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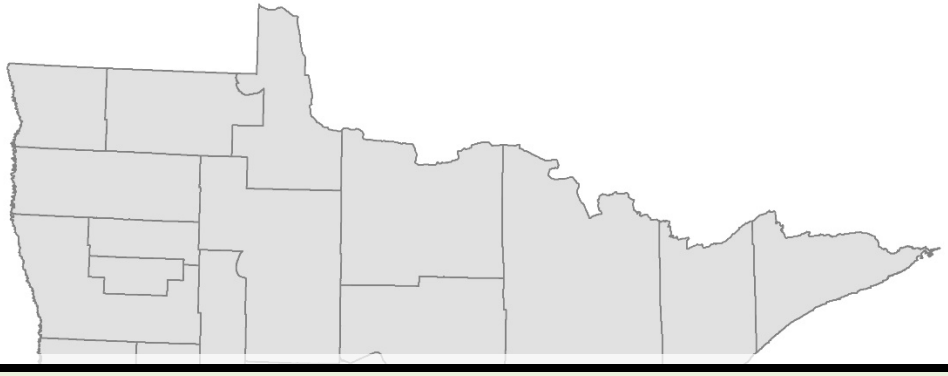
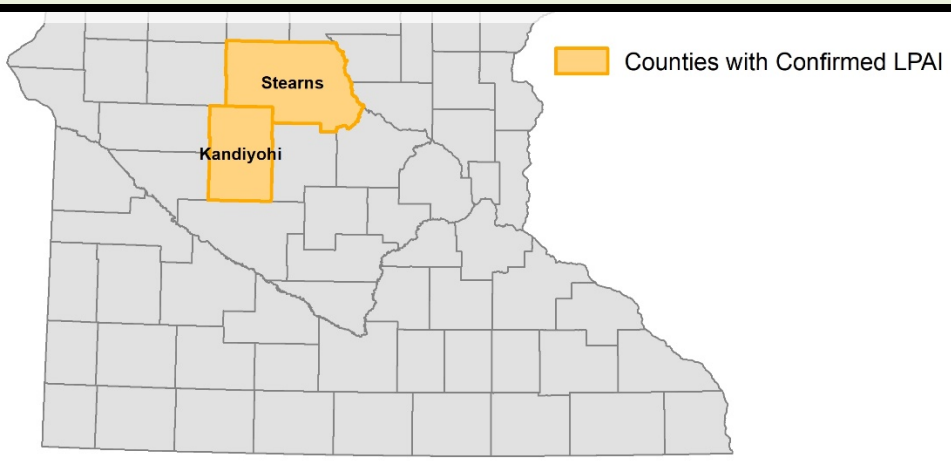


Epidemiologic and Other Analyses of Avian Influenza Affected Poultry Flocks

February 2019 Report



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I. Executive Summary

An outbreak of low pathogenic avian influenza (LPAI) was detected in October-November, 2018, in the State of Minnesota. The index flock was detected through avian influenza surveillance testing as part of an industry-driven enhanced sampling surveillance which has occurred in the spring and fall in certain counties in central Minnesota since the 2015 highly pathogenic avian influenza outbreak. The first detection was on a commercial meat tom turkey flock in Kandiyohi County. Laboratory testing by National Veterinary Services Laboratories (NVSL) confirmed H5N2 LPAI of North American wild bird lineage. Subsequently, between October and November, 2018, H5N2 LPAI virus was detected on a total of eight commercial turkey premises in Kandiyohi and Stearns counties in Minnesota. Controlled marketing was completed for poultry on all infected premises in accordance with Minnesota's Initial State Response and Containment Plan and USDA-APHIS-VS LPAI Guidance Documents.

Following response activities, a series of epidemiologic and genetic investigations were initiated to better understand virus introduction and transmission. These investigations were a collaboration between the poultry industry, the State of Minnesota Board of Animal Health, and the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS). This is a report of findings available to date.

The epidemiologic investigation focused on factors and management practices which have been implicated as risk factors for infection in previous avian influenza outbreaks. Similarly, transmission through the movement of fomites such as people, vehicles, and equipment between farms, were likely. Additionally, breaches in biosecurity, for example, contact with wild birds, may have had a role in introduction and transmission. Epidemiologic, phylogenetic, and estimated time of introduction data supports independent introductions from a common source in the two counties, with limited subsequent spread between farms likely occurring. All recovered viruses have been characterized as North American wild bird lineage H5N2 LPAI, and representative viruses have an intravenous pathogenicity index (IVPI) of zero as defined by OIE. Waterfowl sampling in the U.S. from 1 March to 31 October 2018 was opportunistic and limited to 8 states with the majority of samples collected from live-captured birds in the months of August and September. Only one H5 virus was successfully isolated during this timeframe. Wild bird data shared from the National Center for Foreign Animal Diseases (NCFAD, Winnipeg, Canada), revealed highly similar wild bird viruses collected in July 2018 from the Central/Mississippi Flyway.

Diagnostic testing data was used to estimate the most likely date of virus introduction for all the barns that tested positive by rRT-PCR on the eight LPAI H5N2 virus infected turkey premises. These estimates helped industry veterinarians target their review of visitor logs and other investigation activities to identify possible pathways of introduction and to conduct tracebacks on likely exposures. Predictions from diagnostic test results were requested by some industry veterinarians during the outbreak to support operational planning decisions such as scheduling

birds for processing. The disease transmission simulation model was used to predict the time at which there were no infectious birds in the barn for a given set of diagnostic test results. In the current analysis, predictions were made for 5 barns, Kandiyohi 3/Barn 8, Stearns 2/Barn 1, Stearns 3/Barn 1, Stearns 3/Barn 2, and Stearns 4/Barn 4. The model predictions were consistent with the observed rRT-PCR interval for all 5 barns; however, the model predictions were conservative compared to the observed virus isolation interval, with the predicted interval being later than the observed interval in 3 out of 5 barns. The results of this analysis demonstrate the usefulness of diagnostic testing to better understand the behavior of LPAI in infected poultry flocks.

Previous epidemiologic investigations which employed sampling of wild birds surrounding infected farms have been unable to identify the virus in wild bird reservoirs, suggesting that the virus may have been shed and persisted in the environment prior to being introduced to a poultry farm. To explore this possibility, we identified areas in Minnesota at heightened risk for AIV environmental persistence during the fall migration season using geospatial methods. These results can be used to improve surveillance activities and to inform biosecurity practices and emergency preparedness efforts within Minnesota.

II. Introduction

In response to the H5N2 LPAI outbreak in commercial turkey operations in Kandiyohi and Stearns counties, Minnesota, USDA-APHIS and the Minnesota Board of Animal Health initiated epidemiologic and genetic investigations in an effort to better understand the risk factors associated with introduction of avian influenza into poultry flocks and subsequent transmission.

These investigations included the following:

- A field-based study of infected farms using data collected through site visits and interviews with farm personnel
- Analysis of barn-level mortality and diagnostic test data from the earliest affected commercial farm to estimate the date of virus introduction
- Virus phylogenetic analysis
- Summary of waterfowl surveillance from 1 March 2018 to 31 October 2018
- Geospatial estimation of the relative risks of environmental persistence of avian influenza viruses in the environment in Minnesota.

This report includes the results from those investigations, in an effort to provide producers, industry, and other stakeholders with current epidemiologic information.

Description of Outbreak

On October 19, 2018, drinker biofilm samples[1] were collected as part of the industry-driven enhanced sampling influenza surveillance. When these samples were determined to be non-negative, oropharyngeal (OP) swab samples were collected as part of the follow-up testing to determine the status of the commercial meat-type turkey tom flock in Kandiyohi County, Minnesota. OP samples tested positive by H5 PCR at the Minnesota Poultry Testing Laboratory. The National Veterinary Services Laboratories (NVSL) confirmed H5N2 LPAI on October 20, 2018. A total of eight commercial turkey flocks were confirmed infected by NVSL with H5N2 LPAI in Kandiyohi and Stearns counties, Minnesota, between October 20, 2018 and November 16, 2018 (Table 1, Figure 1). Farms were detected through enhanced surveillance, pre-market testing, or as a result of surveillance testing in zones established around infected premises. Clinical signs were not apparent in most flocks, however, respiratory signs were reported in two flocks. NVSL confirmed North American wild bird lineage H5N2 LPAI on all farms based upon partial HA/NA sequencing. Following detection, surveillance was immediately initiated for commercial and backyard premises located within a 10km zone around all infected farms and for epidemiologically linked premises. In the absence of severe clinical signs and additional laboratory results, Veterinary Services and the Cooperating State Agency agreed that poultry on the infected premises pursue controlled marketing in accordance with Minnesota's Initial State Response and Containment Plan and USDA-APHIS-VS Guidance documents. Controlled marketing was completed for all flocks by January 17, 2019.

Table 1. Location, production type, and confirmation date of flocks infected by LPAI H5N2.

State	County	Production Type	Confirmation Date
Minnesota	Kandiyohi	Commercial Turkey	20 October 2018
Minnesota	Kandiyohi	Commercial Turkey	5 November 2018
Minnesota	Kandiyohi	Commercial Turkey	13 November 2018
Minnesota	Kandiyohi	Commercial Turkey	16 November 2018
Minnesota	Stearns	Commercial Turkey	31 October 2018
Minnesota	Stearns	Commercial Turkey	2 November 2018
Minnesota	Stearns	Commercial Turkey	5 November 2018
Minnesota	Stearns	Commercial Turkey	5 November 2018

[1] While drinker biofilm samples are not considered an "official sample" per NPIP/NAHLN, this sample type has been shown to allow detection of avian influenza RNA up to 24 hours ahead of animal samples. Approved laboratories accepting this sample type for testing are instructed to request appropriate samples from the flock immediately following any non-negative result to determine the virus status of the flock.

Figure 1. Counties with confirmed findings of H5N2 LPAI between October and November 2018.

III. Epidemiologic Study to Investigate the H5N2 Virus in Commercial Poultry in Minnesota

Case Series

In collaboration with State Animal Health Officials, USDA-APHIS conducted a case-series study of the H5N2 LPAI infected commercial turkey operations. A questionnaire was administered to individual(s) on each farm most familiar with the farm's management and operations. Questions focused on the three week period prior to the detection of LPAI (Appendix A). The purpose of this study was to generate hypotheses for potential risk factors for infection with LPAI based on descriptive information about farm characteristics and management practices.

Transmission of virus in previous outbreaks of avian influenza in the United States has been attributed to the movement of live and dead birds, transportation of manure, equipment sharing, and contaminated feed trucks, vehicles, water, and people (McQuiston et al., 2005, Halvorson, 2009, Garber et al., 2016). Results of the questionnaire, focusing on risk factors identified in previous outbreaks, are summarized below and in Table 2.

- Among the 8 infected farms, the median farm capacity was 53,000 birds, with a range from 35,000 to 174,000 birds. The number of barns on farms ranged from 3 to 10 barns, with an average barn capacity of 10,750 birds (range: 7,770 – 37,000).
- Most farms (5/8) were single age, and 3/8 were multi-age. Commercial toms were raised on 5/8 farms and commercial hens were raised on 3/8 farms. 50% of farms were grow only, 50% were brood and grow, and there were no breeders.
- The majority of farms (6/8) reported no clinical signs, however, two farms did report respiratory signs.
- A median reported distance to the closest body of water was 665 yards (range: 0-3,500 yards). Water body types were varied and included pond, lake, stream, river, holding pit, slough, and man-made ditch. All farms reported that the primary water source for the farm was a well. Most respondents (7/8) reported that water is treated prior to delivery to poultry.
- Farms reported a median of two employees (range: 1-3).
- The most frequently reported type of visitor was feed truck delivery. Other visitor types reported included service person, veterinarian, and feed consultant.
- Nearly all farms reported seeing wild birds around the farm (including on adjacent habitats away from facilities and equipment, on farm but not in barns, and on farm and sometimes in the barns). Bird types most commonly reported were gulls and small perching birds.

Results showed that some factors and management practices were shared across infected farms, however, the significance of these similarities is difficult to interpret given the small number of infected farms and the study design. When considered in conjunction with knowledge of practices and risk factors from previous outbreaks, this information may provide insights into trends of management practices over time and elucidate opportunities to implement additional mitigations in the future.

Table 2. Characteristics of H5N2 LPAI infected commercial poultry farms.

Characteristic	Level or response	n
Barn ventilation type	Curtain sided	6/8
	Environmental control	0/8
	Side doors	2/8
	Other	2/8
Workers are employed by other poultry operations	Yes	0/8
Family members are employed by other poultry operations or processing plants	Yes	0/8
Workers are restricted from contact with backyard poultry	Yes	8/8
Equipment sharing: Which equipment is farm specific (owned solely by this farm and not used on other farms?		
Company vehicles/trailers	Yes	5/8
Feed trucks	Yes	2/8
Gates/panels	Yes	5/8
Lawn mowers	Yes	4/8
Live haul loaders	Yes	1/8
Poult trailers	Yes	3/8
Preloaders	Yes	2/5
Pressure sprayers/ washers	Yes	4/8
Skid-steer loaders	Yes	4/8
Tillers	Yes	4/7
Trucks	Yes	4/8
Litter shed is present on farm	Yes	4/8
Farm does partial cleanouts	Yes	2/8
Litter disposal site	On farm	0/8
	Taken offsite	6/8
	Both on farm and taken offsite	2/8
Dead bird disposal method	On farm: burial pit/incinerator/composted/other	5/8
	Off farm: landfill/rendering/other	4/8
	Off farm: disposal performed by owner/employee/other	0/7
Type of carcass bin	Covered	2/5
	Uncovered	3/5

IV. Estimating the Time of H5N2 LPAI Infection in Commercial Meat-type Turkey Flocks using Diagnostic Test Results

Summary

Determining the time of LPAI virus introduction in a flock is an important part of outbreak investigations. By narrowing the time window of possible virus introduction, we can better identify the potential routes of virus introduction and enhance our understanding of the pattern of disease spread. In this analysis, diagnostic testing data was used to estimate the most likely date of virus introduction for all the barns that tested positive by rRT-PCR on the eight LPAI H5N2 virus infected turkey premises in Minnesota in 2018. The analysis was performed using a simulation-based method in which the likelihood of observing the diagnostic test results was estimated from a within-house disease transmission model for various candidate times of exposure.

Using this method, Kandiyohi 1 and Stearns 1 were estimated to have been the first premises infected with a most likely date of introduction of October 7th. Stearns 2 was estimated to have been infected only shortly afterwards, on October 8th. The most likely dates of virus introduction estimated for the subsequent premises was October 19th for Kandiyohi 2, October 26th for Stearns 3, and October 28th for both Stearns 4 and Kandiyohi 4. The last premises estimated to have been infected was Kandiyohi 3 on November 6th. The last barn estimated to have been infected was Kandiyohi 4/Barn 4, with a likely date of virus introduction of November 23rd. These estimates helped industry veterinarians target their review of visitor logs and other activity on the infected premises to identify possible pathways of introduction and to conduct tracebacks on likely exposures.

Predictions from diagnostic test results were requested by some industry veterinarians during the outbreak to support operational planning decisions such as scheduling birds for processing. The disease transmission simulation model was used to predict the time at which there were no infectious birds in the barn based on diagnostic test results. As an example, predictions were made based on a test result of 10/10 seropositive serum samples and 1/1 rRT-PCR positive samples with the samples for both tests taken on the same day. This test result was observed in 5 barns during the outbreak, including Kandiyohi 3/Barn 8, Stearns 2/Barn 1, Stearns 3/Barn1, Stearns 3/Barn 2, and Stearns 4/Barn 4. For model validation, the predicted time range was compared to two different observed intervals in the diagnostic testing data, the time until negative rRT-PCR test results were obtained and the time until virus isolation was not successful. The model predictions were consistent with the observed rRT-PCR interval for all 5 barns. The model predictions were conservative with respect to negative virus isolation results, with the predicted time to stop shedding being later than the observed interval in 3 out of 5 barns.

The results of this analysis are subject to some uncertainty due in part to a lack of information from infection and transmission studies with the LPAI H5N2 strain. Proxy H5 and H7 LPAI strains were used instead in the transmission simulation model. Furthermore, the time between diagnostic tests was in some cases several days, which can also introduce uncertainty into the results. Nevertheless, the results of this analysis demonstrate the usefulness of diagnostic testing to better understand the behavior of LPAI in infected poultry flocks.

Methods Overview

Summary of Sampling Data

The time of virus introduction was estimated for each barn that tested positive by rRT-PCR on the eight LPAI virus infected meat-type turkey premises in Minnesota. In total, 33 barns tested positive across the eight premises. The data consisted of the results for oropharyngeal swabs tested by rRT-PCR and serum samples tested by AGID or ELISA for each barn. There is some uncertainty in the number of swabs in each rRT-PCR pooled sample. For the purposes of this analysis, each rRT-PCR pool was assumed to consist of 11 swab samples. A sensitivity analysis performed assuming 10 swabs per pool produced minor differences in the results, suggesting the results are robust to this change in the pool size. The results of the sensitivity analysis are given in Appendix B. A summary of the testing dates, type of testing, amount of sampling performed, and test results are given for two barns on two different premises in Table 3 and Table 4. The testing schedules for the other 31 barns are provided in Appendix B.

Table 3. Summary of surveillance protocols and test results from a barn on an LPAI-infected commercial meat-type turkey premises (Stearns 1) in Minnesota.

Stearns 1: Barn 2				
Date	Test type	Sample[a]	Test result	Average (+) Ct value/ELISA titer
10/30/2018	rRT-PCR	2 pooled samples	2/2 positive	19
10/30/2018	AGID	10 serum samples	7/10 positive	NA
11/05/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/08/2018	rRT-PCR	3 pooled samples	1/3 positive	36
11/08/2018	ELISA	10 serum samples	10/10 positive	2233
11/12/2018	rRT-PCR	3 pooled samples	0/3 positive	NA

[a]Pooled samples consisted of 10 or 11 oropharyngeal swabs.

