United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Epidemiologic and Other Analyses of HPAI-Affected Poultry Flocks: June 15, 2015 Report

Contents

EXECUTIVE SUMMARY

For the past several months, the USDA's Animal and Plant Health Inspection Service (APHIS) has conducted epidemiological investigations and other studies with the goal of identifying transmission pathways of highly pathogenic avian influenza (HPAI). This report includes the results to date of investigations spanning more than 80 commercial poultry facilities, as well as other in-depth studies and analyses performed with the assistance of academic, Federal, State, and industry partners. APHIS will update this report regularly as more analyses are completed.

APHIS concludes that at present, there is not substantial or significant enough evidence to point to a specific pathway or pathways for the current spread of the virus. We have collected data on the characteristics and biosecurity measures of infected farms and studied wind and airborne viruses as possible causes of viral spread, and conducted a genetic analysis of the viruses detected in the United States.

APHIS scientists believe wild birds were responsible for introducing HPAI into commercial poultry. However, given the number and proximity of farms affected by HPAI, it appears the virus is spreading in other ways as well. For instance, one analysis provides evidence that a certain cluster of farms was affected by identical viruses, pointing to possible transmission among those farms. In addition, genetic analyses of the HPAI viruses suggest that independent introductions as well as transmission between farms are occurring in several States concurrently.

Although APHIS cannot at present point to a single statistically significant pathway for the current spread of HPAI, a likely cause of some virus transmission is insufficient application of recommended biosecurity practices. For example, APHIS has observed sharing of equipment between an infected and noninfected farm, employees moving between infected and noninfected farms, lack of cleaning and disinfection of vehicles moving between farms, and reports of rodents or small wild birds inside poultry houses. We are compiling these observations and will present our findings in a subsequent update of this report. Until then, USDA is collaborating with affected industries and States to implement more stringent biosecurity procedures while continuing to work on identifying and mitigating other possible disease pathways in poultry farms nationwide.

Environmental factors may also play a part in transmitting HPAI. APHIS found that genetic material from the HPAI virus could be detected in air samples taken inside and outside infected poultry houses, supporting the idea that the virus can be transmitted through air. Further reinforcing this concept is preliminary analysis of wind data that shows a relationship between sustained high winds (25 mph or greater for 2 days or longer) and an increase in the number of infected farms 5 to 7 days later.

APHIS will continue to investigate how the HPAI virus is introduced and spread and will provide updated results regularly. Comprehensive and stringent biosecurity practices will remain crucial to reducing the risk of HPAI infection.

INTRODUCTION

Since the expansion of highly pathogenic avian influenza (HPAI) viruses into commercial poultry occurred in January 2015, APHIS Veterinary Services (VS) has initiated a number of epidemiologic and laboratory based investigations to better understand the factors associated with HPAI virus transmission. These investigations include:

- field-based observational studies with data collected through surveys and site visits;
- geospatial analyses;
- on-farm sampling efforts; and
- phylogenetic investigations.

This report summarizes the preliminary findings from these studies. As investigation and analysis efforts continue, this report will be updated with recent results in an effort to provide producers, industry, and other stakeholders tangible and effective ways to mitigate initial introduction of HPAI viruses into commercial poultry operations and transmission of virus between operations.

I. FIELD-BASED OBSERVATIONAL STUDIES

A. Descriptive Analysis of Epidemiologic Findings for Turkey Flocks Infected with HPAI in IA, MN, ND, SD and WI

Project Background

The purpose of the analysis is to describe demographics and management on affected premises that were part of the HPAI outbreak in late 2014 and early 2015. The survey was designed as an assessment tool to provide an in-depth review of the current biosecurity and management practices and exposure risks on an infected farm.

Project Status

Data collection and analyses continue.

Methods

A survey instrument continues to be administered by State and Federal animal health officials in multiple states affected by HPAI strain H5N2 (see Appendix A). Survey administrators are requesting that respondents be individual(s) most familiar with the farm's management and operations. Instructions request responses be provided for the two-week period prior to HPAI detection. Investigators have been asked to complete the investigation within one week of detection. Additionally, for each survey completed for an infected barn/farm, investigators were requested to complete a survey for at least one non-infected barn/farm within the same complex or as near as possible to the infected flock.

Completed questionnaires are delivered via secure email to VS. Analytical epidemiologists are responsible for questionnaire review, data entry, and analysis.

The questionnaire includes both closed- and open-ended questions focused on the following categories: premises description, farm biosecurity, farm help/workers, farm equipment, litter handling, dead bird disposal, farm visitors, and presence of wild animals, including birds. Additionally, respondents have been asked to provide mortality data (charted over the duration since placement of turkeys in a barn), a copy of the most recent biosecurity audit or assessment if available, and a farm diagram.

Updated Analyses and Findings

Turkey Farm Case Series

In this report, we provide a preliminary case series report on HPAI-infected turkey farms in five states: IA, MN, ND, SD, and WI (Tables 1 – 7). This report can be used to generate hypotheses about disease predilection based on descriptive information, but it cannot be used to identify HPAI risk factors due to the lack of a comparison group.

Interpretation and Limitations

These results are preliminary, and several limitations should be recognized. The numbers of infected and non-infected farms available for the initial descriptive analysis presented in this document were small and thus did not allow for a statistical comparison of infected and non-infected farms. Many of the analysis variables were collected at the farm level (e.g., other animals located on the farm, premises biosecurity, dead bird disposal), and therefore will be the same for both infected and non-infected barns.

Analysts will continue to review, enter and analyze surveys and update results regularly. Variables will continue to be evaluated in more detail as more data become available, and results could represent areas of focus for a more rigorous follow-up study to evaluate risk factors for virus introduction and transmission. Information collected in the written responses and mortality data will also continue to be evaluated in conjunction with the analysis for purposes of hypothesis generation and to inform the next steps of the investigation. Case control studies for turkey and layer operations are currently in the data collection phase and additional geospatial characteristics are being collected to support multivariate model building.

As of June 5, 2015, 81 questionnaires from infected turkey farms had been completed, reviewed, and analyzed by analysts. The locations for these infected flocks were IA (2), MN (67), ND (2), SD (6), and WI (4). About ¾ of HPAI-infected turkey farms were meat production farms (74%), while 20% were breeder farms. Farms that reported "other" production types raised both commercial turkeys and commercial chickens.

