DRAFT Interagency Risk Assessment for the Public Health Impact of Highly Pathogenic Avian Influenza Virus in Poultry, Shell Eggs, and Egg Products

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1 Executive Summary

Background

The United States Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) developed a quantitative risk assessment for the highly pathogenic avian influenza virus(es) (HPAIV) in food in collaboration with the Department of Health and Human Services' (DHHS) Food and Drug Administration (FDA) and USDA's Animal and Plant Health Inspection Service (APHIS). The risk assessment was developed by an Interagency Workgroup formed from representatives of each of these three agencies. The purpose of this risk assessment was to 1) estimate the exposure and potential human illness from consumption of HPAIV-contaminated poultry, shell eggs, and egg products from the index flock, and 2) examine the effectiveness of mitigation strategies to control HPAIV if detected in the United States.

Public Health Context

Avian influenza viruses are typically species-specific, causing disease in birds. However, H5N1 and other H5 and H7 HPAI subtypes have recently become a zoonotic concern. In June 2008, the World Health Organization reported that worldwide from 2003-2008, there have been 385 confirmed HPAIV human illnesses, resulting in 243 deaths. Retrospective studies have determined that the majority of these cases are associated with close contact with live or dead HPAIV-infected birds likely caused by respiratory inhalation of infective droplets or self-inoculation (*e.g.*, by a human handler touching mucous membranes or conjunctiva after contact with avian fecal contamination, avian respiratory secretions, or avian body fluids), rather than consumption of poultry or shell eggs or egg products. Currently, there is no compelling epidemiological evidence linking the consumption of cooked poultry meat, shell eggs, or egg products to human illness caused by HPAIV. HPAIV is not considered to be a foodborne pathogen although the virus has been isolated from poultry muscle and the interior of eggs. Two HPAIV-confirmed human illnesses may have been related to the consumption of infected raw duck blood products, although contact with live or dead HPAIV-infected poultry could not be epidemiologically excluded. Despite this lack of evidence, the possibility of poultry and egg consumption as an exposure route to HPAIV remains a concern to food safety experts. In light of this and the recent HPAIV poultry and human illnesses in Asia, Africa, Europe, and the Middle East, the Interagency Workgroup developed this food safety risk assessment for HPAIV exposure and illnesses in humans from consumption of poultry meat, shell eggs, and egg products.

Model Approach

The risk assessment model simulates human exposure and potential illness from consumption of H5 and H7 HPAI strains that can make humans ill and lead to death. Exposure from HPAIV is modeled separately for poultry meat and for shell eggs. Each model consists of three modules representing production, processing, and consumer preparation. The production module assumes introduction of HPAIV into the index flock of a single U.S. meat or egg poultry house following

HPAIV entry into the U.S. A bird-to-bird transmission model simulates HPAIV spread to estimate within-flock prevalence of HPAIV at the farm and for poultry meat production, during transportation. For an infected flock destined for meat production the transmission model simulates an increase in the prevalence of HPAIV in the flock until substantial bird mortality would allow the disease to be detected or the undetected flock is sent to slaughter. In the processing module, it was assumed birds sent to slaughter are subject to federal inspection, which could result in the removal of infected birds. The likelihood an infected bird is identified due to visible pathology was dependent on how long the bird was infected before slaughter. The amount of HPAIV in each serving of poultry is related to the time between infection and slaughter. For the shell egg model, egg production continues to be simulated until substantial bird mortality would allow the disease to be detected. Routine inspection of shell eggs sent to processing prior to flock detection would not detect HPAIV within shell eggs, but may identify non-specific markers of HPAI. Therefore, for the purpose of the model, shell eggs with visible pathology (*e.g.*, thin-shelled, soft-shelled, or abnormally small) are removed from commerce and are not included in the risk estimates. The consumer preparation module examines the impact of cooking and cross-contamination on levels of HPAIV, thereby resulting in estimates of the level of HPAIV ingested by the consumer. The predicted amount of contaminated poultry meat or number of infected eggs available for human consumption is used along with a dose-response function to estimate the number of potential human illnesses. Other routes of exposure such as inhalation, mucosal contact, and wound exposures by food preparers and consumer contact with contaminated raw poultry and shell eggs, as well as farm and processing occupational exposures, are not addressed in this risk assessment.

Results

This risk assessment has been developed as a tool to evaluate mitigation scenarios should HPAIV be identified in the U.S. The number of human illnesses predicted serves as a basis to assess the magnitude and effectiveness of mitigation strategies. Given the uncertainty regarding the dose-response relationship and the uncertainty regarding the likelihood of human illness from consumption of poultry and eggs, the model-predicted number of human illnesses should not be considered an absolute value, and it should not be used outside of the context of the scenario analysis described below. Using scenario analysis, the following outputs were identified:

1. *Poultry Model*

- If a flock is exposed to HPAIV, the model predicts a 94 and 98% probability that a chicken and turkey flock, respectively, would be identified as HPAIV-positive before slaughter and not enter commerce. This is because flocks infected early in the grow-out period will have enough time to demonstrate significant mortality (≥ 2% flock morality over a single day) on the farm, resulting in identification of the flock as HPAIV-positive.

- There is a 6 and 2% probability that an HPAIV-infected chicken or turkey flock, respectively, may go to slaughter without detection of the disease. This would happen when HPAIV infects a flock that is approaching market weight with not enough time for

the flock to demonstrate significant mortality on the farm. In these instances, some fraction of HPAIV-contaminated poultry meat may enter commerce.

- On-farm HPAIV testing as a potential mitigation strategy has the greatest impact of lowering predicted illnesses. Approximately 95% of illnesses are mitigated if flocks are tested immediately before being sent for slaughter.

- Increased on-farm surveillance of daily flock mortality is predicted to reduce human exposure and illness. However, the model predicts that relying on a single day of flock mortality to detect all HPAIV-infected, but undetected flocks is impractical. This is because a flock may have few dead birds if infected late in its grow-out period as about 36 to 42 hours are required before infected birds die from HPAI.

- Increased surveillance at processing during FSIS' antemortem inspection is predicted to reduce human exposure and illness. However, the model predicts that using the number of dead birds following transportation to trigger detection of all HPAIV-positive flocks is impractical given that a flock may have few dead birds if infected late in its grow-out period.

- Cooking poultry to the FSIS recommendation of 165°F is predicted to inactivate the virus and result in negligible risk to public health from HPAIV-contaminated poultry meat.

- Cross-contamination of HPAIV from contaminated poultry to foods not likely to be cooked resulting in oral uptake increased the number of predicted illnesses by approximately 2.5%. Consumer messages should continue to emphasize measures to prevent the potential cross-contamination of HPAIV and other microbiological hazards.

2. Shell Egg and Egg Products Model

- If a 100,000 hen flock becomes infected with HPAIV, the baseline scenario predicts that 11,293 HPAIV-contaminated eggs are produced before the flock is discovered as HPAIV-positive. However, the baseline model predicts no human illnesses because > 99.99% of HPAIV-positive eggs would still be in the distribution chain at the time of diagnosis and not yet be available for consumers to purchase. This assumes that all HPAIV-positive eggs can be removed from distribution.

- If the risk assessment model baseline assumptions are changed using scenario analysis, HPAIV-positive shell eggs may enter commerce. Thorough cooking of eggs (150 ^{o}F) results in inactivation of the virus; however, if eggs are not cooked completely, the model predicts a few illnesses are possible. For example, the model predicts two illnesses may occur before a flock is identified as HPAIV-positive in a scenario where eggs reach market within 24-hours.

- As a mitigation strategy, removing HPAIV-positive shell eggs from commerce will reduce potential exposure. Effectiveness is dependent on how many days of eggs production are removed. The model predicts that greater than 97% of potentially contaminated shell eggs can be removed from commerce given a 2 day market withdrawal.

- In-shell pasteurization of HPAIV-positive eggs is predicted to inactivate the virus and result in negligible risk to public health.

- Data from USDA's Agricultural Research Service show that FSIS time and temperature recommendations for egg product processing are sufficient to inactivate HPAIV, therefore this risk assessment model does not quantitatively assess the risk of illness from HPAIV-contaminated egg products. Only dried egg white processing may not completely inactive HPAIV; however, the process of preparing dried egg whites requires a minimum of 7 days. It is likely that the hen flock that produced the contaminated eggs would have been identified as HPAIV-positive before the process is completed, and egg products processors would be alerted to the problem. APHIS is currently developing a separate risk assessment to assess the risk of illness from HPAIV-contaminated egg products.

Summary

This quantitative risk assessment provides a science-based, analytical approach to collate and incorporate available data into a mathematical model, and it provides risk managers a decision-support tool to evaluate the effectiveness of interventions to reduce or prevent foodborne illness from HPAIV in the U.S. This risk assessment can also be used to target risk communication messages, identify and prioritize research needs, and provide a framework for coordinating efforts with stakeholders. The risk assessment is being used to help guide APHIS's HPAI emergency response planning and FDA's HPAI preparedness.

Although unlikely, the risk assessment demonstrates that some amount of HPAIV-contaminated poultry and shell eggs could enter commerce. The data indicate that there is a 3.5- or 6-day window during which potentially HPAIV-positive poultry or shell eggs, respectively, could escape detection. The model predicts that consumption of HPAIV-contaminated poultry and shell eggs poses a negligible risk if properly cooked. At the same time, data suggest that some people will undercook these products and could become exposed and possibly ill.

The model shows that preventive measures, such as HPAIV-flock testing and increased inspection, would result in increased detection of HPAIV-contaminated flocks and reduce the risk of HPAIV illnesses by preventing the consumption of contaminated poultry. In addition, effectively recalling shell eggs would substantially reduce the risk to consumers from HPAIV-contaminated shell eggs.

2 Introduction

2.1 Scope

Due to recent poultry outbreaks of HPAI associated with poultry in Asia, Africa, Europe, and the Middle East and the subsequent heightened awareness of the public for food safety, the Interagency Workgroup developed a farm-to-table food safety risk assessment for HPAIV from consumption of poultry, shell eggs, and egg products. Though consumption of HPAIV–contaminated poultry, shell eggs, and egg products is not epidemiologically associated with human illness, the possibility of illness from such an exposure route remains. Given the potential public health risk, the risk assessment focuses on the H5 and H7 HPAI strains that can make humans ill and occasionally lead to death. FSIS initiated the planning and development of this risk assessment to 1) estimate the exposure and potential human illness from consumption of HPAIV-contaminated poultry, shell eggs, and egg products[1], and 2) examine the effectiveness of current and future policy to control HPAIV if detected in the U.S. The poultry and egg models only assess exposure and risk associated with a single flock, the index flock, and does not address transmission among houses or flocks.

The risk assessment was intended to answer the following risk management questions:

1. What is the risk of human exposure to and subsequent illness from consumption of HPAIV-contaminated poultry meat, shell eggs, and egg products?

2. What is the effectiveness of interventions to reduce human exposure and illness from the introduction of HPAIV into commerce from shell eggs? The following scenario analyses were also addressed:

a. Evaluate the reduction in human exposure to HPAIV from contaminated shell eggs assuming the infected flock is identified and closed[2] following various days after infection.

b. Evaluate the reduction in human exposure to HPAIV from contaminated shell eggs following market withdrawals/recalls of eggs laid various hours (*e.g.* 12, 24, 48, 72, and 96 hours) before the house was identified as infected and closed.

The scope of this assessment is limited to where the Food Safety and Inspection Service (FSIS), Food and Drug Administration (FDA), and to a lesser extent, the Animal and Plant Health Inspection Service (APHIS) have direct statutory authority that could impact HPAIV as a food

[1] Egg products are qualitatively evaluated while poultry and shell eggs are quantitatively evaluated. APHIS is currently developing an egg products risk assessment.

[2] A closed flock is defined as a flock where poultry or poultry products are held before the suspected flock is tested, reported, and officially quarantined.

safety concern. This analysis is limited to strains of HPAIV currently causing poultry outbreaks in Asia, Africa, Europe, and the Middle East, and which occasionally result in human morbidity and mortality. This analysis does not apply to HPAI strains that are occasionally detected in U.S. poultry flocks but are rarely associated with human infection. This risk assessment quantitatively assesses direct foodborne exposure and potential illness of humans to HPAIV from consumption of HPAIV-contaminated poultry and shell eggs prepared by consumers and qualitatively addresses egg products. Poultry includes chickens and turkeys and does not include other fowl, such as ducks or geese. Poultry and shell eggs designated for preparation and cooking at foodservice outlets, such as restaurants, and other institutions, such as hospitals, schools, etc., are not assessed. The risk assessment addresses only those products purchased by consumers for home preparation and consumption and does not include poultry processed and sold as ready-to-eat or partially cooked. In addition, this assessment, in general, does not address other routes of exposure beyond oral exposure, such as occupational exposure during poultry processing, or retail or home food preparers that could be exposed to HPAIV during preparation (see Possible routes of exposure for food preparers to HPAIV). For further discussion, see Exposure pathways not addressed.

2.2 Public Health Regulatory Context

2.2.1 Public Health Background

Avian influenza (AI) viruses are Influenza A viruses of the family *Orthomyxoviridae* and are endemic in wild waterfowl and shore birds of most countries. AI viruses are classified into subtypes based upon the combination of the 16 different types of hemagglutinin (H) and 9 different types of neuraminidase (N) proteins found on their surfaces. Therefore, there are 144 possible subtypes (16 x 9). Each of the 144 subtypes can be further subdivided into two categories: low pathogenic avian influenza virus(es) (LPAIV) or HPAIV. HPAIV are AI viruses that meet the World Organization for Animal Health (OIE) definition for highly pathogenic, *i.e.*, a 75% or greater mortality rate in chickens inoculated intravenously and/or the presence of a specific type of amino acid sequence at the cleavage site of the virus' hemagglutinin molecule. AI viruses of all subtypes that do not meet either of these criteria are called LPAIV. AI viruses have been shown to infect mammals such as cats, horses, pigs, seals, and whales and vary in their ability to cause disease in humans (Kamps et al., 2006). LPAIV rarely infect humans and do not pose a significant human health threat. On the other hand, HPAIV currently circulating in other countries can occasionally infect and cause serious disease in humans. Of most recent identification, HPAI subtypes H5 and H7 are of greatest zoonotic concern (Swayne and Halvorson, 2003). However, it is the H5 subtype that is usually epidemiologically associated with severe human illness and death.

Very little epidemiological evidence supports a foodborne transmission route of influenza viruses to humans. However, HPAIV-infected poultry have been shown to disseminate the virus to poultry muscle and to internal egg contents (Swayne, 2006a; Thomas and Swayne, 2007a;

Mase et al., 2005b; Swayne and Beck, 2005; Tumpey et al., 2003; Tumpey et al., 2002; Bean et al., 1985; Beard et al., 1984), suggesting human exposure to HPAIV through consumption of contaminated poultry, shell eggs, and egg products is possible.

2.2.2 Policy Context

To address the possibility of HPAIV in poultry, shell eggs, and egg products, FSIS initiated this risk assessment. The risk assessment evaluates the public health risk associated with changes in the prevalence or level of HPAIV in contaminated poultry products, shell eggs, and egg products. Specifically, the scenarios evaluated are:

1. Changes in the level of HPAIV in contaminated poultry products and shell eggs,
2. Altering the effectiveness of in-shell pasteurization of shell eggs,
3. Altering the effectiveness of cooking of poultry and shell eggs,
4. Evaluating the effectiveness of on-farm HPAIV testing,
5. Evaluating the effectiveness of antemortem and postmortem poultry inspection, and
6. Altering the amount of HPAIV transferred from contaminated poultry to foods not likely to undergo cooking (*e.g.*, ready-to-eat foods and salads) during home food preparation.

APHIS is developing a risk assessment to evaluate the economic and animal health impact on the U.S. egg and poultry industries should HPAIV be introduced in the U.S. Ultimately, it is anticipated that the outputs of the APHIS risk assessment model can be used as inputs to this food safety risk assessment model

These efforts, along with the development of a comprehensive food safety HPAIV risk assessment, will help prepare Agencies should HPAIV be introduced in poultry flocks into the U.S. Development of this risk assessment, while HPAIV is not currently in the U.S., allows Agencies to be proactive in evaluating the effectiveness of various federal measures to mitigate public health risk associated with poultry and eggs should HPAIV enter the U.S. This risk assessment is being performed in conjunction with other federal efforts (*e.g.*, emergency planning, development of product testing protocols, etc.) to further enhance policy, communication, and outreach efforts.

2.3 Review of AI Risk Assessments

Few risk assessments have been developed to evaluate the human health impact from exposure to HPAIV from ingestion. The work performed has generally been in the form of qualitative risk assessments, which provide a summary of the literature and evaluation of the risk in descriptive terms (*e.g.*, "low" risk). Development of these types of risk assessments is a direct result of the paucity of data for HPAIV. Nevertheless, several countries have developed risk assessments to explore consumption as an exposure pathway, concluding that the risk of human exposure to HPAIV is low and the likelihood of such an exposure resulting in illness is very low (Table 1).

These conclusions are supported by epidemiological evidence that strongly suggests most known human illnesses are due to close contact from dead and infected birds. To date, a quantitative risk assessment to evaluate resultant illness from exposure to HPAIV from consumption of poultry, shell eggs and egg products has not been conducted.

Table 1. HPAIV risk assessments for ingestion of food or water.

RISK ASSESSMENT	COUNTRY	PRODUCT	ANALYSIS	MODEL
Greiner et al., 2007	Germany	poultry	quantitative (expert opinion)	*Monte Carlo* probabilistic
World Health Organization, 2006	Netherlands	water and sewage	qualitative	None
European Food Safety Authority, 2006	NA	domestic poultry meat and shell eggs	qualitative	None
Schijven et al., 2005	Netherlands	water	quantitative	*Monte Carlo* probabilistic
French Agency for Medical Safety of Food, 2005	France	domestic poultry and shell eggs	qualitative	None
Advisory Committee on the Microbiological Safety of food, 2005	United Kingdom	domestic poultry	qualitative	None
Pharo, 2003	New Zealand	imported chicken meat	qualitative	None

3 Hazard Identification for HPAIV

3.1 Avian Influenza Virus

Avian influenza viruses are Influenza A Viruses of the family *Orthomyxoviridae* and are endemic in birds of many countries. Avian influenza viruses have also been shown to infect mammalian species such as horses, pigs, and sea mammals (Hinshaw et al., 1981; Capua and Alexander, 2002; Ito et al., 1998). They are enveloped single-stranded segmented RNA viruses expressing two variant surface glycoproteins: hemagglutinin (H) and neuraminidase (N). There are 16 different H and 9 different N proteins (de Jong et al., 2005). Each influenza virus subtype contains one H and one N protein on their envelope surface, therefore giving influenza viruses their unique nomenclature (*e.g.*, H5N1 or H7N2). Influenza viruses vary in their ability to cause disease in both humans and birds. LPAIV cause mild disease in avian species, such as respiratory distress, low morbidity with low mortality rates, and rarely cause human disease. HPAIV cause a more severe clinical disease, with high morbidity and mortality rates in avian species. HPAIV can also infect humans and other mammalian species.

Of most recent identification, HPAI subtypes H5 and H7 are of greatest zoonotic concern (Swayne and Halvorson, 2003). However, it is the H5 subtype that is usually epidemiologically associated with severe human illness and death, while the H7 subtype is associated less frequently with severe human illness and death (CDC, 2008). In 2003, an epidemic of H7N7 in the Netherlands led to 89 confirmed H7N7 confirmed cases, including the death of a veterinary practitioner (Fouchier et al., 2004). As of June 2008, the H5N1 epidemic is known to have spread to 15 countries (WHO, 2008).

LPAIV is infrequently associated with human illness and has not resulted in human death (Swayne, 2006b). LPAIV outbreaks in domestic poultry have occurred, among other locations, in Pennsylvania (1983-1984 and 1997-1998) and Minnesota (1978) (Davison et al., 1999; USDA, 2006); however, LPAIV are less of a public health concern[3] and have not been associated with consumption of poultry products from infected birds. It is possible for LPAIV infecting a flock to mutate into an HPAI strain, as has been documented for H5 and H7 subtypes[4]. This occurred during the 1983-1984 Pennsylvania outbreaks and in an outbreak of LPAIV in Italy (Henzler et al., 2003; Mannelli et al., 2006).

LPAIV has not been associated with contamination of poultry or shell egg/egg products. Research has shown that experimental LPAIV infection of chickens was unable to infect the

[3] Human infection with LPAI has been observed in the U.S. (2 cases of H7N2), China (7 cases of H9N2), and the U.K. (1 case of H7N7) (Swayne, 2006b).

[4] For purposes of international trade, outbreaks of serotype H5 and H7 LPAI are reportable to national authorities because of the possibility of mutation to HPAI. See Article 2.7.12.1 Terrestrial Animal Health Code http://www.oie.int/eng/normes/mcode/en_chapitre_2.7.12.htm.

muscle or eggs produced by these birds (Swayne and Beck, 2004; 2005). It is therefore very unlikely that food could serve as a vehicle for LPAIV human exposure.

3.2 AI Epidemiology

Avian influenza, or "fowl plague," was first identified in the late 1800's. Despite the presence of AI among wild fowl and domesticated poultry, relatively few cases of severe human illness have been associated with this virus until recently (Swayne and Halvorson, 2003). In 1997, the first human cases of HPAI subtype H5N1 were detected in Southeast Asia (Buxton Bridges *et al*., 2000; Gao *et al*., 1999; Hiromoto *et al*., 2000; Katz *et al*., 1999; Lu *et al*., 1999), resulting in 18 hospitalizations and six deaths. This H5N1 strain, formally only detected in avian species, was able to cross the species barrier into humans and cause severe and sometimes fatal disease. In 2003, H5N1 reemerged in Southeast Asia causing multiple outbreaks in domesticated poultry and the World Health Organization (WHO) began to record the number of confirmed H5N1 human infections and deaths. From 2003, reports indicate 385 human cases and 243 deaths from HPAIV H5N1 between 2003 and June 2008 (WHO, 2008).

Appendix D presents a summary of relevant published epidemiological studies, including the uncertainties related to human infection rates and exposure pathways. The following are key points of the epidemiological evidence linking HPAIV infection in poultry with human cases:

- Given the very high virus burden among poultry in some areas of the world, particularly Indonesia, the number of documented human infections given the high probability of exposure is small and sporadic in nature
- The majority of known human cases are epidemiologically linked to close contact with live or dead poultry (Sedyaningsih *et al*., 2007; Beigel *et al*., 2005; Lye *et al*., 2007; Peiris *et al*., 2007)
- Most human infections most likely can be attributed to respiratory mucosal exposure due to inhalation of infective droplets or self-inoculation (touching mucous membranes, conjunctiva) via avian fecal contamination, avian respiratory secretions, or avian body fluids.
- The likelihood that foodborne exposure led to infection and disease is difficult to ascertain. WHO has stated that more than 25% of reported human cases have an unknown source of exposure (WHO, 2008). Precise exposure histories for some persons infected with the Asian strain of HPAIV H5N1 and others in the affected communities are uncertain (many H5N1 diagnoses are made post-mortem or patients are too ill at presentation to provide information on exposures (Abdel-Ghafar et al., 2008; CDC personal communication)). The questionnaires used and the potential for interviewer bias are largely unknown as detailed questions about potential foodborne exposures were generally not asked. The presence of a positive history of some other route of exposure does not rule out food as a potential vehicle.

- Naturally occurring and experimental oral exposure resulting in AI infection of other species, such as poultry and felines, have been well documented (Keawcharoen *et al*., 2004; Kuiken *et al*., 2004; Rimmelzwaan *et al*., 2001; Songserm *et al*., 2006; Swayne and Beck, 2005). However, extrapolating this information to humans is problematic and the absence of clearly foodborne infections among consumers of meat and eggs from infected birds who do not have other more likely routes of exposure suggests that the risk of foodborne transmission is low.
- Although currently there is no conclusive epidemiological evidence linking the consumption of raw or undercooked poultry, shell eggs, or egg products to human illnesss from AI, the possibility of transmission of HPAIV from poultry to humans via oral exposure cannot be epidemiologically excluded.

3.3 Clinical Signs in Poultry and Humans

3.3.1 Poultry

Species susceptible to HPAIV include chicken (*Gallus gallus*), turkey (*Melleagris gallopavo*), emu (*Dromaiidae spp.*), ostrich (*Struthionidae spp.*), and geese (*Anser anser domesticus*). Asymptomatic carriers of HPAIV H5N1 include duck (*Anas platyrhyncha*) and pigeon (*Columbia livia*) (Perkins and Swayne, 2002). Infected susceptible birds suffer a morbid viscero-hemorrhagic disease and die within 3-4 days of exposure. In chickens, clinical signs are characterized by diarrhea, swollen wattle and head, apathy, depression, reduced vocalization, and decreased feed and water intake. For turkeys, clinical signs also may include neurological signs such as staggering, head tilting, lethargy, and recumbency (lying down). HPAIV infections in ducks and other wild fowl are typically asymptomatic; however clinical signs occasionally occur.

3.3.2 Humans

Epidemiological evidence suggests that most humans infected with HPAIV H5N1 sought hospitalization 4-5 days after the onset of clinical symptoms (Weekly Epidemiology Report, 2006). The incubation period in humans following exposure to infected poultry is reported to be 7 days or less, and in most cases, 2-5 days (Abdel-Ghafar et al., 2008). The age of infected individuals ranged from 3 months to 75 years with a median age of 20 years (Weekly Epidemiology Report, 2006). Symptoms in humans include high fever, pneumonia, acute encephalitis, diarrhea, coma, hemorrhage, and multi-organ failure (Uiprasertkul et al., 2005; de Jong et al., 2005; Dybing et al., 2000; Hayden and Croisier, 2005). Some individuals have been asymptomatic. Conjunctivitis was commonly identified in an H7N7 outbreak in the Netherlands (Fouchier et al., 2004, Koopmans et al., 2003).

3.4 Sources of HPAIV

The natural reservoir of AI viruses is believed to be wild aquatic and shore birds, where the viruses exist as LPAI strains. The transition to HPAIV generally occurs after domestic poultry are infected with LPAIV. HPAIV may be isolated from domesticated poultry from their respiratory secretions, feces, the shells of eggs, internal egg contents, and tissues including blood and muscle. Therefore, it is of concern that exposures to HPAIV could be foodborne, via direct consumption of contaminated poultry, shell eggs, and egg products or by cross-contamination to other products manufactured in the same facility or within the retail establishment and household kitchens. In the U.S., HPAIV H5N1 has not been detected in wild birds or other avian species, and therefore, risk of contracting HPAI from ingestion of processed poultry, shell eggs, and egg products is very low (WHO, 2006d). Other subtypes of HPAIV have been detected in the U.S. as recently as 2004, but these HPAIV have not been associated with serious human disease (USDA, 2006). See CDC website on avian influenza viruses in humans: http://www.cdc.gov/flu/avian/gen-info/avian-flu-humans.htm

3.5 Transmission

Avian influenza is spread among poultry via secretions from the mouth, nares, and eyes. Feces from infected birds are contaminated with high levels of virus and constitute an important route of bird-to-bird transmission. Wild birds are thought to be the natural reservoir of influenza virus and can introduce influenza virus into flocks via contaminated feces. During bird-to-bird transmission of HPAIV within a domestic poultry flock, airborne secretions and feces containing the virus are thought to constitute primary routes of transmission (Swayne and Halvorson, 2003). More recently HPAIV has crossed the species barrier from poultry to humans most likely through inhalation of virus particles. Humans in close proximity to poultry are at highest risk of exposure to HPAIV (Beigel et al., 2005; Mounts et al., 1999; Bridges et al., 2002; Koopmans et al., 2004). Despite this risk, only five subtypes[5] of AI have been known to result in human illness (Kamps et al., 2006; Swayne, 2006b), suggesting that there is a species barrier. Person-to-person transmission has been documented (Katz et al., 1999; Wang et al., 2008) for the Asian strain of HPAIV H5N1, though it appears to be rare. This may have occurred through direct or indirect contact with infected individuals.

3.6 Pathogenicity in Poultry

[5] Note that Kamps et al. 2006 (p. 28, 64) is inconsistent with respect to the 2003 case of H7N2 infection in a New York man. Kamps et al. states on p. 28, "Of the hundreds of strains of avian influenza A virus, only four are known to have caused human infection: H5N1, H7N3, H7N7, and H9N2". The p. 64 table includes the H7N2 case, suggesting 5 subtypes have resulted in human illness, but it is not reported in the cited WHO 2005 report. Swayne, 2006b also states H7N2 resulted in human illness.

HPAIV has been shown to infect many tissues of susceptible avian species, such as chickens and turkeys. The virus has been isolated from avian breast and thigh muscle, spleen, blood, respiratory tissues and secretions, as well as gastro-intestinal tissues and feces (Swayne, 2006a; Swayne and Beck, 2005; Mase et al., 2005b; Tumpey et al., 2003; 2002). The virus has also been isolated from avian ovaries, reproductive tissues, and internal egg contents (Cappucini et al., 1985; Beard et al., 1984; Nakatani et al., 2005).

Studies suggest that experimentally inoculated chickens show clinical signs ranging from depression, facial edema, to shank hemorrhage 24-48 hours post inoculation, some showing diarrhea and swollen, cyanotic combs and wattles. According to pathologists at the USDA APHIS Animal Disease Center at Plum Island, visible pathology can be determined as soon as 48 hours after infection and includes visible reddening of the muscle through the thin avian skin (dark red-maroon birds) and hemorrhage of the internal organs. Some cases are sudden with little to no clinical signs or pathology; however, these instances are rare. Laying hens may show greater clinical signs due to having more expansive combs and wattles that become cyanotic and necrotic at a more observable rate (D. Swayne, personal communication).

For HPAIV H5N2, mortality occurs between 3-4 days post experimental infection and ranges from 90-100% in the flock (W. White, personal communication). However, for experimental HPAIV H5N1 infection of chickens, mortalities can be expected within 2-3 days (intranasal inoculation) and possibly 1 day longer in naturally infected birds, or birds infected with lower doses of the virus (D. Swayne, personal communication). Given clinical signs and the high rate of mortality associated with some strains of HPAIV, this suggests infected flocks would be quickly identified and removed from production. However, clinical signs for HPAIV-infected poultry vary and could possibly delaying identification (Swayne and Halvorson, 2003; Elbers et al., 2005). In the 2003 H7N7 Netherlands outbreak, clinical signs were not found to be an effective means of infected flock identification (Elbers et al., 2007) and the authors state "Clinical signs of AI are difficult to distinguish from a large range of other poultry disease" (Elbers et al., 2005).

3.7 Factors Affecting HPAIV Survival

Factors that could affect HPAIV survival in food include virus strain, temperature, pH, dehydration, salinity, and other microorganisms (Scholtissek et al., 1985; Stallnecht et al., 1990; Shortridge et al., 1998; Lu et al., 2003b; Swayne and Halvorson, 2003; John and Rose, 2005). However, there are relatively little data available to allow for predictive microbiological modeling to estimate how HPAIV may survive in processed poultry and shell eggs or the environment. Shortridge et al (1998) observed that HPAIV H5N1 dried at 25 ^{o}C in poultry feces dropped to nondetectable levels by 1 day; however the virus could survive in wet feces under similar conditions up to 4 days. Similar results were found by Beard et al. (1984) — HPAIV H5N2 survived in wet feces up to 2 and 35 days at 25 and 4 ^{o}C, respectively, and in chicken carcasses up to 4-7 days at 4 ^{o}C. According to the American Veterinary Medical Association, AI viruses may persist in the environment of a poultry facility (broiler house, egg layer complex) for up to 105 days (AVMA, 2006). Other studies show that environmental persistence of H5N1

lasts for at least 35 days at 4 ^{o}C and 6 days at 37 ^{o}C in feces and H5N2 within eggs stored at 10 to18 ^{o}C (INFOSAN, 2005; Cappucci et al., 1985).

Many influenza viruses are acid labile (Scholtissek, 1985), suggesting that the ability for the virus to survive within the human gastrointestinal tract may be limited (EFSA, 2006). However, many biological factors can change individual responses to the virus such as gastric pH alterations due to age, presence of ulcers, antacid use, medications, and the content of recent meals.

A USDA Agricultural Research Service (ARS) study shows that the virus is heat inactivated when exposed to temperatures of 70 ^{o}C (165 ^{o}F) for 1 second and therefore can be eliminated from food by thoroughly cooking to the FSIS recommended temperatures for poultry and shell eggs (Swayne, 2006a; Thomas et al., 2008).

3.8 Oral route as a possible source of HPAIV entry and infection

Though the epidemiology strongly supports transmission of HPAIV to humans through close contact with poultry, the possibility of exposure to the virus through food consumption must be considered. Two potential sites of virus replication in humans are the gastrointestinal (GI) tract and the respiratory tract.

Exposure to HPAIV followed by virus replication is necessary for human illness (Swayne and Pantin-Jackwood, 2006). The location of the replication site will be dictated primarily by the presence of specific human receptors that allow for viral entry into human cells. The GI tract has been proposed as a possible site of replication based on some of the following evidence. As indicated above, patients infected with HPAIV exhibit a range of symptoms, including diarrhea. Patients with confirmed H5N1 had diarrhea 7/10 times (70%), 2/4 times (50%), 7/17 times (41%) and 3/18 times (17%) from 4 reported outbreaks (Beigel et al., 2005; also see Abdel-Ghafar et al., 2008 Table 2). Furthermore, H5N1-infected patients presenting with diarrhea but without respiratory symptoms have been observed (Apisarnthanarak et al., 2004; de Jong et al., 2005). Typically, molecular investigation of tissues from persons infected with HPAIV demonstrate localization of virus to the respiratory tract with little evidence of a broader infection. However, a study detected both positive and negative sense ribonucleic acid (RNA) in the small and large intestine of a patient with progressive viral pneumonia. Negative sense RNA indicates the presence ofthe viral genome and positive sense RNA indicates the presence of viral messenger RNA (mRNA). This result provides evidence for active viral replication (because viral particles do not contain viral mRNA), and suggests that the presence of HPAIV in intestinal tissues was due to both dissemination and active viral replication (Uiprasertkul et al., 2005)[6]. In another study, HPAIV was isolated from fecal specimens suggesting localized intestinal replication and shedding (de Jong et al., 2005). Tissue culture studies suggest human influenza

[6] "Infectious virus and viral RNA have been detected in feces and intestines, suggesting that the virus sometimes replicates in the gastrointestinal tract (Abdel-Ghafar et al., 2008)."

viruses can replicate efficiently in human intestinal cell cultures (Zhirnov et al., 2007). The human intestinal cell line CACO-2 expresses alpha 2,3 linked sialic acid receptors that are preferred by HPAIV and other avian influenza viruses (Zhirnov et al., 2007). Animal studies demonstrate that HPAIV replicates in the GI tracts of poultry (Webster et al., 1978; Shortridge et al., 1998) and cats (Rimmelzwaan et al., 2006). However, extrapolating from chickens infected with a species-specific virus to humans is problematic. Though the GI tract as a site for HPAIV replication remains as a possibility during natural infection, there are no data to support that consumption of HPAIV-infected food can result in viral replication and therefore illness in humans.

The primary site for HPAI viral replication in humans is in the respiratory tract (Uiprasertkul et al., 2005). The preferred receptor for HPAIV is the alpha 2,3 linked sialic acid. These receptors are found on cells of the lower respiratory tract (bronchiole and alveolus) and are only rarely found on epithelial cells lining the trachea and nasal mucosa (Shinya et al., 2006). Therefore, HPAIV preferentially binds to cells of the lower respiratory tract (Shinya et al., 2006; van Riel et al., 2006; Matrosovich et al., 2004). H5N1 HPAI isolates with enhanced binding to the alpha 2-6 linked sialic acid receptor (the most common influenza receptor of the human upper respiratory tract) were isolated from 2 cases in Hong Kong, 2003 (Gambaryan et al., 2006). Molecular studies suggest that very few mutations in the viral hemagglutinin gene are required to alter receptor binding properties (Gambaryan et al., 2006; Stevens et al., 2006).

Though proper cooking of poultry products is effective to inactivate the virus, it should be considered that if HPAIV-infected food is consumed, contact with respiratory tissues may occur (EFSA, 2006). Some poultry and shell eggs will be undercooked, and cross-contamination from the raw product to foods not likely to undergo cooking can occur. However, if the respiratory tract was easily accessible by the virus during oral uptake, it is likely more human HPAIV illness would be associated with consumption of poultry or shell eggs in geographic areas affected by the ongoing epidemic of H5N1 in poultry. This suggests that though this is a potential route, it is not easily exploited by HPAIV.

3.9 Possible routes of exposure for food preparers to HPAIV

In addition to consumption of HPAIV-contaminated foods as a possible exposure route, retail[7] and domestic food preparation of raw HPAIV-contaminated poultry and eggs could constitute a source of exposure. To date, there are few epidemiologic data to support home food preparation behaviors common to U.S. consumers as an exposure pathway that can result in HPAIV illness; however, "butchering and preparation" of poultry by poultry workers was found to be a risk factor during a Hong Kong HPAIV outbreak (WHO, 2006a; INFOSAN, 2005; Bridges et al., 2002). Experience with bacterial foodborne pathogens such as *Salmonella* and *Campylobacter* suggest that raw products combined with unsanitary food preparation could lead to exposure. Safe food handling practices should minimize potential for exposure, however, the possibility of

[7] Retail food preparation includes restaurants, hospitals, supermarkets, and other settings outside of the home.

introduction of the virus during food preparation remains. Exposure to HPAIV could occur though 4 general pathways:

1) Inhalation of aerosolized particles. Virus particles could be aerosolized by various food preparation behaviors including tenderization and grinding of poultry meat and beating and blending of eggs. The virus could then be inhaled by food preparers, directly exposing the respiratory tract to the virus.
2) Contact with human mucosa. During food preparation, hand contact with raw product and subsequent touching/rubbing of eyes and/or nose could result in exposure to the virus to the conjunctiva and the respiratory tract, respectively. In addition, aerosolized virus could result in contact with conjunctiva tissue.
3) Contact with wounds. Open wounds, most likely on uncovered hands, coming in direct contact with contaminated product could serve as an exposure route. In addition, accidents during preparation of raw poultry, for example a knife cutting accident, could result in exposure directly to the blood or other tissues.
4) Behaviors resulting in oral uptake. Unsanitary preparation behaviors such as licking of fingers, accidental splashing of juices from raw contaminated product, and cross-contamination to foods not likely to be cooked could result in deposition of the virus in the oral cavity. Such food preparation behaviors would be considered to result in oral uptake and cross-contamination is addressed using scenario analysis (see Risk Characterization).

Given the comparison with bacterial foodborne pathogens, behaviors resulting in oral uptake through cross-contamination during food preparation are addressed using scenario analysis in this risk assessment. However, for the other three exposure pathways, though the above discussion suggests that these routes of exposure are possible, the frequency at which they occur is unknown. Furthermore, exposure through these potential routes does not necessarily constitute illness and would be dependent on additional dose-response relationships. Subsequent illness from exposure will depend on multiple factors, including dose, route of exposure, host susceptibility/resistance, and HPAI strain factors (see Exposure to food preparers).

4 Exposure Assessment

4.1 Modeling HPAIV exposure from poultry and egg consumption

Separate models to predict human exposure to HPAIV from the index flock[8] were developed for poultry meat and eggs and mathematically described in Appendix C. The models estimate human exposure[9] to HPAIV from consuming poultry, shell eggs, and egg products given the contamination of a single flock with HPAIV. The poultry model estimates exposure from consuming chicken and turkey products (Figure 1) and the egg model estimates exposure from consumption of shell eggs and egg products (Figure 7). The estimated HPAIV exposure is converted to human illnesses by a dose-response relationship. It is important to note that the pathways modeled were exclusively for human exposure from consumption of these products—alternative routes of exposure are not considered here (see Exposure pathways not addressed).

4.2 Criteria for data inclusion

Extensive review of the published literature was the foundation for identification of relevant data to inform the risk assessment. However, when data were not available, personal communications with industry, government, and academia provided useful information, guidance, and unpublished data. The following discussion characterizes how data were generally included or excluded from the risk assessment.

Ideally, the risk assessment model would have been informed primarily with HPAIV H5N1 epidemiological and experimental data. In particular, those strains of HPAIV H5N1 that are known to cause illness and occasional death in humans were used to inform the models. However, given its relatively recent emergence, data for HPAIV H5N1 was often not available. Given the choice between HPAIV H5N1 and equivalent HPAIV data, HPAIV H5N1 was chosen. If such data could not be identified, data from HPAIV H7N7 and other HPAI strains were used. Data generated from other viruses were not used in the model except for comparison.

To inform the baseline model, the average value for each variable was used when multiple data sources were identified; in such cases, the studies were reviewed to determine if methodologies were compatible so that data could be combined. If the data could not be combined or aggregated (*i.e.*, because the methodology was unknown or not compatible), the data were incorporated to encompass the possible range or results. If only a single data point was found, that value was used. If it was unclear how best to choose, and the data could not be combined or

[8] The index flock refers to the first time zoonotic HPAI is discovered and confirmed in a U.S. commercial chicken, turkey, or layer flock.

[9] The risk assessment does not estimate occupational exposure. Guidance for occupational workers is being developed by experts at the Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health (CDC/NIOSH), USDA, FDA, and Occupational Safety and Health Administration (OSHA).

aggregated, the conservative option was chosen (*i.e.*, those data that suggested that model outputs would not be underestimated) and incorporated into the baseline scenario.

4.3 Combinatorial approach to evaluate uncertainty

The combinatorial modeling approach is different from a probabilistic Monte Carlo analysis. In a Monte Carlo simulation each model variable is represented by a probability distribution that describes the likelihood of all possible values of a variable from least to most likely. Monte Carlo analysis then draws from these different probability distributions in each model simulation to characterize the variability inherent in the natural system. However, this type of probabilistic analysis is only useful if data are available to properly characterize the variability within each model variable. For this risk assessment, several of the model variables are not well characterized by probability distributions and it is unclear how accurately some of the data identified represent the true range of values from the natural system. Therefore, the combinatorial approach (described below) was employed to characterize the effect of uncertainty.

The exposure models were based on data and information from published and unpublished sources, including expert opinions provided by a variety of HPAI and poultry science experts (Table 2). Even so, there are data limitations for some of the model parameters, which add to the uncertainty in the model estimates. In some cases, this uncertainty arises from the difficulties inherent to scientific study of human infectious agents which, for ethical reasons, must rely largely on animal models and observed naturally-occurring cases. Moreover, scientific studies to determine HPAIV transmission and pathology parameters within an environment that accurately represents a U.S. commercial poultry house are costly, complex and have only been performed to a limited extent.

The models assess the impact of this uncertainty on the results (reported as estimated human illnesses) through a combinatorial analysis that allows evaluation of many different scenarios with a range of data inputs for each important parameter. Conventionally, the combinatorial approach to uncertainty analysis is a method where each factor is assigned a limited set of discrete values, *e.g.*, low, nominal, and high values (Morgan and Henrion, 1990). Then, while keeping the other inputs at their nominal (*e.g.*, central or expected value) values, we calculate the effect on the output of varying each input from its low to high values. These effects, often called "swing weights," are used to identify important model inputs. The combinatorial approach is also useful for exploratory analysis to identify the combinations of inputs values (scenarios) that lead to the worst (or best) predicted outcomes. Stated differently, this approach allows users to evaluate the effect of each parameter and its accompanying uncertainty on the estimated number of human illnesses due to consumption of poultry or eggs from HPAIV-infected flocks. For instance, there is limited information available on the rate at which birds in a commercial poultry house will succumb to HPAIV once exposed (Das et al., 2006). Although we can not reduce this uncertainty without further scientific study, we can evaluate the impact on model outputs by running scenarios with different input values for each parameter. To do this, we might assume

for our model that the true proportion of birds (the proportion we would observe if we could actually look at every bird) that die after 36 hours following exposure to HPAIV is 0.4. Thus, whenever a bird became infected with HPAIV, it would have a 40% probability of dying at the end of 36 hours. We are uncertain about this proportion, however, since it was extrapolated from a study with a small number of birds infected at a single dose by a single HPAI strain. In reality, 0.4 may represent the mean of a distribution from 0.2 to 0.6. Because we are uncertain of the true proportion of birds that die after 36 hour, every time we run our model we could assume a discrete value from 0.2 to 0.6. By running the model with each different value, *e.g.*, 0.2, 0.4, 0.6, we can measure the impact of these different assumptions on the number of predicted human illnesses.

A baseline scenario was developed by comparing the results from model runs with these different input values (Table 2). The baseline scenario uses a majority of central values and mean values for input variables where sufficient data were available to estimate a mean. The more conservative value was typically used when a mean could not be calculated. Furthermore, for some input variables, state transition probabilities (percentiles) were used (*e.g.*, 17% of poultry is cooked to a temperature of 135ºF or less, 11% is cooked to 135-145 °F, etc.) In the above example, the baseline scenario would have a value of 0.4 and the results from simulations using values from 0.2 to 0.6 would be compared to this scenario. The degree to which these different scenarios impact the baseline scenario is the measure of uncertainty in this combinatorial approach.

The advantage of this approach is that we are modeling an entire series of "what-if" scenarios and can thus closely examine the sensitivity of the output to each of the input parameters, *i.e.*, how important is each parameter for the exposure level of humans to HPAIV through consumption of poultry or eggs. Because we are uncertain about some of our input data, it is important to be able to show the effect of our uncertainty associated with each input by itself *and* in combination with all of the other inputs. A disadvantage of this approach is that if we assume the uncertainties are not correlated, extensive computational time will be required to model extremely rare combinations. For example, if we think of our lower and upper values as being assigned at the 5^{th} and 95^{th} percentiles of our confidence, then having all ten of our inputs at the upper value would only occur once in every ten trillion times of a Monte Carlo simulation—where values are essentially chosen at random along a distribution for each input for each run.

1

2 Table 2. Baseline data and assumptions for the poultry and egg model.

Model Parameter	Source(s)	Model Input	Assumptions
Poultry and Egg Production model			
Flock size, chicken	Steve Pretanik, National Chicken Council (NCC) John E. Starkey, US Poultry & Egg Association (USPEA). Industry survey, August 10, 2006, personal communication	20,000	2001 USPEA industry survey is representative of the mean flock size currently in industry. All chicken equally susceptible.
Flock size, turkey	Christy Marr, National Turkey Federation (NTF), August 23, 2006 personal communication John E. Starkey, USPEA	9,000	2001 USPEA industry survey is representative of the mean flock size currently in industry. All turkeys equally susceptible.
Flock size, layer	Gene W. Gregory, United Egg Producers (UEP), September 05, 2006, personal communication	100,000	The mean flock size used is representative of the mean flock size currently in industry. All hens equally susceptible.
Weeks in house, chickens	Steve Pretanik, NCC, August 15, 2006; October 31, 2006 personal communication John E. Starkey, USPEA, Industry survey	8	Eight weeks of production for chickens is the time required for chickens to reach market weight.
Weeks in house, turkeys	Christy Marr, NTF. Industry survey, September 22, 2006, personal communication	20	Twenty weeks of production for turkeys is the time required for turkeys to reach market weight.
Eggs laid per day	Gene W. Gregory, UEP, September 05, 2006, personal communication	0.7	0.7 eggs per day over the life of a hen. Does not account for variation in egg laying over a hen's cycle.
Contact rate	Marian Bos, unpublished data; van der Goot et al (2005)	8 birds / 6 hrs – chickens and turkeys 2 birds / 6 hrs – laying hens	Based on the 2003 HPAIV H7N7 Netherlands outbreak, authors estimated 1 chicken could infect 32 chickens per day. Assume this effective contact rate (32/24 hrs or rather 8/6 hrs) is representative of HPAI strains causing human illness in Asia, Africa, and the Middle East.
Latency	Das et al., 2006, unpublished data	See Table 4	Presence of HPAIV in chicken trachea, heart, breast, or thigh meat at various infection time intervals is a surrogate for chicken's ability to spread HPAIV.
Mortality per bird	Das et al., 2006, unpublished data	36-42 hrs	These data are representative of chickens, turkeys, and hens.