Table 4 Summary of surveillance protocols and test results from a barn on an LPAI infected commercial meat-type turkey premises (Stearns 4) in Minnesota.

Stearns 4: Barn 1				
Date	Test type	Sample[a]	Test Result	Average (+) Ct value/ELISA titer
11/02/2018	rRT-PCR	2 pooled samples	1/2 positive	37
11/06/2018	ELISA	10 serum samples	0/10 positive	NA
11/12/2018	rRT-PCR	1 pooled sample	1/1 positive	21
11/12/2018	ELISA	10 serum samples	0/10 positive	NA
11/19/2018	rRT-PCR	1 pooled sample	1/1 positive	32
11/19/2018	ELISA	10 serum samples	9/10 positive	2233
11/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/03/2018	rRT-PCR	1 pooled sample	0/1 positive	NA

[a]Pooled samples consisted of 10 or 11 oropharyngeal swabs.

Overview of Modeling Approach

The possible dates of virus introduction for each barn were estimated using a simulation-based approach. The number of infectious and seroconverted birds over time were estimated from a within-house stochastic disease transmission model. These numbers were then used to estimate the likelihood of observing the test results for a given range of potential disease introduction dates. The most likely date of LPAI virus introduction and its 95% CI were estimated from the overall combined likelihood values for all the observed test results.

The stochastic disease transmission model was parameterized specifically for turkeys infected with LPAI virus. Distributions for the length of the latent and infectious periods at the bird level were estimated from inoculation studies in which turkeys were infected with H5 or H7 LPAI virus strains using a maximum likelihood approach (Comin et al, 2011; Iqbal et al, 2012; Pantin-Jackwood et al., 2017; Pillai et al., 2010; Saenz et al., 2012; Spackman et al., 2010). The same approach was used to estimate a distribution for the length of time from infection until seroconversion. However, due limited data on H5 or H7 LPAI virus strains, inoculation studies involving any LPAI virus strain were included in the estimation of the time to seroconversion distribution (Dundon et al., 2007; Homme et al., 1970; Morales, 2008; Preskenis, 2010). Detailed information on model parameters is provided in Appendix B.

The disease transmission model was also used to predict the time at which there were no infectious birds in the barn given a set of available diagnostic test results. For model validation, the predicted time until no infectious birds was compared to both the observed time period to obtain negative rRT-PCR test results and the observed time period until virus could not be isolated from the rRT-PCR sample. The observed range of dates from the rRT-PCR test results consisted of the date of the last rRT-PCR positive result and the date of the following rRT-PCR

negative. The range of dates from the virus isolation results consisted of the date of the last instance where virus could be isolated from an rRT-PCR sample and the following date when virus could not be isolated from an rRT-PCR sample. The test results considered consisted of 10/10 seropositive serum samples and 1/1 positive pooled tracheal swab sample (up to 11 swabs per pooled sample) tested by rRT-PCR, with both the serum and oropharyngeal swab samples taken on the same day. Predictions were made for the 5 barns in which these test results were observed during the outbreak.

Results

The estimated most likely date of virus introduction (95% CI) for each barn ranged from 7 October to 23 November 2018 (Table 5). Two barns were estimated to have been infected on 7 October (Kandiyohi 1/Barn 2 and Stearns 1/Barn 3). Stearns 2/Barn 1 was estimated to have been infected next with a likely date of virus introduction only one day later, on 8 October. Next, Kandiyohi 2 was estimated to have been infected on 19 October. Stearns 3 was estimated to have been infected on 26 October, while Kandiyohi 4 and Stearns 4 were estimated to have been infected on 28 October. The premises estimated to have the latest date of virus introduction was Kandiyohi 3, which was estimated to have been infected on 6 November. However, the barn with the latest date of virus introduction was Kandiyohi4/Barn1, which was estimated to have been infected on 23 November.

The estimates for the time of virus introduction in barns on a single premises were in some cases quite variable. For example, the number of days between the earliest and latest most likely date of virus introduction for houses on Kandiyohi 4 was 26 days, the largest difference for any premises. In contrast, the difference between the first and last infected barns was estimated to be only 7 days on Kandiyohi 2 and Stearns 1. The average difference between the times of the first and last infected barns was estimated to be 13 days for the seven premises where more than one barn tested positive.

In order to visualize how well the actual test results align with the estimated introduction times, the likelihood of obtaining the test result on the date it was observed for a given time of virus introduction for individual rRT-PCR and serology tests, as well as the combined likelihood for all test events, are shown for Stearns1/Barn2 in Figure 2 and Stearns4/Barn1 in Figure 3. These figures illustrate how the likelihoods of individual test results vary across different possible introduction times and impact the overall likelihood considering all the test results.

Understanding the relationship between seroconversion and the time at which virus cannot be detected or isolated from an rRT-PCR test sample can improve our understanding of how LPAI spreads through flocks and the length of time until no infectious birds remain in the barn. In 12 out of the 33 barns, at least one positive rRT-PCR test result was observed on or after the date when 100% of the serum samples tested positive. In comparison, virus isolation was successful on or after the date of the 100% positive serum samples in only 4 barns. Table 6 provides the number of days between the first instance when 100% of the serum samples tested positive and the date when all rRT-PCR samples tested negative, as well as the number of days between the date of 100% positive serum sample results and the first date when virus could not be isolated. The median number of days between the 100% seropositive and negative rRT-PCR result was 4

days (0-15 days, 5-95% CI respectively). The median number of days between the 100% seropositive and negative virus isolation result was 0 days (0-7 days, 5-95% CI, respectively).

The average number of days between the estimated most likely date of virus introduction and the date of the first positive diagnostic test result across all the barns was 9 days. Similarly, the average number of days between the estimated most likely date of virus introduction and the first rRT-PCR negative test result, with all subsequent rRT-PCR samples also testing negative, was 26 days. The average time between the estimated most likely date of virus introduction and first virus isolation negative test result was 20 days.

The relationship between the adequate contact rate parameter and the day of virus introduction is shown in Figure 4 for Stearns1/Barn2 and in Figure 5 for Stearns 4/Barn1. The figures display the likelihood for each evaluated contact rate and day of virus introduction pair. Lower contact rates represent slower within-house infection spread, which lead to earlier estimated virus introduction times. In both figures there is a well-defined high likelihood region for the day of virus introduction, which means both an upper and lower confidence bound could be obtained for the parameter estimate. In the case of the contact rate, only in Figure 5 is there a well-defined region with high likelihoods. In Figure 4, high likelihoods persist at the upper bound of the evaluated contact rate values. Figure 4 was the most commonly observed result in the barns, where a lower and upper bound could be estimated for the day of virus introduction, but only a lower bound could be estimated for the contact rate.

The predicted and observed ranges for the date when infectious birds were no longer present in the barn following the test result of 10/10 seropositive serum samples and 1/1 positive samples by rRT-PCR are given in Table 7 for the five barns in which this test result was observed on a single day of testing. The predicted range compares favorably to the observed rRT-PCR test results with the 95% prediction interval (PI) overlapping with the observed range in all five barns. Conversely, the PI and observed range from the virus isolation test results overlap in only two of the five barns.

Table 5 Mostly likely date of LPAI virus introduction (95% CI) for the barns that tested positive during the 2018 H5N2 outbreak in Minnesota, estimated from rRT-PCR and serology diagnostic testing data.

Premises	Most likely date of virus introduction (95% CI)	Barn
Kandiyohi 1	October 7 (Sep 13 – Oct 09)	Barn 2
	October 18 (Oct 14 – Oct 19)	Barn 1
	October 23 (Oct 20 – Oct 24)	Barn 4
	October 24 (Oct 22 – Oct 25)	Barn 3
Stearns 1	October 7 (Sep 22 – Oct 11)	Barn 3
	October 11 (Sep 29 – Oct 15)	Barn 1
	October 14 (Sep 27 – Oct 18)	Barn 2
Stearns 2	October 8 (Sep 22 – Oct 13)	Barn 1
Kandiyohi 2	October 19 (Oct 12 – Oct 22)	Barn 1
	October 19 (Oct 13 – Oct 23)	Barn 5
	October 24 (Oct 14 – Oct 28)	Barn 3
	October 25 (Oct 14 – Oct 28)	Barn 2
	October 26 (Oct 18 – Oct 30)	Barn 4
Stearns 3	October 26 (Oct 19 – Oct 28)	Barn 3
	October 31 (Oct 27 – Nov 4)	Barn 4
	November 8 (Nov 2- Nov 10)	Barn 1
	November 8 (Nov 2 – Nov 10)	Barn 2
Stearns 4	October 28 (Oct 14 – Oct 29)	Barn 4
	October 28 (Oct 18 – Oct 31)	Barn 1
	November 2 (Oct 27 – Nov 6)	Barn 2
	November 9 (Oct 27 – Nov 12)	Barn 3
Kandiyohi 4	October 28 (Oct 19 – Nov 8)	Barn 2
	November 6 (Oct 23 – Nov 11)	Barn 4
	November 19 (Nov 10 – Nov 22)	Barn 3
	November 23 (Nov 15 – Nov 29)	Barn 1
Kandiyohi 3	November 6 (Oct 31 – Nov 8)	Barn 8
	November 11 (Oct 31 – Nov 13)	Barn 1
	November 11 (Oct 31 – Nov 13)	Barn 3
	November 11 (Oct 31 – Nov 13)	Barn 7
	November 15 (Nov 9 – Nov 17)	Barn 2
	November 15 (Nov 9 – Nov 17)	Barn 4
	November 15 (Nov 9 – Nov 17)	Barn 6
	November 16 (Nov 9 – Nov 18)	Barn 5

Table 6 The number of days from the first 100% seropositive test result until the first rRT-PCR negative test result with no rRT-PCR positive test results following, and the number of days from the first 100% seropositive test result until the first rRT-PCR test.[2]

Premises	Barn	Days from the first 100% seropositive test result to first rRT-PCR negative, no subsequent positives	Days from the first 100% seropositive test result until the first negative virus isolation
Kandiyohi 1	Barn 1	11 days	0 days
	Barn 2	15 days	8 days
	Barn 3	14 days	0 days
	Barn 4	14 days	0 days
Kandiyohi 2	Barn 1	4 days	4 days
	Barn 2	3 days	0 days
	Barn 3	11 days	4 days
	Barn 4	4 days	0 days
	Barn 5	4 days	0 days
Kandiyohi 3	Barn 1	0 days	0 days
	Barn 2	0 days	0 days
	Barn 3	0 days	0 days
	Barn 4	0 days	0 days
	Barn 5	0 days	0 days
	Barn 6	0 days	0 days
	Barn 7	0 days	0 days
	Barn 8	5 days	0 days
Kandiyohi 4	Barn 1	0 days	0 days
	Barn 2	0 days	0 days
	Barn 3	NA	NA
	Barn 4	6 days	6 days
Stearns 1	Barn 1	0 days	0 days
	Barn 2	4 days	0 days
	Barn 3	6 days	0 days
Stearns 2	Barn 1	6 days	1 days
Stearns 3	Barn 1	8 days	7 days
	Barn 2	7 days	7 days
	Barn 3	0 days	0 days
	Barn 4	0 days	0 days
Stearns 4	Barn 1	NA	NA
	Barn 2	NA	NA
	Barn 3	NA	NA
	Barn 4	21 days	0 days

[2] Cases where the negative rRT-PCR test or negative virus isolation result was observed prior to the 100% seropositive test result were set to zero. No 100% seropositive test result was observed in Barn 3 on Kandiyohi 4 and Barns 1-3 on Stearns 4.

Table 7 Predicted and observed dates when infectious birds were no longer present in the flock following test results of 10/10 seropositive and 1/1 rRT-PCR positive samples for the five barns in which this test result was observed.

Premises/ Barn	Sampling day for positive test results	Predicted most likely date no infectious birds in the flock (95% CI)	Observed date rRT-PCR negative	Observed date virus isolation negative
Kandiyohi 3/ Barn 8	11/21/2018	12/02 (11/25 – 12/11)	11/21 – 11/26	11/15 – 11/21
Stearns 2/ Barn 1	10/31/2018	11/11 (11/03 – 11/21)	11/01 – 11/06	10/31 – 11/1
Stearns 3/ Barn 1	11/20/2018	12/01 (11/23 – 12/11)	11/27 – 11/28	11/20 – 11/27
Stearns 3/ Barn 2	11/20/2018	12/01 (11/23 – 12/11)	11/20 – 11/27	11/20 – 11/27
Stearns 4/ Barn 4	11/12/2018	11/23 (11/16 – 12/02)	11/26 – 12/03	11/2 – 11/11

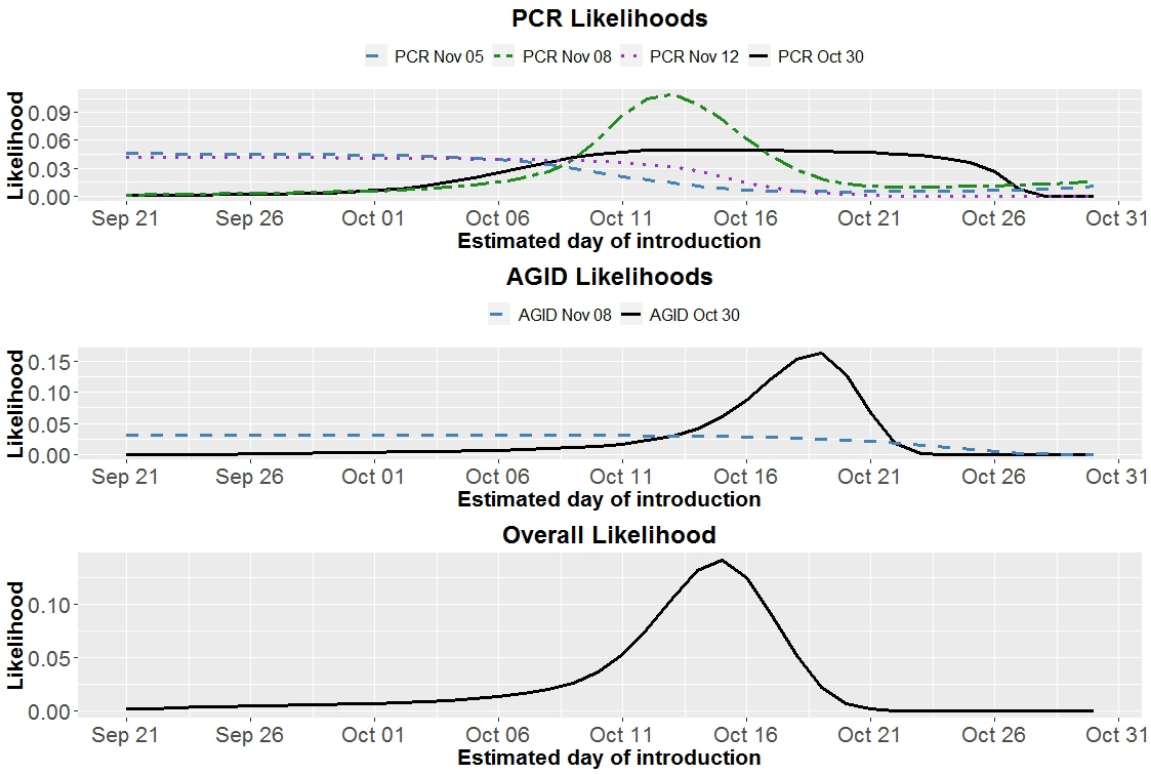


Figure 2 Likelihood of obtaining the test result on the date it was observed in the data for a given day of introduction for individual rRT-PCR test results, individual AGID test results, and the overall likelihood of the combined test results estimated from the Stearns 1/Barn 2 diagnostic test data.

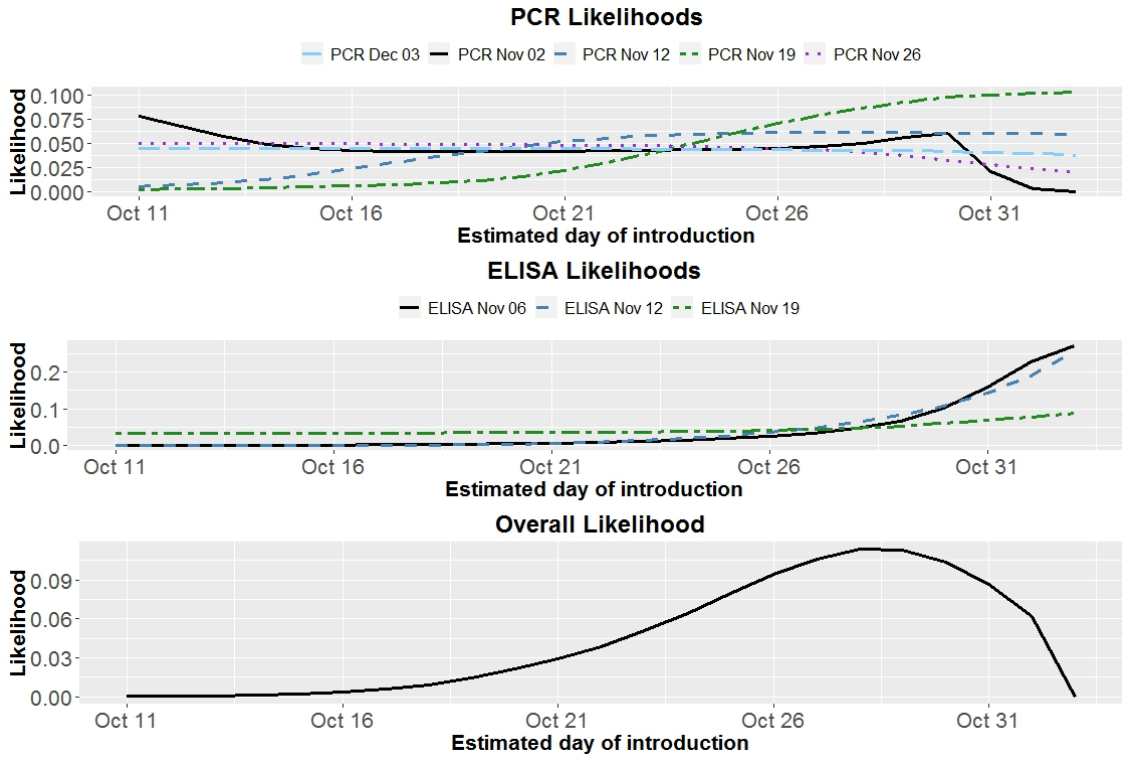


Figure 3 Likelihood of obtaining the test result on the date it was observed in the data for a given day of introduction for individual rRT-PCR test results, individual ELISA test results, and the overall likelihood of the combined test results estimated from the Stearns 4/Barn 1 diagnostic test data.

