Risk Assessment to Estimate the Probability of a Chicken Flock Infected with H5N1 Highly Pathogenic Avian Influenza Virus Reaching Slaughter Undetected*

Neal J. Golden, Wayne D. Schlosser, and Eric D. Ebel

Abstract

Highly pathogenic avian influenza (HPAI) H5N1 is an infectious disease of fowl that can cause rapid and pervasive mortality resulting in complete flock loss. It has also been shown to cause death in humans. Although H5N1 HPAI virus (HPAIV) has not been identified in the United States, there are concerns about whether an infected flock could remain undetected long enough to pose a risk to consumers. This paper considers exposure from an Asian lineage H5N1 HPAIV–infected chicken flock given that no other flocks have been identified as H5N1 HPAIV positive (the index flock). A state-transition model is used to evaluate the probability of an infected flock remaining undetected until slaughter. This model describes three possible states within the flock: susceptible, infected, and dead, and the transition probabilities that predict movements between the possible states. Assuming a 20,000-bird house with 1 bird initially infected, the probability that an H5N1 HPAIV–infected flock would be detected before slaughter is approximately 94%. This is because H5N1 HPAIV spreads rapidly through a flock, and bird mortality quickly reaches high levels. It is assumed that approximately 2% or greater bird mortality due to H5N1 HPAIV would result in on-farm identification of the flock as infected. The only infected flock likely to reach slaughter undetected is one that was infected within approximately 3.5 days of shipment. In this situation, there is not enough time for high mortality to present. These results suggest that the probability of an infected undetected flock going to slaughter is low, yet such an event could occur if a flock is infected at the most opportune time.

Introduction

Avian influenza viruses are typically species-specific; however, H5N1 and other highly pathogenic avian influenza virus (HPAIV) subtypes have emerged as an important zoonotic concern. The World Health Organization reported 387 confirmed H5N1 HPAIV human illnesses worldwide from 2003 to 2008, resulting in 243 deaths (WHO, 2008). Retrospective studies suggest the majority of these cases were associated with close contact with live or dead H5N1 HPAIV–infected birds (Beigel et al., 2005; Lye et al., 2007; Feiris et al., 2007; Sedyaningish et al., 2007). Because of these recent outbreaks of H5N1 HPAIV, which were associated with poultry in Asia, Africa, Europe, and the Middle East, there is heightened public concern regarding the safety of poultry consumption.

There is no direct epidemiologic evidence supporting foodborne transmission of H5N1 HPAIV from contaminated poultry or eggs to humans. Retrospective studies have determined that the majority of known human 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. However, two confirmed cases of H5N1 HPAIV human illness have been linked to ingestion of raw duck blood (EFSA, 2006). H5N1 HPAIV–infected poultry have been shown to disseminate the virus to the muscle and internal egg contents (Beard et al., 1984; Bean et al., 1985; Tumpey et al., 2002; Tumpey et al., 2003; Mase et al., 2005; Swayne and Beck, 2005; Swayne, 2006; Thomas and Swayne, 2007), suggesting that exposure to H5N1 HPAIV through contaminated poultry and eggs is possible. Further, animal studies have indicated consumption of H5N1 HPAIV–contaminated poultry as a viable
exposure pathway (Kuijken et al., 2004; Rimmelzwaan et al., 2006; Lipatov et al., 2008). To examine the possibility of H5N1 HPAIV in the food supply, this study assesses the influence of Asian lineage H5N1 HPAIV—transmission dynamics within a broiler chicken house on the probability that an infected, yet undetected flock, is sent to slaughter. A within-flock transmission model was constructed to represent a hypothetical index flock in the United States. The state-transition model estimates the following outputs: (1) the length of time until the infected flock is detected, and (2) exposure, as determined by the amount of virus presented to a slaughter establishment. Although this study does not directly examine the public health consequences of H5N1 HPAIV in a commercial chicken flock, the exposure output is an important component of risk assessment to estimate H5N1 HPAIV—caused human illnesses from consumption of chicken meat.

