United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Epidemiologic and Other Analyses of HPAI-Affected Poultry Flocks: September 9, 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 updates to earlier reports released by APHIS on June 15 and July 15, 2015.

With the data from these reports, APHIS concludes that there is not substantial or significant enough evidence to point to a specific pathway or pathways for the current spread of the virus. This is further supported by the molecular analysis of the virus. This edition includes data on the characteristics and biosecurity measures of infected turkey farms and a case-control study to compare these measures between infected and non-infected farms. We have also sampled wildlife near affected and unaffected farms. This report also describes an analysis of wind plumes and the potential for airborne transmission of HPAI virus.

In an update of the case-control study focused on egg layer flocks in Iowa and Nebraska, a number of risk factors for HPAI introduction and factors associated with lowering the risk of introduction were identified in our multivariable analysis at both the farm and barn levels. At the farm level, being located in an existing control zone was highly associated with farm status. Rendering dead birds was a risk factor; 39% of case farms (compared to 13% of control farms) reported that the renderer came onto the farm. Although a similar percentage of case and control farms reported that garbage trucks came to the farm, 61% of case farms (compared to 23% of control farms) reported that the garbage trucks came near the barns. Having visitors change clothing was protective. Visits in the past 14 days (see prior report for the definitions of time periods for data collection) by a company service person were associated with farm status.

At the barn level, three variables remained statistically significant in the final multivariable model. Having a hard-surface barn entry pad that was cleaned and disinfected was protective when compared with all other levels combined (i.e., not having a hard surface, or no cleaning or no disinfection). Dead bird disposal within 30 yards of a barn remained a statistically significant risk factor. Although we identified a ventilation type that was protective, we are continuing to analyze that data due to a number of related factors that influence the effect of ventilation type.

We investigated the potential for airborne transmission by multiple methods. When aerosol exposure indices and distance measures were assessed together, the effect of the aerosol exposure index was often no longer statistically significant. These two variables are by nature correlated, as distance is an inherent part of the aerosol exposure index in addition to wind direction and speed. As a result, it was not possible to separate their effects in this analysis, and we were not able to determine with certainty whether aerosol transmission was responsible for a farm becoming infected. Other mechanisms associated with proximity could also have resulted in HPAI spread between nearby farms.

Also in this edition are updated results of a study of wildlife near affected and unaffected premises. Testing is ongoing on more than 2,600 samples collected, but some small perching-type birds were found serologically positive for H5 virus.

APHIS will continue to investigate how the HPAI virus is introduced and spread using both epidemiologic and molecular methods and will provide updated results regularly. We are also 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. With the results of this report and the two previous reports, we have identified several possible pathways. Comprehensive and stringent biosecurity practices remain crucial to reducing the risk of HPAI infection.

INTRODUCTION

Since the expansion of 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 provides an update of findings from these studies. Updated and new information is identified in the table of contents in red. As investigation and analysis efforts continue, this report will be updated with recent results 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

Background and Summary

This case series describes 81 turkey farms in Iowa, Minnesota, North Dakota, South Dakota, and Wisconsin with infections of HPAI: 63 meat production farms (grow and/or brood), 11 breeder farms, 6 farms that raised breeder candidate birds to breeding age, and 1 turkey farm that did not provide information on production type. Birds on these farms developed clinical signs of HPAI between March 30 and May 2, 2015. The median farm capacity was 46,000 birds and the median number of barns per farm was four.

The purpose of this case series is to describe farms with HPAI infections and generate hypotheses about disease predilection based on the descriptive information about the facilities or management on the farm. The case series cannot identify HPAI risk factors due to the lack of a negative comparison group.

In previous AI outbreaks in the United States, transmission occurred through movement of people between farms, transporting live and dead birds, equipment sharing, and transporting manure (Halvorson, 2009).

For several farms in this case series, fomites appear to have transmitted HPAI. The fomites were a person, farm equipment, farm vehicles, and a shared mortality bin. For these farms, 7 to 11 days passed between the potential exposure event and the onset of HPAI clinical signs. As expected, feed trucks and renderers were frequent visitors to the farms in this case series. Because feed trucks and renderers usually service more than one farm, they should be further explored as potential fomites for HPAI spread in this outbreak. Some observational evidence indicated airborne transmission of HPAI; further research should be done to determine if airborne transmission has been contributing to spread of the virus. For farms where airborne transmission was suspected, the incubation period was 3 to 8 days (somewhat shorter than those where fomites transmission was suspected).

There was a potential age predilection for HPAI. Almost half of infected tom farms had 13- to 16-week-old birds when the outbreak occurred, while half of hen farms had 9- to 12-week-old birds. Extra vigilance may be indicated when birds are at these life stages. Importantly, only 43% of case farms reported that biosecurity audits or assessments were conducted on the farm by the company or a third party. Farms can decrease their HPAI risk by verifying that biosecurity procedures are being followed properly.

Methods

State and Federal animal health officials in multiple States affected by HPAI virus strain H5N2 (HPAI-H5N2) continue to administer a survey instrument (Appendix A). Survey administrators are requesting that respondents be individual(s) most familiar with the farm's management and operations. Instructions request responses for the 2-week period prior to HPAI detection. Investigators have been asked to complete the investigation within 1 week of detection.

Completed questionnaires are delivered via secure email to USDA-APHIS-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.

Farm Characteristics

This case series of 81 turkey farms is comprised of 63 meat production farms (grow and/or brood), 11 breeder farms, 6 farms that raised breeder candidate birds to breeding age, and 1 turkey farm that did not provide information on production type (Table 1). It is interesting to note the relatively high number of breeder farms (14+7%=21% of all cases) involved in the outbreak. Breeder farms typically have very good biosecurity due to the higher value of the birds; many breeder farms are shower-in, shower-out facilities. The median farm capacity was 46,000 birds, and the median number of barns per farm was four (Table 2). Most of the farms (76%) had barns that were uniform in orientation (i.e., parallel to each other; Table 3).

Table 1. Percent HPAI-positive turkey farms by production type

Production type (type_code)	Number Farms	Percent Farms
Grower Only – Toms	27	33
Grower Only – Hens	5	6
Grower Only – Toms and Hens	1	1
Brooder Only – Toms	1	1
Brooder Only – Hens	1	1
Brooder Only – Toms and Hens	0	0
Grow and Brood – Toms	18	22
Grow and Brood- Hens	3	4
Grow and Brood – Toms and Hens	7	9
Breeders	11	14
Grow Breeder Candidate Poults	6	7
Not Specified	1	1
Total	**81**	**100**

Table 2. Descriptive statistics for HPAI-positive turkey farms

Characteristic	Median	Min	Max
Farm Capacity (h313)	46,000	5,000	488,000
Number of Barns (h314)	4	1	24
Barn Capacity (h315)	12,000	2,500	90,000
Distance to Closest Body of Water (yd) (h319)	800	15	8,800

Table 3. Percent HPAI-positive turkey farms by farm characteristics

Characteristic	Number Respondents	Level or Response	Percent Farms
Age type (h303)	80	Multiple ages on farm	45
		Single age on farm	55
Brooder & grower in same house (for the subset of farms that brood and grow) (h312)	28		25
Ventilation (h316)	78	Curtain sided	47
		Environ. Control	5
		Side doors	9
		Other*	38
Cool cell pads (h317)	79		4
Closest body of water (type) (h320-h324)	81	Pond	38
	81	Lake	22
	81	Stream	20
	81	River	15
	81	Other	30
Other animals on farm (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
	79	Other	4
Drinking water source (h335)	81	Municipal	5
		Well	93
		Surface	0
		Other	2
Water treated (h336)	80		71
Orientation of barns on premises (orientation)	70	Uniform	76
		Mixed	24
**mostly curtains plus other*			

Outbreak Characteristics

Epidemic Curve

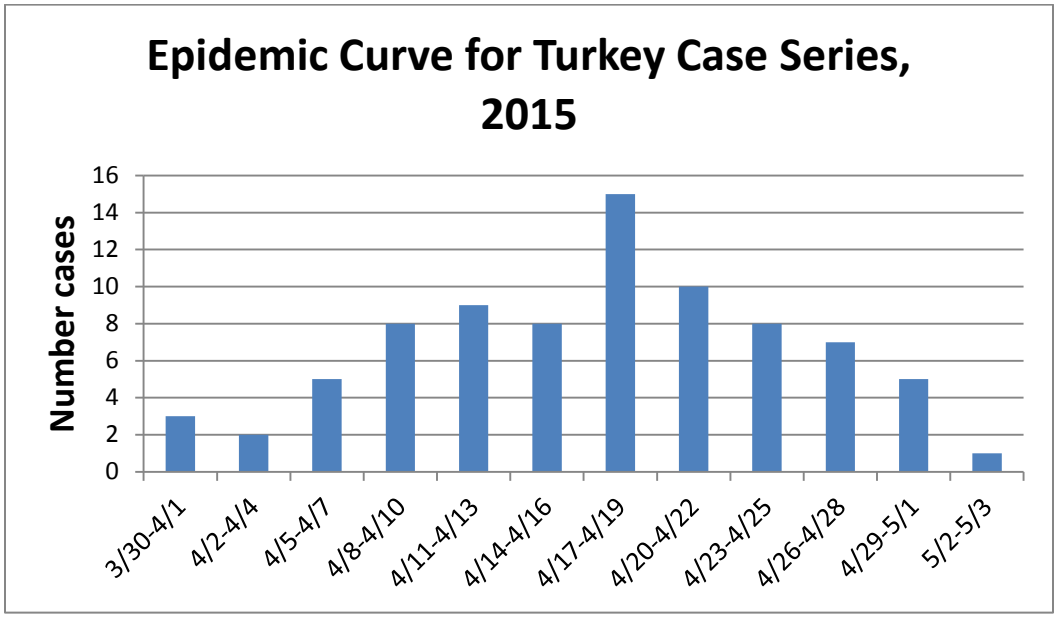

Figure 1. Epidemic curve for turkey case series farms

Bird Age

None of the case farms that provided bird age information had birds younger than 4 weeks old (Table 4). The median ages at the time of the outbreak were 11, 14, and 30.5 weeks for hen farms, tom farms, and breeder farms, respectively.

Almost half of infected tom farms had 13- to 16-week-old birds when the outbreak occurred, while half of hen farms had 9- to 12-week-old birds. The incidence of disease was slightly skewed toward older toms (Figure 2). The apparent age predilection may indicate changes in bird susceptibility at different ages, or could be related to changes in traffic and farm activities at different bird ages.

Table 4. Percent farms by bird age at time of outbreak

Production type	Age (weeks)	Percent Farms
Hens (n=10)	<4	0
	4-8	20
	9-12	50
	>12	30
Toms (n=34)	<4	0
	4-8	11
	9-12	17
	13-16	46
	>16	26
Breeder (n=14)	≤16	7
	17-36	64
	>36	29

**not all farms provided this information*

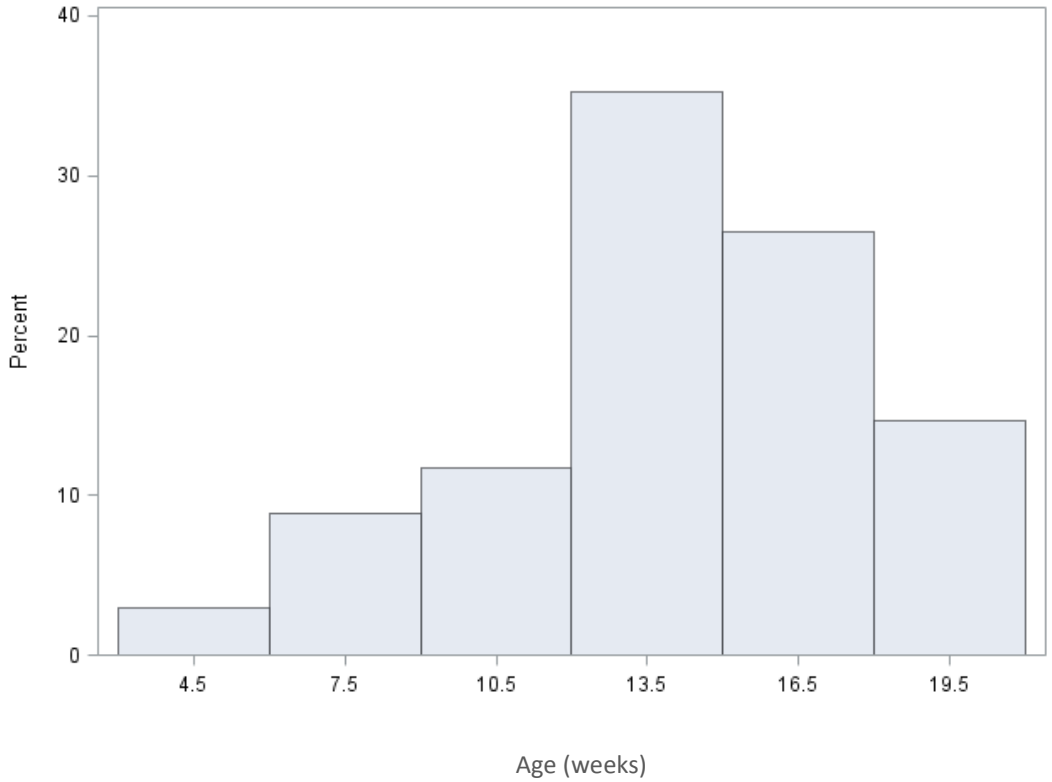

Figure 2. Histogram of bird age at time of outbreak on tom farms

Time since Placement

The median time from bird placement to the date when HPAI clinical signs began was 63 days, with a range of 1-416 days (Table 5).

Table 5. Percent farms by time from bird placement to outbreak date* (n=59)

Time (days)	Percent farms
< 7	5
7-30	14
31-60	31
61-90	17
>90	34

**not all farms provided this information*

Outbreak Pattern

Information about the first barn where birds developed clinical signs was extracted for each farm; however, not all respondents provided enough supplemental information to determine barn details (see number reporting in Table 6). On the majority of case farms, the first affected barn had an east-west orientation (73% of farms), was at the end of a row or standing alone (**not** surrounded on 2 sides by other barns, 63%), and was **not** the closest barn to a water body (59%, n=17). The majority of all turkey barns in the area may be oriented E-W to reduce barn heating during summer months.

Table 6. Percent farms by orientation patterns of barns*

Barn characteristic	Level	Percent farms
Orientation of first affected barn (n=70) (orientation_first)	N-S	23
	E-W	73
	Diagonal	4
First affected barn surrounded on 2 sides by other barns (internal) (n=68) (barn_surr)	Yes	37
First infected barn closest to nearest water body (n=17) (closest)	Yes	41

**not all farms provided this information*

Farm Biosecurity

Turkey farms typically follow biosecurity protocols, which are established by the company with which they work. Common procedures include spraying vehicle tires with disinfectant at the farm entrance, requiring visitors and employees to wear coveralls and disposable boot covers (or dedicated footwear) before entering the barns, using disinfectant footbaths at barn entrances, using rodent control, and caring for younger birds before caring for older birds. The objective is to establish a clean-dirty line where outside contaminants are not carried into the barn. Showering before entering the barn is commonly required on breeding farms.

It is important to note that the results in Table 7 are based on answers to a questionnaire and not necessarily observation of routine biosecurity practices used on farms. Therefore, the findings are a reflection of farm policies, but may not reflect the practices that were actually in use. Importantly, only 43% of case farms reported that biosecurity audits or assessments were conducted on the farm by the company or a third party. Farms can decrease their HPAI risk by verifying that biosecurity procedures are being followed properly.

In this case series, 46% of farms had a wash/spray area for vehicles, 73% used dedicated coveralls for workers before entering each house, 100% used boots or boot covers for workers, and 99% had footbaths at barn entrances (Table 7). The most commonly used footbath disinfectants were phenolic compounds, oxidizing agents and iodophors. A few farms used quaternary ammonium compounds or chlorine compounds in footbaths. For washing vehicles, most farms used oxidizing agents or chlorhexidine.

Statistics on the use of biosecurity practices on U.S. turkey farms in general are not widely available. VS' National Animal Health Monitoring System (NAHMS) conducts periodic studies to characterize animal health and management on farms throughout the United States. Unpublished data from a 2010 NAHMS study, in which a small number of turkey farms (n=34) serving as controls for a study on clostridial dermatitis (USDA, 2012), were compared to the case series farms. Among these control turkey farms from 2010, the use of the above biosecurity practices was similar to the percentages reported for the case series farms. Therefore, biosecurity policies on the farms in this case series may be typical for the industry.

Table 7. Percent HPAI-positive turkey farms by biosecurity practices

Biosecurity	Number Respondents	Level or Response	Percent Farms
House with family on property (h401)	81	Yes, common drive	38
		Yes, no common drive	22
		No	40
Signage (“no admittance” or “biosecure area”) (h403)	80		83
Gate to farm entrance (h404)	79	Yes, locked	9
		Yes, not locked	18
		No	73
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 (h412)	80		49
Changing area for workers (h413)	81	Yes, shower	27
		Yes, no shower	46
		No	27
Workers wear dedicated coveralls (h415)	81		73
Workers wear rubber boots or Boot covers (h416)	81		100
Barn doors lockable (hh417/h418)	81	Yes, routinely locked	40
		Yes, not routinely lock	22
		No	38
Foot pans at barn entrances (h419)	81	Yes, in use	99
Footbath type (h421, h422)	81	Dry	12
	81	Liquid	98
Ante area (h425)	81		98
Rodent bait station (h426, h427)	81	Yes, checked q 6 weeks	95
Fly control (h428)	81		41
Raccoons, possums, foxes seen in or around barns (h433)	81		28
Wild turkeys, pheasants, quail seen around poultry (h434)	81		26
Biosecurity audits (h435)	81		43

Employee Characteristics

People are potential fomites for transmitting HPAI, particularly if they move from farm to farm on the same day. None of the farms in this case series had employees who worked at multiple farms, and 94% had rules restricting workers from having contact with backyard poultry. These findings are typical for the turkey industry. However, 16% had family members who were employed by other poultry operations (Table 8). This is not surprising considering the density of

poultry operations in the area. Several steps of virus transfer would be required for disease to pass from farm to farm via family members who work at different farms, so the risk for this transmission route is likely to be fairly low.

Table 8. Percent HPAI-positive turkey farms by employee characteristics

Employee Characteristics	Number Respondents	Level or Response	Percent Farms
Total number (h501)	81	< 3	52
			48
Any nonfamily workers living on premises (h503)	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

Equipment Sharing

Equipment sharing is very common in the poultry industry. In the majority of cases, feed trucks, live haul loaders, pre-loaders, and other items were shared by multiple farms (Table 9). Equipment is typically disinfected between farms, but not all items are easy to disinfect (e.g., vehicles). In addition, disinfectants need sufficient contact time, and are less effective if organic matter and feces are present. Respondents were asked to describe their cleaning and disinfection procedures for pre-loaders. Most respondents described power washing followed by a disinfectant. If done correctly, this procedure should be very effective at inactivating HPAI. The power washing stage to remove all organic matter is particularly important, and is sometimes done inadequately in actual practice. A few respondents noted the importance of removing organic material, manure, and feathers.

Equipment sharing makes economical and logistical sense, but it also increases the risk of lateral spread of HPAI between farms. Fomites, such as equipment, are probably playing a role in this outbreak.

Table 9. Percent HPAI-positive turkey farms by equipment characteristics

Equipment	Number Respondents	Level or Response	Percent Farms
Farm specific (NOT shared with other farms)	75	Company vehicles/ trailers (h601)	65
	77	Feed trucks (h604)	19
	80	Gates/panels (h607)	91
	80	Lawn mowers (h610)	63
	78	Live haul loaders (h613)	8

Equipment	Number Respondents	Level or Response	Percent Farms
	68	Poultry trailers (h616)	31
	72	Pre-loaders (h619)	15
	79	Pressure sprayer/washer (h623)	57
	77	Skid-steer loader (h626)	61
	67	Tillers (h629)	87
	70	Trucks (h632)	56
	58	Other (h636)	66

Litter Characteristics and Carcass Disposal

Movement of manure and dead birds have both caused transmission of AI in previous outbreaks (Halvorson, 2009). When litter and carcasses are transported, infectious material may be spread to nearby farms as trucks travel down the road. In this case series, 89% of farms disposed of litter off-farm, and 47% used off-site disposal for carcasses (e.g., renderer, landfill; Table 10). Litter that was moved off site was most often applied to cropland or fields, while some farms moved litter off-site to be used as fuel at a power plant. It is important to reiterate that these were practices of producers in the 14 days prior to disease detection. Once detected, all movement of litter or manure was strictly controlled by federal and state regulatory officials.

Litter and carcass disposal methods were compared to the turkey flocks in the NAHMS 2010 clostridial dermatitis study (USDA, 2012). Although carcass disposal methods were comparable, farms in this series may have been more likely to use off-farm litter disposal. The comparison should be interpreted cautiously because the study was not designed to provide a control group for HPAI cases. Nonetheless, off-farm litter disposal may be a risk factor in the current outbreak.