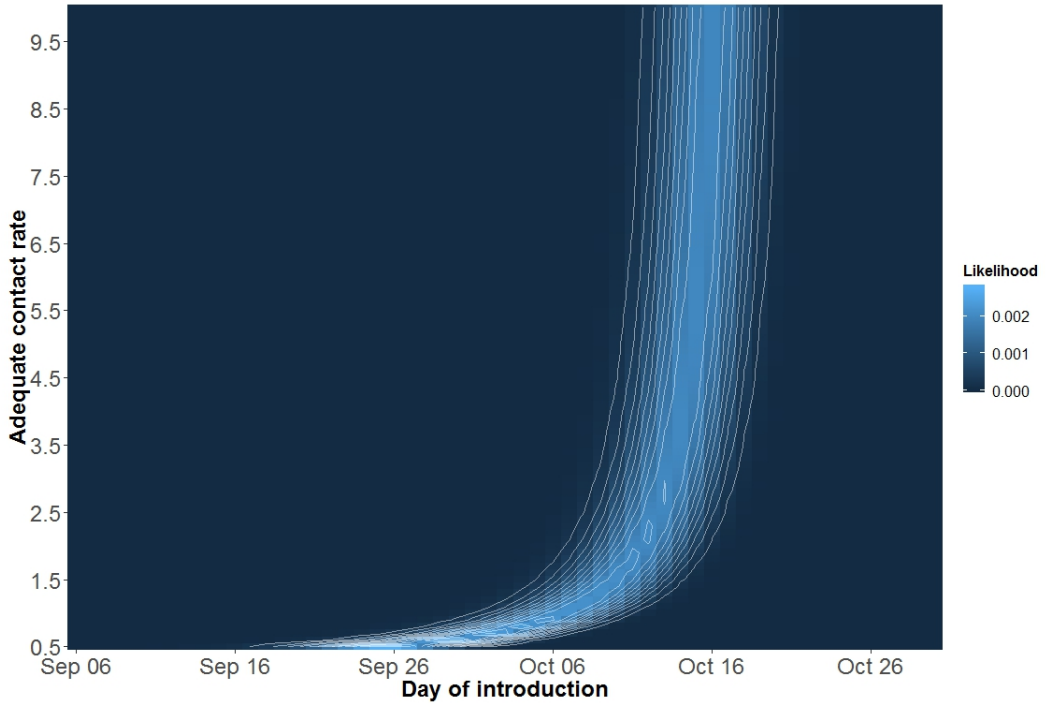


Figure 4 Contour plot of the likelihood of observing the diagnostic test results for Stearns 1/Barn2 given different adequate contact rate and date of virus introduction pairs.

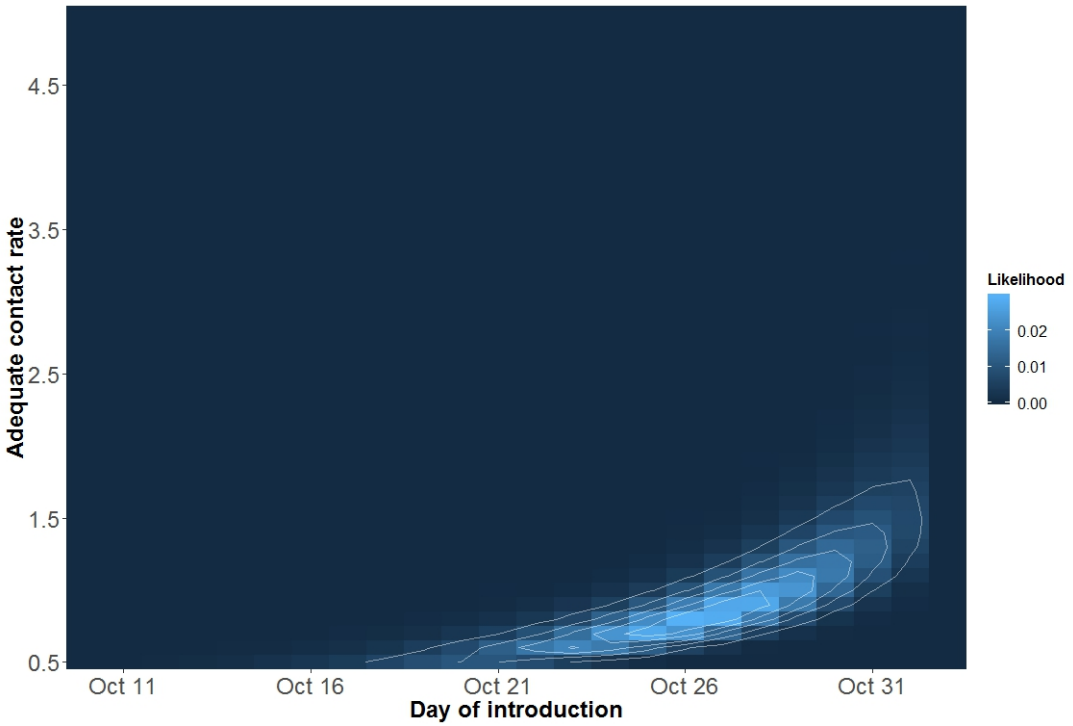


Figure 5 Contour plot of the likelihood of observing the diagnostic test results for Stearns 4/Barn1 given different adequate contact rate and date of virus introduction pairs.

Discussion

Diagnostic test results can be used to estimate the day of LPAI virus introduction by providing insight into the stage of infection in the flock. Understanding of the transmission dynamics of the disease and of the flock must be considered as well; for example, caged birds would be expected to have a different transmission pattern than floor raised birds. Generally, where no seroconversion is detected in the face of virus detection suggest that the infection is in the earlier stages, when few birds have seroconverted. Conversely, if all serum samples test positive, then the infection is more likely to be in the later stages or no longer present in the flock. Where seropositivity (not all serum samples test positive) is incomplete, the rRT-PCR results from swabs aid in understanding the potential time of virus exposure. Baseline model results indicate that it may take 9 to 16 days post exposure for 50 percent of the flock to seroconvert depending on the rate of within house disease spread.

As can be observed in Table 5, Kandiyohi 1 and Stearns 1 had the earliest likely date of virus introduction of 7 October 2018. However, the presumptive index barn on Kandiyohi 1 had an earlier lower confidence bound of 13 September 2018 as compared to the presumptive index barn on Stearns 1, which had a lower confidence bound of 22 September 2018. Stearns 2 was estimated to have a highly similar most likely date of virus introduction of 8 October 2018. The similar estimated times of introductions for the index premises in these two counties is consistent with independent introductions from a common source, as also indicated by the phylogenetic data. The most likely date of virus introduction for the remaining premises, given in chronological order, was estimated to be 19 October for Kandiyohi 2, 26 October for Stearns 3, 28 October for Kandiyohi 4 and Stearns 4, and 6 November for Kandiyohi 3. Narrowing the

time window of possible LPAI virus introduction can help in the identification of potential routes of virus introduction and improve the understanding of the pattern of disease spread. In this Minnesota outbreak, the model results were used by industry veterinarians to focus on potential epidemiological contacts during the estimated range of virus introduction times while screening out those occurring outside the window of potential virus introduction dates.

Diagnostic test results provide information on the flock status and suggests that such data could be used to make predictions in real-time during an outbreak to help assess risk and aid in decision-making. As an example, the time until no more infectious birds were present in the flock was predicted using the disease transmission model from a test result of 10/10 seropositive and 1/1 rRT-PCR positive samples from five barns where this result was observed with the samples for both tests taken on the same day. The results in Table 7 indicate that the model provided reasonable predictions in all 5 barns for the observed range related to the rRT-PCR test results. The model predictions were conservative with respect to negative virus isolation results with the predicted time to stop shedding being later than the observed interval in 3 out of 5 barns. The predictions may be improved by using a more informative contact rate distribution, which determines the rate of disease spread in the transmission model.

In general, negative rRT-PCR and virus isolation results were observed within a short interval of observing a 100% seropositivity. However, viral RNA (e.g. rRT-PCR results) was detected in some barns up to 2 weeks after the 100% seropositive result. This is not an unexpected finding as experimental studies confirm that viral RNA may still be detected by rRT-PCR after seroconversion. In the current outbreak, no viable virus was recovered from any of the positive rRT-PCR samples collected between 3 and 13 days after the 100% seropositive result.

For rRT-PCR positive samples with Ct values above 35 (1 out of 31 rRT-PCR samples) virus was recovered only from one (Ct 37.7). Where the observed diagnostic test results, as well as the disease transmission simulation model, indicate that the flock would likely be at a later stage of infection several days after obtaining a 100% seropositive result, the prevalence of infectious birds would be lower. Further risk assessment is required to address how diagnostic testing data can be applied to support product movement decisions in conjunction with other considerations such as clinical presentation, environmental conditions, and applicable mitigations. Strain-specific experimental and outbreak data will further help reduce uncertainty and inform risk assessments.

Factors contributing to the uncertainty in the estimated time of virus introduction include uncertainty in model parameters such as the adequate contact rate, the inherent variability in disease spread dynamics, and the number and frequency of diagnostic tests results. Data from infection and transmission studies were not available on the LPAI H5N2 strain involved in the outbreak, leading to different H5 and H7 LPAI virus strains being used in the parameterization of the disease transmission model. The behavior of individual LPAI virus strains can differ substantially and the characteristics of the 2018 Minnesota H5N2 strain are uncertain. Nonetheless, the analysis presented here demonstrates the value of diagnostic testing data and its ability to provide information on disease dynamics within a poultry flock.

V. Phylogenetic Analysis and Diagnostics

Phylogenetic Analysis and Diagnostics

North American H5N2 LPAI from turkeys (AM H5N2 2018)

This section describes H5N2 LPAI from turkeys confirmed by the National Veterinary Services Laboratories (NVSL) in October 2018. The first detection in Kandiyohi County was based upon industry-driven enhanced sampling protocol which incorporates testing of drinker biofilm samples. A non-negative drinker biofilm result[3] triggered collection of oropharyngeal (OP) swabs from turkeys on the premises which were tested the same day (19 October 2018) at MPTL with presumptive results by PCR for influenza A and subtype H5. Samples were hand carried to NVSL the next day (20 October 2018), and NVSL confirmed H5N2 LPAI that evening by partial gene sequencing. Additional detections in Kandiyohi County followed.

On 30 October 2018, non-negative results from routine blood samples collected for routine pre-movement testing in Stearns County triggered collection of OP swab samples from the animals. OP swab samples were collected and after samples were tested at MPTL they were sent to NVSL for confirmation. An H5N2 LPAI virus was confirmed on the evening of 31 October 2018 by partial gene sequencing. Although the hemagglutinin (HA) and neuraminidase (NA) gene partial sequence was found to share 99% identity with the virus from Kandiyohi, the full genome data revealed differences across the internal genes and analysis showed that the HA genes clustered separately by county (Figure 6).

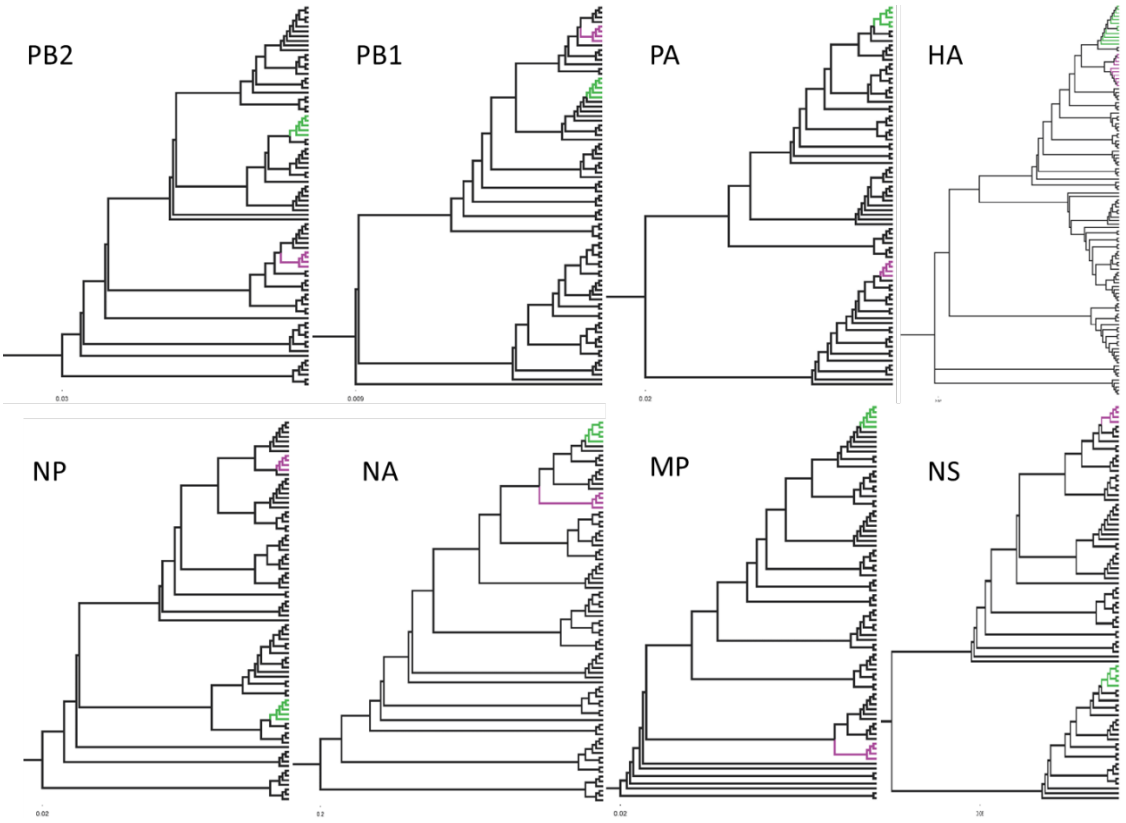
All recovered viruses have been characterized as North American wild bird lineage H5N2 LPAI, and representative viruses have an intravenous pathogenicity index (IVPI) of zero as defined by OIE. The current phylogenetic analysis of viruses from the two counties supports independent introductions from a common source. Wild bird data shared from the National Center for Foreign Animal Diseases (NCFAD, Winnipeg, Canada), revealed highly similar wild bird viruses collected in July 2018 from the Central/Mississippi Flyway representing both introductions (Figure 6, Table 8). The lack of epidemiologic links between the counties further supports this finding.

Weekly virus monitoring activities conducted in both counties during quarantine prior to control marketing revealed no changes in the HA gene cleavage site for any of the viruses obtained. The presence of an NA stalk deletion, which may be associated with virus adaptation in poultry, was observed from Kandiyohi 03 (refer to Figure 6b), and acquisition of an NA stalk deletion was noted from the fourth sampling (and the last from which virus was recovered) for Kandiyohi 01.

NOTE: The outcomes of phylogenetic analysis should be interpreted in context of all available virus and epidemiologic information and should not be used directly to infer transmission.

[3] While drinker biofilm samples are not considered an "official sample" per NPIP/NAHLN, this sample type has been shown to allow detection of avian influenza RNA up to 24 hours ahead of animal samples. Approved laboratories accepting this sample type for testing are instructed to request appropriate samples from the flock immediately following any non-negative result to determine the virus status of the flock.

a.



b.

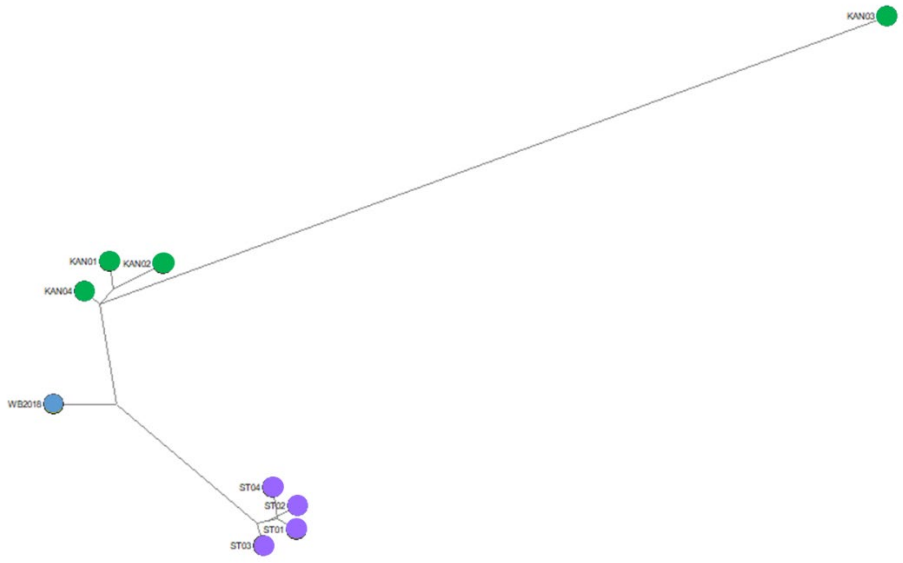


Figure 6 a) Phylogenetic analysis by gene for viruses from Kandiyohi (green) and Stearns (purple) counties; b) concatenated analysis of common genes (HA and NA) of viruses from Kandiyohi[4] (green), Stearns (purple), and Canadian wild birds[5] (blue).

