement permitting system provide for review of and compliance with these procedures as well as industry biosecurity measures already in practice. The risk that the driver carries HPAI virus off the premises may be effectively mitigated if such PPE or biosecurity guidelines are followed. The UEP/UEA – USDA APHIS VS Movement Control Model Plan also requires the cab interior be cleaned and disinfected if the driver steps out of the cab.

12.3.3 Risk of Cleaning and Disinfection not Inactivating the HPAI Virus on the Vehicle

The UEP/UEA – USDA APHIS VS Movement Control Model Plan contains provisions for cleaning and disinfection during an outbreak. These plans are similar to those developed to control the Exotic Newcastle Disease (END) outbreak in California and were found to be effective in that situation. The movement control plan requires cleaning and disinfection of the cargo interior, exterior, tires and wheel wells of the transportation vehicles. An EPA registered disinfectant against avian influenza should be used while disinfecting the trailer interior. Similarly, other relevant guidelines such as the NAHEMS guidelines[104] also address the cleaning and disinfection of vehicles in detail.

As discussed in section 11.3.3.1, we expect the amount/frequency of HPAI virus contamination of vehicles transporting shell eggs to be low. Given that several disinfectants and detergents can inactivate avian influenza virus, we conclude that the risk of HPAI virus on a cleaned and disinfected vehicle is also low.

12.3.4 Risk that Susceptible Poultry are Exposed to HPAI Virus via a Contaminated Vehicle or Driver

As mentioned earlier, most washed and sanitized shell eggs are transported in closed vehicles to end customers and other locations without poultry on the premises. Industry experts estimate that more than 99 percent of shell eggs are transported to locations that do not have poultry on the premises.[91] In these cases, there is no clear pathway for exposure to susceptible poultry.

Let us consider the 1 percent of the cases where washed and graded shell eggs are moved to breaking and other processing plants that might have poultry on the premises. Egg breaking plants are required to be under continuous FSIS inspection and come under additional sanitary measures as stated in 9CFR590. A survey of 77 egg processing plants found that 82 percent of the plants inspected nonproduct contact zones daily and more than 90 percent have cleanup shifts and mid-shift cleanups.[94] Also, 73 percent of the plants perform environmental microbial testing. These additional precautions at processing plants are mostly aimed at reducing bacterial contamination with pathogens such as *Salmonella*, *E. coli* and *Listeria monocytogenes*. Egg breaking or processing plants with poultry on the premises usually have workers dedicated to working in specific areas of the plants such as the processing area or the henhouse and thus have limited potential for cross-contamination of the henhouse via personnel.

From the above discussion, we conclude that the risk that a susceptible flock would be exposed to shell eggs via the transportation vehicle or driver is low.

In summary, the risk that shell egg transportation vehicles are contaminated with HPAI virus from the premises of origin is low, and the amount of infectivity on the vehicle due to cross-contamination is also expected to be low. The cleaning and disinfection protocols described in the UEP/UEA – USDA APHIS VS Movement Control Model Plan would likely inactivate any possible HPAI virus from cross-contamination from the premises. Considering this information, we conclude that in the situation where shell eggs are transported to locations without poultry (99 percent of shell egg transportation), the overall risk that a susceptible flock is infected due to cross-contamination via the vehicle or driver transporting the shell eggs is *negligible* provided the cleaning and disinfection guidelines specified in the UEP/UEA – USDA APHIS VS Movement Control Model Plan are followed.

In the situation where shell eggs are transported to a location with poultry on the premises (1 percent or less of shell egg transportation), we conclude that the overall risk that a susceptible flock is infected due to cross-contamination via the vehicle or driver transporting the shell eggs is *low*.

12.4 Conclusion

Most shell eggs are transported to locations without poultry on the premises. The probability that vehicles carrying shell eggs are contaminated with HPAI virus from infected but undetected premises is low. The cleaning and disinfection protocols

described in the UEP/UEA – USDA APHIS VS Movement Control Model Plan would effectively inactivate HPAI virus on the truck. The risk that the movement of a vehicle carrying shell eggs would mechanically transmit HPAI virus and result in infection of a susceptible flock is *negligible* if the eggs are transported to locations without poultry and applicable guidelines present in the UEP/UEA – USDA APHIS VS Movement Control Model Plan are followed.

If shell eggs are transported to a location with poultry located on the premises, the overall risk that a susceptible flock is infected due to cross-contamination via the vehicle or driver transporting the shell eggs is *low.* The determination of "low risk" suggests further evaluation of this risk onsite or additional resources to mitigate this risk may be considered depending on circumstances, although this is not a strict requirement (see Appendix 13).

13. Summary

The objective of this risk assessment was to evaluate the risk that the movement of shell eggs during a highly pathogenic avian influenza (HPAI) outbreak in the poultry egg industry in the United States will result in HPAI infection on another poultry premises. The assessment is applicable to all eggs processed under Federal regulations, State regulations, or industry-generated protocols that are equivalent to 7CFR56.76(f) parts (3) and (11),[ee] which specify the temperature of the water used for washing and sanitizing, and mandate the concentration of sanitizer (chlorine or equivalent) to be not less than 100 ppm or more than 200 ppm. Additionally, layer farms included in this risk assessment must be participants in the active surveillance protocol to be implemented by industry in conjunction with APHIS during an outbreak.

To estimate the risk of HPAI spread to susceptible poultry via contaminated shell eggs from an infected but undetected premises, we first estimated the maximum daily number of contaminated eggs from an infected but undetected premises. The HPAI prevalence in an infected flock changes over time, increasing exponentially until detection. The number of contaminated eggs from an infected but undetected flock thus varies with the time to disease detection (i.e. the level of surveillance effort). Our simulation models predicted that the number of internally contaminated shell eggs that may be moved from the premises before infection is detected is 11 (90 percent probability interval 0-44) when the active surveillance protocol described in the UEP/UEA – USDA APHIS VS Movement Control Model Plan is followed. Using published literature, we utilized $10^{4.5}$ EID_{50}/ml as the viral titer in the internal contents of HPAI virus contaminated eggs.

Next, we evaluated whether HPAI virus on the surfaces of washed and sanitized eggs would present a risk for HPAI spread. We assumed that the frequency of external contamination of eggs laid by HPAI infected hens is similar to that of internal content contamination as observed in experimental studies. Based upon the disease transmission and the active surveillance models, we estimated that the number of eggs moved per day from an infected but undetected flock that would have been externally contaminated before washing and sanitizing is 11 (90 percent probability interval 0-44). We found that the egg washing and sanitizing procedures as outlined in section 7.2.3 would inactivate HPAI virus on the eggshell surface by a factor of 1,000 (a 3-log reduction). Given the estimated viral titer on the eggshell of $10^{3.6}$ EID_{50}/eggshell, we estimated that the residual HPAI viral titer after washing and sanitizing would be $10^{0.6}$ EID_{50}/eggshell.

We also examined the possibility of recontamination of washed and sanitized eggs via aerosolized HPAI virus. Exposure to aerosols could occur when henhouses are naturally ventilated without positive airflow from the packing room to the henhouse. Based on conservative upper bounds derived from HPAI outbreak data and experimental studies of shell egg microbial quality at various points in egg processing, we conclude that the risk of recontamination of eggshells after sanitizing via aerosols leading to infection of susceptible poultry is negligible.

[ee] See Appendix 1.

Then, we proceeded with an evaluation of risk that susceptible poultry are infected with HPAI virus from washed and sanitized eggs transported from the premises. When shell eggs are transported to retail or food service, there is no plausible pathway for exposure to susceptible poultry. We conclude that the risk of a susceptible flock being infected via HPAI virus from washed and sanitized eggs that are moved to locations without poultry is negligible.

For the scenario where shell eggs are transported to an inline breaking facility, we evaluated the possibility of HPAI virus from washed and sanitized eggs being transmitted into the henhouse via personnel or pests. Assuming the preventive measures in 9CFR590 and 21CFR110 are strictly enforced, we concluded that the risk of a susceptible flock being infected via HPAI virus on the shell surface of contaminated eggs is negligible. We found the risk of a susceptible flock being infected from HPAI virus within the contents of contaminated eggs to be low.

Finally in chapter 12, we evaluated the risk of HPAI spread to susceptible poultry through the vehicle or the driver transporting the shell eggs. We estimated the likelihood of the vehicle being contaminated with HPAI virus via personnel (while loading), pests and aerosol to be low. The cleaning and disinfection protocols described in the UEP/UEA – USDA APHIS VS Movement Control Model Plan would effectively inactivate HPAI virus on the truck. Most shell eggs are transported to locations without poultry on the premises. For this scenario, we concluded that the risk of HPAI spread to susceptible poultry through the vehicle or the driver transporting the shell eggs is negligible. When shell eggs are transported to a location with poultry on the premises, we concluded that risk of HPAI spread to susceptible poultry through the vehicle or the driver transporting the shell eggs is low.

Table 8. Estimates of key variables and probabilities for the risk that movement of shell eggs from an HPAI infected but undetected flock will result in infection of susceptible poultry.

Parameter Description	*Value*
Maximum daily number of internally contaminated eggs that may be moved from infected but undetected premises.	11 (90 percent P.I. 0-44) eggs/day
HPAI viral titer in contents of contaminated eggs.	$10^{4.5}$ EID_{50}/ml
Maximum daily number of eggs moved per day from an infected but undetected premises that would have been externally contaminated before washing and sanitizing.	11 (90 percent P.I .0-44) eggs/day
Degree of HPAI virus inactivation with egg washing and sanitizing operations as specified in 7CFR56.76.	1000 factor or 3-log reduction with 97 percent probability
Residual HPAI viral titer on the shell surface of contaminated eggs after washing and sanitizing.	$10^{0.6}$ EID_{50}/eggshell
The risk that a susceptible flock is infected via HPAI virus from washed and sanitized eggs moved to premises without poultry.	negligible
The risk of susceptible flock being infected via HPAI virus on the shell surface of washed and sanitized eggs moved to premises with poultry.	negligible
Probability of infection in a chicken exposed to 0.1 ml of HPAI contaminated egg contents through the oral route.	0.88 (90 percent P.I. 0.47-0.999)
The risk of susceptible flock being infected via HPAI virus from the internal contents of shell eggs moved to premises with poultry.	low
The risk that a susceptible flock is infected via the vehicle or driver when shell eggs are transported to premises without poultry.	negligible
The risk that a susceptible flock is infected via the vehicle or driver when shell eggs are transported to premises with poultry.	low

14. Overall Conclusions

The objective of this assessment was to estimate the risk that the movement of washed and sanitized shell eggs into, within, and outside of a control area during a highly pathogenic avian influenza outbreak in the poultry industry in the United States will result in HPAI infection of other poultry premises. With respect to the major component risks that were analyzed, this document concludes the following:

a) For an infected but undetected flock following the active surveillance protocol described in UEP/UEA – USDA APHIS VS Movement Control Model Plan,

   i. The number of internally contaminated eggs may be moved per day prior to detection is 11 (90 percent probability interval 0-44).

   ii. The number of eggs moved per day prior to detection that would have been externally contaminated before washing and sanitizing is 11 (90 percent probability interval 0-44).

b) The risk that a susceptible flock is infected via HPAI virus on the shell surface of eggs that are washed and sanitized as specified in 7CFR56.76 is *negligible*.

It is concluded that the overall risk of moving washed and sanitized shell eggs into, within, and outside of a control area during an HPAI outbreak is *negligible* if there are no poultry on the destination premises provided that,

- The active surveillance protocol is followed for the flock.
- The truck cleaning and disinfection guidelines in the UEP/UEA – USDA APHIS VS Movement Control Model Plan are followed.

It is concluded that the overall risk of moving washed and sanitized shell eggs into, within, and outside of a control area during an HPAI outbreak is *low* if there are poultry on the destination premises provided that,

- The active surveillance protocol is followed for the flock.
- The cleaning and disinfection guidelines in the UEP/UEA – USDA APHIS VS Movement Control Model Plan are followed.
- The sanitary measures and good manufacturing practices stated in 9CFR590 and 21CFR110 are implemented.
- Applicable current industry preventive measures described herein are strictly followed.

However, it should be remembered that:

a) This assessment is based on current (August 2009) information and will need to be reviewed and revised as circumstances warrant.

b) The assessment aids, but does not replace, the judgment of on-scene officials.

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Appendix 1. Selected portions of 7CFR56.76

56.76 Minimum facility and operating requirements for shell egg grading and packing plants.

…

(f) Shell egg cleaning operations. (1) Shell egg washing equipment must be sanitarily designed, maintained in a clean and sanitary manner, and thoroughly cleaned at the end of each operating day.

(2) Shell egg drying equipment must be sanitarily designed and maintained in a clean and sanitary manner. Air used for drying purposes must be filtered. These filters shall be cleaned or replaced as needed to maintain a sanitary process.

(3) The temperature of the wash water shall be maintained at 90 °F (32.2 °C) or higher, and shall be at least 20 °F (6.7 °C) warmer than the internal temperature of the eggs to be washed. These temperatures shall be maintained throughout the cleaning cycle. Accurate thermometers shall be provided for monitoring wash water temperatures.

(4) Approved cleaning compounds shall be used in the wash water.

(5) Wash water shall be changed approximately every 4 hours or more often if needed to maintain sanitary conditions, and at the end of each shift. Remedial measures shall be taken to prevent excess foaming during the egg washing operation.

(6) Replacement water shall be added continuously to the wash water of washers. Chlorine or quaternary sanitizing rinse water may be used as part of the replacement water, provided, they are compatible with the washing compound. Iodine sanitizing rinse water may not be used as part of the replacement water.

(7) Only potable water may be used to wash eggs. Each official plant shall submit certification to the national office stating that their water supply is potable. An analysis of the iron content of the water supply, stated in parts per million, is also required. When the iron content exceeds 2 parts per million, equipment shall be provided to reduce the iron content below the maximum allowed level. Frequency of testing for potability and iron content shall be determined by the Administrator. When the water source is changed, new tests are required.

(8) Waste water from the egg washing operation shall be piped directly to drains.

(9) The washing, rinsing, and drying operations shall be continuous and shall be completed as rapidly as possible to maximize conservation of the egg's quality and to prevent sweating of eggs. Eggs shall not be allowed to stand or soak in water. Immersion-type washers shall not be used.