Table 10. Percent HPAI-positive turkey farms by litter characteristics

Litter Characteristics	Number Respondents	Level or Response	Percent Farms
Litter shed present (h703)	81		37
Partial cleanouts (h704)	80		23
Who does cleanout (h708)	78	Grower	71
		Contractor	29
Litter disposal (h711)	79	On-farm	11
		Offsite	89
Dead bird disposal (h802-h804)	81	Burial pit/incinerator/composted on farm	51
	81	Off farm (landfill/renderer/other)	47
	81	Off-farm by owner/employee	20
Render (h803, h808, h809)	78	Yes, no bin cover	22
		Yes, bin cover not	4
		Yes, bin cover routinely	19
		No rendering	55

For the majority of farms, the barns were cleaned out more than 6 weeks before HPAI clinical signs began. None of the breeder farms (n=3) had a delivery of shavings less than 2 weeks before clinical signs began, but 36% of the meat farms did (Table 11).

Table 11. Percent farms by time from last cleanout to outbreak date, and time from most recent bedding delivery to outbreak date *

	Cleanout (a103)		Bedding delivery (a105)	
Time (weeks)	Percent breeder farms (n=15)	Percent meat farms (n=57)	Percent breeder farms (n=3)	Percent meat farms (n=14)
<2	0	7	0	36
2-6	20	14	33	43
>6	80	79	67	21

**not all farms provided this information*

Farm Visitors

Farm visitors are potential fomites for transmitting HPAI, particularly if they move from farm to farm on the same day. About half of farms (53%) had a visitor log, and 68% provided outer clothing for visitors (Table 12). For each farm, we examined visitor and vehicle traffic in the 3 to 10 days before HPAI clinical signs began, because HPAI probably arrived on the farm during this period (Table 13). There were no unusual patterns in visitors or vehicle traffic. The most common visitors/vehicles entering the farms were feed delivery vehicles and renderers. Because of the frequency of these visitors, and because they usually service more than one farm, they should be further explored as potential fomites for HPAI spread. Other vehicles or visitors may have been important in HPAI spread in this case series of farms, but information was not available on every type of visitor.

Table 12. Percent HPAI-positive turkey farms by visitor characteristics

Visitor Characteristics	Number Respondents	Level or Response	Percent Farms
Number of Daily visitors (h901)	79	0	89
Visitor log (h902)	80		53
Outer clothing provided (h904)	75		68
Feed covers kept closed (h963)	78		95

Table 13. Percentage of farms that had the following visitors/vehicle traffic 3 to 10 days before clinical signs began*

Visitor/Vehicle	n	Percent Farms
Feed delivery (feed)	41	83
Service person (service_person)	47	15
Litter services (litter)	43	12
Bird removal (load out)	48	4
Bird delivery(poult_delivery)	49	10
Cleanout services (lastcleanout)	52	0
Renderer/carcass removal (render)	53	38

**not all farms provided this information*

Wild Birds

Wild birds can transmit a variety of diseases to poultry. In particular, wild waterfowl are considered the primary reservoir for avian influenza viruses. Other wild bird species vary in their susceptibility to AI and their ability to transmit the virus. For instance, sparrows are highly susceptible to HPAI and can shed virus, while pigeons are unlikely to transmit virus (Brown et al., 2009).

Wild birds were observed inside the barns on 35 percent of the farms (Table 14). Starlings and sparrows were the most common type of bird seen in barns, and respondents reported seeing them in the barns from daily to occasionally. Eighty-four percent of farms reported that certain wild birds were present seasonally – particularly waterfowl migrating in Spring and Fall. Many respondents reported that small perching birds were seen year round.

Table 14. Percent HPAI-positive turkey farms by wild bird presence

Wild Bird Characteristics	Number Respondents	Level or Response	Percent Farms
Wild birds around farm (h1001-h1006)	78	Waterfowl	63
	79	Gulls	33
	78	Small perching birds	96
	78	Other water birds	15
	78	Other birds	28
Houses bird proof (h430)	79		62
Wild birds seen in house (h431)	81		35
Birds seen year round (h1007)	77		90
Seasonality to presence of some birds (h1009)	79		84
Bird location (h1011-h1013)	76	Away from facilities	49
	77	On farm, not in barns	66
	76	On farm, in barns	26

Impressions from Narrative Responses in Questionnaire

This section summarizes material provided as narratives in the questionnaire. While this can be valuable information to capture, it may be subject to the biases of the data collector and respondent.

Airborne Transmission

A number of producers expressed a suspicion about airborne HPAI transmission and noted very windy conditions prior to HPAI diagnosis. The following are some for air/wind-related spread mechanisms:

- Two grower farms suspected that birds were exposed to HPAI during placement on the farm in windy conditions. The flocks developed clinical signs 5 to 8 days post-placement. Neither farm reported any equipment sharing, farm visitors or vehicle traffic (not even feed trucks) in the 3 to 10 days before clinical signs began (except for the delivery of the birds).
- One farm observed an unusual pattern of disease spread. The birds were kept in multiple pens. Disease started in the pen closest to a ventilation window, and moved along the path of air flow from the ventilation window to the exhaust fans.

- One producer (Farm A) suspected that transmission occurred from sawdust blowing off the road onto his farm. The sawdust likely came from birds that were transported for processing on April 9 from Farm B (1.25 miles away). Farm B was diagnosed as HPAI positive on April 11. The blowing sawdust was seen the week of April 12, and Farm A developed clinical signs on April 17.
- Two breeder farms developed clinical signs 3-4 days after depopulation of a nearby positive premises. Both farms had very good biosecurity policies (e.g., shower in/out). The depopulated premises was about 500 yards away from 1 farm, and about 1,200 yards away from the other (2 different positive premises). In one case, the barns closest to the depopulated premises became infected while the barn farthest away did not.
- A brooder farm became infected 6 days after depopulation of a nearby premises. Distance between farms was less than one-quarter mile. The barn closest to the depopulated premises became infected first; this barn draws ventilation from the direction facing the depopulated premises. Both premises were under the same ownership, so it is possible other contacts caused transmission rather than airborne.

Other Modes of Transmission

For most farms, it was not possible to definitively identify the specific mechanism by which HPAI was transmitted to the farm. However, for a few farms, a particular transmission route was highly likely. The likely transmission mechanisms are listed below. The numbers in parentheses indicate number of days between the potential exposure event and the start of clinical signs on the exposed farm.

- A person who traveled back and forth between two farms (10-11 days).
- A piece of equipment that was borrowed from a pre-clinical positive farm (10 days).
- Two farms in close proximity that shared equipment and vehicles daily (11 days).
- Two farms in close proximity that shared a mortality bin. Farm 1 may have become infected due to waterfowl in standing water near the barn. Farm 2, which shared a mortality bin with Farm 1, developed clinical signs 7 days after Farm 1 (7 days).
- Five farms in a single State used the same company for rendering and/or load out services. These farms all developed clinical signs within a 10-day period.

These findings demonstrate potential important fomites in lateral transmission of HPAI – including equipment, vehicles, and people. The time periods in parentheses (7 to 11 days) are longer than the expected 3- to 5-day incubation period for some AI viruses. The incubation for this virus appears to be longer than 3 to 5 days based on experimental work conducted by the USDA Agricultural Research Service (ARS) Southeast Poultry Research Laboratory (SEPRL). In addition, fomites might carry the virus around an exposed farm for several days before it reaches the birds. In the observations of potential airborne spread (last section), the incubation period tended to be shorter (3-8 days).

Several farms noted that birds were being treated for other diseases at the time of HPAI diagnosis, such as clostridial dermatitis and cholera. Therefore, stress may play a role in susceptibility to HPAI.

One farm employs workers who commute together with other workers to a crowded communal housing facility that they rent together. These workers who live in the same house work for

multiple poultry operations in the area. Virus would need to survive several transfer steps (farm 1 (infected) → worker 1 → house surfaces at shared housing → worker 2 → survive biosecurity measures such as coveralls and footbaths → farm 2) for disease transmission to occur via this route, making it fairly unlikely, but not impossible. Certain practices by the workers could make this route more likely, such as having gross fecal contamination on shoes they wear home or sharing clothing/shoes/fomites with other workers. These details were not available.

We examined questionnaires carefully for farms that were geographically isolated from other infected farms. These farms may provide clues about spread via fomites. We identified the following potential HPAI sources:

- A load-out crew
    - The live haul loader was shared between multiple farms, some of which were in the most concentrated outbreak area. The affected farm was far from other cases, and the live haul crew visited 4 days before clinical signs began.
- Renderer or family member employed on another turkey operation
    - One farm had 2 risk factors: a renderer visit 5 days before clinical signs began, and a family member who was employed at another turkey farm (HPAI status unknown). The same rendering company was used on the same day by a farm that developed HPAI clinical signs 3 days later; however, data were not available to determine if the same rendering truck visited both farms.
- Sparrows or load-out crew
    - Another geographically isolated farm had two risk factors: sparrows inside the barns and a visit from a load-out crew 3 days before clinical signs began.
- Sparrows or day-old poult delivery
        - An independent farm had very little outside traffic. Poults were delivered very near the date clinical signs began. Sparrows were also seen inside the barns.

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USDA, 2012. Poultry 2010, Clostridial Dermatitis on U.S. Turkey-Grower Farms. USDA–APHIS–VS–CEAH–NAHMS. Fort Collins, CO #645.0612 (http://www.aphis.usda.gov/animal_health/nahms/poultry/downloads/poultry10/Poultry10_dr_ClostridialDermatitis.pdf)

B. Multivariable Analyses of Iowa Layer Case-Control Study – UPDATED

Background

A full description of the Iowa layer case-control study, including data collection methods and preliminary univariate data analysis, is reported in the USDA-APHIS-VS document "Epidemiologic and Other Analyses of HPAI-Affected Poultry Flocks: July 15, 2015, Report" (available at http://www.aphis.usda.gov/animal_health/animal_dis_spec/poultry/downloads/Epidemiologic-Analysis-July-15-2015.pdf. This document updates the earlier report and contains results of multivariable modeling at the farm and barn levels.

Statistical Methods

In brief, we performed two case-control analyses: the first was a farm-level comparison of case farms versus control farms, and the second a barn-level comparison of case barns on case farms with control barns on control farms. Variables were screened individually (methods previously described in July 15 report). Variables with *p*-values ≤0.20, and for which every level of the variable met a criteria of a minimum of eight observations, were considered for entry into candidate multivariable models. Multivariable logistic regression models were fit using a forward-selection procedure. Variables with *p*-values ≤0.05 were considered to be statistically significant.

Results

Respondents representing 28 case farms/barns participated in the study, with a set of 31 control farms/barns selected within a defined time period relative to case farms. Interviews were conducted from May 14 to June 3, 2015.

Farm-Level Analysis

Five variables were statistically significant in the final multivariable model. Being located in an existing control zone was highly associated with farm status (Table 15). Half of case farms were located in an existing control zone compared to only 10% of control farms (OR=28.8, p=0.002).

Rendering dead birds was a risk factor; 39% of case farms (compared to 13% of control farms) reported that the renderer came onto the farm. Additionally, 29% of case farms (and only 3% of control farms) reported that rendering trucks came near the barns (OR=21.4, p<.001).

Although a similar percentage of case and control farms reported that garbage trucks came to the farm, 61% of case farms (compared to 23% of control farms) reported that the garbage trucks came near the barns (OR=14.0, p<.001).

Having visitors change clothing was protective (OR=0.10, p=0.01). Many other variables related to visitor and employee precautions (such as hand washing, footwear protection, etc.) were significant in the univariate analysis but did not remain in the final model, due to high levels of collinearity and the likelihood that these variables were measuring similar biosecurity-related behaviors.

Visits in the past 14 days (see prior report for the definitions of time periods for data collection) by a company service person was associated with farm status: 50% of case farms and 19% of control farms had a company service person visit (OR=4.3, p<.001). Additionally, 43% of case farms and 16% of control farms reported that the service person entered the barn. We note that causation relative to infection cannot be determined via this study; therefore, we can't know whether the increased risk was due to the company service person's visit, or if this variable is a proxy for another risk, such as the initiating reason for requesting a service person visit.

Other variables may be associated with farm status, but could not be included in the multivariable model due to sparse data causing model instability. The variables included sharing racks and pallets as a potential risk factor, and employee hand washing and fly control as potential protective factors.

Table 15. Results of multivariable logistic regression of farm level analysis.

FACTOR	Percent case farms	Percent control farms	Adjusted Odds Ratio	P-value
In an existing control zone	50	10	28.8	.002
Garbage trucks near barns	61	23	14.0	<.001
Rendering trucks near barns	29	3	21.4	<.001
Visitors change clothes	77	93	0.10	.01
Company service person visit in past 14 days	50	19	4.3	<.001

Barn-Level Analysis

Three variables remained statistically significant in the final multivariable model (Table 16). Having a hard-surface barn entry pad that was cleaned and disinfected was protective when compared with all other levels combined (i.e., not having a hard surface, or no cleaning or no disinfection) (adjusted OR=0.16, p=0.01). A higher percentage of control barns (53.6%) than case barns (28.6%) had hard surface entry pads that were cleaned and disinfected.

Dead bird disposal within 30 yards of a barn remained a statistically significant risk factor (adjusted OR=2.78, p=0.002). Case barns (60.7%) were much more likely to have dead disposal within 30 yards than were control barns (35.5%). This corresponds with results in the farm level analysis, where we found higher risk of farm infection when rendering trucks entered the farm and came near barns.

When ventilation type was dichotomized into two levels, ceiling/eaves versus all other types, where other types included tunnel, curtain, and sidewall ventilation, we found the ceiling/eaves type to be protective (adjusted OR=0.33, p<0.001). Control barns were more likely to have ceiling or eaves inlets (67.7%) compared with case barns (48.2%).

Table 16. Results of multivariable logistic regression of barn level analysis.

FACTOR	Percent case barns	Percent control barns	Adjusted Odds Ratio	P-value
Barn entry with a hard surface entry pad cleaned and disinfected	28.6	53.6	0.16	0.01
Disposing of dead birds near a barn (within 30 yards)	60.7	35.5	2.78	0.002
Having ceiling or eaves inlet ventilation type (compared with curtain, sidewall or tunnel types)	48.2	67.7	0.33	<0.001

C. Qualitative Analysis of Interviews Conducted Among HPAI Case and Control Layer Farms in Iowa

Project background

A case-control study for HPAI was conducted among layer and pullet operations in Iowa. The study included all detected cases as of May 15, 2015, in Iowa or Nebraska, and controls were recruited from the surrounding geographic area for each case farm. Respondents representing 28 cases participated in the study, with a matching set of 30 controls. A 28-page questionnaire was administered to each participant; the questionnaire focused on the 2-week period leading up to detection of disease on a case farm (either via clinical signs/increased mortality or detection through surveillance). This 2-week period was defined as the reference period. Case participants responded to the survey for the reference period of the matched case survey.

During the interview, producers answered a number of open-ended questions regarding how they thought disease was spreading, if and how trucks and traffic were being re-routed, the pattern of spread within their barns (cases only), and the layout and structure of their facilities. Responses to these questions were analyzed along with interviewers' notes captured during discussions with the producers, using a qualitative framework approach (Pope et al., 2000). The goal of this analysis was not to repeat the information collected on the questionnaires, but rather to capture the narrative responses producers may have offered and determine common themes.

Approach

The team of interviewers involved in the initial data collection conducted the qualitative analysis on case farms only. Following the method described by Pope et al., the interviewers first familiarized themselves with the questionnaires and identified key issues, concepts, and themes to examine. Four open-ended questions (see Table 17) were used to define the four topical areas analyzed: producer comments on possible disease spread mechanisms, changes to truck routing due to the outbreak, pattern of spread within barns, and layout/structural issues of farms possibly affecting disease spread. The analysts identified a series of themes within each of

these topical areas (see Table 18). Each investigator applied this thematic framework to the surveys and assigned themes to the notes on each questionnaire. Single notes could include multiple themes. Once indexing was complete, the team obtained a count of responses within each theme (see Table 19).

Table 17. Open-Ended Questions Used to Define the Topical Areas

Questions
How do you think HPAI is spreading within your geographic area?
Inquire about truck routing. Are feed trucks, egg trucks, and live haul trucks routed in particular ways? (E.g., to avoid driving past a known positive farm, to avoid delivering to a known positive farm, or to visit known positive farms last.) Please explain.
For the first infected barn, attach a diagram including proximity of initial infection to vents, doors, personnel entrances, manure storage, and other potential contributing factors.
If possible, attach a diagram, farm map, or photographs showing orientation of barn(s) including barn numbers, water location, feed storage, rendering bin, litter storage, ventilation, and windbreaks.

Results

HPAI spread within the geographical area

Predominant themes emerged from the four identified topical areas. Producers most commonly identified airborne spread (20 out of 28 cases responding) as the most likely route of disease introduction onto their facility or general area. It should be noted that at the time of this survey, the news on TV and radio was generally indicating that airborne transmission was a possible contributing factor to widespread cases throughout Iowa.

Some producer comment highlights:

- "I think it's in the air; when the soil gets tilled by the farmers all that dust is blowing around. Plus, infected producers are keeping their fans going and blowing all that virus out into the air." (AD003)
- "I feel it is airborne. It has been very windy the previous 14 days prior to [HPAI test positive] confirmation. Farmers have been working [the] ground and there aren't any natural filters yet without crops in the ground." (BMC003)
- "It blew across the road from [nearest positive] facility. Really windy days after [that farm] broke. That brought it over." (BMC008)

Nine producers indicated that the disease potentially spread through their shared management areas in which supervisors or other employees visited most if not all of the company's production sites, sometimes within the same day.

Truck re-routing due to disease

When asked whether trucking routes for vehicles coming onto the property were changed and/or managed in some way, two main themes appeared. In the case of large companies that owned their own trucks and managed their own feed mills, the truck routes were managed to avoid passing positive farms once they were identified. However, during the incubation period, trucks generally continued to move back and forth between positive and negative sites until either the farm experienced clinical signs and/or a positive diagnosis was made.

One company manager commented that as soon as HPAI broke in Iowa, he "spoke with the owner of the feed elevator nightly and tried to avoid positive sites." This company kept one "clean" feed truck that only serviced HPAI-free company sites and one "dirty" feed truck that served positive HPAI company sites. (SA015)

The second major theme applied to smaller or independent farm owners who believed that trucks were being re-routed away from positive sites but had no way to confirm this information. Due to their smaller size/independent status, they have no control over their contracted truck management and could not monitor trucking routes. Therefore, they ultimately did not know how effectively trucks were rerouted; this response was categorized as "Limited Knowledge."

A typical response from managers/owners of these smaller farms who were unable to direct their own trucks was, "For all trucks that were not owned by the company, [we] tried to ask for dedicated trucks." However, they would then indicate that they had "no way of knowing" whether or not the trucks were dedicated and/or if they avoided driving near positive premises.

Disease spread within the first infected barn

Respondents noted that within an infected barn, most often birds near a ventilation fan (which brought air into the barn) first appeared sick and then the disease spread out from that area to other birds. The other common theme was that the first birds to appear sick were those near the back of the barn, away from the entrance. This was often linked to the ventilation system of the bird houses with large fans located toward the back of the barn.

"The way that it started in the house (in the middle) and then looking at the temperature and ventilation graphs from the days leading up to the break, I firmly believe that it came in from the intakes of Barn # (# omitted to protect anonymity – referred to first infected barn)." (CA001)

Layout of farm or particular barn

When evaluating the farm layout itself, respondents reported no striking differences. Four producers noted that they believed the barns that were impacted first were more at risk due to their environmental exposure, such as dust or irrigation aerosols from the nearest road that experienced more company traffic, exposure to the prevailing wind, and/or proximity to nearby fields being irrigated. One such producer commented, "We have excellent biosecurity – shower in, shower out, and a consistent crew. Barn #X, on the northwest corner of the property, is just south of the manure barn. Wind comes from the northwest right over the manure barn and into Barn #X (where infection first broke)."

Despite no consistent major themes for farm or barn layout in the narrative, the interviewers noticed a strong relationship between the company layer farms and their related pullet sites. This study included four large companies and all had a high degree of in-company connectedness among their feed trucks, company personnel, and other factors such as common rendering trucks coming on-site. The reviewers evaluated each survey for its connectedness and 18 of the 28 had a company connection that potentially increased their exposure and/or risk for contracting the disease by virtue of that connection to a company system.