Model Parameter	Source(s)	Model Input	Assumptions
Daily detection threshold	David E. Swayne; March 20, 2006, personal communication Steve Pretanik, NCC, November 28, 2006, personal communication Christy Marr, NTF, December 08, 2006, personal communication	0.5 – 2 % daily flock mortality	A 0.5 to 2% threshold, after which a flock would not go to slaughter, is assumed based on the D. Swayne personal communication (NCC and NTF did not provide an estimate). From 0.5%, an increasing linear relationship is assumed. Below 0.5%, a flock is not detected as HPAIV-positive. Data are representative of turkeys. For hens, 2% is assumed.
Live weight chicken	Steve Pretanik, NCC, October 31, 2006, personal communication	2,722 g (6.0 lbs)	
Live weight turkey	Christy Marr, NTF. Industry survey, September 22, 2006, personal communication NTF Sourcebook, May 2006	18,461 g (40.7 lbs)	
Egg weight	FSIS. *Salmonella* Enteritidis Risk Assessment (SERA, 2005)	60 g	
Poultry and Egg Processing Model			
FSIS postmortem inspection	Das et al., 2006, unpublished data	See Table 4	Unpublished data are representative of when chickens and turkeys present visible pathology.
Carcass weight chicken	Steve Pretanik, NCC , October 31, 2006, personal communication	1,336 g	Assumed 70% of live weight for dressed carcass (up to and including evisceration) and 70% for final yield (deboned).
Carcass weight turkey	Christy Marr, NTF, 2006 Sourcebook	9,062 g	Dressing and yield assumed same as chickens.
HPAIV levels in poultry meat	Thomas and Swayne, 2007a, Thomas and Swayne, 2007b, Swayne, 2006a, Swayne and Beck, 2005 Tumpey et al., 2002; 2003C. Thomas, personal communication	$10^{7.7}$ $\log_{10}$ EID_{50}/g[10]	Represents average level within all chicken meat parts (breast, thigh, leg, etc. meat) during peak infection. Levels pre-peak infection are assumed to exponentially decrease. Data are representative of turkey meat.
Tissue infectivity (% contaminated meat over time)	Das et al., 2006, unpublished data	See Table 4	Chicken meat parts (breast, thigh, leg, etc. meat) become infected at same point in time. Data are representative of turkey meat.
HPAIV levels in eggs	Swayne and Beck, 2004	$10^{4.9}$ $\log_{10}$ EID_{50}/mL	These data are representative of the level of HPAIV in the albumen and yolk at any time during hen infection.

[10] Embryo Infectious Dose 50% (EID_{50}) which are the level of virus required to infect 50% of embryonated eggs.

Model Parameter	Source(s)	Model Input	Assumptions
Egg inspection (% eggs with visible pathology)	Cappucci et al., 1985	5%	These H5N2 data are representative of HPAI strains causing human illnesses in Asia, Africa, and the Middle East.
Poultry and Egg Preparation Model			
Fraction of eggs that are processed as shell eggs and egg products	NA	NA	Model does not assess egg products. Assumed all eggs produced by a hen flock are processed for table egg consumption.
Serving size, chicken	Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998	83 gms.	
Serving size, turkey	CSFII 1994-1996, 1998	61 gms.	
Serving size, egg	*Salmonella* Enteritidis Risk Assessment (SERA), 2005	60 gms.	Assumes one egg feeds one person.
Frequency of HPAIV-positive eggs	Bean et al., 1985; D. Swayne, personal communication	last hen egg produced could be positive	Assumes all HPAIV-positive hens are capable of producing an HPAIV-positive egg. Assumes that there is not a drop in egg production.
Time for eggs to reach consumers	SERA, 2005	6 days	
Cooking temperatures for poultry	Audits International/FDA 1999 U.S. Food Temperature Evaluation Home Cooking Temperature Summary	See Table 6	Data are representative of the peak temperature achieved by consumer cooking of chicken and turkey. Data are not representative of hospital, restaurant, and institution cooking.
Cooking temperatures for eggs	Fleischman, 2006, unpublished data	See Table 11	FDA unpublished data are representative of peak cooking temperature achieved by consumer cooking of eggs.
Cooking time for poultry and eggs	No data identified.	10 sec	Ten seconds are representative of the duration at which the peak temperature achieved is held.
Fraction of egg cooking styles	SERA, 2005	See Table 12	Data are representative of how consumers cook eggs.
Log_{10} reduction cooking of poultry and eggs	Thomas and Swayne, 2007a; SERA, 2005	See Table 7; Table 12	Data are representative of log_{10} for chicken, turkey, and, eggs.

4.4 Poultry model

The poultry model estimates the likelihood an HPAIV-infected chicken and turkey flock is sent to slaughter and, if so, the resulting human health impact of consumption of HPAIV-contaminated poultry (Figure 1). The model is divided into three modules: *production*, *processing* and *preparation*.

- *Production*: The production module estimates the likelihood that an HPAIV-infected flock will go to slaughter and, if an infected flock is slaughtered, the total number of infected birds to be processed for human consumption.

- *Processing*: The processing module estimates the effect of transportation and holding prior to slaughter. This module then estimates if an infected bird will be undetected during slaughter/processing and, the number of grams of infected poultry allowed into commerce with the level of HPAIV per gram of meat

- *Preparation*: The preparation module estimates human exposure to HPAIV based on the number of servings consumed, viral level per serving and the level reduction from cooking.

Each module utilizes a number of parameter inputs (Figure 1; Table 2). Many of the model inputs can be easily adjusted by the user (see Appendix A: User's Manual). This feature allows the model user to select a range of values for any given input and observe the estimated impact on human exposure and illness.

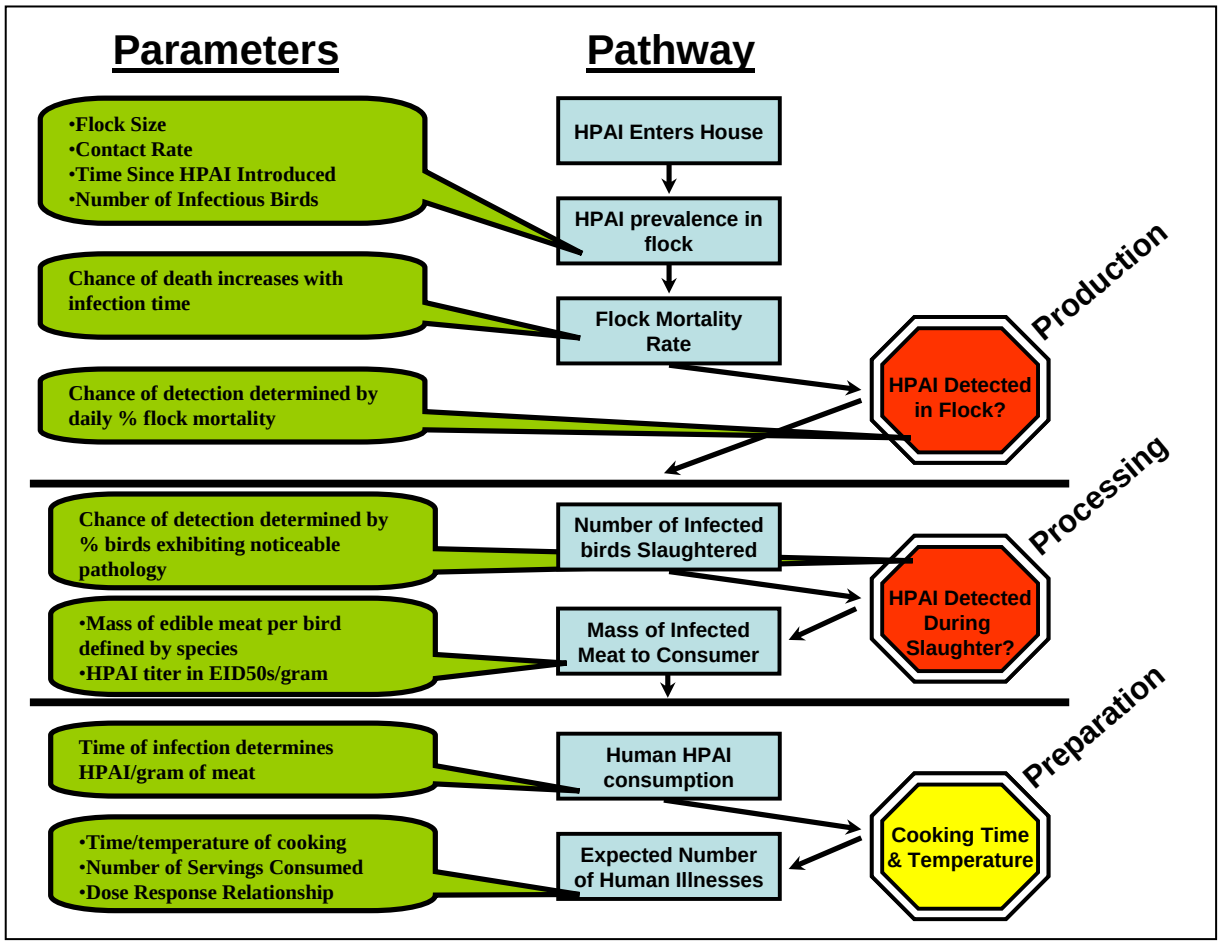

Figure 1. Poultry model diagram.

4.4.1 Production Module: Probability of infected flock being identified before slaughter

The production module estimates the likelihood that an HPAIV-infected flock will go to slaughter. The two primary factors that predict if an HPAIV-infected flock will be sent to slaughter or not are: 1) when a flock is infected with HPAIV, and 2) the time required for a flock to show significant mortality.

The time at which a flock becomes infected is simulated as a random event. If a flock is infected early during the grow-out process, the chance the flock is sent to slaughter is very low because the disease will have time to manifest and be observed by poultry/farm managers. However, if a flock is infected while the birds are near market weight, there may not be enough time for the disease to spread and cause significant levels of poultry mortality before the birds are sent to slaughter. Therefore, a flock infected with HPAIV is only a concern if it is randomly infected near the time the flock should reach market weight.

When a flock is infected late in its grow-out period, the time required for a flock to show substantial mortality becomes important. The transmission model (see below) estimates the number of birds that become infected and die every 6 hours over the course of the simulated outbreak. Thus, the model computes a mortality rate at each 6-hour interval which can be stated as # dead birds / # total birds /6hrs.

There are no data to indicate when poultry workers would detect a flock as being unfit to send to slaughter[11]. This would likely be dependent on the strain of virus and bird, grow-out conditions, HPAIV-outbreak history, season, poultry/farm work experience, and other factors. Therefore, the model uses a daily mortality threshold 0.5 to 2.0% for detection of an infected flock (D. Swayne, personal communication) (Figure 2). At 0.5% there is a small chance of detection and at 2% mortality there is 100% detection.

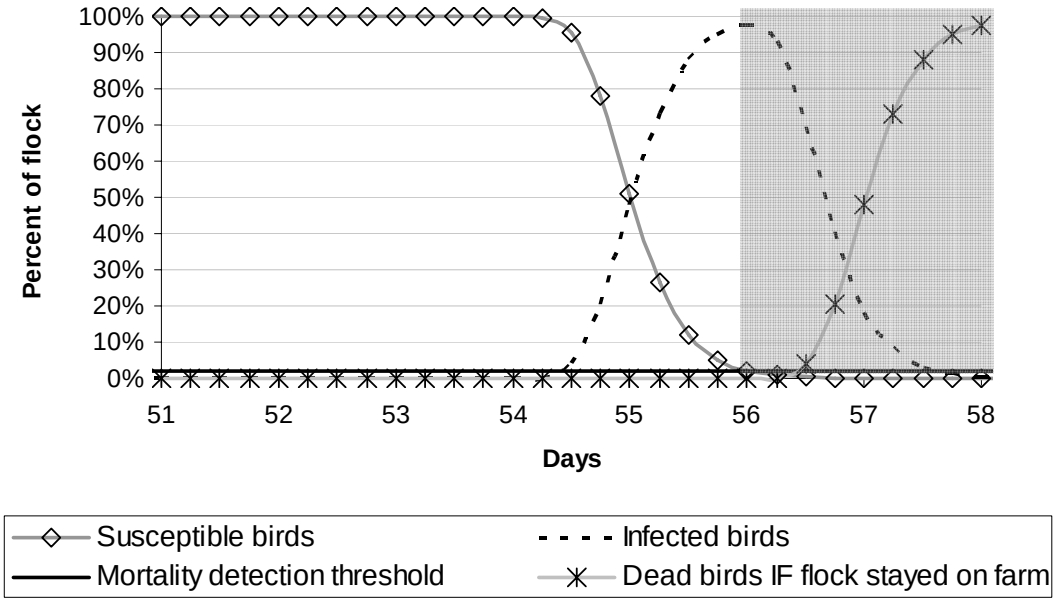

Figure 2. A chicken flock grown out for 56 days (8 weeks) could be infected with HPAIV within the last days before they reach market weight and are sent to slaughter (at 56 days). In this example, the flock is infected at 53 days. The progression of the disease shows that by the time the flock reaches 56 days, though many birds are infected with HPAIV, few have died given that it take 36-42 hours for infected birds to succumb to HPAI. This flock would therefore be sent to slaughter without discovery of the virus. In the shaded gray section is the progression of the disease had the birds stayed longer on the farm.

[11] Personal communications through the National Turkey Federation and National Chicken Council did not provide an estimate of threshold detection.

4.4.2 Production Module Output

The initial output of the production module is whether or not an HPAIV-infected flock will be detected (based on mortality rate). If the flock goes undetected, the model estimates the number of HPAIV-infected birds sent to slaughter. The model tracks the length of infection and the final state (percent birds that are infectious, latent, etc.) of the flock at the time it leaves for slaughter.

4.4.3 Production Module: Transmission model

The production module characterizes the likelihood of HPAIV-infected birds being sent to slaughter, and if so, how many. To estimate the probability of an HPAIV-infected flock being sent to slaughter the model first simulates the number of infected birds at a given point in time. If this number exceeds a threshold value the flock is detected and not sent to slaughter. If this number is below threshold then the flock is sent to slaughter with an additional chance for birds to be detected based on pathogenesis observed at the slaughter house. In order to begin the simulation, a single bird is assumed to be infected with HPAIV at a random time during grow-out. This bird then becomes infectious and can spread the disease to neighboring birds. As the disease progresses, some birds will remain susceptible, some will become infected, some will proceed to being infectious, and others will die.

A disease transmission model was developed to simulate the spread of disease within the flock once a single bird is infected. The disease transmission model uses state-transition to simulate the number of birds within a flock that are at each of the following four states at any given time: susceptible, latent (infected but not yet infectious), infectious (infected and infectious) or dead.

- Susceptible birds are those birds that have not been exposed to HPAIV. Susceptibility is assumed equal for all birds.
- Latent birds are those birds that have become infected following HPAIV exposure, but cannot yet spread the disease.
- Infectious birds are those birds that are HPAIV-infected and can spread the disease.
- Finally, dead birds are assumed to have died from HPAI.

The model tracks the number of birds in each state in 6-hour intervals (*i.e.*, the model updates every 6 hours of simulated time to assess the change in state of birds). In addition, the transmission model follows and records the length of time each bird has been infected. This is important as the movement from one state to another is based in part on the duration of infection. Several factors will affect the rate of transmission, the movement from state to state, and whether an infected flock is sent to slaughter including: the initial number of birds infected, flock size, weeks in house before the first infection, contact rate, latency, bird mortality rate, and daily mortality threshold.

4.4.3.1 Birds Initially Infected

The number of birds initially infected is assumed to be one for the baseline scenario. However, depending on how a flock becomes infected, more than one bird may initially be infected. For instance, contaminated feed could result in many birds becoming infected with HPAIV at or about the same time. To estimate the impact of this uncertainty, a range of 1 to the total flock size can be used as an input to the model.

4.4.3.2 Flock Size

Farms typically divide a flock of birds into several houses. To reflect this, the model addresses transmission of HPAIV from bird-to-bird within a single house. Thus, flock size refers to the number of chickens or turkeys in one house. This house represents the maximum number of birds that can be exposed to HPAIV in any one simulation since the model does not simulate inter-house transmission. Also, poultry managers place birds into a house at once and they go to slaughter at once (this is called all-in-all out management). The model uses an average value of 20,000 for chickens and 9,000 for turkeys to represent the average flock size for the baseline scenario (Table 2). However, the number of birds within a single poultry house can vary. A range of 100-120,000 birds that could be exposed to HPAIV can be used as an input to the model.

4.4.3.3 Weeks in House

"Weeks in House" refers to the number of weeks a flock is reared for production (the grow-out period). The duration of the grow-out period is dependent on the type of subspecies of poultry (Table 3). The longer birds are reared, in general, the larger they will grow. Therefore, as different grow-out periods are chosen, the model automatically simulates an individual bird weight commensurate with the grow-out period. The average grow-out period for chicken and turkey are 8 and 20 weeks, respectively; these values are used for the baseline scenario. The range of weeks that can be simulated by the model is 4-32 (NASS, personal communication) (Table 3).

Table 3. Number of weeks of production for chickens and turkeys.

WEEKS IN A HOUSE	CHICKEN FLOCK	LIVE WEIGHT (LBS)	TURKEY FLOCK	LIVE WEIGHT (LBS)
4	CGH[1]	2		
5	CGH	3		
6	Broiler	4		
7	Broiler	5		
8	Broiler	6		
9	Broiler	7		
10	Broiler	8		
11	Roaster	9		

12	Roaster	9		
13	Capon	9		
14	Capon	9	Light	28.8
15	Capon	9	Light	28.8
16	Capon	9	Light	28.8
17	Capon	9	Medium	37
18	Capon	9	Medium	37
19	Capon	9	Medium	37
20	Capon	9	Heavy	40.7
21	Capon	9	Heavy	40.7
22	Capon	9	Heavy	40.7
23	Capon	9	Heavy	40.7
24	Capon	9	Heavy	40.7
25	Capon	9	Heavy	40.7
26	Capon	9		
27	Capon	9		
28	Capon	9		
29	Capon	9		
30	Capon	9		
31	Capon	9		
32	Capon	9		

[1] Cornish game hen

4.4.3.4 Contact Rate

The contact rate dictates the speed at which HPAIV can spread through a house. Here, contact rate refers to the *effective contact rate* or the number of contacts that produce a new infection per unit time. For example, an effective contact rate of 10 indicates that per unit time, an infected bird can infect 10 susceptible birds. Therefore, 1 infectious bird can contact 10 susceptible birds resulting in 10 latent birds.

To estimate the baseline contact rate for chickens and turkeys, a personal communication based on the 2003 H7N7 HPAIV Netherlands outbreak indicated that the contact rate during that outbreak was 32 birds within a 24-hour period (M. Bos, personal communication). Because bird mortality and prevalence of HPAIV in poultry data are given every 6 hours (Table 4), for the baseline scenario, an effective contact rate of 8 birds every 6 hours is assumed for both chicken and turkeys. The range of potential contact rates is 1 to 64.

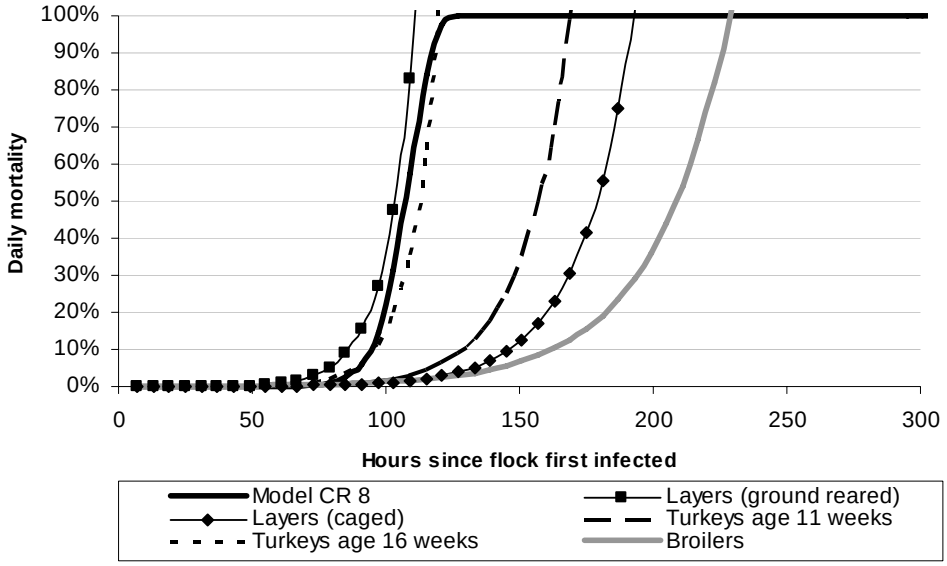

Figure 3. Percent daily mortality as predicted by the model at an effective contact rate of 8 compared with 2003 H7N7 HPAIV Netherlands outbreak data.

To help validate this assumption, using mortality as a surrogate for infection, data from Elbers et al., 2007 were considered. Within-flock mortality prevalence data from a total of 192 HPAIV infected flocks were used to estimate regression coefficients by non-linear regression (Elbers et al., 2007). Mean estimated coefficients were used in the poultry transmission model to compare actual HPAIV-infected flock data to model predictions at different contact rates. Figure 3 shows a contact rate of 8 and how it compares to the actual outbreak data. As can be seen, a contact rate of 8 represents the ground-reared layers and the turkeys aged greater than 16 weeks. Interestingly, the broiler data demonstrated a much slower spread of HPAIV. These data are based from only 4 flocks and compared with 124 and 6 flocks for ground-reared layers and turkeys, respectively.

4.4.3.5 Latency

Once a bird becomes infected with HPAIV it is not immediately infectious to other birds. The virus requires time to replicate before it can be shed and infect susceptible birds. To estimate the length of time a bird remains latent, the point at which HPAIV is first experimentally detected in internal tissues from HPAIV-infected chickens was used (Das et al., 2006). Researchers exposed chickens to HPAIV and measured HPAIV levels within the trachea, breast, thigh, and heart using three different detection methods every six hours (Table 4). The time point at which HPAIV is detected in any of the tissues is assumed to represent the point at which latent birds can now spread the virus. Ideally, a time course evaluating the presence of HPAIV in tissues that are directly related to spread of the virus would be more representative. HPAIV is known to replicate to high levels in the lung and intestinal compartments of the bird, resulting in shedding

of the virus through respiratory secretions and feces, respectively. Given the lack of this information, the data in Table 4 are assumed representative for chickens and turkeys.

Table 4. Pathogenesis and detection of HPAIV at different post-inoculation time points in chickens intranasally inoculated with A/WhooperSwan/ Mongolia/244L/05 H5N1.

s = number positive n = number examined					Detection of AI Virus															
					Trachea[c]				Breast Meat				Thigh Meat				Heart			
Time (Hour)	Morbidity[a]		Mortality[b]		RRT-PCR[d]		AI Antigen[e]		RRT-PCR		VI[f]		RRT-PCR		VI		RRT-PCR		VI	
	s	n	s	n	s	n	s	n	s	N	s	n	s	n	S	n	s	n	s	n
0	0	10	0	10	0	10	0	10	0	10	0	10	0	10	0	10	0	10	0	10
6	0	10	0	10	2	10	0	10	0	10	0	10	3	10	1	10	4	10	2	10
12	0	10	0	10	1	10	0	10	3	10	2	10	3	10	1	10	2	10	1	10
18	0	10	0	10	4	10	1	10	4	10	5	10	6	10	7	10	4	10	10	10
24	9	10	0	10	9	10	6	10	9	10	10	10	9	10	10	10	10	10	10	10
30	10	10	0	10	10	10	8	10	10	10	10	10	10	10	10	10	10	10	10	10
36	6	10	4	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
42			19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
48			11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11

[a] The number of sick per number of birds sampled at each time point.
[b] The number of deaths per number of birds sampled at each time point.
[c] The number of positive samples per the number of samples tested. All control chickens were negative for AI virus by all test methods and are not included in these numbers.
[d] real-time reverse transcriptase PCR assay (Spackman et al., 2002)
[e] commercial type A influenza antigen capture immunoassay kit
[f] embryonated chicken eggs

The data in Table 4 could be combined in several different ways to estimate when and how frequent virus is detected. For example, all or a portion of tested tissues could be combined, or all or just one detection method could be considered representative, or a combination of the detection methods, etc. Given that it is unclear how to best utilize the data in Table 4, the data were aggregated in several different ways to estimate the impact of handling the data in these different ways (see Appendix B: Chicken and Turkey Model Options). For the chicken and turkey baseline scenario, it is assumed that the highest frequency HPAIV-positives observed in any of the tested tissues by any method indicates that the virus has had sufficient time to replicate to numbers that could be shed by birds (Figure 4).

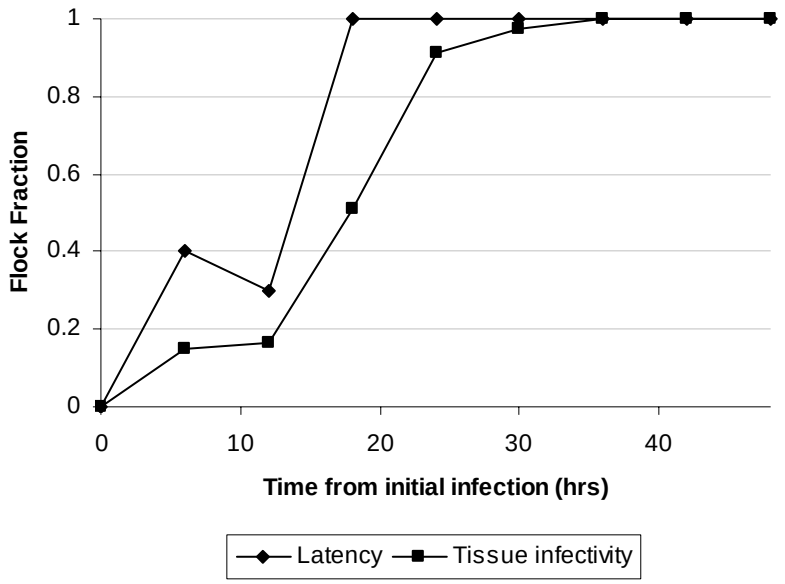

Figure 4. Fraction of flock that is infectious and contains HPAIV in the muscle over time (Das et al., 2006).

4.4.3.6 Tissue infectivity

Once a bird becomes infected with HPAIV, time is required for the virus to spread internally and begin to replicate in the muscle. To estimate when the muscle of HPAIV infected birds could be infective to human, the data in Table 4 were used (Das et al., 2006). Again, given that it is unclear how to best utilize the data, the data were aggregated in several different ways to estimate the impact of handling the data in these different ways (see Appendix B: Chicken and Turkey Model Options). For the chicken and turkey baseline scenario, it is assumed that the average detection of HPAIV in any of the tested tissues by any method indicates that the virus is present in any muscle meat (Figure 4).

4.4.3.7 Mortality Rate

Daily mortality rate is the number of infectious birds in a flock that die each day. The transmission model measures daily mortality rate as the percent mortality/day. The time it takes from when a bird is infected to when it dies is informed by unpublished ARS data (Das et al., 2006) (Table 4). Data indicate that approximately 40% of infected birds will die by 36 hours and 100% mortality can be expected by 42 hrs. These data are used in the chicken baseline scenario and are considered representative of turkeys.

4.4.3.8 Daily Mortality Threshold

A daily mortality threshold is used to determine if an HPAIV-infected flock is detected as positive or not. The transmission model calculates a daily mortality percentage and compares

this to the flock detection threshold. The threshold for detection is set at a mortality rate of 0.5%. Below 0.5%, a flock is never detected as HPAIV-positive. At 0.5%, a flock is unlikely to be discovered with the probability linearly increasing up to 100% detection when the daily mortality reaches 2% (D. Swayne, personal communication). The impact of this assumption is tested by sensitivity analysis.

4.4.4 Processing Module

The processing module simulates the effect of transportation and holding on the various states of birds within the flock prior to slaughter, whether an infected bird will be detected during inspection, the number of grams of infected meat allowed into commerce and the viral titer per gram of poultry. The processing module includes 1) transportation/holding, 2) FSIS antemortem and postmortem inspection, and 3) the level of HPAIV in poultry meat.

4.4.4.1 Transportation and Holding

Birds transported to the slaughter/processing facility can be in a susceptible, latent or infectious state when leaving the house. The processing module addresses the additional time transportation and holding contribute by updating the transition states accordingly. Personal communications indicated 0.25 to 4 hours and 0.5 to 8 hours for transportation and holding for chicken, respectively (J. Starkey, personal communication) and 0.75-1 hour and 6 hours for transportation and holding for turkey, respectively (C. Marr, personal communication). For the baseline scenario, the model assumes 6 hours for transportation and holding for both chickens and turkeys. Birds that have died during transportation are subtracted from the total number of birds entering slaughter.

4.4.4.2 FSIS Inspection

Transported birds are subject to FSIS antemortem inspection prior to receipt at slaughter. If enough birds died during transportation, this may alert individuals to hold the transportation vehicles prior to slaughter. The transmission model calculates the number of additional birds that have died due to HPAIV during transportation and holding and treats detection the same as the production module (see Daily Mortality Threshold). Given data were not identified as to the number of dead birds that would trigger holding of the flock during antemortem inspection, the effect of antemortem inspection is not part of the baseline evaluation. However, the model can be used to estimate the impact of antemortem inspection through scenario analysis.

FSIS postmortem inspection could non-specifically remove HPAIV-positive carcasses due to visible abnormalities caused by HPAIV infection (FSIS, 2007). To estimate the fraction of carcasses that would show visible pathology given various lengths of infection prior to slaughter, unpublished ARS data were used (Das et al., 2006). HPAIV infection of chickens with 10^6 $\log_{10}$ EID_{50} indicated that by 18 hrs, none of the birds demonstrate signs. By 24 hrs post-infection,

90% (9/10) demonstrated visible signs of infection and by 30 hrs, all birds were visibly ill (Table 4). Data were not found demonstrating the fraction of HPAIV-positive chicken carcasses over time showing visible pathology and it was therefore assumed that the live bird analysis performed by Das et al., 2006 was representative of slaughtered birds. The carcasses of infected birds slaughtered that were visibly ill are subtracted from the total number of carcasses processed. There are no data to indicate a time course of visible signs for turkeys infected with HPAIV. These data are assumed representative of turkeys[12].

4.4.4.3 HPAIV levels in poultry

The processing module estimates the level of HPAIV in contaminated poultry meat. The extent to which the poultry meat is contaminated is dependent on the length of time the bird has been infected. Table 5 lists the levels of HPAIV in poultry meat reported from infected bird tissues predominantly during peak infection or postmortem. HPAIV levels are likely lower given less time of infection. This has been evidenced by identifying levels of HPAIV from chicken organ tissues taken at different times post infection (1.7 $\log_{10}$ EID_{50} present at 8 hrs in brain while 7.5 $\log_{10}$ in brain at 48 hrs; D. Swayne, personal communication). Therefore, HPAIV levels in poultry meat appear to be a function of the duration of infection. Levels may also be a function of species of infected bird, HPAI strain, and the type of muscle meat (Table 5)[13].

Although HPAIV levels are occasionally measured in number of viral particles, level is more typically measured in units based on viral activity such as Infectious Dose 50% (ID_{50}) and Lethal Dose 50% (LD_{50}). HPAIV tissue levels are commonly measured as Embryo Infectious Dose 50% (EID_{50}) which are the level of virus required to infect 50% of fertilized eggs. This measure is used for this risk assessment.

Table 5. HPAIV titers in poultry.

REFERENCE	PRODUCT (MUSCLE)	HPAI STRAIN: TITER (EID_{50}/g)
Thomas and Swayne, 2007a	chicken thigh	H5N1: $10^{7.8}$; $10^{7.8}$; $10^{8.5}$
	chicken breast	H5N1: $10^{7.5}$; $10^{7.5}$; $10^{7.5}$
Thomas and Swayne, 2007b	chicken thigh	H5N1: $10^{8.0}$
	chicken breast	H5N1: $10^{7.5}$ H5N2: $10^{4.9}$; $10^{6.4}$; $10^{6.9}$
Swayne, 2006a	chicken thigh	H5N1: $10^{6.8}$ H5N2: $10^{2.8}$
	chicken breast	H5N1: $10^{5.6}$ H5N2: $10^{2.3}$
Swayne and Beck, 2005	chicken	H5N1: $10^{7.3}$ H5N2: $10^{2.7\text{-}3.2}$
Tumpey et al., 2003	chicken thigh[1]	H5N1: $10^{6.0\text{-}6.7}$
	chicken breast	H5N1: $10^{5.3\text{-}5.5}$

[12] Studies indicate that turkeys and chicken demonstrate different types of pathology during peak infection.
[13] HPAI levels, following slaughter, are assumed not to change.

Tumpey et al., 2002	chicken thigh[1]	H5N1: $10^{6.2\text{-}6.7}$
	chicken breast	H5N1: $10^{5.3\text{-}5.5}$
C. Thomas, personal communication	chicken thigh	H5N1: $10^{7.5}$
	chicken breast	H5N1: $10^{7.7}$; $10^{7.9}$; $10^{8.1}$

[1] Text is unclear to which value within the range is muscle compared to other tissues tested.

Data were not identified to estimate the level of HPAIV in poultry meat as a function of duration of infection. Therefore, the 21 H5N1 level estimates for chicken breast and thigh meat were averaged to determine the expected value for the level of HPAIV in chicken meat (Table 5). $10^{7.7}$ EID_{50}/g H5N1 was used as an upper bound to indicate the level of HPAIV in poultry meat of dead birds at 43 hours. Das et al, 2006 observed HPAIV-infected birds dead at the latest by 42 hours. Forty-three hours was the next appropriate 6-hour time interval and was therefore chosen to represent the maximum level of virus. Working from this maximum level, the level of HPAIV was decreased exponentially every six hours until the 7-hour time interval was reached. (Figure 5)[14]. Das et al., 2006 indicate that HPAIV could be detected at 6 hours post-infection by testing of embryonated eggs. The level of detection for this method was 1.9 EID_{50}/g HPAIV and is used to estimate the level of HPAIV in muscle of 7 to 12-hour old infected birds. Model options allow 10-fold increases or decreases to the level of HPAIV in infected poultry meat to estimate the impact of this assumption (see Appendix B: Chicken and Turkey Model Options). These data are assumed representative of turkeys.

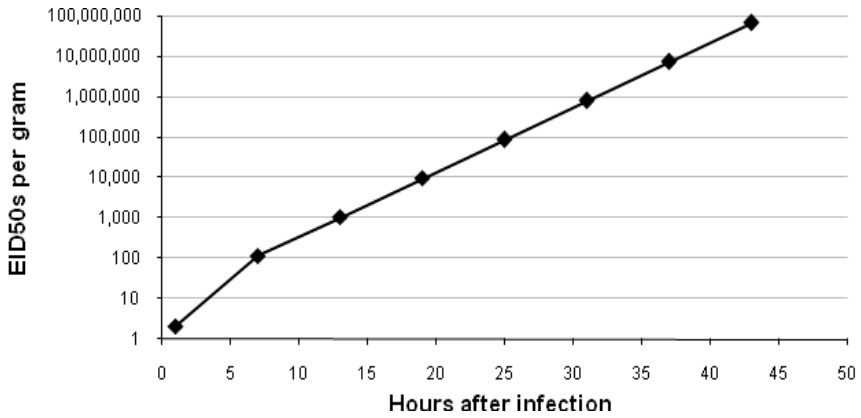

Figure 5. Level of HPAIV EID_{50}/g of infected poultry meat as a function of infection length.

4.4.5 Poultry Preparation Module

The preparation module estimates human exposure to HPAIV based on the number of servings consumed, viral level per serving and the reduction of viral activity from cooking. In addition, the preparation module includes the impact of cross-contamination from raw poultry resulting in

[14] The rate of decrease was chosen given that HPAI virus will likely replicate exponentially given optimal growth conditions. However, few data were identified to estimate the magnitude of this change.

ingestion of the virus. Consumer storage is not considered in this risk assessment as it is assumed that virus will not grow or decay in post -slaughtered meat (see HPAIV survival during storage).

4.4.5.1 Cooking of poultry

Poultry is assumed to be subject to a consumer cooking step prior to consumption. The destruction of virus at high temperatures is characterized by D-values. At a fixed temperature, the D-value is the length of time taken for the level of virus to decrease by a factor of 10 (1 $\log_{10}$). ARS data are used to estimate D-values in seconds at various temperatures representative of consumer cooking (Swayne and Beck, 2004; Thomas and Swayne, 2007a)[15].

4.4.5.2 Cooking temperature and time

To estimate the effect of cooking HPAIV-contaminated poultry, it was necessary to identify data that are representative of the temperature and time U.S. consumers use to cook their poultry. A nationwide survey conducted by Audits International/FDA (1999) was used in the baseline scenario to represent the range of temperature consumers use to cook their chicken and turkey. Among the different type of foods measured, 570 samples of poultry were considered representative of the temperatures in which consumer cook poultry[16].

The proportion of poultry cooked at various temperatures is given in Figure 6 and is used to estimate the fraction of temperatures used to cooked poultry. Given that the data are aggregated into ranges of temperatures, the midpoint of each temperature range was used to represent the range of temperature. To estimate the fraction of poultry cooked at various temperature ranges, four categories of cooking were defined and are given in Table 6. These categories are assumed representative of the temperature at which U.S. consumers' cook their poultry.

[15] It is assumed that the level of HPAI is homogeneous throughout the poultry meat given that HPAI replicates in the endothelium of the muscle.

[16] These measurements are not representative of retail (hospitals, schools, restaurants, etc.) cooking.

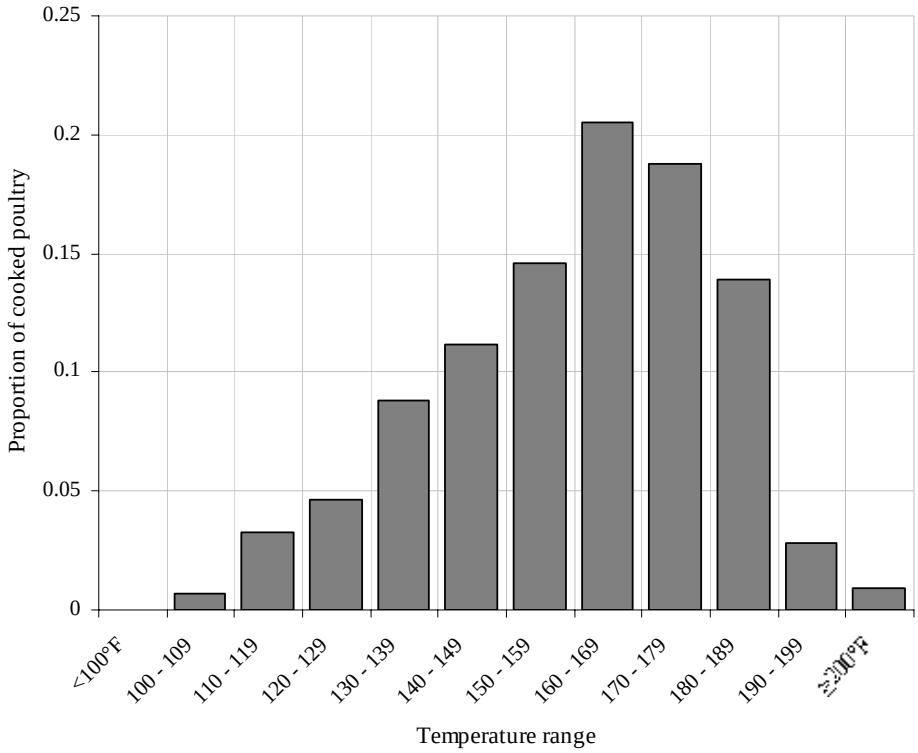

Figure 6. Proportion of poultry cooked at various temperatures (Audits International/FDA, 1999).

No data were identified to estimate the distribution of time that consumers cook poultry. As a result, it was assumed that Audits International/FDA (1999) data represented the peak temperature held for 10 seconds. No data were identified to estimate the time/temperature combination for heat-up or cool-down during cooking and therefore the effect of this additional time and temperature on the level of HPAIV could not be estimated.

Table 6. Baseline cooking temperature ranges (Audits International/FDA, 1999).

CATEGORY	TEMPERATURE RANGE (°F) [17]	PERCENT OF COOKED POULTRY	PREDICTED LOG_{10} REDUCTION IN 10 SECS
1	<100-139	17.4	0.048
2	140-149	11.2	0.791
3	150-159	14.6	12.92
4	160-≥200	56.9	211.1

[17] The temperature is given as the peak temperature achieved during cooking.

4.4.5.3 Heat lethality

The analysis of Thomas and Swayne, 2007a was used to estimate the HPAIV log_{10} reduction from heat applied to poultry. The authors developed line equations by use of linear regression from graphs of the time to reduce infectious titer by 10-fold vs. temperature. These regression line equations were used to predict the D-value for other temperatures (Table 7.) The D-value for chicken thigh meat was used in the baseline model to represent the effect of cooking HPAIV-contaminated poultry[18]. Log_{10} reductions were estimated by assuming a 10 second cook (for example: a reduction of ~13 log_{10} EID_{50} can be expected if poultry is cooked at 155 ^{o}F for 10 seconds (10/0.77)) (Table 6).

Table 7. Heat lethality D-values for HPAIV in chicken.

Temperature range	Midpoint	Temperature ^{o}C	Percent of cooked poultry	Chicken breast meat =10^(14.54628-0.21306*^{o}C) D-value (seconds)	Chicken thigh meat =10^(14.80834-0.21834* ^{o}C) D-value (seconds)
<100°F	95	35.0	0	12,279,481	14,670,334
100 - 109	105	40.6	0.7	804,476	898,338
110 - 119	115	46.1	3.3	52,704	55,010
120 - 129	125	51.7	4.6	3,453	3,369
130 - 139	135	57.2	8.8	226	206
140 - 149	145	62.8	11.2	14.8	12.6
150 - 159	155	68.3	14.6	0.97	0.77
160 - 169	165	73.9	20.5	0.064	0.047
170 - 179	175	79.4	18.8	0.0042	0.0029
180 - 189	185	85.0	13.9	0.00027	0.00018
190 - 199	195	90.6	2.8	0.000018	0.000011
≥200°F	205	96.1	0.9	0.0000012	0.0000007

4.4.5.4 Cross-contamination

During preparation of HPAIV-contaminated raw chicken and turkey, HPAIV could be transferred from the product to foods not likely to be cooked. Such an exposure route could lead to consumption of HPAIV and potential illness. Cross-contamination of bacterial pathogens during preparation of poultry is a known exposure route for surface-adhered pathogens such as *Campylobacter* and *Salmonella*. However, as HPAIV is not surface-adhered, but rather is found within the muscle cells throughout the meat, the role of cross-contamination of HPAIV as an exposure route is unclear.

[18] Unpublished data indicate the following parameters for lethality of HPAI: =10^(14.6773-0.2157* ^{o}C) (Thomas and Swayne, 2007b). Log_{10} reductions assuming a 10-second cook were 0.046 for 135 ^{o}C; 0.73 for 145 ^{o}C; 11.53 for 155 ^{o}C; and 182.2 for 165 ^{o}C. These data have little effect on model outputs.

Despite this, the conceptual approach used by the "drip fluid model" was used to estimate the impact of cross-contamination. This model was originally developed to describe the cross-contamination of *Campylobacter* during preparation of chicken products (Fazil et al., 1999). The model is largely heuristic, but the underlying concept is that each product will have an associated amount of fluid (purge) which is gained during the processing of the product. This purge is assumed to contain HPAIV that may have been released from muscle cells. The fluid may be transferred to other foods prepared and consumed at the same meal, or may contaminate hands or utensils that subsequently contact the mouth.

To estimate the effect of cross-contamination, two parameters[19] are used: 1) fraction of the total level of HPAIV in purge, and 2) proportion consumed. The fraction of the total HPAIV assumed to be in the purge gives an estimate of the level of HPAIV in the drip fluid. The following data were identified to estimate the proportion of HPAIV that may be present in chicken and turkey purge. Swayne and Beck (2005) took pre- and post-chill body cavity rinses from chickens infected with either LPAIV or HPAIV (H5N2). Levels ranged from 3.9 to 5.4 and 3.0 to 3.3 $\log_{10}$ EID_{50}/mL for LPAIV and HPAIV, respectively. Carcasses were submerged for 1 hour in 30 ppm chlorine and lower levels in post-chill rinse compared with pre-chill were observed for LPAIV samples. No significant change was observed for HPAIV, suggesting that under these experimental conditions, chlorine can be effective against LPAIV but not HPAIV[20]. Given that these data may not be representative of industry practices and of H5N1, the highest level observed will be used for the baseline scenario. Therefore, the fraction of HPAIV in the purge is 5.4 $\log_{10}$ EID_{50}/mL divided by the peak level of HPAIV observed in poultry meat, 7.7 $\log_{10}$ EID_{50}/g, or 0.0053[21] (0.53%)[22].

Of the HPAIV that is cross-contaminated, the model estimates what fraction is ingested. There were no data identified to estimate the proportion consumed. Therefore, the model allows the user to input different values to estimate the impact of consuming cross-contaminated HPAIV.

4.4.5.5 Consumption

To convert probability of exposures to number of predicted illnesses, it is necessary to estimate number of servings from a single chicken or turkey in the U.S. Specifically, the number of

[19] The risk model does not specify what fraction of servings is expected to be cross-contaminated. Therefore, when this component of the model is "on," all HPAI-positive servings are assumed to cross-contaminate. The cross-contamination component of the risk model is therefore "off" for the baseline analysis.

[20] Active chlorine level were not tested (D. Swayne, personal communication).

[21] The level of transferred HPAI is subtracted from the poultry meat to account for the removal of the transferred HPAI.

[22] Additional data were identified using calicivirus cross-contamination experiments to estimate the proportion that could be cross-contaminated (Bidawid et al., 2004; D'Souza et al., 2006.) Unfortunately, Bidawid et al. inoculated virus directly onto the surface of ham to test transfer to hands. This does not characterize the expected level of virus in purge or what would be expected on the surface of HPAI-contaminated poultry muscle. D'Souza et al. employed similar methodology when testing transfer from stainless steel to wet lettuce. These studies found, on average, that 6.0 and 6.45%, respectively, of the starting virus inoculum could be transferred.

servings for each product type that could come from an average sized chicken or turkey carcass and the average number of servings consumed by an individual. To estimate the average number of servings consumed, the 1994-1996, 1998 USDA Continuing Survey Food Intake by Individuals (CSFII) was used. The grams per serving was derived from consumers only and are an average of a two survey days. The population group is male and female, ages 2 and older. Foods were excluded if they did not contain poultry and/or eggs or if they were ready-to-eat, such as jarred and canned foods, including baby foods (Table 8).

Table 8. Turkey, chicken, and egg consumption.

	Turkey	Chicken	Egg
mean (g)	60.6	83	19.8
Percentile	grams		
10	15.8	9.4	0.5
20	21.3	26.7	1.2
25	24.7	36.2	1.6
30	28	44.7	2.1
40	32.6	56.7	3.4
50	42.6	72.3	5.3
60	53	85.5	8.4
70	59.6	97.4	14.9
75	67.2	103.1	21.6
80	83.5	119	37
90	113.9	170	73.3
95	169.8	209.8	87.4
97.5	224.1	254.3	101.2
99	336.1	338.8	139.8
99.5	425.6	379.2	168.7
99.9	859.5	582.3	257.5
100	1220	928.8	450.6

4.5 Egg Model

A single egg production flock is modeled in a similar manner as meat birds in that both use the transmission model to estimate the within-flock prevalence of infected birds over time. With meat birds, the public health concern is due to an undiagnosed flock going to slaughter. There is only a short window when the flock is near market weight in which a flock could pose a risk to humans and the flock only goes to slaughter one time. With egg birds, however, the flock stays in the house for a year or more while producing eggs for human consumption. In this case, the risk is posed by eggs produced by an HPAIV-infected flock before the flock is diagnosed. Thus, the egg model starts at the point of diagnosis and estimates the number of eggs that could have gone to processing prior to detection.