(10) Prewetting shell eggs prior to washing may be accomplished by spraying a continuous flow of water over the eggs in a manner which permits the water to drain away or other methods which may be approved by the Administrator. The temperature of the water shall be the same as prescribed in this section.

(11) Washed eggs shall be spray-rinsed with water having a temperature equal to, or warmer than, the temperature of the wash water. The spray-rinse water shall contain a sanitizer that has been determined acceptable for the intended use by the national supervisor and of not less than 100 p/m nor more than 200 p/m of available chlorine or its equivalent. Alternate procedures, in lieu of a sanitizer rinse, may be approved by the national supervisor.

(12) Test kits shall be provided and used to determine the strength of the sanitizing solution.

(13) During non-processing periods, eggs shall be removed from the washing and rinsing area of the egg washer and from the scanning area whenever there is a buildup of heat that may diminish the quality of the egg.

(14) Washed eggs shall be reasonably dry before packaging and packing.

(15) Steam, vapors, or odors originating from the washing and rinsing operation shall be continuously and directly exhausted to the outside of the building.

Appendix 2. Selected 9CFR References

Bold-type sections are included in this Appendix

Title 9: Animals and Animal Products
PART 590—INSPECTION OF EGGS AND EGG PRODUCTS
(EGG PRODUCTS INSPECTION ACT)

Scope of Inspection

§ 590.20 Inspection in accordance with methods prescribed or approved.
§ 590.22 Basis of service.
§ 590.24 Egg products plants requiring continuous inspection.
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§ 590.550 Washing and sanitizing room or area facilities.
§ 590.552 Cleaning and sanitizing requirements.
§ 590.560 Health and hygiene of personnel.

Appendix 2: Selected portions of 9CFR590 (continued)

Scope of Inspection

§ 590.24 Egg products plants requiring continuous inspection.

No plant in which egg products processing operations are conducted shall process egg products without continuous inspection under these regulations, except as expressly exempted in §590.100.

§ 590.26 Egg products entering or prepared in official plants.

Eggs and egg products processed in an official plant shall be inspected, processed, marked, and labeled as required by these regulations. Egg products entering an official plant shall have been inspected, processed, marked, and labeled as required by these regulations.

Sanitary, Processing, and Facility Requirements

§ 590.504 General operating procedures.

(a) Operations involving processing, storing, and handling of shell eggs, ingredients, and egg products shall be strictly in accord with clean and sanitary methods and shall be conducted as rapidly as practicable. Pasteurization, heat treatment, stabilization, and other processes shall be in accord with this part and as approved by the Administrator. Processing methods and temperatures in all operations shall be such as will prevent a deterioration of the egg products.

(b) Shell eggs and egg products processed in official plants shall be subjected to constant and continuous inspection throughout each and every processing operation. Any shell egg or egg product which was not processed in accordance with these regulations or is not fit for human food shall be removed and segregated.

(c) All loss and inedible eggs or egg products shall be placed in a container clearly labeled “inedible” and containing a sufficient amount of approved denaturant or decharacterant, such as FD&C brown, blue, black, or green colors, meat and fish by-products, grain and milling by-products, or any other substance, as approved by the Administrator, that will accomplish the purposes of this section. Shell eggs shall be crushed and the substance shall be dispersed through the product in amounts sufficient to give the product a distinctive appearance or odor. Notwithstanding the foregoing, and upon permission of the Inspector, the applicant may hold inedible product in containers clearly labeled inedible which do not contain a denaturant if such inedible product is denatured or decharacterized prior to shipment from the official plant: Provided, That such product is properly packaged, labeled, segregated, and inventory controls are maintained. In addition, product shipped from the official plant for industrial use or animal food need not be denatured or decharacterized, provided, that such product is properly packaged, labeled, segregated, and inventory controls are maintained, and that such product is shipped under Government seal and certificate and received at the destination location by an inspector or grader as defined in this part.

(d) The inspector may, prior to receipt of laboratory results for salmonella, or for other reasons such as labeling as to solids content, permit egg products to be shipped from the official plant when he has no reason to suspect noncompliance with any of the provisions of this part. However, such shipments shall be made under circumstances which will assure the return of the product to the plant for reprocessing, relabeling, or under such other conditions as the Administrator may determine to assure compliance with this part.

(e) Pasteurizing, stabilizing, or drying operations shall start as soon as practicable after breaking to prevent deterioration of product, preferably within 72 hours from time of breaking for egg products other than whites which are to be desugared.

(f) Each person who is to handle any exposed or unpacked egg products or any utensils or container which may come into contact with egg product, shall wash his hands and maintain them in a clean condition.

(g) No product or material which creates an objectionable condition shall be processed, stored, or handled in any room, compartment, or place where any shell eggs or egg products are processed, stored or handled.

(h) Only germicides, insecticides, rodenticides, detergents, or wetting agents or other similar compounds which will not deleteriously affect the eggs or egg products when used in an approved manner and which have been approved by the Administrator, may be used in an official plant. The identification, storage, and use of such compounds shall be in a manner approved by the Administrator.

(i) Utensils and equipment which are contaminated during the course of processing any shell eggs or egg products shall be removed from use immediately and shall not be used again until cleaned and sanitized.

(j) Any substance or ingredient added in the processing of any egg products shall be clean and fit for human food.

(k) Packages or containers for egg products shall be of sanitary design and clean when being filled with any egg products; and all reasonable precautions shall be taken to avoid soiling or contaminating the surface of any package or container liner which is, or will be, in direct contact with such egg products. Only new containers or used containers that are clean, in sound condition and lined with suitable inner liners shall be used for packaging edible egg products. Fiber containers used without liners require the approval of the Administrator.

(l) Egg products shall be inspected to determine the wholesomeness of the finished product.

(m) Egg products shall be processed in such a manner as to insure the immediate removal of blood and meat spots, shell particles, and foreign materials.

(n) Utensils and equipment, except drying units, powder conveyors, sifters, blenders, and mechanical powder coolers shall be clean and sanitized at the start of processing operations. Equipment and utensils shall be kept clean and sanitary during all processing operations.

(o) Egg products prior to being released into consuming channels shall be pasteurized in accordance with §590.570 except that dried whites prepared from nonpasteurized liquid shall be heat treated in accordance with §590.575.

(1) To assure adequate pasteurization, egg products shall be sampled and tested for the presence of salmonella. Sampling for the presence of salmonella shall be in accordance with §590.580 and product found to be salmonella positive shall be reprocessed, pasteurized, and analyzed for the presence of salmonella, or denatured.

(2) Nonpasteurized or salmonella positive egg product may be shipped from an official plant only when it is to be pasteurized, repasteurized, or heat treated in another official plant. Shipments of products from one official plant to another for pasteurization, repasteurization, or heat treatment shall be in sealed cars or trucks with an accompanying certificate stating that the product is not pasteurized or is salmonella positive. If nonpasteurized or salmonella positive products are to be stored in other than the official plant facilities, the inspector at the consignee's and consignor's plants shall be given full knowledge of the disposition of the product, including warehouse inventory receipts, until such time as product is pasteurized, repasteurized, or heat treated. The containers of such nonpasteurized or salmonella positive product shall be marked with the identification mark shown in Figure 3 of §590.415.

(3) Notwithstanding the provision of paragraph (o)(2) of this section, nonpasteurized salted egg products containing 10 percent or more salt added may be shipped from an official plant directly to a manufacturer of acidic dressings only under the following provisions:

(i) Before such shipment is made, the manufacturer of the acidic dressing shall apply in writing and receive permission from the Administrator to receive and use unpasteurized egg products. The applicant shall sign a written statement containing the specification for the treatment of the nonpasteurized egg product in a manner that will insure that viable salmonella microorganisms are destroyed, and such processing treatment shall be approved by the Administrator prior to use.

(ii) Product shall be shipped under seal from the official plant, accompanied by an official USDA certificate stating that the product is nonpasteurized and for use in acidic dressings only.

(iii) The applicant shall acknowledge receipt of each shipment by indicating on the reverse side of the USDA certificate. “The quantity of nonpasteurized egg product stated on this certificate was received at _____,” the blank being filled in with the name and address of the receiving company and the date and signature of the person completing the form. The certificate shall be returned to the USDA inspector at the origin plant.

(iv) The acidic dressing manufacturer shall maintain processing records indicating the use of each shipment of unpasteurized salted product and the code lots of acidic dressing into which it was processed. Records of the pH and the acidity expressed as percent acetic acid of each code lot shall be maintained. The records shall also demonstrate that the acidic dressing was held 72 hours prior to shipment. These records shall be maintained for 2 years and shall be available for inspection by a representative of the Department.

(v) Each container of salted egg product shipped from the official plant shall be labeled as required in §590.411, and shall bear the words “Caution—this egg product has not been pasteurized or otherwise treated to destroy viable salmonella microorganisms,” and shall bear the official identification shown in figure 4 of §590.415.

(p) Air which is to come in contact with product or with product contact surfaces shall come from approved filtered outside air sources.

(q) All liquid and solid waste material in the official plant shall be disposed of in a manner approved by the Administrator to prevent product contamination and in accordance with acceptable environmental protection practices.

[36 FR 9814, May 28, 1971, as amended at 37 FR 6658, Apr. 1, 1972; 40 FR 20059, May 8, 1975. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981, as amended at 47 FR 745, Jan. 7, 1982; 60 FR 49170, Sept. 21, 1995]

§ 590.510 Classifications of shell eggs used in the processing of egg products.

(a) The shell eggs shall be sorted and classified into the following categories in a manner approved by the National Supervisor:

(1) Eggs listed in paragraph (d) of this section.

(2) Dirty.

(3) Leakers as described in paragraph (c)(2) of this section.

(4) Eggs from other than chicken; duck, turkey, guinea, and goose eggs.

(5) Other eggs—satisfactory for use as breaking stock.

(b) Shell eggs having strong odors or eggs received in cases having strong odors shall be candled and broken separately to determine their acceptability.

(c) Shell eggs, when presented for breaking, shall be of edible interior quality and the shell shall be sound and free of adhering dirt and foreign material, except that:

(1) Checks and eggs with a portion of the shell missing may be used when the shell is free of adhering dirt and foreign material and the shell membranes are not ruptured.

(2) Eggs with clean shells which are damaged in candling and/or transfer and have a portion of the shell and shell membranes missing may be used only when the yolk is unbroken and the contents of the egg are not exuding over the outside shell. Such eggs shall be placed in leaker trays and be broken promptly.

(3) Eggs with meat or blood spots may be used if the spots are removed in an acceptable manner.

(d) All loss or inedible eggs shall be placed in a designated container and be handled as required in §590.504(c). Inedible and loss eggs for the purpose of this section and §590.522 are defined to include black rots, white rots, mixed rots, green whites, eggs with diffused blood in the albumen or on the yolk, crusted yolks, stuck yolks, developed embryos at or beyond the blood ring state, moldy eggs, sour eggs, any eggs that are adulterated as such term is defined pursuant to this part, and any other filthy and decomposed eggs including the following:

(1) Any egg with visible foreign matter other than removable blood and meat spots in the egg meat.

(2) Any egg with a portion of the shell and shell membranes missing and with egg meat adhering to or in contact with the outside of the shell.

(3) Any egg with dirt or foreign material adhering to the shell and with cracks in the shell and shell membranes.

(4) Liquid egg recovered from shell egg containers and leaker trays.

(5) Open leakers made in the washing operation.

(6) Any egg which shows evidence that the contents are or have been exuding prior to transfer from the case.

(e) Incubator reject eggs shall not be brought into the official plant.

[36 FR 9814, May 28, 1971, as amended at 40 FR 20059, May 8, 1975. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981]

§ 590.515 Egg cleaning operations.

(a) The following requirements shall be met when washing shell eggs to be presented for breaking:

(1) Shell egg cleaning equipment shall be kept in good repair and shall be cleaned after each day's use or more frequently if necessary.

(2) The temperature of the wash water shall be maintained at 90 °F or higher, and shall be at least 20 °F warmer than the temperature of the eggs to be washed. These temperatures shall be maintained throughout the cleaning cycle.

(3) An approved cleaning compound shall be used in the wash water. (The use of metered equipment for dispensing the compound into solution is recommended.)

(4) Wash water shall be changed approximately every 4 hours or more often if needed to maintain sanitary conditions and at the end of each shift. Remedial measures shall be taken to prevent excess foaming during the egg washing operation.

(5) Replacement water shall be added continuously to the wash water of washers to maintain a continuous overflow. Rinse water and chlorine sanitizing rinse may be used as part of the replacement water. Iodine sanitizing rinse may not be used as part of the replacement water.

(6) Waste water from the egg washing operation shall be piped directly to drains.

(7) The washing operation shall be continuous and shall be completed as rapidly as possible. Eggs shall not be allowed to stand or soak in water. Immersion-type washers shall not be used.

(8) Prewetting shell eggs prior to washing may be accomplished by spraying a continuous flow of water over the eggs in a manner which permits the water to drain away, or by other methods which may be approved by the Administrator.

(b) Shell eggs shall not be washed in the breaking room or any room where edible products are processed.

[36 FR 9814, May 28, 1971, as amended at 40 FR 20059, May 8, 1975. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981, as amended at 60 FR 49170, Sept. 21, 1995]

§ 590.516 Sanitizing and drying of shell eggs prior to breaking.

(a) Immediately prior to breaking, all shell eggs shall be spray rinsed with potable water containing an approved sanitizer of not less than 100 ppm nor more than 200 ppm of available chlorine or its equivalent. Alternative procedures may be approved by the Administrator in lieu of sanitizing shell eggs washed in the plant.

(b) Shell eggs shall be sufficiently dry at time of breaking to prevent contamination or adulteration of the liquid egg product from free moisture on the shell.

[60 FR 49170, Sept. 21, 1995]

§ 590.520 Breaking room facilities.

(a) The breaking room shall have at least 30 foot-candles of light on all working surfaces except that light intensity shall be at least 50 foot-candles at breaking and inspection stations. Lights shall be protected with adequate safety devices.

(b) The surface of the ceiling and walls shall be smooth and made of a water-resistant material.

(c) The floor shall be of water-proof composition, reasonably free from cracks or rough surfaces, sloped for adequate drainage, and the intersections with walls and curbing shall be impervious to water.

(d) Ventilation shall provide for:

(1) A positive flow of outside filtered air through the room;

(2) Air of suitable working temperature during operations.