Table 1. Percent HPAI-infected turkey farms by premises characteristics

Premises Characteristic	Number of Respondents	Level or Response	Percent farms
Production type (H302)	80	Meat	74
		Egg	0
		Breeding	20
		Other	6
Age type (H303)	80	Multiple age	41
		Single age	59
Sex (H304)	80	Hen	31
		Tom	56
		Both	13
Flock size (H305)	79	<20,000 birds	27
		20,000 +	73
Facility type (H306-H311)	81	Brood	19
	81	Grow	64
	81	Other	6
	81	Both brood and grow	22
	81	Breeder	16
	81	Commercial	22
Brooder & grower same house (H312)	47		15
Farm capacity (H313)	77	<50,000 birds	53
		50,000 +	47
Number of barns (H314)	79	1 – 4	65
		5+	35
Ventilation (H316)	78	Curtain sided	47
		Environ. control	5
		Side doors	9
		Mostly curtains, plus other	38
Cool cell pads (H317)	79		4
Closest body of water (yds) (H319)	79	< 350 yds	39
		350 +	61
Water body type (H320-H324)	81	Pond	38
	81	Lake	22

Premises Characteristic	Number of Respondents	Level or Response	Percent farms
	81	Stream	20
	81	River	15
	81	Other	30
Other animals (H325-H334)	79	Beef cattle	6
	79	Dairy cattle	4
	79	Horses	4
	79	Sheep	3
	79	Goats	1
	79	Pigs	8
	79	Dogs	30
	79	Cats	24
	79	Poultry or domestic waterfowl	6
	70	Other	4
Water source (H335)	81	Municipal	5
		Well	93
		Surface	0
		Other	2
Water treated (H336)	80		71

Table 2. Percent HPAI-infected turkey farms by biosecurity factors

Biosecurity	Number of Respondents	Level or Response	Percent farms
House with family on property (H401)	81	Yes, common drive	42
		Yes, no common drive	22
		No	36
Signage (H403)	80		83
Gate to farm entrance (H404)	79	Yes, locked	10
		Yes, not locked	18
		No	72
Farm area fenced in (H407)	81		11
Freq veg. mowed (per month) (H408)	81	< 4	40
		4 +	60
Facility free of debris/trash (H409)	81		89
Wash/spray area for vehicles (H410)	81		46
Designated parking workers/visitors (412)	80		49
Changing area for workers (H423)	81	Yes, shower	28
		Yes, no shower	46
		No	26
Dedicated coveralls (H415)	81		73
Rubber boots or boot covers (H416)	81		100
Barn doors locked (H417)	81	Yes, routinely locked	40
		Yes, not routinely locked	22

Biosecurity	Number of Respondents	Level or Response	Percent farms
		No	38
Foot pans (H419)	81	Yes, in use	96
Footbath type (H421)	81	Dry	12
	81	Liquid	98
Ante area (H425)	81		98
Rodent bait station (H427)	81	Yes, checked every 6 weeks	96
Fly control (H428)	81		41
Houses bird proof (H430)	79		72
Wild birds in house (H431)	81		35
Raccoons, possums, foxes (H433)	81		28
Wild turkeys, pheasants, quail (H434)	81		26
Biosecurity audits (H435)	81		43

Table 3. Percent HPAI-infected turkey farms by employee characteristics

Employee Characteristics	Number of Respondents	Level or Response	Percent farms
Total number (H501)	81	< 3	52
		3+	48
Any nonfamily (H502)	48		29
Worker assigned to: (H504)	81	Entire farm	62
		Specific barn/area	38
Common break area (H505)	78		69
Workers employed by other poultry operation (H507)	81		0
Biosecurity training sessions per yr (H508)	72	1+	94
Family members employed by other poultry operation (H509)	80		16
Part-time/weekend help (H511)	79		28
Restrict contact with backyard poultry (H512)	81		94

Table 4. Percent HPAI-infected turkey farms by equipment on farm

Farm Equipment	Number of Respondents	Level or Response	Percent farms
Farm specific (H601)	75	Company trucks	65
(H604)	77	Feed trucks	19
(H607)	80	Gates/panels	91

Farm Equipment	Number of Respondents	Level or Response	Percent farms
(H610)	80	Lawn mowers	63
(H613)	78	Live haul loaders	8
(H616)	68	Poult trailers	31
(H619)	72	Pre-loaders	15
(H623)	79	Pressure sprayer/washer	57
(H626)	77	Skid-steer loader	61
(H629)	67	Tillers	87
(H632)	70	Trucks	56
(H636)	58	Other	66

Table 5. Percent HPAI-infected turkey farms by litter characteristics and carcass disposal

Litter Characteristics and Carcass Disposal	Number of Respondents	Level or Response	Percent farms
Shed (H703)	81		37
Partial cleanouts (H704)	80		23
Who does cleanout (H708)	78	Grower	71
		Contractor	29
Litter disposal (H710)	79	On-farm	11
		Offsite	89
Dead bird disposal	81	On farm	51
	81	Off farm	47
	81	Off-farm by	20
Render	78	Yes, no bin cover	22
		Yes, bin cover not routinely	4
		Yes, bin cover routinely	19
		No rendering	55

Table 6. Percent HPAI-infected turkey farms by visitor characteristics

Visitor Characteristics	Number of Respondents	Level or Response	Percent farms
Number of Daily visitors	79	0	89
Visitor log	80		53
Outer clothing provided	75		68
Visitor:			
Service person	78	Yes, bird contact	35
		Yes, no bird contact	27
		No	38
Vaccination crew	76	Yes, bird contact	12
		Yes, no bird contact	7
		No	82
Moving crew	75	Yes, bird contact	36
		Yes, no bird contact	7
		No	57

Visitor Characteristics	Number of Respondents	Level or Response	Percent farms
Poult delivery	71	Yes, bird contact	41
		Yes, no bird contact	17
		No	42
Rendering pickup	71	Yes, bird contact	0
		Yes, no bird contact	42
		No	58
Litter service	72	Yes, bird contact	1
		Yes, no bird contact	56
		No	43
Cleanout service	71	Yes, bird contact	1
		Yes, no bird contact	20
		No	79
Equipment shared	60	Yes, bird contact	7
		Yes, no bird contact	12
		No	82
Feed delivery	76	Yes, bird contact	5
		Yes, no bird contact	84
		No	11
Feed covers kept closed	78		95

Table 7. Percent HPAI-infected turkey farms by wild bird characteristics

Wild Bird Characteristics	Number of Respondents	Level or Response	Percent farms
Wild birds around farm	81	Waterfowl	60
	81	Gulls	32
	81	Small perching	93
	81	Other water birds	15
	81	Other birds	27
Birds year round	77		90
Seasonality	79		84
Bird location	81	Away from facilities	46
	81	On farm, not in barns	63
	81	On farm, in barns	25

II. GEOSPATIAL ANALYSES

A. Comparison of General Wind Direction and Direction of HPAI Spread in One Cluster of HPAI in Minnesota

Project Background

This portion of the spatial analysis investigates the hypothesis that HPAI (EA/AM-H5N2) in MN is spread by air. To test this hypothesis we compared a directional analysis of positive premises in one cluster of positive HPAI premises in MN using ClusterSeer software with a generalized compass rose based on weather stations in the area. The results suggest very little alignment of general wind direction to disease spread direction although the data and methods used were very limited.