[4] NOTE: Kandiyohi 03 has a long branch due to the presence of a stalk deletion in the NA gene.

[5] Courtesy of ML Killian NVSL, wild bird data from National Center for Foreign Animal Diseases, Winnipeg, Canada

Table 8 Percent sequence similarity between H5N2 LPAI in MN turkeys to the highly similar wild bird viruses from Canada supports independent introductions from a common source.[6]

	Kandiyohi Co. to A/mallard/Sasketchewan/ OTH30-2/2018 (H5N2)	Stearns Co. to A/mallard/Sasketchewan/ OTH30-2/2018 (H5N2)
PB2	96%	95%
PB1	94%	95%
PA	99%	89%
HA	99%	99%
NP	99%	93%
NA	99%	99%
MP	99%	97%
NS (MALL)	99%	78%
NS (NOPI)	79%	99%

Comparison to Other Viruses/Lineages

The H5N2 virus clusters with recent wild bird viruses and apart from other recent H5 poultry detections (2016 Missouri H5N1 LPAI, 2017 Wisconsin H5N2 LPAI). Highly similar viruses were identified from wild bird surveillance in Canada during July 2018 collected in the Central/Mississippi Flyway.

Public Health Aspects

To date, there have been no reports of the H5N2 LPAI 2018 virus infection in humans. The health of response workers and on-farm personnel was monitored at the state level. The virus sequences have been shared with CDC for analysis which indicated that the viruses to date lack key molecular features associated with human receptor binding, increased virulence, or transmission in mammals; no known markers of neuraminidase inhibitor (Oseltamivir) or polymerase inhibitor (baloxavir) resistance have been identified.

Diagnostics and Characterization for Influenza A Viruses

The NVSL rapidly shares genetic and biological materials in collaboration with the Southeast Poultry Research Laboratory, the Influenza Division of the Centers for Disease Control and Prevention, USDA-APHIS Wildlife Services, as well as other key partners. Consensus data from whole genome sequencing are used to monitor the virus evolution and assess the risk to veterinary and public health based upon the presence/absence of specific amino acid substitutions or protein motifs. Analysis of sequence data includes phylogeny of all eight segments and determination of amino acid substitutions across the HA1 protein. Genetic data are also used to confirm that diagnostic assays are fit for purpose. *In silico* analysis confirmed high similarity between the H5N2 virus sequences and the relevant primers and probes used for the IAV and H5 diagnostic rRT-PCR tests.

[6] Courtesy of ML Killian NVSL, wild bird data from National Center for Foreign Animal Diseases, Winnipeg, Canada.

General Information

Avian influenza subtypes H5 and H7 are reportable worldwide because of their potential for mutation to high pathogenicity during replication in poultry. The presence of basic amino acids at the cleavage site contribute to the mutation from low to high pathogenicity. Mechanisms by which H5/H7 mutate from LPAI to HPAI include the gradual accumulation of basic amino acids (AA), insertion of repeated basic AA, and insertion of non-homologous genetic material (only reported for H7 viruses).

Molecular diagnostic tests for influenza A virus (IAV) are used across the U.S. National Animal Health Laboratory Network (NAHLN). The most sensitive and specific tool for influenza A detection is the Type A-specific rRT-PCR, which targets at least the matrix gene (IAV-M); this is the primary surveillance tool used and provides a semi-quantitative result. The NAHLN tests samples first by the IAV-M test and further by the NAHLN H5 and H7 tests where IAV is detected.

All poultry samples with a non-negative test result for IAV (serology or PCR) are forwarded to NVSL for confirmatory testing. The NVSL uses Sanger sequencing protocols to generate partial HA/NA gene sequence directly from the sample for subtype and pathotype determination, when sufficient viral RNA is present. Whole genome sequencing is conducted on all isolated viruses, and select viruses are further characterized by pathotype assay in specific pathogen-free chickens.

NVSL confirms the virus HA and NA subtype through molecular sequencing and/or antibody subtyping, and the pathotype (LPAI vs HPAI); where no virus can be recovered nor sequence obtained directly from sample(s), the pathotype is determined by the clinical presentation of the flock compared to the USDA-APHIS HPAI case definition.

VI. Waterfowl Surveillance

Waterfowl are natural reservoir hosts for influenza A viruses (IAV; subtypes H1-H16), but not usually HPAI. Influenza A viruses in wild birds tend to circulate seasonally within migratory flyways, and subtype prevalence can wax/wane in multiyear cycles. Areas where birds from different flyways congregate provide opportunities for viruses to mix across flyways.

Waterfowl migration in North America generally consists of north-south seasonal movements between breeding grounds and wintering areas. There are four major flyways in North America (Figure 7). These flyways are broadly defined corridors where the migratory paths of many species of interest tend to converge and are associated with major topographical features in North America, which also tend to be aligned along a north-south axis. The four North American flyways have areas of overlap and convergence, particularly at the north and south ends. Flyway boundaries are defined administratively, and are not biologically fixed or sharply defined.

The U.S. National Surveillance Plan for Highly Pathogenic Avian Influenza in Wild Birds was implemented in 2015 to maximize our ability to detect IAV in wild waterfowl. Surveillance helps to: 1) understand how IAV is distributed in the United States, 2) detect the spread of IAV to new areas of concern, 3) monitor wild dabbling duck populations for introductions of novel viruses, and 4) estimate the apparent prevalence of IAVs of concern (e.g., Eurasian lineage H5 and H7). The surveillance plan targets areas with extensive mixing of wild bird populations and a history of IAV detection.

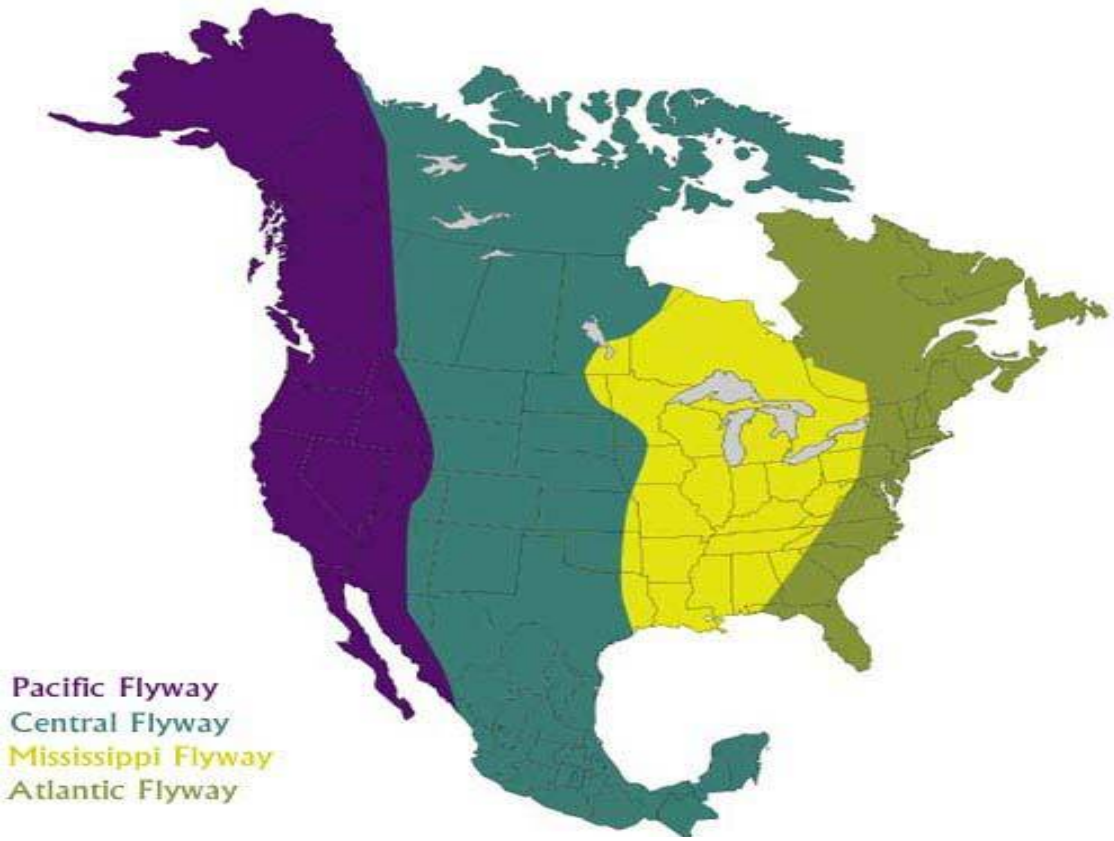


Figure 7 The four primary North American waterfowl flyways.

From March 1 to October 31, 2018, 1996 wild waterfowl and gulls were sampled and tested by rRT-PCR for avian influenza A infection. (Information on the sampling in January and February, 2018, was summarized in the report *Epidemiological and Other Analyses of Avian Influenza Affected Poultry Flocks, May 25, 2018*)

Sampling was opportunistic and limited to eight states: Alaska, Oregon and Idaho in the Pacific Flyway; Colorado and North Dakota in the Central Flyway; Minnesota and Ohio in the Mississippi Flyway; and Connecticut in the Atlantic flyway (Figure 8, Figure 10). The majority of samples were collected from live-captured birds in the months of August and September (Figure 9). Gulls in North Dakota were sampled at landfill facilities in the course of management activities (n=206).

Avian influenza A was detected in a total of 602 samples, or 30% of the total. This is a high prevalence, and is skewed because, with limited resources for sampling, we targeted areas with historically high prevalence in the late summer, when the occurrence of AI in wild birds is highest.

In spite of the high prevalence of Influenza A, only one H5 sample was successfully isolated (Table 9). This was a blue-winged teal in Colorado with a low pathogenic H5N2 infection. One other bird was identified with a H5 infection, but no isolate was obtained. Six samples were presumptively positive for H7, but no isolates were obtained. All H5 and H7 samples were from dabbling ducks, sampled in August and September with the exceptions of one H5 being sampled on October 31 in Minnesota.

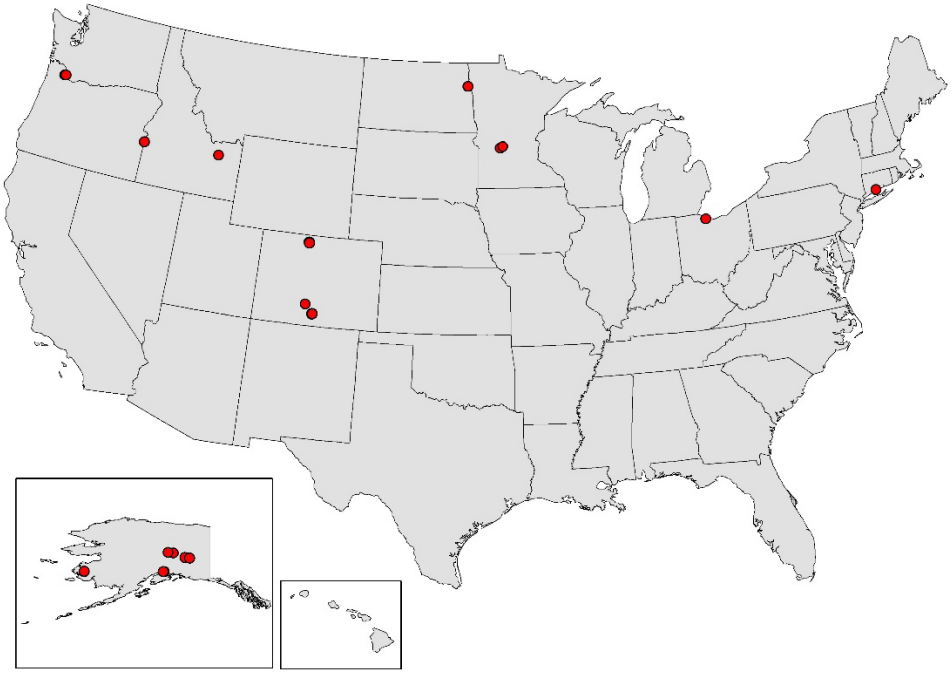


Figure 8 Sampling locations for avian influenza virus A in wild birds from March – October 2018.

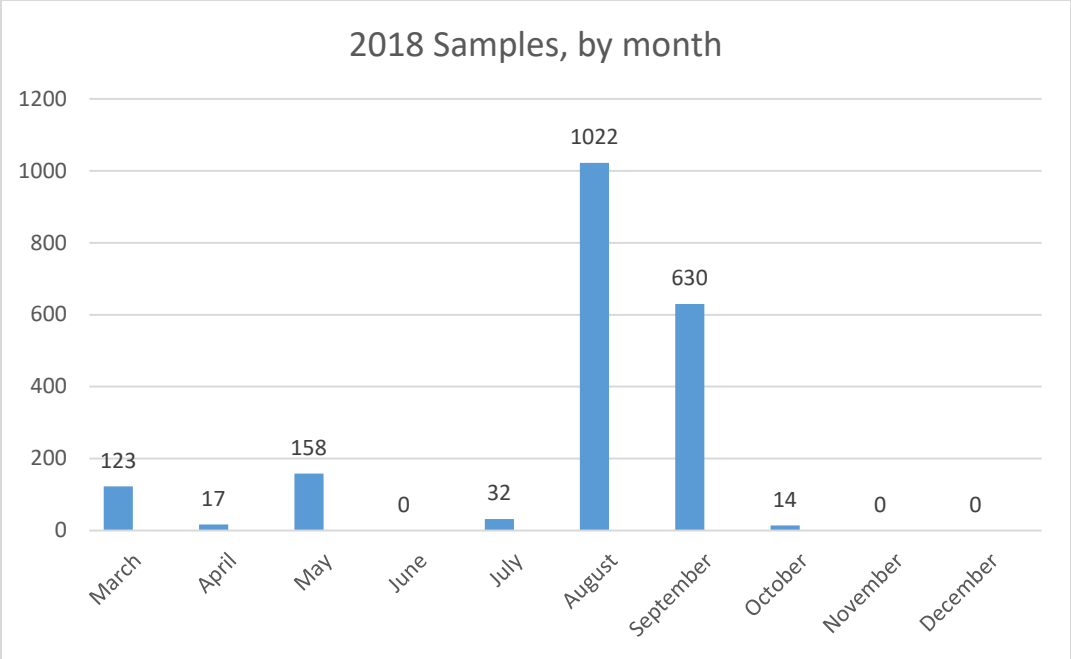


Figure 9 Total samples collected by month between March and December, 2018.

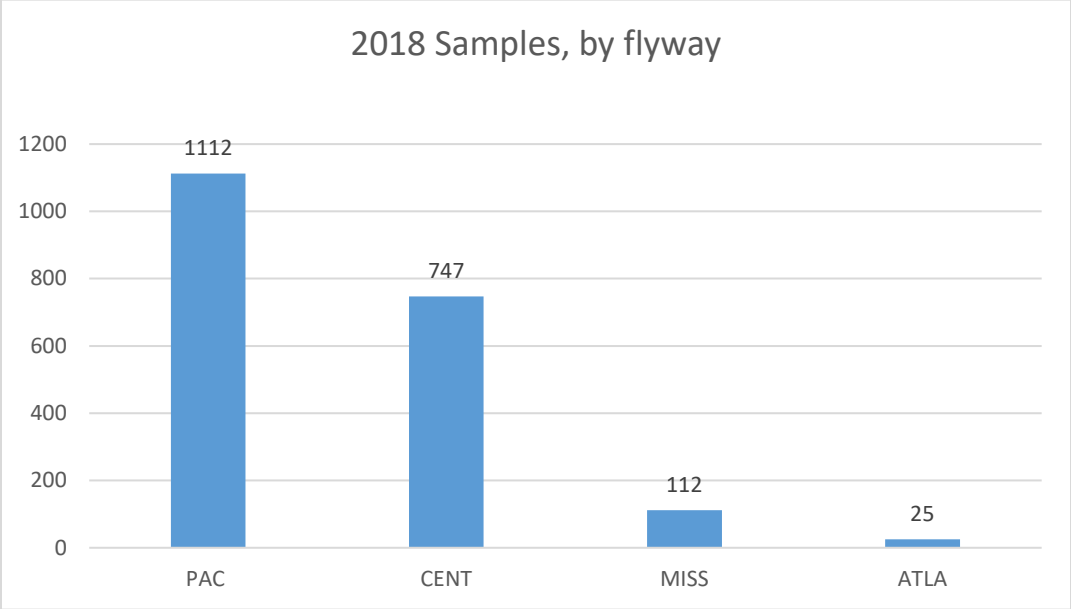


Figure 10 Wild bird sample numbers by flyway between March and December, 2018.