(e) There shall be provided adequate hand washing facilities which are easily accessible to all breaking personnel, an adequate supply of warm water, clean towels or other facilities for drying hands, odorless soap, and containers for used towels. Hand washing facilities shall be operated by other than hand operated controls.

(f) Containers for packaging egg products are not acceptable as liquid egg buckets.

(g) A suitable container conspicuously identified shall be provided for the disposal of rejected liquid.

(h) Strainers, filters, or centrifugal clarifiers of approved construction shall be provided for the effective removal of shell particles and foreign material, unless specific approval is obtained from the National Supervisor for other mechanical devices.

(i) A separate drawoff room with a filtered positive air ventilation system shall be provided for packaging liquid egg product, except product packaged by automatic, closed packaging systems.

[36 FR 9814, May 28, 1971, as amended at 37 FR 6659, Apr. 1, 1972. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981]

§ 590.522 Breaking room operations.

(a) The breaking room shall be kept in a dust-free clean condition and free from flies, insects, and rodents. The floor shall be kept clean and reasonably dry during breaking operations and free of egg meat and shells.

(b) All breaking room personnel shall wash their hands thoroughly with odorless soap and water each time they enter the breaking room and prior to receiving clean equipment after breaking an inedible egg.

(c) Paper towels or tissues shall be used at breaking tables, and shall not be reused. Cloth towels are not permitted.

(d) Breakers shall use a complete set of clean equipment when starting work and after lunch periods. All table equipment shall be rotated with clean equipment every 21/2hours.

(e) Cups shall not be filled to overflowing.

(f) Each shell egg shall be broken in a satisfactory and sanitary manner and inspected for wholesomeness by smelling the shell or the egg meat and by visual examination at the time of breaking. All egg meat shall be reexamined by a person qualified to perform such functions before being emptied into the tank or churn, except as otherwise approved by the National Supervisor.

(g) Shell particles, meat and blood spots, and other foreign material accidentally falling into the cups or trays shall be removed with a spoon or other approved instrument.

(h) Whenever an inedible egg is broken, the affected breaking equipment shall be cleaned and sanitized.

(i) Inedible and loss eggs as defined in §590.510 apply to this section.

(j) The contents of any cup or other liquid egg receptacle containing one or more inedible or loss eggs shall be rejected.

(k) Contents of drip trays shall be emptied into a cup and smelled carefully before pouring into liquid egg bucket. Drip trays shall be emptied at least once for each 15 dozen eggs or every 15 minutes.

(l) Edible leakers as defined in §590.510(c)(2) and checks which are liable to be smashed in the breaking operation shall be broken at a separate station by specially trained personnel.

(m) Ingredients and additives used in, or for, processing egg products, shall be handled in a clean and sanitary manner.

(n) Liquid egg containers shall not pass through the candling room.

(o) Test kits shall be provided and used to determine the strength of the sanitizing solution. (See §§590.515(a)(9) and 590.552.)

(p) Leaker trays shall be washed and sanitized whenever they become soiled and at the end of each shift.

(q) Shell egg containers whenever dirty shall be cleaned and drained; and shall be cleaned, sanitized, and drained at the end of each shift.

(r) Belt-type shell egg conveyors shall be cleaned and sanitized approximately every 4 hours in addition to continuous cleaning during operation. When not in use, belts shall be raised to permit air drying.

(s) Cups, knives, racks, separators, trays, spoons, liquid egg pails, and other breaking equipment, except for mechanical egg breaking equipment, shall be cleaned and sanitized at least every 21/2hours. This equipment shall be cleaned at the end of each shift and shall be clean and sanitized immediately prior to use.

(t) Utensils and dismantled equipment shall be drained and air dried on approved self-draining metal racks and shall not be nested.

(u) Dump tanks, drawoff tanks, and churns shall be cleaned approximately every 4 hours. All such equipment and all other liquid handling equipment, unless cleaned by acceptable cleaned in-place methods, shall be dismantled and cleaned after each shift. Pasteurization equipment shall be cleaned at the end of each day's use or more often if necessary. All such equipment shall be clean and shall be sanitized prior to placing in use.

(v) Strainers, clarifiers, filtering and other devices used for removal of shell particles and other foreign material shall be cleaned and sanitized each time it is necessary to change such equipment, but at least once each 4 hours of operation.

(w) Breaking room processing equipment shall not be stored on the floor.

(x) Metal containers and lids for other than dried products shall be thoroughly washed, rinsed, sanitized, and drained immediately prior to filling. The foregoing sequence shall not be required if equally effective measures approved by the National Supervisor in writing are followed to assure clean and sanitary containers at the time of filling.

(y) Liquid egg holding vats and containers (including tank trucks) used for transporting liquid eggs shall be cleaned after each use. Such equipment shall be clean and sanitized immediately prior to placing in use.

(z) Tables, shell conveyors, and containers for inedible egg product shall be cleaned at the end of each shift.

(aa) Mechanical egg breaking machines shall be operated at a rate to maintain complete control and accurately inspect and segregate each egg to insure the removal of all loss and inedible eggs. The machine shall be operated in a sanitary manner.

(1) When an inedible egg is encountered on mechanical egg breaking equipment, the inedible egg and contaminated liquid shall be removed. The machine shall be cleaned and sanitized, or contaminated parts replaced with clean ones in the manner prescribed by the Administrator for the type of inedible egg encountered and the kind of egg breaking machine.

(2) Systems for pumping egg liquid directly from egg breaking machines shall be of approved sanitary design and construction, and designed to minimize the entrance of shells into the system and be disconnected when inedible eggs are encountered. The pipelines of the pumping system shall be cleaned or flushed as often as needed to maintain them in a sanitary condition, and they shall be cleaned and sanitized at the end of each shift. Other pumping system equipment shall be cleaned and sanitized approximately every 4 hours or as often as needed to maintain it in a sanitary condition. All liquid egg pumped directly from egg breaking machines shall be reexamined, except as otherwise prescribed and approved by the Administrator.

(3) Mechanical egg breaking equipment shall be clean and sanitized prior to use, and during operations the machines shall be cleaned and sanitized approximately every 4 hours or more often if needed to maintain them in a sanitary condition. This equipment shall be cleaned at the end of each shift.

[36 FR 9814, May 28, 1971, as amended at 37 FR 6659, Apr. 1, 1972; 40 FR 20059, May 8, 1975; 40 FR 20941, May 14, 1975. Redesignated at 42 FR 32514, June 27, 1977, and further redesignated at 46 FR 63203, Dec. 31, 1981]

Appendix 3. Disease Transmission Model

Introduction

In this appendix, we provide the details of the simulation analysis for estimating the proportion of HPAI contaminated eggs from an infected but undetected flock. We conducted this simulation analysis in two parts. We first estimated the HPAI prevalence, the disease mortality and the proportion of internally contaminated eggs at various time points post infection of the flock using a stochastic disease transmission model. We then used a simulation model of the planned surveillance protocol in the event of an outbreak along with the disease transmission model results to estimate the number of days to detect infection and the maximum daily proportion of contaminated eggs before infection is detected.

Modeling the Spread of HPAI in an Infected but Undetected Flock Using a Stochastic Disease Transmission Model

The disease transmission model simulates the spread of HPAI disease within a flock to estimate the HPAI prevalence and the disease mortality at various time points post infection of the flock. The deterministic version of the disease transmission model was developed by an FSIS-APHIS-FDA interagency group for use in various risk assessments. The deterministic version of the model is available online as part of a draft of the FSIS risk assessment[47] of the public health risks due to HPAI infection in poultry.

In the following, we first provide an overview of the deterministic disease transmission model and then describe the extension of the disease transmission model to consider the variability in the number of birds in various stages of infection.

Deterministic Disease Transmission Model

The disease transmission model is an extension of the Reed-Frost Susceptible-Latent-Infectious-Died (or Removed) model to include more infectious states. The model calculates the number of birds at various disease stages at discrete 6-hour time intervals following the infection in the flock. The primary advantage of having more infected stages is that differences in the probability of death and the infectivity of chickens infected for different durations can be considered. We have 8 infected stages in our disease transmission model: infected 1 hour (I1), infected 7 hours (I7),…, infected 43 hours (I43), in addition to the susceptible (S), and dead states.

As is often assumed in HPAI transmission models, (Vandergoot *et al.,*[42], Bouma *et al.,*[41]) we assume frequency dependent transmission where each individual makes a fixed number of contacts with other individuals per unit time regardless of the population size. Alternatively, in density dependent transmission, the number of contacts each individual makes per unit time is dependent on the population size. In reality, the type of disease transmission can depend on the mode of transmission such as fecal-oral or aerosol. Frequency dependent transmissions might be more appropriate for animals that are held

at a fixed stocking density (such as caged layers) Bouma *et al.*[41] In addition, frequency dependent transmission models provided reasonable fits to the outbreak data as described in Bos *et al.*[44] and Tiensen *et al.*[43]

The latent state was not modeled explicitly in the transmission model. Instead it was considered that chickens infected for different periods have different chances of being infectious. For instance, in the baseline scenario, it was assumed that chickens in the first 6 hours post infection are all latently infected whereas chickens infected for more than 19 hours post infection are 100 percent infectious. Refer to draft FSIS HPAI risk assessment[47] for formal presentation of the mathematical equations related to the deterministic transmission model.

Stochastic version of the disease transmission model to consider additional variability

The draft FSIS HPAI risk assessment[47] utilized deterministic calculations to estimate the number of chickens in the susceptible, dead and various infectious stages. The number of susceptible chickens is *S*, the number of infectious birds is *I*, and the total number *(S+I)* is *N*. In the deterministic calculations, the number of susceptible birds in period *t*+1 is calculated as

$$S_{t+1} = S_t(e^{-\beta\frac{I_t}{N_t}})$$

Where β is the effective contact rate

As in various chain binomial models, we used the binomial distribution to introduce variability in the number of birds moving from the susceptible to the first stage of infection (equivalent to a latent stage for the parameters used in the baseline scenario of the FSIS risk assessment). Let *p* be the probability that a susceptible bird in period *t* becomes infected in period *t+1*. We estimated *p* according to the following equation

$$p = (1 - e^{-\beta\frac{I_t}{N_t}})$$

Given *p*, we used the binomial(*S*, *p)* distribution to compute the number of birds moving from susceptible to the first infection stage (latent) between periods *t* and *t*+1. When the number of trials was greater than 1000, we used the Poisson approximation when $Sp < 15$. The Normal approximation to the Binomial distribution was used for other cases.

We also used the binomial distribution to estimate the number of chickens transitioning between various infected stages from one period to the next. For example, a binomial distribution with a 10 percent probability (p=0.1) was used to estimate the number of chicken in the 31st hour of infection that would die by the next time period.

Estimation of the disease transmission model parameters

(i) Expected length of latently infected period

Data on the latently infected period and mean time to death from chickens inoculated HPAI H5N1 virus are summarized in Appendix 2 Table 2. To our knowledge, only a couple of studies, Bouma *et al.*[41] and Das *et al.*[27] estimated the latent period for HPAI H5N1 infected chicken. Bouma *et al.*[41] utilized Bayesian analysis and estimated a latent period of 0.24 (0.099-0.48) days based on daily testing of artificially inoculated chickens (1 sample per day). Das *et al*.[27] tested tracheal and tissue (breast thigh and heart) samples of intranasal inoculated chickens every 6 hours. We decided to use this study for the estimation of latent and infectious periods as the sampling performed every 6 hours compared with Bouma *et al.*[41], where sampling was performed on a daily basis and the latent period was approximated via statistical analysis.

In our model, the probability of being latently infected is 1, 0.6, and 0.7 for I1, I7 and I13 states respectively. The probability of being latently infected is zero for the states I19 to I37. Given these probabilities, the expected length of latently infected period is 13.8 hours.

(ii) Estimation of the mean time to death and the infectious period

The expected infectious period in our model is 29.58 hours. The expected time to death is 43.38 hours. We employed methods of Das *et al.*[27] to estimate the mean time to death as the data had a greater precision with a 6-hour sampling compared with most studies which report the mean time to death in days (see Table 2). Pfeiffer *et al.*[110] estimated a time to death between 36-48 hours. Other studies, shown in Table 2, estimated the time to death to vary between 1-3 days.

Appendix 3 Table 2: Latently infected period, infectious period and the mean time to death for HPAI H5N1 infections in chicken from experimental studies

Source	Latent period	Infectious period
Bouma *et al.* (2009)[41]	6 hours	48 hours
Das *et al.* (2008) [27]	6-24 hours	12-36 hours
Source	Time to death	
Pfeiffer *et al.* (2009)[110]	36-48 hours	
Shortridge *et al.* (1998)[16]	2-3 days, most likely value was 2 days	
Lee *et al.* (2008)[111]	1-2 days	
Tsukomoto *et al.* (2007)[81]	2 days	

(*iii*) *State transition probabilities*

As discussed above, the expected time to death for an infected bird in our transmission model is 43.38 hours. The expected time to death and infectious period are functions of the state transition probabilities that represent the likelihood that a bird in a particular

infected state in one period either dies or transitions to another infected state in the next period. We utilized data from Das et al., 2008 to estimate these transition probabilities. For example 4 out of 40 birds died between 30-36 hours post inoculation in Das et al., 2008. We estimated the probability that a chicken in the 31st hour of infection would die in the next 6 hour time period is 0.1. The probabilities of transitioning between various possible states (infected 1-hour (I1), infected 7 hours (I7), …, infected 43 hours (I43), susceptible (S), and dead (D) states) from one period to the next are summarized in Appendix 3 Table 1.

