Application of Diagnostic Tests to Inform Disposal Option Choice for Diseases Where Animals Can Recover

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Abstract. *Early depopulation and disposal have been recognized as key factors in the control of animal disease outbreaks. However, there are additional considerations when managing outbreaks of diseases where animals may recover from an acute viral infection. In this case, the proportion of animals that are actively shedding virus would be lower at later stages of disease spread in the population when most of the animals would have recovered. We discuss how the prevalence of infectious and recovered birds varies over time, and how this information can be used to guide decisions regarding off-site disposal options, based on an example scenario of low pathogenicity avian influenza (LPAI) infection in a broiler-breeder flock. First, we used stochastic simulation models to predict the proportion of infectious and recovered birds over time in an LPAI infected broiler-breeder flock. We then simulated detection using various diagnostic testing options, including serological testing of 15 samples using the Agar Gel Immunodiffusion (AGID) assay, and an influenza A matrix-gene real-time reversed transcriptase polymerase chain reaction (RRT-PCR) testing protocol using pooled samples of 11 swabs each, and combinations of RRT-PCR and AGID. The simulation models were used to predict the range of time to detect LPAI post exposure and the proportion of infectious birds in the flock at the time of detection under various active surveillance protocol options. We then used the simulation model results to show the benefit of additional diagnostic testing in reducing uncertainty in the outcome variables and providing confidence that the number of infectious birds at the time of movement to disposal are likely to be very low (acceptable). Our results indicate that a combination of RRT-PCR and AGID provides the most information regarding the prevalence of infectious birds and the additional number of days required for the flock to stop shedding. Finally, we discuss how the concepts and approach illustrated through the LPAI example may be generalized to other diseases where the animal populations would eventually recover.*

Keywords. Disposal, Low Pathogenicity Avian Influenza, Active Surveillance, Agar Gel Immunodiffusion

Introduction

Timely access to multiple carcass disposal options is important for the management of animal disease outbreaks or other mass mortality events. The limiting factors impacting the choice of a disposal option include logistical feasibility, environmental considerations, disposal costs, and risks associated with handling infectious animal waste. While off-site disposal options such as rendering may have potential economic benefits (e.g., reducing downtime before production can be resumed, partial compensation to offset disposal costs, etc.), off-site disposal may also be perceived to be a higher risk for disease transmission.

Early detection and rapid depopulation are common strategies used to manage outbreaks of highly contagious foreign animal diseases. These strategies are well suited for outbreaks of highly pathogenic avian influenza (HPAI) in commercial poultry, which typically causes acute clinical signs, including mortality. In this case, dead-bird targeted active surveillance has been shown to reduce time to detection in infected flocks, ensuring a lower prevalence of infectious birds at the time of depopulation and disposal, and thus reducing the potential risk of further disease spread. However, there are additional considerations when managing outbreaks of diseases where animals may recover from an acute viral infection. In the case of low pathogenicity avian influenza (LPAI), detection would likely occur at a later stage of disease progression in the flock because of milder clinical signs and much lower disease mortality. Moreover, the prevalence of infectious birds would be much lower at later stages of disease spread in the flock, as most birds would have recovered. The potential risk of disease spread associated with off-site disposal would also be correspondingly lower during the later stages of infection, given the lower prevalence of actively shedding birds.

Therefore, additional disposal options may be considered in situations where it can be determined that most of the birds have recovered, and are not actively shedding virus. Results from active surveillance testing using different types of diagnostic tests can be used to predict the prevalence of infectious birds in the flock, and the time until most birds in the flock stop shedding.

As an example, we consider the case of an LPAI infected broiler-breeder flock, detected through routine surveillance testing for H5/H7 LPAI. First, we used a within-flock disease transmission model to predict the prevalence of infectious and recovered birds over time. Next, we simulated detection via various diagnostic testing options[1] that include; 1) serological testing of 15 samples using the Agar Gel Immunodiffusion (AGID) assay, and 2) influenza A matrix-gene real-time reversed transcriptase polymerase chain reaction (RRT-PCR) testing using pooled samples of 11 swabs. We use the simulation model results to show the benefit of additional diagnostic testing to reduce uncertainty in outcome variables, providing confidence that the number of infectious birds at the time of movement to disposal would be very low. We discuss how the prevalence of infectious and recovered birds varies over time, and how this information can be used to guide decisions regarding off-site disposal options, based on an example scenario of low pathogenicity avian influenza (LPAI) infection in a broiler-breeder flock.