HPAIV-contaminated shell eggs sent for washing, sorting, and packing are subject to inspection and a fraction of eggs may be removed from the market. Eggs that are not removed, continue

through with the assumption that no changes occur to their level of HPAIV. The path of eggs then splits: shell eggs for table egg consumption enter the preparation module where they can be cooked, while shell eggs destined for egg products will receive a pasteurization step prior to entry into commerce[23] (Figure 7).

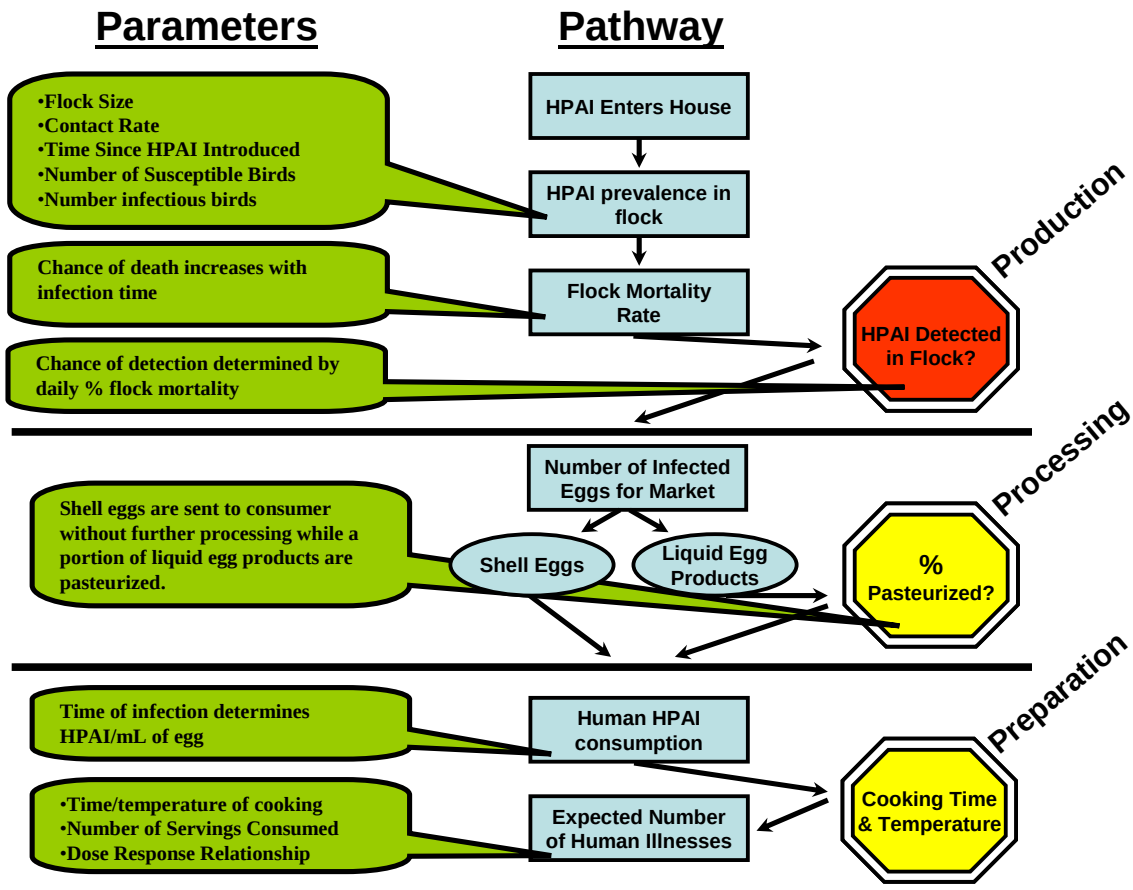

Figure 7. Egg model diagram.

There are three modules that make up the egg model:

- *Production*: The production module estimates the point at which an HPAIV-infected laying flock would be identified as HPAIV-positive, and the number of contaminated eggs that would have been sent into commerce prior to identification of the flock.

- *Processing*: The processing module estimates the number of contaminated eggs that would be passed during processing, the number of contaminated eggs that would go to market, and the level of HPAIV per gram of contaminated egg.

[23] Egg products are not quantitatively evaluated in this version of the risk model.

- *Preparation*: The preparation module estimates human exposure to HPAIV based on the number of servings consumed, viral level per serving, and the effect of cooking on reducing the amount of virus.

4.5.1 Egg Production Module: Transmission model

The transmission model is used to estimate the number of infected hens over time given one hen initially infected with HPAIV. The model then estimates the number of eggs that could have been produced by those infected birds to simulate the total number of infected eggs released for public consumption. Just as for the poultry model, several factors[24] will affect the rate of transmission and movement of birds from state to state: flock size, contact rate, and daily mortality threshold. Birds initially infected, latency, tissue infectivity, and mortality rate are the same for both models and not reiterated here.

4.5.1.1 Flock size

As with meat-type birds, the size of a hen flock can vary. The baseline model uses a value of 100,000 hens in a single house to represent the average hen flock size (G. Gregory, personal communication).

4.5.1.2 Contact rate

Hens are typically caged during their egg production life cycle. Given the reduced bird-to-bird contact, it is possible that HPAIV-infected caged birds would spread the virus more slowly compared with non-caged or ground raised meat-type birds (Elbers et al., 2004; 2006, 2007). To estimate the rate of spread of HPAIV among caged birds the data from HPAIV infected caged layers (Elbers et al., 2007) were used (see Contact Rate). An effective contact rate of 2 is assumed in the baseline scenario (Figure 8). This suggests that 1 infectious bird can expose 2 susceptible birds to HPAIV every 6 hours. The model allows input of different contact rates ranging from 1 to 64.

[24] Unlike the poultry model, "weeks in house" and bird type are not needed for the egg model. This is because for chickens and turkeys, the size of the bird increases as the bird is reared for longer periods of time. The more meat associated with a carcass, the greater the potential exposure. "Weeks in house" and "bird type" are associated with changes in the magnitude of the exposure (bird size) and are therefore not needed for the egg model

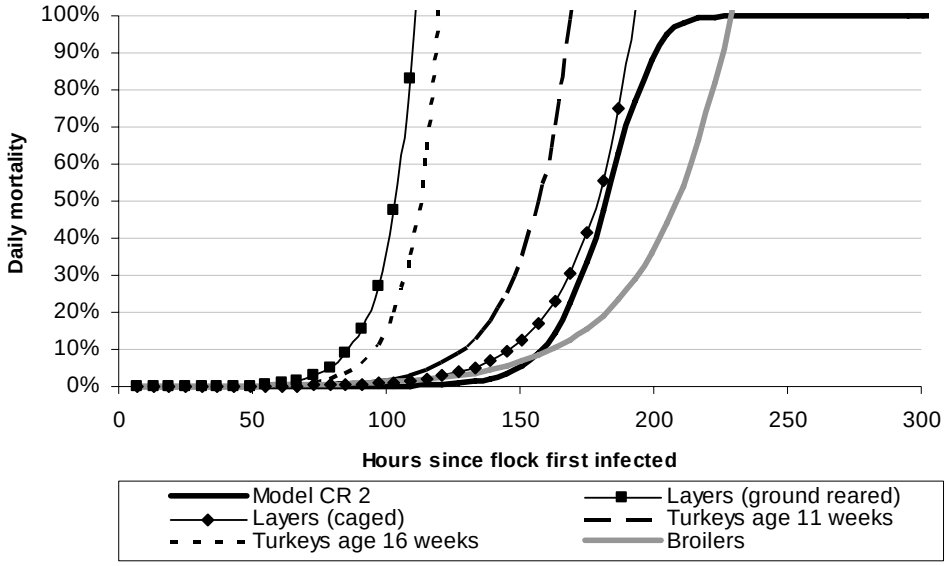

Figure 8. Percent daily mortality as predicted by the model at an effective contact rate of 2 compared with 2003 H7N7 HPAIV Netherlands outbreak data (based on 55 caged layer flocks).

4.5.1.3 Daily mortality threshold

The egg transmission model uses daily flock mortality to indicate when a flock is diagnosed as HPAIV-positive. In the baseline egg model, HPAIV infected flocks with a daily flock mortality of 2% or greater are always assumed to be identified.

4.5.1.4 Frequency of HPAIV-positive eggs

Internal contents of shell eggs from infected birds may or may not be contaminated. Few data were identified to estimate the number of HPAIV-positive eggs that may be produced from flocks infected with different HPAI strains. Three issues were considered to estimate the number of contaminated eggs from infected birds: 1) egg production rate, 2) time course of contaminated eggs, and 3) frequency of egg contamination.

Egg production rate. The rate at which healthy layers produce eggs is on average 0.7 eggs/day/hen (American Egg Board, 2006). However, HPAIV-infected hens could produce eggs at a lower frequency. When egg production might drop and to what extent is unknown; however, data from natural outbreak studies of HPAIV and LPAIV suggest a drop in egg production of 2-40% given length of infection and strain (Mutinelli et al., 2003; Henzler et al., 2003; Zanella, 2003; Kinde et al., 2003; Bowes, et al., 2004). In addition, Bean et al., 1985 estimated a 31% egg production drop during an H5N2 outbreak while Elbers et al., 2004 and 2005 indicated that for naturally H7N7 infected flocks, "egg production drops quickly to almost 0% in only a few days." Furthermore, Swayne and Halvorson, 2003 mention "Precipitous drops in egg production occur in breeders and layers with typical declines including total cessation of egg production within six days."

For this risk assessment, a drop in egg production is not modeled as it is unclear if the data identified are representative of more virulent strains causing outbreaks in Asia, Africa, and the Middle East. In addition, the data do not indicate when this drop may begin (see below). LPAI and HPAI strains from the data above appear to kill birds more slowly than more recent H5N1 HPAI strains. This could have allowed a drop in egg production to be observed. HPAI strains of concern in this risk assessment may kill birds too quickly for there to be a practical drop in egg production[25].

Time course of contaminated eggs. When eggs become contaminated is important. It is assumed that eggs are not immediately contaminated following exposure to HPAIV and thus not all eggs laid by an HPAIV-infected hen will be positive. Eggs collected from flocks naturally infected with H5N2 HPAIV and experimentally infected chickens 1 and 2 days[26] post-infection did not contain the virus internally (Cappucci et al., 1985; Beard et al, 1984). Only by the third day was virus detected within internal egg contents.

In addition, Bean et al., 1985 observed that among 37 eggs produced by H5N2 infected hens, only the last egg produced by 3 hens was HPAIV-positive. All other eggs were free of the virus. This observation is supported by a personal communication from ARS (D. Swayne, personal communication) that suggested only the last egg or last two eggs produced by a bird infected with HPAIV would be HPAIV-positive.

Frequency of egg contamination. Bean et al., 1985 estimated ~8% egg contamination, while Cappucci et al., 1985 estimated ~ 7-57% egg contamination for different flocks. Beard et al., 1984 observed ~35% of eggs HPAIV-positive. These estimates are based on the entire course of the infection or the number of eggs produced daily.

For this risk assessment, the following approaches were taken to estimate the number of contaminated eggs laid by infected hens. Each method is a model option and the effect on the model output of choosing an option can be evaluated.

1) Option 1: The probability of egg contamination by an infected hen is 50%. Hens typically produce 0.7 eggs/day and given the short life expectancy of an HPAIV-infected hen (36-42 hrs (Das et al., 2006)), hens are likely on average to produce no more than 2 eggs. Based on the data of Bean et al., 1985 data and the ARS personal communication, in which only the last egg produced by an infected hen could be positive, half of those eggs would be infected. In addition, 50% represents an upper bound similar to the 57% observed by Cappucci et al., 1985. This option is currently used in the baseline model.

[25] Despite this possibility, Elbers et al., 2007 used LPAI and HPAI data to develop recommendations for when poultry managers should consult a veterinary practitioner: if a depression in egg production of ≥ 5%/day is seen for two consecutive days.

[26] Interval of time between onset of clinical illness and the collection of the eggs that were tested (Cappucci et al., 1985).

2) Option 2: The probability of egg contamination is the same as the probability of tissues being infected (Das et al., 2006). ARS researchers exposed chickens to HPAIV and measured HPAIV levels within the trachea, breast, thigh, and heart using three different detection methods every six hours (Table 4). Ideally, a time course evaluating the presence of HPAIV in ovary and oviduct tissue would be needed (virus has been found in both organs (Nakatani et al., 2005)). However, given the lack of information, these data are used as a proxy to estimate the frequency of egg contamination over the course of infection. If this option is chosen for the baseline model, the data in Table 4 are aggregated by averaging the recovery for all organs by either detection method.

3) Option 3: The probability of egg contamination is based on a threshold distribution. This approach is represented by the Cappucci et al., 1985 and Beard et al., 1984 studies that observed H5N2-infected hens did not lay contaminated eggs until 3 days post-infection. These data appear to indicate that there is a threshold point below which a hen can not produce contaminated eggs. If this option is chosen for the baseline model, it is assumed that hens infected for 1-13 hours would not produce contaminated eggs; hens infected for 19 hrs or more produce contaminated eggs 100% of the time. The threshold time is different from the above studies given the likelihood that more virulent H5N1 strains would contaminate eggs more quickly than H5N2 strains[27].

4.5.2 Eggs Processing Module

The egg processing module estimates the number of contaminated eggs that would be passed during processing, the number of contaminated eggs that would go to market, and the level of HPAIV per gram of contaminated egg. Currently, the egg processing module does not partition eggs into shell eggs and egg products. The model assumes that all infected eggs from an infected flock are shell eggs. The model parameters that determine the amount of HPAIV reaching consumers through shell eggs are the level of HPAIV deposited in eggs, egg inspection, and the time required for transport and storage before consumption.

4.5.2.1 HPAIV level in eggs

Few studies were identified to estimate the distribution of level of HPAIV in shell eggs laid by a hen over the time course of the infection (Table 9). All measurements appear to be taken during peak infection and do not provide data to estimate the level of HPAIV in an egg laid during pre-peak infection. The risk assessment therefore assumes that regardless of when an egg is laid, the level of HPAIV is the same.

[27] If the data of Cappucci et al., 1985; Beard et al, 1984 are considered representative of more virulent HPAI strains, hens would be predicted to produce very few HPAI-positive egg because all hens are predicted to be dead within 36-42 hours.

Table 9. HPAIV levels in shell eggs.

REFERENCE	PRODUCT	HPAI STRAIN: TITER	TIME SAMPLE TAKEN
Promkuntod et al., 2006	internal contents of quail eggs	H5N2: $10^{4.6\text{-}6.2}$ ELD_{50}/mL	unknown
Swayne and Beck, 2004	internal contents of hen eggs	H5N2: $10^{4.9}$ EID_{50}/mL	3 and 4 days PI
Bean et al., 1985	egg albumen	H5N2: $10^{5.6}$ EID_{50}/mL	day of death
	egg yolk	H5N2: $10^{3.6}$ EID_{50}/mL	
Beard et al., 1984	internal contents of hen eggs	H5N2: > $10^{4.0}$ ELD_{50}/mL	3 and 4 days PI

Of those eggs that are HPAIV-positive, data indicate that the albumen compartment is more frequently contaminated with HPAIV than the yolk or yolk and albumen combined. From studies of naturally H5N2-infected commercial layers and breeders 30% (53/175) of albumens were HPAIV-positive compared with 20% (35/175) of yolks and albumen and yolks (Cappucci et al., 1985). For hens experimentally infected with H5N2, 11/14 albumens were identified as HPAIV-positive compared with 9/14 of yolks (Beard et al., 1985).

Only one study was identified that separated the yolk from the albumen to estimate the level of HPAIV in each (Bean et al., 1985). Therefore, it is possible to incorporate into the risk assessment the different levels for the albumen and yolk. However, given the very limited data to estimate these distributions, the data are not incorporated in this version of the risk assessment. Currently, it is assumed that any contaminated egg contains a point estimate of $10^{4.9}$ EID_{50}/mL.

The data in Table 8 are assumed to be representative of HPAI strains that can make humans ill. However, the data are based on the H5N2 and an unknown HPAI strain. This strain of H5N2 has been shown to be less virulent to birds and result in lower virus levels in poultry meat (Swayne and Beck, 2005; Swayne, 2006a). Therefore, it is possible that HPAI strains that result in severe human illness may result in more or less[28] HPAIV in contaminated eggs.

4.5.2.2 Egg inspection

The processing module estimates the number of contaminated eggs that will pass inspection during processing. Inspectors remove eggs that do not meet quality standards. Given healthy birds, a baseline level of eggs can be expected to be removed; this baseline level will not be modeled. For birds that are infected with HPAIV, data suggest that an increased number of eggs will be removed. Cappucci et al., 1985 observed that 10% of eggs produced by HPAIV H5N2 naturally-infected hens were "thin-shelled, soft-shelled, or abnormally small". Beard et al., 1984 observed of 15 HPAIV H5N2 eggs, 3 were soft-shelled. Assuming these eggs would have been removed during processing, the data suggest 20% of HPAIV-positive eggs could be abnormal. Hens infected with HPAIV H5N1 may show a similar level or greater. The baseline model assumes that of HPAIV-contaminated eggs produced by infected layers, 5% will not pass

[28] H5N1 strains may result in more HPAI in eggs by growing rapidly in the ovaries and oviduct. However, it is possible that H5N1 strains may result in lower egg contamination given the speed of hen mortality and that approximately 17 hrs are required for egg formation.

inspection. The effect of higher or lower rejection levels can also be tested by the model (see Appendix B: Chicken and Turkey Model Options).

4.5.2.3 Time for eggs to reach consumers

The time required for eggs to reach the market shelf following processing will vary. Some factors affecting this include in-line or off-line processing, duration of packing and storing eggs, and transportation to and from and storage at various facilities before eggs are placed on the market shelf. To estimate the length of time required for the average egg to reach the market shelf, a combination of published studies, surveys, and expert opinion were used. Figure 9 shows the median and upper and lower bounds of the estimate egg age at various stages in the food supply system. A survey of egg layer flocks was used to estimate the age of an egg at the end of "On farm Storage" (Figure 9) (NAHMS Layers 99, 2000). The time at processing (transportation thought retail transportation) is informed by expert elicitation (USDA, 2004). The time eggs remain at 'Retail Storage" is informed by Bell et al., 2001. The data used are described in the Risk Assessment for *Salmonella* Enteritidis in Shell Eggs and *Salmonella* spp. in Egg Products (SERA, 2005).

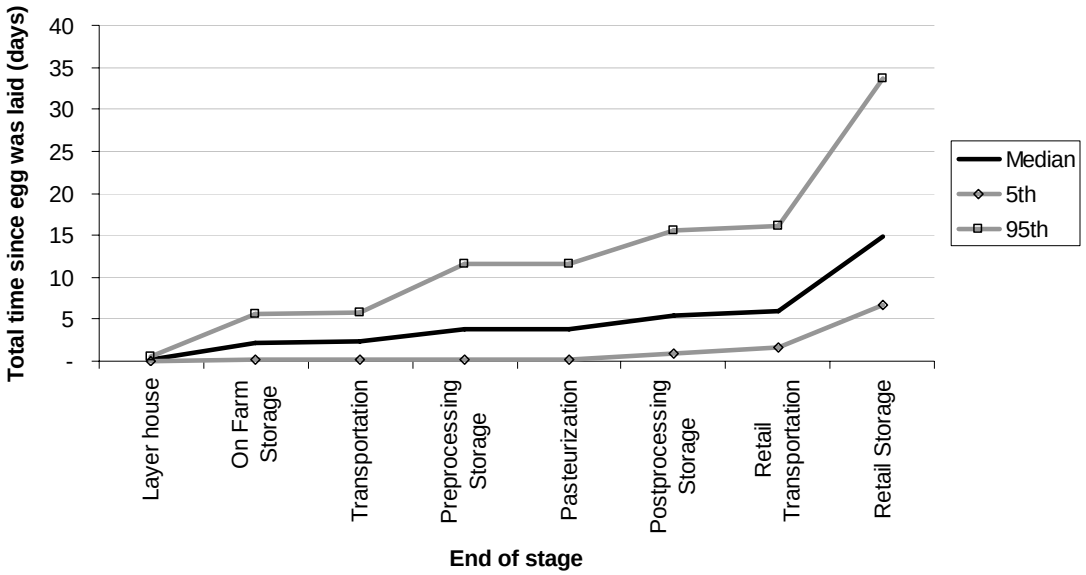

Figure 9: Age of eggs at various stages of storage, transportation, and processing.

The chart graphically represents the cumulative age of eggs from lay to consumer purchase. The solid line indicates the average age of eggs at the various stages in distribution. Stages are represented by the "end of a stage". For example, the average age of eggs that reach a retail establishment is represented by the "retail transportation" stage on the x-axis, this is approximately 6 days. Six to 15 days represents the time eggs are at the retail establishment; 6 days being the average age of eggs when they arrive at retail and 15 days being the average age of eggs when they are purchased. Therefore, it was assumed for the baseline scenario that eggs

reach market shelves by 6 days following lay. The sensitivity of the model outputs to this assumption is evaluated by the model.

4.5.3 Egg Products pasteurization

A plant pasteurization step for egg products is not modeled. This risk assessment assumes that the experimental data of Swayne and Beck, 2004 are representative of industry practices and would result in elimination of the virus under most processing time and temperate profiles (Table 10).

Table 10. Time to inactivate HPAIV in egg products (Swayne and Beck, 2004).

EGG PRODUCT	INDUSTRY TEMPERATURE (^{o}C)	INDUSTRY TIME	TIME TO INACTIVATE HPAIV IN EGGS
Whole egg	60	210 sec	133 sec
Whole egg blends	60	372 sec	133 sec
Whole egg blends	61.1	210 sec	67 sec
Liquid egg white	55.6	372 sec	182 sec
Liquid egg white	56.7	210 sec	162 sec
10% Salted yolk	62.2	372 sec	>98 sec
10% Salted yolk	63.3	210 sec	>98 sec
Dried egg white	54.4	7 to 10 days	15.2 days
Dried egg white	67	15 days	0.59 days

4.5.4 Egg Preparation Module

The preparation module estimates human exposure to HPAIV based on the number of eggs consumed and the level reduction from preparation (cooking).

4.5.4.1 Cooking time and temperature

To estimate the effect of cooking HPAIV-contaminated eggs, it was necessary to identify data that are representative of the temperature and time U.S. consumers use to cook their eggs. Unpublished data using an infrared camera to estimate the surface temperature of yolk and albumen during different styles of cooking were used to estimate the temperature that eggs reach during cooking by consumers (Fleischman, 2006) (Table 11). Linear regression analysis developed by Swayne and Beck, 2004 were used to estimate $\log_{10}$ reductions at different cook temperatures (Table 11).

Table 11. Effect of cooking eggs (Fleischman, 2006; Swayne and Beck, 2004).

	°C	°F	Homogenized Whole Eggs (=(-0.274[x-52°C])+3.626)	
Scrambled			D-value (secs)	Log_{10} reduction (10 secs)
Min	72	161.6	0.014	714
Mean	78.5	173.3	0.000	43,151
Max	85.8	186.44	0.000	4,317,178
Sunny side up (20 min. cook time)				
Yolk	46.6	115.88	41,465	0.0
Thick albumen	63.6	146.48	2.803	3.6
Thin albumen	78.5	173.3	0.000	43,151
Over easy (8 min. cook time)				
	Before flipping			
Yolk	24.7	76.46	1,921,754,912	0.0
Thick albumen	53.9	129.02	1,274	0.0
Thin albumen	65.9	150.62	0.657	15.2
	After flipping			
Yolk	75.2	167.36	0.002	5,380
Thick albumen	75.5	167.9	0.002	6,501
Thin albumen	72.7	162.86	0.009	1,111

No data were identified to estimate the distribution of time that consumers cook eggs. As a result, it was assumed that the temperature data represented the peak temperatures held for 10 seconds. Data were identified to estimate the time/temperature combination for heat-up (but not cool-down) during cooking (P. Curtis, personal communication). However, the effect of this additional complexity on the level of HPAIV was not estimated.

Fleischman 2006 did not investigate egg cooking temperatures for other cook styles including soft boiled/poached, hard boiled, beverages, and mixtures. To estimate the log_{10} reductions associated with other cook styles and to estimate the fraction of egg cooking styles, data from the Risk Assessment for *Salmonella* Enteritidis in Shell Eggs and *Salmonella* spp. in Egg Products were used (SERA, 2005). The styles of cooking, the fraction of eggs that are assumed to be cooked in that style, and the log_{10} reductions assumed for the baseline model are given in Table 12.

Table 12. Egg baseline model inputs.

Type of Product	Soft boiled and poached	Sunny side up	Scrambled and omelettes	Over easy	Hard boiled	Beverages	Mixtures
Fraction	0.120	0.135	0.470	0.135	0.140	0.003	0.530
Log_{10} reduction	0.94	1.20	2144.62	0.04	8.00	0.00	12.00

4.5.4.2 Consumption

To convert probability of exposures to number of predicted illnesses, it is necessary to estimate number of servings from shell eggs and egg products. Specifically, the data needed are the number of servings for each product type that could come from an average sized egg and the average number of servings consumed by an individual. The egg model assumes that one egg results in one exposure and an egg represent 60 mL.

4.6 Exposure pathways not addressed

4.6.1 Ducks and Geese

Exposure to HPAIV-contaminated ducks and geese processed by FSIS plants will not be evaluated by this risk assessment. Though some data are available, in general, there are less data for these two bird-types than that available for chicken and turkeys. Also, ducks and geese constitute approximately 0.3% of the total poultry production mass slaughtered in FSIS inspected plants according to the electronic Animal Disposition Report System (eADRS, 2002). Therefore, chickens and turkeys comprise the majority of domestic commercial poultry processed for human consumption.

The level of HPAIV in goose muscle from infected geese is unknown. For ducks, HPAIV-contaminated muscle appears to harbor significantly less virus than that associated with chicken muscle (Table 13), suggesting that cooking will be more effective and consumer cross-contamination less frequent leading to a safer product. However, the consumption of duck and goose could pose some level of risk. Morbidity and mortality associated with ducks and geese infected with HPAIV are sporadic. Ducks and geese typically present without signs (Shortridge *et al*., 1998) and with limited mortality, though HPAI strains resulting in significant mortality for both species have been identified (Zhao *et al*., 2006). As a result, a low level of mortality may be associated with HPAIV infection for ducks and geese resulting in difficulties in detection of an HPAIV-infected duck or goose flock.

Table 13. HPAIV levels in duck.

REFERENCE	PRODUCT	HPAI STRAIN: TITER
Tumpey et al., 2003	duck muscle	H5N1: $10^{2.7\text{-}3.5}$ EID_{50}/g
Tumpey et al., 2002	duck muscle	H5N1: ~$10^{3.5}$ EID_{50}/g

In addition, consumption of ducks is associated with specific ethnic groups suggesting that exposure to some groups may be relatively greater than the general population. However, despite this, the Advisory Committee on Microbiological safety of Food (2005) and French

Agency for Medical Safety of Food (2005) determined that the risk is anticipated to be low for the UK and French population, respectively.

4.6.2 Ready-to-Eat and Partially Cooked Poultry

Cooked ready-to-eat (RTE) poultry are assumed HPAIV-free given the required heat lethality for RTE products of 7.0 $\log_{10}$ (9 CFR 381.150) at the plant and the additional heating step often applied by the consumer. ARS researchers demonstrated that high titers of HPAIV ($10^{8.5}$ EID_{50}/g) in contaminated chicken muscle were eliminated following a process that delivered a 7.0 $\log_{10}$ reduction in *Salmonella* (Thomas and Swayne, 2007a). The authors' state, "Calculations with the conservative D-values predicted that cooking chicken meat according to current U.S. Department of Agriculture Food Safety and Inspection Service time-temperature guidelines will inactivate Korea/03 in a heavily contaminated meat sample, such as those tested in this study, with a large margin of safety." Therefore, RTE products cooked in accordance with FSIS regulations will pose a negligible risk.

Partially cooked poultry (breaded chicken patties, nuggets, etc.) are not modeled as well. These products undergo a partial heating step within the plant that may reduce some level of HPAIV if present. These products are often purchased frozen and cooked by the consumer as they are not considered RTE. These products are assumed to constitute a low level of risk given the relatively small volume of production and consumer reheating.

Occasionally, RTE foods are consumed directly without consumer cooking. The most common RTE food that may be eaten cold is hot dogs. A USDA hotline questionnaire obtained some information on eating of hot dogs cold, directly from the package. The data indicate that between 14 and 46 of 223 persons in the families of the 84 people responding ate hot dogs cold under some circumstances. In addition, the American Meat Institute (AMI) survey of 1000 persons (American Meat Institute, 2001) obtained information on the fraction of hot dogs eaten cold. Among the AMI survey respondents, 134 indicated that they sometimes ate hot dogs without reheating, 97 indicated that other members of their household sometimes did so, and 657 indicated that they always reheated them. These data indicate that there is a fraction of the U.S. population that consumes hot dogs without reheating. However, given the compliance guideline recommended time/temperature combinations for production of RTE poultry (http://www.fsis.usda.gov/OPPDE/rdad/FSISNotices/RTE_Poultry_Tables.pdf[29]), hot dogs are predicted to be free of virus.

4.6.3 In-plant Cross-Contamination

Changes in the level and prevalence of HPAIV will not be explicitly modeled within the processing environment as it will be assumed that HPAIV will not grow in non-living tissue.

[29] Accessed 9/5/07.

HPAIV within the poultry environment are likely to decline, however this will be dependent on temperature, presence of water, and other factors (see Factors Affecting HPAIV Survival). However, during the processing of an HPAIV-infected flock, virus can spread to previously uncontaminated poultry or other meat species thereby increasing the prevalence of HPAIV contaminated product. These events will not be modeled by the current risk assessment as they are likely to result in surface contamination susceptible to consumer cooking. In addition, the amount of virus transferred is likely relatively low compared to the level in naturally infected poultry meat, again allowing the virus to be susceptible to cooking. Product that is cross-contaminated then ground is likely to be of a relatively higher risk given that surface contamination would be folded into the interior of the product. However, compared to naturally HPAIV-infected meat where the virus is throughout the product, the risk, if any, should be low.

4.6.4 Egg shell contamination

The egg shell surface can be contaminated with HPAIV, typically from HPAIV-positive feces (Cappucini et al., 1985; Beard et al., 1984). The model will not assess the impact of cross-contamination from contaminated egg shell surfaces nor direct contact, as it will be assumed industry procedures to wash shell eggs eliminate external virus (Hutchison et al., 2004).

4.6.5 Exposure to food preparers

Inhalation, mucosal contact, and wound exposures to food preparers from handling contaminated raw poultry and eggs during food preparation will not be addressed by this version of the risk assessment model. Exposure to food preparers could occur through multiple pathways as described above (see Possible routes of exposure for food preparers to HPAIV). The following are a list a data needs that would be required to effectively model different routes of exposure and subsequent illness for food preparers.

1. The most daunting data need would be the requirement of multiple dose-response relationships based on the route of exposure. Data would be needed to inform three additional dose-response models to assess the probability of illness for food preparers exposure to HPAIV. Dose-response relationships for inhalation exposure would be needed for aerosolized virus and contact with conjunctiva tissue and intranasal dose-response relationships would also be required. In addition, contact with open wounds or direct contact with blood or other tissues would be needed given a kitchen accident such a slicing with a knife.
2. The amount of HPAIV food preparers could be exposed to would be needed. For instance, how much aerosolized virus particles can be expected given tenderization of poultry? How much virus could be transferred from raw product to a food preparers' hand and then subsequently transferred to their eye or nose? Transfer coefficients would be needed to model the possible exposure dose for individuals, given various preparation behaviors.

3. Data would also be needed on the frequency of various food preparation behaviors. For example, the frequency of eye rubbing without washing hands between handling of raw product and facial contact.

Methods and some data are available to address some of the issues above. For example, unsanitary food preparation behaviors could be grouped into non-specific behaviors. The frequency of each event leading to exposure would not be defined per se, but a single distribution representing all unsanitary food preparation behaviors could be used. In addition, the amount of virus transferred from surface to surface could be extrapolated from bacterial studies assuming such studies are representative of transfer of HPAIV within the kitchen environment. However, these techniques would result in a substantial amount of uncertainty being introduced into the model outputs and would still not be able to address the dose-response. As the route of exposure is critical for infectivity of the virus, there simply are no data to provide the needed resolution to inform multiple dose-response relationships.

4.6.6 HPAIV survival during storage

Inactivation during storage of HPAIV-contaminated poultry and eggs was not modeled as data to estimate a daily inactivation rate under various storage conditions were not identified. Therefore, it was assumed that the level of HPAIV does not decrease during storage as product should be maintained at about 4 ^{o}C. Using other viruses, the following data support this assumption: Pearson, 1944 conducted several experiment using influenza type A and showed that the virus suspended in alloantoic fluid "at 4-6 C retained its original titer" for at least 15 days. In addition, Lynt, 1966 found that poliovirus and coxsackievirus stability was depended on the food matrix in which it was tested— surviving well in potato salad for up to a month but not pizza or shrimp. A more recent study using feline calicivirus as a surrogate for norovirus inactivation on the surface of ham found about a 1 $\log_{10}$ decline in virus titer over 1 week at 4 ^{o}C (Mattison et al., 2007). However, these studies tested the effect of drying and the authors attribute the relative stability of virus on ham due to seepage "through the surface of the ham to an inner matrix, thereby being protected against dryness." Given that most HPAIV will be internal, this study is not likely representative. A review paper indicated Sobsey et al., 1986 stated "HAV [hepatitis A virus] did not decline over 8 weeks in groundwater or soil samples and none of the three viruses [HAV, poliovirus, echovirus] declined in the effluent samples at 5 ^{o}C" (John and Rose, 2005). Alternatively, virus titers have been observed to decline in ground water and other matrices over time. This is largely dependent on temperature where refrigeration temperatures reduce or inhibit inactivation (John and Rose, 2005). Collation of data from six virus types suggests that the mean rate of decline ranges from 0.03 to 0.2 $\log_{10}$ per day (data from experiments using 3-30 ^{o}C) (John and Rose, 2005). H5N2 titers in poultry organs were found to decline during composting, however, temperatures reached > 55 ^{o}C over the 20 day time course (Senne et al., 1994). A similar result was found for H13N7 stored on porous and nonporous surfaces at room temperature (Tiwari et al., 2006). Interestingly, one study was identified that demonstrated decline of H1N1 in experimentally contaminated swine meat held at

4 and -20 °C. At the higher temperature, H1N1 remained relatively stable for 2 days post inoculation, however by day 15, no virus was detected in the samples (Romijn et al., 1989).

5 Hazard Characterization (Dose-response)

In microbiological risk assessment, hazard characterization includes the evaluation of the nature of the adverse effects associated with a microbiological agent. In a quantitative assessment, the dose-response relationship between the magnitude of the dose of an infectious agent and the magnitude and/or severity of the effect (*i.e.*, the likelihood of becoming ill) is used to estimate the likelihood of illness for a given level of exposure. Currently there are no published studies on the human HPAI dose-response relationship from oral exposure to HPAIV (*i.e.*, consumption of infected poultry or egg products). This section discusses the available data and methodological options for estimating a human HPAI dose-response relationship.

5.1 Available Data

Human illness from HPAIV is likely to vary widely given differences in exposure route, dose, host factors, and HPAI strains. Five sets of data were identified for potential use in developing a dose-response function. The data included: epidemiological studies, intranasal animal, intranasal human, human vaccine trials, and animal feeding/gavage studies. Dose-response data from human oral exposure was not identified in the published literature. Furthermore, choosing among the available data to use for a human oral dose-response for HPAI is confounded by the lack of strong epidemiologic data to support consumption as possible HPAIV exposure pathway.

Criteria were developed to assist in the selection of the most appropriate data to use for the dose response module for this risk assessment. The criteria included consideration of the study design relative to the appropriateness of the test subject, dose delivery route, virus subtype, the use of multiple strains and if they were unaltered, the metric to measure level, the range of doses employed, and if susceptible subpopulations were tested. Table 14 summarizes the advantages and disadvantages of four of the potential data set (see Appendix D: Review of Selected Epidemiological Studies). None of the options satisfied all of the criteria and practical considerations limited the use of the vaccine trial data (see Summary of Influenza Vaccine Trial Data). Ultimately data from the Beare and Webster (1991) study was selected because this choice eliminated the uncertainty in extrapolating from animals to humans. It cannot be understated however, that extrapolation from human intranasal studies to human consumption of HPAIV introduces considerable but unquantifiable uncertainty in the risk estimates resulting from this approach. Therefore, the scenario analysis results are most appropriately considered as percentage changes and not absolute values.

Table 14. Advantages and disadvantages of various dose-response data.

ISSUES	HUMAN INTRANASAL[1]	VACCINE TRIAL DATA[2]	ANIMAL FEEDING DATA[5]	ANIMAL INTRANASAL DATA[4]

Consumption exposure pathway	No	No	Yes	No
Human species barrier	Yes	Yes	No	No
Relevant physiology	Yes	Yes	No	No
Relevant H5 strain	Unknown	Unknown	Yes	Yes
Strains of avian origin	Yes	Unknown	Yes	Yes
Multiple strains tested	Yes	Yes	Yes	Yes
Wildtype (unaltered) strains	Yes	No	Yes	Yes
Virus measured in EID_{50}	Yes	No	Yes	Yes
Broad range of doses tested	No	Yes	No	Yes
Susceptible population tested	No	Yes	No	No
More data forthcoming	No	Possibly[3]	Unknown	Yes[5]

[1] Beare nnd Webster, 1991
[2] see Table 16.
[3] Unpublished data may exist from clinical data for vaccines with avian surface proteins (H5N1, H9N2).
[4] See Table 17.
[5] http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=409883 (Accessed 5/20/08).

5.1.1 Summary of Epidemiological Studies

While epidemiological studies with data on human exposure and subsequent illness are preferred, epidemiological studies were not used to estimate a dose-response relationship for this risk assessment since: 1) outbreak studies were not identified where consumption was the exposure pathway, and 2) data to estimate the HPAIV dose resulting in human illness from outbreaks was not available. To estimate the dose-response relationship (*i.e,* the probability of human illness from oral exposure to HPAIV), epidemiological studies in which consumers were exposed to and became ill from the virus through a consumption exposure pathway would be needed. Such studies were not identified. Two cases in Asia suggested a possible link of infection to the consumption of raw duck blood (EFSA, 2006); however, these cases are not useful given that the amount of virus consumed is unknown.

Given this, epidemiological studies where other exposure pathways were evident could be used as a proxy (Appendix D, Table 1). For these studies to be useful for a dose-response relationship, the number of human illnesses caused by HPAIV, the number of exposed individuals, and the HPAIV dose would be needed. Several studies were identified with HPAIV confirmed or estimated human illnesses; however, few of these studies also estimated the population that could have been exposed during the outbreak (Appendix D, Table 1, column 4). None of the outbreak studies estimated the dose of HPAIV to which sick individuals were exposed. Without this information, a dose-response relationship could not be developed using these epidemiological studies. For a more complete review of human epidemiological studies, please see Appendix D: Review of Selected Epidemiological Studies.

5.1.2 Summary of Beare and Webster (1991) Data

A human AI intranasal exposure trial was conducted to evaluate human susceptibility to AI (Beare and Webster, 1991). In this study, 82 volunteers were exposed with a minimum of $10^{6.8}$

EID_{50} intranasally. Clinical symptoms were recorded and identified as severe, moderate, mild, and very mild (Table 15).

Table 15. Responses of human volunteers to infection with avian influenza viruses (Beare and Webster, 1991)

Surface antigens	Virus Dose (log_{10} EID_{50})	Severe	Moderate	Mild	Very mild	No response
H1N1	7.5	0	1	0	3	6
H1N1	7.7	0	0	0	0	5
H1N1	7.5	0	0	0	0	6
H3N8	7.7	0	0	0	1	5
H3N2	8.0	0	0	1	0	2
H6N2	9.2	0	0	0	1	4
H6N1	9.0	0	0	1	2	8
H9N2	8.2	0	0	0	1	6
H4N8	7.5	0	1	2	3	8
H10N7	6.8	0	0	2	6	7

Eight different AI subtypes were employed including H1N1, H3N2, H3N8, H4N8, H6N1, H6N2, H9N2, and H10N7. Moderate, mild, and very mild symptoms were observed to varying extents, suggesting that AI can infect humans exposed intranasally with high virus titers. The limitations of this study for human oral dose response include the following: 1) Uncertainty due to the need to extrapolate from the relatively high dose levels used in this study to the lower dose-response region, 2) Uncertainty relative to the unknown representativeness of the virus strains used, and 3) Uncertainty concerning the tissue specificity of receptor distribution.

5.1.3 Summary of Influenza Vaccine Trial Data

Attenuated flu vaccine trial studies were identified for possible use as a source of dose-response data. Attenuated flu vaccines consist of reassortant[30] viruses containing six RNA segments encoding "internal" viral proteins derived from an attenuated virus strain These strains include viruses of avian origin, or cold-adapted (ca) human strains which cannot replicate outside of the human nares or upper respiratory tract. The remaining two RNA segments encode "external" viral proteins derived from a virulent virus strain in current circulation (*e.g.*, H1N1, H3N2).

Clinical studies evaluating these reassortants as human influenza vaccines were identified (Table 16). In these studies, human subjects were infected, by intranasal infection, with attenuated virus at doses ranging from 3.0 to 7.5 log $TCID_{50}$ (tissue culture infectious dose 50% endpoint). Outcome was judged by viral shedding, clinical signs (fever) or immune response. Human ID_{50} was measured at 2.9 to 6.5 $TCID_{50}$ depending on source of internal viral genes, external viral genes, and age of recipient. These data were not selected for use in the dose-response model. A

[30] A virus with gene segments derived from more than one virus http://www.nature.com/nrmicro/journal/v3/n8/glossary/nrmicro1208_glossary.html.

key limitation is that the dose was determined as $TCID_{50}$ and not EID_{50}. No data were identified to determine the relationship between the two metrics.

Attenuated vaccines incorporating external viral proteins from AI viruses with pandemic potential have been produced and tested in animals. Vaccines combining H9 and N2 coding external segments with the internal segments from a ca strain or another source (strain PR8) was developed and tested in mice and chicken (Chen et al., 2003a; Chen et al., 2003b). A reassortant containing genetically-altered H5 and N1 coding external segments and PR8 internal segments was also engineered using "reverse genetics" and evaluated in mice and chicken (Subbarao et al., 2003). Based on a review of the publicly-available literature, these vaccine candidates have not been evaluated in human subjects (Subbarao and Luke, 2007).

Table 16. Influenza Vaccine Trial Data.

Reference	Population	Virus-int	Virus-ext	Human ID_{50}, log_{10} $TCID_{50}$	Dose range, log_{10} $TCID_{50}$
Sears et al.,1988	adults	ah and ca	H1N1 and H3N2	4.9 (ca, H1N1); 5.4 (ah, H1N1); 6.4 (ca, H3N2); 6.5 (ah, H3N2)	4.5, 5.5, 6.5, 7.5
Snyder et al.,1986	adults	ah	H1N1 and H3N2	4.9 (H1N1), 5.4 (H3N2)	4.5, 5.5, 6.5, 7.5, 8.5
Clements et al., 1989	adults	ah	H3N2	5.8 and 6.3 (2 AI strains providing internal genes)	4.5, 5.5, 6.5, 7.5
Clements et al., 1983	adults	ca	H3N2	5.3	4.5, 5.5, 6.5, 7.5
Clements et al., 1986	adults	ah	H3N2	6.2	5.5, 6.5, 7.5
Belshe et al.,1984	children; 12-48 months	ca	H1N1	3.5	3.2 - 7.2
Steinhoff et al., 1991	children; 6-48 months	ah and ca	H1N1	2.9 (ah), 2.6 (ca)	3, 4, 5, 6
Gruber et al.,1997	children; 2-36 months	ca	H1N1 and H3N2	Nc	4, 6, 7
Steinhoff et al., 1990	children; 6-48 months	ah and ca	H3N2	4.6 (ah), 4.4 (ca)	3, 4, 5, 6, 7

virus-int	source of internal virus genes (ca strain, avian strain)
virus-ext	source of external virus genes (H and N)
ca	cold-adapted
ah	avian-human reassortants (human contribute HA and NA coding segments)
in	intranasal
nc	not calculated

5.1.4 Summary of Animal Data

A host of scientific studies have introduced HPAIV into live birds or mammals by intravenous, intra-tracheal, or oral routes (Table 17). Although many of these studies provide important

observations about the pathology and tissue distribution of HPAIV, unfortunately, they are less useful for directly informing the probability of human illness from poultry, shell eggs and egg products consumption for at least three reasons.

1. The vast majority of studies exposed animals through a single large dose of a limited number of HPAI strains (Table 17), which does not allow for calculation of illness over a range of possible doses.
2. The majority of existing studies exposed the animal model through intranasal injection rather than orally.
3. Even if a study exposed mammals via an oral route using multiple strains and was properly designed to calculate the probability of illness for that animal model, translating the probability of illness to humans would involve a large amount of uncertainty. This is because we currently lack a sufficient scientific understanding of host specificity to interpret, to any reasonable extent, probability of illness results from animal models to probabilities of human illness. In other words, even if HPAIV were infective through oral uptake in a mouse, the degree to which the same strain may be infective to a human is simply unknown.

Table 17. Experimental Animal AI Exposure Data.

REFERENCE	STRAIN	ANIMAL SPECIES	EXPOSURE ROUTE	INFECTION TITER
Swayne, 2008[1]	H5N1	Pigs, ferrets, mice	Intranasal, feeding, gavage	10^6 EID_{50}[2]
Swayne, 2006a	H5N1; H5N2	chicken	Intranasal	10^6 EID_{50}
Rimmelzwaan et al., 2006	H5N1	cats	Intranasal feeding	2.5×10^4 $TCID_{50}$ $>10^9$ $TCID_{50}$/g
Swayne and Beck, 2005	H5N1; H5N2	chicken	oral feeding of infected chicken muscle	$10^{7.8}$; $10^{3.5-3.6}$/bird
Nguyen et a., 2005	H5N1; H5N2	mouse	Intranasal	$10^{0.5}$, $10^{4.3}$; $10^{4.8}$ MID_{50}[1]
Mase et al., 2005a	H5N1	chicken	Intranasal	10^6 EID_{50}
Mase et al., 2005b	H5N1	chicken; mouse	intravenous; intranasal	10^6 EID_{50}; 1.6×10^6 MID_{50}
Govorkova et al., 2005	H5N1	ferret	Intranasal	10^6 EID_{50}
Maines et al., 2005	H5N1	mouse, ferret	Intranasal	10^7 MID_{50}
Kuiken et al., 2004	H5N1 H3N2	cats	intratracheal horizontal transmission feeding	2.5×10^4 $TCID_{50}$ NA $> 2.5 \times 10^4$ $TCID_{50}$
Lu et al., 2003c	H5N1	mouse, ferret	Intranasal	$10^{4.5}$ MID_{50}; 10^6 EID_{50}
Lu et al., 2003a	H5N1	mouse	Intranasal	$10^{4.5}$ MID_{50}
Kuiken et al., 2003	H5N1	macaque	tonsils, conjunctively	2.5×10^4 $TCID_{50}$[1]
Tumpey et al., 2003	H5N1	chicken; mouse; duck	intravenous; intranasal	10^8; 10^6 EID_{50}
Zitzow et al., 2002	H5N1; H3N2	ferret	Intranasal	10^7 EID_{50}
Tumpey et al., 2002	H5N1, variants	chicken; mouse; duck	intravenous; intranasal	10^8; 10^6 EID_{50}
Rimmelzwaan et al., 2001	H5N1	macaque	intranasal, conjunctival, direct exposure on tonsils	2.5×10^4 $TCID_{50}$
Nishimura et al., 2000	H5N1	mouse	Intranasal	5 and 200 PFU[1]
Gao et al., 1999	H5N1	mouse	Intranasal	$0.6\text{-}4.2 \times 10^5$ PFU
Lu et al., 1999	H5N1	mouse	Intranasal	10^2, 10^3, 10^4 MID_{50}
Beare and Webster, 1991	several strains, no H5 or H7	human	Intranasal	$10^{6.8-9.2}$ EID_{50}
Murphy et al., 1982	several strains, no H5 or H7	squirrel monkey; hamster	transtracheal; intranasal	10^7; 10^5 $TCID_{50}$
Hinshaw et al., 1981	several strains, no H5 or H7	ferrets, cats, pigs	Intranasal	10^{6-8} EID_{50}

[1] Unpublished data. D. Swayne, personal communication. http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=409883 (Accessed 5/20/08).
[2] EID_{50} = Embryo Infectious Dose 50%, MID_{50} = Mouse Infectious Dose 50%, $TCID_{50}$ = Tissue Culture Infectious Dose 50%, and PFU= Plaque Forming Units

Though several animal models studies were identified, there are limited data as to which animal model would be most representative of human response. Research at USDA/ARS's Southeast Poultry Research Laboratory on pigs could provide a suitable model given that the

gastrointestinal tracts of pigs are thought to be more similar to humans compared to ferrets and mice. These data are promising as pigs were exposed though feeding and oral gavage. However, these data are currently being developed and suitability as dose-response data will be dependent on the number of pigs infected, number of strains tested, and other factors.