Appendix 3 Table 1: Probability of a chicken being in a specific state in period (t+1) given its state in period (t) in the disease transmission model

	S_{t+1}	$I1_{t+1}$	$I7_{t+1}$	$I13_{t+1}$	$I19_{t+1}$	$I25_{t+1}$	$I31_{t+1}$	$I37_{t+1}$	$I43_{t+1}$	Dead
S_t	$e^{-\beta\frac{I_t}{N}}$	$(1-e^{-\beta\frac{I}{N}})$	0	0	0	0	0	0	0	0
$I1_t$	0	0	1	0	0	0	0	0	0	0
$I7_t$	0	0	0	1	0	0	0	0	0	0
$I13_t$	0	0	0	0	1	0	0	0	0	0
$I19_t$	0	0	0	0	0	1	0	0	0	0
$I25_t$	0	0	0	0	0	0	1	0	0	0
$I31_t$	0	0	0	0	0	0	0	0.9	0	0.1
$I37_t$	0	0	0	0	0	0	0	0	0.366	0.633
$I43_t$	0	0	0	0	0	0	0	0	0	1
Dead	0	0	0	0	0	0	0	0	0	1

(iii) Effective contact rate and associated parameters

The rate of the exponential increase in prevalence from the disease transmission model is largely determined by the effective contact rate parameter (also referred to as the transmission parameter). The effective contact rate is defined as the number of birds an infectious chicken comes into contact with that is sufficient to transmit infection per unit time. To estimate the effective contact rate directly, we would require data on the number of susceptible, infectious and dead birds over time from an HPAI infected flock in conditions reflective of commercial operations. Such data are rare. Consequently, approximate methods have to be utilized. In the following, we summarize the estimates for the contact rate for HPAI in caged layers from the literature.

Several articles estimated the contact rate from experiments on transmission of HPAI infection between pairs of chickens consisting of an inoculated bird and a contact bird. The small population of just two birds is justified on the assumption of frequency dependent transmission where the transmission rate is not dependent on the population size. However, in practice, the contact rate might increase with the population (density dependent transmission) when the population size is very low.[41] For instance, the contact rate might be higher if there are a greater number of chickens within a single cage. Furthermore, considering the impact of layer management practices such as airflow, feed delivery systems, and stocking density, extrapolating the contact rate from small scale experiments to that of a commercial flock might be unreliable.[42] Utilizing this approach Vandergoot *et al.*[42] estimated a contact rate of 33 birds/day for HPAI H7N7 infection in

chickens. Recently, Bouma *et al.*[41] estimated the contact rate as 0.72 (0.42-1.2)/day for HPAI H5N1 infections in chicken.

Investigators have estimated the contact rate from data on the increase in daily mortality over time from HPAI infected flocks. Typically, such outbreak data are inadequate for estimating the contact rate directly and hence several assumptions or back calculations must be made. Some of the issues associated with natural outbreak data are that the day when the flock was first infected and the numbers of infected birds at various points in time are not known. Two studies estimated the contact rate by back calculating the number of infected birds over time from the number of dead birds assuming specific values for latently infected and infectious periods. Using 2004 HPAI H5N1 epidemic data from Thailand, Tiensen *et al.*[43] estimated a contact rate of 2.30 birds/per day for laying hens and broiler chicken with a one day infectious period and no latent period. Based upon 2003 Netherlands HPAI H7N7 outbreak data, Bos *et al.*[44] estimated a contact rate of 4.50 birds per day (95 percent CI 2.68-7.57) with no latent period and a 4 day infectious period. In an alternate generalized linear model in Bos *et al.*[44] (not the best fit model) with a latent period of 1 or 2 days, the contact rate was 19.9 per day. Hence, inclusion of latent period can significantly impact the contact rate estimated from the data. Also, including the type of housing (caged or loose) did not have a significant influence on the model results.

Savill *et al.*[45] developed a simulation model of HPAI H5N1 infection spread in caged layers via feces or aerosol. In their model, the contact rate was 17 birds per day with an infectious period of 34.8 hours and a basic reproductive number R_0 of 25.

In summary, the effective contact rate typically is not directly estimable from the data without significant assumptions or approximations. There is considerable variation in the effective contact rate estimates from alternate approaches and from different models using the same approach. The contact rates from the literature ranged from 0.72 birds per day to 33 birds per day. Although, it is logically plausible that the contact rate is lower for caged layers, some recent studies of outbreak data report that the inclusion of housing type did not improve the fit of the data to model.[43,44] Given the significant uncertainty in the effective contact rate estimates, it is essential to choose a conservative value for the effective contact rate. Sensitivity analysis of the deterministic as well as the stochastic versions of the disease transmission models indicated that a higher contact rate might lead to greater number of contaminated eggs. In this respect, using a higher effective rate is more conservative. We conservatively assumed a contact rate of 2-birds/6-hour interval. We address the impact of uncertainty associated with the contact rate with sensitivity analysis later on in the appendix.

Given an expected infectious period of 29.58 hours, the basic reproductive number R_0 for our transmission model is 9.9. The expected generation interval (the mean duration between time of infection of a secondary infected hen and the time of infection of its primary infector) is 16.8 hours.[112]

(iv) Frequency and Timing of contaminated eggs

To estimate the daily number of contaminated eggs, we used the egg production Option 3 of the disease transmission model as presented in the draft FSIS interagency risk assessment.[47] In this option, no contaminated eggs are laid before the first 19 hours post infection while all the eggs laid after 19 hours post infection are considered to be contaminated. This option corresponds well with empirical data from laboratory studies[17,38] that show that eggs laid on the first day post infection are not contaminated (see FSIS risk assessment[47] for details on transmission model).

Transmission model assumptions

The main assumptions of the disease transmission model are summarized below:

i. *The effective contact rate for disease transmission estimated from HPAI outbreaks in Netherlands and Thailand (2 birds/6 hours) is applicable for layer flocks in the US.* Differences in the layer management practices between the United States and other countries, and the characteristics of the HPAI strain causing the outbreak may result in a different contact rate than that used in this assessment. Sensitivity analysis with respect to the contact rate indicates that our risk estimates are robust and conservative with respect to the range of contact rates estimated in the literature for caged layers.

ii. *The mean infectious period and latent period for the HPAI strain are similar to those for Asian H5N1 strains*. We estimated a latent period of 13.8 hours and an infectious period of 29.6 hours using experimental data from HPAI H5N1 inoculated hens. The transmission model results are sensitive to the infectious period of the HPAI strain causing the outbreak and may vary considerably for strains with longer infectious periods (e.g., Netherlands HPAI H7N7).

iii. *A flock size of 100,000 represents the mean flock size currently in the industry.* A greater flock size would likely result in higher normal mortality and decreased chance of detection in a given time. We consider the flock size of 100,000 as a conservative estimate given that the USDA APHIS Layer 1999 survey of layer farms in the U.S. indicates that the mean and median flock sizes are less than 83,000 hens.[46]

Simulation Results

The above model was coded in Excel using Visual Basic for Applications and @RISK software. An effective contact rate (β) of 2 chicken/6 hour time period and a flock size of 100,000 layers were used as input parameter values. We conducted simulations for 12000 iterations with Latin Hypercube sampling.

From Appendix 3 Table 3, we observe that the 90 percent probability intervals for the daily mortalities in various days are relatively wide. Outbreak data from Netherlands showed similarly high variance in the daily mortality.[40] Depending on normal flock mortality (mortality independent of HPAI infection), this variance in the daily mortality may result in increased uncertainty in the day HPAI is detected in the flock.

Appendix 3 Table 3: Daily mortality predicted by the transmission model in a flock of 100,000 layers starting with one infected bird (I=1)

	Daily Mortality					
Parameter	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Deterministic	0	1	4	31	235	1757
Stochastic mean	0	0.67	3.8	31	235	1761
Stochastic two sided 90% interval	0	1	1-8	11-59	82-442	617-3,228

We noted that the daily mortality values were quite sensitive to the time period in the day when the mortality was enumerated. Given that the unit time period in the transmission model is 6 hours, there are 4 time periods in the day. For instance, in the deterministic transmission model, the daily mortality for the 5th day is 66 if mortality is checked in the first 6-hour period of the day and 109 if mortality is checked in the second 6-hour period of the day. The sensitivity of the outputs shows the importance of considering the variability in the transmission model.

Simulation Model to Estimate the Time to Detect Infection and the Maximum Daily Proportion of Contaminated Eggs under the Active Surveillance Protocol

In the targeted active surveillance described in the UEP/UEA – USDA APHIS VS Movement Control Model Plan, swabs from 5 randomly selected birds among the daily mortality sample are pooled together and tested via RRT-PCR each day. The number of days to detect infection under this protocol depends on the variability in the normal mortality independent of HPAI and the variability in the mortality due to HPAI. We used outputs from the transmission model in conjunction with the simulation model of the active surveillance protocol to estimate the number of days to detect infection in a flock.

According to the UEP/UEA – USDA APHIS VS Movement Control Model Plan, eggs or egg products from flocks within the control area will be allowed to move with a permit only after the flock tests negative with RRT-PCR testing as described above. This protocol implies that if infection from a flock is detected on a particular day by RRT-PCR testing, then the eggs or egg products from the flock on that day would not be moved. Therefore, for this analysis we assumed that eggs produced on the day on which infection is detected are not relevant for risks associated with movement of shell eggs. Given this assumption, we defined the maximum daily proportion of contaminated eggs as the highest daily proportion of contaminated eggs among all the days starting from the day the flock is infected to one day before the infection in the flock is detected. In the following, we present the details of the simulation analysis of the active surveillance protocol.

Notation

t = index of days, $t = 1,\ldots,t_d$; where t=1 is the first day on which the flock is infected and $t = t_d$ is the day on which infection is detected in the flock.

$M^d(t)$ = mortality due to HPAI on day t (birds/flock of 100,000 layers).

$M^n(t)$ = normal mortality independent of HPAI on day t (birds/flock of 100,000 layers).

$M^t(t)$ = total mortality on day t (birds/flock of 100,000 layers). $M^t(t) = M^d(t) + M^n(t)$

M_{lim} = threshold for total mortality above which HPAI infection in the flock would be detected due to increased mortality regardless of diagnostic testing.

n = number of swabs in the pooled sample submitted for RRT-PCR testing each day (swabs).

$$X^{pool}(t) = \begin{cases} 1 \text{ if a swab from HPAI mortality is included in pooled sample for RRT - PCR on day } t \\ 0 \text{ otherwise} \end{cases}$$

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

$$X(t) = \begin{cases} 1 \text{ if infection in the flock is detected on day } t \text{ by either RRT PCR or increased mortality} \\ 0 \text{ otherwise} \end{cases}$$

$$Y(t) = \begin{cases} 1 \text{ if infection in the flock is detected on day } t. \text{ Y(t) = 1 implies day t = } t_d. \\ 0 \text{ otherwise} \end{cases}$$

Se = sensitivity of the RRT-PCR testing procedure.

$E(t)$ = calculated number of HPAI contaminated eggs produced on day t (contaminated eggs/flock of 100,000).

E_{max} = maximum number of contaminated eggs per day that may be moved among all days $t = 1,\ldots,t_d - 1$ prior to detection of infection in the flock.

Assumptions

- Apart from active surveillance of mortality via RRT-PCR, other clinical indicators of HPAI infection such as drop in egg production and decreased feed intake are not considered towards detecting infection.
- The sampling of daily mortality is random i.e., swabs from dead birds with clinical signs are not more likely to be included in pooled samples tested via RRT-PCR.
- Weekly mortality data were used to estimate the normal daily mortality. This assumption can result in some underestimation of the variability in the daily mortality.

- The active surveillance protocol with daily RRT-PCR testing of a pooled sample from the flock was implemented before the flock became infected with HPAI. The assumption is well justified after the initial few days of RRT-PCR testing in the HPAI outbreak. However, the differences in the model results without this assumption are not significant as discussed in appendix 11.

Simulation Model

The number of diseased birds present in the 5 randomly chosen birds from the pool of daily mortality was assumed follow a hypergeometric distribution. Specifically,

$$X^{pool}(t) = HyperGeometric(M^t(t), n, M^d(t))$$

Depending on the sensitivity of the test, there is a chance that infection may not be detected even if the 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.*

$$X^{pcr}(t) = \begin{Bmatrix} 0 \ if \ X^{pool}(t) = 0 \\ Bernoulli(Se) \ if \ X^{pool}(t) = 1 \end{Bmatrix}$$

Finally, disease is detected on the day *t* either by RRT-PCR testing or due to the daily mortality being higher than the threshold, $M_{lim.}$

$$X(t) = \begin{Bmatrix} 1 \ if \ X^{pcr}(t) = 1 or M^t(t) > M_{\lim} \\ 0 \ otherwise \end{Bmatrix}$$

The probability that infection is detected on a particular day *t*, $P(Y(t) = 1)$ or equivalently $P(t = t_d)$ is given by

$$P\{Y(t) = 1\} = P\left\{\sum_{k=1}^{k=t-1} X(k) < 1\right\} P\{X(t) = 1\}$$

The maximum number of contaminated eggs/day before infection in the flock is detected is given by,

$$E_{\max} = Max(E(t) \mid t \le t_d - 1)$$

Input Data

Flock size: 100,000 to represent the average flock size currently in the industry. The flock size of 100,000 is conservative as data from the USDA APHIS Layer 1999 survey indicate that the mean and median layer flock sizes are less than 83,000 hens. [46] Additionally, only 13.5 percent of the flocks had more than 120,000 hens.

Daily mortality due to HPAI in different days in an infected flock (**$M^d(t)$**): We estimated this parameter from the output of the disease transmission model.

Normal mortality independent of disease (**$M^n(t)$**): Normal mortality data were provided by AgriStats Inc. through Iowa State University Veterinary Extension. The data consisted of weekly mortality numbers for 27 layer flocks for the entire production cycle (60-100 weeks). The data are from a representative sample of 9 flocks each from the 3 major egg layer breeds in the US and 9 flocks each from 3 geographic regions (Iowa and Minnesota, California and, Pennsylvania). We calculated daily mortality in a flock of 100,000 layers based on the weekly mortality numbers, i.e, daily mortality = weekly mortality/7. The mean daily mortality rate was 28 deaths per 100,000 birds with a standard deviation of 33 deaths/100,000 birds. Given a sample size of 1801 weekly average mortalities, the standard deviation of the expected mortality was 0.78. The 97.5th percentile for this data was 91, i.e., the P(number of deaths <=91)=0.975). We note a higher flock size would lead to a greater normal mortality and can result in increased time to detection and a greater number of contaminated eggs before detection. The flock size of 100,000 is conservative given the results of the USDA APHIA Layers 1999 survey (see above reasoning for flock size estimate).[46] For the simulation, we utilized an empirical distribution (sampled directly from data) as the sample size is relatively large.

Egg Production Rate: 0.7 eggs/hen/day[47]. AgriStats, Inc., data, described in the preceding section indicate that the egg production rate may vary from 0.6 eggs/hen/day to 0.9 eggs/hen/day depending on the age of the flock and other factors. The number of contaminated eggs from an infected but undetected flock is directly proportional to the egg production rate.