Data and Methods - Generalized Wind Rose

The generalized wind rose was developed based on wind direction and speed from the four weather stations found in Stearns, Meeker, and Kandiyohi counties, Minnesota (Figures 1 and 2). We chose to group wind direction for the four stations to get a view of how wind behaves across the area of interest used in the analysis. Combining would also reduce localized variations that could affect the directional analysis across the larger area of infections. Dates used to create the generalized wind rose were March 23 through April 2, 2015. These data are collected through the Automated Surface Observing System (ASOS). The data used were downloaded from the Iowa Environmental Mesonet website: http://mesonet.agron.iastate.edu/

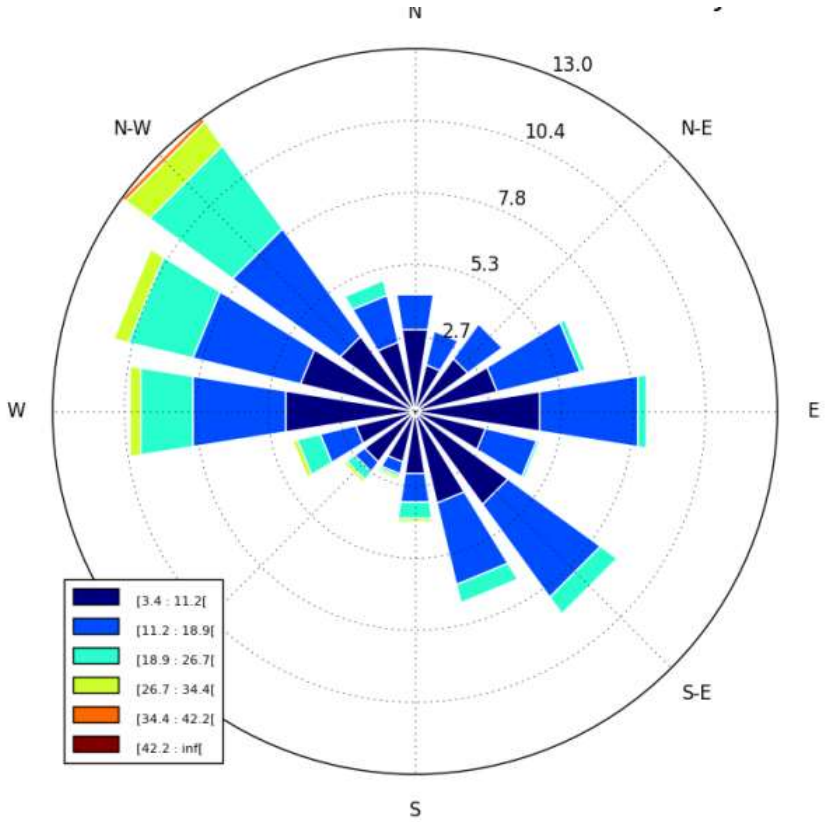

Figure 1. Wind Rose Minnesota: Combined BDH D39 LJF PEX

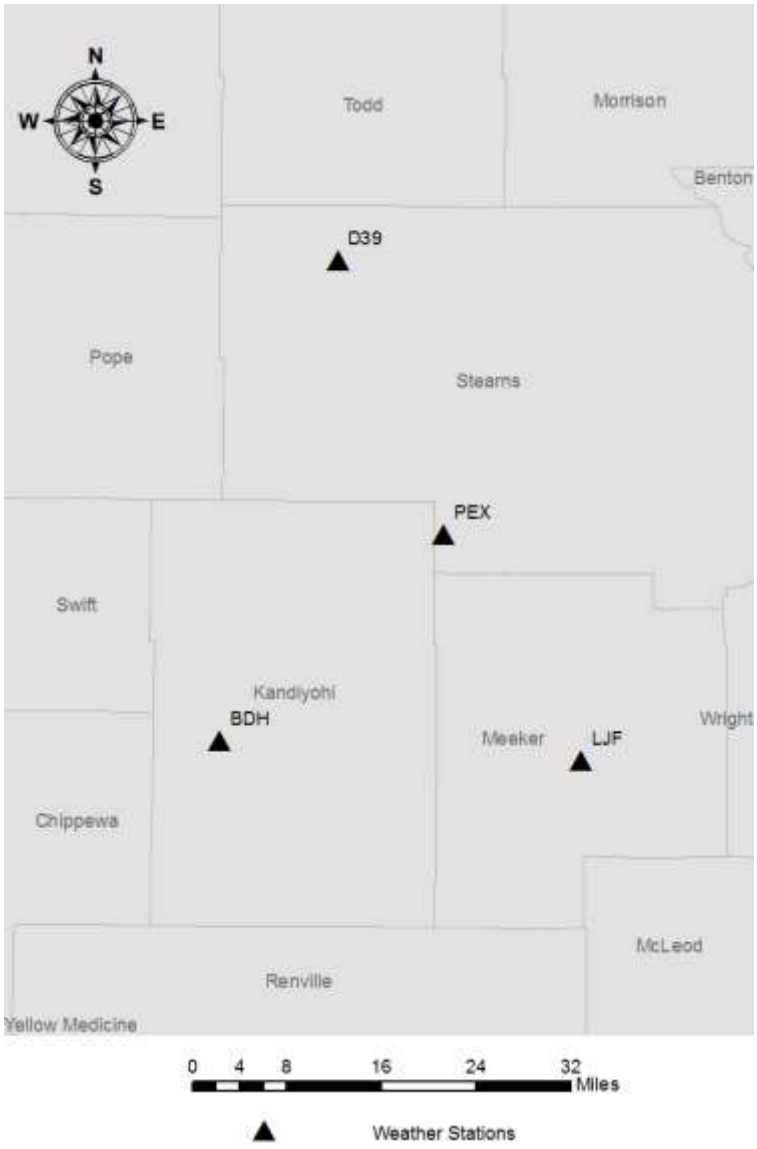

Figure 2. Location of weather stations used to create wind rose and resulting wind rose integrating data from all four stations.

Data and Methods - ClusterSeer Analysis

ClusterSeer is a software package developed for spatio-temporal analysis of disease. Within ClusterSeer we used the direction method to evaluate the direction of disease spread in one area of clustered HPAI cases in Minnesota. The Direction Method tests for a space-time interaction and calculates the average direction of disease spread. A relative model was used, which connects each case to all subsequent cases. This method was chosen since each positive case had the potential to infect all subsequent cases throughout the period of time for the cluster (approximately 3 weeks). The null hypothesis is that cases following (in a temporal sense) a given case are located in a random direction. The alternative hypothesis is that subsequent cases are located in a specific direction. ClusterSeer provides the following results: a significance test for the above hypothesis, the average direction of disease spread, and a measure of the variance in the angles between connected cases.

Case data for the ClusterSeer analysis were extracted from the APHIS EMRS (Emergency Management Response System) and imported into ArcGIS software. The spatial locations of all confirmed positive premise were validated using geocoding and aerial imagery interpretation to

ensure accuracy of the locations using ArcGIS software. Next, we identified a cluster of 35 cases in Kandiyohi, Stearns, and Meeker counties. The start date of the premise status represents the date premises were confirmed positive by NVSL and these dates were used for ClusterSeer analysis. The selected set of 35 cases were exported from ArcGIS as a text file and then prepared for input to ClusterSeer.