Table 18. Topical areas and themes

Topical Area	Themes
Producer comments on possible disease spread mechanisms	Airborne spread
	Irrigation-related aerosols
	Shared management
	Absence of clinical signs prior to detection
	Worker behavior – related risks
	Feed trucks
Truck routing due to the outbreak	Limited knowledge of truck routes
	Lack control or limited route options
	Information difficult to obtain on safe routes
	Managed routing
	No change to truck routing
Layout/structural issues of farms possibly affecting disease introduction/spread	Presence of wild bird attractants (lagoons, feed access, etc.)
	Perceived “high risk” barn with more environmental exposure to wind, traffic, etc.
	Connectedness of farms both geographically and through business
Pattern of spread within barns	Clinical signs began near ventilation fan
	Clinical signs began near area of temperature extreme (hottest or coldest part of barn)
	Clinical signs began in back of barn
	Clinical signs began near area of greatest human activity

Table 19. Qualitative analysis matrix of topical areas and themes by individual survey

	Possible Disease Spread Mechanisms						Truck Routing Due to the Outbreak					Pattern of Spread Within the Barns				Layout/Structural Issues of Farms Possibly Affecting		
Survey ID (n=28)	Airborne Spread	Irrigation Related Aerosols	Shared Management	Absence Clinical Signs	Worker Behavior Related	Feed Trucks	Limited Knowledge	Lack Control	Information Difficult	Managed Routing	No Change	Near Fan	Temp Barn	Back of Barn	Human Activity Area	Perceived Wild Bird Attractants	High Risk Barn	Connectedness
AD004	1	0	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0
AD006	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0	1
AD001	1	0	1	0	1	1	0	1	0	0	1	0	1	0	0	0	0	0
AD003	1	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0
SA006	0	0	0	1	0	0	1	0	0	0	0	1	1	1	0	0	0	0
AD002	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
BMC003	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
BMC001	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
BMC008	1	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	1
BMC009	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1
BMC010	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1
SA004	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1
SA002	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1
SA007	1	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1
SA014	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
SA008	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1
SA005	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
CA001	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
SA015	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1
SA013	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1
SA001	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1
CA003	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
CA010	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
CA008	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
CA009	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
GS002	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1
GS001	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1
ISU007	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
Total	20	2	9	3	3	1	10	1	1	15	3	13	4	8	2	1	4	18

Conclusion

When analyzing the qualitative comments and questions of the case surveys, the team noted multiple themes either directly identified by the producer or inferred by the interviewer who gathered both measurable and contextual data during the interview. Based on this survey, it can be said that many producers believed that the virus was being spread via the air and that in some cases it may have spread by aerosolization of virus present on nearby, recently irrigated land. It was noted that many producers had no definite knowledge of whether trucking routes were being managed, but, conversely, larger companies had the ability to manage trucks and the routes that were taken. A high proportion of producers mentioned specifically that the first ill birds on the barn were near a fan, and in most cases this was an intake fan bringing air into the barn. For nine respondents, the first sick birds being near a fan and the participant believing that the virus was airborne were compatible responses.

Perhaps the most striking theme to the interviewers was the noteworthy connectedness within four of the companies. Companies with four or more operations represented 16 of the 28 case surveys and 7 of the 30 control surveys. This company model is a common production type in the Iowa layer system and those surveyed here are representative of the greater layer-hen industry in Iowa.

Sharing of feed and other company trucks that make several trips back and forth from the main company site, which houses hens and often feed mills, to serve smaller pullet sites is one potential route of spread within an organization. In addition, the sharing of other pieces of equipment and common personnel cannot be ignored as a risk factor.

Future network analyses may provide stronger data and support to indicate significantly increased risks among highly connected companies. Certainly the layer-hen industry in Iowa is a highly networked system with both large and small operations interacting with many other companies via common feed trucks, feed routes, egg trucks, and egg processing or breaker facilities. Risk from these activities cannot be defined by this analysis, but greater risk can be inferred.

Reference

Pope, C.; Ziebland, S.; Mays, N. 2000. Analysing qualitative data. BMJ 2000; 320:114.

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 (Figure 3 and Figure 4). 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/

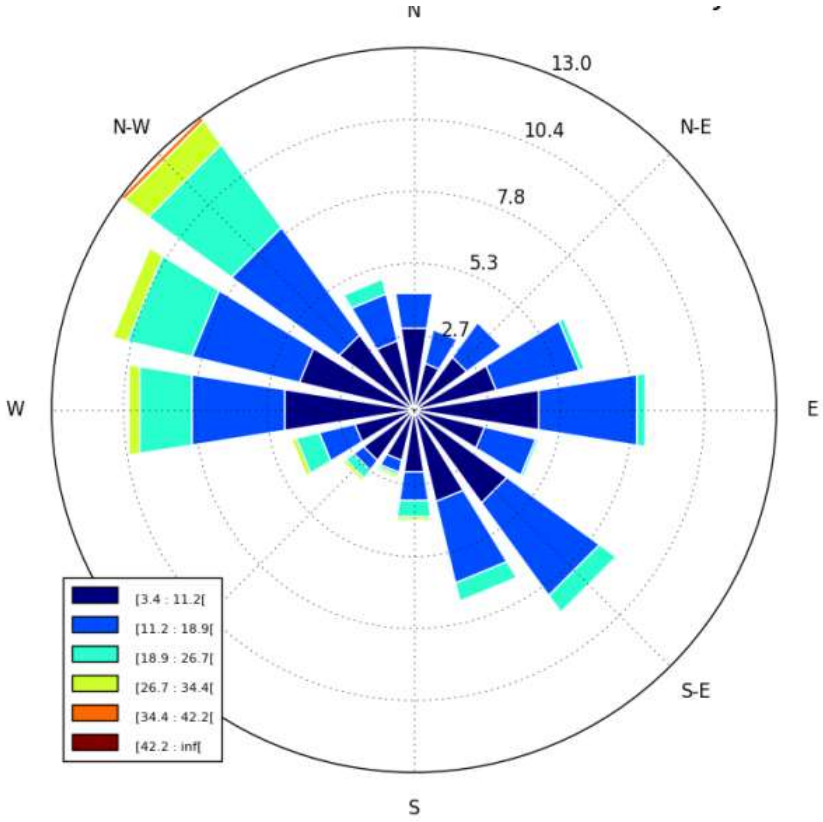

Figure 3. Wind Rose Minnesota: Combined BDH D39 LJF PEX

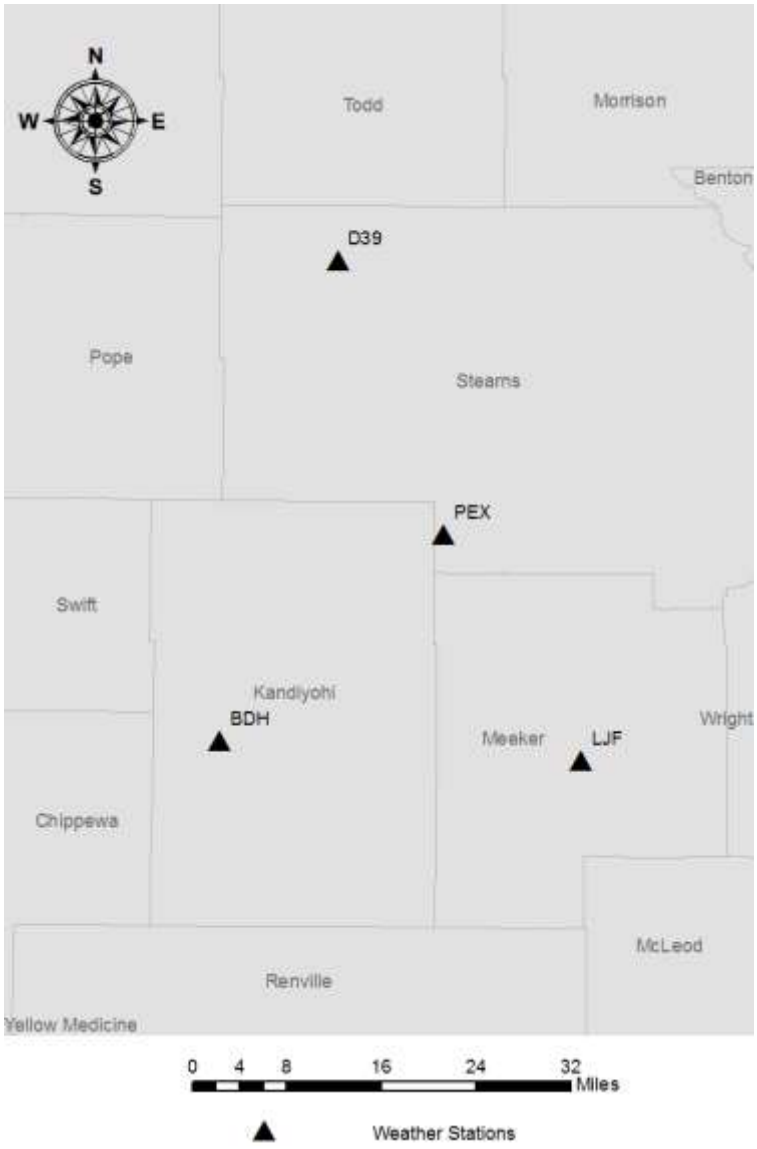

Figure 4. Location of weather stations used to create wind rose and resulting wind rose integrating data from all four stations.

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 premises status represents the date premises were confirmed positive by USDA-APHIS National Veterinary Services Laboratories (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 5). 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.

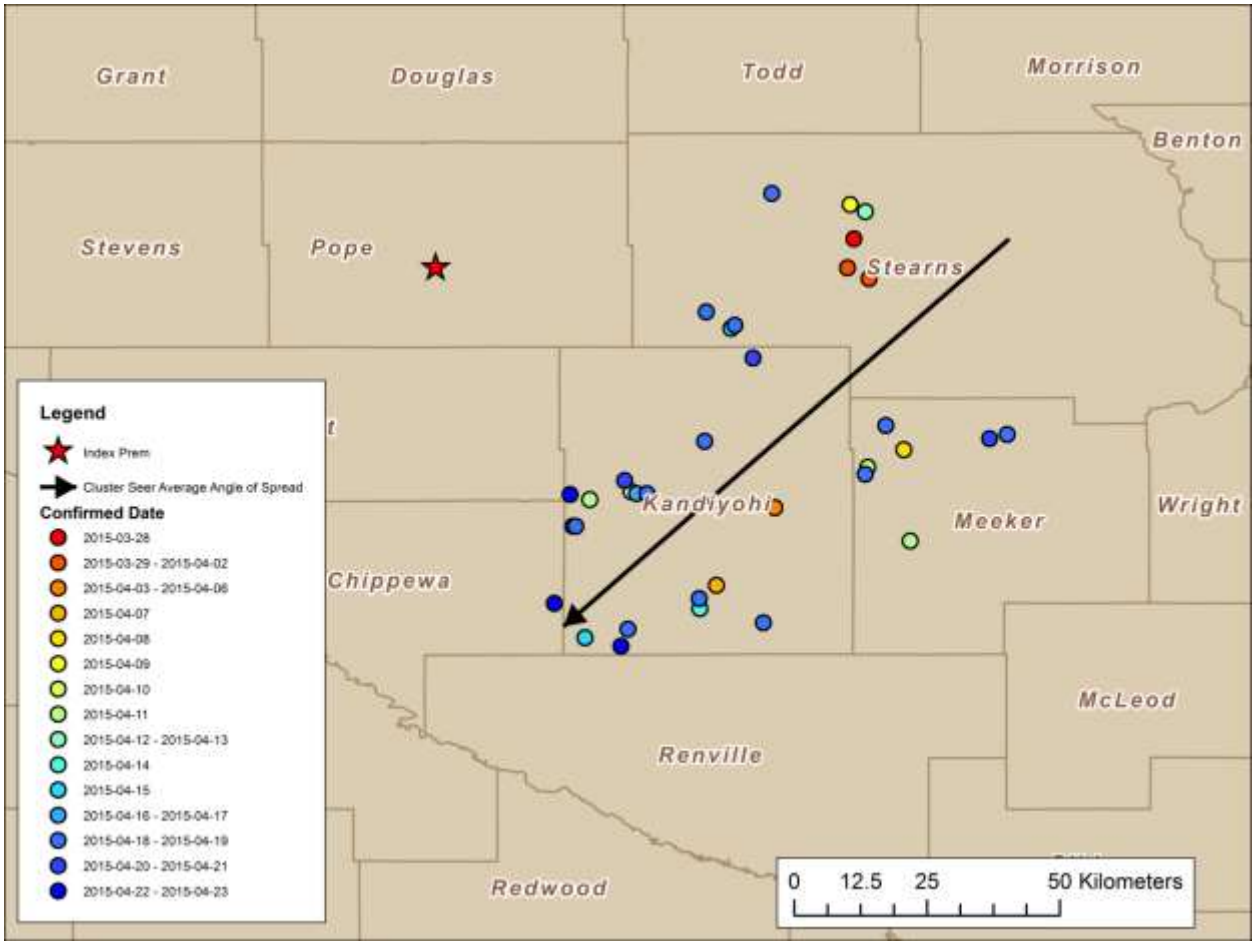

Figure 5. Positive premises used in ClusterSeer analysis and direction of spread as reported by ClusterSeer.

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 6).

- 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 6. 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. The H5 clade 2.3.4.4 HPAI viruses have a longer incubation period for gallinaceous poultry based upon current laboratory studies; therefore, onset of clinical signs is likely to occur several days after actual introduction of the virus. 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 near Kandiyohi and Stearns counties. A more robust analysis is ongoing.

C. Wind-Related Spread of EA/AM H5N2 HPAI Virus between Commercial Turkey Flocks in Minnesota - NEW

Background

Aerosol transmission of avian influenza virus is an active area of research. Aerosol generation from the respiratory tract is an important mode of within-flock avian influenza virus transmission because of high virus concentrations in the respiratory tract (Swayne and Halvorson, 2012). Aerosol spread of avian influenza virus between commercial poultry farms has been implicated in some outbreaks, although its role is considered limited in most instances (Selleck et al., 2003; Ssematimba et al., 2012). Previous analyses suggested very little alignment of general wind direction to disease spread direction based on space-time clustering analysis. The need was identified for a large-scale case-by-case analysis of disease spread and wind patterns using commonly employed "plume models." This approach enables a shorter period of wind data for use in highlighting predominant wind directions. This type of analysis also enables more accurate temporal modeling of virus shedding and periods of infectivity.

To evaluate the likelihood of aerosol transmission between commercial poultry operations, we used a combination of approaches including a review of past outbreak experiences (see Appendix C) and experimental laboratory transmission studies, and exploratory scenario analysis using aerosol dispersion models in conjunction with epidemiological analyses.

Objective

The objective of this analysis was to evaluate the potential role of aerosol transmission in the spread of EA/AM H5N2 HPAI virus between commercial turkey operations in Minnesota from February 16 to June 12, 2015. There were 98 infected commercial turkey farms in the dataset.

Aerosol Dispersion Modeling

Aerosol dispersion models have been extensively used to predict aerosol particle concentrations at different distances from a generating source. These models predict the dilution of aerosol concentration with distance from a generating source due to dispersion in air or gravitational settling, considering the meteorological conditions. The concentration of bioaerosols at a specific distance from a source depends on factors such as:

1) Source emission rate, which is the relative amount of particles emitted by the source per-unit-time
2) Dispersion or dilution of the particles, given the local meteorological conditions and topography
3) Depletion of particles from the air column due to settling or precipitation, given the particle size distribution
4) Decay of aerosolized microorganisms with time due to environmental factors acting upon them

Materials and Methods

To predict the HPAI virus concentration at various distances from an infected turkey farm, we used the U.S. Environmental Protection Agency's (EPA) AERMOD Modeling System. Wind speed and direction data were obtained from 105 National Weather Service Automated Surface Observing System (ASOS) stations across Minnesota. Other meteorological parameters such as relative humidity, cloud cover, and temperature were obtained for Renville County, Minnesota from the

Minnesota Pollution Control Agency. The HPAI virus aerosol emission rate for turkey barns ($10^{4.62}$ Embryo Infectious $Dose_{50}$/second) was calculated based on the average number of birds in a turkey barn (14,000 birds), the HPAI virus concentration in poultry manure, and the suspended dust particle emission rate. Distributions of the number of particles of different size fractions generated in a turkey barn were estimated from published literature.

In addition to the concentration of HPAI virus, the chances of HPAI spread to a susceptible downwind flock depend on the flock size, the aerosol infectious dose in turkeys, and the volume of air inhaled per bird, per day. We used a 50 percent turkey infectious dose of $10^{4.6}$ EID_{50} for the EA/AM H5N2 HPAI virus in turkeys based on recent unpublished data for the intranasal route (personal communication, Erica Spackman, David E. Swayne, Southeast Poultry Research Laboratory, Athens, GA). The volume of air inhaled per bird-day was estimated to be 2.4 m^3 per day for a bird weight of 10.7 kg. An exponential dose response model was used to predict the likelihood of infection of a susceptible flock via aerosols.

Aerosol exposure regions around infected farms (the source of HPAI virus aerosol emissions) were computed using the AERMOD dispersion model (Figure 7).

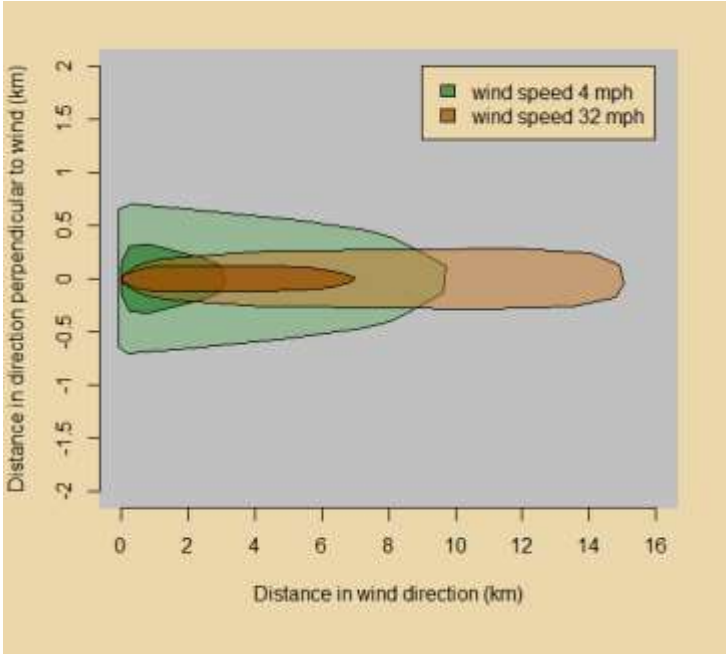

Figure 7. Aerosol exposure regions representing a 0.01 (lighter region) of low to moderate daily risk and 0.05 (darker region) high daily risk of transmission from an infected farm at 4 mph and 32 mph wind speeds

The area around each infected farm with a predicted daily transmission likelihood of 5 percent was defined to be a high-risk region. Aerosol exposure occurring over multiple days would increase the cumulative risk of a susceptible flock becoming infected. For example, if a premises was in a 0.05 exposure region of a shedding infected premises for 14 days, the cumulative probability of infection over the 14-day interval would be 0.51 (Table 20). We defined the 0.05 exposure region to be *high risk* considering, the high cumulative probability of infection for a susceptible flock present in this region over multiple days.

Calculations on a wider region, with a daily transmission likelihood greater than 1 percent, captured the impact of low HPAI virus concentration exposures; it was defined to be a *low to moderate risk* region. In this case, a premises under a 0.01 plume exposure region for the entire 14-day period would have a predicted cumulative probability of infection of 0.13, meaning that it would have had a 13% chance of aerosol exposure. The predicted probability of infection for a premises under a 0.01 or 0.05 exposure regions for different durations and the corresponding qualitative descriptors are provided in Table 21.

Table 21. Qualitative likelihood scale used to describe the risk of HPAI virus aerosol exposure in this assessment. A cumulative probability of infection for the premises was calculated based on the duration of exposure to a plume containing HPAI virus generated by a shedding premises at 4 different wind speeds.

Aerosol plume exposure region	Days at risk	Cumulative probability of infection	Qualitative descriptor
0.05	14	0.51	High risk, meaning that the event has a reasonable probability of occurring
0.05	6	0.26	High risk, meaning that the event has a reasonable probability of occurring
0.01	14	0.13	Moderate risk, meaning that the event is unlikely but does occur
0.01	6	0.06	Low risk, meaning that the event is very unlikely to occur

Interpretation and Limitations

Aerosol dispersion modeling predicted that susceptible turkey flocks located up to 7 km (4.35 miles) from an HPAI-infected farm could be at a high risk of infection via aerosol transmission depending on wind speed, wind direction, and other meteorological parameters. The model predicted that farms located between 7 and 15 km (4.35 and 9.3 miles) from an infected farm could be at a low to moderate risk of aerosol transmission. Dispersion modeling showed that wind speed can have a considerable impact on the distance at which aerosol transmission may occur. However, there is considerable uncertainty associated with several of the model parameters such as aerosol dose response for the EA/AM H5N2 HPAI virus strain in turkeys, particle size distribution, and aerosol source emission rate once a flock becomes infected. The potential decay of HPAI virus in bioaerosol particles due to environmental factors such as sunlight exposure was not considered in the current analysis. Given the uncertainty in model parameters and assumptions, further epidemiological analysis was needed to evaluate whether the presence of a farm in a predicted aerosol exposure region was associated with disease.