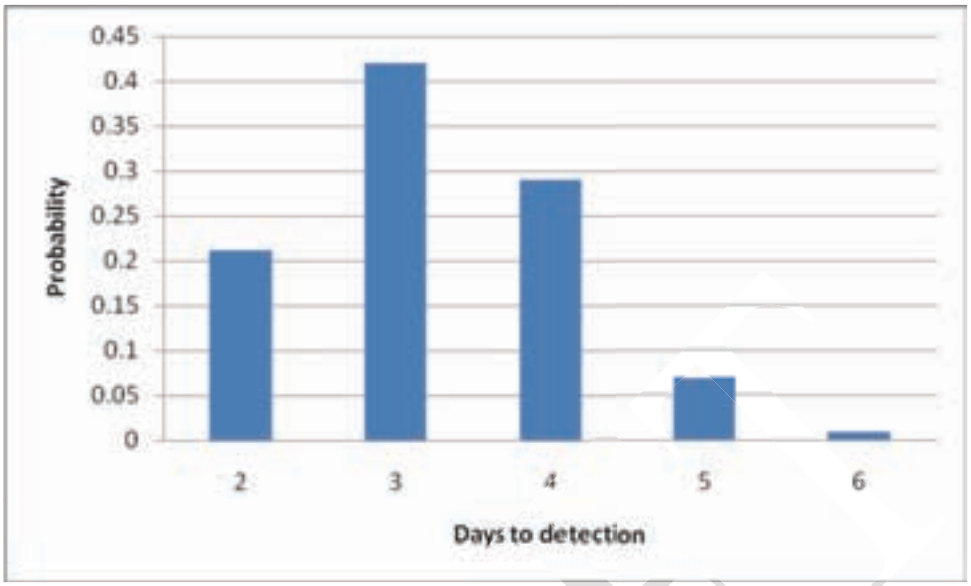
Sensitivity of the PCR testing (*Se):* Based on the published results for H7N2 LPAI [49]and personal communication, Dr. Erika Spackman, we used a sensitivity of 86.5 percent for the RRT-PCR testing.

Mortality threshold M_{lim}: 0.5 percent daily mortality. The threshold is based on the analysis of Agristats, Inc., and other daily mortality data (Details are provided in Appendix 10).

Results

Number of days to detect HPAI after the first chicken is infected t_d: The distribution for t_d from the simulation is shown in Appendix 3 Figure 1. The mean t_d was 3.3 days. However, there is a 0.074 probability that 5 or more days are required to detect the infection. One reason for the late detection of HPAI (>4 days) is high normal mortality rate. A high normal mortality implies that a swab from a chicken death due to HPAI is less likely to be included in the pooled sample for RRT-PCR testing. Another factor

leading to the late detection is that the RRT-PCR is 86.5 percent sensitive and it may therefore fail to detect some cases.



Appendix 3 Figure 1. Simulation output distribution for the number of days to detect HPAI in a flock of 100,000 layers with active surveillance via PCR testing.

Maximum number of contaminated eggs per day that may be moved prior to detection of infection in the flock E_{max} **:** The mean E_{max} from simulation was 11 contaminated eggs/day. The two sided 90 percent probability interval for E_{max} from simulation was 0-44 contaminated eggs/day. However, in 5 percent of the simulation iterations, 44-973 contaminated eggs were produced prior to detection. Typically, these cases when a greater number of contaminated eggs were produced prior to detection in the simulation results were associated with late detection of infection.

Sensitivity Analysis with Respect to Effective Contact Rate

We selected the effective contact rate for sensitivity analysis considering the higher uncertainty associated with its estimation compared with other transmission parameters such as the latent and infectious periods that are more directly estimable from experimental studies. An increase in the effective contact rate would have two competing effects that impact the number of contaminated eggs produced from a flock before detection. First, given a higher contact rate, a greater number of chicken are expected to become infected and die with HPAI within a specific time. The higher HPAI mortality likely results in reduced time to detect infection in the flock. However, increased contact rate would also result in a greater number of infected chickens within a given time and thus potentially higher number of contaminated eggs.

For our sensitivity analysis, we performed 6000 simulation iterations of the disease transmission and the active surveillance models for the effective contact rates of 1, 2 and 4 birds/6 hours. The contact rate of 4 birds/6 hours can be considered an upper bound estimate for caged layers as predicted by the simulation model of Savill *et al.*[45] As

detailed in appendix 2, the contact rates from HPAI outbreak data were mostly less than 2-birds/6 hours. The results shown in Appendix 3 Table 5 indicate that a higher effective contact rate may result in a greater number of contaminated eggs being moved before infection is detected in the flock.

Appendix 3 Table 5. Variation of E_{max} with the effective contact rate.

	Estimated maximum number of contaminated eggs per day among all the days prior to detection from a flock of 100,000 layers		
Effective contact rate (birds/6 hours)	1	2*	4
Mean (eggs/day)	4	11	24
90% interval (eggs/day)	0-15	0-44	0-49
Max (eggs/day)	273	1018	1385

*We used this contact rate as the baseline for our analysis.

The above sensitivity analysis indicates that the number of contaminated eggs from an infected, undetected flock of 100,000 birds (within a single layer house) following the active surveillance protocol increases with the effective contact rate. The effective contact rate of 2-birds/6 hours used in our assessment is higher than estimates from HPAI outbreak data in the literature and is therefore a conservative estimate.

Appendix 4. Estimating the Probability of Infection in a Chicken Exposed to Various Doses of HPAI Virus

The exponential dose response model

A dose response model is useful in estimating the probability of infection in a chicken exposed to a certain dose of virus. We used the single parameter exponential dose response model which has been used previously to model avian influenza and other viruses such as foot and mouth disease.[113] The exponential model assumes that each unit dose of virus has an identical probability (p) of causing infection. The most commonly used dosage unit is the 50 percent embryo infectious dose or EID_{50}. The dose response curve is generated from data on the proportion of chickens developing infection given varying doses. A recent article by Swayne and Selmons (2008)[114] provides experimental data from eleven HPAI strains and also summarizes the relevant data in the literature. We used data from this article to fit the dose response curve.

Estimating the probability that a single embryo infectious dose of HPAI infects a chicken

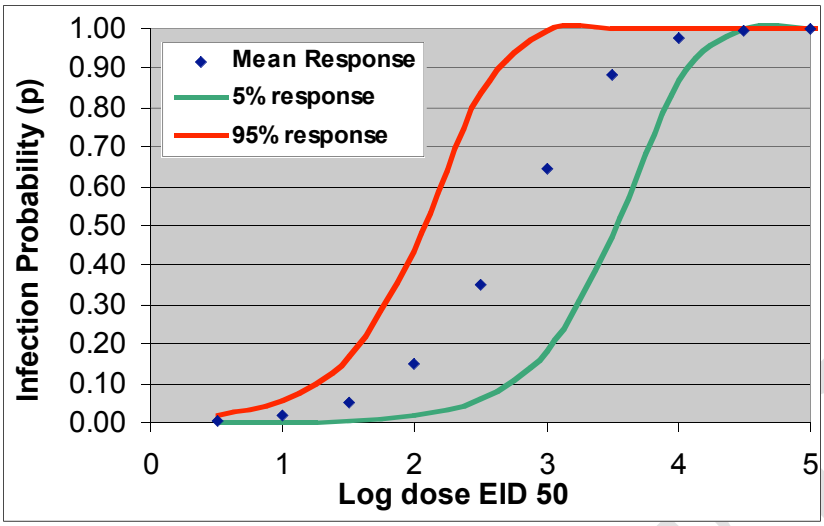
Data on infectivity of various HPAI strains is often presented as the 50 percent chicken infectious dose (CID_{50}). For example, a single CID_{50} of 10^3 EID_{50} implies that one dose of 10^3 EID_{50} would infect 50 percent of exposed chickens. The CID_{50}s of 17 HPAI strains reviewed in Swayne and Selmons [114] were mostly between 10^2 EID_{50} to $10^{3.9}$ EID_{50}. We utilized @RISK to fit various distributions to this data. We selected a logistic distribution for these data based on the Kolmogorov-Smirnov goodness of fit test.[115] We used a logistic distribution with a mean of $10^{2.80}$,and a standard deviation of $10^{0.43}$ EID_{50} as the distribution of CID_{50} for HPAI. According to the exponential dose response model, the probability of infection in a chicken (P_{inf}) given a dose (D) is given by the following equation, where p is defined as the probability of 1 EID_{50} causing infection:

$$P_{\text{inf}} = 1 - e^{-pD}$$

Given that a dose (D) is equal to the CID_{50}, the probability (P_{inf}) is 0.5 by definition, and p can be derived as follows:

$$p = -\ln(0.5) \Big/ D$$

We utilized @RISK to simulate the distribution of CID_{50} and obtain the output distribution for the parameter p. Given the uncertainty in p via simulation, we also obtained the distributions for the probability of infection for various doses in the range $10^{0.5}$ to 10^7 EID_{50}. We used Latin Hypercube sampling with 50000 iterations for the simulation settings. The resulting dose response curve with the mean probability of infection at various doses and two sided 90 percent probability interval is shown in Appendix 4 Figure 1.



Appendix 4 Figure 1. Dose response curve for chickens exposed to HPAI virus.

Appendix 5. Simulation Model to Evaluate the Effectiveness of the Chlorine Based Sanitizing Spray in Inactivating HPAI

Overview

We developed an @RISK[116] simulation model to evaluate the potential degree of inactivation resulting from the sanitizing spray. A commonly used measure of exposure to disinfecting agents is *Ct* defined as the product of the average concentration C_a and exposure time *t*. In the simulation model, we first estimated a probability distribution for *Ct*, using exposure times t=1,…,8 seconds. We considered that chlorine concentration would exponentially decay (Equation 1) due to reaction with organic material on the eggshell surface.

$$C = C_o e^{-kt} \qquad (1)$$

Where *C* is the chlorine concentration at time *t,* C_0 is the initial chlorine concentration and *k* is the decay rate (min^{-1}). *Ct* can then be calculated as shown in equation (2).

$$Ct = \frac{C_o}{k}(1 - e^{-kt}) \qquad (2)$$

We compared *Ct* values from the simulation output with values required to achieve a 1000 factor inactivation as reported in various sources.[61,117]

Model Assumptions

a) Eggs have been washed and cleaned prior to sanitizing, leaving no soil or feces on the shell egg surface.
b) HPAI virus is present on the shell egg surface[ff]
c) Eggs are in contact with the sanitizing spray for 1 to 8 seconds
d) Chlorine concentration is maintained at 100-200 ppm
e) Hepatitis A is less sensitive to inactivation by chlorine compared to the enveloped HPAI. As Hepatitis A is a non-enveloped virus, it is likely less sensitive to chlorine inactivation than the enveloped HPAI virus. Hepatitis A virus is relatively resistant to inactivation and has been used as an indicator virus for disinfection of water by the EPA.
f) The *Ct* values and chlorine decay rates given in the above reference are reasonable values to use for egg processing operations

Model Inputs

a) The exposure time *t* =time (minutes) that the eggshell is in contact with the sanitizing rinse. The exposure time is not specified by regulations and may vary according to conveyer belt speed and timing of sanitizing-rinse application. Industry experts suggest a minimum contact time of 8 seconds. We used a conservative distribution, t~Uniform (1/60-8/60), in minutes, for this parameter.

[ff] The amount of virus present was not specified in the model, as there has been no research on this issue. However, HPAI has been detected on egg shells [8,22,50]

b) k is the exponential decay rate constant for chlorine concentration. This decay rate could be relatively low if washing is effective and all eggshells are relatively clean. We conservatively chose to utilize the k from experiments with chlorine demand (reduction of chlorine concentration due to reaction with organic material) from chicken egg aminoallantoic fluid.[61] We used Uniform (1.24, 2.33) (min^{-1})as the distribution for k.
c) Initial chlorine concentration C_0: We used Uniform (100, 200) mg/l as the distribution for initial chlorine concentration given that regulation mandates a chlorine concentration between 100-200 ppm.
d) Reported *Ct*: the *Ct* required for achieving a 3-log inactivation of HPAI virus as reported from various literature sources. The reported *Ct* depends on the temperature and effective pH of the sanitizing rinse. For neutral pH (7-8), we used the *Ct* value from Rice *et al.*[61] of 0.79 mg-min/L. For pH greater than 10, we used reported values based on hepatitis A inactivation at 25° C [65,117]. Uniform 1-5 mg-min/L was used as the distribution for reported *Ct* when sanitizing rinse pH is greater than 10.

Simulation Results

We used @RISK to simulate the above input distributions. We utilized Latin hypercube sampling with 50000 iterations. The mean *Ct* value from the simulation was 11.9 mg-min/L. The *Ct* value was greater than 4.2 mg-min/L with 95 percent confidence. For neutral pH(7-8) all simulated values were greater than the reported *Ct* implying that a 3-log inactivation would be achieved. For pH greater than 10, there is 0.97 probability that the simulated *Ct* is greater than the reported *Ct* for achieving 3-log inactivation of HPAI.

Appendix 6. Leakage of HPAI Contaminated Eggs: Expert Opinion

We obtained expert opinion to help quantify parameters requisite in HPAI related risk assessments. The elicitation process consisted of a web conference introducing risk assessments followed by responses to individual questionnaires by the experts. We sought expert opinion on the ratio of the probability that HPAI contaminated eggs leak during transport to the probability that virus free eggs leak as data on this parameter was not available in the literature. Excerpts from responses to the questionnaires by the experts are provided below. A caveat is that the experts indicated that their responses could be subjective.

Miscellaneous question:

How many times more likely is it for an HPAI contaminated egg with no visual defects to leak during transportation as compared to a virus free egg?

1. Response from Dr. David Swayne, Laboratory Director, Southeast Poultry Research Laboratory; USDA/Agricultural Research Service

	Maximum	Minimum	Most likely
Number times more likely to leak during transportation	400%	200%	400%

2. Response from Dr. Eric Benson, Associate Professor, Bioresources Engineering Department, Animal and Food Science Department, University of Delaware

	Maximum	Minimum	Most likely
Number times more likely to leak during transportation	X10	X1	X5

3. Response from Dr. Dave Halvorson, Diplomate, ACPV, Professor Emeritus College of Veterinary Medicine, University of Minnesota.