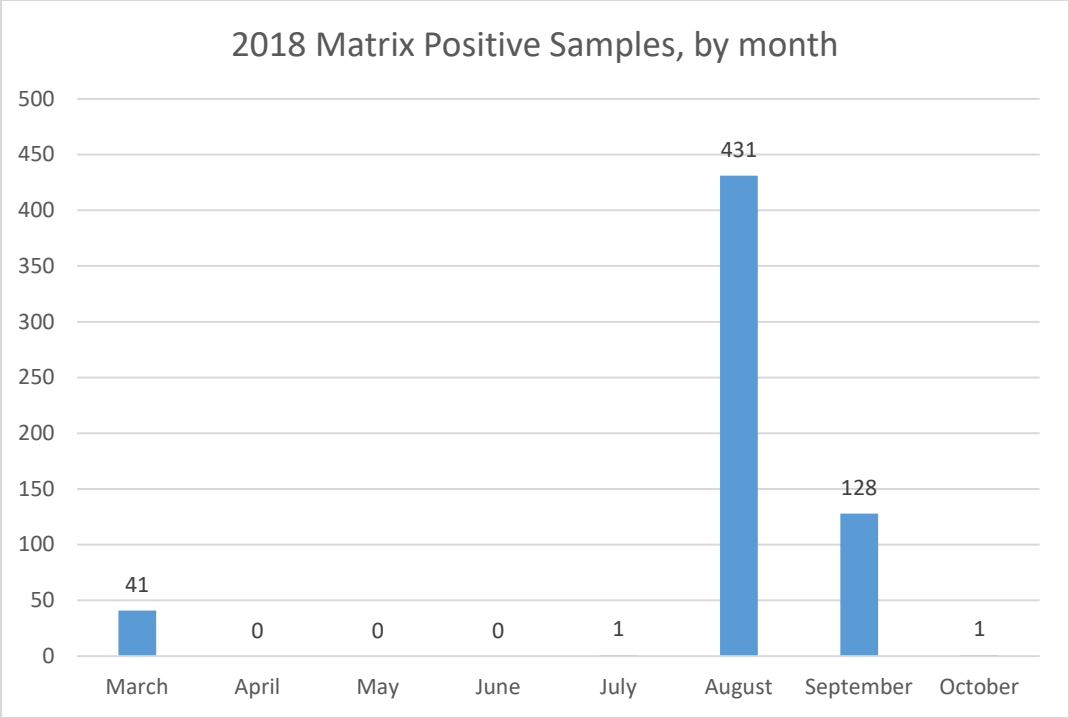


Figure 11 Wild bird matrix positive samples by month from March to October, 2018.

Table 9 2018 H5 and H7 positive samples by state and date collected.

State	Date Collected	Subtype	Species
Ohio	Mar. 22	H7 LPAI	Mallard
Alaska	Aug. 3	H7 LPAI	Mallard
Alaska	Aug. 3	H7 LPAI	Mallard
Alaska	Aug. 3	H7 LPAI	Mallard
Colorado	Aug. 15	H7 LPAI	American Green-winged Teal
Colorado	Aug. 28	H7 LPAI	Mallard
Colorado	Sept. 17	H5N2 LPAI	Blue-winged Teal
Minnesota	Oct. 31	H5 LPAI	Mallard

VII. Using geospatial methods to measure the relative risk of environmental persistence of avian influenza virus in Minnesota

Summary

Introduction of AIV into domestic poultry can be initiated by exposures to infectious wild birds or to virus surviving in the environment. This analysis aimed to define areas in Minnesota at heightened risk for AIV environmental persistence using geospatial methods. Environmental factors known to influence AIV survival were identified through an evaluation of the published literature, and corresponding data were located and downloaded from publicly available sources. Data layers were resampled to a 1km resolution and weighted based on their influence on virus survivability. Maps showing the relative risk of AIV persistence in the Minnesota environment were created from these layers using ESRI's ArcGIS Predictive Analysis Tool. Five categories defined in the World Organization for Animal Health (OIE) Risk Assessment Guidelines were assigned to each 1km cell ranging from very low/negligible to very high. The results presented in this report focus on risk during the fall migration season (September 1 – November 30) which completely encompasses the 2018 LPAI outbreak period from October 20 through November 14. A majority filter was then employed to summarize risk for each county in Minnesota. Finally, maps were produced showing the number of commercial and backyard poultry premises in relation to highest risk counties. These results can be used to improve surveillance activities and to inform biosecurity practices and emergency preparedness efforts within Minnesota.

Model Inputs and Development

Factors known to influence AIV introduction and survival in the ambient environment, focusing on H5 and H7 subtypes, were identified through an evaluation of the peer-reviewed literature. This information was combined with an assessment of data availability and a total of five factors (water presence, water temperature, wetland cover, presence of wildlife refuges, and presence of wild birds) were selected as inputs to develop risk models. These factors, their corresponding data sources, trends relative to AIV survival, and rationale for model inclusion are summarized in Table 10. A detailed description of data processing for each factor is available in Appendix C.

Table 10 Environmental factors selected as model inputs, data sources, trends relative to AIV survival, and rationale for model inclusion.

Factor	Data Source	Trends with AIV	Rationale
Water Presence	USGS Gap Analysis Program	*AIV particles survive well in water compared to air or other dry media*	AIV survives in water more prominently than dry land
Water Temperature	USGS Moderate Resolution Imaging Spectroradiometer (MODIS) remote sensing data	*Inverse association between persistence and increasing temperature: optimal temperatures are near freezing and sub-optimal temperatures are 17-28 °C*	Temperature of water sources greatly impacts the rate of survival of AIV
Wild Bird Presence	Dabbling Duck Occurrence and Abundance model (USGS Patuxent Wildlife Research Center)	*Wild birds are a main reservoir for AIV, and introduce the virus to their surrounding environment*	When carrying the virus, wild birds deposit viral particles into water and other habitat locations
Wetland Cover	United States Fish and Wildlife Service	*Wetland cover provides ideal habitat for migratory birds, and contains aquatic zones where AIV can thrive for long periods*	Previous studies show that wetland areas are associated with AIV presence
Wildlife Refuges	United States Fish and Wildlife Service	*Wildlife refuges are preservation zones of wild bird habitat*	Refuges provide ideal habitat for AIV reservoir birds

Model Execution

Risk of environmental AIV persistence was determined using the Esri Predictive Analysis (PA) Tools Add-In within ArcGIS 10.6 (PA Add-In: http://appsforms.esri.com/products/download/). The PA Add-In is a collection of tools used to create models to predict the location of suitable sites based on input factors, in this study environmental or wild bird conditions. The PA tool was used to build a simple additive expression with environmental factors weighted based on their relative contribution to the persistence of AIV, as determined through subject matter expert (SME) consultation and literature review (Table 11).

The 1km risk model was then aggregated to a county level using a majority statistic tool in ArcMap. Additional analyses were performed to determine the proximity of LPAI-infected operations to moderate and high risk 1km cells within 3km and 10km.

Table 11 Environmental factors, layer values, and PA add-in query expression weights used to develop the final predictive model for the persistence of avian influenza virus in the environment.

Factor	Layer Values	Weighted PA Values
Wild Birds	1 = Presence	3
	0 = Absence	0
Water Temperature	1 = < 10°C	3
	2 = 10°C ≤ > 20°C	2
	3 = ≥20°C	1
Water Presence	1 = Fresh water	3
	0 = No water	0
Wetlands	1 = Presence	2
	0 = Absence	0
Wildlife Refuges	1 = Presence	3
	0 = Absence	0

Results and Discussion

The raw model output values (ranging from 0 – 14) were reclassified by quantile to illustrate relative risk (OIE categories ranging from 0 – 5) (Figure 12). The quantile classification method distributes roughly the same number of 1km cells into each of the five risk categories. The five risk categories displayed correspond to the OIE Risk Assessment Guidelines for describing risk: negligible/very low, low, moderate, high, and extremely high risk (OIE, 2017). Reclassification followed the scheme outlined in Table 12.

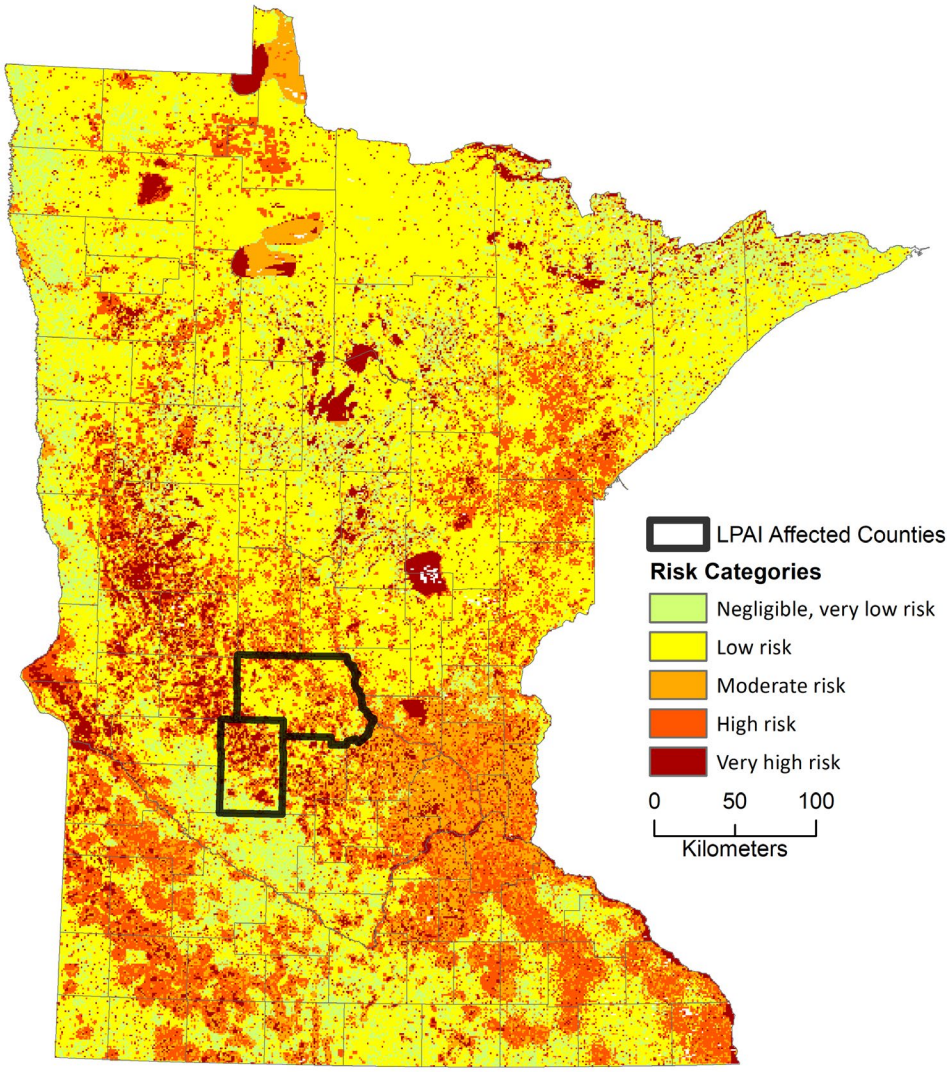


Figure 12 Risk (1 km resolution) of fall (September 1 – November 30) environmental persistence of AIV in Minnesota and LPAI-affected counties (October 20 – November 14, 2018).

Table 12 PA model values and reclassification into OIE risk categories.

Original model value	Risk category (value)
0	*Negligible/Very low risk (0)*
1-2	*Low risk (1)*
3-4	*Moderate risk (2)*
5	*High risk (3)*
6-14	*Very high risk (4)*

The risk of environmental persistence of AIV derived from the 1km resolution models was then aggregated to the county level using the majority zonal statistic in ArcMap (Figure 13; http://desktop.arcgis.com/en/arcmap/10.3/tools/spatial-analyst-toolbox/zonal-statistics.htm). The majority zonal statistic computes the most frequently occurring original model value within each zone, in this case, the county. The resulting majority values for each county were reclassified into one of three risk categories following the scheme in Table 13. Out of 87 counties in Minnesota, 61 counties, including those affected by LPAI in fall 2018 (Kandiyohi and Stearns), had low risk, 11 counties had moderate risk, and 15 counties had high risk.

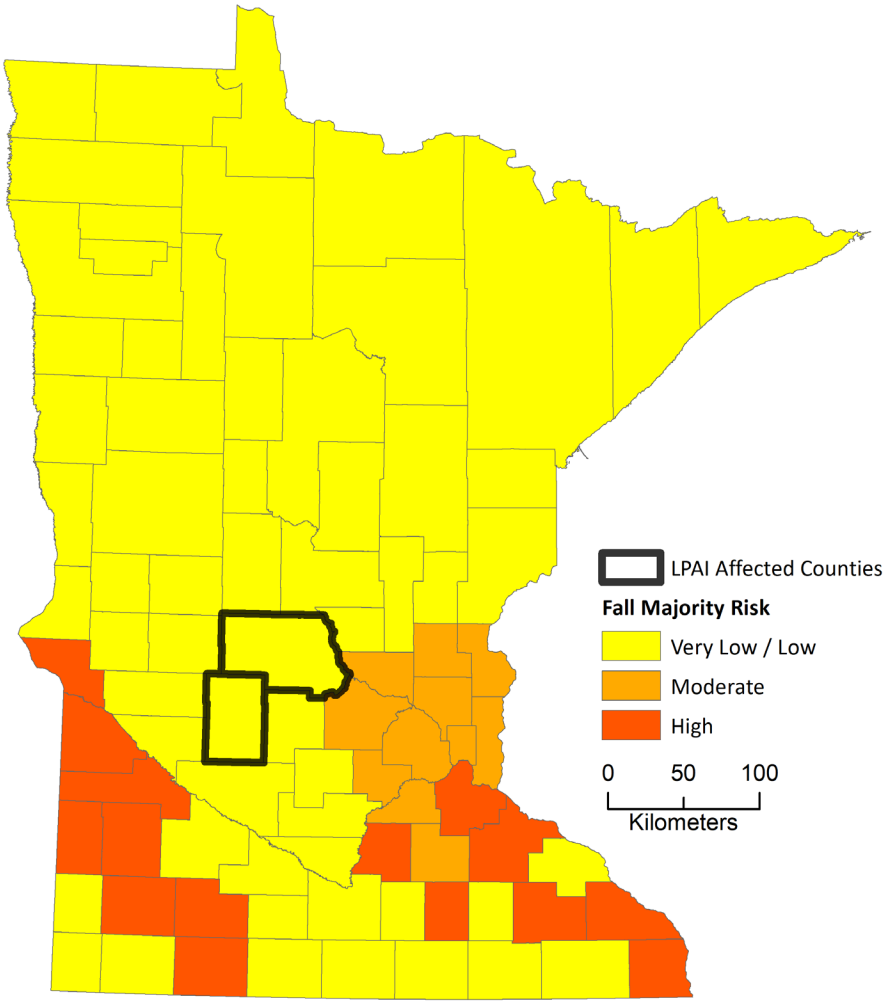


Figure 13 County level AIV environmental persistence risk in Minnesota in fall (September 1 – November 30) and LPAI-affected counties (October 20 – November 14, 2018).

Table 13 County-level AIV environmental persistence risk reclassification scheme.

Majority value	Risk category (value)
0 -2	*Very Low / Low*
3	*Moderate*
5	*High*

Figures 14 and 15 show commercial and backyard poultry operations in combination with counties having high risk for AIV persistence in the environment. Counties in the central portion of the state with the highest number of commercial operations, do not have the highest levels of AIV environmental persistence. Likewise, the highest concentrations of backyard operations are also in the central portions of the state where counties have lower risk of AIV persistence in the environment.

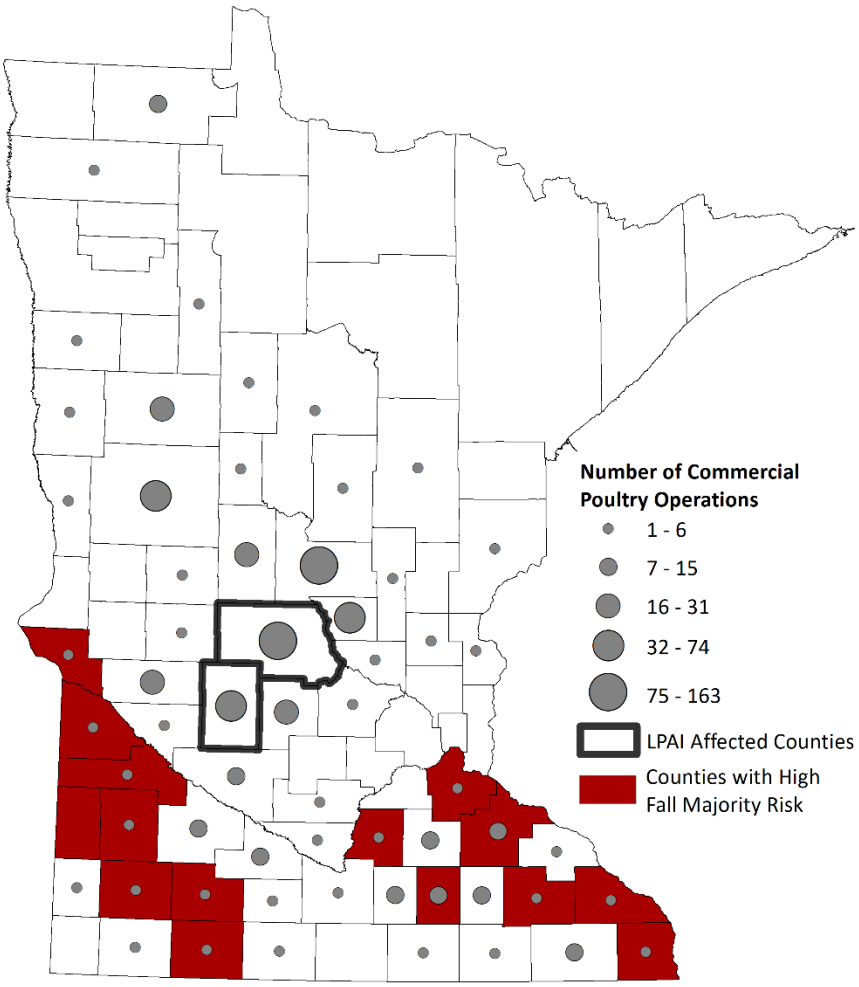


Figure 14 Number of commercial poultry operations, counties with high fall season AIV environmental persistence risk, and LPAI-affected counties (October 20 – November 14, 2018).