Regarding other animal models, Van Riel (2006) observed H5N1 attachment within the lower human respiratory tract was most similar to that of cats and, to a lesser extent, ferrets and dissimilar to that of mice and macaques[31]. Cats are an attractive dose-response model as feeding trials exist indicating consumption is a possible route (Rimmelzwaan et al., 2006; Kuiken et al., 2004). Unfortunately, the study methodology could not rule out that cats were simultaneously exposed to HPAIV by a respiratory pathway. In addition, these studies did not quantitate the level of HPAIV exposure making it very difficult to estimate a dose-response relationship (Table 17). The ferret animal model is another possible animal model given some similarity to the human respiratory tract (van Riel, 2006); however, it was not considered for this risk assessment. Another limitation of some animal studies is that many are not measured in EID_{50} and therefore are not compatible with the exposure data. This is the case with the primate studies.

Schijven et al., 2005 developed a dose-response model using mouse study data to estimate the probability of human illness from ingestion of HPAIV-contaminated water. The model uses data from Nguyen et al. (2005) and Lu et al. (1999). In those studies, groups of mice were given doses of HPAIV EID_{50} ranging from 0 to 10^7 and MID_{50} (50 percentile mouse infectious dose) was determined. These studies found a large range of MID_{50}, over 5 orders of magnitude difference, among the strains tested. Schijven et al. assumed that the least virulent strain in mice, H5N2 (Dk/VN/342/01), would represent their human dose-response relationship. Figure 10 illustrates the dose-response function. The dark black line represents the dose-response function developed by Schijven et al. (2005), where $r = 10^{-5}$ for strain H5N2 (Dk/VN/342/01). The gray lines indicate a dose-response using two other strains for which an MID_{50} was determined (Nguyen et al., 2005): $r = 10^{-3.4}$ (H5N1 (Gs/VN/113/01)) and $10^{-1.7}$ (H5N1 (HK/483/97)). These curves were generated using Equation 1 (see below) and substituting different dose values (D) and solving for probability of illness (p)[32].

Despite choosing the least virulent HPAI strain, it is still likely that the average dose needed to infect humans through consumption is higher then what was estimated using the mouse data. Mice appear to be fairly susceptible to zoonotic H5N1 strains. Shortridge et al., 1998 found "H5N1 viruses have a surprisingly high pathogenicity for mice" and Dybing et al., 2000 suggest that mouse models may be problematic due to the fact that H5N1 "viruses were nearly 100% infectious and lethal in the mice, whereas infection and lethality rates in humans were much lower."

[31] Comparative infection between the different models was not addressed nor other factors, such as immunological difference and viral replication between cats, ferrets, mice, and macaques.

[32] Note: These dose-response curves are not based on original data, but rather the reported MID_{50} and the dose units were EID_{50}.

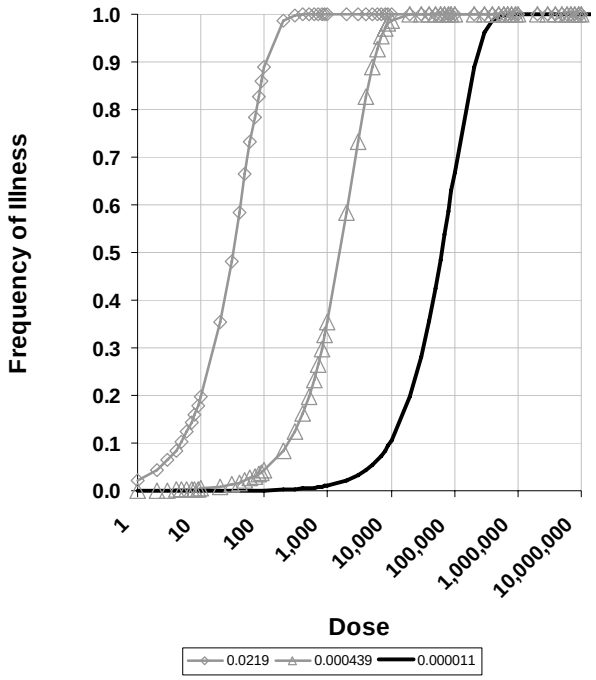

Figure 10. Human dose-response extrapolated from mouse intranasal data. Dark line indicates an *r*-value of 10^{-5} for strain H5N2 (Dk/VN/342/01) Gray lines indicate $r = 10^{-3.4}$ (H5N1 (Gs/VN/113/01), triangle) and $10^{-1.7}$ (H5N1 (HK/483/97), diamond). Dose units: $EID_{50.}$

5.2 Dose-Response using a Human Intranasal Study

No generally accepted dose-response function models are available to estimate human illness from consumption of HPAIV using intranasally exposed human or animal data. Therefore, the following approach was taken:

1) The dose and associated frequency of illness symptoms data from Beare and Webster, 1991 (Table 15) were plotted without weighting severity of the symptoms to develop dose-response relationship (Figure 11).

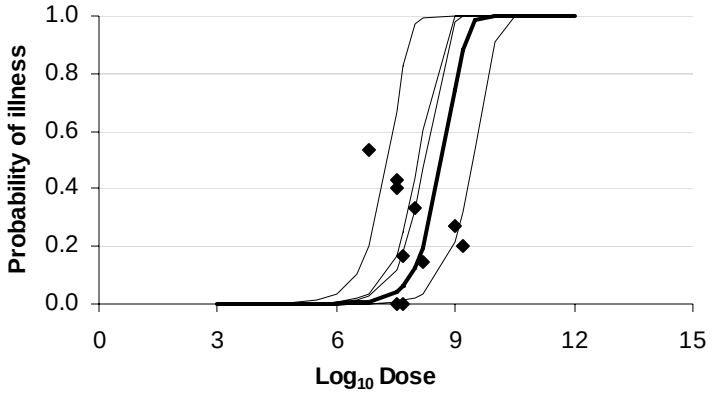

Figure 11. Exponential dose-response using data from Beare and Webster, 1991. The dark line was developed using the entire data set.

2) These data were sub-grouped into 4 data sets (Table 18) based on the H-antigen of each strain to characterize the variability of *r*-values (measure of virulence).

3) The exponential dose-response function (Equation 1) was selected and used to fit each data set, where the probability of illness is:

$$p = 1\text{-}e^{-rD} \qquad (1)$$

and r = measure of virulence and D = dose .

The exponential dose-response function was chosen for the following reasons: a) it is biologically plausible, b) it contains only one parameter that can be used as a measure of virulence (*r*-value), and c) it had been previously employed in a risk assessment to estimate human illnesses from ingestion of HPAIV-contaminated water (Schijven et al., 2005).

4) The parameter values, *r*, of the exponential models were determined using the Maximum Likelihood Estimation (MLE), which is based on the likelihood function (Equation 2) associated with each data set. This estimates the optimal *r*-value where $L(\Theta)$ can be maximized. Assuming that the number of people with illness, x_i, is distributed binomially, the likelihood function is expressed as:

$$L(\theta) = \prod_{i=1}^{I} \binom{n_i}{x_i} P(d_i;\theta)^{x_i} [1 - P(d_i;\theta)]^{n_i - x_i} \qquad (2)$$

where, I = independent dose groups (i = 1, 2, 3, …. I)

n_i = the number of people tested or dosed organisms in a group I

x_i = the number of people with illness
$P(d_i; \theta)$ = the fraction of predicted by the model

The OptQuest option in the software package Crystal Ball® (Denver, Colorado) was used to search for the optimal parameters in which the likelihood function associated with the model of each data set was maximized. Given the subgroup data sets, the associated *r*-values were estimated (Table 18). From these *r*-values, dose-response curves in Figure 11 are realized.

Table 18. Estimated *r*-values of the exponential model for different HPAI strains

Strains	Others (H4, H9, H10)	H1	H3	H6	All (H1, H3, H6, and others)
r =	1.19E-08	5.79E-09	3.83E-09	2.42E-10	1.35E-9

Results show that the *r*-values range from 2.42×10^{-10} to 1.19×10^{-08}. This suggests that a human ID_{50} for intranasal exposure could range from approximately 7.8 to 9.5 $\log_{10}$ EID_{50}. Given the uncertainty in using these data to represent a human dose-response relationship, the unweighted *r*-value from all strains, 1.35×10^{-9}, was used for the baseline model poultry and egg model (intranasal exposure human ID_{50} of ~ 8.7 $\log_{10}$ EID_{50}). No attempt was made to extrapolate to a human consumption dose-response; however, based on animal models, a consumption exposure pathway is expected to be less sensitive (*i.e.*, requiring more virus for the same frequency of illness) (Swayne and Beck, 2005). The impact of using other *r*-values is described in Hazard Characterization Sensitivity Analysis.

6 Risk Characterization

6.1 Poultry Model

The following section is designed to answer the following question: "What is the risk of human illnesses from consumption of poultry if one chicken or turkey flock is infected with HPAIV in the United States?" The response to this question is divided into several sections that explore the different issues related to HPAIV exposure and potential human illness. The risk characterization first estimates the probability that an HPAIV-infected flock would be sent to slaughter. If such an event occurs, mitigations that could help limit both exposure and illnesses are expressed in terms of relative risk reduction. As a secondary focus, the number of contaminated bird carcasses is estimated to characterize exposure from consumption of contaminated servings. The model then estimates the number of illnesses that could occur given a certain level of exposure.

6.1.1 The probability an HPAIV-infected flock goes to slaughter

The poultry model estimates the probability that an index flock infected with HPAIV is sent or not sent to slaughter. The model estimates this by simulating different times when a flock was first exposed. For example, the model asks if a single flock was infected 1 hour before it was sent to slaughter, would the flock still be sent to slaughter. This simulation is followed by another simulation that asks: had this same flock been infected 7 hours before it should go to slaughter, would this flock still have gone to slaughter? The process is repeated for each 6- hour interval within the last 2 weeks (336 hours) of grow-out (*e.g.*, 1, 7, 13 … 331, 337 hours)[33]. The number of 6-hour time intervals[34] the HPAIV-positive flock went to slaughter divided by the total number of 6-hour time intervals during a flock grow-out period is the approximate probability that a single infected flock would go to slaughter (see below).

Given the baseline scenario assumptions (Table 2), the model predicts an HPAIV-positive chicken flock has approximately a 94% probability it will *not* be sent to slaughter. Fourteen 6-hour intervals were predicted to result in the infected flock being sent to slaughter out of 224 possible 6-hour intervals within 8 grow-out weeks; 14/224 * 100 = 6.25%. For a turkey flock reared for 20 weeks (560 6-hour intervals), there is approximately a 98 % chance the flock will *not* go to slaughter; 13/560 * 100 = 2.3%.

[33] Only the last 2 production weeks are evaluated. A flock infected before the last 2 weeks is predicted to not go to slaughter because there is time for the disease to spread resulting in high bird-mortality, thus indicating a flock problem.

[34] The time interval of 6 hours was used given the data of Das et al., 2006 (Table 4). Data were not identified for other time intervals.

The low probability that the index-HPAIV flock will be sent to slaughter is because a chicken or turkey flock infected early in the grow-out process will have enough time to demonstrate significant mortality on the farm resulting in identification of the flock as HPAIV-positive. However, there is approximately a 6 and 2% predicted chance that a chicken or turkey flock will be sent to slaughter without detection of the disease, respectively. Chicken flocks are more likely to go to slaughter compared with turkey flocks because chickens are simulated to be reared for 8 weeks compared with 20 weeks for turkeys.

If either a chicken or turkey flock is infected at 79 hours or less before the birds are supposed to be sent to slaughter, the flock is predicted not to be detected and is subsequently sent to slaughter. A flock infected before 79 hours will have enough time to demonstrate daily mortality within the rage of 0.5 and 2% or greater and would therefore not be sent to slaughter. Therefore, the model predicts that a chicken or turkey flock infected with HPAIV is only a risk if the birds are infected when they are close to market weight (within 3.5 days before the flock is supposed to go to slaughter).

6.1.2 Relative risk reduction through mitigation scenarios

The poultry model is a tool to estimate the effectiveness and relative risk reduction of governmental and/or industry mitigations. In this risk assessment, specific mitigations strategies are not mechanistically modeled; however the *effect* of a mitigation strategy can be estimated. For example, a consumer outreach campaign aimed at greater awareness of the risk associated with undercooking poultry may be an effective mitigation strategy at changing consumer cooking behavior. Using scenario analysis, the model estimates what if more consumers cook to the FSIS recommended poultry temperature than is currently assumed by the baseline scenario. By considering different cooking temperatures, the model can show if such an outreach campaign were effective, if potential human illnesses would be lessened and if so, to what degree. The model does not assess the feasibility of this mitigation strategy, but rather by showing the degree of effectiveness, demonstrates the potential usefulness, or lack thereof, of such an approach.

6.1.2.1 Testing flocks prior to slaughter

The risk assessment can be used to look at the effectiveness of when to test a flock for HPAIV. Testing a flock long before it is to reach market weight is not predicted to be effective at preventing the index flock from being sent to slaughter[35]. This is because such flocks would have enough time to demonstrate significant mortality and therefore the flock would be identified regardless of testing. However, testing can be beneficial when a flock has been infected near market weight. Such a flock may not be detected by observing daily mortality

[35] HPAI testing of flocks earlier in the grow-out period may be useful at preventing or limiting the spread of HPAI to neighboring flocks; however, as this risk assessment does not evaluate flock-to-flock transmission, the benefit of on-farm testing on neighboring flocks is not addressed.

because the disease will not have time to manifest itself prior to the flock being sent to slaughter. In this case, testing for HPAIV could help identify the flock as HPAIV-positive.

Table 19 demonstrates testing programs where an infected chicken flock is sampled for HPAIV before it goes to slaughter. These scenarios make the following assumptions: 1) 65 birds die per day due to other causes besides HPAIV (~0.3% daily mortality (Tabler et al., 2004)) for a 20,000 bird chicken flock within the last week prior to slaughter, 2) a 0.882 probability of detecting 1 positive sample using the RRT-PCR (APHIS, personal communication), and 3) test results are immediate and actionable. Testing of 7 to 9 dead chickens immediately before a flock is supposed to be sent to slaughter can reduce the number of HPAIV-positive flocks entering slaughter and the relative risk associated with the HPAIV-infected index flock (~95%). The relative risk reduction levels off at ~97% because not all 6-hour time intervals in which a flock could be infected with HPAIV can be detected as HPAIV-positive by testing. That is, flocks infected within 38 hours of being sent to slaughter will not be detected given that there are no dead birds from HPAIV. Details of this analysis can be found in Appendix E.

Table 19. Effect of testing flock for HPAIV prior to slaughter.

# DEAD BIRDS TESTED PRIOR TO SLAUGHTER	RELATIVE RISK REDUCTION (%)
5	91
7	94
9	96
11	97
13	97

6.1.2.2 Use of morbidity to identify an HPAIV flock

The probability that a flock is sent to slaughter is based on using 0.5-2.0% daily bird mortality as an indicator for identification of a flock and subsequent holding. Other means of identifying a flock as HPAIV-positive, such as morbidity or reduced feed/water intake are not addressed in the baseline model. Percent daily morbidity is not used for the following reasons: HPAI, in general, is not pathognomonic and therefore clinical signs may be confused with other non-notifiable poultry diseases (Elbers et al., 2005; 2007). Swayne and Halvorson (2003) report, "Clinical manifestations vary depending on the extent of damage to specific organs and tissues (*i.e.*, not all clinical signs are present in every bird). In most cases in chicken and turkeys, the disease is fulminating with some bird being found dead prior to observance of any clinical signs." Furthermore, unpublished morbidity data from infecting 10 chickens with H7N7 showed 2 birds with general signs, 6 with non-specific signs, and 2 with no signs 24 to 48 hours before death (J. van der Goot, personal communication). These data suggest that the majority of infected birds did not show specific signs of HPAI prior to death. In addition, detection of signs is a subjective measure and is likely variable.

Nevertheless, Agriculture Research Service (ARS) H5N1 morbidity data over time do exist. The ARS data demonstrate that 90% of chickens intranasally inoculated with high levels of HPAIV (10^5 $\log_{10}$ EID_{50}) have clinical signs at 24 hours post-inoculation. At 30 hours, all birds were observed to possess clinical signs. To assess the effect of these data, a what-if scenario was conducted by incorporating into the model the number of infected birds that become morbidly ill at each 6-hour time interval. This was estimated given the morbidity probabilities in Table 4. This was then added to the number of dead birds from HPAIV predicted for each time interval. Therefore, if a poultry manager were to count the number of morbid and dead birds immediately prior to when the flock is supposed to be sent to slaughter, what would be the probability that such an infected flock would go to slaughter (given a threshold of 0.5-2%)? The model predicts that for a chicken flock, on average, there would be a 95% probability that flock would not go to slaughter, resulting in a relative risk reduction of 84%. For a turkey flock, on average, there would be a 98% probability that flock would not go to slaughter, resulting in a relative risk reduction of about 90%.

6.1.2.3 Effect of cooking outreach campaign

The risk assessment estimates the effect of a cooking outreach campaign by simulating what would happened if the estimated 28.6% of consumers (Table 6) that cook poultry less than 150 ^{o}F increased their current peak cooking temperature by 10 ^{o}F (28.6 - 11.2 = 17.4%) or 20 ^{o}F (17.4 - 8.8 = 8.6%). An increase of 10 ^{o}F or 20 ^{o}F results in a 48% and 75% relative risk reduction, respectively (Table 20). Therefore, cooking poultry properly reduces potential illnesses from HPAIV.

Table 20. Relative risk reduction through consumer cooking mitigation.

% OF CONSUMERS THAT COOK POULTRY < 150°F	RELATIVE RISK REDUCTION
28.6%	N/A
17.4%	48%
8.6%[36]	75%
All poultry cooked ≥ 150°F	100%

6.1.2.4 Effect of multiple mitigations

The risk assessment demonstrates that multiple mitigations are more effective than most single mitigations. Table 21 shows the combined effect of 3 mitigations resulting in approximately 97% reduction in relative risk. The first mitigation sets the daily mortality threshold that poultry

[36] 8.6% is the percent of individuals observed to cook poultry at <100-129 ^{o}F. Therefore an increase of 20 ^{o}F would still result in cook step of 149 ^{o}F or less.

managers would not send a flock onto slaughter at 0.5 to 1%, where at 1% or greater, flocks are always identified (baseline assumption is 0.5 to 2%). The second mitigation says that a chemical application to poultry carcasses such as chlorine[37] or other antimicrobial will have the effect of lowering levels of HPAIV by 10-fold. Finally, the last mitigation specifies an outreach campaign that has the effect of reducing the percentage of consumers that undercook poultry to less than 150 ^{o}F to 17.4% (see Table 20).

Table 21. Relative risk reduction from multiple mitigation strategies.

MITIGATION	RELATIVE RISK REDUCTION	COMBINED RELATIVE RISK REDUCTION
Increased on-farm surveillance	41%	97%
Chemical treatment of poultry carcasses	90%	
Effective cooking outreach	48%	

6.1.2.5 Recall mitigation

A recall is an important risk management option to reduce consumer exposure following the discovery of an HPAIV-positive flock; however, recalling a chicken or turkey flock once it has entered commerce was not modeled as a mitigation strategy in this risk assessment. To do this would require modeling the transmission of HPAIV between flocks, which was outside of the scope of this risk assessment. If the index flock was identified as HPAIV-positive on the farm, it will not be sent to slaughter and a recall is unnecessary. However, if this index flock is not identified on the farm, it could be slaughtered and enter the food supply. To recall product from the index flock, an event would be needed to discover the presence of HPAIV. Given that the virus would likely have spread on-farm, it appears likely that a second flock would become noticeably sick. The time required for the "second" flock to be discovered as HPAIV-positive will determine the extent of human exposure from the index flock. Without modeling the inter-flock transmission, the time to discovery for the second flock is unknown.

6.1.3 Number of contaminated poultry servings

The risk assessment estimates the fraction of contaminated poultry servings associated with various levels of HPAIV. As indicated in Figure 12, the majority of exposures that result in illnesses range from 10^3 to 10^9 $\log_{10}$ EID_{50} of chicken. This is approximately 7% of the total number of servings that were originally contaminated with HPAIV. Because most contaminated

[37] Chlorine treatment of a solution of H5N1 reduces titers by >3 $\log_{10}$ $TCID_{50}$ at 5 ^{o}C for 60 seconds. However, poultry chiller conditions were not used and therefore these data are not used in the risk assessment (Rice et al., 2007, unpublished).

servings are cooked properly, the vast majority of servings, approximately 93% are less than 3 $\log_{10}$ EID_{50}.

As less than 1 percent of the contaminated servings contain between -4 to 2 $\log_{10}$ $EID_{50,}$ there are few low dose exposures within a reasonable dose range for both chicken and turkey exposures. This suggests that the majority of illnesses predicted by the risk assessment is due to high dose exposures, around 6 $\log_{10}$ EID_{50} for chicken and 7 $\log_{10}$ EID_{50} for turkey.

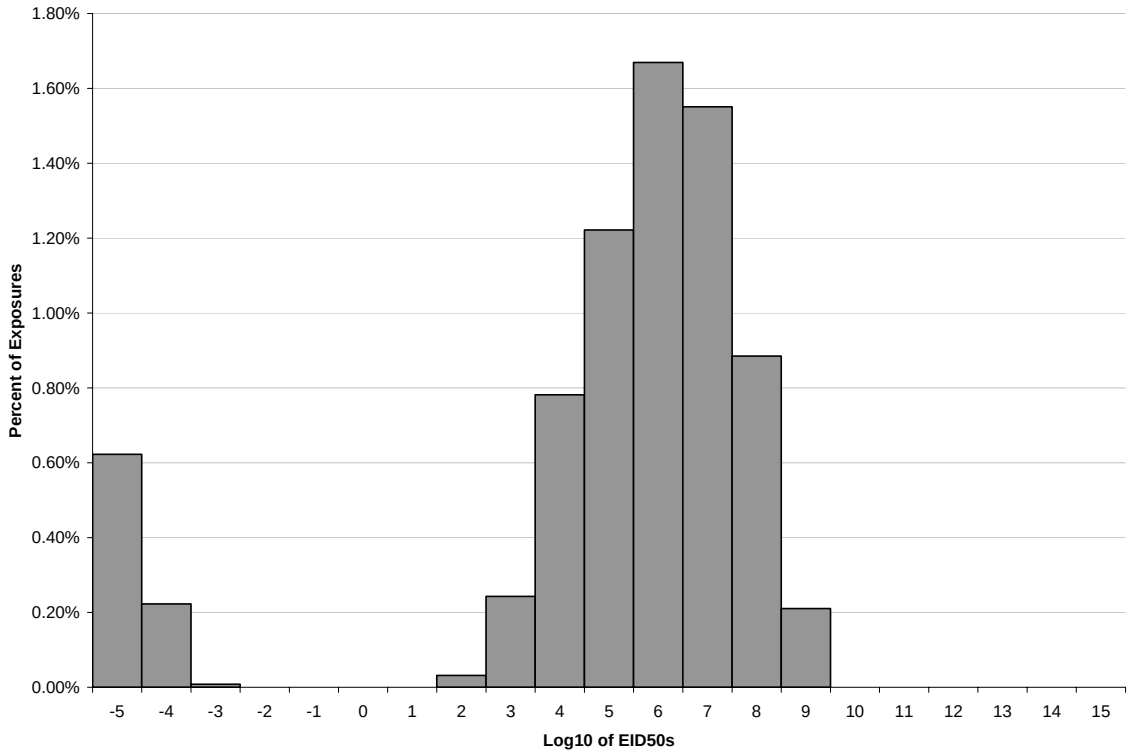

Figure 12. Percent of exposures as a function of HPAIV level in chicken meat. Percent exposures represent the average for those time intervals that resulted in infected flocks sent to slaughter.

6.1.4 Number of predicted illnesses from consumption of poultry

The risk assessment was not designed to predict the absolute number of human illnesses from consumption of HPAIV, given the large uncertainties associated with some model inputs. However, to determine the relative effectiveness of various mitigation strategies, it was necessary to establish a baseline scenario upon which mitigations can be compared. Therefore, the risk assessment estimates the number of illnesses associated with each 6-hour interval of time in which a flock could have become infected. A chicken or turkey flock infected within about a day (25 hours) of being sent to slaughter pose little risk to consumers because there is little time for HPAIV to spread resulting in few infected chickens contaminated with a relatively low level of HPAIV in the poultry meat. However, a flock infected for longer periods (up to 79 hours) has

a greater within-flock prevalence of infected birds with higher levels of HPAIV in the meat. These two factors result in more predicted human illnesses for both chicken and turkey (Table 22; Table 23). A chicken flock infected for more than 79 hours prior to slaughter and a turkey flock infected for more than 73 hours prior to slaughter is predicted to not pose a human health risk.

Table 22. Expected number of human illnesses from a chicken flock infected within 3.5 days of being sent to slaughter (20,000 chicken flock, 8 week grow-out).

Number of hours that flock is infected before slaughter	Daily mortality	Infected birds to slaughter	EID_{50} at consumption total	Expected number of illnesses total
1	0.00%	1	0.00E+00	0
7	0.00%	1	4.00E+03	5.4E-06
13	0.00%	4	3.98E+04	5.3E-05
19	0.00%	7	1.16E+06	0.0015
25	0.00%	25	2.02E+06	0.0027
31	0.00%	48	3.85E+06	0.0051
37	0.00%	145	9.59E+06	0.012
43	0.01%	310	2.68E+07	0.036
49	0.01%	850	6.56E+07	0.088
55	0.03%	1901	1.64E+08	0.22
61	0.07%	4608	3.99E+08	0.53
67	0.17%	9159	9.87E+08	1.3
73	0.42%	15475	2.35E+09	3.1
79	1.03%	11906	3.32E+09	4.4

Table 23. Expected number of human illnesses from a turkey flock infected within 3.5 days of being sent to slaughter (9,000 turkey flock, 20 week grow-out)[38].

Number of hours that flock is infected before slaughter	Daily mortality	Infected birds to slaughter	EID50s at consumption total	Expected number of illnesses total
1	0.00%	1	0.00E+00	0
7	0.00%	1	2.71E+04	3.6E-05
13	0.00%	4	2.70E+05	0.00036
19	0.00%	7	7.89E+06	0.010
25	0.00%	25	1.37E+07	0.018
31	0.00%	48	2.61E+07	0.035
37	0.00%	144	6.50E+07	0.087
43	0.01%	307	1.81E+08	0.24
49	0.03%	826	4.44E+08	0.59

[38] The 79 hours row has been omitted because for turkeys as the model does not predict that a turkey flock will be sent to slaughter if infected 79 hours prior to the end of is grow-out period.

55	0.06%	1780	1.11E+09	1.4
61	0.15%	3916	2.67E+09	3.5
67	0.37%	6551	6.46E+09	8.7
73	0.94%	5834	1.01E+10	13

The risk assessment predicts that given an infected flock is sent to slaughter, no human illnesses to a few illnesses may result from consumption of contaminated chicken or turkey. It is important to note that each time interval is just as likely as the next and therefore the number of illnesses that can be expected from an HPAIV-positive flock sent to slaughter is dependent on when the flock was infected prior to slaughter. Given this, predicted human illnesses as a function of when the flock is infected can be collapsed to show the average number of predicted illnesses over the 6-hour time intervals that result in flocks being sent to slaughter. The risk assessment estimates an average of 1 and 2 human illnesses from an infected chicken and turkey flock, respectively (average of Table 22 or Table 23, column 5). The risk assessment predicts all human illnesses are due to an HPAIV-infected chicken or turkey flock sent to slaughter and subsequent inadequate consumer cooking of poultry. Of these illnesses, 89% arise from the 17% of poultry that is cooked to temperatures of 139°F or less. Eleven percent of illnesses are from 11 % of poultry that is cooked to above 139°F but less than 150°F. No illnesses from HPAIV were associated with the 72% of poultry cooked to 150°F or above. FSIS recommended consumer cooking of poultry to 165°F is predicted to result in negligible risk to public health.

6.1.4.1 Predicted incidence of illness

While epidemiological studies with data on human exposure and subsequent illness are preferred, epidemiological studies could not be used to predict potential illnesses because outbreak studies were not identified where consumption was the exposure pathway and data to estimate the HPAIV dose resulting in human illness from outbreaks was not available.

Despite that predicted illness estimates should only be used in the context of scenario analysis, there is some utility in attempting to validate model outputs using epidemiological studies. However, this cannot be done directly given that there are no known human HPAI outbreaks attributed to consumption of contaminated food. However, we can compare human epidemiological studies that have been attributed to other exposure pathways to the model outputs predicted for consumption. To do this, the following information is needed from the model: 1) estimated number of predicted illnesses, and 2) estimated number of food exposures. From epidemiological studies the following is needed: 1) number of HPAIV human illnesses, and 2) estimated number of exposures.

The model estimates on average approximately 0.7 human illnesses from exposure to a single chicken flock (Table 22, average of column 5). To estimate the average number of human exposures from a 20,000 bird flock, it is assumed that all chicken from an infected flock that went to commerce is consumed and that one serving is consumed per person. The average number of human exposures from a 20,000 bird chicken flock is therefore estimated to be

268,000[39]. Thus, the number of illnesses per serving would be 0.7/268,000 = 2.6 x 10^{-6} or an illness incidence of about 1 predicted illness in 400,000 exposures.

Epidemiologic data suggests an illness incidence of between about 1% and 10% for individuals exposed to HPAIV-infected flocks, or rather about 1 in 100 or 1 in 10, respectively (Bridges et al., 2002 ; Koopman et al., 2004; Puzelli et al., 2005; Thorson et al., 2006) (see Appendix D, Table 1 column 4).

The estimated incidence of illness from consumption of contaminated chicken meat (1 in 400,000) from an infected flock is 3 to 4 orders of magnitude below what has been observed in epidemiological studies of other modes of exposure to HPAIV (1 in 10 or 100). Again, the model predictions cannot be validated using these epidemiological studies; however, they do validate the expectation that frequency of human illness from exposure to HPAIV through consumption would be much lower than that resulting from human HPAIV outbreaks attributed to close contact with live or dead poultry.

6.1.5 Cross-contamination

Consumers handling raw poultry during preparation may be exposed to HPAIV through cross-contamination. There are many possible exposure pathways that could describe cross-contamination from raw product to food not likely to undergo cooking (*e.g.,* salads and ready-to-eat foods). Many of these pathways are poorly described creating a significant challenge to mechanistically model cross-contamination mechanistically. Given this complexity, a cross-contamination component was added to the model using only two variables: 1) fraction of the total level of HPAIV in poultry meat that could be cross-contaminated, and 2) proportion consumed.

At the fraction HPAIV is assumed to be cross-contaminated (~0.53%) from poultry and subsequently ingested (see Cross-contamination), cross-contamination of HPAIV is not a substantial source of human illnesses in comparison to the number of predicted illnesses from direct-consumption. On average, an increase of approximately 2.5% in the estimated average number of illnesses is predicted. However, this is likely an overestimate. The model does not

[39] To estimate number of human exposures from a 20,000 bird chicken flock that was not detected on-farm and sent to slaughter, the following was done. A flock of 20,000 chickens with a daily average farm mortality of 0.3% (Tabler et al., 2004) will have approximately 17,000 birds available for slaughter. The transmission model was then used to estimate the average number of dead birds due to transportation and the average number of carcasses removed due to post-mortem inspection. Assuming this represents approximately 250 birds on average, approximately 16,750 carcasses are estimated to enter commerce. Given that consumers will consume some fraction of a carcass (a serving), to estimate the total number of individuals exposed it was necessary to estimate the number of serving consumed. At 83 grams per serving, each processed chicken carcass (1336 g) would produce about 16 servings or 268,000 (16 x 16,750) servings of chicken from the flock. Assuming that all servings (both contaminated and not contaminated) are consumed and that one serving is consumed per person, the average number of human exposures is estimated to be 268,000.

allow the user to specify what fraction of servings is cross-contaminated[40]. Therefore, all HPAIV-positive servings are assumed to result in cross-contamination for this scenario analysis. Furthermore, 100% of the cross-contaminated virus is assumed to be consumed. In reality, only a portion of the virus would likely be cross-contaminated to a surface, and then only a sub-portion would then be transferred to a food not likely to be cooked.

6.2 Egg Model

The following section is designed to answer the following question: "What is the effectiveness of interventions to reduce human exposure and illness from the introduction of HPAIV into commerce from shell eggs?" To respond to the effectiveness of interventions, a baseline model must be developed from which we can estimate a change in relative risk following application of mitigations. The risk assessment can then be used to specifically address the additional risk management questions through scenario analysis: "1) Evaluate the reduction in human exposure to HPAIV from infected shell eggs assuming the infected flock is identified and closed following various days after infection, and 2) Evaluate the reduction in human exposure to HPAIV from contaminated shell eggs following market withdrawals/recalls of eggs laid various hours (*e.g.* 12, 24, 48, 72, and 96 hours) before the house was identified as infected and closed." The effectiveness of these mitigations as it pertains to exposure and illnesses is described below.

6.2.1 Probability eggs sent to processing

The egg model, unlike the poultry model, does not estimate the probability that an HPAIV-infected hen flock will go to slaughter. The risk from a hen flock is not from the infected meat, but rather from the contaminated eggs. Given this, it is still not practical to estimate the probability that contaminated eggs would go to market in the same manner that the poultry model estimated contaminated poultry meat would go to slaughter. Unlike poultry, egg production is not based on an "all-in all-out" model; eggs are continuously sent to processing. For eggs, the probability that an egg goes to processing is variable and depends on when the egg was laid. A contaminated egg laid soon after the hen flock is infected will likely go to processing (be washed, examined, and packed for market), while an egg laid near the time the flock is diagnosed with HPAIV will probably not. The risk assessment estimates the number of eggs sent to consumers by tracking the number of HPAIV-positive eggs produced by infected birds over time. Following each 6 hours of egg production, the model updates the number of contaminated eggs. These eggs are simulated to be sent to processing and commerce. Once the

[40] An analysis to estimate the frequency of cross-contamination events during preparation poultry was not attempted. Therefore, when this component of the model it turned on, all serving are assumed to result in cross-contamination. The impact of this assumption is proportional. For example, if 25% of consumers are expected to cross-contaminate during poultry preparation, the number of predicted illnesses due to cross-contamination will be a fourth of that predicted by the model.

flock reaches a specific threshold percent daily mortality, it is identified as HPAIV-positive and eggs are no longer sent to processing.

6.2.2 Time to flock detection

The risk assessment predicts that if a hen flock becomes infected with HPAIV, some contaminated eggs will be sent to processing and possibly onto commerce. This is because the risk assessment estimates that on average, a hen flock infected with HPAIV will require 6 days before the daily mortality threshold reaches 2% or greater and the flock is discovered as HPAIV-positive (Figure 13). This is about twice as long as predicted for a poultry flock (3.5 days).

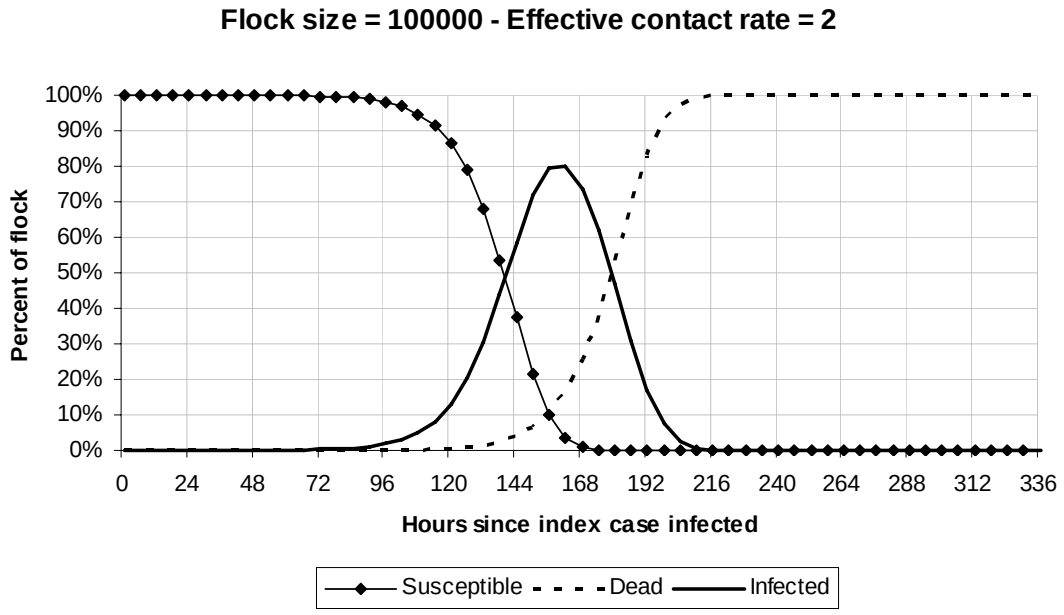

Figure 13. The various states (susceptible, infected, and dead) of birds within a hen flock during an HPAIV infection with an effective contact rate of 2. The hen flock is detected as HPAIV-positive at 2.16% daily mortality at 144 hours (6 days) following initial infection at time zero.

The primary reason for this difference between the egg and poultry models is the effective contact rate used in the respective transmission models. An effective contact rate of 2 is assumed for the current egg baseline; therefore, the egg model estimates that each HPAIV-infected bird will infect 2 additional birds every 6 hours. The poultry model assumes an effective contact rate of 8. The rate at which HPAIV spreads within a hen flock is likely lower than the rate for a chicken flock. Hen flocks are typically cage-reared, minimizing the spread of the virus. This is in contrast to ground-reared chickens that can roam freely. Observations during actual HPAIV poultry outbreaks support this assumption (Elbers et al., 2004; 2006; 2007); however, there is no experimental evidence to suggest what the actual contact rate is for

caged birds infected with H5N1. For a more complete discussion of contact rate, see Sensitivity Analysis, Contact rate.

6.2.3 Egg mitigations

6.2.3.1 Removing eggs from market

Removing eggs that were produced by an HPAIV-positive hen flock from commerce will reduce exposure and subsequent illnesses. The effectiveness of a recall/withdrawal is dependent on how many days of eggs production are recalled. Removing of the most recent days of egg production from when the hen flock was discovered as HPAIV-positive will be the most effective. This is because the longer the infection, more birds become infected with HPAIV and more contaminated eggs will be produced. As hens begin to die from the virus, flock managers will be alerted to the presence of HPAIV once a daily flock mortality of 2% or greater is reached. The number of eggs produced prior to discovery of the flock as HPAIV-positive is of concern (Figure 14).

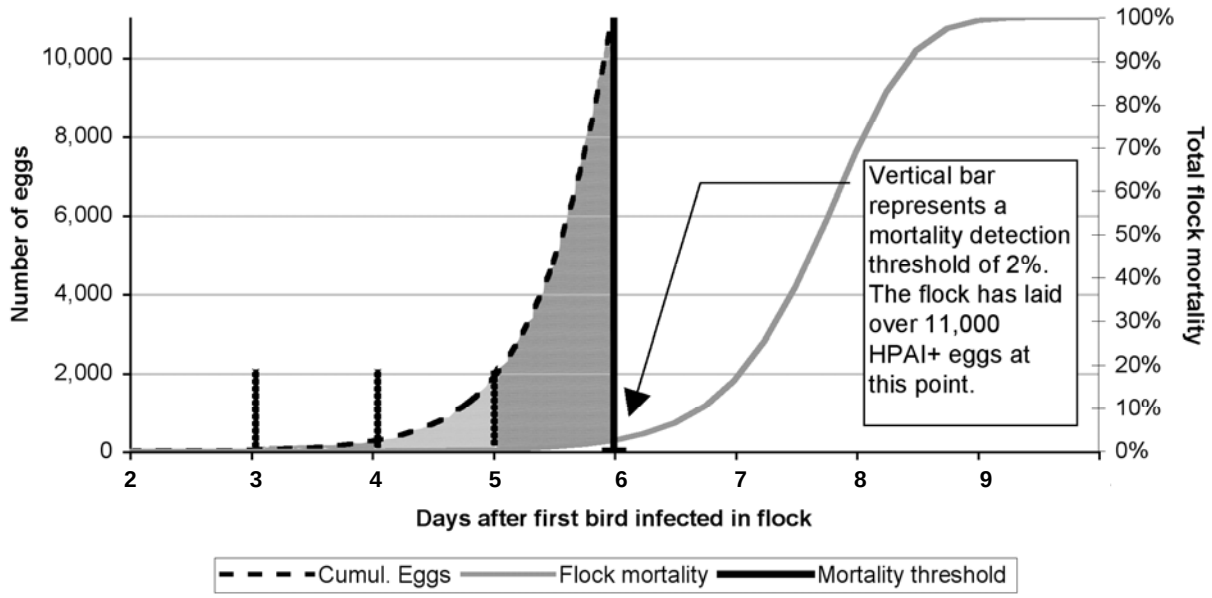

Figure 14. Number of contaminated eggs prior to discovery of HPAIV-infected hen flock. The dark grey shared area represents the total number of contaminated eggs produced by an HPAIV-infected flock within the most recent 24-hours of egg production, prior to discovery of the flock as HPAIV-positive. The light grey shaded area represents the total number of contaminated eggs produced between 24 and 48 hours. The short vertical black lines demarcate the 24-hour time periods of egg production.

Greater than 97% of HPAIV-contaminated eggs produced by an infected hen flock can be accounted for given a 2 day removal (Table 24). The baseline scenario predicts that recalling

contaminated eggs that had been produced within 2 days of the hen flock being discovered with HPAIV removes 11,033 of the 11,293 contaminated eggs that were laid by the flock.

Table 24. Relative exposure reduction of recalling eggs.

Recall most recent egg production day	# of HPAIV+ eggs removed	HPAIV+ eggs available for consumption	Relative exposure reduction (%)
1 day	9,431	1,862	83.5
2 days	11,033	260	97.69
3 days	11,258	35	99.69
4 days	11,288	5	99.95
5 days	11,292	1	99.99
6 days	11,293	0	100

When estimating the effectiveness of a recall, the model assumes that all contaminated eggs can be recalled, regardless of when the egg was produced. In reality, an actual recall will not likely be able to recover all eggs produced by the HPAIV-infected flock. Though there are many factors that will influence the success of a recall, the primary factor is likely to be when a contaminated egg was laid in relation to when the flock was identified as positive. For example, HPAIV-positive eggs recently laid by a discovered layer flock and still on the farm will have a high probability of being successfully recalled, where HPAIV-positive eggs produced 4 or 5 day ago, may have a low probability of recall (these eggs may be somewhere in the distribution chain and difficult to track). Data to inform these probabilities were not identified, though is not likely to change the outcome of the scenario analysis. This is because most HPAIV-positive eggs are predicted to be laid very near when the flock is identified as HPAIV-positive (Figure 14). These eggs will easily be recalled given that it requires about 6 days on average for eggs to reach the market. HPAIV-positive eggs produced earlier in the infection may not be recalled, but these constitute a small fraction of the total HPAIV-positive eggs produced by the flock.

6.2.3.2 In-shell pasteurization

In-shell pasteurization could serve as a mitigation strategy to inactivate HPAIV-internally contaminated eggs. To estimate the effect of in-shell pasteurization, a preliminary analysis was completed to estimate the magnitude of decreasing the level of HPAIV in contaminated eggs on the predicted number of illnesses (Table 25). A 3 $\log_{10}$ reduction in the level of HPAIV in contaminated eggs results in an approximate 99% relative risk reduction in predicted illnesses.

Table 25. Relative risk reduction of in-shell pasteurization.

Log_{10} reduction	Relative risk reduction
0	NA
1	44.24

2	86.93
3	98.89
4	99.89
5	99.99

Though experimental evidence on the impact of heat lethality on HPAIV in shell eggs has not been completed, HPAIV heat lethality data in chicken meat can be used as a surrogate to estimate the expected HPAIV $\log_{10}$ reductions given an in-shell pasteurization time and temperature profile. A personal communication with National Pasteurized Eggs, Inc. provided that shell eggs can be pasteurized at 133 ^{o}F for 53 minutes (R. W. D. Cox, personal communication). Using this time/temperature profile and the chicken breast meat regression parameters (=10^(14.54628-0.21306* 56.1 ^{o}C) an approximate 8 $\log_{10}$ reduction of HPAIV within eggs can be expected.

6.2.4 Scenario analysis

The risk assessment can be used to evaluate the impact of different data by developing scenario or what-if analysis. For example, 25 white leghorn hens were inoculated via intranasal and the conjunctival sac with a strain of H5N2 identified during the 1983 Pennsylvania HPAIV outbreak (Beard et al., 1984). The rate of mortality caused by this H5N2 strain is different compared with the mortality data that is considered representative of H5N1 in this risk assessment (H5N1 mortality within 36-42 hrs compared with a minimum of 96 hours for this H5N2); however, scenario analysis can be performed by asking "what would be the impact of this HPAI strain infecting a layer flock that resulted in the following flock characteristics (Table 26)?"

Table 26. Characteristics of H5N2 experimental study in hens

Days post-inoculation of H5N2	Clinical signs	Dead	Eggs total	HPAIV+ eggs	Thin/soft shelled
2	1	0	14	0%	
3	23	0	14	86%	3
4		16%	3	100%	2
5		48%	0		
6		72%	0		
7		76%	0		
10		80%	0		
11		84%	0		
12		88%	0		
20		92%	0		

The effect of replacing the baseline data with the data in Table 26 (columns 1, 3-5) are given in Table 27. The uncertainty in the data can also be reflected by further scenario analysis (columns 3-6, Table 27).