Epidemiological Analyses

Three separate approaches were used to evaluate the association between the predicted degree of aerosol exposure of a farm and its infection status, while considering wind speed, wind direction, relevant meteorological parameters, and the location of all nearby infected farms during the same period. In each analysis, we also explored the impact of proximity to infected farms regardless of the wind direction.

The first approach used the full database of poultry farm locations in Minnesota. Plumes of virus-associated particles were modeled for all infected farms based on the average wind speed and direction over various time frames. The odds of becoming infected were estimated for farms located downwind of infected, shedding farms using logistic regression. The second approach was a case-control study examining the effects of cumulative daily exposure to modeled plumes of virus-associated particles, while taking into account the age and susceptibility of the birds located on each farm. The third approach used a repeated measures analysis to examine the daily and cumulative risk of disease associated with exposure to modeled plumes of virus-associated particles. The specific methods and results for each of these approaches are presented.

Odds of Disease Associated with Average Wind Speed and Direction over Varying Timeframes

Objective

The objective of this analysis was to evaluate if a farm downwind of another farm infected with HPAI is at increased risk of becoming infected, based on average wind speed and direction. We examined multiple time periods that were hypothesized to produce plumes of virus-associated particles. For each period, the average wind speed and direction were used in combination with aerosol-dispersion modeling to identify which farms were located in plumes. The resulting dataset was explored using logistic regression modeling to estimate the odds ratio of being in a plume and becoming infected.

Materials and Methods

Data on wind speed and direction were obtained from 105 National Weather Service ASOS stations across Minnesota. We obtained other meteorological parameters, such as relative humidity, cloud cover, and temperature, for Renville County, MN, from the Minnesota Pollution Control Agency. For each time frame, average wind speed and wind direction were calculated for each infected farm based on data from the closest weather station.

We obtained a statewide dataset of poultry operations from the Minnesota Poultry Laboratory. Data were merged with records from the VS Emergency Management Response System to obtain dates for the onset of clinical signs, confirmation of infection based on laboratory testing, and the depopulation of all infected farms. Using the average wind speed and wind direction across each time frame, we created plumes representing a 0.01 and 0.05 daily likelihood of disease transmission for each infected farm and identified any farms located within the plumes.

We identified seven time frames (Figure 8) when plumes of virus-associated particles could be produced:

1) Entire length of time virus may be present on a farm, ranging from 10 days before the onset of clinical signs to 17 days after depopulation began
2) Entire length of time viable virus was most likely on the farm, ranging from 10 days before the onset of clinical signs to 3 days after depopulation began
3) Time near depopulation, from 2 days before to 2 days after depopulation
4) Period when depopulation activities are ongoing until disposal begins
5) Ten days before the onset of clinical signs
6) Time near the onset of clinical signs, when viral replication is rapidly increasing

7) Time from the onset of clinical signs, when birds are actively producing virus, until depopulation starts

Multiple time frames were explored due to the uncertainty in the amount of virus shedding over time from infected flocks and the quantity and viral load of dust and particulates released during depopulation and disposal activities.

The relationship between infection status and location within a plume was explored using logistic regression for each time period and plume size. For any time frames deemed significant, we included the distance to the nearest infected farm in the analysis to assess whether distance or direction (e.g., being downwind) was the better predictor of infection status.

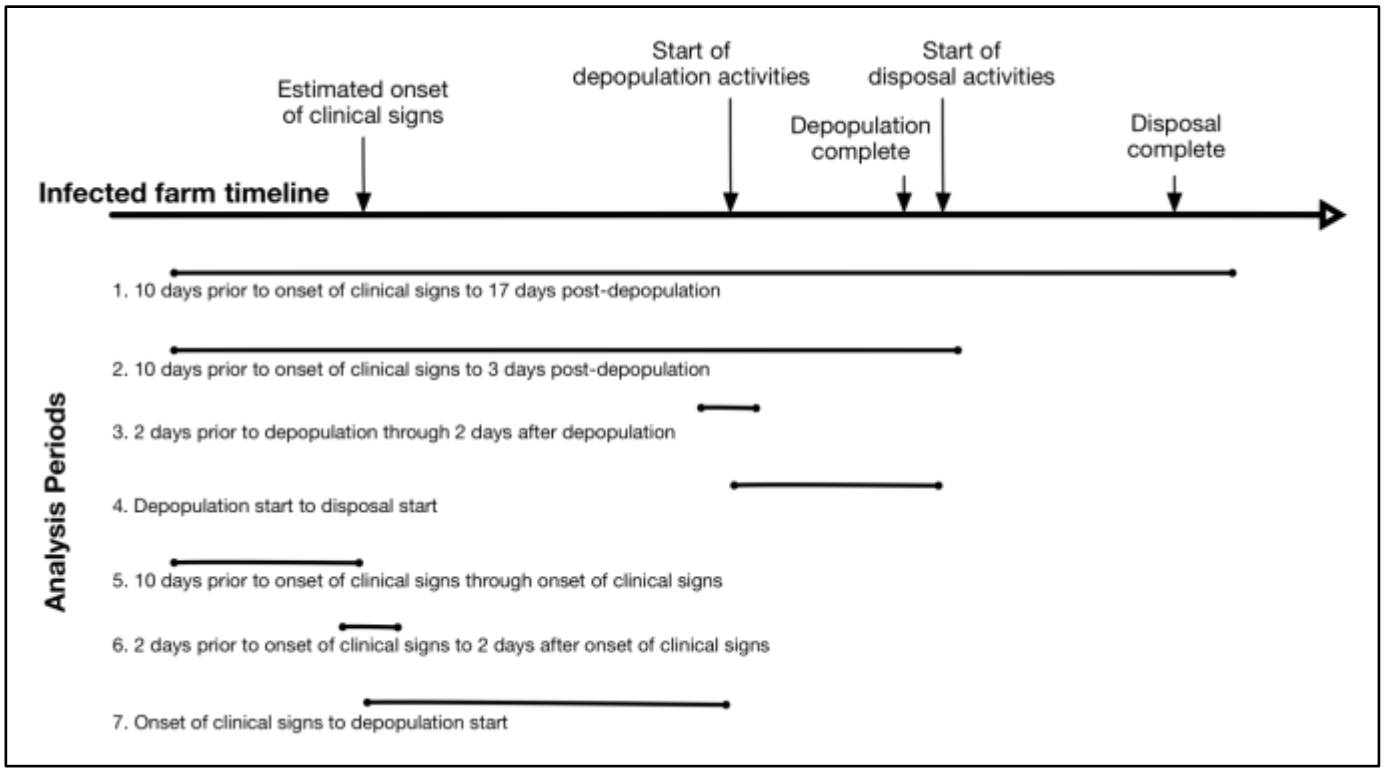

Figure 8. Time frames examined. The timeline for each infected farm was unique, and there was variability in the length of time required for beginning and completing depopulation. This diagram was designed for illustrative purposes only.

Results

The location of an operation in estimated plume areas with a 5 percent probability of transmission did not result in an increased risk of infection for any of the time frames examined (Table 22). This result was primarily due to the very low numbers of infected farms located within these plume areas, which suggests that a static plume based on average wind speed and direction may not have adequately captured this exposure region.

Table 22. Unadjusted odds ratio for the effect of being downwind of an infected farm during each time frame, based on univariate logistic regression analysis

Time period	Plume size based on probability of transmission (POT)	N	Unadjusted odds ratios	95% CI	p-value	No. positive farms in a plume/all farms in plume
10 days before onset of clinical signs to 17 days after depopulation began	5% POT	810	2.5	(0.66, 9.39)	0.18	3/12
	1% POT	810	5.4	(2.57, 11.17)	<0.001	13/33
10 days before onset of clinical signs to 3 days after depopulation began	5% POT	810	2.0	(0.55, 7.43)	0.282	3/14
	1% POT	810	2.8	(1.27, 6.22)	0.011	9/34
2 days before to 2 days after depopulation	5% POT	803	---		---	0/3
	1% POT	806	1.3	(0.37, 4.46)	0.69	3/21
Depopulation start to disposal start	5% POT	796	---		---	0/12
	1% POT	781	---		---	0/27
10 days before onset of clinical signs to onset of clinical signs	5% POT	809	1.5	(0.32, 6.93)	0.6	2/12
	1% POT	809	3.1	(1.43, 6.59)	0.004	10/36
2 days before onset of clinical signs to 2 days after onset of clinical signs	5% POT	808	2.5	(0.5, 12.74)	0.259	2/8
	1% POT	808	1.4	(0.41, 4.97)	0.58	3/19
Onset of clinical signs to start of depopulation	5% POT	809	0.7	(0.09, 5.26)	0.7	1/12
	1% POT	809	1.3	(0.44, 3.86)	0.63	4/27

For estimated plume areas with a 1 percent probability of transmission, the odds of becoming infected were significantly increased when farms were downwind of an infected farm, based on average wind speed and direction. The three time frames included:

- The entire length of time virus may be present on a farm, ranging from 10 days before onset of clinical signs to 17 days after depopulation began ($p<0.001$)
- The entire length of time viable virus was most likely on the farm, ranging from 10 days before onset of clinical signs to 3 days after depopulation began ($p=0.011$); and
- The 10 days before onset of clinical signs ($p=0.004$)

Each time frame was then explored using multivariate regression to adjust for bird species present (chickens vs. turkeys) and to determine whether distance or wind direction was a better predictor. The results of the final multivariate models are shown in Table 23.

In all cases, being located within 5 km (3.1 miles) of an infected farm increased the odds of becoming infected, and being a turkey operation significantly increased the odds of becoming infected. When distance to the nearest infected farm was included in the analysis, the effect of being downwind of an infected farm became nonsignificant (p>0.05). This would suggest that in this analysis, proximity to an infected farm during the timeframes examined regardless of wind speed or wind direction was a better predictor of infection status.

Table 23. Multivariate modeling results of the effect of being located downwind of an infected farm, adjusted for species of bird present and being within 5 km of an infected farm

Time period	Predictor Variable	Adjusted odds ratios	95% CI	p-value
10 days before onset of clinical signs to 17 days after depopulation began	Located in a 1% POT plume	1.19	(0.54, 2.64)	0.662
	< 5 km from an infected farm	5.55	(3.37, 9.15)	<0.001
	Turkey operation	8.07	(2.88, 22.65)	<0.001
10 days before onset of clinical signs to 3 days after depopulation began	Located in a 1% POT plume	0.70	(0.30, 1.65)	0.414
	< 5 km from an infected farm	4.97	(3.03, 8.13)	<0.001
	Turkey operation	8.26	(2.95, 23.14)	<0.001
10 days before onset of clinical signs to onset of clinical signs	Located in a 1% POT plume	1.26	(0.54, 2.95)	0.591
	< 5 km from an infected farm	2.21	(1.31, 3.73)	0.003
	Turkey operation	10.41	(3.75, 28.95)	<0.001

Conclusions

This analysis focused on the risk associated with being downwind of an infected farm during various periods during the infection and disease control process. A basic analysis examining infection status and location within a plume found increased odds of becoming infected during three time frames:

- The entire length of time virus may be present on a farm, ranging from 10 days before onset of clinical signs to 17 days after depopulation began;
- The entire length of time viable virus was most likely on the farm, ranging from 10 days before onset of clinical signs to 3 days after depopulation began; and
- The 10 days before onset of clinical signs. However, when we accounted for the distance to the nearest infected farm, the effect of being downwind became nonsignificant.

This finding suggests that being located within a control area, less than 5km (3.1 miles) from an infected farm, increased the odds of becoming infected, regardless of wind speed or direction.

This approach utilized an average wind speed and direction across each time frame. In areas where the wind speed and direction are highly variable within short time periods, the use of averaging has numerous drawbacks. When wind speed and direction vary greatly, infected farms are more likely to produce plumes of virus-associated particles in multiple directions and at various distances throughout the day. As a result, the farms surrounding an infected farm could be located within a plume of virus-associated particles for only portions of each period. The second epidemiologic approach focused on examining the risk associated with cumulative exposure of surrounding farms using a much smaller time interval to address these limitations.

Case-Control Study to Evaluate Association Between Plume Exposure and Disease

A matched case-control study was designed to compare the aerosol exposure index for cases during an averaging period prior to the onset of clinical signs with an exposure index for controls during the same time period. The exposure index was cumulative so that the aerosol exposure of a farm occurring over multiple days could increase the overall chances of a turkey flock becoming infected. Conditional logistic regression was used to evaluate the potential association between the location of a farm within an aerosol exposure region and infection.

We calculated six different aerosol indices for each poultry farm in Minnesota on each day throughout the outbreak. For all aerosol index calculations, only infected farms that were within their shedding period were considered to contribute to aerosol spread. The shedding period for each actual infected farm was defined to start 10 days prior to the onset of clinical signs and end on the day that composting was started. The direction of the aerosol exposure region from a shedding infected farm was varied according to wind direction, and the average wind direction for each 2-hour period was used in order to capture variability in wind direction. A description of each index calculation examined is shown in Table 24.

Table 24. Summary of aerosol exposure indexes evaluated in this analysis

Aerosol index	Description	Interval for calculating cumulative index	Dispersion model exposure regions
Index A	14-day aerosol exposure index	Past 14 days	Both high risk (0.05) and low to moderate risk (0.01) regions
Index B	14-day 0.01 region index	Past 14 days	Low to moderate risk (0.01) region
Index C	14-day 0.05 region index	Past 14 days	High risk (0.05) region
Index D	6- to 11- day aerosol exposure index	11 days prior to 6 days prior	Both high risk (0.05) and low to moderate risk (0.01) regions
Index E	6- to 11- day 0.01 region index	11 days prior to 6 days prior	Low to moderate risk (0.01) region
Index F	6- to 11- day 0.05 region index	11 days prior to 6 days prior	High risk (0.05) region

First, we calculated a cumulative 14-day aerosol exposure index value (Index A), representing the daily aerosol exposure for a farm over the previous 14 days (first row of**Error! Reference source not found.**). The daily exposure index signifies the number of exposure regions from other infected farms that the farm was under, the exposure region category (0.01 vs 0.05 regions), and the duration the farm was under those exposure regions. For example, if a farm fell under the 0.05 exposure region (Figure 7) of one shedding farm on a day, its daily index would be 0.05. Note that if a farm was under the 0.05 exposure region, it would also be under the wider 0.01 exposure region. If however, the farm was not under the 0.05 region and was only under the 0.01 region of one shedding farm (Figure 7), its daily index would be 0.01. The cumulative 14-day index was then calculated by summing up the daily index values for a farm over the past 14 days (second row of Table 25). The 14-day period before the onset of clinical signs for an infected farm represented an "at risk" period during which it could have become infected.

Table 25. Example aerosol indices for one case farm where the date of onset of clinical signs was on April 25th. For the 14-day index, the aerosol index values were summed over a 14-day period (including the date of onset of clinical signs). For the 6- to 11-day index, daily index values were summed from 6 to 11 days prior to the onset of clinical signs in the case flock.

Aerosol index	Index value on Apr 25	Apr 12	**Apr 13**	**Apr 14**	**Apr 15**	**Apr 16**	**Apr 17**	**Apr 18**	**Apr 19**	**Apr 20**	**Apr 21**	**Apr 22**	**Apr 23**	**Apr 24**	**Apr 25**
Daily	**0.05**	0.01	0.01	0.02	0.01	0.01	0.01	0.05	0.05	0.05	0.01	0.05	0.05	0.10	0.05
14- day	**0.49**	0.01	0.01	0.02	0.01	0.01	0.01	0.05	0.05	0.05	0.01	0.05	0.05	0.10	0.05
6- to 11- day	**0.18**	0.01	0.01	0.02	0.01	0.01	0.01	0.05	0.05	0.05	0.01	0.05	0.05	0.10	0.05

For the second and third indices (Indexes B and C in Table 24), a cumulative 14-day aerosol exposure index value was calculated separately for the low to moderate risk region (0.01 exposure region) and the high risk region (0.05 exposure region). This approach was taken to separately evaluate the impact of being present in a low to moderate or high-risk region.

The next set of indices (Indexes D, E, and F in Table 24) restricted aerosol exposure to the time interval when a farm could most likely have become infected prior to onset of clinical signs. Simulation models of within-flock HPAI virus transmission have indicated that it would take 6- to 11-days post-infection to detect HPAI virus in turkey houses with enhanced passive surveillance (i.e., detection when elevated mortality exceeds a pre-determined threshold). A 6- to 11-day aerosol exposure index was calculated, similar to the 14-day indices, except that aerosol exposure starting from 11 days prior to onset of clinical signs to 6 days prior to onset was summed to represent cumulative aerosol exposure (third row in Table 25). The 6- to 11- day low- to moderate-risk region index (0.01 exposure region) and the high-risk region index (0.05 exposure region) were also calculated.

Two measures of proximity to other infected farms during their shedding periods were incorporated into the analysis as a separate risk factor. For the first distance measure, a distance index for a farm on a day was defined as the sum of the inverse-squared distance from every infected farm within 15 km that was in a shedding state over the past 14 days. If a farm is located near two shedding

premises, the distance index for the farm at risk would be the sum of the individual premises distance indices. For example, if an at-risk premises (Premises C) is located near two shedding premises (Premises A at 2 km, and Premises B at 3 km), the total distance index for Premises C would be 5.06 over the past 14 days (Table 26). The second distance measure was defined as the shortest distance to an infected, shedding farm during the past 14 days.

Table 26. An example total distance index calculation for an at-risk premises (Premises C) located near 2 shedding premises at different distances

Premises	Distance (km)	Shedding period duration (days)	Individual premises contribution to index
A	2	14	$14/(2)^2 = 3.50$
B	3	14	$14/(3)^2 = 1.56$

Matched case control analyses

We performed a matched case-control analysis to test for an association between aerosol exposure and case status while controlling for distance. Due to the complexity of wind patterns and HPAI virus shedding periods for infected farms, we explored two scenarios for the definitions of cases and controls. In both scenarios, cases were defined as having a positive laboratory confirmation of HPAI while the definition for controls was varied. A shedding farm was defined as an infected farm during the time interval starting from 10 days prior to date of onset of clinical signs and ending on the date that carcass composting began.

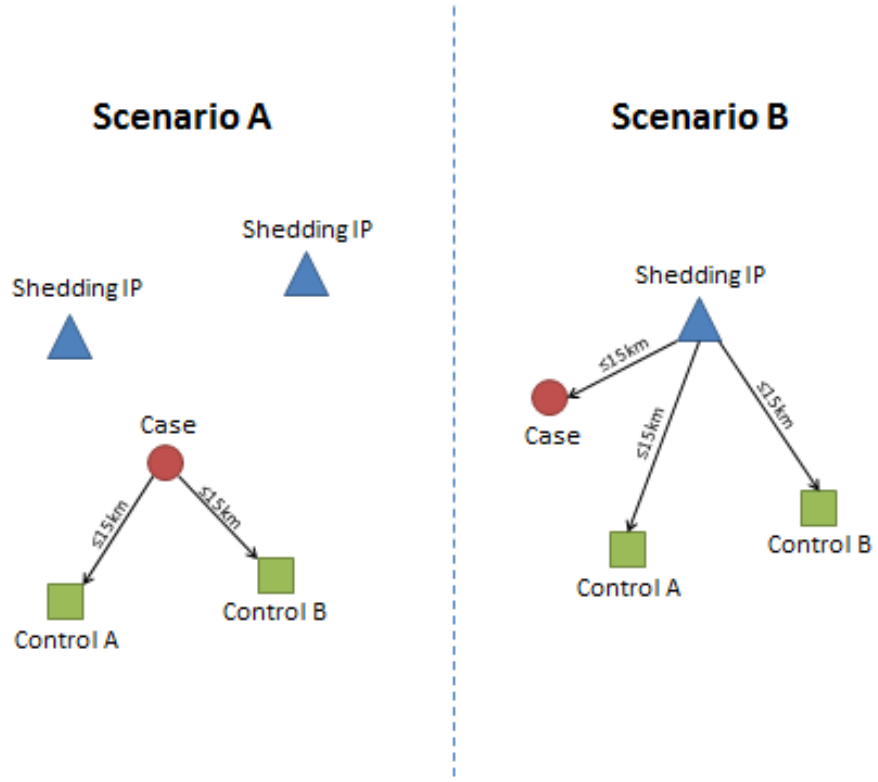

Figure 9. Two of six scenarios evaluated for exposure to HPAI virus through aerosol. In scenario A, controls are within 15 km of a case. In scenario B, case and controls are both required to be within 15 km of a shedding infected farm.