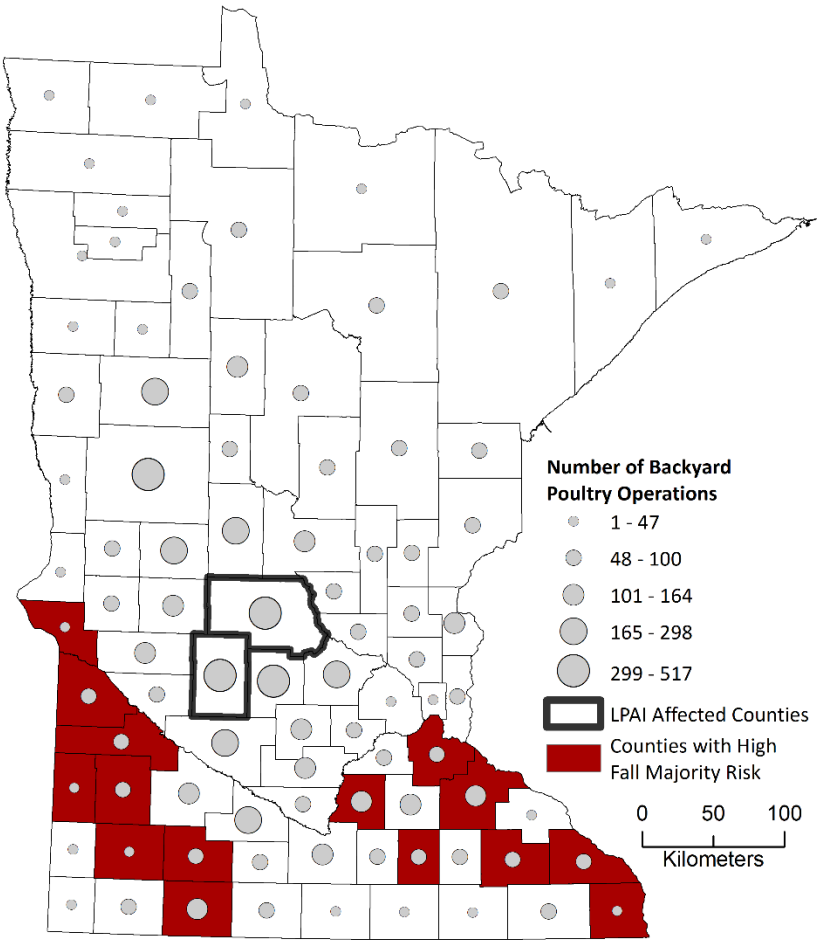


Figure 15 Number of backyard poultry operations, counties with high fall season AIV environmental persistence risk, and LPAI-affected counties (October 20 – November 14, 2018).

Using the majority statistic to aggregate risk to the county level has limitations and may not accurately capture risk at the poultry operation level. In most Minnesota counties, the 1km model shows diversity; i.e., within a given county, AIV environmental persistence risk ranges from low to very high. To better understand risk at the operation level, the diversity and range of 1km risk was evaluated within 3km and 10km buffers surrounding each of the recent Minnesota LPAI-infected operations. These buffer distances correspond to the typical zones used for emergency response and surveillance.

This additional evaluation of diversity in buffers illustrates that even in low risk counties, an individual poultry operation may be in close proximity to moderate and high risk geographic areas at 1km spatial resolution. For example, as illustrated in Figure 16, both 3km and 10km buffer zones around an operation include moderate to very high risk 1km cells. Majority risk, at the county level in which this operation is located, is low. When evaluating the risk of AIV persistence in the environment, scale should be considered. Where feasible, it may be more applicable to use the higher resolution risk model (1km) to evaluate risk of environmental persistence near individual poultry operations.

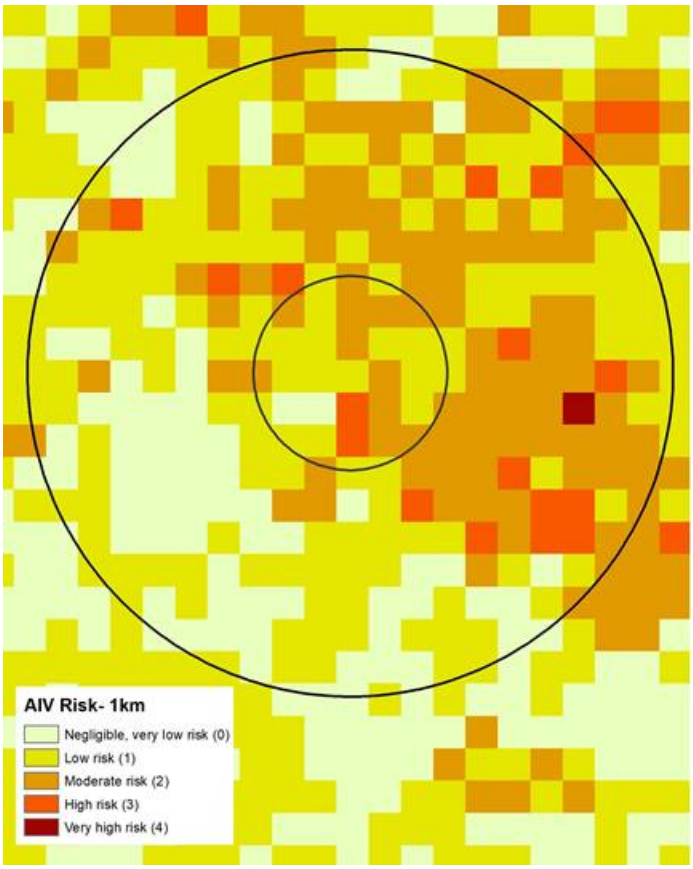


Figure 16 3km and 10km buffer zones around an individual commercial poultry operation (not shown) in the recent Minnesota LPAI outbreak (October 20 – November 14, 2018). Buffers are overlaid with the 1km AIV environmental persistence risk model.

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Appendix A: Case Series Questionnaire

USDA

Animal and Plant Health Inspection Service

Veterinary Services

HPAI Investigation - Questionnaire

INSTRUCTIONS

The purposes of these investigations are to assess potential pathways of initial introduction of HPAI viruses onto commercial poultry operations and potential lateral transmission routes of HPAI viruses from infected premises to noninfected premises.

Following confirmation of an HPAI virus introduction into a commercial flock, an investigation should be initiated as soon as possible, no later than 1 week following detection. The investigator(s) assigned should be integrated into other response activities but their primary focus is on completion of the introduction investigation.

The investigation form provided is a guide for conducting a systematic and standardized assessment of potential pathways of initial virus movement onto the farm and potential movement of the virus off the farm. All sections of the form should be completed through direct conversation with the individual(s) most familiar with the farm's management and operations and questions are to be answered for the period 2 weeks prior to the detection of HPAI. Where applicable, direct observation of the biosecurity or management practice asked about should be conducted. This is not a box-checking exercise but an in-depth review of the current biosecurity and management practices and exposure risks on an affected farm. For example, direct observation of the farm employee donning and doffing procedures and compliance with company biosecurity practices is more important than checking the box on the form that indicates workers wear coveralls into the poultry houses. Investigators are encouraged to take notes and include them with the investigation form when completed.

An investigation form should be completed for the infected house or farm and **at least one** noninfected house or farm within the same complex as near as possible to the index infected flock.

Version 1.0 -March 2015 Page 1

Date: _____

Interviewer name/organization: _____

Interviewee name/organization: _____

A. PREMISES INFORMATION

Farm name: _____

Farm address: _____

Farm (premises) ID: _____ County: _____

Township: _____ Range: _____ Section: _____

Is facility enrolled in NPIP?..... ☐1 Yes ☐3 No

B. PREMISES CONTACT INFORMATION

1. Contact name: _____

   Phone: _____ Cell phone: _____ Email: _____

2. Contact name: _____

   Phone: _____ Cell phone: _____ Email: _____

3. Contact name: _____

   Phone: _____ Cell phone: _____ Email: _____

4. Flock Veterinarian: _____

   Phone: _____ Cell phone: _____ Email: _____

C. PREMISES DESCRIPTION

1. Poultry type: ☐$_1$ Broiler ☐$_2$ Layer ☐$_3$ Turkey ☐$_4$ Other (specify: _____)

2. Production type: ☐$_1$ Meat ☐$_2$ Egg ☐$_3$ Breeding ☐$_4$ Other (specify: _____)

3. Age: ☐$_1$ Multiple age ☐$_2$ Single age

4. Sex: ☐$_1$ Hen ☐$_2$ Tom ☐$_3$ Both

5. Flock size: ..... _____ # birds

6. Facility type: *[Check all that apply]*

   ☐ Brood

   ☐ Grow

   ☐ Other (specify: _____)

   ☐ Both brooder & grower houses are present on the same premises

   ☐ Breeder

   ☐ Commercial

7. If brooder and grower houses are present on the same premises, are there multiple stages of management (brooding and growing), in the same house? ..... ☐$_1$ Yes ☐$_3$ No

8. Farm capacity ..... _____ # birds

   Number of barns ..... _____ # barns

   Barn capacity ..... _____ # birds

9. What is the **primary** barn type/ventilation: *[Check one only.]*

   ☐$_1$ Curtain sided

   ☐$_2$ Environmental control

   ☐$_3$ Side doors

   ☐$_4$ Other (specify: _____)

10. Are cool cell pads used? ..... ☐$_1$ Yes ☐$_3$ No

    If Yes, what is the source of water for these pads? _____

11. Distance in yards of closest body of water near farm: ..... _____ yd

12. Water body type: *[Check all that apply.]*

☐ Pond

☐ Lake

☐ Stream

☐ River

☐ Other (specify: _____)

13. What other types of animals are present on the farm?

a. Beef cattle ..... ☐$_1$ Yes ☐$_3$ No

b. Dairy cattle ..... ☐$_1$ Yes ☐$_3$ No

c. Horses ..... ☐$_1$ Yes ☐$_3$ No

d. Sheep ..... ☐$_1$ Yes ☐$_3$ No

e. Goats ..... ☐$_1$ Yes ☐$_3$ No

f. Pigs ..... ☐$_1$ Yes ☐$_3$ No

g. Dogs ..... ☐$_1$ Yes ☐$_3$ No

h. Cats ..... ☐$_1$ Yes ☐$_3$ No

i. Poultry or domesticated waterfowl ..... ☐$_1$ Yes ☐$_3$ No

j. Other (specify: _____) ..... ☐$_1$ Yes ☐$_3$ No

14. What is the **primary** water source for poultry? *[Check one only.]*

☐$_1$ Municipal

☐$_2$ Well

☐$_3$ Surface water (e.g., pond)

☐$_4$ Other (specify: _____)

15. Is water treated prior to delivery to poultry? ..... ☐$_1$ Yes ☐$_3$ No

If Yes, how is it treated and with what? _____

D. FARM BIOSECURITY

1. Is there a house with a family living in it on the property? ..... ☐1 Yes ☐3 No
2. Is there a common drive entrance to farm and residence?..... ☐1 Yes ☐3 No
3. Do you have signage of "no admittance" or "biosecure area" on this property? .. ☐1 Yes ☐3 No
4. Is there a gate to this farm entrance?..... ☐1 Yes ☐3 No
5. Is the gate secured/locked? ..... ☐1 Yes ☐3 No
   If Yes, what hours is it secured? _____
6. Is the farm area fenced in?..... ☐1 Yes ☐3 No
7. How frequently is vegetation mowed/bush hogged on the premises?..... _____ times/month
8. Is facility free of debris/clutter/trash piles? ..... ☐1 Yes ☐3 No
9. Is there a wash station/spray area available for vehicles? ..... ☐1 Yes ☐3 No
   If Yes, what disinfectant is used? _____
10. Is there a designated parking area for workers and visitors away from the barns/pens? ..... ☐1 Yes ☐3 No
11. Is there a changing area for workers?..... ☐1 Yes ☐3 No
    Do they shower?..... ☐1 Yes ☐3 No
12. Do workers don dedicated laundered coveralls before entering each house on the premises?..... ☐1 Yes ☐3 No
13. Do worker wear rubber boots or boot covers in poultry houses?..... ☐1 Yes ☐3 No
14. Are the barn/pen doors lockable?..... ☐1 Yes ☐3 No
    Are they routinely locked? ..... ☐1 Yes ☐3 No
15. Are foot pans available at barn/pen entrances?..... ☐1 Yes ☐3 No
    Are they in use?..... ☐1 Yes ☐3 No
16. Are foot baths dry (powdered or particulate disinfectant)? ..... ☐1 Yes ☐3 No
17. Are foot baths liquid disinfectant? ..... ☐1 Yes ☐3 No
18. Frequency foot pan solutions are changed? ..... _____ times/month

What disinfectant is used? _____

19. Is there an entry area in the barns/pens before entering the bird area? ..... ☐1 Yes ☐3 No

20. What pest and wildlife control measures are used on this farm?

a. Rat and mouse bait stations ..... ☐1 Yes ☐3 No

b. Bait stations checked at least every 6 weeks ..... ☐1 Yes ☐3 No

c. Fly control used..... ☐1 Yes ☐3 No

If Yes, type and frequency: _____

d. Houses are bird proof..... ☐1 Yes ☐3 No

e. Wild birds seen in house..... ☐1 Yes ☐3 No

If Yes, type, number and frequency: _____

f. Raccoons, possums, foxes seen in or around poultry houses ..... ☐1 Yes ☐3 No

g. Wild turkeys, pheasants, quail seen around poultry..... ☐1 Yes ☐3 No

21. Are biosecurity audits or assessments (company or third party) conducted on this farm? ..... ☐1 Yes ☐3 No

If Yes, when was the last audit or assessment conducted? _____
(Obtain a copy of the result of the audit or assessment if available.)

22. Has this farm been confirmed positive for HPAI? ..... ☐1 Yes ☐3 No

E. FARM HELP/WORKERS

1. Total number of persons working on farm ..... _____ #

2. Number of workers living on the farm premises who are:

a. Family..... _____ #

b. Nonfamily..... _____ #

3. Workers are assigned to: *[Check one only.]*

☐1 Entire farm

☐2 Specific barns/areas

4. Do the workers have a common break area? ..... ☐1 Yes ☐3 No

If Yes, location: _____

5. Are workers employed by other poultry operations?..... ☐1 Yes ☐3 No

6. How often are training sessions held on biosecurity for workers?..... _____ times/year

7. Are family members employed by other poultry operations or processing plants? ☐1 Yes ☐3 No

   If Yes, poultry operation or processing plant: _____

8. Do part-time/weekend help and other extended family members on holidays and vacations? ..... ☐1 Yes ☐3 No

9. Are workers (full & part-time) restricted from being in contact with backyard poultry?..... ☐1 Yes ☐3 No

   How is this communicated? _____

F. FARM EQUIPMENT

Is the equipment used on this premises farm specific, under joint ownership that remains on this premises, or under joint ownership and used on other farm premises? A list of equipment follows.

1. Company vehicles/trailers:

   Farm specific? ..... ☐1 Yes ☐3 No

   If No, by whom is equipment jointly used: _____

   Dates: _____

2. Feed trucks (excess feed):

   Farm specific? ..... ☐1 Yes ☐3 No

   If No, by whom is equipment jointly used: _____

   Dates: _____

3. Gates/panels:

   Farm specific? ..... ☐1 Yes ☐3 No

   If No, by whom is equipment jointly used: _____

   Dates: _____

4. Lawn mowers:

   Farm specific? ..... ☐1 Yes ☐3 No

   If No, by whom is equipment jointly used: _____

   Dates: _____

5. Live haul loaders:

   Farm specific? ..... ☐1 Yes ☐3 No

   If No, by whom is equipment jointly used: _____

   Dates: _____

6. Poult trailers: Farm specific?

   Farm specific? ..... ☐1 Yes ☐3 No

   If No, by whom is equipment jointly used: _____

   Dates: _____

7. Pre-loaders:

   Farm specific? ..... ☐1 Yes ☐3 No

   If No, by whom is equipment jointly used: _____

   Dates: _____

   Describe pre-loader cleaning and disinfection procedures:

   _____

   _____

8. Pressure sprayers/washers:

   Farm specific? ..... ☐1 Yes ☐3 No

   If No, by whom is equipment jointly used: _____

   Dates: _____

9. Skid-steer loaders:

   Farm specific? ..... ☐1 Yes ☐3 No

   If No, by whom is equipment jointly used: _____

   Dates: _____

10. Tillers:

    Farm specific? ..... ☐1 Yes ☐3 No

    If No, by whom is equipment jointly used: _____

    Dates: _____

11. Trucks:

    Farm specific? ..... ☐1 Yes ☐3 No

    If No, by whom is equipment jointly used: _____

    Dates: _____

12. Other equipment: _____

    Farm specific? ..... ☐1 Yes ☐3 No

    If No, by whom is equipment jointly used: _____

    Dates: _____

G. LITTER HANDLING

1. Litter type: _____
2. Supplier/source: _____
3. Is a litter shed present?..... ☐1 Yes ☐3 No
4. Do you do partial cleanouts?..... ☐1 Yes ☐3 No

   If Yes, give dates of last partial cleanout: _____
5. Date of last cleanout: ..... _____ date

   Frequency of cleanout:..... _____ times/month
6. Who does the cleanout?

   ☐1 Grower

   ☐2 Contractor

   If contractor, name and location_____
7. Litter is disposed of:

   ☐1 On farm

   ☐2 Taken off site

   If taken offsite, name and location: _____

H. DEAD BIRD DISPOSAL

1. Approximate normal daily mortality ..... _____ # birds

2. How is daily mortality handled?

   a. On-farm: Burial pit/incinerator/composted/other (specify: _____)

   b. Off-farm: Landfill/rendering/other (specify: _____)

   c. Off-farm disposal performed by: Owner/employee/other (specify: _____)

   d. If burial or compost pits are used, are carcasses covered with soil on a daily basis? ..... ☐1 Yes ☐3 No

3. Contact name of company or individual responsible for disposal:

   _____

   If rendering is used, include location of carcass bin on the farm map.