Results

Based on the ClusterSeer directional test, subsequent cases typically occurred in the southwest direction (221.288 degrees) to previous cases (Figure 3). The analytic results were statistically significant (p = 0.001), and the results were weakly consistent (ClusterSeer "concentration" value of 0.35, with 0 being randomly spread and 1.0 being strongly consistent in directional spread.) The generalized wind rose shows wind direction during this time window to be predominantly in the west-northwest direction but highly variable throughout the period. Based on this comparison, the two do not match and suggest that a simple wind movement of infection based on predominant wind direction during this time window does not explain the spread of avian influenza in this cluster of positive cases in Minnesota.

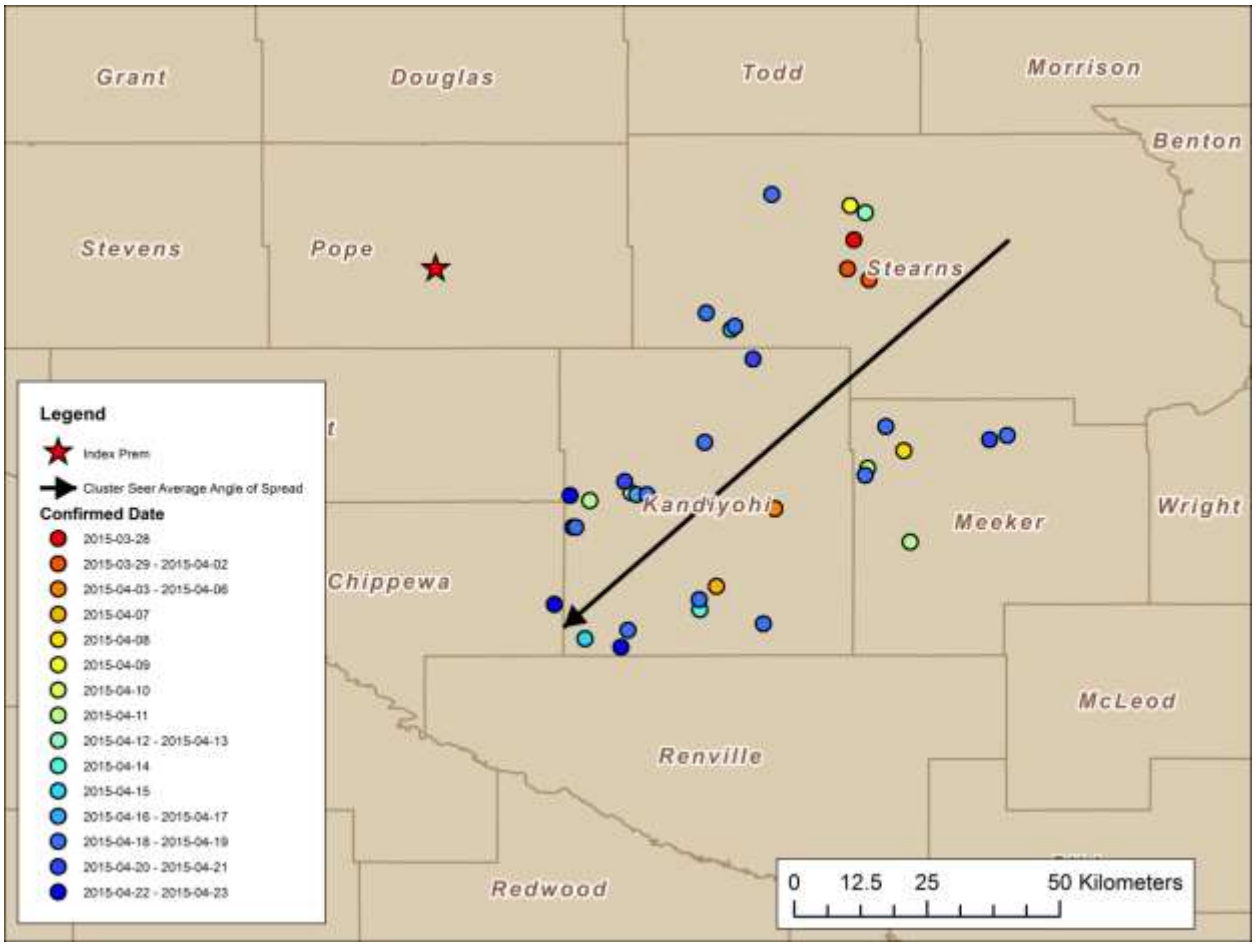

Figure 3. Positive premises used in ClusterSeer analysis and direction of spread as reported by ClusterSeer.

Limitations

The evidence suggests that there are likely multiple routes of disease spread for HPAI. Possible routes of disease spread include direct and indirect contacts between premises, such as movement of trucks, feed, people, and equipment. Movement of wild birds carrying HPAI can spread the virus to new areas and interactions between wild and domestic birds can cause infection. This analysis does not account for these methods of disease spread. The potential for HPAI to be spread by air is dependent on the period of viral shedding and the distance that HPAI can travel on dust particles and survive in the atmosphere. Detailed information on the survival characteristics of EA/AM-H5N2 HPAI may not be available at this time.

The generalize approach to measuring wind direction over the entire period of a cluster of cases used here makes it difficult to identify a predominant wind direction. A large-scale case-by-case analysis of disease spread and wind patterns using commonly employed "plume models" would enable a shorter time period of wind data to be used and highlight predominant wind directions. The large-scale case-by-case analysis would also enable more accurate temporal modeling of virus shedding and periods of infectivity. This approach has been used by other researchers to evaluate wind-borne spread of HPAI between farms. Plume model development is currently ongoing.

B. Wind Speed and Outbreak Clusters

Project Background

Based on field veterinarian observations, sustained high wind speeds over two days appeared to be related to clusters of outbreaks 5-7 days later.

Data and Methods

To investigate this hypothesis, wind speed data in Minnesota were collected from the ASOS weather station data network (http://mesonet.agron.iastate.edu/request/download.phtml?network=MN_ASOS). Stations close to the cluster of outbreaks around Kandiyohi and Stearns counties were used for the analysis. The chosen stations were Paynesville, Willmar, and Sauk Center.

Wind speed data from these three stations were processed to calculate 2-day minimums, medians, means, and maximums. The processed data were put into Tableau software for visual comparison of high sustained wind time periods and clusters of cases 5-7 days later.

Results

There appears to be some evidence for periods of sustained winds associated with new cases 5 to 7 days later. The clearest patterns can be found in the minimum two-day winds, where winds did not stop blowing (no zeroes) (Figure 4).

- The first strongly sustained wind of the season was around March 22. The first batch of investigations was March 29 and April 1, 7 and 9 days later.
- The second strongly sustained wind occurred around April 5. There are a large number of investigations around April 12, 7 days later.
- There was not a strong wind around April 12, but median values indicate a moderately sustained wind April 11 and 12. There was a very large number of investigations initiated on April 19.
- There was another very strong sustained wind around April 19. There were a large number of investigations initiated on April 26, 7 days later.