Materials and Methods

A transmission model, based on Markov Chain principles and programmed in Excel 2003 (with Visual Basic for Applications), was developed to simulate the spread of disease within the flock once a single bird is infected. This transmission model uses state-transition probabilities to simulate the number of birds within a flock in each of three general states at any given time, that is, susceptible, infected, or dead (Fig. 1). Susceptible birds are those birds that have not been exposed to H5N1 HPAIV. Susceptibility is assumed equal for all birds. Infected birds are those birds that are H5N1 HPAIV infected; some infected birds are capable of spreading infection (i.e., infectious). Although not explicitly modeled, there is an implied latency effect included in the model to account for infected birds in the early states of infection that do not shed virus. Finally, dead birds are assumed to have died from H5N1 HPAIV.

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 the status of the 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, effective contact rate, latency, bird mortality rate, flock size, when the first infection occurs, the duration of the grow-out, and daily mortality threshold. Model parameters and baseline inputs are described in Table 1.

To estimate the probability of an H5N1 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. To begin the simulation, a single bird is assumed to be infected with H5N1 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. The model utilizes primarily the data from Das et al. (2008).

Markov chain model

To simplify this analysis, the H5N1 HPAIV—infected broiler flock was assumed to begin with a 20,000 bird house (J. Starkey, personal communication) scheduled for slaughter at 8-weeks of age (both assumptions can be varied to explore the impact of house size and grow-out duration). Further, given the available evidence, it was assumed that this flock was not infected before 6 weeks of age. Such an assumption is supported by preliminary modeling that demonstrated that any flock infected earlier than its sixth week of production would be detected as infected with H5N1 HPAIV (data not shown).

The model focuses on the final 2 weeks of production during which it is feasible that a flock could become infected, remain undetected, and eventually sent to slaughter. This 2-week window for possible infection represents 336 hours or fifty-six 6-hour intervals.

The model is defined as the cumulative number of 6-hour intervals a flock is infected before slaughter (1 < T < 56). To predict the status of a flock at the time of slaughter, t is defined as an index of time of infection within a flock. It represents the cumulative time between initial infection and the time the flock is slaughtered (i.e., 1 < t < T). For purposes of exposition, each time interval is considered an integer index value representing a 6-hour interval of infection. In the results, these index values are converted back to the first hour of the interval. For example, t = 1 is an index representing the time interval of 1–6 hours after infection entered the flock and will be denoted as 1 hour in the results; t = 2 represents the time interval of 7–12 hours after infection entered the flock and will be denoted as 7 hours in the results.

The dynamics of H5N1 HPAIV infection within a broiler flock are modeled for each time step as:

\[ P \times n_t = n_{t+1} \] (1)

We define \( n_t \) as a 26 × 1 vector. Its first row is the number of susceptible birds in the flock at time \( t \) (\( S_t \)), the last row is the number of dead birds in the flock at time \( t \) (\( D_t \)), and the intervening rows represent the number of birds in 24 possible states of infection at time \( t \) (\( I_t \)). Each of the infection states represents a 6-hour time of infection (e.g., \( I_t^1 = 6 \), \( I_t^2 = 12 \), ..., \( I_t^{35} = 144 \)). It is necessary to model multiple infection states because the amount of virus within an infected bird depends on the length of time the bird is infected. The number of infection states was simply chosen to be large enough that no infected birds would survive beyond the last state. Based on available data (Das et al., 2008), the probability of an infected bird surviving to \( I_t^{35} = 144 \) is nil.