Scenario A:

In this scenario, controls were defined as uninfected farms within 15 km (9.3 miles) of a case. Two controls were chosen for each case. Cases that could not be matched to two controls were excluded from the analysis. The exposure regions predicted through dispersion modeling in the previous step indicated that aerosol transmission was unlikely to occur over distances greater than 15 km. In this scenario, it is possible that some of the cases were located more than 15 km from a shedding farm where aerosol transmission would be unlikely regardless of the wind direction and speed (Figure 9). Similarly, some of the controls could potentially be located more than 15 km from a shedding farm (other than the case). We selected 44 cases and 88 controls, then evaluated the index values for aerosol exposure and distance on the date of onset of clinical signs for a case and its matched controls using conditional logistic regression.

Scenario B:

In this scenario, the cases and controls were defined with respect to proximity to a shedding, infected farm. In this analysis, cases were defined as infected farms within 15 km of another shedding farm during its at-risk period (i.e., the 14-day interval prior to the onset of clinical signs). For example, if the date of onset of clinical signs for an infected farm is April 25, it would need to be within 15 km of a shedding infected farm between April 12 and 25 to meet the criteria for a case (see Figure 9). The case and its matched control both had to be within 15 km of the same shedding infected premises (Figure 9). This definition ensures that cases and controls were both at risk of aerosol exposure from another infected farm depending on wind speed and direction.

In addition, control farms were required to have at least one flock present on the farm with age greater than or equal to 7 weeks on the date of onset of clinical signs for its matched case. Age was considered in defining controls as very few flocks less than 7 weeks of age became infected in the HPAI outbreak in Minnesota. Although the definition of case and controls is more complex in this scenario, it ensures that control flocks were eligible to become cases based on exposure to aerosols and the presence of susceptible birds on the farm. We selected 31 cases and 62 controls. The index values for aerosol exposure and distance on the date of onset of clinical signs for a case and its matched controls were then evaluated using conditional logistic regression.

We tested all predictor variables for linearity in the logit and entered them into the model as continuous or categorical variables accordingly. Bivariate models with both the aerosol index and a distance measure were also evaluated. The aerosol index was categorized at different cut points (threshold values) as the distribution of the aerosol indices was significantly right skewed. Each aerosol index was categorized into two levels at specific cut points. The cut point values evaluated for aerosol indices were 0, 0.01, 0.02, 0.03, 0.04, and 0.05. The minimum distance was also similarly categorized using cut points from 0 to 5 km in steps of 0.5 km.

Results

We evaluated the case control data from scenarios A and B using conditional logistic regression. None of the variants of the aerosol exposure index were significantly associated with disease when evaluated as continuous variables in both scenarios. Some of the aerosol index variables categorized at specific cut points were significant when evaluated individually (Table 27). In both scenarios, farms located less than 3.5 km (2.17 miles) from an infected farm had increased odds of being a case. When a significant aerosol exposure index variable and minimum distances were evaluated together in a bivariate model, only minimum distance remained significant (AOR 2.8; p-0.04 in Scenario A and AOR 4.2, p-0.02 in scenario B). The aerosol indexes were correlated with minimum distance from an infected farm. These results suggested that further examination of cumulative exposure at various time points was warranted, which led to the third epidemiologic analysis, a repeated measures analysis.

Table 27. Summary of significant variables in the univariate analysis

Scenario	Variables	Categorization cut-point	Odds Ratio	95% CI	p-value
Scenario A	Index D: 6- to 11- day aerosol exposure index	>0.04	4.2	(1.1-16.6)	0.04
	Index E: 6- to 11- day 0.01 region index	>0.02	12	(1.5, 99)	0.02
	Minimum Distance	<3.5 km	3.4	(1.3-8.8)	0.01
Scenario B	Index A: 14-day aerosol exposure index	>0.03	4.2	(1.1,15.8)	0.03
	Index F: 6- to 11- day 0.05 region index	>0.01	4	(1.1,14.9)	0.04
	Minimum distance	<3.5 km	5.1	(1.7,15.6)	0.005

Repeated Measures Analysis to Examine the Daily and Cumulative Risk of Disease Associated With Exposure to Modeled Plumes of Virus-Associated Particles

Objective

The objective of this analysis was to evaluate if cumulative changes in the amount of time a farm is exposed in an aerosol exposure region each day increased the odds of becoming infected. We also explored the effect of distance to other infected, shedding farms, and various time lags between exposure and the onset of clinical signs.

Materials and Methods

The daily aerosol index, the daily distance index, and the shortest distance to a shedding neighbor were computed as described in the case control study for each of 811 susceptible farms in Minnesota each day between February 16 and June 8, 2015. Based on the results from the case control study, the value of these three variables was lagged from six to 11 days to explore when the indices or distance measure had the greatest effect prior to the onset of clinical signs. Two to six-day cumulative sums of the aerosol and distance indices were generated to capture the number of days of exposure of a farm over time, and these cumulative sums were also lagged from six to 11 days to explore how many days prior to onset of clinical signs the cumulative exposures had the greatest effect. Daily data for each farm was included in the dataset, and once a farm became infected (on the date of onset of clinical signs), it was removed from the set of susceptible farms.

The variables examined as predictors of disease were: aerosol and distance indices (original, original lagged 6-11 days, two- to six-day cumulative sums, and two- to six-day cumulative sums lagged 6-11 days), and the set of minimum distance measures (original plus lagged 6-11 days). Each of these variables was fit into univariate repeated measures logistic regression models. A first-order autoregressive covariance structure was assumed to account for correlation between daily measures within the same farm. The best lagged or cumulative sum lagged variable for each of the measures (daily aerosol exposure index, daily distance index, and minimum distance measure) was found and an attempt was made to fit all possible combinations of these three variables in multivariate logistic repeated measures models.

Results

Accumulated exposure in an aerosol exposure region for 6 to 11 days was associated with increased odds of becoming infected (OR 1.4). This result would suggest that for every 1% increase in the cumulative sum of probability of transmission for all aerosol exposures regions that a farm is located within over a course of 6 to 11 days, the odds of becoming infected are increased 1.4 times.

Daily exposure to plumes of virus-associated particles was associated with disease risk, regardless of when that exposure occurred relative to the onset of clinical signs (Table 28**Error! Reference source not found.**). Some combinations of aerosol exposure provided more stable estimates of increased odds of disease, according to model fit criterion. If only a few farms experienced either extremely high or extremely low amounts of aerosol exposure during a time period (outliers) the model estimates would become unstable. Accumulated exposure in an aerosol exposure region for 6 to 11 days was the most stable model according to fit statistics. Model fit decreased and was more influenced by outliers as the number of days of exposure decreased.

When we examined the effect of a single day of exposure 7, 8, 9, or 10 days prior to the date examined, the models and odds ratios were very similar, with no day predicting odds of disease

better than another. Models of the effect of exposure for each of these days were more stable than the models examining exposure 6 or 11 days before the date examined.

As noted in previous analyses, multivariate modeling of the effect of exposure and distance identified strong correlation between these two types of variables, which could not be controlled for in the analysis. A comparison of the model fit statistics for exposure predictors versus distance predictors found that almost all of the aerosol exposure predictors provided more stable estimates of increased odds of disease. Simply evaluating the effect of minimum distance to an infected farm did not result in a stable estimate of disease risk in this repeated measures framework, most likely because the minimum distance remained fairly constant through time.

Table 28. Unadjusted odds ratios for the effect of the daily aerosol and distance indices based on a univariate repeated measures logistic regression analysis

Number of days exposure prior to date of interest	Unadjusted odds ratios	95% CI	p-value
6 days	2.5	(2.2, 2.9)	<.0001
7 days	2.8	(2.4, 3.2)	<.0001
8 days	2.7	(2.3, 3.2)	<.0001
9 days	2.7	(2.3, 3.3)	<.0001
10 days	2.9	(2.3, 3.5)	<.0001
11 days	2.4	(2.0, 3.1)	<.0001
6 to 7 days	1.8	(1.7, 2.0)	<.0001
6 to 8 days	1.6	(1.5, 1.7)	<.0001
6 to 9 days	1.5	(1.4, 1.6)	<.0001
6 to 10 days	1.4	(1.3, 1.5)	<.0001
6 to 11 days	1.4	(1.3, 1.5)	<.0001

Interpretation and Limitations from All Epidemiological Analyses

When examined individually, certain thresholds of aerosol exposure indices and daily values of the aerosol exposure index were significantly associated with increased odds of a farm becoming infected. The cumulative exposure of a farm to multiple plumes over a 6- to 11-day period created the most stable predictor of increased odds of disease. However, proximity to other infected farms had a consistent association with increased odds of becoming a case in all of the epidemiologic analyses. The significant association between infection and being within a short distance such as 3.5 km (2.17 miles) of a shedding farm indicates that local spread mechanisms besides plumes of virus-associated particles could be contributing to spread. Given the current data, it is difficult to confirm or rule out the contribution of aerosol transmission toward the increased likelihood of infection with proximity of farms up to 3.5 km (2.17 miles) from a shedding farm. For example, some farms were estimated to have very high aerosol exposure indices and yet never became infected.

When aerosol exposure indices and distance measures were assessed together, the effect of the aerosol exposure index was often no longer statistically significant. These two variables are by nature correlated, as distance is an inherent part of the aerosol exposure index in addition to wind direction and speed. As a result, it was not possible to separate their effects in this analysis, and we were not able to determine with certainty whether aerosol transmission was responsible for a farm becoming infected. Other mechanisms associated with proximity could also have resulted in HPAI spread between nearby farms.

The deposition of dust contaminated with HPAI virus on ground areas around infected farms has to be considered as a potential source of environmental contamination contributing to local area spread. Aerosol sampling studies during the current as well as previous outbreaks identified the presence of HPAI virus in air up to 1,000 meters from an infected farm in some cases. The possibility that boots, hands, and clothing of farm workers and contractors may have become contaminated with HPAI virus contaminated dust from walking across contaminated ground areas outside of infected farms, resulting in spread to other operations should be considered.

Several important limitations apply to the current analyses. First, the wind direction in the affected counties was highly variable. The clustering of cases in high poultry density areas in conjunction with variable winds resulted in most farms (cases and controls) being present in predicted aerosol exposure regions of one or more shedding farms over the outbreak timeframe. This inability to separate farm density and exposure regions makes it difficult to differentiate the impact of wind speed and direction in the analysis.

Second, there is considerable uncertainty in several key aerosol transmission model parameters. These include the aerosol exposure dose for the field EA/AM H5N2 HPAI virus strain, the particle size distribution for aerosols generated by turkey houses, the potential decay of HPAI virus in bioaerosols due to environmental factors such as ultraviolet light, and temperature and the shedding period for an infected farm. Further studies on these parameters would improve aerosol dispersion model estimates. In addition, the use of phylogenetic information along with epidemiological and meteorological data may be useful to evaluate the potential association with wind direction.

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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

- Liquid cyclonic collector (Midwest Micro-tek, Brookings, SD, USA) capable to process 200 liters of air per minute (l/min);
- Andersen Cascade Impactor (ACI) (Thermo Electron Corporation, Waltham, MA, USA) able to process 28.3 l/min; and
- 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. Surface 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 reverse-transcriptase polymerase chain reaction (RT-PCR) for influenza viruses and, if positive, were re-tested using specific H5 and N2 PCRs. To assess the infectivity of RT-PCR positive and suspect air samples, virus isolation in embryonated eggs was attempted at NVSL 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 29). 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 30. 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 10). 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 31). 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.

Table 29. 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 30. 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 31. 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*				

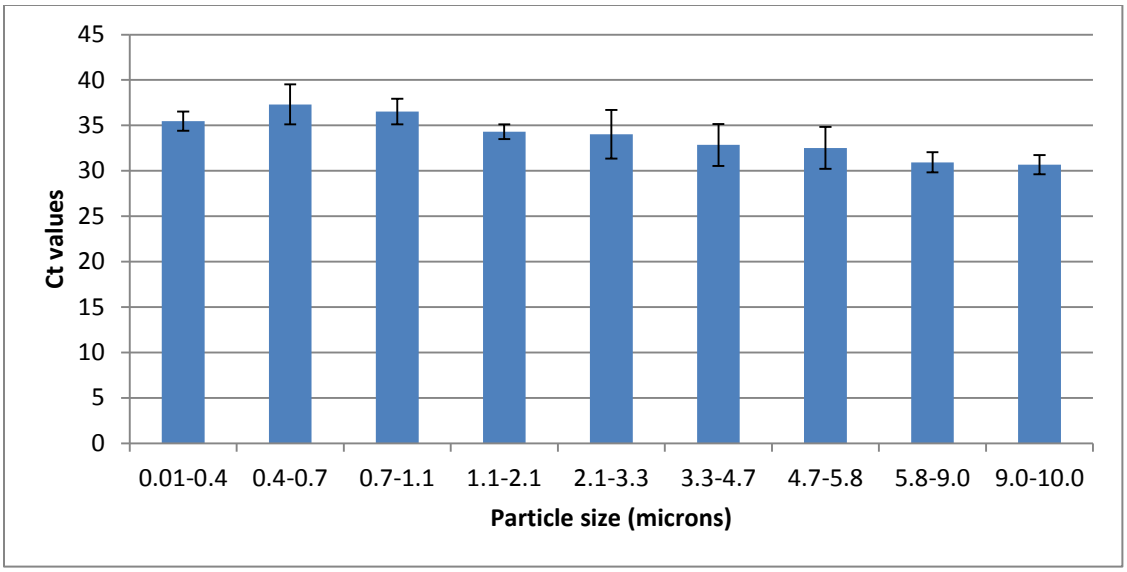

Figure 10. Average RT-PCR cycle threshold (Ct) values by particle size of air samples collected inside and immediately outside of turkey and layer flocks using the Anderson Cascade Impactor. Ct <35: positive; Ct 35-40: suspect; Ct >40 negative.

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.

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B. Sampling for HPAI Virus in Synanthropic Wildlife at Affected and Unaffected Premises – UPDATED

Objective

To evaluate the potential for synanthropic wildlife associated with egg-layer chicken flocks to become exposed or infected with HPAI H5N2 virus, we sampled peri-domestic birds and mammals on farms that had been infected with H5N2 and flocks with no known exposure to H5N2.

Materials and Methods

Flocks

Five farms with confirmed H5N2 HPAI infections and five farms with no known infections with H5N2 HPAI were investigated (Table 32). All flocks were located in northwest Iowa. Sampling at confirmed infected sites was conducted 2-4 weeks after clinical signs were evident in poultry. Four of the five infected flocks were depopulated prior to wildlife sampling and one of the flocks was being depopulated during sampling. Sampled farms with no known infections exhibited a similar flock size range to the sampled infected farms (i.e., two small, one medium, and two large flocks).

Table 32. Summary of Infected Flocks

Site	Approximate Flock Size	Date of Clinical Signs	Date H5N2 Confirmed by NVSL	Wildlife Sampling Period
Farm 1	3.7M	4/24/15	4/28/15	5/23-27/2015
Farm 2	574K	4/28/15	5/11/15	5/13-15/2015
Farm 3	4.1M	4/16/28	4/20/15	5/15-19/2015
Farm 4	275K	4/22/15	4/29/15	5/21-23/2015
Farm 5	275K	5/6/15	5/7/15	5/20-21/2015

Sampling Procedures

Wild birds and wild mammals were captured on farms, primarily in and around farm structures. Birds were captured using mist nets, baited funnel traps, and air guns. Mammals were trapped using baited collapsible Sherman traps (mice and voles) and baited Tomahawk traps (5”x5”x16” for cottontails, 10”x12”x32” for raccoons and skunks). Some Sherman traps were placed inside poultry houses, but only on infected farms.

Captured individuals were sampled for infection with influenza-A viruses, swabs, washes, and tissues and prior exposures (blood). For birds, an oral swab, cloacal swab, and external swab was collected. For targeted avian species (e.g., house sparrows, European starlings), we also collected a blood sample and lung tissue. For mammals, an oral swab, nasal swab/wash, and external swab were collected. For targeted species (e.g., mice), we collected a blood sample and lung and/or trachea tissue samples. Further, we also collected any observed aberrant tissue (e.g., lesion, abnormal mass). Swabs, washes, and tissue samples were placed in 1-3mL of viral transport media (BHI: brain-heart infusion broth) and stored on ice. Blood was collected into serum separator tubes, allowed to clot, and centrifuged prior to shipping. We shipped samples overnight on ice to testing laboratories within 24 hours during the week or stored them in a refrigerator and shipped overnight on ice.

Laboratory Procedures

Swabs, washes, and tissue samples were screened for influenza A virus (IAV) matrix gene RNA via real-time reverse transcriptase polymerase chain reaction (RRT-PCR). The Avian Veterinary

Diagnostic Laboratory at Colorado State University conducted matrix gene RRT-PCR testing of avian oral and cloacal swabs, while the National Wildlife Research Center Virology Laboratory conducted all other matrix gene RRT-PCR. Per the National Animal Health Laboratory Network (NAHLN) protocol, any sample with a cycle threshold (Ct) value >0 is considered positive for viral RNA. Samples with Ct>0 by matrix gene RRT-PCR were submitted to the USDA's NVSL in Ames, IA, for confirmatory testing. Confirmatory testing included subtype confirmation using H5 and H7 2014 assays targeting Eurasian and Americas lineage viruses, as well as an H5 (icA) specific assay which targets the Eurasian H5 clade 2.3.4.4 viruses first detected in the United States in December 2014. Virus isolation in embryonated chicken eggs was conducted in parallel. Avian serum samples with adequate sample volumes were screened for antibodies to influenza A virus using the IDEXX AI Multi-S Screen Ab test, which is a multi-species blocking enzyme linked immunosorbent assay (ELISA) targeting an epitope of the nucleoprotein. All avian and mammalian serum samples were submitted to NVSL for hemagglutinin inhibition (HI) assay testing using the Eurasian H5 icA as the antigen. Avian samples that were antibody-positive by the IDEXX Multi-S test, but negative for the Eurasian H5 (and had sufficient serum available) via HI, were additionally tested using a standard North American panel of H1-H16.

Results

Across the 10 sampled farms (5 infected, 5 uninfected), we collected 2,627 samples from 426 individuals (Table 33Table 33). On infected farms, we collected samples from 190 individual mammals from 3 species (primarily house mice) and on uninfected farms, we collected samples from 39 individuals from 5 species (primarily mice, Table 34). On infected farms, we sampled 220 individual birds across 17 species and on uninfected farms, we sampled 199 individuals across 18 species (Table 35). House sparrows, European starlings, rock pigeons, swallows, and American robins were the most commonly sampled bird species.

All PCR testing is complete. Of the 2,184 swab, wash, and tissue samples tested, a single sample was confirmed for influenza A matrix gene RNA and was confirmed by RRT-PCR as the Eurasian H5 (icA) associated with clade 2.3.4.4. The positive sample was from lung tissue collected from a juvenile European starling captured on an infected premises. The starling was captured using a mist net that targeted a cavity nest built in a breach at the bottom of a walkway between two poultry barns.

Table 33. Summary of Samples Collected

Sample Type	Number Collected from Birds on Infected Sites	Number Collected from Birds on Uninfected Sites	Number Collected from Mammals on Infected Sites	Number Collected from Mammals on Uninfected Sites	Total
Serum	153	99	153	38	443
Oral Swab	217	199	188	38	642
Cloacal Swab	204	196	--	--	400
Nasal Swab/Wash	--	--	188	39	227
External Swab	135	197	26	38	396
Tissue	118	155	207	39	519

Table 34. Summary of sampled mammals

Species	Scientific Name	Number Captured on Infected Farms	Number Captured on Uninfected Farms	Total
House mouse	*Mus musculus*	185	10	195
Deer mouse	*Peromycus maniculatus*	3	19	22
Eastern cottontail	*Sylvilagus floridanus*	2	3	5
Northern short-tailed shrew	*Blarina brevicauda*	0	4	4
Raccoon	*Procyon lotor*	0	3	3

Table 35. Summary of sampled birds

Species	Scientific Name	Number Captured on Infected Farms	Number Captured on Uninfected Farms	Total
House sparrow	*Passer domesticus*	112	68	180
European starling	*Sturnus vulgaris*	15	54	69
Rock pigeon	*Columba livia*	19	19	38
American robin	*Turdus migratorius*	21	8	29
Common grackle	*Quiscalus quiscula*	12	6	18
Cliff swallow	*Petrochelidon pyrrhonota*	13	1	14
Barn swallow	*Hirundo rustica*	5	11	16
Red-winged blackbird	*Agelaius phoeniceus*	1	8	9
Chipping sparrow	*Spizella passerine*	5	4	9
American goldfinch	*Spinus tristis*	2	4	6
Brown-headed cowbird	*Molothrus ater*	0	6	6
Common yellowthroat	*Geothlypis trichas*	5	0	5
Killdeer	*Charadrius vociferous*	0	4	4
Least flycatcher	*Empidonax minimus*	2	1	3
Vesper sparrow	*Pooecetes gramineus*	0	3	3
American redstart	*Setophaga ruticilla*	2	0	2
Gray catbird	*Dumetella carolinensis*	2	0	2
Eastern bluebird	*Sialia sialis*	0	1	1
Blue jay	*Cyanocitta cristata*	0	1	1
Eastern kingbird	*Tyrannus tyrannus*	1	0	1
Ring-necked pheasant	*Phasianus colchicus*	1	0	1
Savannah sparrow	*Passerculus sandwichensis*	1	0	1
Yellow warbler	*Setophaga petechia*	1	0	1

Serological screening of avian samples using the IDEXX ELISA identified seven positive samples (Table 33) and several samples were suspect positive (within 0.1 of the manufacturer's recommended cutoff threshold). Because the IDEXX test is not validated for the avian species sampled, all serum samples were forwarded to NVSL for confirmatory testing via HI using the Eurasian icA H5. Three samples were positive for exposure to icA H5 influenza A. Two additional samples were positive for North American H5, with equivocal results for the icA strain. Both of these samples also showed low reactivity to one or both of the H7N2 and H9N2, which may indicate steric

inhibition by the N2 glycoprotein. Each of the ELISA or HI positive samples were collected from a single infected premises. Serological testing of mammalian samples is ongoing.