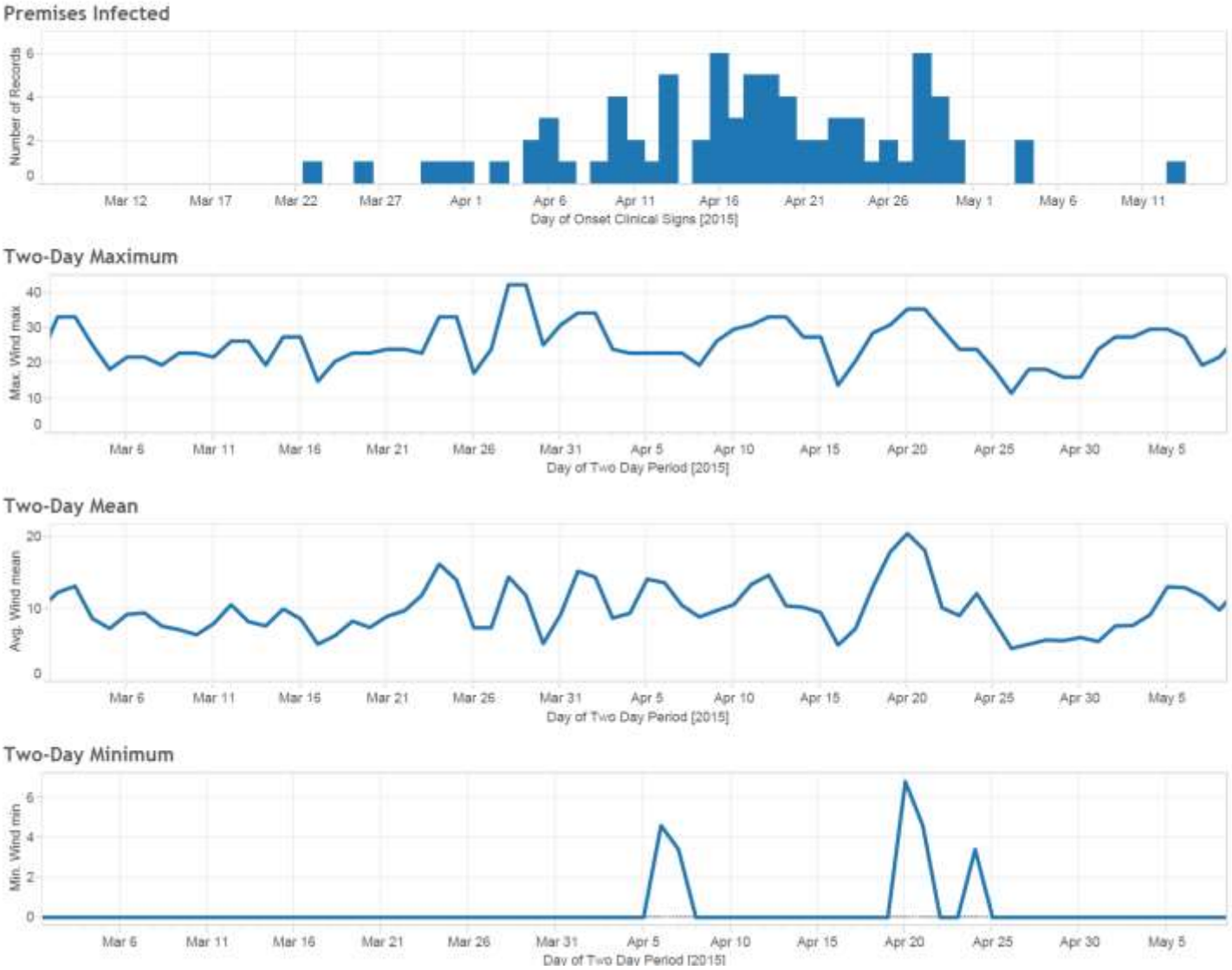

Figure 4. Associations between wind speed and clusters of HPAI cases in Kandiyohi and Stearns Counties, Minnesota

Limitations

This analysis is preliminary as an investigation of wind velocity as a component of disease spread. This is only a visual comparison, not a statistical analysis. The analysis is based on data from three stations and can only be applicable to infected premises in the vicinity of Kandiyohi and Stearns counties. A more robust analysis is ongoing.

III. ON-FARM SAMPLING

A. Detection of HPAI Virus in Air at Affected Premises

Objective

In order to evaluate the potential for airborne transmission of HPAI virus in turkey and layer flocks, a series of investigations was conducted in flocks with known H5N2 infection status.

Materials and methods

Affected Flocks

Six flocks with confirmed H5N2 HPAI infections were investigated: three turkey flocks located in Minnesota and three layer flocks located in Iowa and Nebraska. Sampling in most flocks was conducted within 3 to 10 days after diagnostic confirmation. Flocks had mortality rates ranging between 5 to 80% at the time of sampling and one flock had already disposed of a large proportion of dead birds.

Sampling Procedures

Air samples were collected inside and immediately outside (5 meters) of affected barns, and at extended distances ranging from approximately 70 to 1000 meters downwind from the barns. Air samples were collected using a (a) liquid cyclonic collector (Midwest Micro-tek, Brookings, SD, USA) capable to process 200 liters of air per minute (l/min); (b) Andersen Cascade Impactor (ACI) (Thermo Electron Corporation, Waltham, MA, USA) able to process 28.3 l/min; and (c) Tisch Cascade Impactor (TCI) (Tisch Environmental, Inc., Village of Cleves, OH) a high volume cascade impactor capable to process 1,100 l/min. Both the ACI and the TCI separate particles by size into several stages (0.4 to >9.0 µm) to determine the size particles that HPAI virus is associated with. For each air sampling event, there were 9 stages assayed for the ACI, 5 for the TCI and 1 sample for the cyclonic air collector (according to the design of each collector). Samples were collected for 30 (cyclonic and TCI) or 60 minutes (ACI) into collection media appropriate for each collector as per manufacturer' instructions. Negative controls were included to confirm absence of cross-contamination of collectors between samplings.

Environmental samples were also collected from surfaces in locations at high risk of direct exposure to the air exhausted from layer flocks. Surfaces samples were collected using disposable gloves with gauzes dipped into sterile media. Surfaces tested included both farm fixtures (e.g., silos, walls, fans, door handles) and temporary fomites exposed to exhaust air for approximately 2 hours (e.g., sampling equipment, plastic containers).

All samples were processed, aliquoted and submitted for diagnostic testing to the University of Minnesota Veterinary Diagnostic Laboratory. Air samples were screened using the matrix AI RT-PCR for influenza viruses and, if positive, were re-tested using specific H5 and N2 PCRs. Ct values < 35 were considered positive, 35-40 suspect, and >40 negative. To assess the infectivity of RT-PCR positive and suspect air samples, virus isolation in embryonated eggs was attempted at the National Veterinary Services Laboratory in Ames, Iowa. Positive samples were characterized as HPAI per cleavage site analysis from partial gene sequence as defined by OIE (sequence >99% similar to the index case A/Northern pintail/Washington/40964/2014).