Materials and Methods

[1] We used these surveillance options as examples, in an effort to be consistent with current NPIP minimum requirements outlined in 9 CFR 145.33(l) U.S. Avian Influenza Clean. As applied to breeding, a flock is all poultry of one kind of mating and of one classification on one farm. Therefore, if 15 samples are required, 1 pool of 11 swabs for antigen testing would meet these minimum standards.

We evaluated two routine (i.e., in the absence of an outbreak) surveillance-testing protocol options (Options A and B) for detecting LPAI in broiler-breeder flocks.

Protocol Option A: 15 blood samples taken from live birds in one flock and tested via AGID at 90 day intervals.

Protocol Option B: One 11-swab pooled sample per-flock is tested via RRT-PCR at 90 day intervals. The available dead birds are sampled first, followed by live birds as necessary to get a total of 11 swabs.

We used stochastic simulation models to predict the prevalence of infectious, dead, and recovered birds over time in an LPAI infected broiler-breeder flock. Some of the models used are adaptations of those described in earlier work (Weaver et al., 2015). The transmission model output was then used to simulate detection via active surveillance, and to predict the time post-exposure when LPAI is detected. Details of these procedures are as follows:

Protocol Option A used the transmission model output for both the number of live and seropositive birds at the time of sampling. The number of samples from seropositive birds was first simulated based on the flock sero-prevalence at the time of sampling and testing. The number of positive serology results from tests on seropositive bird samples was then simulated using a binomial distribution, with probability of detection equal to the serological test sensitivity. If at least one of the results was positive, LPAI was detected in the flock.

Under Protocol Option B, the proportion of infectious birds actively shedding virus in the sample pool was simulated from the transmission model-predicted disease mortality and normal daily mortality data from 8 broiler-breeder flocks. Given a virus-positive sample, the detection process via RRT-PCR testing was simulated as a Bernoulli trial, with the probability of success equal to the RRT-PCR test sensitivity.

In addition to detection, diagnostic test results are useful in establishing the stage of LPAI disease progression, the proportion of infected birds actively shedding virus, and the number of days for the flock to stop shedding virus. We evaluated the benefit of diagnostic testing's ability to provide information on these relevant epidemiological outcomes. We predicted the proportion of infectious birds actively shedding virus, and the time to stop shedding virus, conditional on the number of positive results from 15 blood samples tested via AGID. We performed 100,000 iterations of the simulation model implemented in the software R (R Core Team, 2015) and extracted the simulations where the specific test results were obtained. The distributions of the relevant epidemiological variables conditional on the test results were then estimated from the extracted simulations.

One critical input in the disease transmission simulation model is the adequate contact rate, which determines the rate of within-flock infection spread. There is considerable uncertainty regarding this parameter for LPAI spread in chickens, given the limited number of estimates from outbreak data in the published literature. To address the uncertainty associated with this parameter, we used two scenarios for the adequate contact rate. In the slow contact rate scenario, we used Uniform (0.69 - 0.77) per-day as the adequate contact rate distribution based on the estimates for LPAI infected cage-free egg-layer flocks in the Netherlands (Gonzales et al., 2012). We also evaluated a fast contact rate scenario using contact rate estimates from HPAI infected flocks (Uniform (2.68 - 7.57) per-day), where field data was available from a greater number of outbreak flocks (Bos et al., 2009). The parameters of the disease transmission and surveillance simulation models are summarized in **Table 1**. The test-day was allowed to vary from 1 to 40 days and 1 to 65 days post-exposure of the flock in the faster and slower LPAI within-flock disease spread scenarios respectively (i.e., it would take much longer for LPAI infection to spread through the entire flock in the slower spread scenario).

Results

The model-predicted time post-exposure (days) given detection of LPAI in a broiler-breeder flock under routine surveillance at 90 day intervals under the slow spread scenario is shown in **Figure 1**. Mean post-exposure time given that 1 pool of 11 swabs tested positive by RRT-PCR was predicted to be 22.97 (90% Prediction Interval (P.I.) 11 to 30) days, and 41 (90% P.I. 19 to 62.5) days when at least one of 15 samples was positive using AGID. The relatively wider interval for time post-exposure for detection via AGID is because detection could occur over a wide range of possible times, including days when the flock has completely recovered.