Approximately 30% of "infected" eggs will be abnormal; of these, half will be soft shelled or leakers and they will be discarded. The other half of the 30% will be thin shelled and will crack or break at a rate of 10X a normal egg. The other 70% will crack or break at the same rate as a normal egg.

Appendix 7. Federal and State Regulation of Shell Eggs

Federal and State Regulation of Shell Eggs
USDA:APHIS:VS
June 2008

Executive Summary

The regulation of shell eggs is shared between various Federal agencies and state governments. At the Federal level, the Agricultural Marketing Service (AMS), the Food Safety Inspection Service (FSIS), and the U.S. Food and Drug Administration (FDA) all regulate shell eggs in some manner. There are no mandatory Federal regulations that detail specific requirements for washing shell eggs (Table 1, page 3). However, the AMS has a voluntary shell egg grading program, (7CFR56), which includes specific egg washing requirements. AMS also oversees a shell egg surveillance program that places limitations on restricted eggs (7CFR57). Both the FSIS and FDA have identical temperature requirements for shell egg storage, but FSIS regulations are directed at egg handlers[gg] and FDA requirements are directed toward retail establishments. In addition, both agencies have labeling requirements. FSIS requires a label that indicates refrigeration is required and the FDA label includes safe handling instructions.

Regulations for shell eggs in the top 5 egg producing states (Iowa, Ohio, Pennsylvania, Indiana, and California)[hh] are similar: all contain regulations on grades, temperatures, and labeling. Ohio, Indiana, and California have all adopted Federal standards, grades, and weight classes for shell eggs, while Pennsylvania and Iowa have State regulations that are consistent with the Federal ones. Only Iowa and Indiana codes contain specific requirements for egg washing (Table 1, page 3). Ohio, Pennsylvania, and California all have voluntary State egg quality assurance programs, with California claiming enrollment of 95% of the state's egg production.[ii] The state

[gg] Defined in the 7CFR: Egg handler means any person, excluding the ultimate consumer, who engages in any business in commerce that involves buying or selling any eggs (as a poultry producer or otherwise), or processing any egg products, or otherwise using any eggs in the preparation of human food.

[hh] Top egg producing states, ranked by number of layers. Egg Industry Facts Sheet, American Egg Board website

[ii] http://animalscience.ucdavis.edu/Avian/qap.htm

regulations controlling shell egg temperature, labeling, and grades generally follow those issued by AMS, FSIS, and FDA.
Based on our review of the regulations, using egg washing as a generally applicable mitigation during an HPAI outbreak is not feasible. Egg washing regulations issued by AMS and the various state governments lack uniformity, and their effectiveness in reducing the presence of HPAI on eggshells will have to be evaluated at time of the outbreak.

Table 1. Federal and State Regulations for Shell Egg Washing

Agency	**Regulations for Shell Egg Washing? (Yes/No)**
AMS	Voluntary
FSIS	No
FDA	No
Iowa Dept. of Inspection and Appeals	Yes
Ohio Dept. of Agriculture	No
Pennsylvania Dept. of Agriculture	No
Indiana State Egg Board	Yes
California Dept. of Agriculture	No

Federal Regulations

Regulating shell eggs at the Federal level is divided between the Agriculture Marketing Service, the Food Safety Inspection Service (which are both part of USDA), and the Food and Drug Administration (which is part of HHS).

Agricultural Marketing Service

While AMS's voluntary shell egg grading program includes washing and sanitizing requirements, the sanitizing requirements specify only temperature and chlorine concentrations. There are no requirements for a minimum sanitizing time. AMS oversees the Voluntary Grading of Shell Eggs program (7CFR56). This is a fee for service program based on the official U.S. standards, grades, and weight classes for shell eggs.[jj] Included in the Code of Federal Regulations are requirements for egg quality, weight, and condition, as well as facility requirements. Incorporated in the facility requirements are guidelines for shell egg cleaning operations, which include:

[jj] The U.S. standards, grades and weight classes for shell eggs were removed from the CFR on December 4, 1995, and are maintained by the Agricultural Marketing Service as the AMS 56.

- Design, cleaning and maintenance requirements for washing and drying equipment;
- Temperature, replacement and source requirements for wash water;
- Approved cleaning compounds for wash water and replacement water;
- Requirements to maximize conservation of the egg's quality and to prevent sweating of eggs;
- Temperature and chlorine concentration requirements[kk] for sanitizing after washing; and,
- Testing to determine the strength of the sanitizing solution.

In fiscal year 2007, the USDA (AMS) officially graded approximately 40% of table eggs processed and packaged in the U.S.[ll]

In addition to the voluntary grading program, AMS is also responsible for a mandatory shell egg surveillance program in order to carry out certain provisions of the Egg Products Inspection Act (EPIA), as amended (21 U.S.C. 1031-1056). Responsibilities for carrying out the provisions of the EPIA are shared between AMS and FSIS. The shell egg surveillance program focuses on inspection and disposition of restricted eggs (7CFR57). Restricted eggs include checks, dirties, incubator rejects, inedibles, leakers or loss (7CFR57).[mm] The shell egg surveillance program inspects business premises, facilities, inventories, operations, transport vehicles, and records of all egg handlers and shell egg packers. Egg handlers are inspected periodically and shell egg packers are inspected a minimum of once each calendar quarter. Producers with an annual egg production from a flock of 3,000 hens or less are exempt from the regulations on restricted eggs but still must maintain their records according to the regulations (7CFR57.100).

Food Safety Inspection Service

FSIS's focus is on egg products. However, FSIS has also issued regulations that specify temperature and labeling requirements for shell eggs (the FSIS regulations are in 9CFR590).[nn,oo] The regulations include the following requirements:

- No shell egg handler shall possess any shell eggs that are packed into containers destined for the ultimate consumer unless they are stored and transported under refrigeration at an ambient temperature of no greater than 45°F (7.2°C); and,

- No shell egg handler shall possess any shell eggs that are packed into containers destined for the ultimate consumer unless they are labeled to indicate that refrigeration is required.

[kk] It should be noted that the regulations do not specify a minimum time for sanitization.
[ll] From email correspondence with Roger Glasshoff, National Supervisor, Shell Eggs, AMS.
[mm] These terms are defined in the CFR.
[nn] It should be noted that while FSIS has issued regulations for washing and sanitizing eggs destined for use in egg products, it does not have similar regulations for shell eggs.
[oo] Producers with an annual egg production from a flock of 3,000 hens or less are exempt from the temperature and labeling requirements of this regulation

Food and Drug Administration

FDA has regulations for the temperature and labeling of shell eggs that are similar to those issued by FSIS. The FDA regulations for refrigeration temperature are directed at retail establishments, but have the same temperature requirement of "no greater than 45°F (7.2°C)" (21CFR115.50).

State Regulations

Regulations for shell eggs on the State level vary. The following is a summary of the regulations from the top 5 egg producing States.[pp]

Iowa

The Iowa Department of Inspection and Appeals regulates shell eggs in Iowa. Temperature requirements are found in both the Iowa code (Chapter 196) and the Iowa administrative code (481IAC36) and are almost identical with the Federal requirements. The administrative code (481IAC36) contains regulations for grades, restricted eggs, and minimum sanitation and operating requirements. Included in the minimum sanitation and operating requirements (481IAC36.3) are the following regulations for egg washing:

- Egg cleaning equipment shall be kept in good repair and shall be thoroughly cleaned after each day's use or more often if necessary to maintain a sanitary condition.
- The wash water shall be potable and maintained at a temperature of 90°F minimum. The wash water temperature must be at least 20°F greater than the egg temperature.
- The wash water shall be replaced frequently and the detergent and sanitizer shall be kept at an effective level at all times.
- During any rest period, or at any time when the equipment is not in operation, the eggs shall be removed from the washing and rinsing area of the egg washer and from the scanning area whenever there is a buildup of heat.
- Only United States Department of Agriculture (USDA) or federally approved cleaning compounds and sanitizers may be used.

Ohio

The Food Safety Division of the Ohio Department of Agriculture is responsible for shell egg regulation. The regulations are limited to standards, grades, refrigeration requirements, and container labels. There are no specific regulations for shell egg washing. Ohio Code 925.02 adopts the Federal standards, grades, and weight classes for shell eggs, found in AMS 56. The refrigeration requirements (Ohio Code 925.03)

[pp] Top egg producing states, ranked by number of layers. Egg Industry Facts Sheet, American Egg Board website

are identical to the Federal requirements and apply to all segments of the shell egg industry from producer (>500 birds) to retail food store. Ohio also has a voluntary Egg Quality Assurance Program administered by the Ohio Department of Agriculture and the Ohio Poultry Association.

Pennsylvania

The egg division of the Pennsylvania Department of Agriculture regulates shell eggs for the State. Temperature and labeling requirements are consistent with the Federal requirements (Title 7 chapter 88 and 46.243). There are no specific regulations for shell egg washing, only requirements that shell eggs be "received clean and sound" and do not exceed the restricted egg tolerances (Title 7, 46.243). In addition, the Pennsylvania code includes guidelines for egg certification and standards for grading and marketing eggs (Title 7 chapters 85, 87). Pennsylvania also has a voluntary egg quality assurance program with support from the Pennsylvania Department of Agriculture and the Pennsylvania Department of Health.

Indiana

Shell eggs are regulated by the Indiana State Egg Board. Temperature requirements for dealer facilities (370IAC 1-2-1), retail stores (371IAC 1-2-2), and transportation (370IAC 1-2-3) are identical to Federal regulations. The Indiana State egg board adopted the United States standards, grades and weight classes for shell eggs found in AMS 56 as well as the regulations governing the inspection of eggs, 7CFR57. The Indiana administrative code contains minimum sanitation and operating requirements for shell egg packers (370IAC1-10-1). For egg washing, the following requirements apply:

- Egg cleaning equipment shall be kept in good repair and shall be thoroughly cleaned after each day's use or more often if necessary to maintain a sanitary condition;
- The wash water shall be potable and maintained at a temperature of ninety (90) degrees Fahrenheit minimum. The wash water temperature must be at least twenty (20) degrees Fahrenheit greater than the egg temperature;
- The wash water shall be replaced frequently, a minimum of once a day, and the detergent and sanitizer shall be kept at an effective level at all times; and,
- Only approved cleaning and sanitizing compounds may be used in shell egg processing plants.

California

The California Department of Food and Agriculture regulates shell eggs through the shell egg quality control program. Under the California Food and Agricultural Code, sections 27532-27533 have the State standards, the standards for grades and surveillance shall be consistent with the Federal grade standards found in AMS 56 and the shell egg surveillance inspection standards, 7CFR57. There are no specific requirements for shell egg washing. California also has a voluntary egg quality assurance plan which claims enrollment of 95% of the state's egg production.[qq]

[qq] http://animalscience.ucdavis.edu/Avian/qap.htm

Conclusions

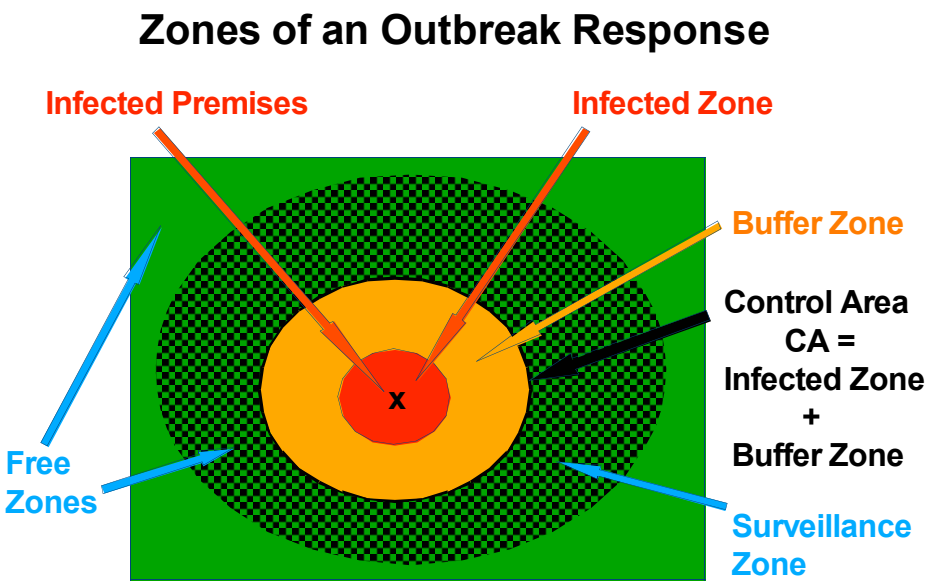
Requirements for shell egg temperature, labeling, and grades are consistent at the State and Federal levels. However, regulations for the washing of shell eggs are lacking at the Federal level and are either lacking or inconsistent at the State level. Given this degree of variation, the use of egg washing requirements as an across-the-board mitigation is not feasible. However, it may be possible to evaluate the effectiveness of washing and sanitizing by individual state.