Results

At least one air sample tested positive in 5 of the 6 flocks investigated. A total of 26% of air samples tested positive, 24% suspect and 50% negative (Table 8). There were 46% positive samples inside and 23% immediately outside. Sampling at distances greater than 70 m and for up to 1000 meters approximately, resulted in 2% positives (70 m) and 23% suspects (70-1000 m). A breakdown by flock type is shown in Table 9. HPAI H5 virus was isolated from one air sample collected inside a turkey flock (results from layer flocks are pending). Positive RT-PCR Ct values ranged between 31 and 35 and between 26 and 32 for samples collected in turkey and layer flocks respectively. These results were indicative of more viral genetic material at a layer flock compared to the turkey flocks. Ct values were also lower (higher viral quantities) in air samples collected inside compared to outside samples. HPAI RNA was associated with particles across multiple size ranges (Figure 5). Average positive Ct values were obtained in particles > 1.1 µm.

Of the two layer sites sampled for surface environmental contamination, one had 45% of suspect results, and the other 63% positives (Table 10). In the latter flock, Ct values ranging between 29 and 32 indicated relatively high amounts of HPAI RNA on the surfaces of farm fixtures and temporary fomites exposed for 60 minutes.

Conclusions

The results obtained to date indicate that HPAI can be aerosolized from infected flocks and remain airborne. HPAI RNA was detected in air samples collected inside and immediately outside of the infected premises. Low levels of genetic material were detected at distances of approximately 70 to 1000 meters. Viable virus was detected in an air sample collected inside an affected barn. The limited detection of viable virus does not necessarily indicate that the virus was not viable since the sampling process could contribute to the inactivation of the virus. In addition, considerable surface environmental contamination (relatively low Ct values) was demonstrated and widespread across multiple surfaces outside the premises of a layer flock.

The implications of these findings in terms of understanding the transmission of HPAI between flocks need further investigation and we hypothesize that both the transport of airborne particles and the deposition of infectious airborne particles on the surfaces around infected premises represents a risk for the spread of HPAI to other locations.

Acknowledgements

This study was possible with the collaboration of members at the College of Veterinary Medicine, School of Public Health and College of Science and Engineering from the University of Minnesota, staff of USDA APHIS, and poultry industry veterinarians.

Table 8. Summary of results obtained from air samples

	Turkeys	Layers	Total
Positive	47 (28%)	51 (24%)	98 (26%)
Suspect	51 (31%)	41 (19%)	92 (24%)
Negative	68 (41%)	124 (57%)	192 (50%)
Total	166 (100%)	216 (100%)	382 (100%)
Ct <35: positive; Ct 35-40: suspect; Ct >40 negative.			

Table 9. Summary of results of air samples obtained by distance

		Inside	5 m	70-150 m	500-1000 m
Turkeys	Positive	40 (36%)	7 (21%)	0%	NT
	Suspect	26 (23%)	17 (50%)	8 (38%)	NT
	Negative	45 (41%)	10 (29%)	13 (62%)	NT
Layers	Positive	28 (78%)	22 (24%)	1 (4%)	0 (0%)
	Suspect	8 (22%)	16 (18%)	9 (32%)	8 (13%)
	Negative	0 (0%)	52 (58%)	18 (64%)	54 (87%)
Total	Positive	68 (46%)	29 (23%)	1 (2%)	0 (0%)
	Suspect	34 (23%)	33 (27%)	17 (35%)	8 (13%)
	Negative	45 (31%)	62 (50%)	31 (63%)	54 (87%)
	Total	147 (100%)	124 (100%)	49 (100%)	62 (100%)
Ct <35: positive; Ct 35-40: suspect; Ct >40 negative.					

Table 10. Summary of surface sample testing

	Layer 1*	Layer 2	Total	Range Ct values
Positive	0 (0%)	7 (63%)	7 (35%)	29.03-32.15
Suspect	4 (45%)	4 (36%)	9 (45%)	35.14-39.15
Negative	5 (55%)	0 (0%)	5 (25%)	>40
Total	9 (100%)	11 (100%)	20 (100%)	
**Layer flock had already disposed of a significant number of dead birds at time of testing* *Ct <35: positive; Ct 35-40: suspect; Ct >40 negative*				

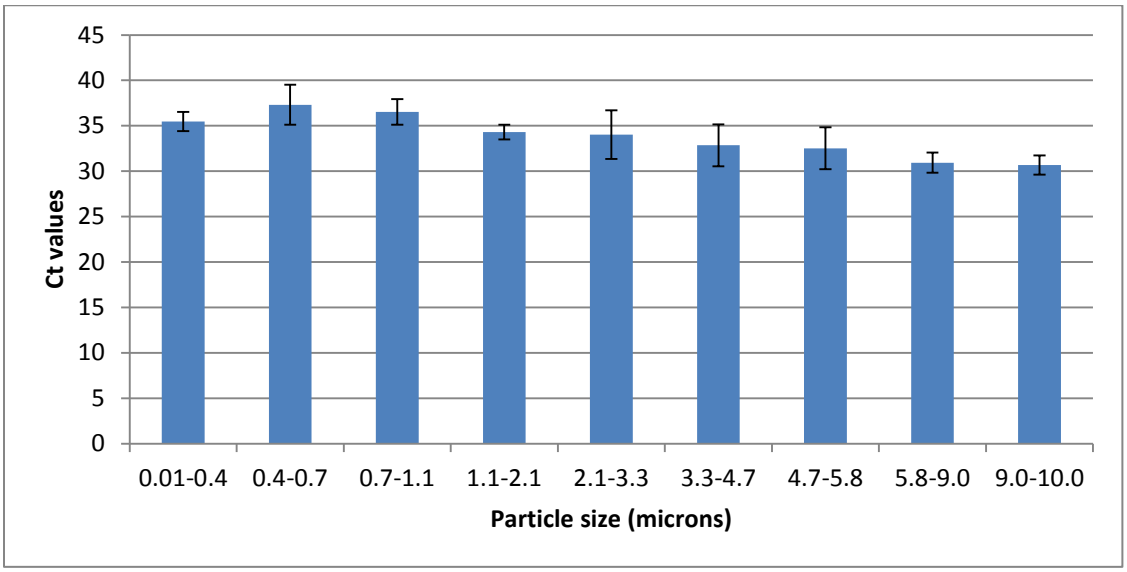

Figure 5. Average RT-PCR cycle threshold (Ct) values by particle size of air samples collected inside and immediately outside of turkey and layer flocks using the Anderson Cascade Impactor. Ct <35: positive; Ct 35-40: suspect; Ct >40 negative.

IV. PHYLOGENETIC ANALYSIS

A. Eurasian H5Nx Virus Overview

HPAI virus (H5N8 clade 2.3.4.4) originating from Eurasia (EA) spread rapidly along wild bird migratory pathways in the Eastern Hemisphere during 2014. Introduction of this virus into the Pacific Flyway of North America sometime during 2014 allowed mixing with North American (AM) origin low pathogenicity avian influenza A viruses generating new (novel) combinations with genes from both EA and AM lineages (so called "reassortant" H5Nx viruses). To date, the H5Nx viruses have been detected in the Pacific, Central, and Mississippi Flyways (Figure 6). These findings are not unexpected as the H5Nx viruses continue to circulate.