Table 27. Results of scenario analysis using Beard et al., 1984 data

Previous hours of egg production once flock is identified as HPAIV-positive	Beard data	Beard data assuming eggs can be contaminated 24 hrs earlier	Beard data assuming eggs can be contaminated 48 hrs earlier	Beard data assuming egg production drop 24 hrs later	Beard data assuming egg production drop 48 hrs later	Current baseline
Past 24 hours	0[1]	0	0	0	0	9,431
Past 48 hours	0	0	0	0	0	1,602
Past 72 hours	0	0	0	0	170	224
Past 96 hours	0	0	0	21	49	31
Past 120 hours	3	26	214	6	6	4
Past 144 hours	1	7	61	1	1	1
Past 168 hours	0	1	8	0	0	0
Past 192 hours	0	0	1	0	0	0
Past 216 hours	0	0	0	0	0	0
Past 240 hours	0	0	0	0	0	0
Past 264 hours	0	0	0	0	0	0
Past 288 hours	0	0	0	0	0	0
Past 312 hours	0	0	0	0	0	0
Past 336 hours	0	0	0	0	0	0
Sum HPAIV+ eggs	3	34	284	28	226	11,293

[1] Numbers indicate HPAIV-positive eggs. Numbers do not include HPAIV-positive eggs that are removed due to visible deformities.

The risk assessment predicts very few exposures compared to the baseline scenario for the following reasons: 1) a drop in egg production limits the number of HPAIV-positive produced (egg production drop is not being modeled in the baseline), and 2) HPAIV-positive eggs are not produced until 3 days post-infection (baseline model allows for 15% of eggs to be HPAIV-positive by 6 hours post-infection).

These data suggest that a drop in egg production would be the key to identifying an infected hen flock *with these characteristics* as positive, and not mortality. This can be seen in Figure 15: there is about a 30% drop in egg production by 5 day post-infection suggesting that if this was enough to alert flock managers to the problem, then the flock would stopped at ~5 days (127 hours, see blue line at 70%, Figure 15) compared with 9 day (as predicted by the mortality data based on the Beard study; ~216 hours, see hatched line at 2%).

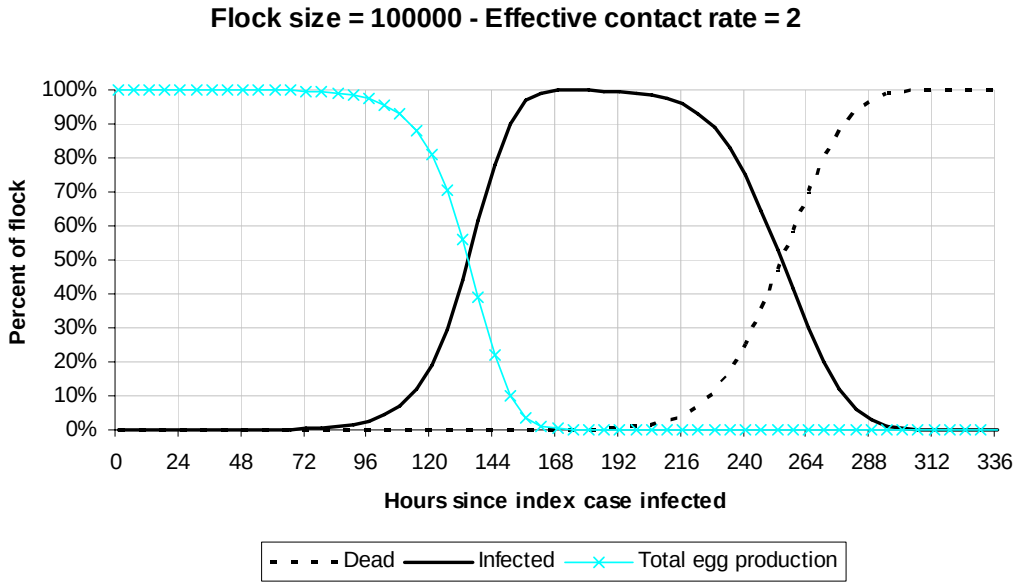

Figure 15. Total egg production, infection, and mortality of a hen flock based on Beard et al., 1984 data.

6.2.5 Egg products

Data from USDA's ARS demonstrates that FSIS time and temperature recommendations for the processing of specific egg products are sufficient to inactivate HPAIV (Swayne and Beck, 2004). Therefore, this risk assessment model currently does not quantitatively assess egg products. However, dried egg whites pasteurization time-temperature protocol was found to be inadequate to eliminate all HPAIV (Table 10) assuming a maximum titer of 4.9 $\log_{10}$/mL (Table 9).

Despite this, egg products are likely to be a very low risk product[41] for the following reasons: 1) It is unlikely that HPAIV-positive dried egg whites could reach consumers because the process of preparing dried egg whites requires a minimum of 7 and a maximum of 10 days. Dried egg whites would then require packaging and storing/transportation. The baseline model predicts

[41] APHIS is developing a risk assessment for pasteurized eggs product (T. Weaver, personal communication). In addition, ARS is examining the issue of dried egg white and HPAI though further experimentation (C. Thomas, personal communication).

that the infected hen flock would be found as HPAIV-positive by 6 days post-infection alerting egg products processors to the problem before dried egg whites were done being processed. 2) HPAIV-contaminated eggs are likely to be mixed with non-contaminated eggs at the processing plant effectively reducing the level of HPAIV per milliliter. This will also help to increase the effectiveness of dried egg white pasteurization process.

6.3 Sensitivity Analysis

Given the lack of certainty and availability of some data needed for this risk assessment, the risk assessment predicts a broad range of potential exposure and subsequent illnesses from consumption of HPAIV-contaminated poultry and shell eggs. The following section demonstrates the effect of various model assumptions using scenario analysis on the outputs of the risk assessment.

6.3.1 Combinatorial approach sensitivity analysis

The combinatorial approach can be used to estimate those variables that have the greatest impact on the number of predicted illnesses and those that have the least impact. The minimum and maximum reasonable inputs were chosen for each variable. All inputs were then run simultaneously. The advantage of such an approach is the effect of interacting variables is addressed. This is distinct from altering one variable while holding others to their baseline values (see below). The results are indicated in Table 28.

Table 28. Combinatorial approach sensitivity analysis

Variable	Fold difference	Minimum input	Maximum input
% of birds showing pathology (postmortem inspection)	70.9	Shift baseline distribution by – 6-hours[42]	Shift baseline distribution by + 6-hours
Dose response (*r*-value)	41.9	1.2E-08[43]	2.4E-10
HPAIV levels in poultry	40.0	Peak level 5.3 EID_{50}/g[44]	Peak level 8.5 EID_{50}/g
Contact rate	10.5	1	64
Mortality rate	7.0	Shift baseline distribution by – 6-hours	Shift baseline distribution by + 6-hours
Latency	2.3	5^{th} percentile of baseline distribution	95^{th} percentile of baseline distribution
Cooking	2.2	Mean of temperature distribution increased by 10 °F	Mean of temperature distribution increased by 20 °F
Daily mortality threshold (on	1.5	0.5%[45]	2%

[42] Six hours is the smallest time interval.
[43] Dose-response values were chosen as these were the minimum and maximum values observed from Beare and Webster, 1991.
[44] Level values were chosen as these were the minimum and maximum values observed from distribution (Table 5).

farm)			
Proportion of cross contaminated virus consumed	1.5	1%	100%
Proportion of virus cross contaminating other product	1.4	0.053%	5.3%
Tissue infectivity (when meat contaminated by HPAIV)	1.3	5^{th} percentile of baseline distribution	95^{th} percentile of baseline distribution
Daily mortality threshold (antemortem inspection)	1.3	0.5%	2%
Birds initially infected	1.1	1	10

6.3.2 On-farm sensitivity analysis

6.3.2.1 Number of birds initially infected

The number of birds initially infected is assumed to be one for the baseline scenario. However, depending on how a flock becomes infected, more than one bird may initially be infected. For instance, contaminated feed could result in many birds becoming infected with HPAIV at or about the same time. To estimate the impact of this uncertainty, the number of birds initially infected can be varied.

Varying the number of meat-type birds from 1 to 10,000 has a two fold effect. Increasing the number of birds initially infected lowers the probability that an infected flock will be sent to slaughter. This is due to the fact that a percent daily mortality of 2.0% or greater is reached sooner thus a flock can be detected by poultry managers in less time (Table 29).

Table 29. Probability an infected flock is not sent to slaughter as a function of initial number of chickens infected.

# birds initially infected	Probability not sent to slaughter
1	93.75
10	94.64
100	95.98
1,000	97.32
10,000	97.32

However, if a flock is still sent to slaughter (daily mortality < 2.0%), more birds will be infected given the fact that more birds were initially infected. Therefore there is greater exposure due to more poultry carcass being infected and more predicted human illnesses from poultry

[45] D. Swayne, personal communication

consumption. Varying the initial number of infected birds 10-fold result in an approximate 10-fold increase in predicted human illnesses (Table 30).

Table 30. Effect of number of chickens initially infected.

Number of hours that flock is infected before slaughter	Number of birds initially infected			
	1	10	100	1,000
	Predicted human illnesses			
1	0	0	0	0
7	5.4E-06	5.4E-05	0.00054	0.0054
13	5.3E-05	0.00053	0.0053	0.05
19	0.0015	0.02	0.2	1.6
25	0.0027	0.03	0.3	2.7
31	0.0051	0.05	0.5	4.5
37	0.012	0.1	1.3	
43	0.036	0.4	3.1	
49	0.088	0.9	4.2	
55	0.22	2.1		
61	0.53	4.3		
67	1.3	2.6		
73	3.1			
79	4.4			
Average	0.69	0.87	1.06	1.47

If an egg-producing flock is infected, again the time to flock detection is decreased given that more birds initially infected results in a daily mortality of 2.0% or greater sooner. However, this has the effect of lowering predicted illnesses from egg consumption given that fewer HPAIV-contaminated eggs can be produced.

6.3.2.2 Weeks in house

The length of time a flock remains on the farm is directly related to the mass of an individual bird (Table 3). Therefore, infected flocks that have been reared for longer periods of time (between 4-12 weeks) will pose a greater risk to public health as more contaminated meat is available from heavier birds (Figure 16). The effect is lost for chickens reared over 12 weeks given that the mass of the birds is assumed not to change. The largest effect is seen between 4 to 6 weeks (2-fold) suggesting that this variable does not significantly impact the number of predicted illnesses.

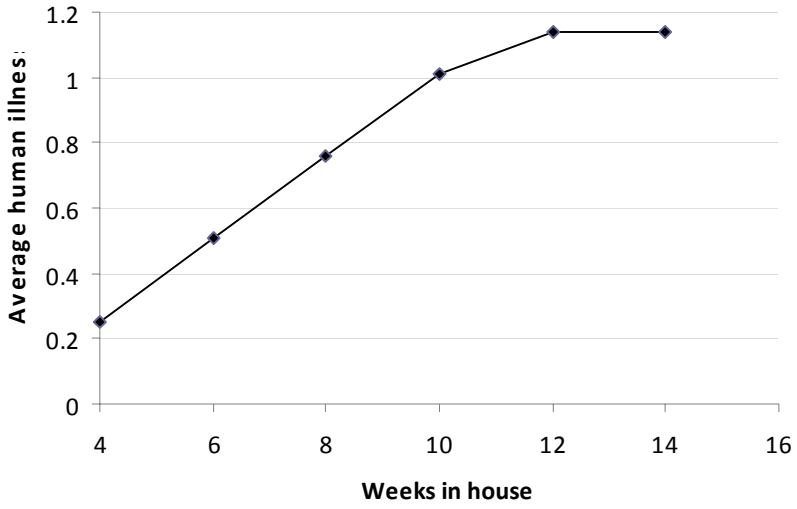

Figure 16. Effect of altering the number of weeks during the chicken grow-out period.

6.3.2.3 Contact rate

The contact rate dictates the speed at which HPAIV can spread through a house. That is, the number of contacts that produce a new infection per unit time. The model allows users to enter different contact rates and evaluate the effect of this parameter on predicted human illnesses. The range of potential contact rates is 1 to 64.

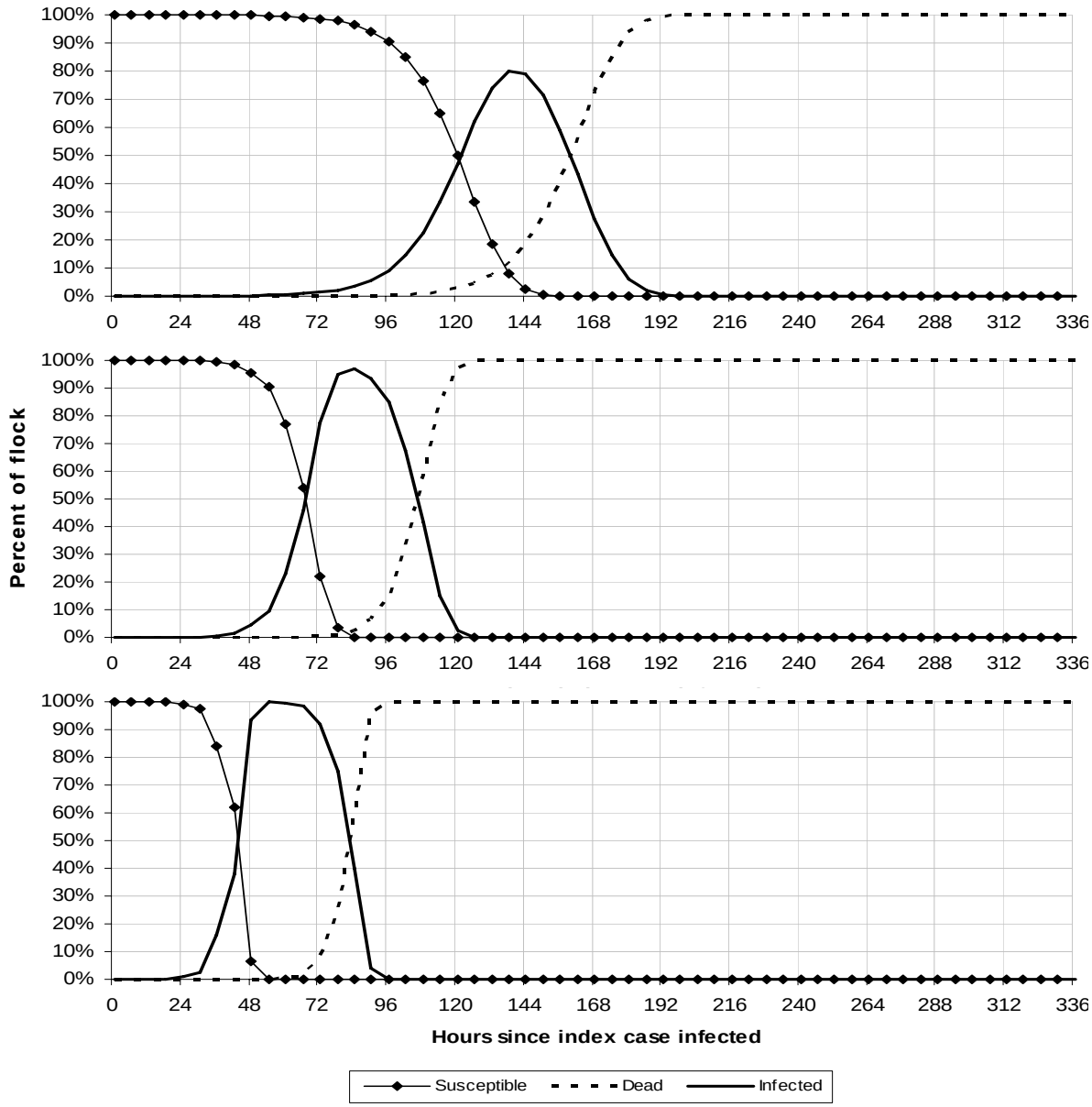

Figure 17. The various states (susceptible, infected, and dead) of birds within a 20,000 chicken flock during an HPAIV infection. Flock is assumed infected with HPAIV at time zero. Effective contact rate of 2, 8 and 32 (top to bottom).

Varying the contact rate has a substantial impact on the number of chickens that are in various states (susceptible, infected, and dead) over the course of an infection. As can be seen in Figure 17, the lower the contact rate (top panel), the slower the number of infected birds increases and the longer a flock will remain undetected. This is due to the slow rate at which the disease is predicted to spread. Alternatively, a higher contact rate results in more infected chicken and a shorter period of time before a flock is detected as positive. This trend applies to both turkey and hen flocks.

In regard to the effect of varying the contact rate on the predicted number of human illnesses from poultry, the contact rate is positively correlated and linearly related to ($R^2 = 0.93$) predicted human illnesses from consumption of HPAIV-contaminated poultry (Figure 18). There is a positive correlation because a higher contact rate results in a faster spread with more birds being infected with HPAIV. The uncertainty surrounding the contact rate has a significant impact on the poultry model results-- varying the contact rate from 1 to 64 results in an approximate 26 fold difference for poultry consumption.

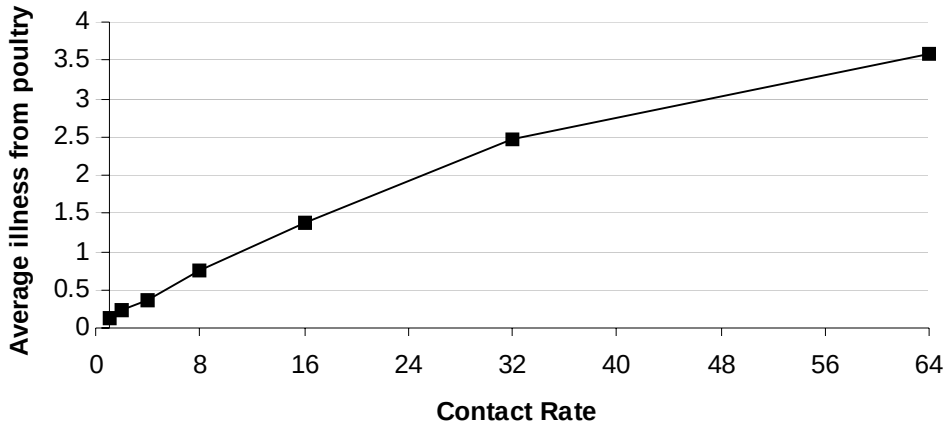

Figure 18. Predicted human illnesses as a function of contact rate. The following contact rates are shown: 1, 2, 4, 8, 16, 32, and 64.

Varying the contact rate has an impact on the number of days to detection of a flock as HPAIV positive. The fastest time an infected flock could be discovered on average (*i.e.*, reach a daily mortality rate of ≥ 2%) is about 2.5 days (Figure 19). This average time will not decrease much further because the model estimates that it takes 36-42 hours for birds to die once infected (Das et al., 2006).

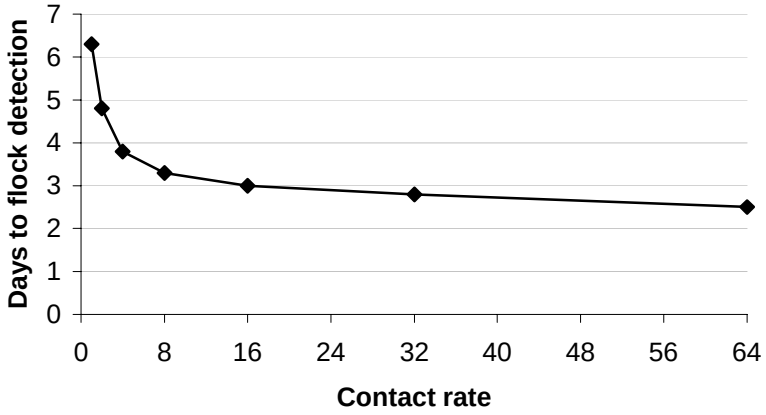

Figure 19. Days to detection of a chicken flock as HPAIV-positive as a function of contact rate. The following contact rates are shown: 1, 2, 4, 8, 16, 32, and 64.

6.3.2.4 Latency

To estimate the effect of latency on the poultry model, the model option inputs were modified as follows: the latency period was treated as the threshold, where before a certain 6-hour time interval there was no possibility of a latent bird becoming infected, and after which all latent birds were capable of being infectious. For example, in Figure 20 at time point 30 hours, it is assumed that birds infected for 30 hours or more are infectious to other birds. Infected birds under 30 hours are still latent and cannot spread the disease. The largest effect is seen between 0 to 6 hours (3.7-fold); however, it is unlikely that this timeframe is realistic. This suggests that this variable does not significantly impact the number of predicted illnesses.

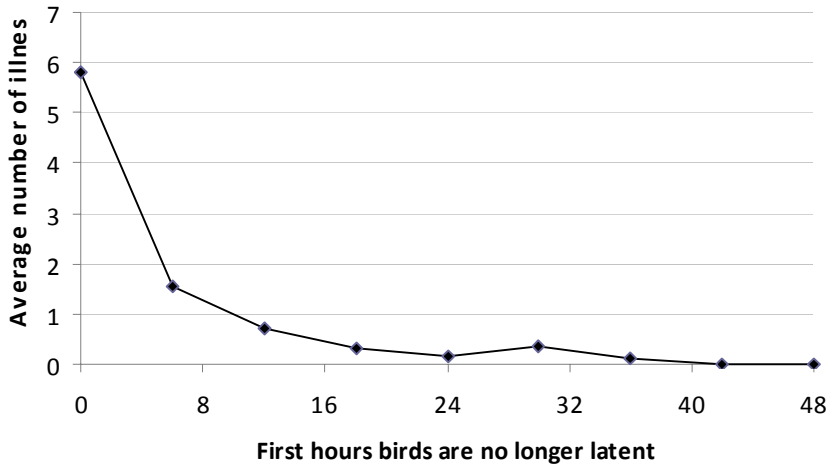

Figure 20. Effect of latency period.

6.3.2.5 Tissue infectivity

To estimate the effect of when the virus is present in muscle, the tissue infectivity model option inputs were modified as follows: tissue infectivity was treated as a threshold event, before a certain 6-hour time interval there was no possibility of a tissue containing virus, and after which all tissues in an infected bird contain virus. For example, in Figure 21 at time point 18 hours, it is assumed that birds infected for 18 hours or more have virus in their muscle. Infected birds under 18 hours do not and could therefore not result in human illnesses if the bird was processed and consumed at this point. The largest effect is seen between 18 and 24 weeks (3-fold) suggesting that this variable does not significantly impact the number of predicted illnesses.

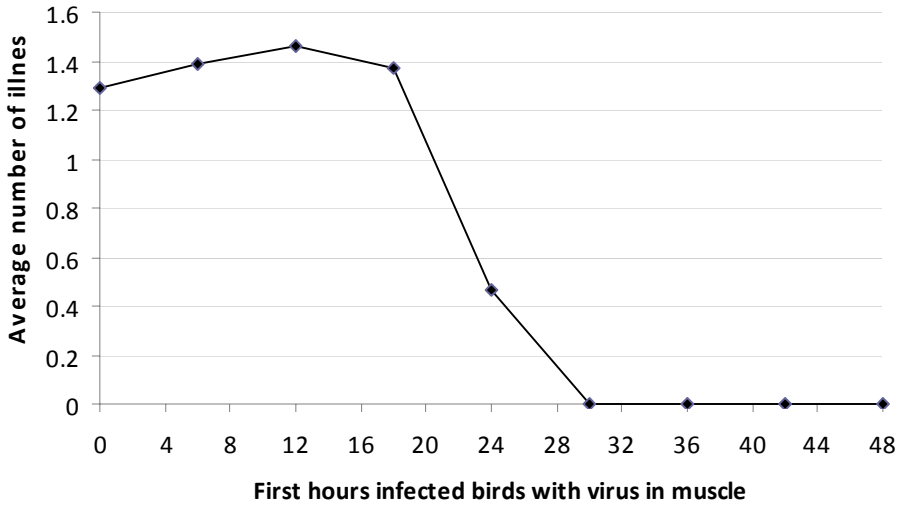

Figure 21. Effect of when muscle can be contaminated within an infected bird.

6.3.2.6 Mortality rate

To estimate the effect of mortality rate, model option inputs were modified as follows: the mortality rate as determined by Das et al., 2006 was shifted by 6-hour time intervals to demonstrate the effect of birds dying sooner or later than expected. The baseline model indicates that 40% of birds infected 31-hours will be dead while birds infected ≤25 and ≥37 hours will all be alive or dead, respectively. This distribution is then shifted by 6, 12, or more hours in either direction to estimate the effect on the model outputs. As indicated in Figure 22, increasing HPAIV-positive bird survival is positively associated with the number of predicted human illnesses. This results from increasing the number of 6-hour time intervals that result in flocks being sent to slaughter. The largest effect is seen between 19 and 25 weeks (4.4-fold).

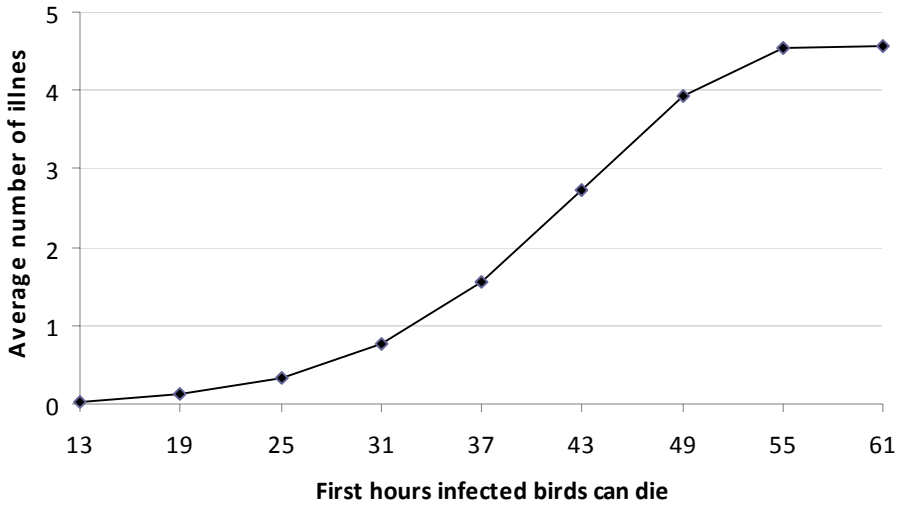

Figure 22. Effect of mortality rate.

6.3.2.7 Daily mortality threshold

The daily bird mortality threshold can be an indicator to farm and poultry managers of HPAIV infection within a flock (see Daily Mortality Threshold). However, the data used in both the egg and poultry models are uncertain. To estimate the effect of altering the daily mortality threshold at which a flock will or will no be sent to slaughter or eggs sent into commerce, various percent detection thresholds were used in the model (Figure 23).

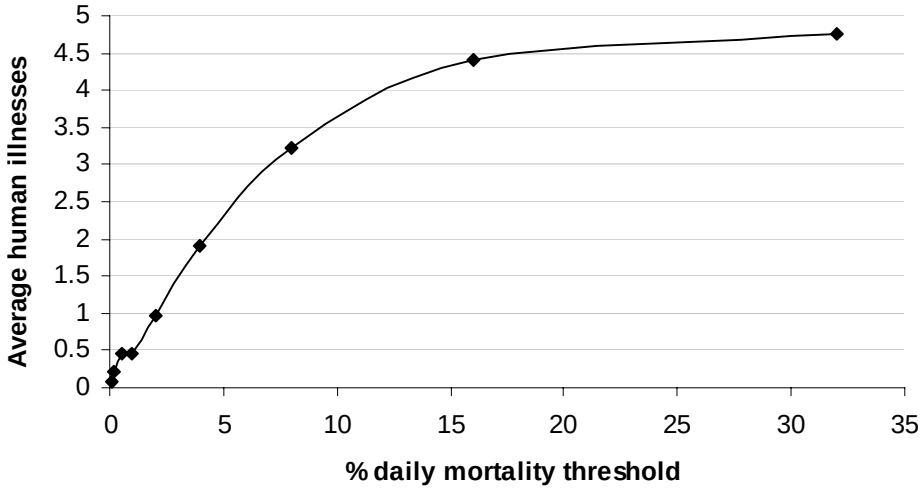

Figure 23. Effect of the daily mortality threshold on predicted human illnesses from consumption of HPAIV-positive chicken. The following percent daily mortality thresholds were analyzed: 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 8.0, 16, and 32.

Altering the percent daily mortality threshold for poultry is positively correlated with human illnesses. This is because as the threshold for the number of dead birds increase, more potentially contaminated product is released for consumption. In addition, there are more 6-hour time intervals that pose a risk for a single HPAIV-positive poultry flock being sent to slaughter. Increasing the percent daily mortality threshold allows flocks that would have been discovered as HPAIV-positive to pass to slaughter. Therefore, the increase in illnesses observed in the poultry model are due to a larger time window in which a flock could have become infected and still been sent to slaughter.

Given the positive correlation, reducing the daily mortality threshold for when a flock is detected will reduce the number of HPAIV-positive birds and eggs that move into slaughter and processing. However, if a reasonable range of daily mortality thresholds is considered (0.5 to 2%, D. Swayne, personal communication), then decreasing the mortality detection threshold by half results in about a 2-fold decrease in illnesses. This suggests that use of a single day mortality estimate as a flock management practice for the detection of HPAIV would not be useful in preventing HPAIV-positives flocks from entering slaughter and subsequently exposing consumers. The daily mortality threshold would have to be set at less than 0.5% or multiple days of mortality would have to be considered.

6.3.3 Processing Sensitivity Analysis

6.3.3.1 Mortality detection threshold at slaughter (antemortem inspection)

Once a flock reaches market weight, it is transported and held before the birds are slaughtered. During this time, HPAIV-infected birds may die due to advanced disease. The percent bird mortality following transportation and holding may therefore be an indicator to slaughter plant inspectors of a problem. To estimate the impact of condemning a flock based on the percent mortality observed at slaughter on predicted human illnesses, a percent daily mortality threshold was added to the model above which the flock would be condemned and below which the flock would be slaughtered.

Because the percent mortality that would cause inspectors to condemn a flock at antemortem inspection is likely variable, the option to vary the detection threshold was used. Varying the detection threshold from 0.5% down to 0.1% reduces the number of predicted illnesses (Figure 24). However, a detection threshold of 1% or greater does not reduce the number of infected carcasses and subsequently the number of predicted illnesses. This is because the number of infected birds that are predicted to die given an additional 6 hours (transportation and holding) is not enough to signal to inspectors that there is a problem and the birds are sent to slaughter. If 12 hours is allowed for transportation and holding, the model predicts a similar result. There simply is not enough mortality over an additional 12 hours to alert flock managers assuming a 1% threshold.

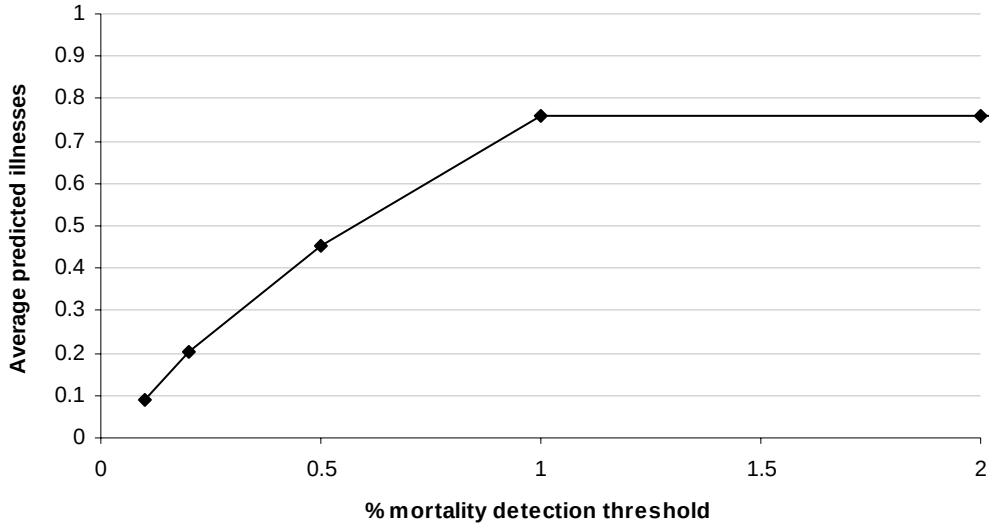

Figure 24. Impact of percent mortality detection threshold at slaughter on average predicted human illnesses from a chicken flock. The following values were used: 0.1, 0.2, 0.5, 1.0, and 2.0 %.

This analysis assumes that infected birds are equally distributed among a flock. Therefore, despite the fact that a flock is partitioned among different trucks during transportation each truck and subsequent holding pen will have the same percent mortality. This analysis also assumes that slaughter plant inspectors have no knowledge of previous bird mortality that likely occurred on the farm. Therefore, percent mortality is not cumulative from the farm to the slaughter plant.

6.3.3.2 Morbidity detection threshold at slaughter (postmortem inspection)

To estimate the effect of postmortem inspection, the "% showing pathology" model option inputs were modified as follows: the mortality rate as determined by Das et al., 2006 was shifted by 6-hour time intervals to demonstrate the effect of birds dying sooner or later than expected. The baseline model indicates that 90% of birds infected 25-hours will present clinical signs while birds infected $\leq$19 and $\geq$31 hours will all be visibly normal or presenting clinical signs, respectively. This distribution is then shifted by 6, 12, or more hours in either direction to estimate the effect on the model outputs. Figure 25 demonstrates that between 31 and 37 hours there is approximately a 7.5-fold effect suggesting this variable has a significant effect on then number of predicted human illnesses.

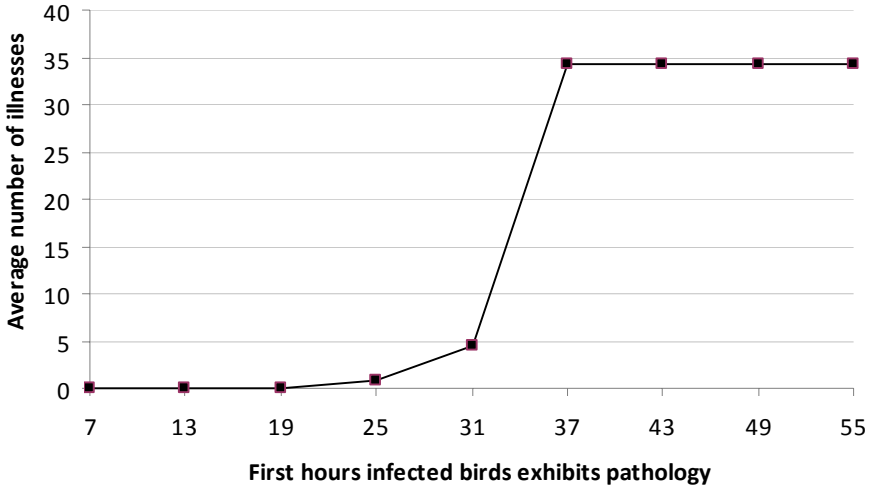

Figure 25. Effect of postmortem inspection to remove visibly ill birds.

6.3.3.3 HPAIV level in poultry meat

The amount of HPAIV present in poultry tissue at varying times of infection is uncertain. The model assumes that $10^{7.7}$ EID_{50}/g are present in poultry meat at 48 hours (peak infection). The model then assumes the levels of HPAIV/g increase by a factor of 5 every 6 hours up to peak infection levels of $10^{7.7}$ EID_{50}/g (see HPAIV levels in poultry). Changing the level of HPAIV in poultry meat at each time point by a factor of 10 decreases the number of predicted illnesses by about 10-fold (Figure 26), suggesting that peaks levels and levels at different points in infection are important inputs.

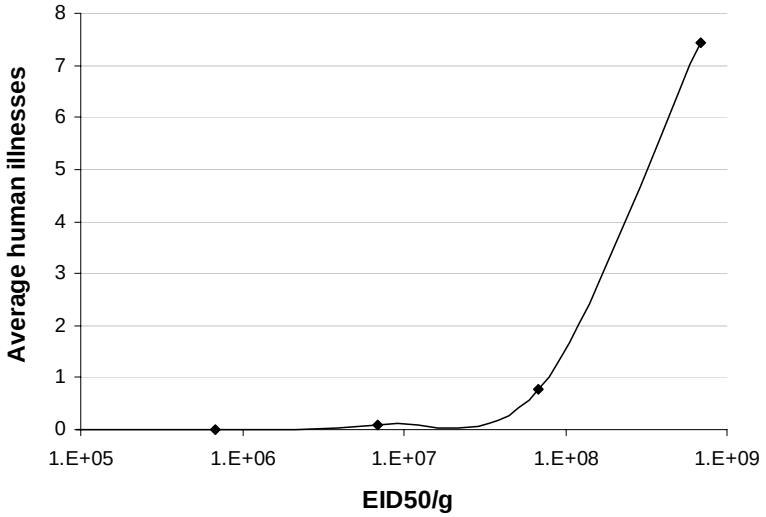

Figure 26. Effect of altering the HPAIV level in chicken and turkey on predicted human illnesses.

6.3.3.4 Time for eggs to reach market shelf

Varying the time the model requires for eggs to reach the market shelf has a significant impact on the number of human illnesses predicted by the model (Figure 27). When an egg is laid, it goes through a series of steps to finally reach the market shelf where it can be purchased. The total duration of these steps will vary from egg to egg. Eggs from flocks that are moved quickly to commerce will result in the greatest number of predicted human illness, while eggs that take longer to reach market will be less of a risk as they will not be available for purchase before the flock of origin in identified as infected. Increasing the time eggs take to reach the market shelf by 24 hours has a 6 to 8-fold effect on the reduction of human illnesses. This suggests that this component of the egg model is an important factor.

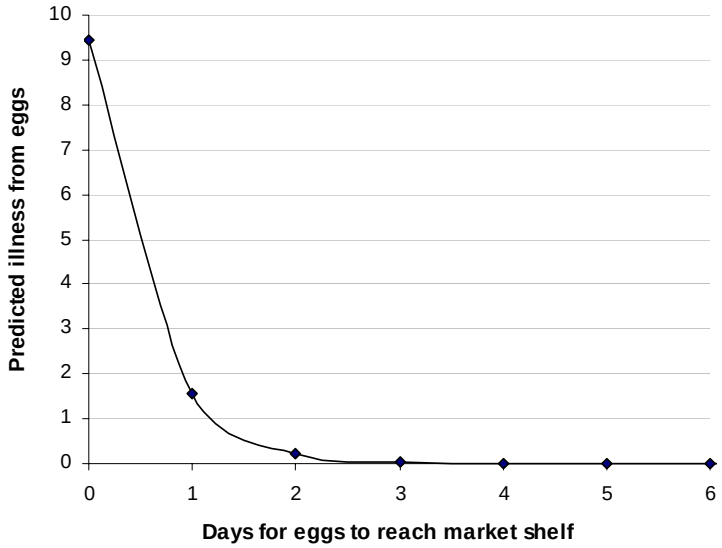

Figure 27. Number of human illnesses as a function of the time required to reach market. A baseline value of 6 days is used in the model.

6.3.4 Preparation Sensitivity Analysis

6.3.4.1 Cooking

To estimate the effect of cooking, cooking model options were modified so that 100% of contaminated servings were cooked at 165, 155, 145, or 135 ^{o}F. Cooking to 165 and 155 ^{o}F results in no predicted illnesses, while cooking at 145 and 135 ^{o}F results in illnesses from consumption of HPAIV-contaminated chicken, respectively. These results suggest that the time and temperature used to cook poultry is a critical input to the model. Changes in estimated $\log_{10}$ reductions and the fraction of servings applied the different reduction would also impact the risk assessment results.

6.3.4.2 Cross-contamination

To estimate the effect of cross-contamination, varying fractions of the total amount of HPAIV in contaminated chicken are used as inputs to the model (Figure 28). Specifically, 0.05, 0.5, 5.0 and 50% of the virus is assumed to be cross-contaminated and consumed for each serving of chicken. Additional illnesses over the baseline estimate of approximately 1 are observed at 5 and 50% of the virus being cross-contaminated, suggesting about a 3-fold effect. Again, this is likely to be an over estimate as it is assumed that all serving result in cross-contamination and all cross-contaminated virus is consumed. Furthermore, 50% of the virus being cross-contaminated is unrealistic.

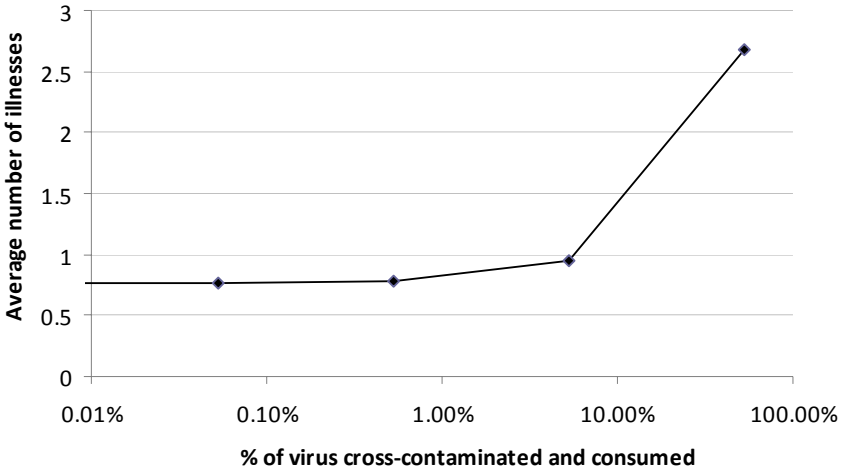

Figure 28. Predicted illnesses from cross-contamination and the cooking pathway.

6.3.5 Hazard Characterization Sensitivity Analysis

6.3.5.1 Dose-Response

No data were identified to adequately estimate the dose-response relationship for human illness from consumption of HPAIV. Therefore, the sensitivity of the baseline dose-response assumption was measured (Figure 29).

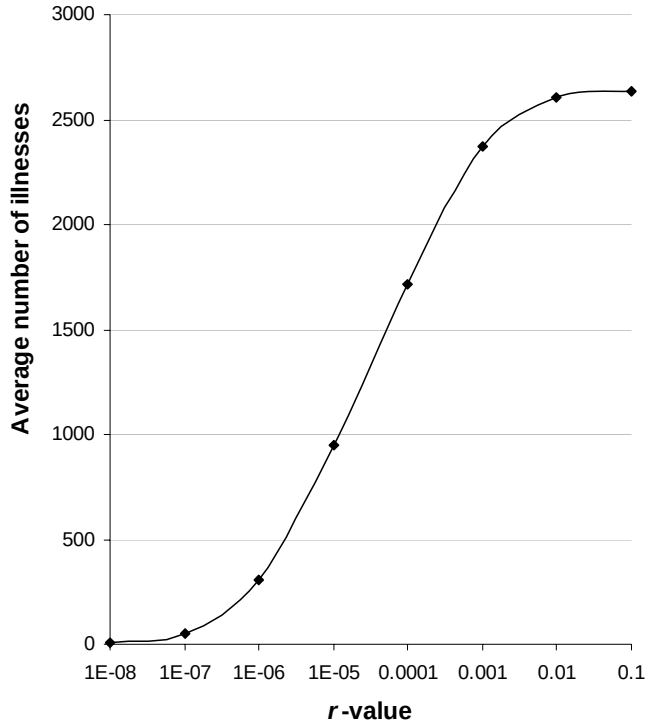

Figure 29. Effect of altering the dose-response on predicted human illnesses.

To estimate the impact of other dose-response data, different *r*-values representing different data were incorporated into the baseline scenario. Table 31 indicates the different strains from mouse and human intranasal studies. Depending on which dose-response data are used, average illnesses range from 0 to 2632 from a single 20,000 bird flock.

Table 31. Sensitivity analysis of various dose-response data.

Study	Strain	Model	ID_{50} (EID_{50})	$r = \ln(2)/ID_{50}$	Average human illnesses
Beare and Webster, 1991	H6	Human	NA	2.40E-10	0
Beare and Webster, 1991 (Baseline)	Average of all strains (H1,H3,H6,H4, H9, H10)	Human	NA	1.35E-09	1
Beare and Webster, 1991	H3	Human	NA	4.00E-09	3
Beare and Webster, 1991	H1	Human	NA	5.80E-09	4
Beare and Webster, 1991	H4, H9, H10	Human	NA	1.20E-08	9
Sears et al.,1988	H1N1 and H3N2	Human	3.16E+06[1]	2.19E-07	139
Sears et al.,1988	H1N1 and H3N2	Human	2.51E+06[1]	2.76E-07	169
Clements et al., 1989	H3N2	Human	2.00E+06[1]	3.47E-07	203
Mase et al., 2005b	H5N1(Dk/ Yokohama/aq10/2003)	Mouse	1.60E+06	4.33E-07	241
Clements et al., 1986	H3N2	Human	1.58E+06[1]	4.37E-07	243
Clements et al., 1989	H3N2	Human	6.31E+05[1]	1.10E-06	463

Maines et al., 2005	H5N1 (CkNCVD8)	Mouse	6.31E+05	1.10E-06	463
Snyder et al.,1986	H1N1 and H3N2	Human	2.51E+05[1]	2.76E-06	771
Sears et al.,1988	H1N1 and H3N2	Human	2.51E+05[1]	2.76E-06	771
Maines et al., 2005	H5N1 (CkIndon)	Mouse	2.00E+05	3.47E-06	858
Clements et al., 1983	H3N2	Human	2.00E+05[1]	3.47E-06	858
Snyder et al.,1986	H1N1 and H3N2	Human	7.94E+04[1]	8.73E-06	1232
Sears et al.,1988	H1N1 and H3N2	Human	7.94E+04[1]	8.73E-06	1232
Nguyen et al., 2005	H5N2 (Dk/VN/342/01)	Mouse	6.31E+04	1.10E-05	1330
Nguyen et al., 2005	H5N1 (Gs/VN/113/01)	Mouse	2.00E+04	3.47E-05	1812
Maines et al., 2005	H5N1 (CkCNVD31)	Mouse	3.16E+03	2.19E-04	2378
Lu et al., 1999	H5N1 (HK/156)	Mouse	1.58E+03	4.39E-04	2494
Maines et al., 2005	H5N1 (VN1204)	Mouse	2.00E+02	3.47E-03	2620
Maines et al., 2005	H5N1 (CkKorea)	Mouse	2.00E+02	3.47E-03	2620
Lu et al., 1999	H5N1 (HK/483)	Mouse	1.58E+02	4.39E-03	2624
Maines et al., 2005	H5N1 (VN1203)	Mouse	6.31E+01	1.10E-02	2631
Maines et al., 2005	H5N1 (SP83)	Mouse	6.31E+01	1.10E-02	2631
Maines et al., 2005	H5N1 (Thai16)	Mouse	2.00E+01	3.47E-02	2632
Lu et al., 1999	H5N1 (HK/486)	Mouse	1.58E+01	4.39E-02	2632
Lu et al., 1999	H5N1 (HK/485)	Mouse	1.26E+01	5.50E-02	2632
Nguyen et al., 2005	H5N1 (HK/483/97)	Mouse	3.16E+00	2.19E-01	2632

[1] Study conducted with $TCID_{50}$. Assume $EID_{50} = TCID_{50}$.

7 Research Needs

The following research needs were identified during the development of the HPAIV risk assessment in poultry meat, shell eggs and egg products. The following list is not prioritized:

1) Time and temperature at which consumers cook poultry and eggs. Proper cooking of HPAIV-contaminated poultry and eggs destroys the virus. However, the temperature data used in this risk assessment suggest that a significant number of consumers undercook poultry and eggs. More recent temperature data are needed to either validate or update the data used in the poultry and egg baseline scenarios. In addition, no data were identified to estimate the duration of cooking. A nationally representative survey measuring paired time and temperatures is needed to better inform the poultry and egg model.

2) Mammalian feeding trial for dose-response relationship. Feeding of infected poultry meat, shell eggs and egg products to relevant animal models (preferably primates) are needed to estimate the amount of virus sufficient to make humans ill via consumption. Studies should use multiple HPAI strains and focus on low dose responses to simulate consumption of contaminated cooked product (WHO, 2006b; EFSA, 2006).
    a. Oral exposures to mammalian species (mice, ferrets, pigs) are currently being studied by researchers at the ARS Southeastern Poultry Research Laboratory in Athens, Georgia, United States under the direction of Dr. David E. Swayne. Completion of the project is scheduled for July 31, 2008. (http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=409883 Accessed 4/03/08).