We define \( P \) as a 26 × 26 stochastic matrix that predicts the transition of the elements in \( n_t \) to \( n_{t+1} \). Each row of \( P \) sums to 1.
### Table 1. Baseline and Lower and Upper Bound Model Inputs

<table>
<thead>
<tr>
<th>Input</th>
<th>Description</th>
<th>Base value</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birds initially infected ( (I_1/C_0) )</td>
<td>Starting number of infected birds for transmission model</td>
<td>1</td>
<td>NA</td>
<td>10</td>
</tr>
<tr>
<td>Effective contact rate ( (\beta) )</td>
<td>How many birds become infected in a 6-hour time block due to exposure to 1 infected bird ( (\text{Bos et al., 2007}^a; \text{Elbers et al., 2007}) )</td>
<td>8</td>
<td>1</td>
<td>64</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Base</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.000</td>
<td>0.000</td>
<td>0.238</td>
</tr>
<tr>
<td>6</td>
<td>0.400</td>
<td>0.200</td>
<td>0.650</td>
</tr>
<tr>
<td>12</td>
<td>0.300</td>
<td>0.135</td>
<td>0.564</td>
</tr>
<tr>
<td>18</td>
<td>1.000</td>
<td>0.762</td>
<td>1.000</td>
</tr>
<tr>
<td>24</td>
<td>1.000</td>
<td>0.762</td>
<td>1.000</td>
</tr>
<tr>
<td>30</td>
<td>1.000</td>
<td>0.762</td>
<td>1.000</td>
</tr>
<tr>
<td>36</td>
<td>1.000</td>
<td>0.762</td>
<td>1.000</td>
</tr>
<tr>
<td>42</td>
<td>1.000</td>
<td>0.861</td>
<td>1.000</td>
</tr>
<tr>
<td>48 and over</td>
<td>1.000</td>
<td>0.779</td>
<td>1.000</td>
</tr>
</tbody>
</table>

<p>| Probability that infected bird is infectious [latency effect] ( (\alpha) ) | Distribution showing proportion of birds becoming infectious over 6-hour time blocks ( (\text{Das et al., 2008}) ) | 0 | 0.000 | 0.036 |
| Probability of virus in muscle ( (p_m) ) | Distribution showing proportion of birds having EID(<em>{50}) in meat over 6-hour time blocks ( (\text{Das et al., 2008}) ) | 6 | 0.150 | 0.229 |
| Levels of EID(</em>{50}) units in breast and thigh muscle ( (e_m) ) | Distribution showing EID(_{50}) levels in meat over 6-hour time blocks ( (\text{Tumpey et al., 2002; Thomas and Swayne, 2007}) ) | 12 | 0.163 | 0.243 |
| Survival probabilities for infected states ( (1-\gamma^{-1+}) ) | Distribution showing probability of bird remaining infected for next 6-hour time block ( (\text{Das et al., 2008}) ) | 0 | 1 | 1 |
| Flock detection probability at time of slaughter ( (\phi_T) ) | Probability that an infected flock will be detected on the farm given a specific daily mortality level ( (\text{D. Swayne, personal communication}) ) | 0.0% | 0.0000 | 0.0000 |</p>
<table>
<thead>
<tr>
<th>% Mortality</th>
<th>Base</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0%</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.1%</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.2%</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.3%</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.4%</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.5%</td>
<td>0.0625</td>
<td>1.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.6%</td>
<td>0.1250</td>
<td>1.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.7%</td>
<td>0.1875</td>
<td>1.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.8%</td>
<td>0.2500</td>
<td>1.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.9%</td>
<td>0.3125</td>
<td>1.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>1.0%</td>
<td>0.3750</td>
<td>1.0000</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

(continued)
indicating that birds in a particular state at time \( t \) must be
apportioned into some state at time \( t + 1 \).

The transition probability for \( S_t \rightarrow I_{t+1} \) is determined using
the Reed-Frost equation:

\[
P(S_t \rightarrow I_{t+1} \mid I_t) = 1 - e^{-\beta I_t \Delta t}
\]

(2)

where \( \beta \) is the effective contact rate for infection, \( I_t \) is the
number of infectious birds in the population at time \( t \), and \( N \) is
the flock size. The number of infectious birds in the flock is
determined as \( I_t = \alpha \cdot m_t \), where \( m_t \) is a \( 24 \times 1 \) vector in
which each row is an element \( I_t \) and \( \alpha \) is a column vector of
probabilities. Each row of \( \alpha \) is the probability that an infected
bird in a given infection state is infectious. It should be recognized
that \( m_t \) is simply \( n_t \) with the \( S_t \) and \( D_t \) elements removed.