Table 36. Summary of positive samples for avian serum samples tested by ELISA and HI

Sample	Species	Site Status	ELISA	HI (icA H5)
Ide000872	American robin	Infected	Suspect Positive	>=1:32
IDe000875	American robin	Infected	Positive	<1:8
IDe000881	American robin	Infected	Positive	>=1:32
IDe000878	Common grackle	Infected	Positive	<1:8
IDe000892	European starling	Infected	Positive	1:16
IDj000870	European starling	Infected	Positive	<1:8*
IDj000891	European starling	Infected	Positive	<1:8
IDe000856	House sparrow	Infected	Positive	<1:8*

Note: an HI value >=1:8 is indicative of exposure to virus

* these samples showed lowed reactivity to one or both of H7N2 and H9N2

Summary

The finding of an influenza A RNA-positive sample from lung tissue extracted from a European starling indicates the need for experimental testing of this species to determine if it could play a role in the epidemiology of highly pathogenic Eurasian H5 viruses. This same individual was also positive for antibodies to influenza A virus via ELISA and HI with the HI confirming exposure to an icA H5 virus. Note that we primarily conducted invasive sampling on European starlings and house sparrows and did not test lung tissues for a majority of other species sampled.

In addition, two American robins were positive for antibodies to the icA H5 strain (one was positive by ELISA and one was suspect positive by ELISA) and a second European starling and a house sparrow were suspect positive for exposure to the icA H5. An additional American robin, an additional European starling, and a house sparrow were positive for exposure to influenza A, but were not confirmed for exposure to the icA H5. Of these three unconfirmed results, only the European starling sample had adequate serum remaining for additional testing. It was tested for exposure to North American H1-H16, but no positive results were identified. All positive and suspect positive samples were collected from a single infected premises. These results indicate that American robins and house sparrows may deserve further scrutiny to elucidate their possible role in Eurasian H5 influenza A viral transmission.

Wildlife sampling at infected farms occurred 2-4 weeks after clinical signs appeared in poultry; some farms had been completely depopulated by the time we collected samples, while others were at varying stages of depopulation. This lag may have reduced the probability of positive results. If future outbreaks occur, real-time wildlife sampling may provide better information on the potential role of synanthropic wildlife in influenza epidemiology.

Acknowledgements

We greatly appreciate the cooperation and support of the poultry industry for allowing us access to their properties.

IV. PHYLOGENETIC ANALYSIS

A. Eurasian H5Nx Virus Overview—UPDATED

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 11). These findings are not unexpected as the H5Nx viruses continue to circulate.

USDA's 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. Consensus data from 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 were implicated in recent poultry outbreaks. Where there is molecular evidence that independent introductions as well as "common source" exposures are occurring concurrently, further field epidemiologic investigation is warranted.

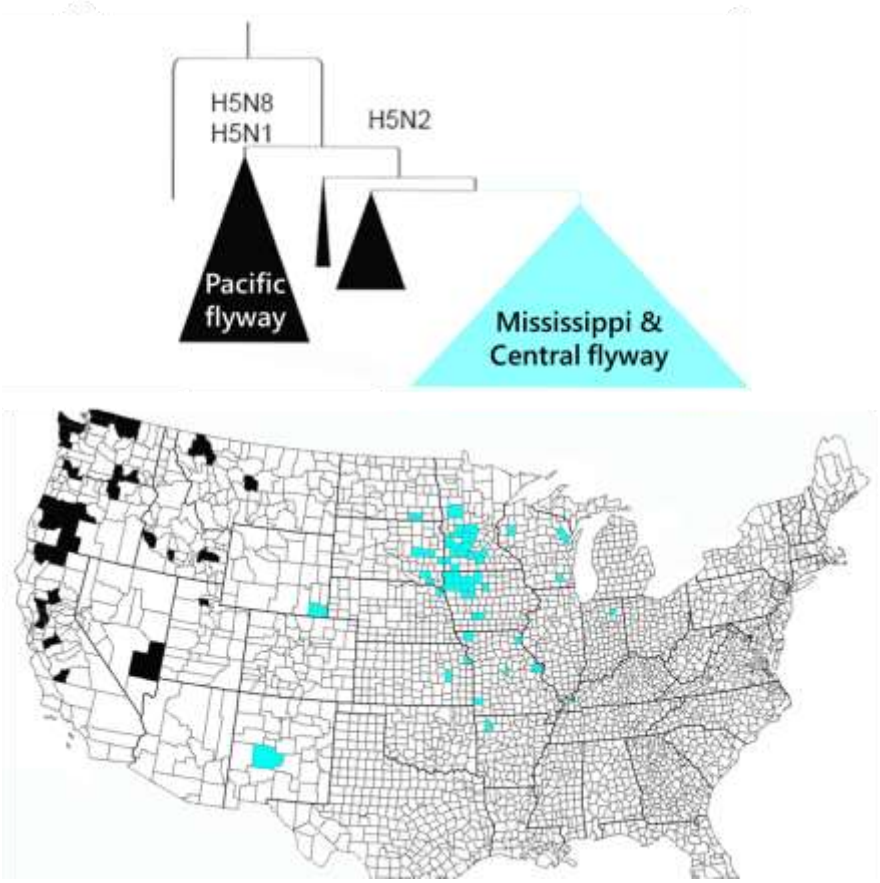


Figure 11. Phylogeny of the PB2, HA, and matrix genes of the H5Nx viruses and geographic distribution by subtype

Poultry events in Pacific Flyway appear to be largely due to point source/independent introductions as were early Midwest events based upon network analysis and available epidemiologic data. Data for later Midwest events suggest point source as well as "common source" exposures occurring concurrently. States affected last appear to be largely due to common source/human activity.

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. However, CDC continues virus monitoring.

This analysis includes samples collected between December 2014 to early June 2015 (Figure 12) from 17 States (>240 viruses distributed as in Table 37). While these viruses remain highly similar overall (>99% similar to the index viruses within subtype as well as to the nearest Asian isolate A/crane/Kagoshima/KU1/2014[H5N8]), analytical tools that identify substitutions along the hemagglutinin (HA), neuraminidase (NA) and internal proteins can improve our understanding of the virologic, antigenic, and epidemiologic features of the virus. The section on Diagnostics and Characterization for H5Nx viruses in this report offers further information.

Table 37. Distribution of viruses by region, subtype or virus group, and sector/type with state/county affected and duration from sample collection

Region	Virus subtype or group	layer, commercial	turkey, commercial	backyard	wild bird+raptor	# states affected	# counties affected	Duration from sample collection	Mode of spread based upon molecular analysis
Midwest H5N2	1					5	16	27 Feb to 20 Apr 2015	independent + limited lateral
	2a					4	16	6 Apr to 4 May 2015	independent + limited lateral
	2b		43			5	18	25 Mar to 4 Jun 2015	ind+lat | 76% MN turkey
	2c	46	30			5	22	13 Mar to 25 May 2015	ind+lat | 85% IA chicken>turkey
	2d		40			4	10	26 Mar to 14 May 2015	ind+lat | 91% MN turkey
Pacific	H5N2				27	4	16	8 Dec 2014 to 11 Feb 2015	independent
	H5N8				15	6	13	7 Dec 2014 to 6 Feb 2015*	independent
	H5N1					1	1	7 Dec 2014 to 6 Feb 2015	independent

* not including Indiana BY 5 May 15

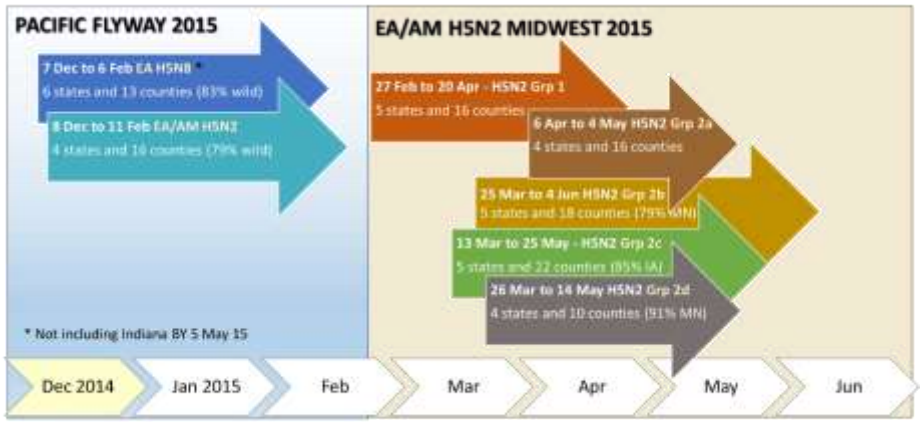


Figure 12. Duration of detection from sample collection date by virus subtype/group

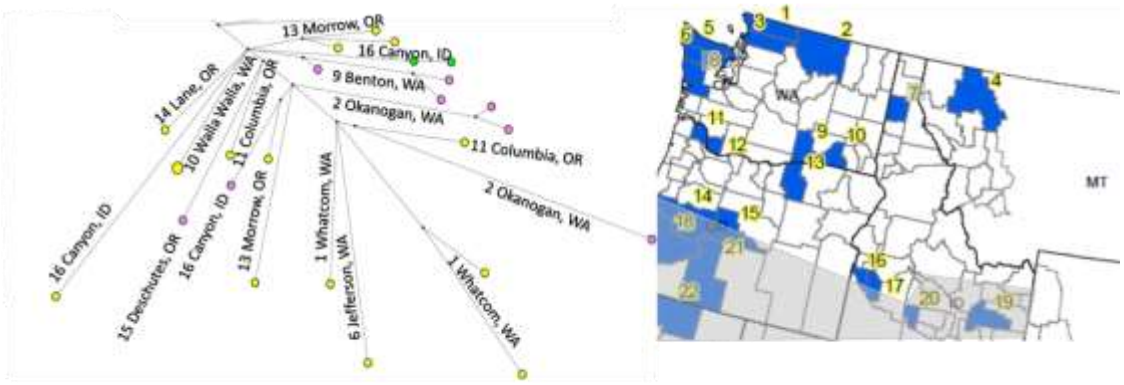
Summary of H5Nx Molecular Analysis

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. Pacific and early Midwest detections appear to be largely independent introductions and later events include potential for human involvement.

Pacific Flyway Findings

- Three different subtypes were detected (Table 37); the EA/AM H5N2 viruses predominated.
- No H5N2 was detected in commercial poultry in the Pacific flyway.
- The H5N8 viruses have wholly Eurasian gene constellations except two from Oregon (Jan2015) with two North American internal genes (PB1 and PA).
- H5N8 was detected in both poultry and wild bird populations in the Pacific flyway.

- Long branches (representing nucleotide differences) observed by network analysis for all H5Nx viruses in the Pacific flyway are suggestive of independent or point source introductions (Figure 13).
- These findings are consistent with both the movement of the virus in wild bird flyways and the low infectivity in gallinaceous poultry.



http://www.aphis.usda.gov/animal_health/downloads/animal_diseases/ai/hpai-incident-map.pdf

Figure 13. 8-gene network: Selection of 24 Pacific flyway detections spanning 3 States and 13 counties from December 8, 2014, to February 11, 2015; long branches suggest independent or point source introductions (greyed area = H5N8). Numbers on network correlate to map, which is available at web site above; yellow circle = wild bird, purple = backyard, red = poultry. Numbering indicates order of county detection; subsequent detections in positive county are not numbered.

Midwest Findings

- The Midwest viruses cluster into major groups 1 and 2 with four subgroups in group 2 indicated in Table 37**.**
- Groups 1 and 2a span several States and counties and contain long branches similar to that observed in the Pacific group suggesting largely independent or point source introductions in addition to limited evidence of lateral spread (Figure 14).
- The remaining groups (2b, c, and d) have a mixture of long branches suggestive of independent or point source introductions alongside shorter branches and highly similar viruses consistent with common source or lateral spread. The network and map in Figure 15 demonstrate the relatedness of the 2d.1 subcluster (ex-Stearns cluster), which gained in number and has confirmed epidemiologic links for many of the premises.
    - — Minnesota viruses are predominantly group 2b, 2d from turkeys
    - — Iowa viruses are predominantly group 2c from layers and turkeys
    - — All Midwest subgroups may be found in turkeys compared to layers (Table 37), suggesting there may be increased risk for a broader range of potential exposures
    - Only a single detection of EA H5N8 has been made outside the Pacific flyway (IN); molecular evidence suggests it may not have been present in the Mississippi, but further data are needed.

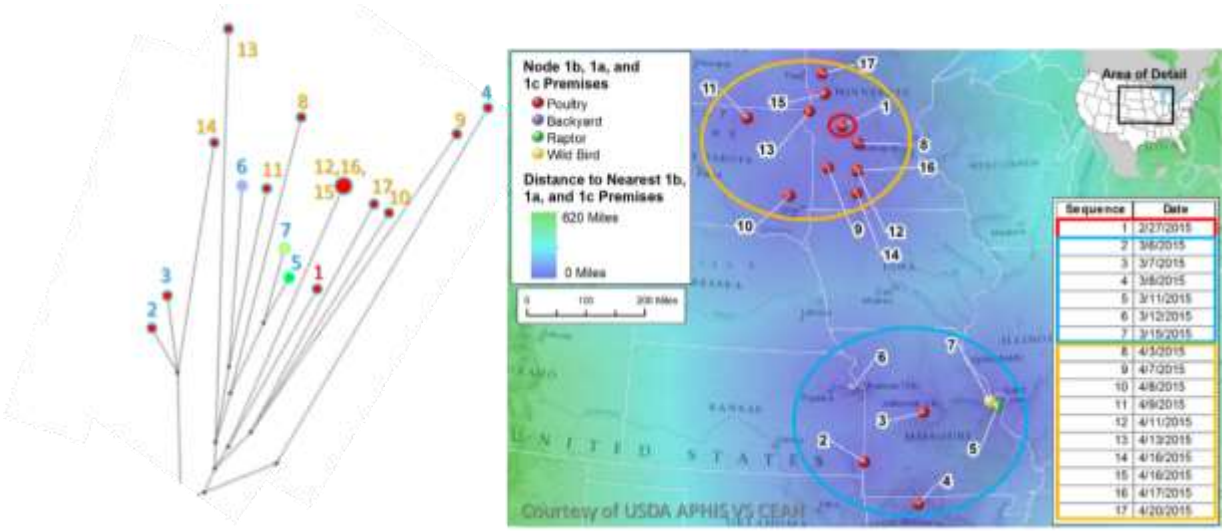


Figure 14. Network analysis (8 gene) of H5N2 Midwest Group 1: 17 detections spanning 5 States and 16 counties from February 27 to April 20, 2015; long branches suggest largely independent or point source introductions with limited evidence of lateral spread. Colored boxes match colored circles on map and colored numbers on network. Yellow circle = wild bird, purple = backyard, red = poultry.

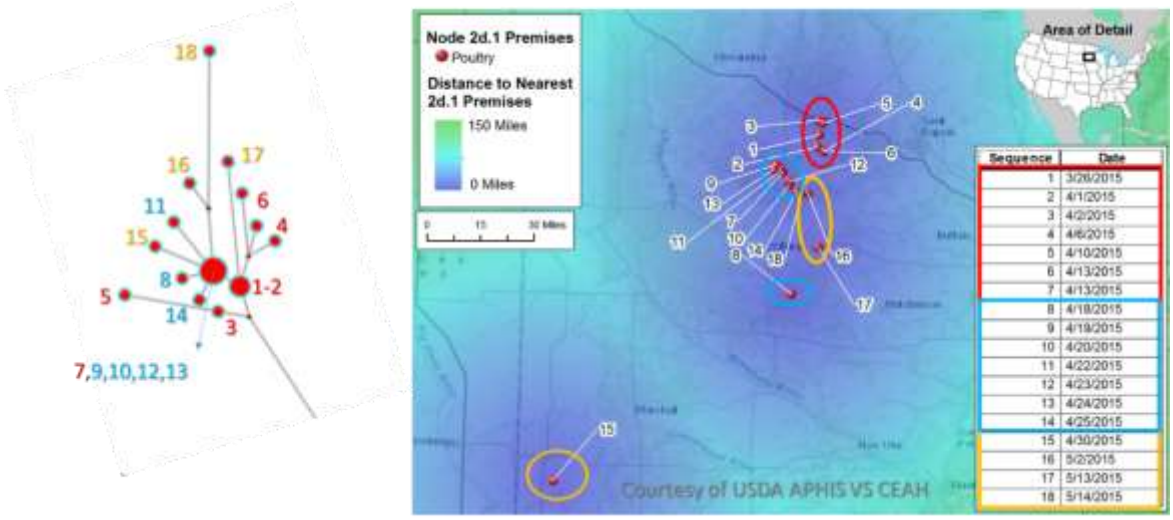


Figure 15. Network analysis (8 gene) of H5N2 Midwest Group 2d.1: 18 detections in single State across 4 counties from March 26 to May 14, 2015; highly similar viruses and shorter branches consistent with common source or lateral spread, viral change is consistent with the date of detection. Colored boxes match colored circles on map and colored numbers on network; red circle = poultry.

Other General Findings

- Over 240 viruses analyzed have been >99% similar to the index case across entire genome within subtype and for HA across subtypes.
- The majority of poultry viruses are nearly identical across the HA1 protein and have a change in the HA1 protein at a putative antigenic site (HA S141P; numbering per mature H5 HA; Table 38). Such substitutions may be more easily sustained in small virus populations (e.g., poultry flock).

- The molecular evidence reported on June 15, 2015, for two viruses that spanned a State boundary between Minnesota and South Dakota was not supported by epidemiologic data, and further molecular analysis across the entire genome suggests they may represent point source events. This emphasizes the challenges of interpreting data from highly similar viruses.
- One H5N2 virus 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.

Where there is molecular evidence that independent introductions, as well as "common source" exposures, are occurring concurrently, further field epidemiologic investigation is warranted.

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 continue to 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. Additionally, the expectation is that the poultry adapted strains have been eradicated and that if viruses return with migratory waterfowl in the fall or spring they would have waterfowl adapted strains. 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 38. 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	IA	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				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 United States during December 2014 and are known to be highly pathogenic to poultry. No other Eurasian H5 viruses have been detected in the United States to date (August 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 NAHLN in the United States 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 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.

[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

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

<u>FARM DIAGRAM -</u>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.

APPENDIX B. HPAI CASE CONTROL QUESTIONNAIRE - LAYERS

Animal and Plant
Health Inspection
Service

Veterinary Services

National Animal Health
Monitoring System

2150 Centre Ave., Bldg B
Fort Collins, CO 80526

Form Approved
OMB Number 0579-0376
Approval Expires:
9/30/2017

Study ID: _____ frmid

Farm (premises) ID: _____

Date: _____ mm/dd/yy

A. PREMISES INFORMATION

Farm name: _____frmname

Farm address: _____frmadd

County: _____frmcty

Township: _____ frmtshp Range: _____ frmrng Section: _____frmsec

1. Supervisor Contact name: _____h201

   Phone: _____ h202 Cell phone: _____ h203 Email: _____ h204

2. Farm manager Contact name: _____ h205

   Phone: _____ h206 Cell phone: _____ h207 Email: _____ h208

3. Flock Veterinarian: _____ h213

   Phone: _____ h214 Cell phone: _____ h215 Email: _____ h216

B. INTERVIEWER INFORMATION

Interviewer name/organization: _____ intrname

Interviewee name/organization: _____ intename

Animal and Plant Health Inspection Service

Veterinary Services

HPAI Case-Control Questionnaire

National Animal Health Monitoring System

2150 Centre Ave., Bldg B
Fort Collins, CO 80526

Form Approved
OMB Number 0579-0376
Approval Expires: 9/30/2017

Study ID: _____ frmid

Date: _____mm/dd/yy

INSTRUCTIONS

The Iowa Poultry Association, Iowa State University, and the United States Department of Agriculture APHIS (USDA APHIS) are conducting a case-control study as part of the highly pathogenic avian influenza (HPAI) investigation efforts to identify factors that may contribute to transmission of H5N2 influenza virus to poultry.