3) The rate at which HPAIV spreads from bird-to-bird. The speed HPAIV can spread among a flock will likely vary given several factors including animal husbandry, HPAI strain, initial number of infected birds, bird species, etc. Experimental studies are needed that quantify the rate of spread of HPAIV infection and the mortality rate among chicken, turkey, and hen flocks when a single bird is initially infected. Experiments should focus on the difference between ground- and cage-reared birds and relevant H5N1 strains.

4) Level of HPAIV in poultry meat and eggs. Though there are some data to estimate the level of virus in product (Table 4), further studies are needed to estimate the distribution of virus in various products at various times of infection. Specifically, the level of various HPAI strains within various parts (breast, thigh, wing, albumen, yolk, etc.) of poultry (chicken, turkey, duck, etc.) and eggs is needed at timed intervals of infection.

5) Stability of HPAIV in poultry meat, shell eggs, and egg products. Data are needed to determine the stability of multiple strains of HPAIV in varies poultry and egg products. Studies quantifying the effect of various food properties including temperature, pH, water activity, fat content, antimicrobials, disinfectants, spoilage organisms, and starting inoculum on the level of HPAIV over timed intervals is needed (WHO, 2006b).

6) Time required for shell eggs to reach market. Shell eggs can reach market in as little as 24 hours; however, the majority to eggs require more time. The distribution of times required for shell eggs to reach the market are needed.

8 Conclusion

Worldwide from 2003-2008, there have been 385 confirmed HPAIV human illnesses, resulting in 243 deaths (WHO, 2008). The majority of these cases are associated with close contact with live or dead HPAIV-infected birds likely caused by respiratory inhalation of infective droplets or self-inoculation (*e.g.*, by a human handler touching mucous membranes or conjunctiva after contact with avian fecal contamination, avian respiratory secretions, or avian body fluids). Currently, there is no compelling epidemiological evidence linking the consumption of cooked poultry meat, shell eggs, or egg products to human illness caused by HPAIV. HPAIV is not considered to be a foodborne pathogen although the virus has been isolated from poultry muscle and the interior of eggs. However, the possibility of poultry and egg consumption as an exposure route of HPAIV remains a concern to food safety experts. In light of this and the recent HPAIV poultry and human illnesses in Asia, Africa, Europe, and the Middle East, the Interagency Workgroup developed this food safety risk assessment for HPAIV exposure and illnesses in humans from consumption of poultry meat, shell eggs, and egg products.

This risk assessment has been developed as a tool to evaluate mitigation scenarios. The risk assessment model simulates human exposure and potential illness from consumption of HPAIV H5 and H7 strains that can make humans ill and lead to death. The number of human illnesses predicted serves as a basis to assess the magnitude and effectiveness of mitigation strategies. Given the uncertainty regarding the dose-response relationship and the uncertainty about the likelihood of human illness from consumption of poultry and eggs, the model-predicted number of human illnesses should not be considered an absolute value, and it should not be used outside of the context of scenario analysis.

The model predicts a 94 and 98% probability that a chicken and turkey flock, respectively, would be identified as HPAIV-positive before slaughter and not enter commerce. This is because flocks infected early in the grow-out period will have enough time to demonstrate significant mortality on the farm, resulting in identification of the flock as HPAIV-positive. There is a 6 and 2% probability that an HPAIV-infected chicken or turkey flock, respectively, may go to slaughter without detection of the disease. This would happen when HPAIV infects a flock that is approaching market weight with not enough time for the flock to demonstrate significant mortality on the farm.

Several mitigation strategies were evaluated. On-farm HPAIV flock testing has the greatest impact of lowering predicted human illnesses, while increased on-farm surveillance of daily flock mortality and increased surveillance during antemortem inspection are relatively less effective. Cooking poultry to the FSIS recommendation of 165°F is predicted to inactivate the virus and result in negligible risk to public health from HPAIV-contaminated poultry meat. Although few illnesses were predicted, consumer messages should continue to emphasize measures to prevent the potential cross-contamination of HPAIV and other microbiological hazards.

As a mitigation strategy, removing HPAIV-positive shell eggs from commerce will reduce potential exposure. Effectiveness is dependent on how many days of eggs production are removed. The model predicts that greater than 97% of potentially contaminated shell eggs can be removed form commerce given a 2-day market withdrawal. Effective recall of shell eggs is predicted to substantially reduce the risk of exposure to consumers from HPAIV-contaminated shell eggs. In addition, in-shell pasteurization of HPAIV-positive eggs is predicted to inactivate the virus and result in negligible risk to public health. Thorough cooking of eggs by consumers (150° F) also inactivates the virus.

This quantitative risk assessment provides a science-based, analytical approach to collate and incorporate available data into a mathematical model, and it provides risk managers a decision-support tool to evaluate the effectiveness of interventions to reduce or prevent foodborne illness from HPAIV in the U.S.

9 Addendum

The draft risk assessment report and model were completed in June 2008. Since that time, additional new data sources that may be useful in a future update of the risk assessment have been identified:

1) EcoSure 2007 Cold Temperature Database (http://foodrisk.org/exclusives/EcoSure/), 2) Lipatov AS, Kwon YK, Sarmento LV, Lager KM, Spackman E, Suarez DL, Swayne DE. Domestic pigs have low susceptibility to H5N1 highly pathogenic avian influenza viruses. PLoS Pathog. 2008 Jul 11;4(7):e1000102, 3) Liptov et al., unpublished, Pathogenesis of H5N1 Influenza Virus Infections in Mice and Ferret Models Differ Between Respiratory and Digestive System Exposure 4) Thomas and Swayne, unpublished, Thermal Inactivation of High Pathogenicity Avian Influenza Virus in Dried Egg White, and Swayne DE, Suarez DL.Current developments in avian influenza vaccines, including safety of vaccinated birds as food. Dev Biol (Basel). 2007;130:123-33.

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11 Appendix A

11.1 User's Manual

11.1.1 Model requirements

The model is written in Microsoft Excel 2003 and has been tested in Microsoft Excel 2007 with Windows XP and Vista. Visual Basic for Applications is used to perform the combinatorial evaluations for different scenarios. The models require macros to be enabled upon startup for program. Simulation run times are recorded in column R of SummaryModel worksheet.

11.1.2 Introduction

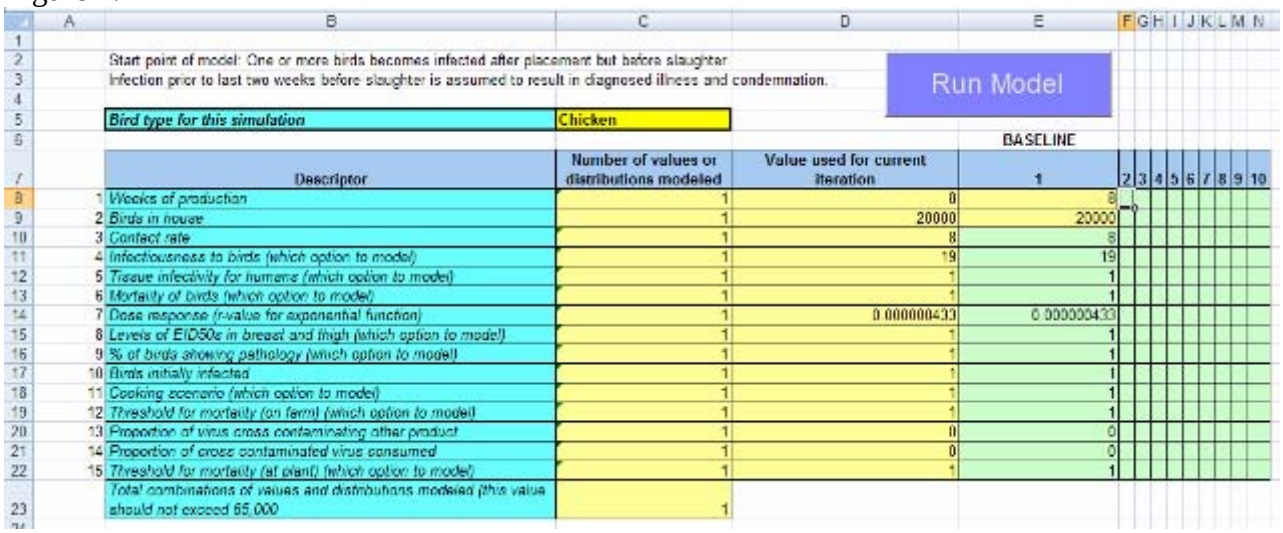
The user's manual describes how to run the baseline model, how to change model options for the purpose of running different model scenarios, and where to find model results. The user's manual does not attempt to describe each worksheet, but rather give the user a basic understanding of how to run the model and record results.

The user's manual is not a stand alone document and requires the risk assessment report and the risk model. The following figures and tables are from versions AI model 070917 Meat b and AI model 071016 Eggs a.

11.1.3 Baseline Poultry Model

The model opens with the SummaryModel Worksheet (Figure 1). Cell C5 (yellow) allows the user to toggle between the chicken and turkey model. The model parameters that can be changed by the user are located under the heading "Descriptor" in column B. There are 15 parameters for the poultry model.

Figure 1.



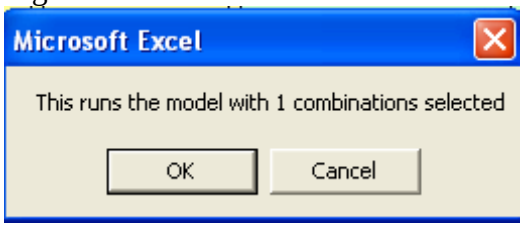
Start point of model: One or more birds becomes infected after placement but before slaughter
Infection prior to last two weeks before slaughter is assumed to result in diagnosed illness and condemnation.

Run Model

	Descriptor	Number of values or distributions modeled	Value used for current iteration	BASELINE 1	2	3	4	5	6	7	8	9	10
	Bird type for this simulation	Chicken											
1	Weeks of production	1	8	8									
2	Birds in house	1	20000	20000									
3	Contact rate	1	8	8									
4	Infectiousness to birds (which option to model)	1	19	19									
5	Tissue infectivity for humans (which option to model)	1	1	1									
6	Mortality of birds (which option to model)	1	1	1									
7	Dose response (r-value for exponential function)	1	0.000000433	0.000000433									
8	Levels of EID50s in breast and thigh (which option to model)	1	1	1									
9	% of birds showing pathology (which option to model)	1	1	1									
10	Birds initially infected	1	1	1									
11	Cooking scenario (which option to model)	1	1	1									
12	Threshold for mortality (on farm) (which option to model)	1	1	1									
13	Proportion of virus cross contaminating other product	1	0	0									
14	Proportion of cross contaminated virus consumed	1	0	0									
15	Threshold for mortality (at plant) (which option to model)	1	1	1									
23	Total combinations of values and distributions modeled (this value should not exceed 65,000	1											

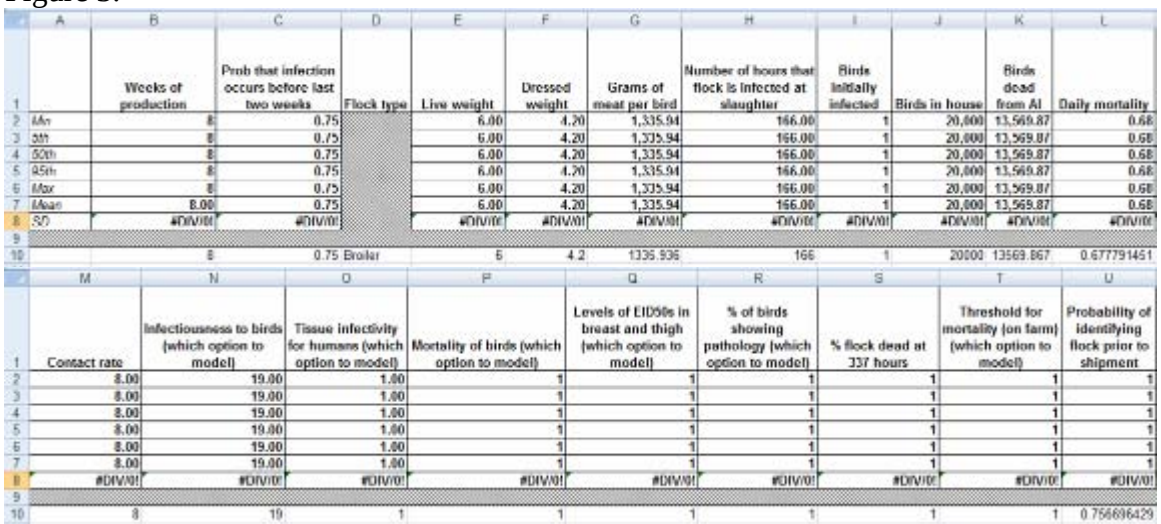
To run the baseline model, click "Run Model". The following message should appear, indicating that only 1 combination will be run (Figure 2).

Figure 2.



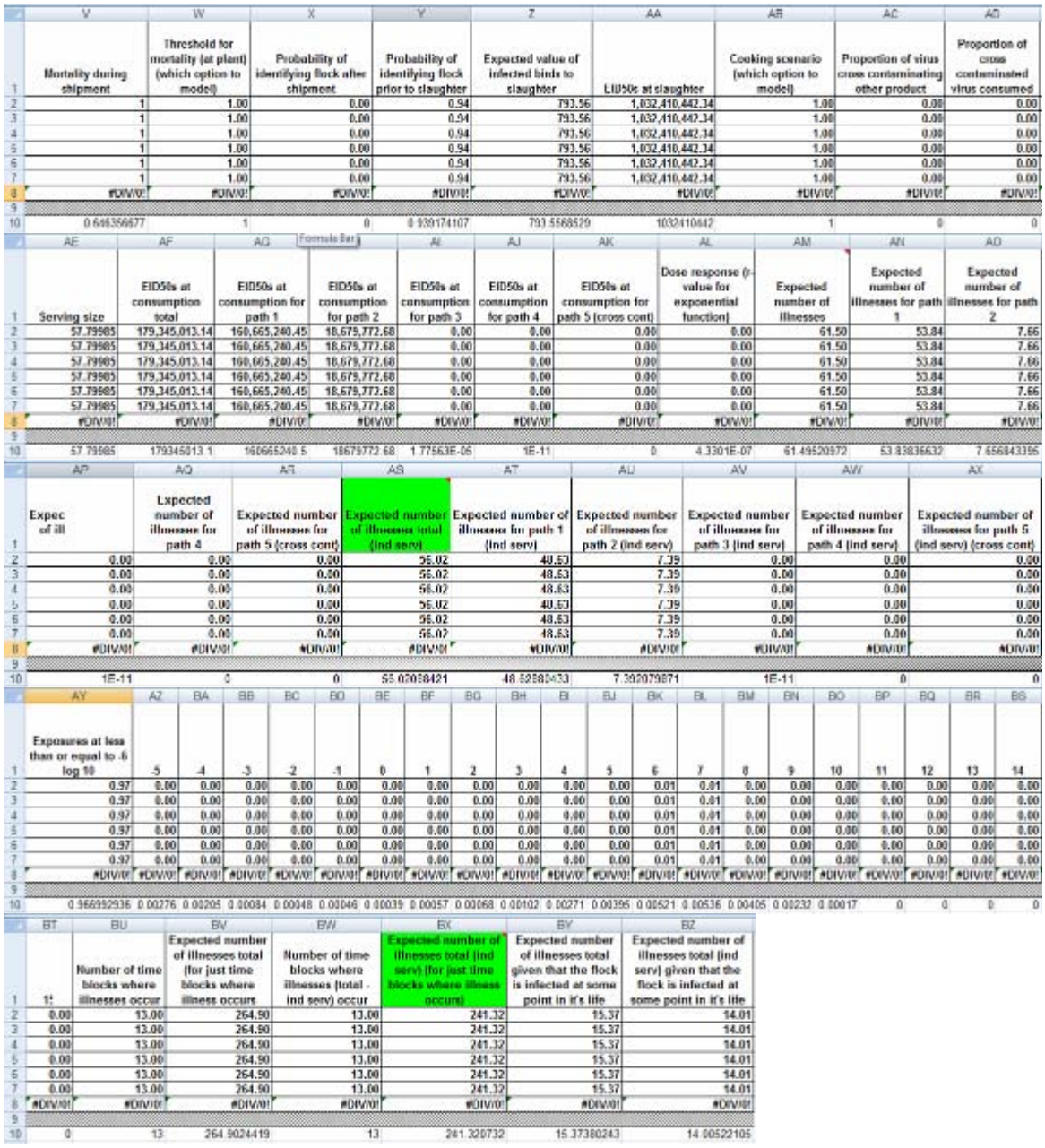
Results for the baseline model can be viewed in worksheet SummaryModelResults (Figure 3).

Figure 3.



	Weeks of production	Prob that infection occurs before last two weeks	Flock type	Live weight	Dressed weight	Grams of meat per bird	Number of hours that flock is infected at slaughter	Birds initially infected	Birds in house	Birds dead from AI	Daily mortality
Min	8	0.75		6.00	4.20	1,335.94	166.00	1	20,000	13,569.87	0.68
5th	8	0.75		6.00	4.20	1,335.94	166.00	1	20,000	13,569.87	0.68
50th	8	0.75		6.00	4.20	1,335.94	166.00	1	20,000	13,569.87	0.68
95th	8	0.75		6.00	4.20	1,335.94	166.00	1	20,000	13,569.87	0.68
Max	8	0.75		6.00	4.20	1,335.94	166.00	1	20,000	13,569.87	0.68
Mean	8.00	0.75		6.00	4.20	1,335.94	166.00	1	20,000	13,569.87	0.68
SD	#DIV/0!	#DIV/0!		#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	8	0.75	Broiler	6	4.2	1335.936	166	1	20000	13569.867	0.677791451

	Contact rate	Infectiousness to birds (which option to model)	Tissue infectivity for humans (which option to model)	Mortality of birds (which option to model)	Levels of EID50s in breast and thigh (which option to model)	% of birds showing pathology (which option to model)	% flock dead at 337 hours	Threshold for mortality (on farm) (which option to model)	Probability of identifying flock prior to shipment
	8.00	19.00	1.00	1	1	1	1	1	1
	8.00	19.00	1.00	1	1	1	1	1	1
	8.00	19.00	1.00	1	1	1	1	1	1
	8.00	19.00	1.00	1	1	1	1	1	1
	8.00	19.00	1.00	1	1	1	1	1	1
	8.00	19.00	1.00	1	1	1	1	1	1
	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	8	19	1	1	1	1	1	1	0.756696429



	V	W	X	Y	Z	AA	AB	AC	AD
1	Mortality during shipment	Threshold for mortality (at plant) (which option to model)	Probability of identifying flock after shipment	Probability of identifying flock prior to slaughter	Expected value of infected birds to slaughter	LID50s at slaughter	Cooking scenario (which option to model)	Proportion of virus cross contaminating other product	Proportion of cross contaminated virus consumed
2	1	1.00	0.00	0.94	793.56	1,032,410,442.34	1.00	0.00	0.00
3	1	1.00	0.00	0.94	793.56	1,032,410,442.34	1.00	0.00	0.00
4	1	1.00	0.00	0.94	793.56	1,032,410,442.34	1.00	0.00	0.00
5	1	1.00	0.00	0.94	793.56	1,032,410,442.34	1.00	0.00	0.00
6	1	1.00	0.00	0.94	793.56	1,032,410,442.34	1.00	0.00	0.00
7	1	1.00	0.00	0.94	793.56	1,032,410,442.34	1.00	0.00	0.00
8	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
9									
10	0.646356677	1	0	0.939174107	793.5668529	1032410442	1	0	0

	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO
1	Serving size	EID50s at consumption total	EID50s at consumption for path 1	EID50s at consumption for path 2	EID50s at consumption for path 3	EID50s at consumption for path 4	EID50s at consumption for path 5 (cross cont)	Dose response (r-value for exponential function)	Expected number of illnesses	Expected number of illnesses for path 1	Expected number of illnesses for path 2
2	57.79585	179,345,013.14	160,665,240.45	18,679,772.68	0.00	0.00	0.00	0.00	61.50	53.84	7.66
3	57.79585	179,345,013.14	160,665,240.45	18,679,772.68	0.00	0.00	0.00	0.00	61.50	53.84	7.66
4	57.79585	179,345,013.14	160,665,240.45	18,679,772.68	0.00	0.00	0.00	0.00	61.50	53.84	7.66
5	57.79585	179,345,013.14	160,665,240.45	18,679,772.68	0.00	0.00	0.00	0.00	61.50	53.84	7.66
6	57.79585	179,345,013.14	160,665,240.45	18,679,772.68	0.00	0.00	0.00	0.00	61.50	53.84	7.66
7	57.79585	179,345,013.14	160,665,240.45	18,679,772.68	0.00	0.00	0.00	0.00	61.50	53.84	7.66
8	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
9											
10	57.79585	179345013.1	160665240.5	18679772.68	1.77563E-05	1E-11	0	4.3301E-07	61.49520972	53.83836632	7.656843395

	AP	AQ	AR	AS	AT	AU	AV	AW	AX
1	Expec of ill	Expected number of illnesses for path 4	Expected number of illnesses for path 5 (cross cont)	Expected number of illnesses total (ind serv)	Expected number of illnesses for path 1 (ind serv)	Expected number of illnesses for path 2 (ind serv)	Expected number of illnesses for path 3 (ind serv)	Expected number of illnesses for path 4 (ind serv)	Expected number of illnesses for path 5 (ind serv) (cross cont)
2	0.00	0.00	0.00	56.02	48.63	7.39	0.00	0.00	0.00
3	0.00	0.00	0.00	56.02	48.63	7.39	0.00	0.00	0.00
4	0.00	0.00	0.00	56.02	48.63	7.39	0.00	0.00	0.00
5	0.00	0.00	0.00	56.02	48.63	7.39	0.00	0.00	0.00
6	0.00	0.00	0.00	56.02	48.63	7.39	0.00	0.00	0.00
7	0.00	0.00	0.00	56.02	48.63	7.39	0.00	0.00	0.00
8	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
9									
10	1E-11	0	0	56.02058421	48.62880433	7.392079871	1E-11	0	0

	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN	BO	BP	BQ	BR	BS
1	Exposures at less than or equal to -6 log 10	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
9																					
10	0.966992936	0.00276	0.00205	0.00084	0.00048	0.00046	0.00039	0.00057	0.00068	0.00102	0.00271	0.00395	0.00521	0.00536	0.00405	0.00232	0.00017	0	0	0	0

	BT	BU	BV	BW	BX	BY	BZ
1	1:	Number of time blocks where illnesses occur	Expected number of illnesses total (for just time blocks where illness occurs	Number of time blocks where illnesses (total - ind serv) occur	Expected number of illnesses total (ind serv) (for just time blocks where illness occurs)	Expected number of illnesses total given that the flock is infected at some point in it's life	Expected number of illnesses total (ind serv) given that the flock is infected at some point in it's life
2	0.00	13.00	264.90	13.00	241.32	15.37	14.01
3	0.00	13.00	264.90	13.00	241.32	15.37	14.01
4	0.00	13.00	264.90	13.00	241.32	15.37	14.01
5	0.00	13.00	264.90	13.00	241.32	15.37	14.01
6	0.00	13.00	264.90	13.00	241.32	15.37	14.01
7	0.00	13.00	264.90	13.00	241.32	15.37	14.01
8	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
9							
10	0	13	264.9024419	13	241.320732	15.37380243	14.00522105

Results for the baseline scenario are given in row 10, columns A-BZ SummaryModelResults worksheet (Figure 3). The SummaryModelResults worksheet also details model option and inputs (reproduced from column D, SummaryModel Worksheet). Many of the results are averages over the last 2 weeks of grow-out in which a flock could be infected by HPAIV. More detailed results can be found in TempSheet worksheet (Figure 4).

Figure 4.

	H	K	L	U	V	Y	Z	AA	AF	AS	BU	BX	BZ
1	Number of hours that flock is infected at slaughter	Birds dead from AI	Daily mortality	Probability of identifying flock prior to shipment	Mortality during shipment	Probability of identifying flock prior to slaughter	Expected value of infected birds to slaughter	EID50s at slaughter	EID50s at consumption total	Expected number of illnesses total (ind serv)	Number of time blocks where illnesses (total) occur	Expected number of illnesses total (ind serv) (for just time blocks where illness occurs)	Expected number of illnesses total (ind serv) given that the flock is infected at some point in it's life
2	**166**	**13570**	**0.67779**	**0.7566964**	**0.6463567**	**0.939174107**	**793.5569**	**1E+09**	**1.8E+08**	**56.02088**	**13**	**241**	**14**
3													
4	1	0	0	0	0	0	1	0	0	0			
5	7	0	0	0	0	0	1	1E+05	21082.6	0.009029			
6	13	0	0	0	0	0	4.199584	7E+05	114197	0.046865			
7	19	0	0	0	0	0	6.598936	1E+07	1868259	0.642571			
8	25	0	0	0	0	0	24.82328	1E+07	2019123	0.47362			
9	31	0	0	0	0	0	48.1376	4E+07	6420039	2.240518			
10	37	0.4	0.00002	0	0.00002	0	144.938	7E+07	1.2E+07	3.503327			
11	43	1	0.00005	0	3E-05	0	310.3587	2E+08	4.1E+07	13.87881			
12	49	2.28	0.00011	0	6.399E-05	0	850.4261	5E+08	8.6E+07	25.86576			
13	55	5.159	0.00026	0	0.000144	0	1900.766	1E+09	2.4E+08	79.35957			
14	61	13.89	0.00067	0	0.0004366	0	4608.187	3E+09	5.4E+08	166.1189			
15	67	34.15	0.00166	0	0.0010137	0	9158.536	8E+09	1.4E+09	456.2377			
16	73	87.02	0.00424	0	0.002648	0	15474.51	2E+10	3.2E+09	978.3935			
17	79	211.7	0.01033	0.375	0.0062637	0.375	11905.71	3E+10	4.5E+09	1410.399			
18	85	527.9	0.02572	1	0.0159767	1	0	0	0	0			
19	91	1274	0.0621	1	0.0383162	1	0	0	0	0			
20	97	2992	0.1459	1	0.091765	1	0	0	0	0			
21	103	6450	0.31527	1	0.2033168	1	0	0	0	0			
22	109	11740	0.57581	1	0.3904079	1	0	0	0	0			
23	115	17041	0.842	1	0.6417901	1	0	0	0	0			
24	121	19546	0.97333	1	0.8466674	1	0	0	0	0			
25	127	19985	0.99887	1	0.9661325	1	0	0	0	0			
26	133	20000	0.99999	1	0.9929637	1	0	0	0	0			
27	139	20000	1	1	0.9987696	1	0	0	0	0			
28	145	20000	1	1	0.9995726	1	0	0	0	0			
29	151	20000	1	1	0.9996755	1	0	0	0	0			
30	157	20000	1	1	0	1	0	0	0	0			
31	163	20000	1	1	1	1	0	0	0	0			
32	169	20000	1	1	1	1	0	0	0	0			

The TempSheet reports the results for each 6-hour time interval, within the last 2 weeks of grow-out (Rows 4-59), in which a flock could randomly be infected by HPAIV. Figure 4 row 1 headings are the same as the previous figure; however, several column have been removed due to space requirement for Figure 4 (column letter are consistent with Figure 3). TempSheet worksheet results are particularly informative as they give both average results over the 2 weeks (row 2) and individual 6-hour time intervals (rows 4-59).

The TempSheet estimates the average number of expected illnesses from HPAIV (column AS, row 2) over 2 weeks. Column BU indicates the number of 6-hour time intervals that resulted in infected flocks being sent to slaughter. Column BX is an average of rows 5-17, Column AS. Column BZ indicates the average number of illness given that a flock is infected at any point in its grow-out period.

11.1.4 Changing baseline inputs

To change a parameter option, choose different numbers in SummaryModel column E (Figure 1). The values that currently reside in column E are the baseline options. The baseline options as seen in Figure 1 and Table 1 are those that are described in the report.

The model is designed to allow the user to easily change the data used in the model to determine which parameters are of importance, test mitigation strategies, and customize the baseline scenario to the users' specific situation and needs. Some model inputs require an actual value, while others require the user to choose from a list of options (Table 1; see ModelOptions and

MortalityModelOptions worksheets). When a model option is required the following text appears after each description: "which option to model" (Figure 1, column B). An actual value is required for model parameter 1, 2, 3, 7, 10, 13, and 14.

All 15 model variables can be changed if desired by altering values in SummaryModel column E. However, if the user desires to change model input within a variable and among variables, this can also easily be performed.

Table 1.

	Poultry Model Variable	Input range	Chicken Baseline inputs	Turkey Baseline inputs
1	*Weeks of production*	4 – 32 weeks	8	20
2	*Birds in house*	100-120,000 birds	20,000	9,000
3	*Contact rate*	1 - 64	8	8
4	*Infectiousness to birds*	Option 1-23	19	19
5	*Tissue infectivity for humans*	Option 1-6	1	1
6	*Mortality of birds*	Option 1-8	1	1
7	*Dose response (r-value for exponential function)*	$10^{0} – 10^{-12}$	1.35E-09	1.35E-09
8	*Levels of EID50s in breast and thigh*	Option 1-3	1	1
9	*% of birds showing pathology*	Option 1-3	1	1
10	*Birds initially infected*	1 - # birds in house	1	1
11	*Cooking scenario*	Option 1-7	1	1
12	*Threshold for mortality (on farm)*	Option 1-3	1	1
13	*Proportion of virus cross contaminating other product*	0 - 1	0	0
14	*Proportion of cross contaminated virus consumed*	0 - 1	0	0
15	*Threshold for mortality (at plant)*	Option 1-4	1	1

Values can be added to columns E-N of SummaryModel worksheet (Figure 5.). In the following example, the user is exploring the effect of changing the contact rate and the level of HPAIV in chicken meat. By adding values to column F-H, the numbers in column C change, resulting in a total of 12 possible combinations and outcomes (cell C23).

Figure 5.

Start point of model: One or more birds becomes infected after placement but before slaughter.
Infection prior to last two weeks before slaughter is assumed to result in diagnosed illness and condemnati

Run Model

		Descriptor	Number of values or distributions modeled	Value used for current	1	2	3	4	5	6	7	8	9	10
		Bird type for this simulation	**Chicken**											
8	1	*Weeks of production*	1	8	8									
9	2	*Birds in house*	1	20000	20000									
10	3	*Contact rate*	4	8	1	4	8	16						
11	4	*Infectiousness to birds (which option to model)*	1	19	19									
12	5	*Tissue infectivity for humans (which option to model)*	1	1	1									
13	6	*Mortality of birds (which option to model)*	1	1	1									
14	7	*Dose response (r-value for exponential function)*	1	0.00001	0.00001									
15	8	*Levels of EID50s in breast and thigh (which option to model)*	3	1	1	2	3							
16	9	*% of birds showing pathology (which option to model)*	1	1	1									
17	10	*Birds initially infected*	1	1	1									
18	11	*Cooking scenario (which option to model)*	1	2	2									
19	12	*Threshold for mortality (on farm) (which option to model)*	1	1	1									
20	13	*Proportion of virus cross contaminating other product*	1	0	0									
21	14	*Proportion of cross contaminated virus consumed*	1	0	0									
22	15	*Threshold for mortality (at plant) (which option to model)*	1	4	4									
23		*Total combinations of values and distributions modeled (this value should not exceed 65,000*	12											

When the model is run with 1 or more combinations the results can be found in SummaryModelResults worksheet (Figure 6). Note column M and Q. Because the model inputs changed for "Contact rate" and "Levels of EID50s", those changes are recorded in row 2-8, column M and Q. All possible combinations and their results are located in rows 10-21. The combination that results in the most predicted illnesses is a combination of the fastest contact rate and highest level of HPAIV (cell AR20). In addition, the highest contact rate tested, 16, was found to have less of an impact on the model output than increasing the level of HPAIV by 1 $\log_{10}$ (cell AR 19 vs. AR 17) compared with the baseline.

Figure 6.

	A	K	L	M	Q	V	Y	Z	AA	AR	AS	AT	AU
1		Birds dead from AI	Daily mortality	Contact rate	Levels of EID50s in breast and	Mortality during shipment	Probability of identifying flock	Expected value of infected birds to	EID50s at slaughter	Expected number of illnesses	Expected number of illnesses	Expected number of illnesses	Expected number of illnesses
2	Min	3676.116	0.139852	1	1	0.05746	0.564732	136.6352	2.41E+08	16.8051	3.190011	7.97E-06	0
3	5th	3676.116	0.139852	1	1	0.05746	0.564732	136.6352	2.42E+08	21.56592	3.590705	8.23E-06	0
4	50th	13323.93	0.664743	6	2	0.627123	0.757254	606.5003	2.86E+09	78.84279	22.4932	0.000103	0
5	95th	14746.73	0.737116	16	3	0.728912	0.800223	1095.576	3.77E+10	482.7703	134.098	0.001414	0
6	Max	14746.73	0.737116	16	3	0.728912	0.800223	1095.576	4.35E+10	596.5356	169.5998	0.001663	0
7	Mean	11267.68	0.551613	7.25	2	0.510155	0.719866	611.303	1.15E+10	161.43	40.54998	0.000418	0
8	SD	4639.339	0.251251	5.879471	0.852803	0.277768	0.096179	380.135	1.53E+10	175.97	50.15082	0.000567	0
9													
10		3676.116	0.139852	1	1	0.05746	0.564732	136.6352	2.43E+09	40.58771	11.5069	7.97E-05	0
11		3676.116	0.139852	1	2	0.05746	0.564732	136.6352	2.43E+10	76.59867	27.27971	0.000797	0
12		3676.116	0.139852	1	3	0.05746	0.564732	136.6352	2.43E+08	16.8051	3.190011	7.97E-06	0
13		12720.86	0.633837	4	1	0.590032	0.739955	419.4616	2.41E+09	81.0869	17.70668	8.44E-05	0
14		12720.86	0.633837	4	2	0.590032	0.739955	419.4616	2.41E+10	198.782	55.41149	0.000844	0
15		12720.86	0.633837	4	3	0.590032	0.739955	419.4616	2.41E+08	25.46114	3.918545	8.44E-06	0
16		13927.01	0.695649	8	1	0.664214	0.774554	793.539	3.29E+09	153.0499	30.36885	0.000121	0
17		13927.01	0.695649	8	2	0.664214	0.774554	793.539	3.29E+10	389.6896	105.0512	0.001209	0
18		13927.01	0.695649	8	3	0.664214	0.774554	793.539	3.29E+08	43.37053	6.046948	1.21E-05	0
19		14746.73	0.737116	16	1	0.728912	0.800223	1095.576	4.35E+09	247.3447	47.69922	0.000166	0
20		14746.73	0.737116	16	2	0.728912	0.800223	1095.576	4.35E+10	596.5356	169.5998	0.001663	0
21		14746.73	0.737116	16	3	0.728912	0.800223	1095.576	4.35E+08	67.84782	8.82046	1.66E-05	0

Model outputs can also be viewed again using the TempSheet. However, only the most right-hand column of combinations will be displayed. Using the above example, the contact rate of 16 was placed in column H and the level of EID50s option 3 was placed in column G (Figure 5). Therefore the TempSheet will only report the results of contact rate 16, level of EID50s 3 with all other variables at baseline. To view other possible combinations, set only 1 combination in column E, SummaryModel.

11.1.5 Changing model options

The user can also relatively easily change the data that are currently used in the model by substituting new data for the previous data. For example, the model currently allows only a 10-fold increase or decrease in the level of HPAIV in poultry. If the user desires to change this, the following can be done: All model options are given in two spreadsheet, ModelOptions and MortalityModelOptions. By changing the data in any of the tables and choosing the corresponding model option in column E SummaryModel spreadsheet, the user can see the effect of their change on the model output.

11.1.6 Baseline Egg Model

The egg baseline model is set up in a similar fashion to that of the poultry model. The baseline model options are given in Figure 7 and the range of options is given in Table 2. Model results are found in SummaryModelResults. There is no TempSheet as the probability of when a hen flock is infected is not considered.

Figure 7.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1														
2		Start point of model. One or more birds becomes infected after placement.		Run Model										
3														
4														
5					BASELINE									
6		*User Changeable Values for Model Runs (Columns Labeled 1 through 10)*												
7	Input number	Descriptor	Number of values or distributions modeled	Value used for current iteration	1	2	3	4	5	6	7	8	9	10
8	1	In-shell pasteurization (Log10 reductions)	1	0	0									
9	2	Flock size (100 to 120000)	1	100000	100000									
10	3	Contact rate	1	2	2									
11	4	Infectiousness to birds (which option to model)	1	19	19									
12	5	Tissue infectivity (whether eggs are contaminated) (which option to model)	1	1	1									
13	6	Mortality of birds (which option to model)	1	1	1									
14	7	Dose response (r-value for exponential function)	1	0.000000433	0.000000433									
15	8	Levels of EID50s in eggs (which option to model)	1	1	1									
16	9	% of eggs showing pathology (which option to model)	1	1	1									
17	10	Birds initially infected	1	1	1									
18	11	Cooking scenario (which option to model)	1	2	2									
19	12	On farm mortality detection threshold	1	0.02	0.02									
20	13	Egg contamination (which option to model)	1	1	1									
21	14	Days eggs held before marketing (0-7)	1	2	2									
22	15	Threshold for egg contamination (only when option 3 for input 13 is modeled)	1	18	18									
23		Total combinations of values and distributions modeled (this value should not exceed 65,000	1											
24		Number of inputs with different choices modeled	0											

Table 2.

	Egg Model Variable	Input range	Hen Baseline inputs
1	*In-shell pasteurization*	0 – 10 $\log_{10}$	0
2	*Flock size*	100-1,000,000 birds	100,000
3	*Contact rate*	1 - 64	2
4	*Infectiousness to birds*	Option 1-23	19
5	*Tissue infectivity (whether eggs are contaminated)*	Option 1-6	1
6	*Mortality of birds*	Option 1-8	1
7	*Dose response (r-value for exponential function)*	10^{0} – 10^{-12}	1.35E-09
8	*Levels of EID50s in eggs*	Option 1-3	1
9	*% of eggs showing pathology*	Option 1-3	1
10	*Birds initially infected*	1 - # birds in house	1
11	*Cooking scenario*	Option 1-6	2
12	*On farm mortality detection threshold*	0.00 – 1.00	0.02
13	*Egg contamination*	Option 1-3	1
14	*Days eggs held before marketing*	0 – 10 days	2
15	*Threshold for egg contamination (only when option 3 for input 13 is modeled)*	0 - 48 hours	18

12 Appendix B

12.1 Chicken and Turkey Model Options

12.1.1 Infectiousness to birds model options

Option	1		
Criteria	Average of recovery for all organs by either method		
Time	**s**	**n**	**Input**
0	0	80	0
6	12	80	0.15
12	13	80	0.1625
18	41	80	0.5125
24	73	80	0.9125
30	78	80	0.975
36	80	80	1
42	152	152	1
48	88	88	1

Option	2		
Criteria	Average of recovery for trachea by either method		
Time	**s**	**n**	**Input**
0	0	20	0
6	2	20	0.1
12	1	20	0.05
18	5	20	0.25
24	15	20	0.75
30	18	20	0.9
36	20	20	1
42	38	38	1
48	22	22	1

Option	3		
Criteria	Maximum of recovery for all organs by either method		
Time	**s**	**n**	**Input**
0	0	40	0
6	9	40	0.225
12	9	40	0.225
18	26	40	0.65
24	39	40	0.975
30	40	40	1
36	40	40	1
42	76	76	1
48	44	44	1

Option	4		
Criteria	Maximum of recovery for trachea by either method		
Time	**s**	**n**	**Input**
0	0	10	0
6	2	10	0.2
12	1	10	0.1
18	4	10	0.4
24	9	10	0.9
30	10	10	1
36	10	10	1
42	19	19	1
48	11	11	1

Option	5		
Criteria	All organs – PCR		
Time	**s**	**n**	**Input**
0	0	40	0
6	9	40	0.225
12	9	40	0.225
18	18	40	0.45
24	37	40	0.925
30	40	40	1
36	40	40	1
42	76	76	1
48	44	44	1

Option	6
Criteria	Trachea - PCR

Time	s	n	Input
0	0	10	0
6	2	10	0.2
12	1	10	0.1
18	4	10	0.4
24	9	10	0.9
30	10	10	1
36	10	10	1
42	19	19	1
48	11	11	1

Option	7		
Criteria	Average of recovery for all organs by either method - 5th %tile		
Time	**s**	**n**	**Input**
0	0	80	0.000
6	12	80	0.098
12	13	80	0.108
18	41	80	0.422
24	73	80	0.844
30	78	80	0.924
36	80	80	0.964
42	152	152	0.981
48	88	88	0.967

Option	8		
Criteria	Average of recovery for trachea by either method – 5th %tile		
Time	**s**	**n**	**Input**
0	0	20	0.000
6	2	20	0.040
12	1	20	0.017
18	5	20	0.132
24	15	20	0.563
30	18	20	0.729
36	20	20	0.867
42	38	38	0.926
48	22	22	0.878

Option	9		
Criteria	Maximum of recovery for all organs by either method - 5th %tile		
Time	**s**	**n**	**Input**
0	0	40	0.000
6	9	40	0.139
12	9	40	0.139
18	26	40	0.519
24	39	40	0.889
30	40	40	0.930
36	40	40	0.930
42	76	76	0.962
48	44	44	0.936

Option	10		
Criteria	Maximum of recovery for trachea by either method - 5th %tile		
Time	**s**	**n**	**Input**
0	0	10	0.000
6	2	10	0.079
12	1	10	0.033
18	4	10	0.200
24	9	10	0.636
30	10	10	0.762
36	10	10	0.762
42	19	19	0.861
48	11	11	0.779

Option	11		
Criteria	All organs – PCR – 5th %tile		
Time	**s**	**n**	**Input**
0	0	40	0.000
6	9	40	0.139
12	9	40	0.139
18	18	40	0.329
24	37	40	0.822
30	40	40	0.930
36	40	40	0.930
42	76	76	0.962
48	44	44	0.936

Option	12		
Criteria	Trachea - PCR - 5th %tile		
Time	**s**	**n**	**Input**
0	0	10	0.000
6	2	10	0.079
12	1	10	0.033
18	4	10	0.200
24	9	10	0.636
30	10	10	0.762
36	10	10	0.762
42	19	19	0.861

48	11	11	0.779

Option	13		
Criteria	Average of recovery for all organs by either method - 95th %tile		
Time	**s**	**n**	**Input**
0	0	80	0.036
6	12	80	0.229
12	13	80	0.243
18	41	80	0.602
24	73	80	0.950
30	78	80	0.990
36	80	80	1.000
42	152	152	1.000
48	88	88	1.000

Option	14		
Criteria	Average of recovery for trachea by either method - 95th %tile		
Time	**s**	**n**	**Input**
0	0	20	0.133
6	2	20	0.271
12	1	20	0.207
18	5	20	0.437
24	15	20	0.868
30	18	20	0.960
36	20	20	1.000
42	38	38	1.000
48	22	22	1.000

Option	15		
Criteria	Maximum of recovery for all organs by either method – 95th %tile		
Time	**s**	**n**	**Input**
0	0	40	0.070
6	9	40	0.352
12	9	40	0.352
18	26	40	0.759
24	39	40	0.991
30	40	40	1.000
36	40	40	1.000
42	76	76	1.000
48	44	44	1.000

Option	16		
Criteria	Maximum of recovery for trachea by either method - 95th %tile		
Time	**s**	**n**	**Input**
0	0	10	0.238
6	2	10	0.470
12	1	10	0.364
18	4	10	0.650
24	9	10	0.967
30	10	10	1.000
36	10	10	1.000
42	19	19	1.000
48	11	11	1.000

Option	17		
Criteria	All organs – PCR – 95th %tile		
Time	**s**	**n**	**Input**
0	0	40	0.070
6	9	40	0.352
12	9	40	0.352
18	18	40	0.579
24	37	40	0.966
30	40	40	1.000
36	40	40	1.000
42	76	76	1.000
48	44	44	1.000

Option	18		
Criteria	Trachea - PCR - 95th %tile		
Time	**s**	**n**	**Input**
0	0	10	0.238
6	2	10	0.470
12	1	10	0.364
18	4	10	0.650
24	9	10	0.967
30	10	10	1.000
36	10	10	1.000
42	19	19	1.000
48	11	11	1.000

Option	19		
Criteria	Max for any method any organ		
Time	**s**	**n**	**Input**
0	0	10	0.000
6	4	10	0.400
12	3	10	0.300
18	10	10	1.000

24	10	10	1.000
30	10	10	1.000
36	10	10	1.000
42	19	19	1.000
48	11	11	1.000

Option	20		
Criteria	Max for any method any organ - 5th %tile		
Time	**s**	**n**	**Input**
0	0	10	0.000
6	4	10	0.200
12	3	10	0.135
18	10	10	0.762
24	10	10	0.762
30	10	10	0.762
36	10	10	0.762
42	19	19	0.861
48	11	11	0.779

Option	21		
Criteria	Max for any method any organ – 95th %tile		
Time	**s**	**n**	**Input**
0	0	10	0.238
6	4	10	0.650
12	3	10	0.564
18	10	10	1.000
24	10	10	1.000
30	10	10	1.000
36	10	10	1.000
42	19	19	1.000
48	11	11	1.000

Option	22		
Criteria	Low infectivity		
Time	**s**	**n**	**Input**
0			0.000
6			0.000
12			0.200
18			0.400
24			0.600
30			0.800
36			1.000
42			1.000
48			1.000

Option	23		
Criteria	High infectivity		
Time	**s**	**n**	**Input**
0			1.000
6			1.000
12			1.000
18			1.000
24			1.000
30			1.000
36			1.000
42			1.000
48			1.000

12.1.2 Tissue infectivity for humans model options

Option	1		
Criteria	Average of recovery for all organs by either method		
Time	**s**	**n**	**Input**
0	0	80	0
6	12	80	0.15
12	13	80	0.1625
18	41	80	0.5125
24	73	80	0.9125
30	78	80	0.975
36	80	80	1
42	152	152	1
48	44	44	1

Option	2		
Criteria	Average of recovery for breast and thigh by either method		
Time	**s**	**n**	**Input**
0	0	40	0
6	4	40	0.1
12	9	40	0.225
18	22	40	0.55
24	38	40	0.95
30	40	40	1
36	40	40	1
42	76	76	1
48	44	44	1

Option	3		
Criteria	Maximum of recovery for all organs by either method		
Time	**s**	**n**	**Input**
0	0	40	0
6	9	40	0.225
12	9	40	0.225
18	26	40	0.65
24	39	40	0.975
30	40	40	1
36	40	40	1
42	76	76	1
48	44	44	1

Option	4		
Criteria	Maximum of recovery for breast or thigh by either method		
Time	**s**	**n**	**Input**
0	0	20	0
6	3	20	0.15
12	6	20	0.3
18	12	20	0.6
24	20	20	1
30	20	20	1
36	20	20	1
42	38	38	1
48	22	22	1

Option	5		
Criteria	Arbitrarily low		
Time	**s**	**n**	**Input**
0	0	0	0
6	0	0	0
12	0	0	0
18	0	0	0
24	0	0	0
30	0	0	0
36	0	0	0.1
42	0	0	0.2
48	0	0	0.3

Option	6		
Criteria	Arbitrarily high		
Time	**s**	**n**	**Input**
0	0	0	1
6	0	0	1
12	0	0	1
18	0	0	1
24	0	0	1
30	0	0	1
36	0	0	1
42	0	0	1
48	0	0	1