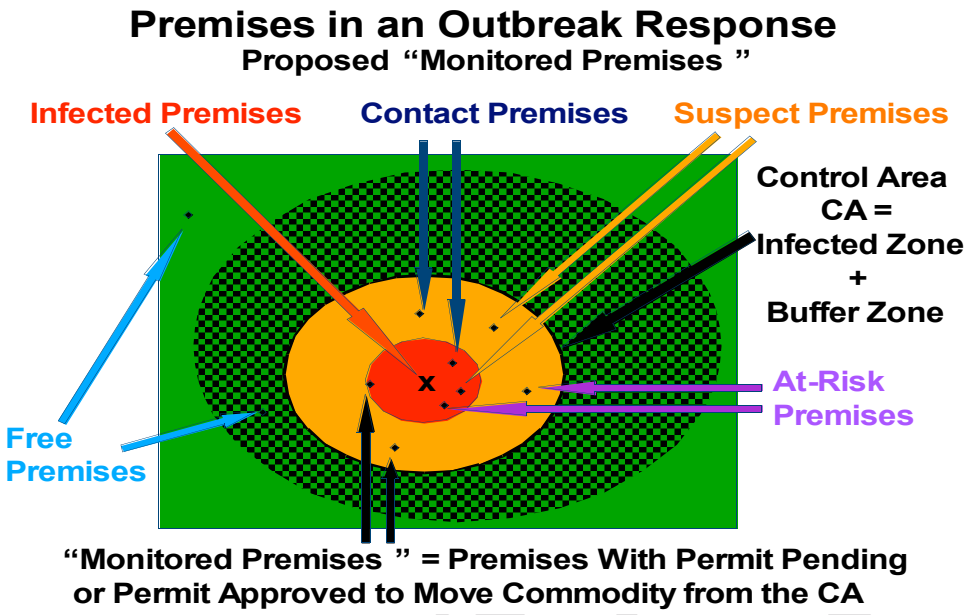
Appendix 8. United Egg Producers/United Egg Association Movement Control Model Plan

United Egg Producers/United Egg Association
USDA APHIS Veterinary Services

Highly Pathogenic Avian Influenza (HPAI)
Movement Control Model Plan: Commercial Layer Industry Operations

Protocol for the Movement of Liquid Egg Product, Further Processed Egg Products, Inedible Egg, Table Eggs and Broken Egg Shells, Egg-Type Hatching Eggs, and Day-Old Chicks Within, Out of, and Into a Defined **"Control Area"**





1. **Flocks that are determined to be "Infected" premises by epidemiological investigation and/or diagnostic testing:**

a. Definition of "Infected" premises: "Infected" premises are premises where HPAI is presumed or confirmed to exist based on laboratory results and compatible clinical signs. All presumed positive premises and confirmed positive premises are classified as infected premises. In addition, all other premises that meet the current case definition for HPAI are classified as "Infected" premises.

b. Disposition of "Infected" premises: "infected" premises are quarantined immediately, and all domesticated birds and other susceptible livestock are depopulated and disposed of in proper biosecure procedures. No movement of susceptible species or their products (e.g., shell eggs, hatching eggs, day old chicks, broken egg shells, unpasteurized liquid egg product, pasteurized egg products) will be allowed off the "Infected Premises" premises, except for disposal and must be moved under permit.

2. **Flocks that are determined to be "Contact" premises by epidemiological investigation:**

a. Definition of "Contact" premises: "Contact" premises are premises with birds or other susceptible animals, *conveyances, or products* that have been exposed directly or indirectly to birds and other animals, products, materials, people, or aerosol from an "Infected" premises. The specific exposure factors to be considered must be appropriate to the epidemiology of HPAI. The commercial layer industry HPAI "Contact" premises" include the following direct or indirect contact sources:

i. Premises with susceptible birds exposed to poultry **manure** from an infected flock (virus in manure).
ii. Premises with susceptible birds exposed to **dead poultry** from an infected flock (virus in carcasses, etc).
iii. Premises with susceptible birds exposed to **live poultry** from an infected flock (virus in bird & secretions & excretions).
iv. Premises with susceptible birds exposed to **eggs or egg handling materials** from an infected flock (HPAI virus in and on egg).
v. Premises with susceptible birds with **unprotected exposure to equipment** that have been in contact with infected birds, manure, carcasses, or eggs. Unprotected means inadequate sanitation procedures for those items/people that come into contact with an infected flock.
vi. Premises with susceptible birds with **unprotected exposure to people** that have been in contact with infected birds, manure, carcasses, or eggs.
vii. Premises involved in depopulation of infected flocks.

b. Disposition of "Contact" premises: "Contact" premises will be quarantined and subject to strict biosecurity measures, daily monitoring of mortality in each house, and intensive surveillance for HPAI viruses in each house by RRT-PCR testing (see 5 below) for at least 42 days or until the Incident Commander is convinced that no HPAI is present on the premises,

i. Following complete epidemiological investigation, biosecurity assessments, and negative diagnostic testing for HPAI, "Contact" premises can be re-designated as "At-Risk" premises or "Monitored" premises.
ii. "Contact" premises with 75,000 hens or more will not be depopulated until a diagnosis of HPAI has been confirmed by case definition or diagnostic testing.
iii. "Contact" premises that are determined to be HPAI infected by case definition or diagnostic testing will be depopulated immediately.
iv. Movement from "Contact" premises is allowed by permit only.

3. Flocks that are determined to be "Suspect" premises by epidemiological investigation:

a. Definition of "Suspect" premises: "Suspect" premises are premises where birds or other susceptible livestock are under epidemiological investigation for a report of clinical signs compatible with HPAI, but the case definition for HPAI has not been met, and HPAI has not been detected or confirmed by diagnostic testing.

b. Disposition of "Suspect" premises": "Suspect" premises will be quarantined and subject to strict biosecurity measures, daily monitoring of mortality in each house, and surveillance for HPAI viruses in each house

by RRT-PCR testing (see 5 below), until the conditions are met to re-designate the "Suspect" premises as an "Infected" premises," a "Contact" premises, an "At Risk" premises, or a "Monitored" premises.

i. "Suspect" premises must have complete epidemiological investigation, biosecurity assessments, and test negative for HPAI before being re-designated a "Monitored" premises (see 6 below).
ii. "Suspect" premises with 75,000 hens or more will not be depopulated depopulated until a diagnosis of HPAI has been confirmed by case definition or diagnostic testing.
iii. "Suspect" premises that are determined to be HPAI infected by case definition or diagnostic testing will be depopulated immediately.
iv. Movement from "Suspect" premises is allowed by permit only.

4. **Flocks that are designated as "At-Risk" premises prior to epidemiological investigation:**

a. Definition of "At-Risk" premises: "At-Risk" premises are those premises in the Infected Zone or Buffer Zone that have susceptible animals, but none of those susceptible animals have clinical signs compatible with HPAI. "At-Risk" premises have not been subject to epidemiological investigation, biosecurity assessments, or diagnostic testing for HPAI to warrant a re-designation to "Contact" premises or "Monitored" premises.

b. Disposition of "At-Risk" premises: After complete epidemiological investigation, biosecurity assessments, and diagnostic testing for HPAI, "At-Risk" premises can be re-designated to "Contact" premises or "Monitored" premises.

i. Movement from "At-Risk" premises is allowed by permit only.

5. **Flocks that are determined to be <u>"Monitored" premises</u> by epidemiological investigation:**

a. Definition of "Monitored" premises: "Monitored" premises are in close proximity to an infected flock, and are located in the Infected Zone or Buffer Zone, which comprise the Control Area. "Monitored premises" can objectively demonstrate that they are not to be designated as "Infected" premises, "Contact" premises, "Suspect" premises, or "At-Risk" premises, following complete epidemiological investigation, biosecurity risk assessments, and diagnostic testing for HPAI. "Monitored" premises objectively demonstrate:

i. "Monitored" premises objectively demonstrate that they do not meet the definitions for "Infected" premises, "Contact" premises "Suspect"

premises or "At-Risk" premises by complete epidemiological investigation and questionnaire.

ii. "Monitored premises" objectively demonstrate that systematic biosecurity measures and precautions have been taken to protect the premises against HPAI.

iii. "Monitored" premises objectively demonstrate flock health parameters

iv. "Monitored" premises objectively demonstrate diagnostic testing negative for HPAI.

b. Disposition of "Monitored" premises:

i. Premises located within the Control Area must only be designated "Monitored" premises by the Incident Commander or their designee.

ii. The designation of "Monitored" premises during an actual incident by the Incident Commander or their designee will be facilitated and accelerated by biosecurity risk assessments conducted prior to the incident, by rapid epidemiological investigation and epidemiological questionnaire at the time of the incident, strategic placement of diagnostic sampling equipment prior to the incident, and tactical execution of diagnostic sample testing at the start of an incident.

iii. "Monitored" premises, depending upon their location, the actual incident circumstances, and epidemiological considerations of the actual outbreak, will be granted permits to move Liquid Egg Product, Further Processed Egg Products, Inedible Egg, Table Eggs and Broken Egg Shells, Egg-Type Hatching Eggs, and Day-Old Chicks Within, Out of, and Into a Defined "Control Area" at the discretion of the incident commander or their designee.

6. Determination of non-infected layer industry flocks in the Control Area.

a. The absence of infection will be documented by requiring chickens from flocks that are not exhibiting signs of the disease and that show no unexpected increase in mortality from each house on the farm to be tested each day and found to be negative by the real time reverse transcriptase – polymerase chain reaction (RRT-PCR) or other suitable procedure as determined by the Incident Command.

i. A minimum or five chickens from the daily mortality and/or from euthanized sick birds from each house (flock) will be placed in a leak proof container (e.g. heavy duty plastic garbage bag) each morning. Each container will be labeled with the farm of origin, house of origin, and the number of birds found dead in the house that day. The containers will be taken to a designated pick-up point, typically the public road closest to the premises.

Rationale: In a large commercial poultry house (100,000 layers) normal" mortality will be about 10 per day. A doubling of normal mortality to 20 due to HPAI (dead bird prevalence of 50 percent and flock prevalence of

0.04 percent) would be detected by sampling 5 dead birds. Historically, APHIS sampled 5 dead birds **per week** to monitor chicken houses in the END outbreak in CA and this plan requires daily monitoring. The proposed AI plan requires daily monitoring and will be 7 times more effective than the monitoring during the END outbreak. It is not unusual for mortality to fluctuate that much from day to day, so sampling dead/sick birds every day is likely more sensitive than monitoring weekly mortality (where a trend over 2 or 3 days might be observed before acting). It is reasonable to assume that 50 percent of the sick and dead birds (in a house that is infected with HPAI) would actually be shedding AI virus then a sample size of 5 birds would allow you to have 95% confidence of finding the virus in the sick or dead birds.

ii. A State or Federal regulatory official or an individual authorized by the Incident Command will take a "oropharyngeal" swab from each chicken. Five oropharyngeal swabs will be pooled in a tube containing brain-heart infusion (BHI) broth. Sample pooling will be done on a per house basis. One BHI tube containing oropharyngeal samples (5 oropharyngeal swabs/BHI tube) will be submitted as directed by the Incident Command to an authorized State Veterinary Diagnostic Laboratory (VDL). These samples must be submitted on the day of sample collection by the State or Federal regulatory official or an individual authorized by the Incident Command. The State VDL and the IC will establish the time of day by which samples must be submitted to an authorized VDL (example, by 12:30 pm). VDL personnel will perform RRT-PCR testing on these samples immediately upon receipt and electronically send test results to the Incident Command (IC) by the end of each day. The IC will report the test result information to the premises as soon as it is available.

7. Movement of liquid egg product, further processed egg products, inedible egg, table eggs and broken egg shells, egg-type hatching eggs, and day-old chicks from "Monitored" premises**.**

a. Movement of liquid egg product, table eggs, egg-type hatching eggs, further processed egg products, and broken egg shells *within and out of* a Control Area from "Monitored" premises will be allowed by permit for those flocks inside the control area testing negative (see Section 5 above) as follows, including any unsold inventories on hand:

i. USDA FSIS inspected pasteurized egg products, or precooked egg products produced by plants within a control area may move within or out of the Control Area by Permit (accompanied by documentation of origin of the products). The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area.

ii. Unpasteurized liquid egg product may move in officially FSIS sealed vehicles per 9 CFR Chapter III Part 590.410 from breaking operations within the Control Area directly to pasteurization plants located within or out of the Control Area by permit. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver willnot be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area.

iii. Inedible egg from graders and/or breaking plants in a Control Area may move by permit for pasteurization or to approved waste disposal sites within or outside the Control Area. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area.

iv, Washed and graded shell eggs destined for food service, retail marketing, further processing, or for breaking may be moved out of the Control Area by permit if they have been washed and sanitized using 100 – 200 ppm chlorine solution. The transport vehicle shall be sealed by farm or company personnel under the authorization of the Incident Command. Egg handling materials used in the transport of eggs to breaking or further processing plants must be destroyed at the plant or cleaned, sanitized (following accepted procedures) and returned to the premises of origin without contacting materials going to other premises. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area.

v. Nest run shell eggs (not washed and sanitized) must be moved directly for washing and grading, further processing, or to an offline breaking operation. The transport vehicle shall be sealed by farm or company personnel under the authorization of the Incident Command. Egg handling materials must be destroyed at the destination plant or cleaned, sanitized (following accepted procedures) and returned to the premise of origin without contacting materials going to other premises. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area.

vi. Broken eggshells on the farm or from breaking plants, pasteurization plants, and/or further processing plants may be moved by permit. The transport vehicle shall be sealed by farm or company personnel under

the authorization of the Incident Command. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area.

vii. Hatching eggs from source flocks tested negative for AI virus by daily mortality sampling may be moved to hatcheries within the Control Area with a permit. Egg handling materials must be destroyed at the hatchery or cleaned, sanitized (following accepted procedures) and returned to the premise of origin without contacting materials going to other premises. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area, and a permit is required to move within and out of the Control Area

viii. Hatching eggs from "Monitored Premises" source flocks tested negative for AI virus by daily mortality sampling may be moved out of the Control Area by permit. The chicks must be placed under a "post-hatch" quarantine for 30 days. Egg handling materials must be destroyed at the premises of destination or cleaned, sanitized (following accepted procedures) and returned to the premise of origin without contacting materials going to other premises. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area. The State Veterinarian of the state of destination must be faxed a copy of the restricted movement permit within 24 hours of issuance, and a permit is required to move within and out of the Control Area.

ix. Day-old chicks from source flocks tested negative for AI virus by daily mortality sampling may be shipped by permit within or out of the Control Area and must be placed under a 30 day quarantine. The State Veterinarian of the State of destination must be faxed a copy of the restricted movement permit within 24 hours of issuance. Hatcheries may receive eggs that originate outside the Control Area (accompanied by documents showing the origin of the eggs and the AI negative status of the source flock) without a permit. The cargo interior and exterior of the transport vehicle must be cleaned and disinfected. The driver will not be allowed outside the cab or else the cab interior must also be cleaned and disinfected. The tires and wheel wells must also be cleaned and disinfected before leaving the premises within the Control Area.

x, The Incident Command or designate will evaluate and approve the risk assessment and risk mitigation procedures necessary to move products

by permit. A permit must be issued and seals placed on the vehicle by a State or Federal regulatory official or a person authorized by the Incident Command. The Incident Command will authorize procedures to break the seals outside of the control area with proper documentation.

b. Movement of liquid egg product, shell eggs, broken egg shells, and hatching eggs *into* a Control Area will be allowed without permit under the following conditions:

i. Pasteurized liquid egg product and unpasteurized liquid egg (and blends) from breaking plants and/or pasteurization plants outside a Control Area (and accompanied by documentation of origin) may move into pasteurization and/or further processing plants located in a Control Area without permit. The driver will not be allowed outside the cab or else the cab interior must be cleaned and disinfected. The exterior of the transport vehicle and the tires and wheel wells must be cleaned and disinfected before leaving the premises in a Control Area, and a permit is required to exit the Control Area.

ii. Shell eggs may move into breaking, grading, pasteurization, and/or further processing plants from outside Control Areas (accompanied by proof of origin) without a permit. Egg handling materials must be destroyed at the plant or cleaned, sanitized (following accepted procedures) and returned to the premise of origin without contacting materials going to other premises. The driver will not be allowed outside the cab or else the cab interior must be cleaned and disinfected. The exterior of the transport vehicle and the tires and wheel wells must be cleaned and disinfected before leaving the premises within a Control Area, and a permit is required to exit the Control Area.

iii. Broken egg shells may move into a Control Area (accompanied by proof of origin) without a permit. The driver will not be allowed outside the cab or else the cab interior must be cleaned and disinfected. The exterior of the transport vehicle and the tires and wheel wells must be cleaned and disinfected before leaving the premises within a Control Area, and a permit is required to exit the Control Area.

iii. Hatching eggs may move into a hatchery from outside Control Areas (accompanied by proof of origin and AI tested negative flocks without a permit. Egg handling materials must be destroyed at the plant or cleaned, sanitized (following accepted procedures) and returned to the premise of origin without contacting materials going to other premises. The driver will not be allowed outside the cab or else the cab interior must be cleaned and disinfected. The cargo interior and exterior of the transport vehicle and the tires and wheel wells must be cleaned and disinfected before leaving the premises within a Control Area, and a permit is required to exit the Control Area.