The USDA APHIS National Veterinary Services Laboratories (NVSL) collaborated with the USDA ARS Southeast Poultry Research Laboratory (SEPRL) and the Influenza Division of the Centers for Disease Control and Prevention (CDC) to generate the analyses for this report. The whole genome sequence is used to monitor the virus evolution and assess risk to veterinary or public health based upon presence/absence of specific amino acid substitutions or protein motifs.

All viruses analyzed to date are highly similar, have an HA gene derived from the EA H5 clade 2.3.4.4, and are highly pathogenic in poultry. Both H5N2 and H5N8 have been implicated in recent poultry outbreaks. There is molecular evidence that independent introductions as well as "common source"

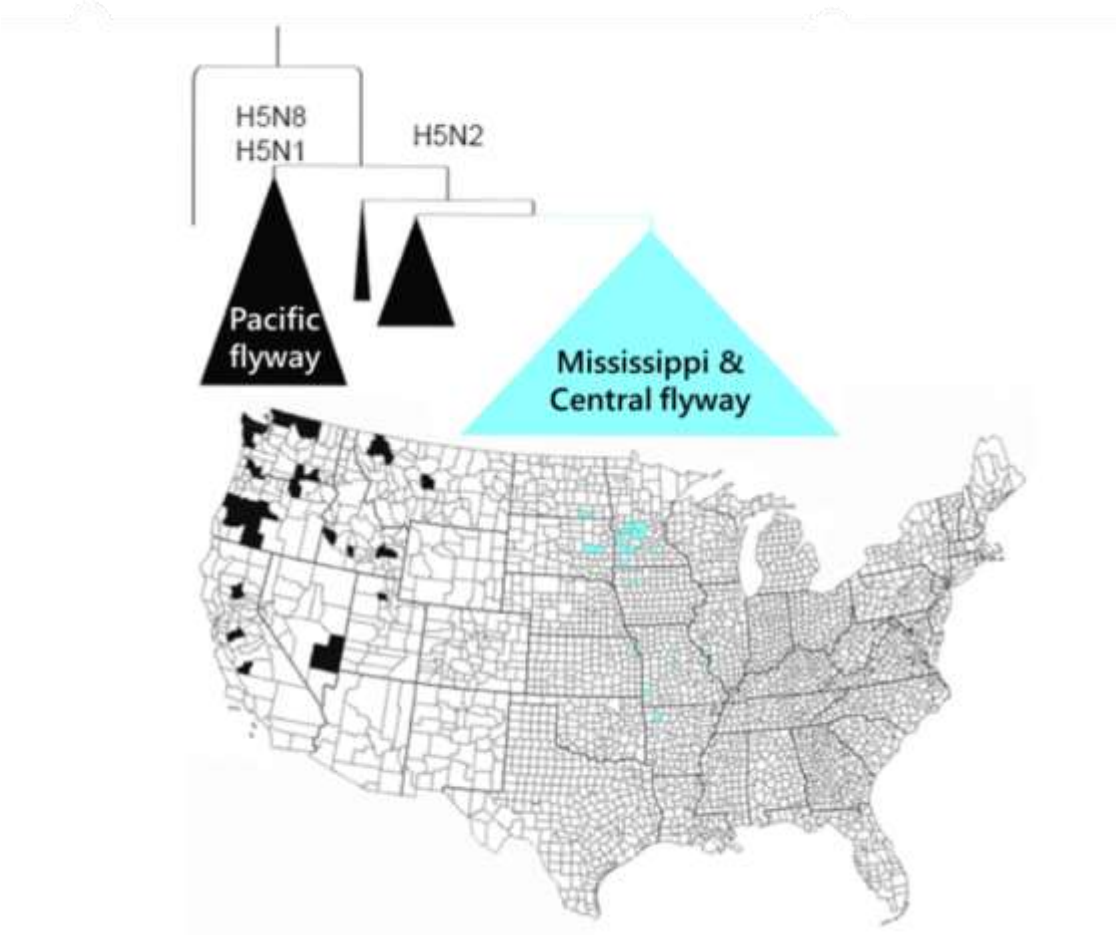

Figure 6. Phylogeny of the PB2, HA, and matrix genes of the H5Nx viruses and geographic distribution by subtype

exposures are occurring in several states concurrently; further field epidemiologic investigation is warranted. Presently the risk to human health remains low; molecular markers associated with antiviral resistance or increased virulence and transmission in mammals have not been detected.

Summary of H5Nx molecular analysis

Both H5N2 and H5N8 have been implicated in recent poultry outbreaks; all viruses detected to date have an HA gene derived from the EA H5 clade 2.3.4.4 and are highly pathogenic for poultry.

This analysis includes viruses detected through early April 2015 from 16 states (n=92 viruses; H5N8=20, H5N2=68, H5N1=4; 13 from backyard, 36 from commercial, and 43 from wild and captive wild birds). While these viruses remain highly similar overall, analytical tools that identify amino acid substitutions along the HA1 protein, the neuraminidase (NA) gene and internal protein genes can improve our understanding of the virologic, antigenic, and epidemiologic features of the virus (refer to section on Diagnostics and Characterization for H5Nx viruses). The findings, depicted in Table 11, are summarized here:

- Viruses are >99% similar across the entire viral genome within subtype.
- More than half of the H5Nx viruses are identical across the HA1 protein (54/92).
- Of viruses with one or more HA1 protein substitutions compared to the A/gyrfalcon virus (index case for H5Nx detection in the U.S. associated with the current outbreak), the majority are from poultry (28/38).
- Turkey H5N2 viruses from AR, IA, MN, ND, SD, and WI contain a change in the HA1 protein at a putative antigenic site (HA S141P; numbering per mature H5 HA) (Table 11); such substitutions may be more easily sustained in small virus populations (e.g. poultry flock) but may or may not persist.
- One H5N2 virus a with a NA stalk deletion (previously associated with poultry adaptation in HPAI H5 viruses) was isolated from a wild Cooper's hawk but has not been seen in U.S. poultry.
- The H5N1 viruses have been detected only in wild birds from Washington in the U.S. and in a backyard flock in British Columbia, Canada.
- Two H5N8 wild bird viruses from Oregon in mid-January have been identified with PB1 and PA internal genes of North American origin suggesting ongoing opportunities for virus reassortment.

Molecular analysis suggests that independent introductions and "common source" exposures are occurring in several states concurrently; interpretation based upon ongoing field investigations is pending

Molecular epidemiology

Evidence for a cluster that may have spanned a state boundary (between Minnesota and South Dakota) appears in APHIS' phylogenetic data. The strongest data links (via network analysis and amino acid substitutions) are for the Minnesota/South Dakota cluster and the Stearns County cluster. Field epidemiologic investigations are ongoing to identify potential indirect contacts between these operations.