12.1.3 Mortality of birds model options

Option	1
Criteria	ARS Study (Das)
Time	Prob of bird remaining infected for next time period
1	1
7	1
13	1
19	1
25	1
31	0.6
37	0
43	0
49	0
55	0
61	0
67	0
73	0
79	0
85	0
91	0
97	0
103	0
109	0
115	0
121	0
127	0
133	0
139	0

Option	2
Criteria	Method 1 shifted by 12 hours
Time	Prob of bird remaining infected for next time period
1	1
7	1
13	1
19	1
25	1
31	1
37	1
43	1
49	1
55	0.6
61	0
67	0
73	0
79	0
85	0
91	0
97	0
103	0
109	0
115	0
121	0
127	0
133	0
139	0

Option	3
Criteria	Method 1 shifted by 22 hours
Time	Prob of bird remaining infected for next time period
1	1
7	1
13	1
19	1
25	1
31	1
37	1
43	0.6
49	0
55	0
61	0
67	0
73	0
79	0
85	0
91	0
97	0
103	0
109	0
115	0
121	0
127	0
133	0
139	0

Option	4
Criteria	Method 1 with some variability
Time	Prob of bird remaining infected for next time period
1	1
7	1
13	1
19	1
25	0.8
31	0.6
37	0.4
43	0
49	0
55	0
61	0
67	0
73	0
79	0
85	0
91	0
97	0
103	0
109	0
115	0
121	0
127	0
133	0
139	0

Option	5
Criteria	Method 2 with some variability
Time	Prob of bird remaining infected for next time period
1	1
7	1
13	1
19	1
25	1
31	1
37	0.8
43	0.6
49	0.4
55	0
61	0
67	0
73	0
79	0
85	0
91	0
97	0
103	0
109	0
115	0
121	0
127	0
133	0
139	0

Option	6
Criteria	Method 3 with some variability
Time	**Prob of bird remaining infected for next time period**
1	1
7	1
13	1
19	1
25	1
31	1
37	1
43	1
49	0.8
55	0.6
61	0.4
67	0
73	0
79	0
85	0
91	0
97	0
103	0
109	0
115	0
121	0
127	0
133	0
139	0

Option	7
Criteria	Slow mortaility
Time	**Prob of bird remaining infected for next time period**
1	1
7	1
13	1
19	1
25	1
31	1
37	1
43	1
49	0.9
55	0.8
61	0.7
67	0.6
73	0.5
79	0.4
85	0.3
91	0.2
97	0.1
103	0
109	0
115	0
121	0
127	0
133	0
139	0

Option	8
Criteria	Method 1 shifted back 12 hours
Time	**Prob of bird remaining infected for next time period**
1	1
7	1
13	1
19	0.6
25	0
31	0
37	0
43	0
49	0
55	0
61	0
67	0
73	0
79	0
85	0
91	0
97	0
103	0
109	0
115	0
121	0
127	0
133	0
139	0

12.1.4 EID50s per gram

EID50s per gram	
Option	1
Criteria	Arbitrary
Time	**EID50s per gram**
1	2
7	115
13	1,054
19	9,670
25	88,699
31	813,611
37	7,463,055
43	68,456,811
49	627,937,857
55	5,759,922,854
61	52,834,386,232
67	484,637,110,520
73	4,445,459,588,766

Option	2
Criteria	Arbitrary higher
Time	**EID50s per gram**
1	21
7	1,149
13	10,542
19	96,698
25	886,986
31	8,136,107
37	74,630,554
43	684,568,114
49	6,279,378,572
55	57,599,228,535
61	528,343,862,323
67	4,846,371,105,198
73	44,454,595,887,661

Option	3
Criteria	Arbitrary lower
Time	**EID50s per gram**
1	0
7	11
13	105
19	967
25	8,870
31	81,361
37	746,306
43	6,845,681
49	62,793,786
55	575,992,285
61	5,283,438,623
67	48,463,711,052
73	444,545,958,877

12.1.5 % showing pathology model options

% showing pathology	
Option	1
Criteria	Das et al., 2006
Time	**% showing pathology**
1	0%
7	0%
13	0%
19	0%
25	90%
31	100%
37	100%
43	100%
49	100%
55	100%
61	100%
67	100%
73	100%

Option	2
Criteria	Method 1 shifted by -12 hours
Time	**% showing pathology**
1	0%
7	0%
13	90%
19	100%
25	100%
31	100%
37	100%
43	100%
49	100%
55	100%
61	100%
67	100%
73	100%

Option	3
Criteria	Method 1 shifted by 12 hours
Time	**% showing pathology**
1	0%
7	0%
13	0%
19	0%
25	0%
31	0%
37	90%
43	100%
49	100%
55	100%
61	100%
67	100%
73	100%

12.1.6 Cooking scenario model options

Option	1		
Criteria	Audits International data (compressed) using ARS analysis		
Pathway	**Temp**	**Proportion**	**LR**[46]
1	135	0.174	0.048479709
2	145	0.112	0.791698824
3	155	0.146	12.92885307
4	165	0.569	211.1348869

Option	2		
Criteria	Audits International data (compressed) using FDA analysis		
Pathway	**Temp**	**Proportion**	**LR**
1	135	0.174	0.176331032
2	145	0.112	1.117194399
3	155	0.146	7.078296483
4	165	0.569	44.84652014

Option	3		
Criteria	Improvement to AI data by 1 category (high ARS analysis)		
Pathway	**Temp**	**Proportion**	**LR**
1	135	0.086	0.048479709
2	145	0.088	0.791698824
3	155	0.112	12.92885307
4	165	0.715	211.1348869

Option	4		
Criteria	Improvement to AI data by 1 category (FDA analysis)		
Pathway	**Temp**	**Proportion**	**LR**
1	135	0.086	0.176331032
2	145	0.088	1.117194399
3	155	0.112	7.078296483
4	165	0.715	44.84652014

[46] LR: Log_{10} reduction

Option	5		
Criteria	Improvement to AI data by 2 categories (high ARS analysis)		
Pathway	**Temp**	**Proportion**	**LR**
1	135	0.04	0.048479709
2	145	0.046	0.791698824
3	155	0.088	12.92885307
4	165	0.827	211.1348869

Option	6		
Criteria	Improvement to AI data by 2 categories (FDA analysis)		
Pathway	**Temp**	**Proportion**	**LR**
1	135	0.04	0.176331032
2	145	0.046	1.117194399
3	155	0.088	7.078296483
4	165	0.827	44.84652014

Option	7		
Criteria	Assumes Option 2 with a 1 second contact time		
Pathway	**Temp**	**Proportion**	**LR**
1	135	0.174	0.017633103
2	145	0.112	0.11171944
3	155	0.146	0.707829648
4	165	0.569	4.484652014

12.1.7 Threshold for mortality on-farm model options

Threshold for mortality	
Option	1
Criteria	D. Swayne, personal communication
%	**Baseline**
0.00%	0.0000
0.10%	0.0000
0.20%	0.0000
0.30%	0.0000
0.40%	0.0000
0.50%	0.0625
0.60%	0.1250
0.70%	0.1875
0.80%	0.2500
0.90%	0.3125
1.00%	0.3750
1.10%	0.4375
1.20%	0.5000
1.30%	0.5625
1.40%	0.6250
1.50%	0.6875
1.60%	0.7500
1.70%	0.8125
1.80%	0.8750
1.90%	0.9375
2.00%	1.0000
3.00%	1.0000

4.00%	1.0000
5.00%	1.0000
6.00%	1.0000
7.00%	1.0000
8.00%	1.0000
9.00%	1.0000
10.00%	1.0000

Threshold for mortality	
Option	2
Criteria	Arbitrary
%	**Lower threshold**
0.00%	0.0000
0.10%	0.0000
0.20%	0.0625
0.30%	0.1250
0.40%	0.1875
0.50%	0.2500
0.60%	0.3125
0.70%	0.3750
0.80%	0.4375
0.90%	0.5000
1.00%	0.5625
1.10%	0.6250
1.20%	0.6875
1.30%	0.7500
1.40%	0.8125
1.50%	0.8750
1.60%	0.9375
1.70%	1.0000
1.80%	1.0000
1.90%	1.0000
2.00%	1.0000
3.00%	1.0000
4.00%	1.0000
5.00%	1.0000
6.00%	1.0000
7.00%	1.0000
8.00%	1.0000
9.00%	1.0000
10.00%	1.0000

Threshold for mortality

Option	3
Criteria	Arbitrary
%	**Higher threshold**
0.00%	0.0000
0.10%	0.0000
0.20%	0.0000
0.30%	0.0000
0.40%	0.0000
0.50%	0.0000
0.60%	0.0000
0.70%	0.0000
0.80%	0.0000
0.90%	0.0000
1.00%	0.0625
1.10%	0.1250
1.20%	0.1875
1.30%	0.2500
1.40%	0.3125
1.50%	0.3750
1.60%	0.4375
1.70%	0.5000
1.80%	0.5625
1.90%	0.6250
2.00%	0.6875
3.00%	0.7500
4.00%	0.8125
5.00%	0.8750
6.00%	0.9375
7.00%	1.0000
8.00%	1.0000
9.00%	1.0000
10.00%	1.0000

Threshold for mortality	
Option	4
Criteria	Arbitrary
%	**With 0.5% as baseline**
0.00%	0.0000
0.10%	0.0625
0.20%	0.1250
0.30%	0.1875

0.40%	0.2500
0.50%	0.3125
0.60%	0.3750
0.70%	0.4375
0.80%	0.5000
0.90%	0.5625
1.00%	0.6250
1.10%	0.6875
1.20%	0.7500
1.30%	0.8125
1.40%	0.8750
1.50%	0.9375
1.60%	1.0000
1.70%	1.0000
1.80%	1.0000
1.90%	1.0000
2.00%	1.0000
3.00%	1.0000
4.00%	1.0000
5.00%	1.0000
6.00%	1.0000
7.00%	1.0000
8.00%	1.0000
9.00%	1.0000
10.00%	1.0000

12.1.8 Threshold for mortality after shipment model options

Threshold for mortality	
Option	1
Criteria	Used for scenario analysis
%	**Baseline**
0.00%	0.0000
0.10%	0.0000
0.20%	0.0000
0.30%	0.0000
0.40%	0.0000
0.50%	0.0000
0.60%	0.0000
0.70%	0.0000
0.80%	0.0000
0.90%	0.0000
1.00%	0.0000
1.10%	0.0000
1.20%	0.0000
1.30%	0.0000
1.40%	0.0000
1.50%	0.0000
1.60%	0.0000
1.70%	0.0000
1.80%	0.0000
1.90%	0.0000
2.00%	0.0000
3.00%	0.0000
4.00%	0.0000
5.00%	0.0000
6.00%	0.0000
7.00%	0.0000

8.00%	0.0000
9.00%	0.0000
10.00%	0.0000

Threshold for mortality	
Option	2
Criteria	Arbitrary
%	**Baseline for on-farm**
0.00%	0.0000
0.10%	0.0000
0.20%	0.0000
0.30%	0.0000
0.40%	0.0000
0.50%	0.0625
0.60%	0.1250
0.70%	0.1875
0.80%	0.2500
0.90%	0.3125
1.00%	0.3750
1.10%	0.4375
1.20%	0.5000
1.30%	0.5625
1.40%	0.6250
1.50%	0.6875
1.60%	0.7500
1.70%	0.8125
1.80%	0.8750
1.90%	0.9375
2.00%	1.0000
3.00%	1.0000
4.00%	1.0000
5.00%	1.0000
6.00%	1.0000
7.00%	1.0000
8.00%	1.0000
9.00%	1.0000
10.00%	1.0000

Threshold for mortality	
Option	3
Criteria	Arbitrary
%	**Lower threshold**
0.00%	0.0000
0.10%	0.0000
0.20%	0.0625
0.30%	0.1250
0.40%	0.1875
0.50%	0.2500
0.60%	0.3125
0.70%	0.3750
0.80%	0.4375
0.90%	0.5000
1.00%	0.5625
1.10%	0.6250
1.20%	0.6875
1.30%	0.7500
1.40%	0.8125
1.50%	0.8750
1.60%	0.9375
1.70%	1.0000
1.80%	1.0000
1.90%	1.0000
2.00%	1.0000
3.00%	1.0000
4.00%	1.0000
5.00%	1.0000
6.00%	1.0000
7.00%	1.0000
8.00%	1.0000
9.00%	1.0000
10.00%	1.0000

Threshold for mortality	
Option	4
Criteria	Arbitrary
%	**Higher threshold**
0.00%	0.0000
0.10%	0.0000
0.20%	0.0000
0.30%	0.0000
0.40%	0.0000
0.50%	0.0000
0.60%	0.0000
0.70%	0.0000

0.80%	0.0000
0.90%	0.0000
1.00%	0.0625
1.10%	0.1250
1.20%	0.1875
1.30%	0.2500
1.40%	0.3125
1.50%	0.3750
1.60%	0.4375
1.70%	0.5000
1.80%	0.5625
1.90%	0.6250
2.00%	0.6875
3.00%	0.7500
4.00%	0.8125
5.00%	0.8750
6.00%	0.9375
7.00%	1.0000
8.00%	1.0000
9.00%	1.0000
10.00%	1.0000

12.2 Egg Model Options

12.2.1 % showing pathology

% showing pathology	
Option	1
Criteria	Arbitrary
Time	**% showing pathology**
1	5%
Option	2
Criteria	Arbitrary high path
Time	**% showing pathology**
1	10%
Option	3
Criteria	Arbitrary low path
Time	**% showing pathology**
1	1%

12.2.2 EID50s per gram

Option	1
Criteria	Arbitrary
Time	**EID50s per gram**
1	79,433

Option	2
Criteria	Arbitrary higher
Time	**EID50s per gram**
1	794,328

Option	3
Criteria	Arbitrary lower
Time	**EID50s per gram**
1	7,943

1.2.2 Log Reductions Due To Cooking

		Served as Eggs					**Other**	
	Type of serving	**Soft boiled and poached**	**Sunny side up**	**Scrambled and omelettes**	**Over easy**	**Hard boiled**	**Beverages**	**Mixtures**
Option 1	**Log reduction**	0.94	1.76	5.51	6.32	8.00	0.00	12.00
	Fraction	0.12	0.14	0.47	0.14	0.14	0.00	0.53

		Served as Eggs					**Other**	
	Type of serving	**Soft boiled and poached**	**Sunny side up**	**Scrambled and omelettes**	**Over easy**	**Hard boiled**	**Beverages**	**Mixtures**
Option 2	**Log reduction**	0.94	1.2	2144	0.04	8.00	0.00	12.00
	Fraction	0.12	0.14	0.47	0.14	0.14	0.00	0.53

		Served as Eggs					Other	
	Type of serving	Soft boiled and poached	Sunny side up	Scrambled and omelettes	Over easy	Hard boiled	Beverages	Mixtures
Option 3	Log reduction	1.47	1.47	2144	0.16	2144	0.00	12.00
	Fraction	0.12	0.14	0.47	0.14	0.14	0.00	0.53

		Served as Eggs					Other	
	Type of serving	Soft boiled and poached	Sunny side up	Scrambled and omelettes	Over easy	Hard boiled	Beverages	Mixtures
Option 4	Log reduction	9.4	146.8	20759.8	15.8	80.0	0.00	120.0
	Fraction	0.12	0.14	0.47	0.14	0.14	0.00	0.53

		Served as Eggs					Other	
	Type of serving	Soft boiled and poached	Sunny side up	Scrambled and omelettes	Over easy	Hard boiled	Beverages	Mixtures
Option 5	Log reduction	14.68	14.68	2075.98	1.58	2075.98	0.00	12.00
	Fraction	0.12	0.14	0.47	0.14	0.14	0.00	0.53

		Served as Eggs					Other	
	Type of serving	Soft boiled and poached	Sunny side up	Scrambled and omelettes	Over easy	Hard boiled	Beverages	Mixtures
Option 6	Log reduction	1.2	1.2	2144	1.2	2144	0.00	12.00
	Fraction	0.12	0.14	0.47	0.14	0.14	0.00	0.53

12.2.3 Egg contamination model options

Egg contamination	
Option	2
Criteria	
Time	Prob of egg contamination same as the prob of tissues infection
1	0
7	0.15
13	0.1625
19	0.5125
25	0.9125
31	0.975
37	1
43	1
49	1
55	1
61	1
67	1
73	1
79	1
85	1

Egg contamination	
Option	3
Criteria	
Time	**Threshold prob of egg contamination**
1	0
7	0
13	0
19	1
25	1
31	1
37	1
43	1
49	1
55	1
61	1
67	1
73	1
79	1
85	1

13 Appendix C

13.1 Mathematical model description

13.1.1 Introduction

This appendix explains the mathematics of modeling the risk of exposure to meat and eggs produced by HPAIV-affected flocks. The development of mathematics in this appendix begins with a simple approach. The complexities needed to more accurately reflect scenarios in the natural world are subsequently added onto this simple model.

The only consequence of HPAIV considered in this risk assessment is human illness. Variability in the severity of illness is not quantified. Thus, the likelihood measured in this risk assessment is the frequency of exposure to doses (contained in servings of poultry meat or eggs) that cause illness.

This is a conditional risk assessment; it only considers risk of exposure given that the meat or eggs consumed came from a HPAIV-affected flock. A more complete risk assessment would include the likelihood of a flock becoming HPAIV-affected; but predicting this likelihood is beyond the scope of this risk assessment. Given the conditional nature of this risk assessment, however, all of its results must be understood to only refer to risk of human exposure if, and only if, an affected flock should occur in the United States. Absent any such flock, the risk of exposure to HPAIV-containing foodstuffs is assumed to be nil.

This appendix strictly covers the mathematics of the risk assessment. It does not consider the data that inform variables and parameters in the model. It also does not address scenarios, results, sensitivity analysis, and uncertainty analysis or policy implications from the model. Data and scenarios actually modeled for this risk assessment are presented in the body of this report.

Explaining the mathematics of the model should inform readers about the general approach used in this risk assessment. The model's math should be independent of specific software used to generate results and, given the math, similar results should result from building the model using different software. Implicit in the development of the mathematics are a number of assumptions; these assumptions are usually employed to simplify the model. If bias is introduced by these assumptions, it is usually preferred that predicted risk is unaffected or increased rather than decreased. Therefore, such assumptions should build conservatism into the model's results.

13.1.2 Simple model for risk of exposure to HPAIV via poultry meat

Assume the prevalence of HPAIV-infected birds (π) in the flock at the time of slaughter is a fixed value greater than zero and less than, or equal to, one. Also, assume there are two independent transformation ratios, both less than one, that describe the fraction of infected birds that pass inspection (δ) and have virus in their meat (τ). Given these parameters, the fraction of the flock that is infected, passes inspection and contain virus in their meat tissue is $\pi \times \delta \times \tau$.

The dose actually delivered to a consumer depends on the density of virus (EID_{50} per gram) in the meat of infected birds (μ), the size of serving consumed (m) and the transformation ratio for the effectiveness of cooking (C). For a given $\log_{10}$ reduction (which is associated with a given time and temperature of cooking), the transformation ratio C equals $10^{-\log \text{ reduction}}$. For example, if cooking is known to reduce contamination by 5 logs, then C equals 0.00001. This transformation ratio explains that one in every 100,000 EID50's survives the cooking process.

Given this model, the exposure distribution ($f(D)$) describes the frequency of servings with various doses to which consumers are exposed. In other words, it represents the variability in exposure dose for consumers. In this simple model, a consumer can either be exposed to a dose of zero (*i.e.*, D=0) or a dose of $d = \mu \times m \times C$. The fraction of all servings with a dose of *d* is $\pi \times \delta \times \tau$ while the fraction of servings with a dose of zero is $1-(\pi \times \delta \times \tau)$. In other words,

$$f(D) = \begin{Bmatrix} 1-\pi\delta\tau, & \text{if D=0} \\ \pi\delta\tau, & \text{if D=d} \end{Bmatrix} \qquad \text{(Equation 1).}$$

An exponential dose-response function predicts the fraction of servings with dose *D* that result in illness (*i.e.*, $f(ill \mid D)$). If we assume that a particular consumer can only be exposed to one contaminated serving[47], then we can calculate the likelihood of illness per serving using the law of total probability [*i.e.*, $f(ill\ per\ serving) = \int_D f(ill \mid D) f(D) dD$].

For this simple model,

$$f(ill\ per\ serving) = \sum_D \left(1-e^{-rD}\right) f(D) = \left(1-e^{-rd}\right) \pi\delta\tau \qquad \text{(Equation 2)}$$

[47] This assumption is necessary for the mathematics to be valid but it is not necessarily robust because consumers often ingest multiple servings from the same bird. If the dose-response relationship is reasonably linear, however, the effect on predicted illnesses may be inconsequential. For example, if the dose per serving is x, the probability of illness is f(x) and the dose-response is linear, then someone who ingests two servings has a probability of illness that is f(2x); but this is just 2f(x).

where $f(ill \mid D) = 1 - e^{rD}$ is the exponential dose-response function with parameter *r*.

Although $f(ill\ per\ serving)$ describes the risk of exposure to a serving, it is generally interesting to also calculate the total number of illnesses that result across a fixed number of servings. For example, we can calculate the total servings of chicken generated from a flock by $N \times {}^{grams}/_{bird} \times m^{-1}$, where *N* is the number of birds slaughtered from the flock. A typical broiler chicken comprises 1336 grams of edible meat and a typical serving of chicken weighs 58 grams; therefore, one bird constitutes 23 servings. If the flock is affected with HPAIV, then the expected number of illnesses from exposure to the $N \times 23$ servings generated from this flock is calculated as;

$$Total\ Ill = N \times 23 \times f(ill\ per\ serving) \qquad \text{(Equation 3).}$$

Alternatively, it could be argued that Equation 3 represents the average number of human cases given the number of independent servings generated from an affected flock and the likelihood of illness per serving. For large values of *N* and small values of $f(ill\ per\ serving)$, the variability in total number of illnesses is predicted by a binomial distribution (*e.g.,* $Binomial(N \times 23, f(ill\ per\ serving))$). Nevertheless, this source of variability is ignored in the risk assessment. An explanation for including some sources of variability in the model, but not others, is offered next.

13.1.3 Sources of variability

Although this mathematical discussion does not include consideration of uncertainty in the model's inputs, decisions on which inputs to model as random variables are influenced by the amount of uncertainty associated with each input (Table 1). Inputs with little uncertainty (*e.g.*, *N* and *m*) are modeled as conservative, fixed values; while inputs with more uncertainty (*e.g.,* μ and π) are modeled as random variables. Anticipating the need for extensive uncertainty analysis, the model is simplified to limit the number of inputs included in that analysis. This simplification reduces the computational difficulties of conducting uncertainty analysis.

Some variables in the risk assessment directly affect the size of dose and other variables primarily influence the number of exposures that occur. For this risk assessment, the variables that influence the exposure dose size are generally emphasized while those variables that influence frequency of exposure (*i.e.*, the number of exposures that occur) are usually represented as average values. This distinction is not always clear, however, because severity and frequency are not always independently derived (*e.g.*, although δ and τ directly influence frequencies of exposure, these inputs are correlated with μ which directly influences dose size). Nevertheless, variables like *N* and the number of servings per bird carcass in Equation 3 primarily the scale the number of

exposures and the effect of their variability is less interesting in the context of this risk assessment's objectives[48].

Table 1. A partial listing of model inputs, their description and variability characteristics is shown.

INPUT NAME	DESCRIPTION	VARIABILITY CHARACTERISTICS
π	Prevalence of infected birds slaughtered	Predicted by transmission model; composed of prevalence by stage of infection (to which other variables are correlated)
π_t	Prevalence of infected birds in stage of infection, t	Predicted by transmission model; describes variability of stage-specific prevalence within an affected flock
δ	Fraction of birds that pass pre-mortem inspection	Varies with stages of infection
τ	Fraction of infected birds that have virus in their meat	Varies with stages of infection
μ	Density of virus per gram of poultry meat in infected birds	Varies with stages of infection
m	Mass of poultry meat serving	Considered a fixed value in the mathematical model development, although variability in this input is examined in the computer model analysis
C	Cooking transformation ratio	Variability explicitly modeled
N	Size of poultry flock at slaughter	Average flock size is used for each production type (e.g., broiler, turkey etc.) but average varies across production type
grams/bird	Average weight of bird carcass following slaughter	Considered a fixed value in model
r	Parameter of exponential dose-response model	Considered a fixed value in model

One reason these types of variables are less interesting is that they tend to represent production characteristics of the industry and its marketing practices. In contrast, variables that directly influence the size of exposure dose tend to represent the biology of HPAIV. These biologic inputs predict the dynamics of virus within infected birds and the effect of mitigations on survival of the HPAIV, but much uncertainty attends these phenomena. Another reason frequency variables are less interesting is that these variables tend to have rather limited and symmetric variability distributions. For example, the number of servings per bird carcass might be modeled as centered around 23 but with equal probability of being 19 or 27. The influence of this symmetric variability on predicted numbers of illnesses will expand the prediction in both directions (lower and higher). Although the influence of this variability may be necessary for precise risk estimates, symmetric variability is less interesting in terms of identifying

[48] The average size of flock, however, varies across poultry classes (*e.g.*, broiler chicken, capon chicken, turkey, etc.). This variability across poultry classes is captured in this risk assessment.

scenarios where risk is asymmetrically influenced by the input variable. In this risk assessment, variables like μ and C are skewed with longer right hand tails. The tails of these distributions tend to be associated with increased risk and their influence on predicted risk per serving is more closely evaluated[49].

13.1.4 Variability in cooking transformation ratio, C

The first complexity to consider is variability in cooking practices applied to a typical serving. Based on available data concerning time and temperature of cooking behaviors of food preparers, the following distribution might be assumed:

$$f(C)=\left\{\begin{array}{l}0.569,\ \text{if C}=10^{-211.135}\\0.146,\ \text{if C}=10^{-12.929}\\0.112,\ \text{if C}=10^{-0.792}\\0.174,\ \text{if C}=10^{-0.048}\end{array}\right\},\ \sum_{j=1}^{4} f(C_j)=1.$$

Although this four point discrete distribution for the effectiveness of cooking is relatively simple, it conveys substantial variability. For this distribution $E(C)=0.174,\ \sigma_C=0.335$ and $skewness=1.64$. A normal distribution has a skewness coefficient of zero, so this distribution is highly skewed to the right. Note that nearly 57% of servings are prepared such that *C* essentially equals zero (*i.e.*, $10^{-211.135}\approx 0$) and no virus is assumed to survive cooking; but 17% of servings are prepared such that C equals 0.89 (*i.e.*, $10^{-0.048}\approx 0.89$) and 89% of viruses in the serving prior to cooking have survived to expose the consumer.

Introducing variability from cooking into the model complicates the exposure distribution compared to the simplest model. The exposure distribution with variable cooking effectiveness is:

$$f(D)=\left\{\begin{array}{ll}1-\pi\delta\tau, & \text{if D}=0\\ \pi\delta\tau(0.569), & \text{if D}=\mu m10^{-211.135}\\ \pi\delta\tau(0.146), & \text{if D}=\mu m10^{-12.929}\\ \pi\delta\tau(0.112), & \text{if D}=\mu m10^{-0.792}\\ \pi\delta\tau(0.174), & \text{if D}=\mu m10^{-0.048}\end{array}\right\}.$$

[49] Although it may not be correct to assume, a priori, that we are only interested in extreme values of these input distributions, it is at least reasonable to recognize that hazards quantified in risk assessments tend to occur infrequently.

To quantify the influence of including variability in cooking effectiveness in the risk calculations, we compare the predictions in total number ill from the simple model and the more complicated model. We make the following assumptions: $\pi = 13.3\%$, δ=98.7%, τ=12.9% . We also assume $\mu = {}^{24,712 EID50's}/_{gram}$, $m = 57.8 grams$, and $r = 10^{-5}$. In the simple model case, we assume C=0.174, which is the expected value of the assumed distribution above. Finally, to calculate the number of illnesses resulting from slaughter of an affected flock, we assume that $N = 20,000$ birds.

In the simple model case (assuming an average cooking effectiveness), the only non-zero dose is:

$$d = \mu \times m \times C = 24,712 \times 57.8 \times 0.174 = 248,130,$$

Using Equation 2, the likelihood of illness per serving is:

$$f_{simple}(ill\ per\ serving) = \left(1 - e^{-10^{-5}(248,130}\right) 0.133 \times 0.987 \times 0.129 = 0.017.$$

Using Equation 3, the number of human illnesses predicted from this flock is:

$$Total\ Ill = 20,000 \times 23 \times 0.017 \approx 7,800.$$

If we incorporate variability of cooking effectiveness into the likelihood of illness per serving calculation, then there are essentially three possible non-zero doses (after noting that C equals zero for 57% of servings) and one of these doses is very nearly zero, too (Table 2).

Table 2. Exposure distribution when variability of C is included.

Values of D	f(D)
0	0.99270
1.68264E-07	0.00247
230,751	0.00190
1,277,507	0.00295

Using Equation 2, the likelihood of illness per serving is:

$$f_{\text{var\_}cook}(ill\ per\ serving) = \left(1 - e^{-10^{-5}(1.68\times10^{-7}}\right)0.133\times0.987\times0.129\times0.146 +$$
$$\left(1 - e^{-10^{-5}(230,751}\right)0.133\times0.987\times0.129\times0.112 +$$
$$\left(1 - e^{-10^{-5}(1,277,507}\right)0.133\times0.987\times0.129\times0.174 =$$
$$0.00465$$

Using Equation 3, the number of human illnesses predicted from this flock is:

$$Total\ Ill = 20,000\times23\times0.00465 \approx 2,150\,.$$

As this simple comparison illustrates, including variability in risk calculations can generate substantially different answers relative to such calculations based only on expected values. In this case comparison, the explicit consideration of variability in cooking effectiveness substantially reduces the risk of exposure from servings of poultry.

13.1.5 Variability in density of virus per gram of meat

At best, the density of virus (*i.e.*, EID_{50} per gram) previously assumed is an average across similar infected birds. These similarly infected birds have passed inspection (*i.e.*, they don't have obvious pathology) and contain virus in their muscle tissue. Nevertheless, it is unlikely that the density of virus is exactly the same for each of these infected birds.

13.1.5.1 Correlation of virus density and pathology with stage of infection

An important covariate with virus density in muscle tissue is the stage of infection. Stage of infection is the time, *t*, between initial infection and slaughter. The longer a bird has been infected, the more virus it has in its muscle tissue at the time of slaughter. Therefore, we can assert some functional relationship between virus density and stage of infection, $\mu = U(t)$.

Positive associations with μ are also assumed for the likelihood that an infected bird is passed for inspection (δ) and the likelihood that an infected bird contains any virus in its muscle tissue (τ). Given the virulence of HPAIV and its association with high rates of morbidity and mortality, it is expected that an infected bird is more likely to develop gross pathologic signs the longer it remains infected and alive. Also, given the increasing density of virus as infection progresses, it is expected that the likelihood of an infected bird having virus in its muscle tissue also increases.

To incorporate these dependencies into the model, we first define the prevalence of infected birds by stage of infection:

π_i, *such that* $\pi = \sum_{all\ i} \pi_i$. In other words, consider the prevalence of infected birds in a flock at the time of slaughter as the sum of the prevalence of infected birds that have been infected for discrete periods of time (*e.g.*, t hours) within that flock. It is convenient to consider the time steps of infection in six hour intervals. For example, the prevalence of infected birds at slaughter might be 10% (*i.e.*, $\pi = 0.10$). This prevalence might result from 5% (π_1) of the flock being infected from 1 to 6 hours, 2.5% (π_2) of the flock being infected from 7 to 12 hours, 1.5% (π_3) of the flock being infected from 13 to 18 hours and 1% (π_4) infected from 19 to 24 hours. It is obvious in this example that the flock was infected approximately 24 hours prior to slaughter. At the time of slaughter, it consists of some birds that have been infected just a few hours, as well as some birds that have been infected for one day. [**Note:** Throughout the remainder of this appendix, stage of infection will be denoted *i* and cumulative time of infection will be denoted *t*. For example, the prevalence of infected birds in stage *i* when the flock has been infected for *t* is denoted $\pi_i(t)$. Both *i* and *t* are typically measured in 6 hour intervals; so *i* = 4 indicates a cohort of birds that have been infected from 19-24 hours and *t* = 4 indicates a flock that became affected 19-24 hours ago. A flock that is affected for *t* periods actually consists of birds that have been infected *i* = 1, 2, 3,…,*t* periods.]

For any stage of infection, we can define corresponding values for virus density, μ = U(*i* = 1, 2, …,T), where T is total number of stages of infection the flock experiences before it is slaughtered. Each of these values of μ has a corresponding likelihood of occurrence, π_i, that describes the proportion of servings with this density of virus per gram. Therefore, we can describe virus density as a random variable ($\mu \sim f(\mu)$) as shown in Table 3.

It is further assumed that δ and τ are also functions (Δ and Υ, respectively) of stage of infection and are perfectly correlated with μ. In other words, for the same stage of infection, $\Delta(i)$, $\Upsilon(i)$, and $U(i)$ all have the same likelihood of occurrence[50] (*e.g.*, π_i) (Table 3).

Table 3. This table describes the discrete random variables for viral density (μ), likelihood of an infected carcass passing inspection (δ), and likelihood of an infected carcass containing virus in its muscle tissue (τ). Each of these variables is a function of stage of infection (*i*) and all three variables have the same likelihood values $f(\bullet)$ for each stage of infection.

[50] Note that we can also specify a likelihood distribution for all three of these variables that is truncated to only consider non-zero values. Because these variables are monotonically increasing with stage of infection *i*, then at i=0 these variables' values are all zero. For non-zero values, the relative frequency is calculated as π_i / π.

STAGE OF INFECTION	$f(\bullet)$	μ	δ	τ
1 to 6 hours	π_1	U(1)	$\Delta(1)$	$\Upsilon(1)$
7 to 12 hours	π_2	U(2)	$\Delta(2)$	$\Upsilon(2)$
13 to 18 hours	π_3	U(3)	$\Delta(3)$	$\Upsilon(3)$
•	•	•	•	•
•	•	•	•	•
•	•	•	•	•
T hours (stages)	π_T	$U(T)$	$\Delta(T)$	$\Upsilon(T)$

Including the variability in virus density, inspection and virus in muscle into the exposure distribution ($f(D)$) increases its complexity substantially. Assuming that cooking effectiveness is independent of virus density[51], there are four possible cooking effectiveness levels ($C_1,...,C_4$) for each possible virus density. The resulting exposure is a convolution of these likelihood values.

To illustrate the convolution process, assume there are just four time periods for stage of infection ($i = 1, 2, 3, 4$). Then the exposure distribution is:

[51] We also assume that virus density and cooking effectiveness are independent of serving size. In other words, we assume there is no correlation between the amount of food consumed in a serving and the density of virus in the serving.

$$f(D)=\left\{\begin{array}{ll} 1-\sum_{1}^{4}\left(\pi_i\Delta(\mathrm{i})\Upsilon(\mathrm{i})\right), & \text{if D=0} \\ \pi_1\Delta(1)\mathrm{T}(1)\mathrm{f}(\mathrm{C}_1), & \text{if D=U(1)}mC_1 \\ \pi_1\Delta(1)\mathrm{T}(1)\mathrm{f}(\mathrm{C}_2), & \text{if D=U(1)}mC_2 \\ \pi_1\Delta(1)\mathrm{T}(1)\mathrm{f}(\mathrm{C}_3), & \text{if D=U(1)}mC_3 \\ \pi_1\Delta(1)\mathrm{T}(1)\mathrm{f}(\mathrm{C}_4), & \text{if D=U(1)}mC_4 \\ \bullet & \\ \bullet & \\ \bullet & \\ \pi_4\Delta(4)\mathrm{T}(4)\mathrm{f}(\mathrm{C}_1), & \text{if D=U(4)}mC_1 \\ \pi_4\Delta(4)\mathrm{T}(4)\mathrm{f}(\mathrm{C}_2), & \text{if D=U(4)}mC_2 \\ \pi_4\Delta(4)\mathrm{T}(4)\mathrm{f}(\mathrm{C}_3), & \text{if D=U(4)}mC_3 \\ \pi_4\Delta(4)\mathrm{T}(4)\mathrm{f}(\mathrm{C}_4), & \text{if D=U(4)}mC_4 \end{array}\right\},$$

where $f(C_j)$ is the likelihood of each cooking effectiveness value and $\sum_{j=1}^{4} f(C_j)=1$.

This exposure distribution can be integrated through Equation 2 to determine the risk of exposure to a random serving of meat generated from this HPAIV-positive flock.

13.1.6 Modeling prevalence and stage of infection

For highly contagious agents like HPAIV, prevalence of infection is generally a monotonically increasing function across time. Because poultry flocks are usually managed in an all-in/all-out style, flock age at slaughter can be considered fixed. If we define A as the age of the flock at slaughter, then we know the length of time a flock can be affected at the time of slaughter (T) is less than (or equal to) A.

HPAIV can enter a flock at any point between zero and age A. Define z as the fraction of A (*i.e.*, 0< z <1) when HPAIV enters the flock. If T equals the total time a flock is affected, then T = (1-z)A. For example, assume a broiler flock is in production for a total of 8 weeks (*i.e.*, A = 8 weeks); if infection enters the flock midway into its production (z = 0.5), then the total time the flock is affected with HPAIV at the time of slaughter is 4 weeks (T = (1-0.5)8 = 4 weeks). It is convenient to consider time in 6 hour intervals, so T = 4 weeks = 28 days = 672 hours = 112 six-hour intervals.

We can define prevalence in the flock as a function of time since the flock became affected; $\pi(t)$, $0< t <T$. So, t is some value between zero (the time of initial infection) and T (the total time the flock was infected when it was slaughtered).

For illustrative purposes only, we can define the prevalence function as: $\pi(t) = \pi_0 e^{g \times t}$, where g is some fixed growth rate for prevalence and π_0 is the initial prevalence of infected birds when infection entered the flock. Usually, we'll assume just one bird initially became infected; so $\pi(0) = 1/N$.

Ignoring some of the complexity we'll introduce later for modeling transmission of HPAIV, we can illustrate how prevalence by stage of infection is predicted using a simple model (Table 4). Assume that $g = 0.8$ for a typical 6-hour transmission interval. Also, for generality, define I(t) as the number of infected birds in a flock as a function of time[52]. If we start with just one infected bird, then after one transmission interval we predict there are 2 infected birds at 6 hours (t = 6 hours). We predict this value from $I(6) = e^{0.8 \times 1}$ and noting that 6 hours equates to one transmission period.

In the next 6 hour interval, these 2 infected birds will move from the first stage of infection (*i.e.*, 1 – 6 hours) to the second stage of infection (7 – 12 hours). But, these 2 birds will also generate 3 new infected birds during the second stage. We can calculate these new infections by calculating how many infected birds there will be at 12 hours (or after two transmission periods) as $I(12) = e^{0.8 \times 2} \approx 5$ and subtracting the 2 birds that generated these new infections. By 18 hours following infection, the 2 originally infected birds have moved into the third stage of infection (13 – 18 hours), the 3 birds that just became infected move into the second stage of infection (7 – 12 hours), and 6 new birds become infected from the 5 (i.e., 2 + 3) that were infected previously. These 6 new infected birds are more obviously determined from $5e^{0.8 \times 1} - 5$; this equation shows that the 5 infected birds at 12 hours generate $e^{0.8 \times 1}$ total infections in the next time interval from which we subtract those 5 infected birds to calculate the infections that are new.

Table 4. The following table provides an illustrative example of how the number of infected birds by stage of infection relates to the evolution of total infected birds across time. In this example, the exponential growth rate per 6-hour transmission interval is 0.8. Note that true values are not integers; this example rounds the results to integer values for simplicity of presentation.

[52] Note that $\pi(t) = \pi_0 e^{g \times t} = 1/N \, e^{g \times t} = I(t)/N$. If we multiply through by N, we have $I(t) = I_0 e^{g \times t}$ and $I_0 = 1$ by assumption.

		Time of infection, t hours				
		0	6	12	18	24
Stage of infection	1 (1 - 6 hours)	1	2	3	6	14
	2 (7 - 12 hours)			2	3	6
	3 (13 - 18 hours)				2	3
	4 (19 - 24 hours)					2
	Total infected	1	2	5	11	25

This example illustrates, while $\pi(t)$ predictably increases (Figure 1), that there is also a consistent pattern to the prevalence of infected birds by stage of infection. Ignoring the effects of an incubation period and mortality for now, we can see that generally $\pi_1 > \pi_2 > ... > \pi_T$, as well as $\pi(t) = \pi_1 + \pi_2 + ... + \pi_t$.

Because stage of infection positively correlates with density of HPAIV in muscle tissue, we can appreciate from this illustrative example that generally the highest fraction of infected birds will be in the first stage of infection; but these infected birds will have the lowest density of HPAIV per gram of meat. Public health risk may be more influenced by those smaller fractions of infected birds that are in later stages of infection.

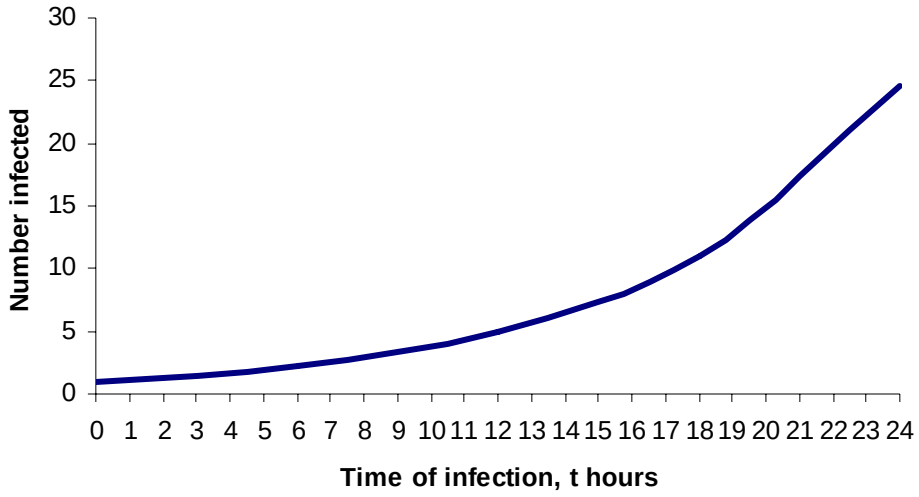


Figure 1. This graph illustrates the number of infected birds predicted between time zero and 24 hours based on the example in Table 4. The exponential rate of growth is evident from this graph.

Before describing the mathematical approach used to model HPAIV transmission within an affected poultry flock, the effect of mortality on detecting a flock prior to its slaughter is explored. The likelihood of a HPAIV-infected bird dying increases as the time it's infected increases; death is the result of extensive pathology and pathology requires time to develop. Mortality fraction (ϕ) is defined as the proportion of the flock dead from HPAIV; like prevalence we can assume the mortality fraction is some monotonically increasing function across time. In fact, it is reasonable to consider $\phi(t)$ as some lagged function of $\pi(t)$ (*i.e.*, $\phi(t) = g(\pi(t-1), \pi(t-2), ..., \pi(t-n))$) because mortality will generally only occur among birds that have been infected prior to t.

For the most part, HPAIV is expected to be detected via a high mortality signal from the flock. If ϕ becomes large enough (ϕ_{crit}), then it is assumed a producer will begin a process that results in the flock being classified as HPAIV-affected. Once detected, a flock will not progress to slaughter and its risk of exposure for human consumers is zero. In other words, if the time of detection $t_{crit} = \phi_{crit}^{-1}(t)$ is less than T, then the flock poses no risk to humans.

Although the simple explanation above may oftentimes apply to highly pathogenic agents such as HPAIV, it is too imprecise to be used for all situations. For example, it is possible that $\pi(t)$ is not always increasing; a typical epidemic curve illustrates that prevalence usually peaks at some level and then decreases as infected individuals either recover or succumb to their infections. Also, if mortality fraction is sufficiently lagged in time, then $\phi(t)$ may be increasing while $\pi(t)$ is decreasing. In fact, in a closed population (*i.e.*, without recruitment of new members) infection will usually peak and decline before mortality achieves its maximum. A more functional approach to modeling transmission is to use a Markov chain model combined with Reed-Frost transmission assumptions. This is the approach actually used in the HPAIV model.

13.1.7 Markov chain model and Reed-Frost transmission equation

A simple state-transition model begins with a description of the various states possible in the flock and the transition probabilities that predict movements between the possible states. One such model with applicability to HPAIV is:

$$S \xrightarrow{\beta S I/N} L \xrightarrow{\alpha L} I \xrightarrow{\gamma I} D$$

where S represents susceptible individuals, L represents latently infected individuals, I represent infectious individuals, and D represents dead individuals. Also,

β = infection rate parameter (number of new infections per infectious individual per unit time)

α = rate of transition from latent to infectious ($1/\text{latency period}$)

γ = mortality rate parameter

This simple model describes all the mutually exclusive states in which birds in a flock can exist. The transition probabilities in this example are fixed for all time periods; except that the probability of moving from susceptible to latently infected per unit time also depends on the number of infectious individuals. This dependency is known as the Reed-Frost assumption in transmission modeling.

Markov chain modeling uses matrix algebra to predict changes across time. The transition matrix is:

FOR ONE TIME STEP		**TO**			
		S	**L**	**I**	**D**
From	**S**	$e^{-\beta I/N}$	$1-e^{-\beta I/N}$	0	0
	L	0	$1-\alpha$	α	0
	I	0	0	$1-\gamma$	γ
	D	0	0	0	1

For one time step, the number of susceptibles, latently infected, etc. are predicted based on the number from the previous time step:

$$\begin{pmatrix} S_{t-1} & L_{t-1} & I_{t-1} & D_{t-1} \end{pmatrix} \times \begin{pmatrix} e^{-\beta I_{t-1}/(N_{t-1}-D_{t-1})} & 1-e^{-\beta I_{t-1}/(N_{t-1}-D_{t-1})} & 0 & 0 \\ 0 & 1-\alpha & \alpha & 0 \\ 0 & 0 & 1-\gamma & \gamma \\ 0 & 0 & 0 & 1 \end{pmatrix} =$$

$$\begin{pmatrix} S_t & L_t & I_t & D_t \end{pmatrix} = \begin{pmatrix} S_t = S_{t-1}(e^{-\beta I_{t-1}/(N_{t-1}-D_{t-1})}) \\ L_t = S_{t-1}(1-e^{-\beta I_{t-1}/(N_{t-1}-D_{t-1})}) + L_{t-1}(1-\alpha) \\ I_t = L_{t-1}\alpha + I_{t-1}(1-\gamma) \\ D_t = I_{t-1}\gamma + D_{t-1} \end{pmatrix}^{\mathrm{T}}$$

This process is repeated for the total number of time steps of infection necessary.

The actual model used for HPAIV is an extension of this simple SLID model. The main extension is the incorporation of infection states that correspond to the stages of infection. As a result of this extension, the state transition probabilities are more complex because, for example, the probability of dying for an infected bird at its 48th hour of infection is different from an infected bird in its 12th hour of infection. Expansion of the dimensions of the matrices also forces a simplifying assumption concerning the transition from latently infected to infectious. An explanation can evolve from a simple example where the model only considers an infection for 24 hours.