We are asking you to fill out this survey, which includes questions about things done daily on the farm, facility and premises condition, deliveries to the farm, and ill birds. We will be asking you questions about a 2 week (14 day) period on the farm starting on a particular date that we will provide. It might be difficult to remember back that far, so please use a pocket calendar or other agenda manager, and any feed and other delivery records that might be available to you.

Term	Case Definition	Control Definition
Premises	Farm location with flocks confirmed to be HPAI H5N2 infected by NVSL, including all barns and buildings; even if not all barns and buildings contain infected birds.	Farm location with no infected birds in any barn or building, in close proximity (less than 10 miles) of the case farm.
Barn	Barn or building that houses HPAI H5N2 infected birds.	On case premise, a barn or building that does not house any infected birds.

Dates of Study Focus:

Case farms answer questions for the timeframe of 14 days prior to the onset of clinical signs or increased mortality. All questions that ask about the past 14 days are referring to this time period. **Control farms** answer questions for the timeframe of 14 days prior to date of first detection on the matched case farm. All questions that ask about the past 14 days are referring to this time period.

NAHMS-349

SEP 2017

A. CASE OR CONTROL

1. Is this a case or control farm? e100 ☐$_1$ Case – ***Go to Question 2.***

   ☐$_3$ Control – ***Go to Question 3.***

2. If this is a **case** farm,
   a. When were clinical signs or increased mortality first observed? …….. e101 _____ mm/dd/yy
   b. 14 days prior to the date of first detection (clarifying timeframe of study focus)…… e102 _____ mm/dd/yy

      All questions regarding the past 14 days are referring to the 14 days prior to this reference date (i.e., the time between "a" and "b").

      c. When was the flock diagnosed as positive?.....e103_____ mm/dd/yy
   d. As of today, how many of the barns on this farm have been confirmed or are suspected to be infected with HPAI?.....e104 _____ # barns
   e. On the reference date, was this farm in an existing control zone?.....e105 ☐$_1$ Yes ☐$_3$ No

   Go to Question 4.

3. If this is a **control** farm,
   a. Enter reference date here (enter date of matched case farm prior to interview)……..e106 _____ mm/dd/yy
   b. Enter the date 14 days prior to the reference date……..e107 _____ mm/dd/yy

      All questions regarding the past 14 days are referring to the 14 days prior to this reference date (i.e., the time between "a" and "b").

   c. Is this farm located in a control zone?..... e108 ☐$_1$ Yes ☐$_3$ No
      i. If "Yes," how long has it been in a control zone? …… e109d/e109w _____ days

         OR

         _____ weeks
   d. What is the distance (in miles) from this farm to the nearest case farm?.....e110 _____ miles

4. How many birds were on this farm on this reference date? ……h313 _____ # birds

B. PREMISES DESCRIPTION

1. Is this a: [*Check one only.*] e201

   ☐1 Company farm?

   ☐2 Contract farm?

   ☐3 Lease farm?

   ☐4 Independent farm?

2. What type(s) of poultry are present on this farm?

   a. Turkey ..... e202 ☐1 Yes ☐3 No

   b. Broiler ..... e203 ☐1 Yes ☐3 No

   c. Layer ..... e204 ☐1 Yes ☐3 No

   d. Other (specify: _____) ..... e205/e205oth ☐1 Yes ☐3 No

3. What poultry production type(s) are present on this farm?

   a. Meat ..... e206 ☐1 Yes ☐3 No

   b. Egg ..... e207 ☐1 Yes ☐3 No

   c. Breeding ..... e208 ☐1 Yes ☐3 No

   d. Other (specify: _____) .....e209/e209 oth ☐1 Yes ☐3 No

4. Is this farm certified organic? e210 ☐1 Yes ☐3 No

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

6. Is this farm multiple age or single age? h303

   ☐1 Multiple age

   ☐2 Single age

7. What stage(s) of production is on this farm?

   a. Pullets ..... e211 ☐1 Yes ☐3 No

   b. Layers ..... e212 ☐1 Yes ☐3 No

   c. Breeders ..... e213 ☐1 Yes ☐3 No

   d. Other (specify: _____) ..... e214 ☐1 Yes ☐3 No

8. How many barns are on this farm? ..... h314 _____ # barns

9. Do any birds on the farm have access to the outdoors? ..... e215 ☐1 Yes ☐3 No

10. How many barns are:
    a. Conventional cage housing?.....e216 _____ #
    b. Enriched caged housing? .....e217 + _____ #
    c. Cage free (certified organic)? .....e218 + _____ #
    d. Cage free (not certified organic)?.....e219 + _____ #
    Total (*must equal Question 8 response*) e219a = _____ #

11. Are any poultry on this farm pastured?.....e220 ☐1 Yes ☐3 No

12. What is the distance (in yards) of the closest body of water (e.g., pond, lake, stream, river, wetland) to this farm? h319 _____ yards

    a. Specify this water body type: _____ h319spe

13. Approximately how many wild waterfowl might have been seen on this body of water at one time? Try to answer the question for the past 14 days. e221

    ☐1 None – ***Skip to Question 15*.**
    ☐2 Tens
    ☐3 Hundreds
    ☐4 Thousands

14. What type(s) of waterfowl were seen on the water in the 14 days?
    a. Ducks.....e222 ☐1 Yes ☐3 No ☐4 Don't Know
    b. Geese.....e223 ☐1 Yes ☐3 No ☐4 Don't Know
    c. Shorebirds (e.g., wading birds, gulls).....e224 ☐1 Yes ☐3 No ☐4 Don't Know
    d. Other (specify: _____)e225/e225oth ☐1 Yes ☐3 No ☐4 Don't Know

15. Are the following water body type(s) visible or within 350 yards (about 3 football fields) of this farm?
    a. Pond ..... e226 ☐1 Yes ☐3 No
    b. Lake ..... e227 ☐1 Yes ☐3 No
    c. Stream ..... e228 ☐1 Yes ☐3 No
    d. River ..... e229 ☐1 Yes ☐3 No
    e. Wetland or swamp ..... e230 ☐1 Yes ☐3 No
    f. Wastewater lagoon ..... e231 ☐1 Yes ☐3 No
    g. Other (specify: _____)..... e234/e234oth ☐1 Yes ☐3 No

16. What is the distance (in yards) to the closest field where crops are harvested?..... e235 _____ yards

17. What crop was last grown in this field? ……..e236

☐$_1$ Corn

☐$_2$ Soybeans

☐$_3$ Alfalfa or grass intended for livestock feed

☐$_4$ Other (specify: _____)e236oth

18. Was this field tilled last fall?.....e237 ☐$_1$ Yes ☐$_3$ No ☐$_4$ Don't Know

19. Was this field actively worked (e.g., tilled or disced) in the past 14 days?.....e238 ☐$_1$ Yes ☐$_3$ No ☐$_4$ Don't Know

20. What was the approximate concentration of wild waterfowl observed at a single view in this field in the past 14 days? e239

☐$_1$ None – ***Skip to Question 22***

☐$_2$ Tens

☐$_3$ Hundreds

☐$_4$ Thousands

21. What type(s) of waterfowl were observed?

a. Ducks ……e240☐$_1$ Yes ☐$_3$ No ☐$_4$ Don't Know

b. Geese ……e241☐$_1$ Yes ☐$_3$ No ☐$_4$ Don't Know

c. Shorebirds ……e242☐$_1$ Yes ☐$_3$ No ☐$_4$ Don't Know

d. Other (specify: _____)e243/e243oth☐$_1$ Yes ☐$_3$ No ☐$_4$ Don't Know

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

a. Beef cattle .....h325 ☐$_1$ Yes ☐$_3$ No

b. Dairy cattle .....h326 ☐$_1$ Yes ☐$_3$ No

c. Horses .....h327 ☐$_1$ Yes ☐$_3$ No

d. Sheep .....h328 ☐$_1$ Yes ☐$_3$ No

e. Goats .....h329 ☐$_1$ Yes ☐$_3$ No

f. Pigs .....h330 ☐$_1$ Yes ☐$_3$ No

g. Dogs .....h331 ☐$_1$ Yes ☐$_3$ No

h. Cats .....h332 ☐$_1$ Yes ☐$_3$ No

i. Poultry or domesticated waterfowl .....h333 ☐$_1$ Yes ☐$_3$ No

j. Other (specify: _____) .....h334/h334oth ☐1 Yes ☐3 No

23. What is the water source for poultry?

a. Municipal .....e244 ☐1 Yes ☐3 No

b. Well .....e245 ☐1 Yes ☐3 No

c. Surface water (e.g., pond) .....e246 ☐1 Yes ☐3 No

d. Other (specify: _____)...e247/247oth ☐1 Yes ☐3 No

24. Are the following water treatments used in the drinking water for the poultry on this farm?

a. Chlorination ..... e248 ☐1 Yes ☐3 No

b. Acidifiers ..... e249 ☐1 Yes ☐3 No

c. Iodine ..... e250 ☐1 Yes ☐3 No

d. Peroxide ..... e251 ☐1 Yes ☐3 No

e. Other (specify: _____)..... e252/e252oth ☐1 Yes ☐3 No

25. Are windbreaks present on this farm? If "Yes," what is the distance (in yards) from the windbreak to the closest poultry barn?

Windbreak type	Present?	If "Yes,", distance to closest poultry barn	
a. Evergreen or juniper windbreak	☐1 Yes ☐3 No	_____ yards	e253/e256
b. Deciduous tree windbreak	☐1 Yes ☐3 No	_____ yards	e254/e257
c. Structural (e.g., hill, natural break)	☐1 Yes ☐3 No	_____ yards	e255/e258

26. Excluding driveways on farm, what is the distance (in yards or miles)

from this farm to the nearest public gravel or dirt road?.....e259y/e259m _____yards OR _____ miles

C. FARM BIOSECURITY

1. Is there a house with people living in it on the property? h401 ☐1 Yes ☐3 No – ***Skip to Question 3***

2. Is there a common drive entrance to farm and residence?.....h402 ☐1 Yes ☐3 No

3. How many entrances are there to the farm that could provide access to the poultry area?.....e301 _____ #

4. Which best describes the road surface on this farm that vehicles coming onto the operation drive on? *[Check one only.]* e302

   ☐$_1$ Hard top/asphalt

   ☐$_2$ Gravel

   ☐$_3$ Dirt

   ☐$_4$ Other (specify: _____)e302oth

5. In general, do the following types of vehicles:

Codes for Question 5
1 = come to the perimeter of the farm only
2 = enter the farm but not near the barns
3 = come near the barns
4 = do not come at all

Enter the codes that apply

a. Garbage/dumpster pick-up? ..... e303 _____ code

b. Propane delivery? ..... e304 _____ code

c. Feed delivery? ..... e305 _____ code

d. Renderer? ..... e306 _____ code

e. Company personnel (e.g., processing plant and barn workers, service person, veterinarian)? ..... e307 _____ code

f. Egg trucks moving eggs *off* the farm (e.g., to processing, for breaking, to the consumer market)? ..... e308 _____ code

g. Egg trucks moving eggs *to* the farm (i.e., sideloading)? ..... e309 _____ code

h. Other business visitors (e.g., meter reader, repairman)? ..... e310 _____ code

6. Is there a gate to this farm entrance?.....h404 ☐$_1$ Yes ☐$_3$ No – ***Skip to Question 8***

7. Is the gate secured/locked? .....h405 ☐$_1$ Always ☐$_2$ After hours only ☐$_3$ Never

8. Is the farm area perimeter surrounded by a security fence? .....h407 ☐$_1$ Yes ☐$_3$ No

9. How frequently is vegetation mowed/bush hogged on the premises (answer for when vegetation is present, e.g., spring and summer) .....h408 _____ times/month

10. Is the facility free of debris/clutter/trash piles? .....h409 ☐$_1$ Yes ☐$_3$ No

11. Is there a wash station/spray area being used

for vehicles?.....h410 ☐$_1$ Yes ☐$_3$ No – ***Skip to Question 13***

12. If "Yes:"
    a. Is it located on the farm? ..... e311 ☐$_1$ Yes ☐$_3$ No
    b. Are the tires washed? ..... e312 ☐$_1$ Yes ☐$_3$ No
    c. Is the vehicle exterior washed? ..... e313 ☐$_1$ Yes ☐$_3$ No
    d. Is the vehicle interior cleaned (e.g., floor mats) ..... e314 ☐$_1$ Yes ☐$_3$ No
    e. Which vehicles are washed:
        i. Worker vehicles? ..... e315 ☐$_1$ Yes ☐$_3$ No
        ii. Feed trucks? ..... e316 ☐$_1$ Yes ☐$_3$ No
        iii. Egg trucks? ..... e317 ☐$_1$ Yes ☐$_3$ No
        iv. Other (specify: _____)? e318/e318oth ☐$_1$ Yes ☐$_3$ No
    f. What disinfectant is used? _____ h411
    g. Was the wash station: [*Check one only.]* e319
        ☐$_1$ Recently put into use as a response to heightened biosecurity concerns?
        ☐$_1$ A permanent station (i.e., in use prior to the HPAI incident)?

13. Do workers and visitors always, sometimes or never park in a restricted area away from the poultry barns?

    a. Workers..... e320 ☐$_1$ Always ☐$_2$ Sometimes ☐$_3$ Never
    b. Visitors ..... e321 ☐$_1$ Always ☐$_2$ Sometimes ☐$_3$ Never

14. What pest and wildlife control measures were used on this farm in the past 14 days?
    a. Rat and mouse bait stations? ..... h426 ☐$_1$ Yes ☐$_3$ No

    If "Yes," how frequently are they checked? ..... e322 _____times/month

    b. Beetle control? ..... e323 ☐$_1$ Yes ☐$_3$ No

    If "Yes," type:
        i. Sprays ..... e324 ☐$_1$ Yes ☐$_3$ No
        ii. Boric acid ..... e325 ☐$_1$ Yes ☐$_3$ No
        iii. Baits ..... e326 ☐$_1$ Yes ☐$_3$ No
        iv. Other (specify: _____)e327/e327oth ☐$_1$ Yes ☐$_3$ No

c. Fly control (other than manure removal)? ..... h428 ☐1 Yes ☐3 No

If "Yes," type:

i. Residual spray ..... e328 ☐1 Yes ☐3 No

ii. Baits ..... e329 ☐1 Yes ☐3 No

iii. Larvacide (spot treatment) ..... e330 ☐1 Yes ☐3 No

iv. Larvacide in feed ..... e331 ☐1 Yes ☐3 No

v. Space sprays/fogger ..... e332 ☐1 Yes ☐3 No

vi. Biological predators ..... e333 ☐1 Yes ☐3 No

vii. Other (specify: _____). e334/e334oth ☐1 Yes ☐3 No

15. Overall, how severe of a problem were rodents during the past 14 days? e335

[*Check one only*.]

☐1 High (e.g., significant damage to building, significant impact on layer health or feed efficiency)

☐2 Moderate (e.g., moderate damage to building, moderate impact on layer health or feed efficiency)

☐3 Low (e.g., minor impact on building or feed efficiency)

☐4 No problem

16. Do you monitor rodent index as part of your rodent control program?.....e336 ☐1 Yes ☐3 No – ***Skip to Question 18***

Note: Rodent index (RI) is the equivalent of number of mice caught in 7 days with 12 traps using the formula:
RI = (number of mice caught) x (7 / days trapped) x (12 / number of traps)

17. Which of the following ranges best describes your rodent index in the past 14 days? [*Check one only.*] e337

☐1 Low (0 to 10 mice)

☐2 Moderate (11 to 25 mice)

☐3 High (26 or more mice)

18. Were wild mammals such as raccoons, opossums, coyotes, or foxes (or evidence of their presence), seen in or around poultry houses in the past 14 days?..... e338 ☐1 Yes ☐3 No

19. Prior to feeding, how frequently do wild birds, wild animals, and rodents have access to poultry feed (i.e., feed spillage, open bag, cover left open)?

	Always/ Nearly always	Most of the time	Sometimes	Never	
a. Wild birds	☐1	☐2	☐3	☐4	e339
b. Wild animals such as raccoons, opossums, coyotes or foxes	☐1	☐2	☐3	☐4	e340
c. Rodents	☐1	☐2	☐3	☐4	e341

20. Describe the protocol or plan for when feed spills on your farm? e342 _____

_____

21. What form of feed is fed to the poultry?

a. ..... Mashe343 ☐1 Yes ☐3 No
b. ..... Pelletse344 ☐1 Yes ☐3 No
c. Other (specify: _____)e345/e345oth ☐1 Yes ☐3 No

22. Is the feed treated with:

a. .....Formaldehyde (i.e., Termin-8)? ☐1 Yes ☐3 No
b. .....Antimicrobial (e.g., ionophores)? ☐1 Yes ☐3 No
c. Other (specify: _____)? ☐1 Yes ☐3 No

23. Is the feed heat treated?..... ☐1 Yes ☐3 No

D. WILD BIRDS

1. How frequently have the following types of wild birds been seen on habitats adjacent to the farm (but not on the farm) in the past 14 days?

Bird type	Daily	Less than daily	Never
a. Waterfowl (e.g., ducks, geese)	☐1	☐2	☐3
b. Gulls	☐1	☐2	☐3
c. Small perching birds (e.g., sparrows, starlings, swallows)	☐1	☐2	☐3
d. Blackbirds and crows	☐1	☐2	☐3
e. Other water birds (e.g., egrets, cormorants)	☐1	☐2	☐3
f. Wild turkeys, pheasants, quail	☐1	☐2	☐3
g. Raptors (e.g., eagles, hawks, owls)	☐1	☐2	☐3
h. Pigeons and doves	☐1	☐2	☐3
i. Other (specify: _____)	☐1	☐2	☐3

a. Do wild waterfowl use this area at other times of the year? ..... e410 ☐1 Yes ☐3 No

2. How frequently have the following types of wild birds been seen on the farm, but outside of the barns (within 100 yards) in the past 14 days?

Bird type	Daily	Less than daily	Never
a. Waterfowl (e.g., ducks, geese)	☐1	☐2	☐3
b. Gulls	☐1	☐2	☐3
c. Small perching birds (e.g., sparrows, starlings, swallows)	☐1	☐2	☐3
d. Blackbirds and crows	☐1	☐2	☐3
e. Other water birds (e.g., egrets, cormorants)	☐1	☐2	☐3
f. Wild turkeys, pheasants, quail	☐1	☐2	☐3
g. Raptors (e.g., eagles, hawks, owls)	☐1	☐2	☐3
h. Pigeons and doves	☐1	☐2	☐3
i. Other (specify: _____)	☐1	☐2	☐3

3. How frequently have the following types of wild birds been seen in the barns in the past 14 days?

Bird type	Daily	Less than daily	Never
a. Large birds (e.g., pigeons, crows)	☐1	☐2	☐3
b. Small birds (e.g., finches, sparrows, starlings)	☐1	☐2	☐3

4. Have you observed any of the following types of ***dead*** wild birds *in* the barns or *outside* of the barns in the past 14 days?

Dead bird type	Inside the barns?	Outside the barns?
a. Large birds (e.g., pigeons, crows)	☐1 Yes ☐3 No	☐1 Yes ☐3 No
b. Small birds (e.g., finches, sparrows, starlings)	☐1 Yes ☐3 No	☐1 Yes ☐3 No
c. Other (specify: _____)	☐1 Yes ☐3 No	☐1 Yes ☐3 No

E. FARM HELP/WORKERS

Questions in this section refer to persons such as the producer, employees, farm help, crews, etc.

1. What is the total number of employees working on this farm that have access to or directly work with poultry (including family, both paid and unpaid)? ..... e501 _____ #

2. Are the following measures always/nearly always, sometimes, or never required for workers entering the poultry houses?

Measure	Always/ Nearly always	Most of the time	Sometimes	Never
a. An established clean/dirty line	☐1	☐2	☐3	☐4
b. Shower	☐1	☐2	☐3	☐4
c. Wash hands before entering and/or before leaving the barn	☐1	☐2	☐3	☐4
d. Different personnel for different houses	☐1	☐2	☐3	☐4
e. Wear disposable coveralls	☐1	☐2	☐3	☐4
f. Change of clothing (washable)	☐1	☐2	☐3	☐4
g. Change of shoes or use of shoe covers	☐1	☐2	☐3	☐4
h. Foot bath (liquid)	☐1	☐2	☐3	☐4
i. Foot bath (dry)	☐1	☐2	☐3	☐4
j. Scrub footwear (bucket and brush)	☐1	☐2	☐3	☐4

3. Do workers on this farm work on other company farms?.....e512 ☐1 Yes ☐3 No

4. Are workers or members of their household employed by other poultry operations, rendering plants, or processing plants? .....e513 ☐1 Yes ☐3 No

   If "Yes," list the poultry operation(s), rendering plant(s), or processing plant(s):

   _____

5. Do any employees own their own poultry, including small backyard flocks?.....e515 ☐1 Yes ☐3 No ☐4 Don't Know

6. Are employees required to stay off farm after exposure to other poultry?.....e516 ☐1 Yes ☐3 No
   If "Yes," for how long (hours)?.....e517 _____ hours

F. FARM VISITORS

1. Did any of the following types of people visit the farm in the past 14 days? If "Yes," how many times did they visit and did they enter the poultry barn?