4. What is the pickup schedule? _____

5. Does the carcass bin have a cover? ..... ☐1 Yes ☐3 No

   Is it routinely kept closed? ..... ☐1 Yes ☐3 No

I. FARM VISITORS

1. How many visitors do you have on a daily basis? ..... _____ #

2. Is there a visitor log to sign in? ..... ☐1 Yes ☐3 No

   Is it current? ..... ☐1 Yes ☐3 No

3. Do you provide any outer clothing to visitors entering the farm? ..... ☐1 Yes ☐3 No

   If Yes, identify items of clothing provided: _____

4. Mark the following services that were on the farm when this flock was on the farm. List date of service and name of person (or contract company) and if they had contact with the birds.

Service		Dates	Name	Contact?
Service person	☐Yes ☐No	_____	_____	☐1 Yes ☐3 No
Vaccination crew	☐Yes ☐No	_____	_____	☐1 Yes ☐3 No

Moving crew (moving from brood to grow, or pullet house to layer house)

☐Yes ☐No _____ _____ ☐$_1$ Yes ☐$_3$ No

Processing plant load out

☐Yes ☐No _____ _____ ☐$_1$ Yes ☐$_3$ No

Load-out crew (positive flock) ☐$_1$ Yes ☐$_3$ No ☐Yes ☐No

If load-out took more than one night, was returning crew the same crew? ..... ☐$_1$ Yes ☐$_3$ No

Truck #/#'s _____

Trailer #/#'s _____

What plant did flock go to? _____

Load-out crew (flock previous to positive flock)

☐Yes ☐No _____ _____ ☐$_1$ Yes ☐$_3$ No

If load-out took more than one night, was returning crew the same crew? ..... ☐$_1$ Yes ☐$_3$ No

Truck #/#'s _____

Trailer #/#'s _____

What plant did flock go to? _____

Poult delivery ☐Yes ☐No _____ _____ ☐$_1$ Yes ☐$_3$ No

Rendering pickup ☐Yes ☐No _____ _____ ☐$_1$ Yes ☐$_3$ No

Litter services ☐Yes ☐No _____ _____ ☐$_1$ Yes ☐$_3$ No

Cleanout services ☐Yes ☐No _____ _____ ☐$_1$ Yes ☐$_3$ No

Equipment shared/rented/loaned/borrowed (each of the categories of visitor is likely to be accompanied by equipment of some sort or another)

☐Yes ☐No _____ _____ ☐$_1$ Yes ☐$_3$ No

Feed delivery ☐Yes ☐No _____ _____ ☐$_1$ Yes ☐$_3$ No

5. Who makes sure covers are closed after delivery? _____

6. Are feed covers kept closed? ..... ☐$_1$ Yes ☐$_3$ No

J. WILD BIRDS

1. Do you see wild birds around your farm? ..... ☐$_1$ Yes ☐$_3$ No

   If Yes, what type of birds? *[Check all that apply.]*

   ☐ Waterfowl

   ☐ Gulls

   ☐ Small perching birds (sparrows, starlings, swallows)

   ☐ Other water birds (egrets, cormorants)

   ☐ Other _____

2. Do you see birds all year round? ..... ☐$_1$ Yes ☐$_3$ No

   If Yes, what type of birds? _____

3. Is there seasonality to the presence of some types of birds? ..... ☐$_1$ Yes ☐$_3$ No

   If Yes, what type of birds and what seasons do you see them? _____

   _____

4. Where are wild birds seen in relation to the farm?

   ☐$_1$ On adjacent habitats away from facilities and equipment (identify location of habitat on photos)

   ☐$_2$ On the farm but not in the barns (identify facilities or equipment birds have contact with)

   ☐$_3$ On the farm and sometimes in the barns (identify facilities or equipment birds have contact with)

Appendix B: Model Parameter Estimation

Barn-level LPAI diagnostic test data

Tables 14-21 give the date of testing, test type, surveillance protocol, and test result for the LPAI-infected barns on eight turkey meat-type premises in Minnesota. Stearns 1/Barn 2 and Stearns 4/ Barn 1 diagnostic test data are given in the main text.

Table 14 Summary of surveillance protocols and test results from the LPAI infected barns on Kandiyohi 1.

Kandiyohi 1 Barn 1				
Date	Test type	Sample[7]	Test result	Average (+) Ct value/ELISA titer
10/19/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/24/2018	rRT-PCR	1 pooled sample	1/1 positive	27
10/24/2018	AGID	10 serum samples	0/10 positive	NA
10/29/2018	rRT-PCR	3 pooled samples	3/3 positive	25
10/29/2018	rRT-PCR	10 individual swabs	10/10 positive	28
10/29/2018	AGID	10 serum samples	6/10 positive	NA
10/30/2018	AGID	30 serum samples	21/30 positive	NA
11/01/2018	rRT-PCR	3 pooled samples	1/3 positive	36
11/01/2018	rRT-PCR	10 individual swabs	9/10 positive	31
11/01/2018	AGID	30 serum samples	30/30 positive	NA
11/05/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/05/2018	rRT-PCR	10 individual swabs	1/10 positive	34
11/08/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/08/2018	rRT-PCR	10 individual swabs	1/10 positive	39
11/12/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/19/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/26/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
House 2				
10/19/2018	rRT-PCR	1 pooled sample	1/1 positive	19

[7] Pooled rRT-PCR samples consisted of either 10 or 11 oropharyngeal swabs.

10/24/2018	rRT-PCR	1 pooled sample	1/1 positive	25
10/24/2018	AGID	10 serum samples	10/10 positive	NA
10/29/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
10/29/2018	rRT-PCR	10 individual swabs	0/10 positive	NA
10/29/2018	AGID	10 serum samples	10/10 positive	NA
10/30/2018	AGID	30 serum samples	30/30 positive	NA
11/01/2018	rRT-PCR	3 pooled samples	2/3 positive	37
11/01/2018	rRT-PCR	10 individual swabs	1/10 positive	37
11/05/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/05/2018	rRT-PCR	10 individual swabs	2/10 positive	38
11/08/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/08/2018	rRT-PCR	10 individual swabs	0/10 positive	NA
11/12/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/19/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/26/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
		House 3		
10/19/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/24/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/24/2018	AGID	10 serum samples	0/10 positive	NA
10/29/2018	rRT-PCR	3 pooled samples	3/3 positive	23
10/29/2018	rRT-PCR	10 individual swabs	10/10 positive	29
10/29/2018	AGID	10 serum samples	0/10 positive	NA
10/30/2018	AGID	30 serum samples	0/30 positive	NA
11/01/2018	rRT-PCR	3 pooled samples	3/3 positive	25
11/01/2018	rRT-PCR	10 individual swabs	10/10 positive	28
11/01/2018	AGID	30 serum samples	0/30 positive	NA
11/05/2018	rRT-PCR	3 pooled samples	2/3 positive	37
11/05/2018	rRT-PCR	10 individual swabs	6/10 positive	31
11/05/2018	AGID	30 serum samples	30/30 positive	NA

11/08/2018	rRT-PCR	3 pooled samples	1/3 positive	39
11/08/2018	rRT-PCR	10 individual swabs	4/10 positive	36
11/12/2018	rRT-PCR	3 pooled samples	1/3 positive	38
11/19/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/26/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
		House 4		
10/19/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/24/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/24/2018	AGID	10 serum samples	0/10 positive	NA
10/29/2018	rRT-PCR	3 pooled samples	3/3 positive	25
10/29/2018	rRT-PCR	10 individual swabs	10/10 positive	29
10/29/2018	AGID	10 serum samples	0/10 positive	NA
10/30/2018	AGID	30 serum samples	0/30 positive	NA
11/01/2018	rRT-PCR	3 pooled samples	3/3 positive	30
11/01/2018	rRT-PCR	10 individual swabs	10/10 positive	31
11/01/2018	AGID	30 serum samples	17/30 positive	NA
11/05/2018	rRT-PCR	3 pooled samples	1/3 positive	38
11/05/2018	rRT-PCR	10 individual swabs	5/10 positive	35
11/05/2018	AGID	30 serum samples	30/30 positive	NA
11/08/2018	rRT-PCR	3 pooled samples	1/3 positive	39
11/08/2018	rRT-PCR	10 individual swabs	0/10 positive	NA
11/12/2018	rRT-PCR	3 pooled samples	2/3 positive	38
11/19/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/26/2018	rRT-PCR	3 pooled samples	0/3 positive	NA

Table 15 Summary of surveillance protocols and test results from the LPAI infected barns on Kandiyohi 2.

Kandiyohi 2 Barn 1				
Date	Test type	Sample[8]	Result	Average (+) Ct value/ELISA titer
10/20/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/25/2018	AGID	9 serum samples	0/9 positive	NA
11/01/2018	rRT-PCR	3 pooled samples	2/3 positive	24
11/05/2018	rRT-PCR	1 pooled sample	1/1 positive	33
11/08/2018	ELISA	10 serum samples	10/10 positive	1741
11/12/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/19/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/03/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
Barn 2				
10/20/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/25/2018	AGID	10 serum samples	0/10 positive	NA
11/01/2018	rRT-PCR	3 pooled samples	3/3 positive	32
11/05/2018	rRT-PCR	1 pooled sample	1/1 positive	22
11/08/2018	ELISA	10 serum samples	1/10 positive	347
11/12/2018	rRT-PCR	1 pooled sample	1/1 positive	35
11/16/2018	ELISA	10 serum samples	10/10 positive	4197
11/19/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/26/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
12/03/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
Barn 3				
10/20/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/25/2018	AGID	9 serum samples	0/9 positive	NA
11/01/2018	rRT-PCR	3 pooled samples	3/3 positive	37

[8] Pooled rRT-PCR samples consisted of either 10 or 11 oropharyngeal swabs.

11/05/2018	rRT-PCR	1 pooled sample	1/1 positive	21
11/08/2018	ELISA	10 serum samples	0/10 positive	NA
11/12/2018	rRT-PCR	1 pooled sample	1/1 positive	32
11/15/2018	ELISA	10 serum samples	10/10 positive	3758
11/19/2018	rRT-PCR	3 pooled samples	1/3 positive	39
11/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/03/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
		Barn 4		
10/20/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/25/2018	AGID	9 serum samples	0/9 positive	NA
11/01/2018	rRT-PCR	3 pooled samples	1/3 positive	37
11/05/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/08/2018	ELISA	10 serum samples	1/10 positive	356
11/12/2018	rRT-PCR	1 pooled sample	1/1 positive	21
11/15/2018	ELISA	10 serum samples	0/10 positive	NA
11/19/2018	rRT-PCR	3 pooled samples	3/3 positive	29
11/26/2018	rRT-PCR	1 pooled sample	1/1 positive	41
11/29/2018	ELISA	10 serum samples	10/10 positive	2237
12/03/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
		Barn 5		
10/20/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/25/2018	AGID	10 serum samples	0/10 positive	NA
11/01/2018	rRT-PCR	3 pooled samples	3/3 positive	23
11/05/2018	rRT-PCR	1 pooled sample	1/1 positive	34
11/08/2018	ELISA	10 serum samples	10/10 positive	1478
11/12/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/19/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/03/2018	rRT-PCR	3 pooled samples	0/3 positive	NA

Table 16 Summary of surveillance protocols and test results from the LPAI infected barns on Kandiyohi 3.

Kandiyohi 3 Barn 1				
Date	Test type	Sample[9]	Test result	Average (+) Ct value/ELISA titer
10/22/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/26/2018	AGID	10 serum samples	0/10 positive	NA
11/02/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/09/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	rRT-PCR	1 pooled sample	1/1 positive	38
11/15/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	rRT-PCR	1 pooled sample	1/1 positive	26
11/26/2018	rRT-PCR	1 pooled sample	1/1 positive	37
11/30/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/07/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/13/2018	ELISA	10 serum samples	10/10 positive	2095
12/14/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/21/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
Barn 2				
10/22/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/26/2018	AGID	10 serum samples	0/10 positive	NA
11/02/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/09/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	rRT-PCR	1 pooled sample	1/1 positive	28
11/26/2018	rRT-PCR	1 pooled sample	1/1 positive	25

[9] Pooled rRT-PCR samples consisted of either 10 or 11 oropharyngeal swabs.

11/30/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/07/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/13/2018	ELISA	10 serum samples	10/10 positive	2779
12/14/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/21/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
		Barn 3		
10/22/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/26/2018	AGID	10 serum samples	0/10 positive	NA
11/02/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/09/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	rRT-PCR	1 pooled sample	1/1 positive	36
11/15/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	rRT-PCR	1 pooled sample	1/1 positive	27
11/26/2018	rRT-PCR	1 pooled sample	1/1 positive	26
11/30/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/07/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/13/2018	ELISA	10 serum samples	10/10 positive	3777
12/14/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/21/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
		Barn 4		
10/22/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/26/2018	AGID	10 serum samples	0/10 positive	NA
11/02/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/09/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	rRT-PCR	1 pooled sample	1/1 positive	24

11/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/30/2018	rRT-PCR	1 pooled sample	1/1 positive	38
12/07/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/13/2018	ELISA	10 serum samples	10/10 positive	3513
12/14/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/21/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
		Barn 5		
10/22/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/26/2018	AGID	10 serum samples	0/10 positive	NA
11/02/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/09/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	rRT-PCR	1 pooled sample	1/1 positive	22
11/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/30/2018	rRT-PCR	1 pooled sample	1/1 positive	33
12/07/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/13/2018	ELISA	10 serum samples	10/10 positive	3527
12/14/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/21/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
		Barn 6		
10/22/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/26/2018	AGID	10 serum samples	0/10 positive	NA
11/02/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/09/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	ELISA	10 serum samples	0/10 positive	NA

11/21/2018	rRT-PCR	1 pooled sample	1/1 positive	27
11/26/2018	rRT-PCR	1 pooled sample	1/1 positive	39
11/30/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/07/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/13/2018	ELISA	10 serum samples	10/10 positive	2937
12/14/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/21/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
		Barn 7		
10/22/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/26/2018	AGID	10 serum samples	0/10 positive	NA
11/02/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/09/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	rRT-PCR	1 pooled sample	1/1 positive	36
11/15/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	rRT-PCR	1 pooled sample	1/1 positive	27
11/26/2018	rRT-PCR	1 pooled sample	1/1 positive	37
11/30/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/07/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/13/2018	ELISA	10 serum samples	10/10 positive	2927
12/14/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/21/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
		Barn 8		
10/22/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/26/2018	AGID	10 serum samples	0/10 positive	NA
11/02/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/09/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/12/2018	rRT-PCR	3 pooled samples	3/3 positive	28
11/15/2018	rRT-PCR	1 pooled sample	1/1 positive	27

11/15/2018	ELISA	10 serum samples	0/10 positive	NA
11/21/2018	ELISA	10 serum samples	10/10 positive	1318
11/21/2018	rRT-PCR	1 pooled sample	1/1 positive	35
11/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/30/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/07/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/13/2018	ELISA	10 serum samples	10/10 positive	3660
12/14/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/21/2018	rRT-PCR	3 pooled samples	0/3 positive	NA

Table 17. Summary of surveillance protocols and test results from the LPAI infected barns on Kandiyohi 4.

Kandiyohi 4 Barn 1				
Date	Test type	Sample[10]	Result	Average (+) Ct value/ELISA titer
10/22/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/23/2018	AGID	8 serum samples	0/8 positive	NA
10/31/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/13/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/20/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/04/2018	rRT-PCR	1 pooled sample	1/1 positive	23
12/11/2018	rRT-PCR	1 pooled sample	1/1 positive	36
12/18/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/20/2018	ELISA	10 serum samples	10/10 positive	1465
12/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
1/03/2019	rRT-PCR	3 pooled samples	0/3 positive	NA
1/07/2019	rRT-PCR	3 pooled samples	0/3 positive	NA
Barn 2				
10/22/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/23/2018	AGID	10 serum samples	0/10 positive	NA
10/31/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/13/2018	rRT-PCR	1 pooled sample	1/1 positive	25
11/20/2018	rRT-PCR	1 pooled sample	1/1 positive	22
11/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/04/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/11/2018	rRT-PCR	1 pooled sample	1/1 positive	38
12/18/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/20/2018	ELISA	9 serum samples	9/9 positive	3146

[10] Pooled rRT-PCR samples consisted of either 10 or 11 oropharyngeal swabs.

12/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
1/03/2019	rRT-PCR	3 pooled samples	0/3 positive	NA
1/07/2019	rRT-PCR	3 pooled samples	0/3 positive	NA
		Barn 3		
10/22/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/23/2018	AGID	10 serum samples	0/10 positive	NA
10/31/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/13/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/20/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/26/2018	rRT-PCR	1 pooled sample	1/1 positive	20
12/04/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/11/2018	rRT-PCR	1 pooled sample	1/1 positive	37
12/18/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/20/2018	ELISA	10 serum samples	9/10 positive	1896
12/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
1/03/2019	rRT-PCR	3 pooled samples	0/3 positive	NA
1/07/2019	rRT-PCR	3 pooled samples	0/3 positive	NA
		Barn 4		
10/22/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
10/23/2018	AGID	9 serum samples	0/9 positive	NA
10/31/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/13/2018	rRT-PCR	1 pooled sample	1/1 positive	38
11/20/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/04/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/11/2018	rRT-PCR	1 pooled sample	1/1 positive	21
12/18/2018	rRT-PCR	1 pooled sample	1/1 positive	31
12/20/2018	ELISA	10 serum samples	10/10 positive	1911
12/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA

1/03/2019	rRT-PCR	3 pooled samples	0/3 positive	NA
1/07/2019	rRT-PCR	3 pooled samples	0/3 positive	NA

Table 18 Summary of surveillance protocols and test results from LPAI infected barns on Stearns 1. The testing summary for Barn 2 is included in the main report.