The transition probability for \( S_t \rightarrow S_{t+1} \) is simply the
complement of the Reed-Frost equation prediction because
susceptible birds can only become infected or remain sus-
ceptible:

\[
P(S_t \rightarrow S_{t+1}) = e^{-\frac{\beta I_t \Delta t}{N}}
\]

(3)

With each time step of the model, infected birds can either
progress to the next infection state or die. Transition proba-
bilities for \( I_t \rightarrow I_{t+1} \) are defined by \( (1 - e^{\gamma t+1}) \), where \( e^{\gamma t+1} \) is the
conditional probability of death given a bird survives to in-
fec tion state \( s \). Correspondingly, transition probabilities for
\( I_t \rightarrow D_{t+1} \) are \( e^{\gamma t+1} \). Transitional probabilities for \( D_t \rightarrow D_{t+1} \)
will equal unity and the probabilities of infected birds moving
to an infection state other than the immediate next state are
zero.

**Determining total embryo infectious dose 50% units
in an infected flock sent to slaughter**

For this model, an output of the total amount of virus
contained in an H5N1 HPAIV–infected broiler flock at the
time it goes to slaughter is used. As a surrogate for total virus
count, the value for embryo infectious dose 50% (EID50) is
used. This value is the level of virus required to infect 50% of
embryonated eggs.

Once an infected flock reaches slaughter age, the vector
\( m_{\tau \rightarrow T} \) contains the number of infected birds in each state
of infection. The EID50 per infected bird varies by infection state.
These EID50 are elements of a \( 24 \times 1 \) vector \( E \). The elements of \( E \)
are derived as the product of the observed EID50 levels in
muscle tissue (conditional on virus being present), \( e_{m_n} \), and the
probability that an infected bird has virus in its muscle tissue,
\( p_m \). Therefore, the total EID50 in an infected flock, given that it
survives to slaughter, is \( E \cdot m_T \).

An infected flock does not necessarily survive to slaughter.
As mentioned previously, if the flock is infected before its final
2 weeks of production, then the probability of detection is
unity. Therefore, an infected flock has a maximum probability of
survival of \((1 - 6/8)\). Further, the probability that the flock
is detected just before its slaughter (\( \phi_T \)) is directly related to its
daily within-flock mortality. As infection progresses in the
flock, the daily mortality is monitored in the model.

The unconditional expectation of the total EID50 in an
infected flock is \( E \cdot m_T (1 - 6/8)(1 - \phi_T) \). This calculation, how-
ever, only considers the expectation for one specific value of \( T \).
To account for all possible values of \( T \) (i.e., considering a flock
that is infected for \( T = 1 \) periods, or \( T = 2 \) periods, ... , or
\( T = 56 \) periods), the expectation for total EID50 is calculated as
follows:

\[
E[\text{Total EID}_{50} \text{ per infected flock}] = E[\text{EID}_{50}]
\]

\[
= \frac{\sum_{T=1}^{56} E \cdot m_T (1 - 6/8)(1 - \phi_T)}{56}
\]

(4)

This equation synthesizes all the model parameters—H5N1
HPAIV transmission dynamics (via the elements in \( m_{\tau \rightarrow T} \)), the
dynamics of EID50 levels by infection state, and the proba-
bility that farm/poultry managers detect H5N1 HPAIV in
their flocks—into a determination of the level of hazard po-
tentially presented to poultry slaughter establishments and,
ultimately, consumers.