To address uncertainty in the rate of disease spread on the range of possible outcomes, results are presented for faster and slower within-flock disease transmission rates. **Figure 2** shows the predicted number of days until all infectious birds stop shedding in an LPAI infected broiler-breeder flock, given results from a set (from 0 to 15 out of 15) of AGID serological test results. In the case of faster within-flock LPAI virus spread (top panel), it took fewer days for the flock to stop shedding given a set of test results.

Results for the percentage of infectious birds in an LPAI infected flock, and the number of days until all birds in the flock stop shedding, given a set of serological test results (AGID), are provided in **Table 2**. Results are interpreted from the perspective that the flock is known to be infected. In the event that there are no positive serological test results out of 15 samples submitted, our model predicts that 8 percent (90% P.I., 0 to 48 percent) of birds in the flock could be shedding virus at that time, and it may take 34.5 days (90% P.I. 25.5 to 43.75 days) on average for all birds in the flock to stop shedding virus. In the event that all 15 serological samples return positive test results, 1 percent (90% P.I. 0 to 6 percent) of the birds in the flock could be shedding virus, where only 2.9 days (0 to 13.25 days) on average are required to complete shedding. As the number of positive serological test results increase, our uncertainty about the level of shedding in flock decreases (i.e., the width of the 90th percentile prediction interval decreases considerably).

Discussion

Timely access to multiple carcass disposal options is important for the management of animal disease outbreaks. However, some off-site disposal options such as rendering, burial in municipal solid waste landfills, or movement to slaughter may be perceived to be a higher risk for disease transmission because of the movement of potentially infectious animals. For diseases where the animals can eventually recover, the proportion of animals that are actively shedding virus would be low in the later stage of diseases progression, when most of the animals would have recovered. For commercial poultry flocks infected with LPAI virus, additional disposal options may be considered in situations where it can be determined that most of the birds have recovered, and are not actively shedding virus. Results from active surveillance testing using different types of diagnostic tests can be used to predict the proportion of birds in the flock actively shedding virus, and the time until most birds in the flock stop shedding. We illustrated these concepts for recoverable diseases based on the case of an LPAI infected broiler-breeder flock, detected through routine surveillance testing for H5/H7 LPAI.

The time interval from detection to when most of the birds have recovered and stopped shedding virus is a key consideration when choosing a disposal option. Our results indicate that LPAI infected flocks may be detected at a later stage of disease progression under routine H5/H7 surveillance protocols used to test broiler-breeder flocks in the United States, given their typically mild clinical signs. In particular, when using serological tests alone, there is a higher likelihood of a detected flock being in a later stage of infection when most of the birds have

recovered. Therefore, for some LPAI infected broiler-breeder flocks detected via routine surveillance, it is possible that the time until all birds in the flock would stop shedding would not be very long.

The results from our analysis indicate that diagnostic test results could be useful when making decisions on a disposal option by reducing the uncertainty in the current stage of infection (i.e., actively shedding versus mostly recovered), and the number of days required for the flock to stop shedding. For instance, positive test results from all 15 serological samples would indicate that the flock has stopped shedding or is likely to stop shedding within a few days. Conversely, 1 or 2 positive serological test results out of 15 samples would indicate that it may take considerably longer for the flock to stop shedding. Therefore, a set of diagnostic test results can provide valuable information, and can be used to partly inform risk management decisions pertaining to the choice and timing of disposal options, along with other key considerations.

Each disposal option has specific advantages and disadvantages. Having access to multiple disposal options during an outbreak provides flexibility for emergency responders. Moving carcasses off-site to a landfill for disposal, for off-site burial, or for rendering may reduce the time needed for an infected premises to resume production, compared within-house composting. Disposal of infected flocks by rendering could potentially increase revenue for the producer and reduce disposal costs, although further economic analysis is needed to address this aspect. Based on the current analysis, waiting until the flock has stopped shedding, based on diagnostic testing, can help make off-site disposal options tenable. However, when choosing a disposal option, risk managers should also consider the additional risk of local spread. This includes spread from the premises during any waiting period, as well as the risk of spread during transportation associated with contamination of equipment and fomites used to move live or dead birds.