Stearns 1 Barn 1				
Date	Test type	Sample[11]	Result	Average (+) Ct value/ELISA titer
10/30/2018	rRT-PCR	2 pooled samples	2/2 positive	20
10/30/2018	AGID	10 serum samples	10/10 positive	NA
11/05/2018	rRT-PCR	1 pooled sample	1/1 positive	36
11/08/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/08/2018	ELISA	10 serum samples	10/10 positive	2465
11/12/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
Barn 3				
10/30/2018	rRT-PCR	2 pooled samples	2/2 positive	36
10/30/2018	AGID	10 serum samples	10/10 positive	NA
11/05/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/08/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/08/2018	ELISA	10 serum samples	10/10 positive	3251
11/12/2018	rRT-PCR	3 pooled samples	0/3 positive	NA

Table 19 Summary of surveillance protocols and test results from the LPAI infected barn on Stearns 2.

Stearns 2 Barn 1				
Date	Test type	Sample[12]	Result	Average (+) Ct value/ELISA titer
10/31/2018	rRT-PCR	1 pooled sample	1/1 positive	23
10/31/2018	ELISA	10 serum samples	10/10 positive	1723

[11] Pooled rRT-PCR samples consisted of either 10 or 11 oropharyngeal swabs.
[12] Pooled rRT-PCR samples consisted of either 10 or 11 oropharyngeal swabs.

11/01/2018	rRT-PCR	1 pooled sample	1/1 positive	24
11/06/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/07/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/12/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/12/2018	ELISA	20 serum samples	19/20 positive	2134

Table 20 Summary of surveillance protocols and test results from the LPAI infected barns on Stearns 3.

Stearns 3 Barn 1				
Date	Test type	Sample[13]	Test result	Average (+) Ct value/ELISA titer
11/01/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/02/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/08/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	rRT-PCR	1 pooled sample	1/1 positive	27
11/16/2018	ELISA	10 serum samples	0/10 positive	NA
11/20/2018	rRT-PCR	1 pooled sample	1/1 positive	23
11/20/2018	ELISA	10 serum samples	10/10 positive	4867
11/27/2018	rRT-PCR	1 pooled sample	1/1 positive	37
11/28/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/03/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
Barn 2				
11/01/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/02/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/08/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/15/2018	rRT-PCR	1 pooled sample	1/1 positive	28
11/16/2018	ELISA	10 serum samples	0/10 positive	NA
11/20/2018	rRT-PCR	1 pooled sample	1/1 positive	21
11/20/2018	ELISA	10 serum samples	10/10 positive	4794

[13] Pooled rRT-PCR samples consisted of either 10 or 11 oropharyngeal swabs.

11/27/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/28/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/03/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
		Barn 3		
11/01/2018	rRT-PCR	1 pooled sample	1/1 positive	37
11/01/2018	ELISA	20 serum samples	0/20 positive	NA
11/02/2018	rRT-PCR	3 pooled samples	3/3 positive	24
11/08/2018	rRT-PCR	1 pooled sample	1/1 positive	28
11/15/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/16/2018	ELISA	10 serum samples	10/10 positive	2744
11/20/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/20/2018	ELISA	10 serum samples	10/10 positive	6812
11/26/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/28/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/03/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
		Barn 4		
11/01/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/02/2018	rRT-PCR	3 pooled samples	0/3 positive	NA
11/08/2018	rRT-PCR	1 pooled sample	1/1 positive	26
11/15/2018	rRT-PCR	1 pooled sample	1/1 positive	27
11/16/2018	ELISA	10 serum samples	8/10 positive	793
11/20/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/20/2018	ELISA	10 serum samples	10/10 positive	4952
11/27/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/28/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/03/2018	rRT-PCR	3 pooled samples	0/3 positive	NA

Table 21 Summary of surveillance protocols and test results from LPAI infected barns on Stearns 4. The testing summary for Barn 1 is provided in the main report.

Stearns 4 Barn 2				
Date	Test type	Sample[14]	Result	Average (+) Ct value/ELISA titer
11/02/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
11/06/2018	ELISA	10 serum samples	0/10 positive	NA
11/14/2018	rRT-PCR	1 pooled sample	1/1 positive	21
11/19/2018	rRT-PCR	1 pooled sample	1/1 positive	22
11/19/2018	ELISA	10 serum samples	9/10 positive	1512
11/26/2018	rRT-PCR	1 pooled sample	0/1 positive	NA
12/03/2018	rRT-PCR	3 pooled samples	0/1 positive	NA
House 3				
11/02/2018	rRT-PCR	2 pooled samples	0/2 positive	NA
11/06/2018	ELISA	10 serum samples	0/10 positive	NA
11/14/2018	rRT-PCR	1 pooled sample	1/1 positive	23
11/19/2018	rRT-PCR	1 pooled sample	1/1 positive	20
11/19/2018	ELISA	10 serum samples	0/10 positive	NA
11/26/2018	rRT-PCR	1 pooled sample	1/1 positive	38
12/03/2018	rRT-PCR	3 pooled samples	0/1 positive	NA
House 4				
11/02/2018	rRT-PCR	2 pooled samples	2/2 positive	27
11/06/2018	ELISA	10 serum samples	0/10 positive	NA
11/12/2018	rRT-PCR	1 pooled sample	1/1 positive	35
11/12/2018	ELISA	10 serum samples	10/10 positive	3298
11/19/2018	rRT-PCR	1 pooled sample	1/1 positive	36
11/26/2018	rRT-PCR	1 pooled sample	1/1 positive	38
12/03/2018	rRT-PCR	3 pooled samples	0/1 positive	NA

[14] Pooled rRT-PCR samples consisted of either 10 or 11 oropharyngeal swabs.

Sensitivity Analysis: Number of Swabs in rRT-PCR Pooled Samples

A sensitivity analysis was performed for the number of swabs included in the pooled samples tested by rRT-PCR. During the outbreak, pooled samples consisted of either 10 or 11 oropharyngeal swabs, though it was often unclear in the data which pool size was used. The baseline pool size used in this analysis was 11 swabs. For the sensitivity analysis the time of virus introduction was estimated for the barns on Kandiyohi 4 and Stearns 3 assuming each rRT-PCR pooled sample consisted of 10 swabs. The comparison of the estimated time of virus introduction assuming rRT-PCR sample pool sizes of 10 and 11 swabs for Kandiyohi 4 and Stearns 3 are given in Table 22. The estimates for the time of virus introduction were highly similar between the two rRT-PCR pool size scenarios, which suggests results are robust to changes in the number of swabs per pooled sample.

Table 22 Most likely date of introduction (95% CI) for the barns that tested positive on Stearns 3 and Kandiyohi 4 during the 2018 H5N2 outbreak in Minnesota considering rRT-PCR pooled samples of size 11 and 10 swabs.

Premises	Barn	Most likely date of virus introduction (95% CI): 11 swabs per rRT-PCR sample	Most likely date of virus introduction (95% CI): 10 swabs per rRT-PCR sample
Stearns 3	Barn 3	October 26 (Oct 19 – Oct 28)	October 26 (Oct 19 – Oct 28)
	Barn 4	October 31 (Oct 27 – Nov 4)	October 31 (Oct 25 – Nov 3)
	Barn 1	November 8 (Nov 2- Nov 10)	November 7 (Oct 31 – Nov 9)
	Barn 2	November 8 (Nov 2 – Nov 10)	November 7 (Oct 31 – Nov 9)
Kandiyohi 4	Barn 2	October 28 (Oct 19 – Nov 8)	October 27 (Oct 19 – Nov 8)
	Barn 4	November 6 (Oct 23 – Nov 11)	November 5 (Oct 23 – Nov 11)
	Barn 3	November 19 (Nov 10 – Nov 22)	November 18 (Nov 7 – Nov 22)
	Barn 1	November 23 (Nov 15 – Nov 29)	November 23 (Nov 13 – Nov 28)

Stochastic Disease Transmission Model Details

The transmission model used to simulate the spread of LPAI within a turkey house was a discrete stochastic individual-based transmission model where the infection status of individual birds was tracked at discrete simulation time steps. The disease states included susceptible, latently infected, infectious, removed/recovered, and seroconverted. The number of birds transitioning from the susceptible to the latently infected state at the beginning of a time step was simulated from a binomial distribution, where the probability of infection was dependent on the adequate contact rate, and proportion of infectious and alive birds. Once infected, the number of time steps each bird would be in the latently infected and then infectious state was simulated from distributions modeling the length of the latent and infectious periods. The number of time steps until the bird seroconverted following infection was also simulated from a distribution. For more details, see Bonney et al. (2018). The parameter estimates for the transmission model are given in Table 23.

Table 23 Parameter estimates for the LPAI transmission model for turkey barns.

Parameter name	Parameter description	Distribution/Value
Contact rate	The mean number of direct or indirect contacts a bird has that are sufficient to transmit infection per unit time	Grid of 0.5 to 10.0 contacts per day discretized at 0.1 intervals
Latent period distribution	Length of the latent period	Gamma distribution (shape 2.5839130, scale 0.2441542; mean 0.63 days)
Infectious period distribution	Length of the infectious period	Gamma distribution (shape 4.040337, scale 2.915678; mean 11.78 days)
Time to seroconversion	Time to seroconvert post-infection	Gamma distribution (shape 3.556011, scale 1.629968; mean 5.80 days)
Proportion seroconverting	Proportion of LPAI infected turkeys that seroconvert	0.99

Estimation of transmission model parameters

There is considerable uncertainty in the within-house adequate contact rate for LPAI in turkeys, a parameter that determines the rate of infection spread in the transmission model. Saenz *et al*. (2012) was the only study identified where a contact rate was estimated for LPAI in turkeys (11). The mean contact rate estimated in Saenz *et al*. (2012) was 2.01 (95% CI: 1.6-2.5) from LPAI H7N1 turkey inoculation data. The contact rate can vary depending on factors such as housing, bird species, and LPAI strain. In light of this uncertainty, a fairly wide range of candidate contact rate values were used, ranging from 0.5 to 10.0 contacts per day.

Parameters for the length of the latent and infectious period were estimated using a maximum likelihood method from experimental data in which turkeys were inoculated with H5 or H7 LPAI strains. Both distributions were assumed to be Gamma distributed. For details on the maximum likelihood method see Appendix 2 of "An Assessment of the Risk Associated with the Movement of Turkey Hatching Eggs Into, Within, and Out of a Control Area during a Highly Pathogenic Avian Influenza Outbreak"(USDA:APHIS:VS, 2015). Insufficient data was available for H5 LPAI strains leading to both H5 and H7 LPAI strains being included in the estimation of the distributions.

The inoculation studies used in the estimation of the latent period distribution include Pillai *et al.* (2010), Iqbal *et al*. (2012), and Pantin-Jackwood *et al*. (2017). Only the turkeys inoculated with H5 LPAI strains isolated from infected farms were used from Pillai *et al*. (2010), as the strains from wild birds and live bird markets appeared to be poorly adapted to turkeys. The data from Iqbal *et al*. (2012) consisted of 20 turkeys, specifically turkeys 81-90 from Table 8 in the article and turkeys 21-30 from Table S1 in the article, that were infected with LPAI H7N1. The data from Pantin-Jackwood *et al*. (2017) consisted of 17 turkeys inoculated with a $10^6 EID_{50}$ dose of LPAI H7N1. The estimated shape parameter of the gamma distribution for the length of the latent period was 2.5839130, while the estimated scale parameter was 0.2441542. The mean of the distribution is 0.63 days.

The inoculation studies used in the estimation of the infectious period distribution include Pillai *et al*. (2010), Saenz *et al*. (2012), Iqbal *et al*. (2012), Comin *et al*. (2011), Pantin-Jackwood *et al*. (2017), and Spackman *et al*. (2010). Pillai *et al*. (2010) was the only study included with H5 data. As in the estimation of the latent period distribution, the only Pillai *et al.* (2010) data used was from those turkeys inoculated with strains from infected farms. In Saenz *et al*. (2012), the data consisted of two separate transmission experiments, one involving 1 turkey inoculated with LPAI H7N1 and 40 contact turkeys, and the other involving 1 inoculated turkey and 41 contact turkeys (Table 5 and Table 6 in the article). The data used from Iqbal *et al*. (2012) consisted of the 10 turkeys inoculated with LPAI H7N1 and 10 contact turkeys given in Table 8 of the article. Similarly, turkeys k10-18 inoculated with LPAI H7N3 from Table 3 in Comin *et al*. (2011) and five turkeys inoculated with a 10^4EID_{50} dose of LPAI H7N1 along with the 17 turkeys inoculated with a 10^6EID_{50} dose of LPAI H7N8 from Pantin-Jackwood *et al*. (2017) were used in the estimation of the infectious period. Lastly, data on 113 turkeys inoculated with 12 different H7 strains were included from Spackman *et al*. (2010). The estimated parameters of the gamma distribution for the length of the infectious period were a shape equal to 4.040337 and scale equal to 2.915678. The mean of the distribution is 11.78 days.

Parameters for the distribution for the time from infection until seroconversion, assumed to be gamma distributed, were estimated using the same maximum likelihood method employed in the estimation of the infectious and latent period distribution parameters. Usable data for the estimation of the time to seroconversion was scarce, leading to data from inoculation studies involving any LPAI strain being included. The studies used were Dundon *et al*. (2007), Morales (2008), Homme *et al*. (1970), and Preskenis (2010), which involved H4, H6, H7, and H9 LPAI strains. Only in Dundon *et al*. (2007) were serum samples taken within 7 days post inoculation. Samples in the other three studies were taken at 7 day increments, which introduces a fair amount of uncertainty into the time to seroconversion distribution. The estimated shape parameter of the distribution was 3.556011 and scale was 1.629968. The mean of the distribution is 5.80 days. The proportion of turkeys that seroconvert was based on Spackman *et al*. (2010) in which 88/89 (99%) turkeys inoculated with twelve North American H7 LPAI virus isolates had detectable antibody by day 18 to 21 post-inoculation. For comparison, 110/116 (~95%) of the surviving chickens had detectable antibody in the same study.

Appendix C: Data Factors and Processing for Geospatial Analysis Predicting Avian Influenza Virus Persistence in the Environment

Data Preparation

All data were prepared using tools within ArcGIS 10.6, and final layers were projected to Universal Transverse Mercator (UTM) Zone 15 North in the datum World Geodetic System 1984 (WGS1984).

Predictive Factors and Data Sources:

- Water Presence

  Avian Influenza Viruses (AIV) have been shown to have improved survival in water compared to dry land (Brown et al., 2009; USGS, 2011). United States Geological Survey (USGS) Gap Analysis Project hydrography data collected between 1994 and 2004, derived from satellite imagery at a 30m resolution, were downloaded and reclassified into two categories of surface water based on suitability for AIV survival: presence of fresh water (high suitability), or no water present (low suitability). The surface water presence layer was aggregated to a one-kilometer (1km) resolution.

- Water Temperature

  Water temperature is inversely associated with the rate of AIV survival (Brown et al., 2009; Keeler et al., 2012; Lang et al., 2008; Stallknecht et al., 1990a; 1990b). USGS Moderate Resolution Imaging Spectroradiometer (MODIS)-derived 8-day land surface and emissivity scenes were downloaded for 2015-2017. Using R version 3.3.3, individual scenes were masked by quality indicators (i.e., cloud cover) and recombined to create summary temperature surfaces by season, at a spatial resolution of 1km (Grim and Knievel, 2013; Ke and Song, 2014; NASA, 2012). Refined MODIS data were then masked with the water presence layer to reflect locations only where surface water was present, and reclassified to represent high suitability (< 10° C), moderate suitability (≥ 10°C and < 20°C), or low suitability (≥ 20°C) for AIV survival (Brown et al., 2007; Brown et al., 2009; Keeler et al., 2014; Nazir et al., 2010).

- Wetlands and Wildlife Refuges

  U.S. Fish and Wildlife Service (USFWS) National Wetland Inventory data were used to identify locations of wetlands and wildlife refuges, which are considered favorable for AIV persistence (USFWS, 2016; Keeler et al., 2012; Belkhiria, et al., 2016; Iglesias et al., 2010; Fuller et al., 2010). Locations classified as 'freshwater emergent wetland' and 'freshwater forested/shrub wetland' were extracted; data were reclassified based on presence or absence of either wetland type and resampled to 1km. For wildlife refuges, USFWS Cadastral data were obtained and reclassified at a 1km resolution based on presence or absence of National Wildlife Refuge (NWR) land.

- Wild Bird Presence

  Data for wild bird presence were provided by the USGS Patuxent Wildlife Research Center. These data were derived using a spatio-temporal model estimating seasonal occurrence (presence) and abundance of 10 dabbling duck species (Table 15) throughout the conterminous United States at a 1km resolution (Humphreys, 2019). The

occurrence model data for each species was combined then reclassified to create a presence/absence dataset for all 10 dabbling duck species in Minnesota.

Table 24 Common and scientific names of dabbling duck species that were included in the wild bird presence model.

Common Name	Scientific Name
American black duck	*Anas rubripes*
American wigeon	*Anas americana*
Blue-winged teal	*Anas discors*
Cinnamon teal	*Anas cyanoptera*
Gadwall	*Anas strepera*
Green-winged teal	*Anas carolinensis*
Mallard	*Anas platyrhynchos*
Mottled duck	*Anas fulvigula*
Northern pintail	*Anas acuta*
Northern shoveler	*Anas clypeata*

- Poultry Operations

  Poultry operation data were provided by the Minnesota Board of Animal Health. The data included location information for 827 commercial operations and 8,581 backyard operations. These data were summarized by county to illustrate the number of commercial and backyard premises in relation to counties at high risk for AIV introduction from wild birds.