**Nondetection window**

The length of time between when the flock is initially in-
fected and when the flock reaches the detection threshold
corresponds to a nondetection window where the flock is
infected but remains undetected. The detection threshold is
the mortality level at which no flock would be missed (100% detection)
for a given scenario. The model simulates the dis-
ease spread and subsequent bird death until a detection
threshold of daily mortality is reached. Once the detection

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**Table 1. (Continued)**

<table>
<thead>
<tr>
<th>Input</th>
<th>Description</th>
<th>% Mortality</th>
<th>Base</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1%</td>
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<td>0.0000</td>
<td></td>
<td></td>
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<td>1.3%</td>
<td>0.5625</td>
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<tr>
<td>1.4%</td>
<td>0.6250</td>
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<td>0.7500</td>
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<tr>
<td>1.7%</td>
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<td>1.8%</td>
<td>0.8750</td>
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<tr>
<td>1.9%</td>
<td>0.9375</td>
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<tr>
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<td>1.0000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*References refer to baseline estimates.

\( ^{b} \)Survival is not expected beyond 48 hours.

EID50, Embryo Infectious Dose 50%.
threshold is reached or surpassed, the number of periods occurring before this threshold is counted to estimate the length of the nondetection window.

Sensitivity analysis

Changing the values of $\beta$, $x$, $\gamma$, $E$, or $\phi_T$ can change the value of $E[EID_{D0}]$ calculated in Equation 4. Sensitivity and scenario analyses examine the effects of changing one or more of these model inputs on $E[EID_{D0}]$.

Baseline, minimum, and maximum values are proposed for each of the model inputs (Table 1). Baseline values are intended to represent best guesses concerning HPAIV if it were introduced into a U.S. broiler flock. Nevertheless, substantial uncertainty surrounds these model inputs, so plausible minima and maxima are also examined via sensitivity analysis. These settings represent an amalgamation of published values, input from industry experts, and reasonable values derived by the analysts.

A univariate sensitivity analysis examines the proportional change in $E[EID_{D0}]$ to changes in each input. Because each input can move from a baseline to a maximum or minimum, sensitivity is calculated for both changes. The sensitivity metric is a unit-less elasticity that describes the proportional change in the model’s output relative to the proportional change in the model’s input;

$$
\text{Sensitivity} = \frac{\% \text{ change in } E[EID_{D0}]}{\% \text{ change in input}} = \frac{(E[EID_{D0}] \text{min or max} - E[EID_{D0}] \text{base})/E[EID_{D0}] \text{base}}{(input \text{min or max} - input \text{base})/input \text{base}}
$$

If the absolute value of the sensitivity is one, then the model’s output changes exactly proportional to the change in the input. If the sensitivity is greater than one, then a change in the model’s input generates a disproportionately larger change in the model’s output (i.e., the model is relatively sensitive to that input). Correspondingly, an absolute value for sensitivity less than one suggests that a change in the model’s input results is something less than a proportional change in the model’s output (i.e., the model is relatively insensitive to that input).

The challenge for sensitivity analysis is the determination of a scalar value to represent those inputs that are vectors (e.g., $x$, $\gamma$, $E$, and $\phi_T$). For each of these inputs, an average value across all possible values within the vector was calculated to represent the entirety of that vector. For example, the vector $E$ is actually derived from the vector $e_m$ that contains the EID$_{60}$ levels in the meat of an infected bird (given that bird has virus in its muscle) across all 24 possible stages of infected birds. For the base case, we calculate the average of these 24 EID$_{60}$ values (i.e., $7.8 \times 10^5$) (Tumpey et al., 2002, 2003; Swayne and Beck, 2005; Swayne, 2006; Thomas and Swayne, 2007). The minimum and maximum inputs for $e_m$ were derived from available information collected from infected moribund and dead birds that suggested possible limits for viral concentrations at various stages of infection. Based on these levels for particular stages, the EID$_{60}$ levels for other stages were derived by extrapolation and tabulated (Table 1). The average EID$_{60}$ for the minimum and maximum $e_m$ inputs are $1.4 \times 10^5$ and $7.3 \times 10^5$, respectively. This method of summarizing a vector of inputs is simple, but it provides a crude indication of magnitude of change for this univariate sensitivity analysis. An alternative approach would involve a separate examination of each element of each vector for its influence on the output, but such an approach was deemed overly complex for this model.