Stearns County Minnesota Cluster

28-Mar	MN	Stearns County	Commercial Turkey	45,140 turkeys
2-Apr	MN	Stearns County (2)	Commercial Turkey	65,698 turkeys
4-Apr	MN	Stearns County (3)	Commercial Turkey	78,000 turkeys
9-Apr	MN	Stearns County (4)	Commercial Turkey	44,800 turkeys

Minnesota/South Dakota Cluster

27-Mar	MN	Lac Qui Parle County	Commercial Turkey	65,800 turkeys
1-Apr	SD	Beadle County	Commercial Turkey	50,587 turkeys

Public health aspects

- All viruses to date lack key amino acid substitutions associated with human-like receptor binding or substitutions in the polymerase or other internal genes associated with increased virulence and transmission in mammals
- No known markers of neuraminidase inhibitor (Oseltamivir) resistance have been identified

Poultry vaccine strain selection considerations

The H5Nx viruses remain highly similar overall, and ongoing detection of both the H5N2 and H5N8 HPAI viruses indicates that a strain with broad antigenic coverage is needed. Genetic, antigenic, and growth characteristics are considered for selection of poultry candidate strains. Experimental studies in poultry indicate that antibody to the neuraminidase protein does not play a significant role in protection. Antigenic characteristics and challenge studies will be used to evaluate protection of candidate vaccines; ongoing evaluation of viruses for antigenic drift will continue.

Table 11. Clade 2.3.4.4 H5Nx viruses through early April 2015 with one or more amino acid substitutions in the HA1 protein (38/92 viruses) compared to the U.S. index virus A/gyrfalcon/Washington/41088-6/2014(H5N8). Month of detection, sector type, and state are listed.

Mature H5 HA1 (reference in red)	12	11	12	12	12	12	12	1	1	1	1	1	1	1	3	3	3	3	4	4	4	4	4	4	4	4	4	3	3	4	4	3	4	4	4	4	4	4	month
	wild bird	wild bird	backyard	backyard	backyard	wild bird	wild bird	wild bird	wild bird	wild bird	wild bird	wild bird	wild bird	backyard	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	turkey	sector
	WA	WA	WA	WA	WA	WA	WA	WA	WA	OR	OR	OR	OR	WA	MN	MN	SD	AR	MN	MN	MN	MN	MN	MN	IA	MN	MN	MN	MN	MN	MN	ND	ND	WI	SD	MN	ND	SD	state
35	K		R	R	R																															Substitutions with potential virologic significance		
73	V												I																									
83	A																									T												
88	D																																			G	G	
114	T	N																																				
122	W					L																																
136	P							Q																		S	S	S	S	S	S								Antigenic site A
141	S								P	P	P			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P				Antigenic site A
162	I																																				V	
194	P												S																									
218	Q								R																													
223	S									G																												Receptor binding; Antigenic site D
234	K																										R	R	R	R								
235	P						Q																															
257	V								I																													
294	I							V																														
309	N																															K	K	K	K			
312	V																																		I			
322	L														Q																								
# Sub	Ref	1	1	1	1	1	1	2	2	2	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	2	3	3	3	3	3	2	2	2	3	1	1	1	vs. A/gyrfalcon/Washington/41088-6/2014_H5N8

Diagnostics and characterization for H5Nx viruses

Eurasian H5 clade 2.3.4.4 viruses (aka H5Nx), more specifically the "Intercontinental Group A viruses"[1] (icA), were initially detected in the U.S. during December 2014 and are known to be highly pathogenic to poultry; no other Eurasian H5 viruses have been detected in the U.S. to date (May 2015). The index viruses are A/gyrfalcon/Washington/41088-6/2014(H5N8) and A/Northern pintail/WA/40964/2014 (H5N2).

Molecular diagnostics for influenza A virus (IAV) used across the National Animal Health Laboratory Network (NAHLN) in the U.S. have been confirmed to work well to detect these Eurasian H5Nx viruses.[2] As a primary surveillance tool, the NAHLN H5 assay is broadly reactive and not intended to distinguish geographic lineage or pathotype. NVSL also uses a highly specific H5-icA assay[3] developed by SEPRL, which targets the Eurasian H5 clade 2.3.4.4 gene and conducts Sanger sequencing protocols to generate partial HA/NA sequence directly from the sample for

[1] 2015 Lee et al, Intercontinental Spread of Asian-origin H5N8 to North America through Beringia by Migratory Birds, epub ahead of print *JVirol* http://jvi.asm.org/content/early/2015/04/02/JVI.00728-15.long

[2] Influenza A protocols including Spackman 2002 targeting the matrix, VetMax Gold AIV and the H5 subtyping assays (2008 and 2014 protocols)

[3] The H5-icA assay protocol is available from SEPRL and positive control is available from NVSL for standard user-fee; note that this assay has a very narrow in spectrum specific to H5 clade 2.3.4.4 viruses and should be used in conjunction with the NAHLN H5 assay, not as a replacement

confirmation, pathotyping, and subtype determination. Select viruses are also processed for in vivo pathotyping in specific pathogen free chickens. Results from in vivo testing is specific to the species tested (e.g., chickens).

Additionally, whole genome sequencing is conducted to monitor viral evolution. Both Ion Torrent and MiSeq technologies are used. A brief summary of the procedure for IAV follows. All eight segments of isolates were amplified using gene-specific and universal primers for each segment. The cDNA was purified and cDNA libraries were prepared for the Ion Torrent using the IonXpress Plus Fragment Library Kit (Life Technologies) with Ion Xpress barcode adapters. Prepared libraries were quantitated using the Bioanalyzer DNA 1000 Kit. Quantitated libraries were diluted and pooled for library amplification using the Ion One Touch 2 and ES systems. Following enrichment, DNA was loaded onto an Ion 314 or Ion 316 chip and sequenced using the Ion PGM 200 v2 Sequencing Kit.

Analysis of sequence data includes phylogeny of all eight segments, determination of amino acid substitutions across the HA1 protein, and network analysis of three gene segments (PB2, HA, MP). Phylogenetic trees are generated using neighbor-joining algorithms with a kimura-2 parameter nucleotide substitution model. Amino acid differences in the HA1 portion of the HA protein compared to the A/gyrfalcon reference virus with potential virologic significance are annotated based on previous experimental studies with HPAI H5 viruses that have demonstrated changes in virus phenotype using various in vivo and in vitro systems. The NA and internal protein genes are aligned to H5N8 and H5N2 reference virus genomes using MUSCLE (i.e., A/gyrfalcon/Washington/41088-6/2014 and A/Northern pintail/WA/40964/2014) and screened for the presence of amino acid substitutions or protein motifs that have previously been associated with either poultry or mammalian host adaptation.

APPENDIX A. HPAI INVESTIGATION – QUESTIONNAIRE

(Version 1.0 – March 2015)

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.

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**	**NameContact?**
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)

K. NARRATIVE/COMMENTS

FARM DIAGRAM -Attach a download from satellite imagery if possible. In addition, draw a simple schematic map of the farm site centering with the poultry houses/pens. Identify where the HPAI positive flocks were housed. Also, include: fan banks on houses, residence, driveways, public roads, bodies of water, feed tanks, gas tanks, out buildings, waster dumpsters, electric meters, dead bird disposal, parking areas, other poultry sites. Digital photographs, if allowed, are excellent supporting documentation.

North

USDA APHIS Veterinary Services

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For more information, contact:

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