The approaches and concepts proposed as options for disposal of LPAI infected flocks put forth in the current analysis can also be generalized for other disease agent-host combinations where animals can recover from infection. Further research and economic analysis of the costs, benefits, and potential economic consequences of various off-site disposal options for flocks or herds affected by recoverable diseases, where decisions on waiting-time and off-site movement are informed based on diagnostic testing, would benefit risk managers who are dealing with animal disease outbreaks.

Table 1. Summary of disease transmission and active surveillance model parameters.

Parameter Name/Notation	Description	Distribution/Value
Adequate contact rate	Mean number of contacts per day each bird has with other birds such that the contact is sufficient to transmit infection	Slow rate ~ Uniform (0.69 - 0.77); Fast rate ~ Uniform (2.68 - 7.57)
Flock size	Number of birds in a broiler breeder house	~ Uniform (9000, 10000)
Latent period distribution	Length in days of the latent period	~ Gamma (shape = 0.82, scale = 0.44)
Infectious period distribution	Length in days of the infectious period	~ Gamma (shape = 8.14, scale = 0.96)

Time to seroconversion distribution	Length in days of time from infection to seroconversion	~ Gamma (shape = 10.03, scale = 0.63)
p_{mort}	Proportion of birds that die following exposure to LPAI	0.005
p_{sero}	Proportion of birds that seroconvert following exposure to LPAI	0.95
Se_{pcr}	rRT-PCR test sensitivity	0.865
Se_{sero}	Serology test sensitivity	0.95

Table 2. The percentage of infectious birds in an LPAI infected flock, and the number of days until all birds in the flock stop shedding, given a set of serological test results (AGID) out of 15 samples submitted. Results are for a slower within-flock disease spread rate.

Number of positive serological test results out of 15 samples tested	**Days until all birds stop shedding LPAI virus**	**Proportion of birds in the flock shedding LPAI virus**
0	34.5 (25.50 – 43.75)	0.08 (0 – 0.48)
1	26.1 (21.50 – 32.00)	0.5 (0.09 – 0.76)
2	24.5 (20.00 – 29.74)	0.65 (0.36 – 0.77)
3	23.4 (19.25 – 28.25)	0.71 (0.59 – 0.77)
4	22.6 (18.36 –27.75)	0.72 (0.6 – 0.77)
5	21.4 (17.80 – 26.20)	0.69 (0.55 – 0.77)
6	21.1 (17.15 – 25.75)	0.66 (0.48 – 0.76)
7	20.3 (16.25 – 25.75)	0.6 (0.43 – 0.74)
8	19.5 (14.75 – 25.31)	0.54 (0.33 – 0.71)
9	17.4 (1.26 – 23.74)	0.43 (0 – 0.69)
10	12.7 (0 – 22.00)	0.26 (0 – 0.61)
11	8.5 (0 – 20.75)	0.13 (0 – 0.52)
12	5.4 (0 – 18.00)	0.05 (0 – 0.33)
13	4 (0 – 15.75)	0.02 (0 – 0.19)
14	3.4 (0 – 14.25)	0.01 (0 – 0.1)
15	2.9 (0 –13.25)	0.01 (0 – 0.06)

[a] Mean and 90th percentile prediction interval.