Sensitivity also depends on $T$, the time an infected flock goes to slaughter. The sensitivity is calculated for each value of $T$ and the average sensitivity across all these values is presented. Such an approach provides a general indication of an input’s effect on the model’s output.

Results

Nondetection window

The transmission model estimates the number of susceptible, infected, and dead birds over time after the infection of a single bird. A daily mortality threshold is then used to estimate when an infected flock could be detected. There are no data to indicate when farm/poultry workers would detect a flock as being unfit to send to slaughter. This would likely be dependent on the strain of virus and bird, grow-out conditions, season, work experience, HPAI-outbreak history, background mortality, and other factors. Therefore, the daily mortality threshold was examined across a range of values. In the baseline scenario it was assumed this threshold could be as low as 0.5% daily mortality (probability of detecting the flock is 0.0625) or as high as 2.0% (probability of detecting the flock is 1.0) (D. Swayne, personal communication). Daily bird mortality within this range or greater was assumed to signify flock detection given farm/poultry managers would be sensitive to unusual mortality.

The baseline transmission model estimates that this nondetection window is 3.5 days for a 20,000 chicken house (Fig. 2). The length of the nondetection window depends on several factors, including the effective contact rate, the latency effect, the time to death, and the daily bird mortality detection threshold. Figure 2 demonstrates the effect of varying the effective contact rate on the nondetection window. The center panel demonstrates the within-flock prevalence of the various states for the baseline assumptions. Decreasing the effective contact rate of eight new infections per 6-hour time interval to two new infections per 6-hour time interval increases the nondetection window to 5 days. Increasing the effective contact rate to 32 decreases the nondetection window to 3 days. Because there is some probability of detection before the detection threshold is reached, an infected flock may be detected prior to reaching the detection threshold. In the baseline scenario this could occur in any period in which the daily mortality was equal to or greater than 0.5% and less than 2.0%. In the baseline scenario this can occur no more than one or two periods before the flock reaches the detection threshold.

Probability an H5N1 HPAIV–infected flock is not sent to slaughter

A flock that is H5N1 HPAIV infected would only go to slaughter if it remains undetected. This would only occur if the flock was initially exposed to H5N1 HPAIV just before the birds reached market weight at the end of the grow-out period. Given that the nondetection window is 3.5 days in the
baseline scenario, the model estimates an H5N1 HPAIV–infected chicken flock with an 8-week (56 days) grow-out period has approximately a 94% probability of not being sent to slaughter.

**Number of EID$_{50}$ units an H5N1 HPAIV–positive flock would present at slaughter**

The number of EID$_{50}$ units that could be presented at slaughter would depend on how long the flock was infected before slaughter (Table 2). A flock infected just before slaughter (< 6 hours) would not have time for the muscle of a single bird to become contaminated. On the other hand, a flock infected more than 6 hours, but less than 3.5 days before slaughter could present a substantial number of EID$_{50}$ units at slaughter. The expectation for the number of EID$_{50}$ units at slaughter across all time periods, $E[Total\ EID_{50} \ per \ infected\ flock]$, is the sum of the values in Table 2 divided by the 56 time periods or $8.63 \times 10^{10}$.

**Sensitivity analysis**

A sensitivity analysis was conducted using the minimum and maximum input for seven model variables found to influence the total virus levels per infected flock presented at slaughter (Table 3). These results indicate that the model’s predictions were generally insensitive to the effective contact rate, the vector of levels in muscle tissue, the vector of probabilities of detecting an infected flock, and the starting number of infected birds. Because an increase in probability of detection actually reduces the $E[EID_{50}]$, this input’s sensitivity value is also negative.