We start with the following transition matrix:

	S	**I_1**	**I_2**	**I_3**	**I_4**	**D**
S	$e^{-\beta\frac{I_{t-1}}{N-D_{t-1}}}$	$1-e^{-\beta\frac{I_{t-1}}{N-D_{t-1}}}$	0	0	0	0
I_1	0	0	$1-\gamma_1$	0	0	γ_1
I_2	0	0	0	$1-\gamma_2$	0	γ_2
I_3	0	0	0	0	$1-\gamma_3$	γ_3
I_4	0	0	0	0	0	1
D	0	0	0	0	0	1

The Reed-Frost expression in this matrix depends on the number of infectious birds at time t-1. Instead of modeling a specific state for latently infected birds, the model simply calculates the average number of infectious birds given the numbers infected for the various time periods. For example,

$$I_4 = \begin{pmatrix} I_1 & I_2 & I_3 \end{pmatrix} \times \begin{pmatrix} \alpha_1 \\ \alpha_2 \\ \alpha_3 \end{pmatrix},$$

where the vector of alphas represents the probabilities that an infected bird transitions from latently infected to infectious during a particular time period. The terminology is also somewhat complicated because the expression on the left hand side of the equation represents the number of infectious birds while the first matrix on the right hand side represents number of infected (but not necessarily infectious) birds. This treatment may not be entirely valid in the context of Markov chain models because it allows for the

possibility that a bird could exist in both the infectious and dead states during the same time period. Nevertheless, this approach was necessary to avoid expanding matrices with dimensions of 26 X 26. Also, for most parameter settings in the model, infected birds cannot die until much later stages of infection than the stage of infection when the likelihood of moving from latently infected to infectious is 100%. In these settings, there is no effect from not explicitly including a latent state in the model and the above calculation is exact.

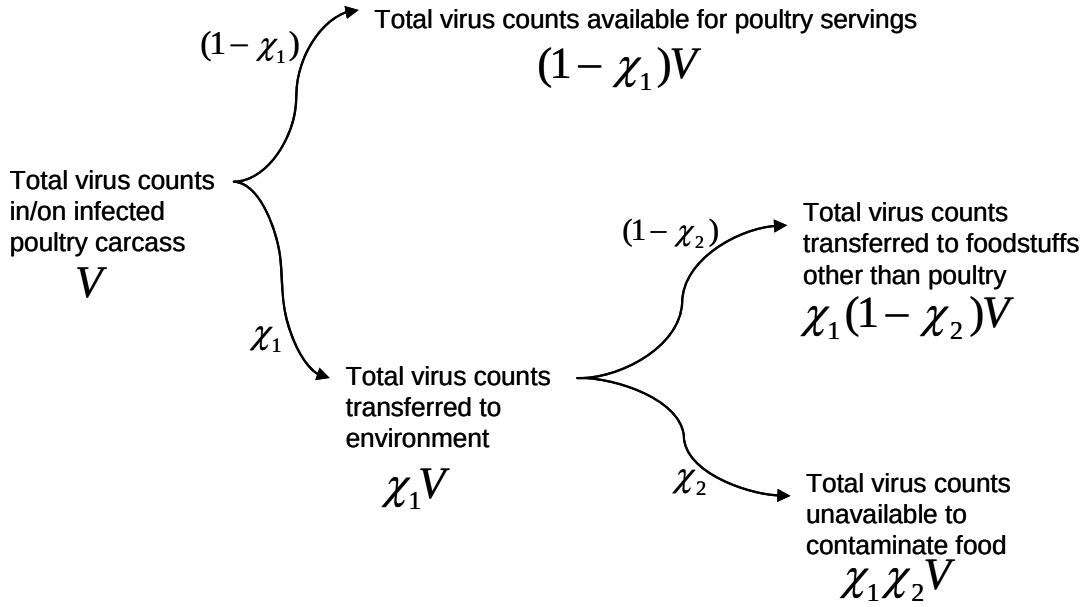
For any flock age when infection is introduced into the flock ($z \times A$), the total time between introduction and slaughter ($T = (1 - z)A$) determines the number of time periods to run the Markov chain model. Because 6 hours time intervals are modeled, there will be $\frac{T}{6}$ Markov chain model iterations. Following each time period's calculations, the mortality fraction is calculated (*i.e.*, $\phi(t) = \frac{D(t)}{N}$) and compared to ϕ_{crit} to determine if the flock's HPAIV infection is detected or not. A non-zero risk of exposure can only occur if the flock remains undetected throughout its productive life and slaughter.

Although the Reed-Frost transmission coefficient is generally considered fixed for a stable population, it can be altered if the environment is changed. Because an affected flock must typically be transported in trucks to a slaughter facility, the transmission dynamics are likely to change during the period of transportation. To account for this change, it is assumed that the 6 hour period just before slaughter has a different β value than for all prior periods.

13.1.8 Incorporation of cross-contamination

As poultry carcasses are processed and handled, some cross-contamination of other foodstuffs can result. Although the pathways to cross-contamination are complex, a simple approach is used to model these phenomena for HPAIV.

Assume that some fraction of virus in/on an infected bird is transferred from the bird to some environmental surface and some fraction of the environmental virus is subsequently transferred into a serving of another foodstuff. In this manner, some of the virus potentially present in servings generated from infected poultry is removed from the poultry serving and transferred to a serving of something else. But, some of the virus removed from the poultry may also be lost in the environment, too. The process can be modeled as;



where V is the total viral count in or on an infected poultry carcass, χ_1 is the fraction of virus transferred from the poultry carcass to the environment and χ_2 is the fraction of environmental viruses that remain (or are destroyed) in the environment and never contact other foodstuffs.

The total viral count per infected carcass can be calculated as $V = \mu \times m \times S$ where μ is the density of virus per gram, m is the mass (in grams) per serving, and S is the number of servings per carcass (previously noted to be, on average, 23). Given this definition of V, the mass balance for viruses on or in an infected poultry carcass is

$$\mu mS = \mu mS\left((1-\chi_1)+\chi_1(1-\chi_2)+\chi_1\chi_2\right)$$

Recognizing that m and S can cancel from both sides of this mass balance equation, the mass balance equation can be written strictly in terms of virus density per gram;

$$\mu = \mu(1-\chi_1)+\mu\left(\chi_1(1-\chi_2)\right)+\mu\chi_1\chi_2$$

In this version of the mass balance equation, the first term on the right hand side is the revised density of virus (per gram) in a poultry serving; the second term is the density of virus per gram in a non-poultry (cross-contaminated) serving; and the third term represents the density of virus lost to the environment through the cross-contamination process. To use this equation in the model, however, it is necessary that the mass per serving and total servings that are assumed applicable to poultry carcasses are also assumed applicable to non-poultry servings. Otherwise, it is possible that virus counts might be inexplicably amplified or reduced in the final tally of risk.

In the simplest model, non-zero doses in poultry servings equaled $\mu \times m \times C$. Incorporating cross-contamination results in at least two doses per serving; $d_1 = \mu \times (1-\chi_1) \times m \times C$ and $d_2 = \mu \times \chi_1 \times (1-\chi_2) \times m \times C$. These doses, however, are not alternative doses. Instead, both doses result from each contaminated carcass and the exposure distribution becomes;

$$f(D) = \begin{cases} 1-\pi\delta\tau, & \text{if D=0} \\ \pi\delta\tau, & \text{if D=}\{d_1 \text{ and } d_2\} \end{cases}$$

Furthermore, the likelihood of illness per serving calculation (Eqn. 2) becomes more complicated because the probability of illness for each non-zero dose must be calculated and summed together to represent the total likelihood of illness. In other words,

$$f(\textit{ill per serving}) = \left[\left(1-e^{-rd_1}\right)+\left(1-e^{-rd_2}\right)\right]\pi\delta\tau$$

Also by using Eqn. 3, the total number of illnesses is predicted (using Eqn. 3) to be:

$$\textit{Total ill} = N \times S \times \left[\left(1-e^{-rd_1}\right)+\left(1-e^{-rd_2}\right)\right]\pi\delta\tau$$

Reorganizing this equation, we get $\textit{Total ill} = NS\left(1-e^{-rd_1}\right)\pi\delta\tau + NS\left(1-e^{-rd_2}\right)\pi\delta\tau$. The first term on the right hand side of the equation is the number of illnesses resulting from contaminated poultry carcasses and the second term is the number of illnesses resulting from servings of non-poultry foodstuffs.

Although cooking effectiveness, C, might be the same for poultry and non-poultry servings, it is more likely that cross-contaminated foods will be cooked differently (*e.g.*, less thoroughly) than poultry. In the simple model, this difference in cooking can be reflected as $d_1 = \mu \times (1-\chi_1) \times m \times C_1$ and $d_2 = \mu \times \chi_1 \times (1-\chi_2) \times m \times C_2$ without further changes.

Finally, the variability in cooking of poultry servings and viral density can be folded into this simple cross-contamination model using the methods described previously. For example, we recognize that virus density is a function of stage of infection. Yet, for any virus density μ_i in an infected poultry carcass, the same mathematics of cross-contamination described in this section will apply.

13.2 Modeling egg contamination

The HPAIV hazard from egg production flocks is constrained to human consumption of eggs containing the HPAIV. It is unlikely that poultry from egg production flocks will be

slaughtered for human consumption. So, the hazard analyzed for meat-type birds is not directly applicable to egg production flocks.

Modeling transmission of HPAIV among egg-producing birds is essentially the same as modeling its transmission among meat-type birds; although the values for some inputs are different. Detection of infection at the flock level, based on unusually high mortality, is also the same for both types of flocks. Nevertheless, the units of production (eggs versus meat) and the type of production (continuous across time for eggs but a fixed point in time for meat) generate differences in modeling HPAIV in egg production flocks relative to meat-type poultry flocks.

13.2.1 Modeling occurrence of egg contamination

Eggs are produced by an egg production flock nearly every day of its productive life. Once such a flock becomes affected with HPAIV, contaminated eggs can be produced by its infected birds as long as those birds remain alive and the flock remains undetected. The cumulative number of contaminated eggs produced by the flock, between the time it became affected and the time it was detected, determines the risk of exposure for humans.

Assume that all affected egg production flocks have natural life-spans that are sufficiently long such that a HPAIV-affected flock will only stop producing eggs because it is detected and ultimately destroyed. Such an assumption is conservative with respect to human health risk because it does not allow an affected flock to go out of production before its infection has run its natural course. So, a flock that otherwise might be scheduled for replacement within 4 days, but becomes infected during that interval, is modeled as continuing its production until it is detected because of high mortality. This is also a simplifying assumption that avoids consideration of molting decisions that apply to a substantial proportion of layer flocks.

For notation, define $\pi(t)$ as the prevalence of infected birds at *t* hours (or *t* 6-hour intervals) following introduction of infection into the flock. Furthermore, define $\pi_i(t)$ as the prevalence of infected birds that have been infected for *i* stages of infection at *t* hours post-infection of the flock. In other words, consider the prevalence of infected birds in a flock at time *t* of its infection as the sum of the prevalence of infected birds that have been infected for discrete periods of time within that flock such that $\pi(t) = \sum_{i \le t} \pi_i(t)$. This definition allows for differential production of contaminated eggs by infected birds as a function of the length of time the birds were infected (or, equivalently, as a function of their stage of infection).

If we assume the average number of eggs produced per bird per day is 0.7 (or $0.7 eggs/24 hrs = 0.175 eggs/6 hrs$)[53] in a flock of size N birds, then the number of eggs produced by HPAIV-infected birds at time *t* of the flock's infection is

$Eggs\ \Pr oduced(t) = N \times 0.175 \times \pi(t) = 0.175N \sum_{i \le t} \pi_i(t)$, where *t* is the number of 6-hour intervals since the flock became infected and *i* indexes the number of 6-hour intervals for stages of infection within which infected birds can exist.

To determine the number of contaminated eggs among these eggs, we consider three options. The first option asserts that all infected birds (regardless of stage of infection) produce contaminated eggs with a fixed probability e (*e.g.*, $e = 0.5$). The second option asserts that the probability of producing a contaminated egg is some (increasing) function of stage of infection. In this case, the probability of tissue infectivity, τ, that was introduced in the poultry meat model is used to predict how egg contamination probability changes with the stage of infection a bird is in. In other words, $\Upsilon(t)$ is some function of stage of infection; it returns a low probability of tissue infectivity and egg contamination in the early stages of infection and a high probability of tissue infectivity and egg contamination in later stages of infection.

The third option asserts that infected birds must pass some stage-of-infection threshold before their eggs are contaminated. In this case, there is some value of stage of infection (i_{crit}) such that the probability of contamination is zero if a bird has not yet reached that stage and the probability of contamination is 100% if a bird is at that stage of infection or beyond. In other words,

$$\Upsilon(i) = \begin{Bmatrix} 0 & i < i_{crit} \\ 1 & i \ge i_{crit} \end{Bmatrix}.$$

For the first option, the total number of contaminated eggs produced before an affected flock is detected is calculated as $TotalContamEggs = 0.175eN \sum_{all\ t} \pi(t)$ because stage of infection does not influence the probability of infected birds producing contaminated eggs.

[53] We note that a constant level of egg production, despite HPAI-infection, is likely conservative because infected birds are expected to suffer reduced production as their illness progresses.

For the second and third options, $TotalContamEggs = 0.175N\sum_{all\ t}\sum_{i=1}^{t}\left(\Upsilon(i)\pi_i(t)\right)$ where $\Upsilon(i)$ is either an increasing function in stage of infection (second option) or a step function that jumps from 0 to 1 at i_{crit} (third option).

Marketing practices can affect the number of contaminated eggs that reach consumers. Once an HPAIV-positive flock is detected, any eggs that have not reached consumers' households can, more or less, be readily recalled by the producer. Therefore, the longer it takes eggs to reach consumers (*i.e.*, the 'holding time'), the fewer contaminated eggs produced by an affected flock that actually are purchased and/or consumed by humans. For example, if a flock is infected on Monday and detected Friday morning, and if it routinely takes 2 days for eggs to reach consumers, then all eggs produced on Wednesday and Thursday are recalled and the only contaminated eggs that could reach consumers are those produced before Wednesday.

We define the hold time as h (*e.g.*, $h = 4$ *or* 8 if the hold time is 24 or 48 hours and we maintain our 6 hour modeling interval). Assume that *t* periods elapse until the flock is detected (*i.e.*, $t \le t_{crit}$). For the first egg contamination option, the number of contaminated eggs that reach consumers is predicted by

$$TotalContamEggs = 0.175eN\sum_{t=0}^{t=t_{crit}-h}\pi(t).$$

For the second and third egg contamination options;

$$TotalContamEggs = 0.175N\sum_{t=0}^{t=t_{cirt}-h}\sum_{i=1}^{i=t_{crit}-h}\left(\Upsilon(i)\pi_i(t)\right)$$

13.2.2 Modeling risk of exposure to HPAIV contaminated eggs

We assume one egg produces one serving for humans and each eggs contains 60 grams of edible material (*i.e.*, $m = 60$). We also assume the density of HPAIV per gram of egg (μ) is constant for all contaminated eggs. The frequency of contaminated eggs is the ratio of the total contaminated eggs produced by the flock to the total eggs produced by the flock during the infection period. Some fraction (δ) of contaminated eggs pass inspection because these eggs do not exhibit overt signs of pathology. Eggs that do not pass inspection do not reach consumers.

If we assume a constant effect of cooking (C) on all exposures, then the exposure distribution for eggs is:

$$f(D) = \left\{ \begin{array}{ll} 1 - \dfrac{TotalContamEggs}{0.175N(t_{crit} - h)}\delta, & \text{if D=0} \\ \dfrac{TotalContamEggs}{0.175N(t_{crit} - h)}\delta, & \text{if D=}\mu \times m \times C \end{array} \right\}.$$

The probability of illness per egg serving is;

$$f(ill\ per\ serving) = \sum_{D} \left(1 - e^{-rD}\right) f(D).$$

The predicted number of total illnesses resulting from contaminated eggs is;

$$Total\ Ill = 0.175 \times N \times (t_{crit} - h) \times f(ill\ per\ serving).$$

These last two equations demonstrate the primary difference between modeling egg contamination and meat contamination. One infected poultry carcass produces a fixed number of potentially contaminated meat servings (*e.g.*, 23 servings per carcass). One infected layer hen produces a variable number of potentially contaminated servings that depends on the number of days the flock remains undetected. Ignoring the effects of stage of infection on contamination levels or frequency, however, these differences suggest that an egg production flock would typically need to remain infected longer than a broiler flock to generate the same number of contaminated servings. Nevertheless, the actual doses per serving for meat or eggs are not necessarily equal.

The preceding equations ignore the effect of variability in cooking effectiveness, as well as the effect of on-going mortality within affected flocks. Nevertheless, these equations reflect the general approach to modeling risk to humans from contaminated egg exposures. Integrating variable cooking effectiveness into the egg model is accomplished exactly like the approach used in the poultry meat model. The Markov Chain transmission model enables predictive calculations for the number of birds remaining in the flock across time; similarly the number of infected birds by stage of infection is an output of that model.

14 Appendix D

14.1 Review of Selected Epidemiological Studies

A literature review of published epidemiological studies reporting information on human infection rates and exposure routes was conducted. A total of 12 studies were identified and are summarized in Table 1. Studies were selected if they contained information useful for (a) estimating HPAIV human infection rates and/or (b) information on probable exposure scenarios. Analysis of the data from these studies highlights the importance of understanding the uncertainties associated with the estimated outcomes and conclusions.

The available data suggest that human cases of HPAIV are likely due to respiratory mucosal exposure due to inhalation of infective droplets or self-inoculation (touching mucous membranes, conjunctiva) via avian fecal contamination, avian respiratory secretions, or avian body fluids. The primary human health concern of infection with HPAIV is related to, and epidemiologically supported by, occupational exposure of poultry industry workers (Bridges *et al*., 2002, Mounts *et al*., 1999, Koopmans *et al*., 2004), and even under these conditions the risk of infection and illness appears low.

Precise exposure histories for persons infected with the Asian strain of HPAIV H5N1 and others in the affected communities are uncertain. The questionnaires used and the potential for interviewer bias are largely unknown. Detailed questions about potential foodborne exposures generally were not asked (especially if another more likely source of exposure was mentioned) and variable amounts of time elapsed between possible exposures and interviews. Therefore, the likelihood that foodborne exposures have led to infection and disease under natural conditions is difficult to ascertain.

Currently, there is no solid epidemiological evidence linking the consumption of raw or undercooked poultry, shell eggs, or egg products to human illness from AI. However, the WHO has stated that more than 25% of reported human cases have an unknown source of exposure (Abdel-Ghafar, et al., 2008) and it is possible that at least some of those cases were exposed through contaminated food even if it seems more likely that some other unreported or unrecognized more biologically plausible route of exposure led to disease. Conversely, the presence of a positive history of some other route of exposure does not rule out food as a potential vehicle, as cases exposed to sick and dead birds almost certainly ate eggs and meat from infected poultry as well. Two cases in Asia have suggested a possible link of infection to the consumption of raw duck blood, though contact with live or dead HPAIV-infected poultry could not be epidemiologically excluded (EFSA, 2006). In addition, naturally occurring and experimental oral exposure resulting in AI infection of other species, such as poultry and felines, have been well

documented (Rimmelzwaan *et al*., 2001; Keawcharoen *et al*., 2004; Kuiken *et al*., 2004; Swayne and Beck, 2005; Songserm *et al*., 2006). This demonstrates that infection through consumption of AI occurs; although extrapolating this information to humans remains problematic.

Seroprevalence studies of occupationally exposed, general populations in endemic areas, and history of diagnosed human infections with both LPAIV and HPAIV indicate that large (but generally unquantified) populations have been exposed to high levels of HPAIV virus (completely unquantified) with few illnesses and seroconversions/seropositive results. These studies are summarized below:

- Bridges *et al*., (2002) found that in a cohort study among 293 government workers who participated in culling exercises, only 1 seroconversion and 3% were seropositive. However, as only 78% of subjects had paired serum samples there is uncertainty as to the exact prevalence rates. Among 1,312 poultry workers, 10% were estimated as seropositive for HPAIV after the avian epidemic was controlled.
- In 2006, a serosurvey was conducted in Nigeria among persons who worked on poultry farms or markets that had suspected or laboratory confirmed HPAIV H5N1 outbreaks among poultry. Of 295 workers tested, none were seropositive (Ortiz J, *et al*. 2007).
- A serosurvey was conducted among 901 residents of 4 Thai villages that had known human cases of HPAIV H5N1 and HPAIV H5N1 among backyard poultry flocks during 2004-2005. One-third of the participants reported contact with sick or dead chickens, yet none had antibody evidence of HPAIV H5N1 infection (Dejpichai R, *et al*. 2007).
- Puzelli *et al*., (2005) did not find any serum samples positive for HPAIV H7N1 neutralizing antibodies among 983 poultry workers during a protracted avian epidemic from 1999-2003, although 7 samples were found positive for LPAIV H7N3.
- Vong *et al*., (2006) collected 351 serum samples in a rural Cambodian area surrounding a known human H5N1 infection and poultry outbreaks. None of the samples were positive for H5N1 antibodies.

In the reviewed studies, the methodology for assessing the risk factors for H5N1 infection employed self- or proxy-reporting (in the event of deceased case subjects) to retrieve information about probable exposure of case patients. Questionnaires, which in certain cases are pre-tested and/or structured, are widely employed in epidemiological studies. However, their use comprises a major source of potential bias towards finding statistical associations with specific risk factors depending on external factors and influences, *e.g.*,, media coverage, education level of participants, time that elapsed between actual facts and interviews. For example, in the majority of the studies, the format of questionnaires is not included in the published article; therefore, one would need to make assumptions regarding the specific risk factors that may have been assessed either directly or indirectly (see Tran Tinh Hien *et al*., 2004, Thorson *et al*., 2006). Particularly regarding

consumption, one would need to assess whether the gastrointestinal route of HPAIV transmission was considered as a risk factor at the time the study was performed. If this was not the case, it is probable that relevant questions were not included in the questionnaires (Dinh *et al.*, 2007). Further uncertainties that may be involved in the assessment of possible HPAIV risk factors refer to the particular methodologies employed for statistical analyses of the results. Although most studies comment on the probable bias carried by the usually small sampling size (*i.e.*, case studies) very few comment on the rationale and applicability of statistical tools employed and assumptions made (for example see Mounts *et al*., 2005 and Dinh *et al*., 2007). The footnotes following Table 1 provides a further analysis of the uncertainties related to these studies, which should be taken into consideration in drawing conclusions about the risk factors associated with HPAIV virus infection in humans.

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Table 1. Summary of Selected Epidemiological Studies.

Reference	HPAI Subtype	Confirmed Cases	Primary Contact Infection Rate	Secondary Contact Infection Rate	Mortality Rate	Exposure Pathway Comments
Koopmans *et al*., 2004	H7N7	89	82/453 (18.1%) or 82/4500 (1.8%)	3/87 (3.4%)	1/89 (1.1%)	Cullers and veterinarians had the highest infection rates (2.8% and 2.9%, respectively). Appeared that the main exposure pathway concerned handling infected birds, poultry farmers did not have relatively high rates of infection (1.1%)
Fielding *et al*., 2005	n/a	n/a	4,220,738 exposed	n/a	n/a	Physical contact with purchased live chicken
Thorson *et al*., 2006	n/a	n/a	650/45,478 (=1.43%) 750/45,478 (=1.6%)	n/a	n/a	Physical contact with sick or dead poultry or having sick or dead poultry in the house
Bridges *et al*., 2002	H5N1	n/a	81/1,312 (6.2%)	0	0	Several occupational related variables were associated statistically with H5 seropositivity (Stratified analysis that controlled for age group)
	H5N1	n/a	9/293 (3.1%)	0	0	Smoking was recognized as a statistically significant risk factor.
Mounts et *al*., 1999	H5N1	18	14/15 (93%) or	1/13 (7.6%)	6/18 (33%)	Exposure to the poultry in the

Reference	HPAI Subtype	Confirmed Cases	Primary Contact Infection Rate	Secondary Contact Infection Rate	Mortality Rate	Exposure Pathway Comments
			15/15 (100%)			market was assessed as statistically significant.
Tran Tinh Hien *et al*., 2004	H5N1	10	8/10 (80%) up to 10/10 (100%)	Possible; 1/10 (10%) or 2/10 (50%)	8/10 (80%)	Authors did not perform a statistical assessment of probable exposure routes; epidemiological links related to direct contact with poultry were listed as probable.
Puzelli *et al*., 2005	H7N3; H7N1	7	7/983 (0.71%)	Not assessed	0	Authors did not perform a statistical assessment of probable exposure routes; epidemiological links related to direct contact with poultry were listed as probable.
Dinh *et al*., 2007	H5N1	28	Could not be estimated	Could not be estimated	21/28 (75%)	Statistically significant associates were found for a number of assessed risk factors.
Buchy *et al*., 2007	H5N1	6	Possibly 5/6 (83%)	Possibly 1/6 (17%)	6/6 (100%)	Authors did not perform a statistical assessment of probable exposure routes; epidemiological links related to direct contact with poultry were listed as probable.
Sedyaningsih *et al*, 2007	H5N1	54	Could not be estimated	Possibly 2/22 (9.1%)	41/54 (76%)	The source of viral infection was identified through interviews with proxies and/or

Reference	HPAI Subtype	Confirmed Cases	Primary Contact Infection Rate	Secondary Contact Infection Rate	Mortality Rate	Exposure Pathway Comments
						medical records. 76% of case patients had direct or indirect contact with poultry; no identifiable exposure route was attributed to the rest.
Vong *et al*., 2006	H5N1	0	n/a	n/a	n/a	Possible poultry exposure was assessed by means of structured questionnaires scanning a period of 12 months before the confirmed H5N1 case in the area.
Ortiz *et al*., 2007	H5N1	0	n/a	n/a	n/a	

n/a = not applicable

Additional Notes:

1) **Koopmans *et al*., 2004**: There are a number of unquantified uncertainties related to the estimates: uncertainties relevant to estimated number of population at risk: *e.g.*, register comprised only people who complained of ill health within the population at risk; also, there is no detailed information in the article on how they estimated the expected number of different groups of population at risk; Uncertainties relevant to communication with subjects: *e.g.*,,, the study employed questionnaires enquiring probable exposure routes - subjective perceptions / recall rates; Laboratory methodology related uncertainties, *e.g.*, cross contamination of samples. The population at risk was defined as "Group of people living or working in the Netherlands after February 28 2003 who had direct contact with poultry or poultry products that could have been infected with H7 (primary contact), or who had a close contact with an H7 infected person (secondary contact)" (n>3410; ~4500). Although 89 HPAIV cases were confirmed in total, 4 were not included in further analysis, 2 did not fit any case definition and 2 did not send a trawling questionnaire. A total of 453 people registered to have health complaints, completed questionnaires and were tested for influenza. It was stated in the report that 87 secondary cases were tested.
2) **Fielding *et al*., 2005**: A number of unquantified uncertainties maybe related to the estimates: Uncertainties relevant to subjective interpretation of wording, *e.g.*, in reporting of buying a chicken point estimates were introduced, *e.g.*, "few times a year" was translated as 4 bought live chickens / year; Uncertainties relevant to reporting all household incidences of physical

contact with bought live chicken; Assumption that only a physical contact with live chickens carries a risk of exposure to HPAIV: it is not clear whether questionnaires (*i.e.*, section 2) included questions regarding consuming and/or home cooking practices. The population of risk was defined as the number of house holds in Hong Kong (n=2,052,890).

3) **Thorson *et al.*, 2006**: This study is unique among the ones listed above; it is the only population-based study. Nevertheless, a number of unquantified uncertainties maybe related to the estimates of incidence rates of "flulike" disease: Uncertainties relevant to communication with subjects during the surveys: it is stated that consistency was greater than 90%, but precise number is not given; Uncertainties relevant to degree of relevance of "flulike" symptoms to HPAIV infection: no serological tests were performed; Uncertainties relevant to assessment of probable exposure to avian influenza: a pre-tested questionnaire was employed; it is not clear from the text what is meant by "direct or indirect contact with well, sick or dead poultry", *e.g.*, whether cooking or consuming was taken into account; other probable exposure routes were not assessed or investigated; Uncertainties relevant to actual number of infected cases; any infected, albeit asymptomatic cases were not concerned. The population at risk is defined as the number of households in FilaBavi, Vietnam (n=11,942) with certain number of inhabitants (n=45,478).
4) **Bridges *et al.*, 2002**: A number of unquantified uncertainties maybe related to the estimates: Uncertainties relevant to possible patterns in selection of samples for performing full set of laboratory testing: methodology employed to randomize samples is not mentioned; Uncertainties relevant to probable timing of infection, *e.g.*, a single serum sample was tested and time of infection is thus difficult to be assessed; this in retrospect may bring more uncertainties regarding probable exposure causal links. The population at risk is defined as the number of poultry workers (PW) in Hong Kong – actual number unknown; sample size in study n=1525). Only 1312 of the 1525 poultry workers who were originally tested were included in the nested case-control analysis. Although a 10% rate was reported; this reported number is based on a series of steps of statistical analysis and only approximately 50% of the samples were tested. Working in retail poultry vs. working in wholesale or a poultry hatchery or farm; >10% mortality among poultry, although it is not clear as to how precise this number could be (*i.e.*, estimates were based on a survey question, *e.g.*, whether >10% mortality was observed among poultry since a certain date); Touching poultry; Butchering poultry; Feeding poultry; Preparing poultry for restaurants, although this activity is not clarified further than generally including butchering chickens.
5) **Bridges *et al.*, 2002**: A number of unquantified uncertainties may be related to the estimates: Uncertainties relevant to laboratory serum tests, *e.g.*, only 78% of subjects had paired serum samples therefore prevalence rates could have been higher. The population at risk is defined as "the number of governmental workers who participated in a particular poultry culling operation" (GW; n=293). Only 78% of subjects had paired serum samples, therefore prevalence rates could have been higher. The one person who seroconverted had an upper respiratory illness, but unfortunately did not have a specimen collected for virologic testing.

6) **Mounts *et al*., 1999**: A number of unquantified uncertainties maybe related to the estimates: Uncertainties relevant to exact timing of exposure events in relation to the onset of illness, *e.g.*, case studies were interviewed by proxy significantly more often (p=0.003); information on probable exposure of case subjects concerned the "week before onset of illness", but it is not clearly stated how onset of illness was defined; Uncertainties relevant to possible correlations of the assessed variables: it is not clear from the text whether an assumption that assessed variables are independent was taken into account. Nevertheless, independency among variables may be difficult to prove, and underlying associations may exist; Number of reported controls: we can estimate 34 (relevant text in p.505-506); instead, a total number of 41 were reported. 18 cases were reported in total; three people with culture confirmed H5N1 illness were excluded from further analysis. The population at risk is unknown. The text that describes the matched analysis (see p. 506) implies that all case studies (n=15) were included in all statistical analyses shown, so it is unclear why only 13 are cited; further, cohort studies conducted to assess the risk of human to human transmission of the virus did not provided any evidence in favor of such an event (Katz *et al*., 1999). The odds ratio was estimated based on a different number of controls for each variable (please, see also p. 506). There were two more statistically significant associations reported. One referred to cleaning procedures in the household; case patients were reported to originate from households that used soap to clean less frequently. Whether this habit could be associated further with HPAIV exposure pathways other than physical contact with live poultry remains uncertain. Although, there has not been detected any statistically significant difference between case patients and controls, the significance of certain consumers' habits may carry underlying associations with other exposure routes. The other statistically significant association found was a negative one between case subjects and playing in an indoor playground. Exposure activities were recalled for "the week before onset of illness" and it is uncertain whether case subjects were already feeling poorly, therefore being less able to resume usual activities.
7) **Tran Tinh Hien *et al*., 2004**: A number of unquantified uncertainties maybe related to the conclusions: epidemiological data were collected through interviews of the relatives in the majority of the cases, and it is not clear whether structured questionnaires were employed, and/or whether questions addressing the possibility of consuming poultry were assessed. The population at risk is unknown.
8) **Puzelli *et al*., 2005**: A number of unquantified uncertainties maybe related to conclusions and estimates: Uncertainties relevant to exposure routes: data on epidemiological links were collected by questionnaires, but the format of these is not included, and the risk factors assessed are not listed; Uncertainties relevant to timing of sampling: serum samples were collected after a number of days after the onset of epizootic events., therefore the number of seropositives may have been underestimated. The article refers to both LPAIV H7N3 and HPAIV H7N1. The population at risk is defined as the number of poultry workers (PW) in Italy – actual number unknown; sample size in study n=983.
9) **Dinh *et al*., 2007**: A number of unquantified uncertainties maybe involved with the estimates: Uncertainties relevant to assessment of probable exposure to avian influenza: a structured questionnaire was employed; not all possible exposure routes

were assessed or investigated, *e.g.*, questions did not address consumption of sick or dead birds; Uncertainties relevant to statistical analyses performed: methodologies employed did provoke a variable response from the scientific community (Lukrafka *et al*., 2007; Horby, 2007). The population at risk is unknown. The level of education (*e.g.*, high school, college or university education); preparing and cooking sick or dead poultry; sick or dead poultry in household; no indoor water source in household.

10) **Buchy *et al*., 2007**: The population at risk is unknown.
11) **Sedyaningsih *et al*., 2007**: The population at risk is unknown.
12) **Vong *et al*., 2006**: The investigation followed a confirmed case in a Cambodian village and its scope was to assess the frequency of poultry to human H5N1 transmission. Certain uncertainties related to that the interviews involved a recall period of 12 months and could not document more precise timings of probable poultry exposure may have influenced these findings.
13) **Ortiz *et al*., 2007**: The investigation followed poultry outbreaks of HPAIV (H5N1) in Nigeria and its scope was to assess the frequency of poultry to human H5N1 transmission. Certain uncertainties related to that (a) confirmed H5N1 virus infection of poultry at the sites enrolled in the study was quite limited (9 farms out of 115 that met suspect H5N1 case definition were tested; 6 of the 9 tested farms were positive for H5N1); (b) the possibility that participants may have been tested before they could mount a detectable H5N1 neutralizing antibody response (in particular referring to the 5 hospitalized workers).

15 Appendix E

15.1 Introduction

To assist APHIS in their HPAIV emergency response plan, FSIS used the HPAIV eggs risk assessment model to address the following question: "Given the enhanced surveillance and testing of daily mortality by PCR as proposed by the industry, how much earlier would disease be detected in the flock and how much would that reduce the number of contaminated eggs produced."

15.2 Approach

To assess an enhanced surveillance program within an HPAI control zone, the transmission portion of the HPAIV model was used. This portion of the model shows the number of susceptible, infected, and dead birds every 6-hours post-initial infection.

For this approach, it was assumed that the number of dead birds is monitored on a daily basis. Therefore, a count of deceased birds by poultry managers was assumed to be taken every 24 hours following initial infection.

Under the enhanced surveillance program, 5 birds would be randomly selected from this daily mortality. These 5 dead birds would then be tested for HPAIV using the RRT-PCR testing method. In order to determine how soon an infected flock would be detected under these conditions, we needed to determine the following: 1) the probability of selecting infected birds out of the total daily mortality, and 2) the probability of detecting an infected sample using the test. The summation of the probabilities products resulted in the overall probability of detecting an infected flock.

15.3 Method

15.3.1 Probability of Selecting Infected Birds

To determine the probability of selecting infected chickens, a hypergeometric distribution was used. A hypergeometric distribution returns the probability of a given number of sample successes, given the sample size, population successes, and population size. In this case, we are determining the probability that x number of birds of the 5 selected will actually be infected. The hypergeometric distribution is defined by 4 input variables: 1) The sample successes, which was equal to the number of selected birds who were actually infected (e.g., 1, 2, 3, 4, or 5), 2) The

sample size, which was always 5, because this is the total number of randomly selected dead birds, 3) The population successes would be the total daily dead birds from HPAIV. This number changes over time, and 4) The population size, which would be the daily dead from HPAIV plus the normal daily mortality. Industry estimates a normal daily morality to range from 5-40 (APHIS, personal communication). In order to provide a conservative scenario, we used 40.

15.3.2 Probability of Detecting an Infected Bird Using RRT-PCR

This determines the probability of at least 1 true positive RRT-PCR result out of x positive samples. RRT-PCR is conservatively assumed have a sensitivity of 88.2% (APHIS, personal communication). This sensitivity reflects the probability of a positive test result given that the test is applied to an infected bird. Further assuming that each test result is independent between infected birds, we can use the following equation to determine the probability of at least one positive test result among x infected birds:

$$P(\geq 1 \text{ positive result}) = 1 - (1 - 0.882)^{x}, \text{ where x is 0,1,2,3,4, or 5.}$$

15.3.3 Probability of a Flock Being Detected Over Time

To determine the probability of an infected flock being detected at Day x, the following was done. First, we determine the average probability of *flock detection* at every 6-hour period (though we were only primarily interested in 24 hour intervals given that poultry managers would probably not check a house more frequently). This probability was obtained by summing the products of 1) the probability of selecting a specific number of infected birds (i.e., 0 to 5 infected birds per sample) from the total daily mortality (HPAIV dead birds + dead birds) and 2) the probability of actually detecting one or more positive samples using RRT-PCR.

The next step was to determine the fraction of an infected that *will be detected* during each day of infection. We chose the probability of detection for hours 19, 43, 67, 91, 115, and 139 and equated them to Day 1, 2, 3, 4, 5, and 6[54]. Therefore, we determine the fraction of all infected flocks that will be detected on Day 1, 2, …, 6 of infection. The following probability tree is helpful in understanding the conceptual approach (Figure 1).

[54] Other hours can be evaluated.

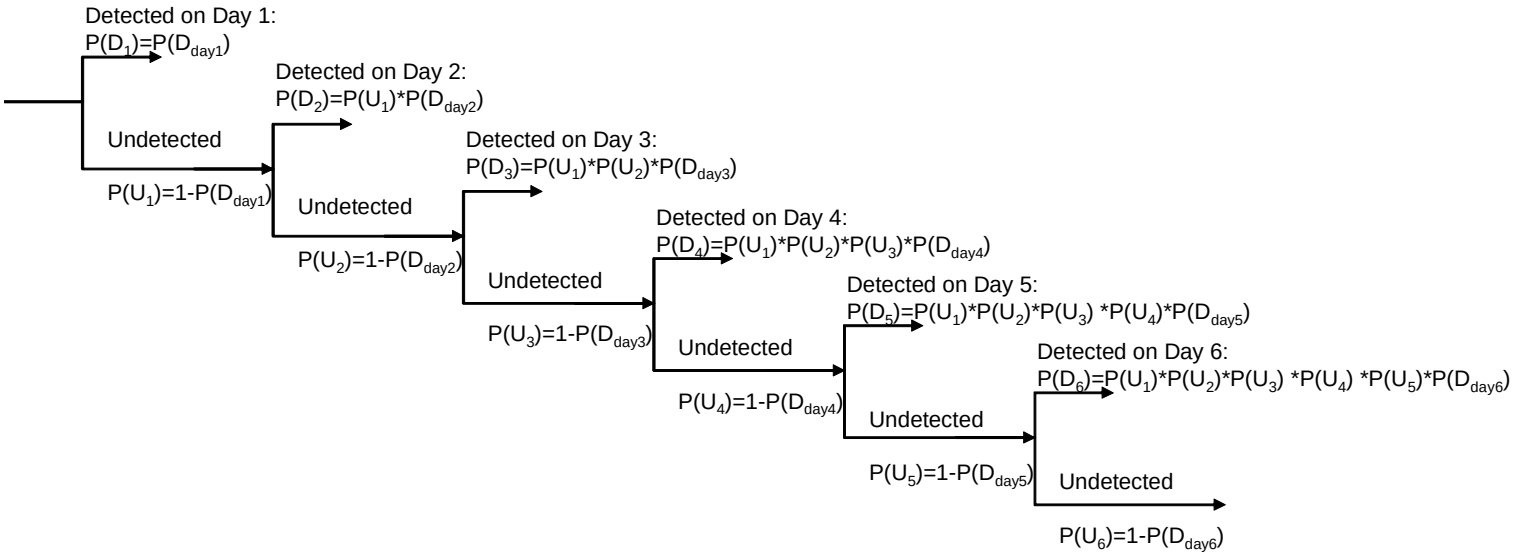


Figure 1. Probability of a *flock being detected* given daily sampling of all flocks using the enhanced sampling program.

Daily mortality from HPAIV increases rapidly during the first 6 days of infection. Elevated mortality increases the daily probability of detection; so the daily detection probability from mortality sampling is variable and increasing with days of infection. Once a flock is detected via mortality sampling, however, it is not eligible for detection at a later time. Furthermore, detection of an infected flock on a given day depends on the probability that it remains undetected through previous days and the daily probability of detection for that day.

In the probability tree, the daily detection probability (that increases with *i* days of infection) is represented as $P(D_{day\ i})$. The probability that a flock is actually detected on a given day of infection is represented as $P(D_i)$. It is $P(D_i)$ that we wish to calculate because it represents the fraction of all infected flocks that will be detected on day *i* of infection.

For example, to determine probability of detection at day 3, $P(D_3)$, the product of remaining undetected at day 2, $P(U_2)$ and day 1, $P(U_1)$ is multiplied by the daily probability of detection, $P(D_{day3})$. By day 6 of infection, mortality from HPAIV is so larger that detection of the flock is inevitable; so we assume $P(D_{day6}) = 1$.

15.3.4 Determining the Risk of Infected Eggs Being Released

To determine the average risk of contaminated eggs being released by an infected flock, the following equation was used:

$$Number\ of\ contaminated\ eggs\ released = \sum_{i=1}^{i=6} \left(P(D_i) \times N_{1 \to i} \right)$$

where $N_{1\to i}$ is the cumulative total number of contaminated eggs produced by the infected from its first day of infection through the day it is detected. This risk calculation is essentially a weighted average of the possible total number of contaminated eggs produced by the flock until it is detected; the weights are the fraction of infected flocks that would be detected on each day of infection.

A more relevant measure is the risk of contaminated eggs within a tanker of liquid egg. Each tanker is assumed to contain just one day's production of eggs; so the number of contaminated eggs included in such a tanker only reflects those eggs produced during the day the flock was detected. The following equation is used to determine this risk;

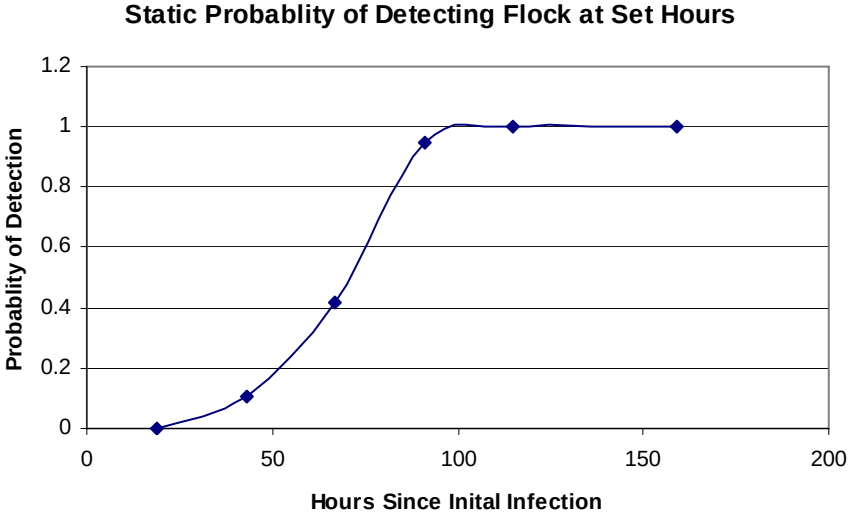
$$Number\ of\ \ contaminated\ \ eggs\ within\ a\ tanker\ \ = \sum_{i=1}^{i=6} \left(P(D_i) \times N_i \right)$$

where N_i is the number of contaminated eggs produced on day *i*.

This equation was performed for every 24 hour period. The daily detection probability of being detected over time was discussed above. The total number of contaminated eggs produced by an infected flock were obtained from the model (see EggContamination worksheet rows 51-64 column E). The numbers of contaminated eggs for four 6-hour periods were summed to give the number of contaminated eggs/day.

15.4 Results

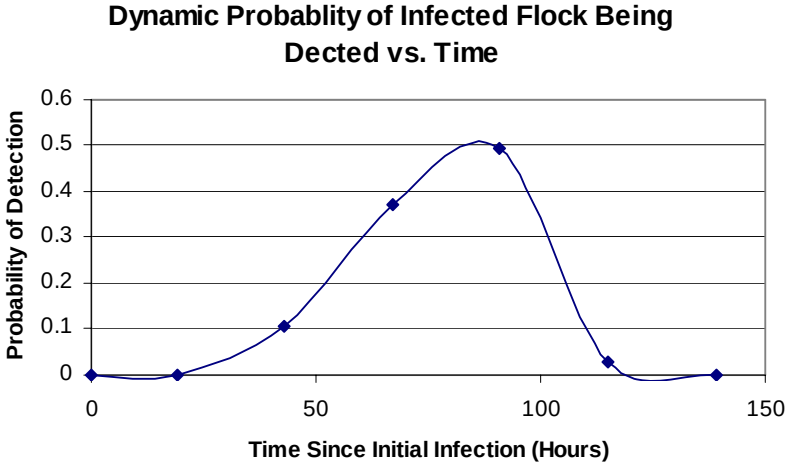
To answer the first part of the question, "how much earlier would disease be detected in the flock," the methodology described in section "Probability of a Flock Being Detected Over Time" was used. First, the probability of *flock detection* was determined (Figure 2). In Figure 1, this represents $P(D_{day1\ldots n})$. Therefore, the probability of *flock detection* based on a sample of 5 dead birds and the RRT-PRC sensitivity = 0% for Day 1 (19 hrs), 10.8% for Day 2 (43 hrs), etc. (Table of Figure 2). Figure 2 represents the static probability of flock detection at set hours.



Probability of Detecting Flock Infection at Set Hrs	
Hours Since Initial Infection	Detection Probability
0	0.0
19	0.0
43	0.107560976
67	0.416156013
91	0.947071061
115	0.999474856
159	0.999956772

Second, the probability of a *flock being detected* was determined (Figure 3). Therefore, the probability of flock being detected on any given day is based on the probability of it not being detected on previous days. $P(D_{1...n})$ = 0% for Day 1 (19 hrs), 10.8% for Day 2 (43 hrs), etc. (Table of Figure 3).

Figure 3 represents the dynamic probability of an inflected flock being detected versus time.



Dynamic Probability of Inflected Flock Being Detected vs. Time	
Time Since Initial Infection	Detection Probability
0	0.0
19	0.0
43	0.10756098
67	0.37139387
91	0.49346679
115	0.02756388
139	1.4482E-05

Given Figure 3, the highest probability of a flock being detected is 91 hours. This is 3.8 days, indicating that the flock would be identified 2 days earlier than relying on mortality alone (recall that using 2% mortality as a cut-off for when a flock would be held and detected as HPAIV-positive, the model predicts that it requires 6 days).

To answer the second part of the question, "and how much would that reduce the number of contaminated eggs produced," we estimate the probability of flocks being detected as discussed above and apply the methodology of section "Determining the Risk of Infected Eggs Being Released". The probability for each day of a flock being detected was determined (Figure 3). This was multiplied by the maximum number of eggs laid each 24 hr period (see EggContamination worksheet rows 51-64 column H, I, J). These products were then summed to give the average number of HPAIV-positive eggs from a single flock given the enhanced sampling plan (Table 1). Table 2 indicates if no sampling was performed and only >2% mortality was used to determine when a flock was HPAIV-positive.

Table 1.

Time (hrs)	P(detected) given enhanced sampling	HPAIV+ eggs laid/day	Column 2 x Column 3
139	0.00001	9,431	0
115	0.02756	1,602	44

91	0.49347	224	111
67	0.37139	31	11
43	0.10756	4	0
19	0.00000	1	0
	Sum = 1	Sum = 11293	Sum = 167

Table 2.

Time (hrs)	P(detected) given no sampling	HPAIV+ eggs laid/day	Column 2 x Column 3
139	1.00000	9,431	9431
115	0.00000	1,602	0
91	0.00000	224	0
67	0.00000	31	0
43	0.00000	4	0
19	0.00000	1	0
	1	11293	9431

Therefore, an HPAIV-infected flock would be limited to167 HPAIV-positive eggs on average using the enhanced surveillance compared to 9,431 HPAIV-positive eggs on average from 2% mortality detection alone. Note, that it is assumed that eggs laid on the day of detection are incorporated in the exposure estimate.