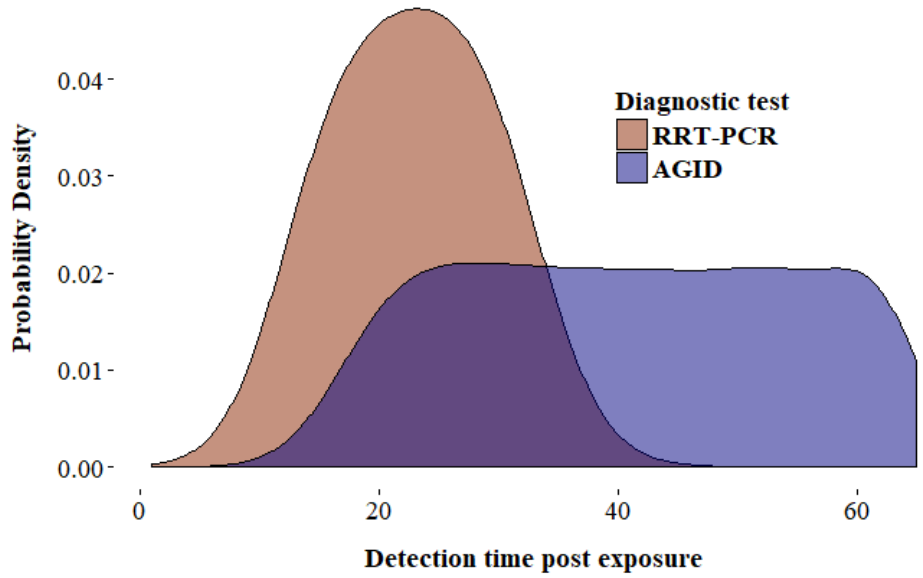

Figure 1. Time post-exposure (days) given detection LPAI in a broiler-breeder flock using either RRT-PCR (1 pool of 11 swabs per-flock) or AGID. Mean detection time post-exposure for RRT-PCR was 22.97 (90% P.I. 11 to 30) days, and 41 (90% P.I. 19 to 62.5) days for AGID. Results are for a slower within-flock disease spread rate.

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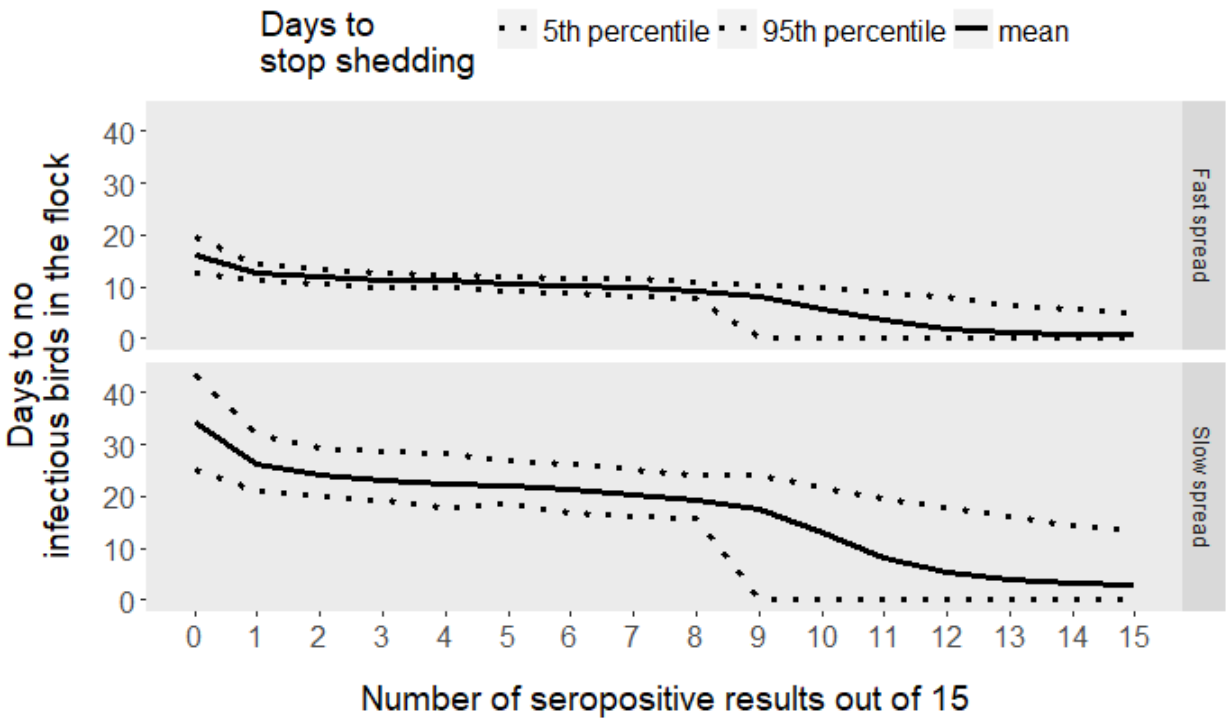

Figure 2. The predicted number of days after test-day until all infectious birds stop shedding LPAI virus in an infected broiler-breeder flock, given results from a set (from 0 to 15 out of 15) of AGID serological test results. The top panel represents faster LPAI spread within a flock, and the lower panel represents slower rates of spread.

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