The model’s output was most sensitive to the vector of probabilities describing the probability of an infected bird being infectious. In particular, the maximum setting for this input vector generated an increase in $E[EID_{50}]$ that was more than 19-fold larger than the change in the average probability. The vector of probabilities describing the transition among infection states to mortality was also influential. This vector

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**FIG. 2.** The various states (susceptible, infected, and dead) of birds within a 20,000 chicken house during an H5N1 HPAIV infection. Flock is assumed infected with H5N1 HPAIV at time zero. Effective contact rates of 2, 8, and 32 are assumed (top to bottom).
essentially reflects the probability of survival as a function of stage of infection; when its average value decreases, the probability of mortality increases and the number of infected birds surviving to slaughter correspondingly decreases. The maximum sensitivity for this input vector also indicates that an increase in this vector allows more infected birds to survive to slaughter and generates a nearly 15-fold increase in $E_{EID_{50}}$.

Sensitivity analysis on the length of the nondetection window indicated that effective contact rate had the strongest influence and was inversely related. The maximum nondetection window of 9.75 days was predicted using the slowest effective contact rate, the lowest mortality rate, the highest detection threshold, and the longest latency effect. The minimum detection window was 1.5 days when these inputs were set at their opposite extremes.

**Discussion**

The transmission model estimates the within-flock prevalence of an H5N1 HPAIV–infected flock. This state-transition model estimates the number of susceptible, infected, and dead birds over time to simulate the progression of disease after infection of a single bird. A single day of bird mortality is then used as a detection threshold to estimate when the flock would not be sent to slaughter, given that farm/poultry managers would recognize atypical bird mortality levels. The time from when the flock is randomly infected to just below the detection threshold is the critical nondetection window, where an infected, yet undetected flock, could be sent to slaughter.

Given the baseline assumptions (Table 1), the model estimates that the nondetection window is 3.5 days for the index flock. During this time, an H5N1 HPAIV–infected flock would not be detected if a single day of mortality is used as the sole indicator of flock infection, because few birds would be dying. Given that the nondetection window is dependent on the effective contact rate, the latency effect, and the time to death, each variable has the potential to increase or decrease the nondetection window.

The effective contact rate is the most important determinant of the length of the nondetection window.
effective contact rate has the effect of decreasing the nondetection window, making it less likely that an H5N1 HPAIV–infected flock would be sent to slaughter. However, because the disease spreads faster, if the flock was sent to slaughter it would carry more EID50 units compared to a flock infected for the same length of time by a slower-spreading virus. Setting the effective contact rate to a maximum of 64 new infections/6 hours and a minimum of 1 new infection/6-hour results in a variable nondetection window of 2.75 and 6.75 days, respectively. In fact, increasing the effective contact rate to 100,000 will result in a minimum nondetection window of 2 days. Such a result suggests that even a fast-spreading H5N1 HPAIV would remain undetectable for 48-hours using a single day of flock mortality as a detection method.

The model predicts that the H5N1 HPAIV–infected chicken index flock has approximately a 94% chance it will not be sent to slaughter. This is dependent on when a flock is infected with H5N1 HPAIV and the length of the nondetection window and grow-out. The baseline assumptions (Table 1) estimate a nondetection window of 3.5 days out of the total flock grow-out of 56 days. Therefore, there is a 6.25% (=3.5/56×100) chance an infected chicken flock will be sent to slaughter undetected. Flocks requiring a longer grow-out to reach market weight have a lower probability of being sent to slaughter, assuming the same nondetection window. For example, a flock kept on the farm for 12 weeks (84 days) would have a 4.17% (=3.5/84×100) chance that it would be sent to slaughter infected and undetected. This apparent lowering of consumer exposure occurs because there would be fewer birds presented to slaughter. Assume a chicken house has 48 weeks available for grow-out. In 48 weeks there is room for six 8-week cycles and four 12-week cycles. Given a nondetection window of 3.5 days in either type of flock, a house with 8 week cycles would have a total of 21 possible days in nondetection windows (6×3.5), whereas a house with 12 week cycles would have a total of 14 days (4×3.5).