8. Determination of Release of Movement Restrictions

a. All premises within the Control Area will be eligible for release from movement restrictions as determined by the Incident Command when:

i. All infected flocks in a Control Area have been depopulated. All depopulated flock premises have been cleaned and disinfected. A minimum of 42 days have passed, or environmental sampling has proven HPAI virus negative status for the depopulated premises.

ii. All contact premises in a control area must have been depopulated or must have been monitored for 42 days.

9. Appendices.

a. APHIS CEAH - Egg Sector Working Group – University of MN CAHFS Pasteurized Liquid Egg Risk Assessement.

This plan has been written by egg industry and university personnel based on their knowledge of the egg industry. Standard Operating Procedures from the Exotic Newcastle Disease (END) outbreak were reviewed as a starting point for developing this plan.

Appendix 9. Email from Roger Glasshoff, AMS National Supervisor Shell eggs

Regarding list of approved egg cleaners and sanitizers:

7/23/08 to Camille Effler

The subject "List" was actually published by the Food Safety and Inspection Service of USDA. However, in 1997, FSIS notified the industry that, as an agency, it would no longer review for approval any compounds for cleaning, sanitizing, lubricating, marking, etc., for use in official processing plants. The Department discontinued publication of the "List" in 1998 and advised industry that a letter of guarantee must be provided musty be provided from the manufacturer of a compound indicating its acceptability as safe for the intended use. In 2001, FSIS stated that although a specific compound appears acceptable on the "1998 List", the industry must have a letter of guarantee that the compound formula has not been changed since approved previously by FSIS. Similarly, AMS established the policy of requiring the letter of guarantee for such compounds for use in plants operating under the Regulations Governing the Voluntary Grading of Shell eggs. Some State programs that are responsible for the processing of eggs for human consumption (non-official plants) may still be referencing the "1998 List." Unfortunately, I do not have a list any longer.

The National Sanitation Foundation (NSF) provides compound manufacturers an approval process on a fee basis in accordance with FDA regulations and posts the approved compounds (with their intended use) on their website – www.nsf.org. The NSF also requires that a manufacturer confirm on an established frequency that the compound formulation has not changed. This practice assures that the NSF list of compounds remains current.

Appendix 10. An Estimate of the Baseline Detection Threshold for Table-Egg Layer Flocks Using Normal Mortality Data

An exponential increase in mortality over time is an important clinical sign of HPAI infection. Although HPAI disease would mostly be detected through diagnostic testing with the active surveillance protocol described in UEP/UEA – USDA APHIS VS Movement Control Model Plan, it might be detected through increased mortality in the flock in some rare instances. We selected a mortality threshold as a complementary detection mechanism in addition to RRT-PCR testing, to consider the cases where HPAI infection might be detected due to increased mortality. In this appendix, we estimate a conservative daily mortality threshold for detecting HPAI disease in the flock based on weekly and daily mortality data for egg layer flocks in the US.

Methods

Normal daily mortality in table-egg layers is experienced throughout the production cycle. In the event of a HPAI outbreak, the total daily mortality pool will contain birds that die as a result of HPAI disease as well as birds that die from other causes. In order to estimate the number of birds that die from other causes, data from commercial table-egg layer flocks were requested for analysis. Weekly mortality totals were initially made available for analysis. Weekly average data cannot be directly used to characterize the variability in daily mortality. However, we used weekly data to derive conservative upper bounds on daily normal mortality. We later refined the analysis and upper bound when daily mortality data were made available.

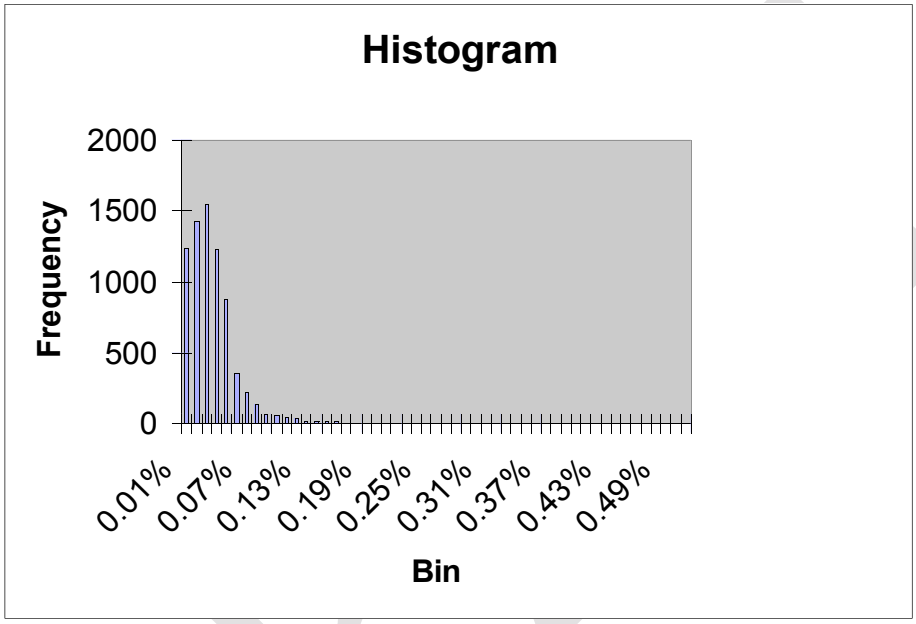
Data sources

Weekly mortality data: Data were provided by AgriStats Inc. through Iowa State University, Veterinary Extension. These data consisted of total weekly mortality (daily mortality summed over 7 days) for 27 layer flocks for an entire production cycle. The duration of production ranged from 60 to100 weeks depending on whether or not the flock was molted. The data represent a sample of 9 flocks each from the 3 major egg layer breeds located in 3 different geographic regions (Iowa and Minnesota, California, and, Pennsylvania).

Daily mortality data: Since daily mortality data records are not commonly kept, these data were provided by the egg industry through Iowa State University by special request. The location of the farms was masked to ensure confidentiality. The data are from 12 flocks: 4 flocks each from the 3 major egg layer breeds in the U.S. The data represent one the entire production cycle (84 weeks on average). These data are less representative of the variation between layer operations across different geographic regions as compared to the data provided by AgriStats Inc.

Summary statistics

Weekly mortality data: There were 1772 data points in this data set. The mean weekly average of daily mortality was 0.028 percent (s.d. 0.038 percent). The daily mortality was less than 0.16 percent in 99 percent of the observations.
Daily mortality data: There were 7113 data points in this data set. The mean daily mortality was 0.031 percent (s.d. 0.027 percent). A histogram of the daily mortality data is provided in appendix 10 figure 1.



Appendix 10 Figure 1. Histogram of daily mortality data.

Results based on Agristats, Inc., data:
We can apply Markov's inequality to derive a conservative upper bound on the probability that the daily mortality is higher than 0.5 percent using Agristats weekly data. Given a mean a mean weekly average of daily mortality of 0.028 percent and applying Markov's inequality, there is 0.944 probability that the daily mortality is less than 0.5 percent.

Results based on daily mortality data:
Given a larger data set with 7113 data points, we used these data directly (empirical distribution) for conducting the analysis. Based on the daily data, the probability that the daily mortality is less than 0.25 percent is 0.999. The probability that the daily mortality is less than 0.5 percent is 0.9997.

Conclusions
We conclude that 0.5 percent is a very conservative limit for normal daily mortality for egg layer flocks in the US. Since normal daily mortality rarely exceeds 0.5 percent each day, this threshold may be used as an upper bound for an extreme event in the disease transmission model.

APPENDIX 11. Simulation of Active Surveillance Protocol for the Scenario where the Flock was infected before the Implementation of the Active Surveillance Protocol

Introduction

In the main document, we assumed that the daily RRT-PCR testing included in the active surveillance protocol was implemented before the flock was initially infected. Under this scenario, there would be multiple days of RRT-PCR testing before the HPAI infection can spreads to a significant portion of the flock. Accordingly, the expected number of contaminated eggs moved before detection would be very low under this scenario. The assumption that daily RRT-PCR testing specified in the active surveillance protocol was being conducted before the flock was initially infected would be well justified after the first day of RRT-PCR testing in the outbreak.

Conversely, if a significant fraction of the flock was already infected by the time daily RRT-PCR testing was implemented, a greater number of contaminated eggs might be moved before detection. For example, suppose the flock had been infected for 5 days when diagnostic testing was first conducted; the chance of detection with the first RRT PCR test would be 86.5 percent. In the 13.5 percent of the cases where HPAI infection was not detected in this example, according to the disease transmission model 511 contaminated eggs would be moved before detection.

In this appendix, we estimate the maximum daily number of contaminated eggs before detection considering that the flock could have been infected for 1-5 days on the day on which the first pooled sample is tested with RRT-PCR. We developed an alternate simulation model of the active surveillance protocol where the flock could have been infected for 1-5 days on the day the first RRT-PCR tested and performed 12000 simulations with @RISK.

Simulation Results

Maximum daily number of contaminated eggs moved among all the days before infection is detected in the flock assuming that the flock was infected for 1-5 days before the active surveillance protocol was implemented.

Assuming that the flock could have been infected for 1-5 days on day 1, the expected value of the maximum daily number of contaminated eggs moved among all days before infection is detected in the flock is 22 (90 percent P.I. 0-85). This estimate is higher than that for the scenario where daily RRT-PCR testing was implemented before the flock was initially infected (as in the main document i.e., 11 with 90 percent P.I. 0-44).

The expected value of the maximum daily number of contaminated eggs moved among all the days from the second day of RRT PCR testing to the day infection is detected is 7 (90 percent P.I. 0-18, max 969).

Conclusions

The estimated maximum number of contaminated eggs moved before HPAI infection is detected in the flock, is greater in the scenario where the flock is assumed to have been infected for 1-5 days before the active surveillance protocol was implemented compared to the case RRT-PCR testing is implemented before the infection of the flock. The difference between the two scenarios is likely related to the greater expected number eggs moved on day 1 after first RRT-PCR test compared to other days for which some account of previous RRT-PCR testing is available.

However, the magnitude of the difference between the cases where the flock has been infected before or after the daily RRT-PCR testing has been implemented is not substantial and does not impact the conclusions of the risk assessment.

Appendix 12. The Use of "Negligible Risk" in this Assessment

Negligible Risk Defined for this Analysis:

For this risk analysis, the term "negligible risk" means there is a very low likelihood that moving shell eggs will cause infection in another poultry production premises. The specific magnitude cannot be determined, as there is no evidence that these products have ever served as a transmission pathway. In quantitative terms, this is defined as a likelihood of less than 1/1,000,000 that moving these products will result in infection in another 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 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. [118-122]While this level has not been formally 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.[123]

Use in Agricultural Risk Analysis

The use of risk analysis for imports of agricultural products became mandatory with the adoption of the SPS Agreement[rr] in 1995.[ss] 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 Organization for Animal Health).[124] The OIE has published standards and guidance [125 126]for conducting risk analysis, but has not formally defined "negligible" in a quantitative sense.[127] However, in a World Trade Organization trade dispute case,[128] negligible risk was considered to be a risk whose probability is very low,[129] 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."[130]

Policy Implications of a Quantitative Definition for Negligible

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,"[131] vague and inconsistent,[122] and has been "used and (abused) in

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

[ss] Risk analysis is also required for moving animals and animal products during an HPAI oubreak.[136]

various policy contexts."[132] However, use of this figure is meant to be a very 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."[125] 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.[133] 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.[134] These opinions by the court system are also consistent with U.S. views expressed in WTO trade disputes.

Appendix 13. The Use of "Low Risk" in this Assessment

Low risk
For this risk analysis, the term "low risk" means it is highly unlikely that moving shell eggs will cause infection in another poultry production premises. The specific magnitude cannot be determined, as there is no evidence that these products have ever served as a transmission pathway. The determination of "low risk" suggests that although not a strict 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 a significant variation in the interpretation of the terms used to describe risk among various risk assessments. For example, in USDA APHIS guidelines on pathway initiated pest risk assessment, the rating of low is interpreted as "the pest will typically not require specific mitigations measures". The FDA guidance document 152 states "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". In a risk rating system used in USDA APHIS template 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. Overall, various definitions of "low risk" have been utilized as appropriate in different situations.

Low risk vs Negligible risk.
There are two related reasons for rating a risk as low vs. negligible. The first is that the risk assessment determined that the level of risk is not negligible and hence additional mitigation measures may be considered in some cases. A second reason is that due to the uncertainty in the risk estimate, it is not clear whether the risk is low or negligible. This uncertainty can be expressed as probability distribution for the risk in a fully quantitative risk assessment. For qualitative risk assessment, there are no formal means of expressing this uncertainty. Therefore, when there is uncertainty about whether the risk is low or negligible, we conservatively rate it as "low risk". In this case, depending on the circumstances, additional mitigation measures or further evaluation to reduce the uncertainty may be considered.