Timing of flock infection is assumed to be a random event. If a flock is infected early during the grow-out, the chance the flock is sent to slaughter is low because the disease will have time to demonstrate mortality. However, if a flock is infected near the end of the grow-out period, there may not be time for the disease to spread and birds to start dying before the flock is sent to slaughter. Therefore, an H5N1 HPAIV–infected flock is only of concern if it is randomly infected at the end of grow-out, just before slaughter.

If a flock is infected late in its grow-out, the time required for a flock to show substantial mortality, that is, the nondetection window, becomes important. The nondetection window is determined by the number of dead birds in the flock. The transmission model estimates the number of birds that become infected and die every 6-hour interval during the course of the simulated infection. As long as this percentage is less than the detection threshold, the model continues to the next 6-hour interval.

For the baseline model assumptions, detection threshold has very little influence on the nondetection window and subsequently on EID90 units presented at slaughter. Because mortality tends to grow exponentially (Fig. 2), the model simulates that an infected flock is either presenting very few dead birds or substantial numbers of dead birds (Table 2, column 3). Decreasing the maximum percent daily mortality (so that the probability of detection is 1.0 for a lower daily mortality) reduces the number of EID90 units presented to slaughter. For example, decreasing the maximum daily mortality from 2% to 0.5% (i.e., a fourfold reduction) decreases the EID90 units presented at slaughter by approximately twofold. However, lowering the detection threshold has practical limitations as other poultry diseases/environmental conditions could result in similar bird mortality levels, thus resulting in falsely labeling a flock as unfit for slaughter (Savill et al., 2008). More than a single day of flock mortality could be considered when mortality is used to detect an H5N1 HPAIV–infected flock (Elbers et al., 2007).

The model demonstrates that undetected infected flocks presented to slaughter do not represent equivalent exposure risks. A flock infected very late in its grow-out, 0 to 6 hours before slaughter, would only contain one infected bird when slaughtered and no EID90 units (H5N1 HPAIV not detected systemically in chickens under 6 hours; Das et al., 2008). However, if a flock is infected just under 3.5 days before the end of grow-out, the flock may consist of 11,906 infected birds containing a total of 2.17×1012 log10 EID90 units when slaughtered (Table 2).

The risk assessment output, total flock EID90 units, presents an exposure distribution of infectivity as a function of when the flock was infected. This output is an important beginning to understanding the impact of poultry slaughter and processing, storage, transportation, consumer preparation and handling, and dose–response on the probability of human exposure and infection from an H5N1 HPAIV–infected, yet undetected, flock sent to slaughter. Additional risk assessment to quantify the plant-to-fork continuum will allow guidance of interventions to mitigate human exposure at both the level of poultry processing and consumption.

Conclusion

The baseline transmission model predicts that there is a high probability (0.94) that an undetected H5N1 HPAIV–infected chicken flock would not be sent to slaughter. This probability is inversely related to the length of the nondetection window, which is a function of the effective contact rate, latency, bird mortality rate, and daily mortality threshold. If a flock is randomly infected within 3.5 days of the end of grow-out, then the flock is expected to remain undetected and sent to slaughter. The total number of EID90 units presented at slaughter will primarily depend on when the flock was infected, the virus levels in the meat of infected birds, and the mortality rate. Use of a single day of mortality as an indicator of H5N1 HPAIV–flock infection is inadequate to exclude all H5N1 HPAIV–infected flocks. Alternative modeling considerations, such as the inclusion of a morbidity or a feed/water intake threshold, for example, could decrease the length of the nondetection window and therefore increase the probability of flock detection. For additional information, please see http://www.fsis.usda.gov/Science/Risk_Assessments/index.asp.

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