Visitor type	Did they visit the farm?	If "Yes,"	
		How many times did they visit?	Did this visitor enter the poultry barn?
a. Federal/state veterinary or animal health worker	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
b. Extension agent or university veterinarian	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No

c. Private or company veterinarian	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
d. Company service person	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
e. Nutritionist or feed company consultant	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
f. Pullet delivery	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
g. Vaccination crew	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
h. Catch crew	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
i. Feed delivery personnel	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
j. Egg truck personnel	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
k. Litter services (delivery, pick-up)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
l. Customer (private individual)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
m. Wholesaler, buyer, or dealer	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
n. Renderer	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
o Occasional worker (e.g., family member, part time help over holiday)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
p. Construction workers	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
q. Other business visitors (including other producers, meter readers, package delivery (UPS), repair person, wildlife services, and service personnel)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No
r. Other nonbusiness visitors (including neighbors, friends, and school field trips)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No

2. Is a visitor log used to record visitor traffic onto the farm?..... e655 ☐1 Yes ☐3 No

3. For those visitors who entered the poultry barn in the past 14 days, did you always/nearly always, sometimes or never require the following?

	Always/ Nearly always	Sometimes	Never
a. Change of outer clothing/farm specific clothing	☐1	☐2	☐3
b. Foot covers or change of footwear	☐1	☐2	☐3
c. Mask	☐1	☐2	☐3
d. Hand sanitizing or gloves	☐1	☐2	☐3
e. Not visit multiple farms in the same day	☐1	☐2	☐3
f. Other (specify:_____)	☐1	☐2	☐3

G. FARM VEHICLES AND EQUIPMENT

1. Were the following vehicles on this farm in the past 14 days? If "Yes," was the vehicle shared with another farm? If "Yes," was it disinfected prior to returning to this farm and who was the vehicle shared with?

Vehicle type	On farm in past 14 days?	If "Yes", was it shared with another farm?	If "Yes,"	
			Was it disinfected prior to returning to this farm?	Who was it shared with? [*Enter DK if don't know.*]
a. Company trucks/trailers (e.g., pickup truck, trailer with supplies, supervisor truck, etc.)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
b. Feed trucks	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
c. Pullet delivery vehicles (i.e., placing pullets)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
d. Bird removal vehicles	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
e. Egg delivery vehicles	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
f. Egg removal vehicles	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
g. Manure/litter hauling	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
h. ATV/4-wheeler	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
i. Other (specify: _____)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	

2. Were the following pieces of equipment on this farm in the past 14 days? If "Yes," was the equipment shared with another farm? If "Yes," was it disinfected prior to returning to this farm and who was the equipment shared with?

Equipment type	On farm in past 14 days?	If "Yes", was it shared with another farm?	If "Yes,"	
			Was it disinfected prior to returning to this farm?	Who was it shared with? [*Enter DK if don't know.*]
a. Gates/panels	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
b. Lawn mowers	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
c. Live haul loaders	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
d. Egg racks or pallets	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
e. Egg flats	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
f. Pressure sprayers/washers	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
g. Skid-steer loaders	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
h. Litter handling	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
i. Manure handling	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	
j. Other (specify: _____)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No	

H. EGG HANDLING

1.Were any eggs from this farm marketed in the past 14 days as:

a.Shell eggs?.....e801 ☐1 Yes ☐3 No – ***Skip to 1b***
i. Washed and sanitized eggs?.....e802 ☐1 Yes ☐3 No
ii.Nest runs?.....e803 ☐1 Yes ☐3 No
b.Liquid eggs (sent to further processing)?.....e804 ☐1 Yes ☐3 No

2. Which best describes the ***primary*** location for shell egg processing (washing, grading, and packing into cartons)? [*Check one only*.].....e805

☐1 On-farm
☐2 Off-farm – ***Skip to Question 4***

3.Are shell eggs from other farms processed on this farm (i.e., side-loading)?.....e806 ☐1 Yes ☐3 No

Go to Section I.

4. When shell eggs are processed off-farm, what is the:

a. Average number of days between egg pickups from the farm?.....e807 _____days
b. Distance (in miles) to the processing plant where the majority of the eggs are processed?.....e808 _____miles
c. What is name of the processing plant?.....e809 _____

I. LITTER AND MANURE HANDLING

1. Is litter (bedding) used on this farm?.....e901 ☐1 Yes ☐3 No – ***Skip to Question 10***

2. What was the last day that litter was brought onto the farm?.....e902_____ mm/dd/yy

3. Who brought the litter onto the farm:
☐1 Company personnel?
☐2 Litter provider?
☐3 Other (specify: _____)? e903oth

4. What is the source (i.e., company name) of the litter?
_____

5. Is the litter heat treated prior to delivery?.....e905 ☐1 Yes ☐3 No ☐4 Don't Know

6. Is litter stored on the farm prior to use:

a. Outside? e906	☐1 Yes	☐3 No
i. If "Yes," is it covered? e907	☐1 Yes	☐3 No
b. In a shed? e908	☐1 Yes	☐3 No
i. If "Yes," is the shed closed? e909	☐1 Yes	☐3 No

If both 6a and 6b are "No," skip to Question 8.

7. What is the minimum distance (in yards) from the on-site litter storage area to the nearest barn?.....e910 _____ yards

8. Prior to use, is litter accessible to:

a. Wild birds? e911	☐1 Yes	☐3 No
b. Wild animals (e.g., raccoons, opossum, coyotes, foxes)?..... e912	☐1 Yes	☐3 No
c. Domestic animals (e.g., dogs, cats)? e913	☐1 Yes	☐3 No

9. What was the date that litter was last removed from any barn on this farm? ..... e914 _____ mm/dd/yy

10. Has manure or used litter from other farms been spread on this farm or adjacent farms? ..... e915 ☐1 Yes ☐3 No ☐4 Don't Know

If "Yes," what was the last date: ..... e916 _____ mm/dd/yy

11. Which of the following manure handling methods are used for barns on this operation?

a. High rise (pit at ground level with house above).....e917 ☐1 Yes ☐3 No
b. Deep pit (below ground).....e918 ☐1 Yes ☐3 No
c. Shallow pit (ground level).....e919 ☐1 Yes ☐3 No
d. Raised slats over floor (no manure belt).....e920 ☐1 Yes ☐3 No
e. Flush system to a lagoon or slurry pit.....e921 ☐1 Yes ☐3 No
   i. If "Yes," is lagoon water used to flush barns?.....e922 ☐1 Yes ☐3 No
f. Manure belt.....e923 ☐1 Yes ☐3 No
g. Scraper system (not flush or pit).....e924 ☐1 Yes ☐3 No
h. Drop board.....e925 ☐1 Yes ☐3 No

12. Excluding belt system, how often is manure removed from the barn?..e926m/e926y _____ # / month
OR
_____ # / year

13. Is manure stored on farm (not including high rise pits)?...e927 ☐1 Yes ☐3 No – ***Skip to Question 16***

14. Is manure stored:
a. In an enclosed building?.....e928 ☐1 Yes ☐3 No
b. In an open structure (e.g., 3 sided building)?.....e929 ☐1 Yes ☐3 No
c. In a lagoon?.....e930 ☐1 Yes ☐3 No
d. Outside other than lagoon?.....e931 ☐1 Yes ☐3 No

15. What is the minimum distance (in yards) from the on-site manure storage area to the nearest barn?.....e932 _____ yards

16. How was manure most recently disposed of?

a. Composted on farm.....e933 ☐1 Yes ☐3 No

If "Yes,"

   i. What is the distance (in yards) to the nearest poultry house?.....e934 ____ yards
   ii. Is manure composted in a composting building?.....e935 ☐1 Yes ☐3 No

b. Applied to land on this farm.....e936 ☐1 Yes ☐3 No

If "Yes," what was the date manure was applied to land?.....e937 _____ mm/dd/yy

c. Taken off site.....e938 ☐1 Yes ☐3 No

If "Yes," name and location: _____ h711

J. DEAD BIRD DISPOSAL

1. What is the approximate normal daily mortality on this farm?.....e1001 _____ # / 1000 birds

2. What are the method(s) of dead bird (daily mortality) disposal on this farm?
   a. Composting .....e1002 ☐1 Yes ☐3 No
   b. Burial .....e1003 ☐1 Yes ☐3 No
   c. Incineration .....e1004 ☐1 Yes ☐3 No
   d. Rendering .....e1005 ☐1 Yes ☐3 No
   e. Landfill .....e1006 ☐1 Yes ☐3 No
   f. Other (specify:_____) ..e1007/ e1007oth ☐1 Yes ☐3 No

3. If 2a (composting) or 2b (burial) are "Yes," how frequently are carcasses covered with:
   a. Soil? .....e1008 ☐1 Daily ☐2 Every 2 or more days ☐3 Never
   b. Manure? .....e1009 ☐1 Daily ☐2 Every 2 or more days ☐3 Never

4. If 2d (rendering) is "Yes,"
   a. Is the carcass bin kept covered? .....e1010 ☐1 Yes ☐3 No
   b. Are carcasses *[Check one only.]* e1011
      ☐1 Taken by the producer/worker to the renderer?
      ☐2 Picked up by the renderer from the farm?
   c. How frequently are carcasses moved to the renderer?.....e1012 _____ # times/week
   d. What were the dates of the pick-ups in the past 14 days?
      _____ mm/dd/yy
      e1013
   e. What is the name of the company that handles this farm's rendering?
      _____

5. What do workers do after handling the carcass bin before returning to the live poultry area?
   _____
   _____
   _____

6. Have any wild birds or wild mammals been observed around the dead bird collection area (i.e., burial, compost pile, rendering, etc.) in the past 14 days?
   a. Wild birds .....e1016 ☐1 Yes ☐3 No
   b. Wild mammals .....e1017 ☐1 Yes ☐3 No

7. Is there a common collection point (i.e., located off the farm) for dead bird disposal?.....e1018 ☐1 Yes ☐3 No

   If "Yes," where is the common collection point located? _____

K. WEATHER CONDITIONS

1. In the past 14 days, how would you describe the wind?

   ☐1 Windier than normal ☐2 Normal ☐3 Less windy than normal ☐4 Not sure

2. In the past 14 days, how would you describe the humidity?
   e1102

   ☐1 Drier than normal ☐2 Normal ☐3 Wetter than normal ☐4 Not sure

BARN LEVEL QUESTIONS

INSTRUCTIONS:

1. **Control farm**: Select one barn to complete this section. Answer questions for the 14 days prior to the reference date specified on page 4. Complete *only* the "Control Barn" column.

2. **Case farm:** 1) Select the *first* barn on this premises that was confirmed to be HPAI positive. Answer questions in the "Case Barn" column for the 14 days prior to the onset of clinical signs or increased mortality. 2) Select one barn at random on this premises that is not HPAI positive. Select a barn that has birds present and is experiencing normal mortality. The Control Barn should physically be a separate structure from any infected barns. Answer questions in the "Control Barn" column for the same 14 day time period (i.e., the 14 days prior to the onset of clinical signs or increased mortality in any barn on this premises). If all barns on the premises are infected, leave "Control Barn" column blank.

	CASE BARN	CONTROL BARN
1. What is the barn ID?		
2. What type(s) of poultry are present in this barn?		
a. Pullet	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No
b. Layer	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No
c. Breeder	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No
d. Other	☐$_1$ Yes ☐$_3$ No If "Yes," specify: _____	☐$_1$ Yes ☐$_3$ No If "Yes," specify: _____
3. How many birds were placed in this barn?	_____ # birds	_____ # birds
4. What was the date of placement in this barn?	_____ mm/dd/yy	_____ mm/dd/yy
5. How old were birds when placed in this barn?	_____ weeks	_____ weeks
6. Which of the following strains were in the layer flock? [*Check one only.*]	☐$_1$ White egg strain ☐$_2$ Brown egg strain	☐$_1$ White egg strain ☐$_2$ Brown egg strain

	CASE BARN	CONTROL BARN
7. Which of the following breeds were in the layer flock? [*Check one only.*]	☐$_1$ Hyline ☐$_2$ Lohmann ☐$_3$ Centurion ☐$_4$ Other (specify: _____ ___)	☐$_1$ Hyline ☐$_2$ Lohmann ☐$_3$ Centurion ☐$_4$ Other (specify: _____ ___)
8. Has this flock been molted?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No

	CASE BARN	CONTROL BARN
9. Did birds in this barn have outside access?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No
10. What was the bird density in the barn?	_____ sq in/bird	_____ sq in/bird
11. Was there another health concern in this flock in the past 14 days?	☐$_1$ Yes ☐$_3$ No If "Yes," specify condition: _____ _____	☐$_1$ Yes ☐$_3$ No If "Yes," specify condition: _____ _ _____ _
12. Was this flock being treated for a condition or health concern in the past 14 days?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No
13. Was this flock vaccinated in the past 14 days?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No
14. How are birds housed in this barn? *[Enter code 1, 2, or 3.]* 1. Conventional cage 2. Enriched cage 3. Cage free	_____ code **If "3, Cage free," Skip to Question 16.**	_____ code **If "3, Cage free," Skip to Question 16.**
15. Are cages curtain backed?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No
16. Do birds have access to droppings from other birds (e.g., manure belt running across top tier of cage)?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No

17. How old is this barn structure?	_____ years	_____ years
18. How long has it been since the last remodel of the barn structure?	_____ years	_____ years
	CASE BARN	**CONTROL BARN**
19. How well has the barn structure been maintained? [*Enter code 1, 2, or 3.*] 1. Well E.g., Concrete foundation, no visible daylight, the barn is tight, intact inlet vent screens, doors well sealed 2. Moderate E.g., Barn tin could have rust or small holes, intact inlet vent screens, doors not completely sealed 3. Poor E.g., Holes in walls are apparent, tin is rusted, may have leaks in roof, there might be some holes large enough for wild birds to enter, multiple areas with daylight visible, inlet vent screens not intact, doors not sealed	_____ code	_____ code
20. Is there a buffer area between the barn and the outdoors which limits movement of air flow from the outside to the birds?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No
21. What is the type of ventilation for this barn? *[Enter Code 1-4.]* 1. Curtain ventilated 2. Sidewall inlet 3. Ceiling or eaves inlet 4. Tunnel ventilation (may have side wall or ceiling inlets)	_____ code	_____ code

22. Where are fans located?	☐$_1$ Sidewall ☐$_2$ End of barn ☐$_3$ Both	☐$_1$ Sidewall ☐$_2$ End of barn ☐$_3$ Both
23. Is intake air filtered?	☐$_1$ Yes ☐$_3$ No If "Yes," specify type of filter: _____	☐$_1$ Yes ☐$_3$ No If "Yes," specify type of filter: _____
	CASE BARN	**CONTROL BARN**
24. Describe ventilation protocol for the past 14 days.		
25. Which best describes the ground surface immediately surrounding (within 1 yard) this barn (excluding vehicle approach and loading area)? *[Enter Code 1-4.]* 1. Gravel or hard surface 2. Dirt 3. Short grass 4. Tall grass or brush	_____ code	_____ code
26. Does this barn have a hard surface entry pad (e.g., concrete, asphalt)?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No
If "Yes," a. Is the entry pad cleaned and how frequently?	☐$_1$ Yes, ☐$_3$ No If "Yes," specify frequency: _____	☐$_1$ Yes, ☐$_3$ No If "Yes," specify frequency: _____
b. Is disinfectant used?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No
27. Does this barn have:		
a. Locks on the doors?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No

b. A service room that personnel must enter through that separates "outside area" from "inside area"?	☐1 Yes ☐3 No	☐1 Yes ☐3 No
c. Changing area for employees	☐1 Yes ☐3 No	☐1 Yes ☐3 No
d. A shower for employees?	☐1 Yes ☐3 No	☐1 Yes ☐3 No
e. Cool cell pads?	☐1 Yes ☐3 No	☐1 Yes ☐3 No
f. Misters?	☐1 Yes ☐3 No	☐1 Yes ☐3 No
28. What type of footbath is in use at this barn? *[Enter Code 1-4.]* 1. Dry (i.e., powdered or particulate) 2. Liquid 3. Other 4. None	_____ code *If "3-Other," specify:* _____ ***If "4 – None," Skip to Question 31.***	_____ code *If "3-Other," specify:* _____ ***If "4 – None," Skip to Question 31.***
29. What is the frequency that footbath solutions are changed?	_____ times/ ☐1 day, ☐2 week, or ☐3 month	_____ times/ ☐1 day, ☐2 week, or ☐3 month
30. What disinfectant is used in the footbaths?	specify: _____	specify: _____
31. Does this barn have drop boards?	☐1 Yes ☐3 No	☐1 Yes ☐3 No
32. Is litter used in this barn?	☐1 Yes ☐3 No ***If "No," skip to Question 38.***	☐1 Yes ☐3 No ***If "No," skip to Question 38.***

33. What type(s) of litter is used in this barn? *[Enter Code 1-4.]* 1. Wood shavings 2. Hulls (e.g., oat, rice, sunflower, other) 3. Straw 4. Other	_____ code *If "4 - Other," specify:* _____	_____ code *If "4 - Other," specify:* _____
34. Is the litter bagged (i.e., bailed) or bulk (i.e., load from shavings mill)?	☐$_1$ Bag ☐$_3$ Bulk	☐$_1$ Bag ☐$_3$ Bulk
35. Who are the supplier(s)/source(s) of litter?		
36. Was litter "tilled" since it was placed in the barn?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No
If "Yes," when was it tilled?	_____ mm/dd/yy	_____ mm/dd/yy
37. How many times was litter added to the barn in the past 14 days?	_____ times	_____ times
38. When was the last full clean out of litter or manure?	_____ mm/dd/yy	_____ mm/dd/yy
39. Were birds present during the last full cleanout?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No
40. Who performed the last full cleanout? *[Enter Code 1 or 2.]* 1. Producer 2. Contractor	_____ code	_____ code
If contractor, specify name and location.	specify: n_____ l_____	specify: n_____ l_____
41. Were the following wild birds seen in this barn in the past 14 days?		
a. Large birds (e.g., pigeons, crows)	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No

b. Small birds (e.g., finches, sparrows, starlings)	☐1 Yes ☐3 No	☐1 Yes ☐3 No
42. What is the distance (in yards) of the closest body of water to this barn?	_____ yards	_____ yards
43. Were wild waterfowl observed on this body of water in the past 14 days?	☐1 Yes ☐3 No	☐1 Yes ☐3 No
44. How far is this barn (in yards) from:		
a. Dead bird disposal/holding area including carcass bin for rendering	____ yards	____ yards
b. Nearest road	____ yards	____ yards
45. Did any of the following types of people enter this barn in the past 14 days?		
a. Federal/state veterinary or animal health worker	☐1 Yes ☐3 No	☐1 Yes ☐3 No
b. Extension agent or university veterinarian	☐1 Yes ☐3 No	☐1 Yes ☐3 No
c. Private or company veterinarian	☐1 Yes ☐3 No	☐1 Yes ☐3 No
d. Company service person	☐1 Yes ☐3 No	☐1 Yes ☐3 No
e. Nutritionist or feed company consultant	☐1 Yes ☐3 No	☐1 Yes ☐3 No
f. Pullet delivery	☐1 Yes ☐3 No	☐1 Yes ☐3 No
g. Vaccination crew	☐1 Yes ☐3 No	☐1 Yes ☐3 No
h. Catch crew	☐1 Yes ☐3 No	☐1 Yes ☐3 No
i. Feed delivery personnel	☐1 Yes ☐3 No	☐1 Yes ☐3 No
j. Egg truck personnel	☐1 Yes ☐3 No	☐1 Yes ☐3 No
k. Litter services (delivery, pick-up)	☐1 Yes ☐3 No	☐1 Yes ☐3 No
l. Customer (private individual)	☐1 Yes ☐3 No	☐1 Yes ☐3 No
m. Wholesaler, buyer, or dealer	☐1 Yes ☐3 No	☐1 Yes ☐3 No
n. Renderer	☐1 Yes ☐3 No	☐1 Yes ☐3 No
o Occasional worker (e.g., family member, part time help over holiday)	☐1 Yes ☐3 No	☐1 Yes ☐3 No

p. Construction workers	☐1 Yes ☐3 No	☐1 Yes ☐3 No
q. Other business visitors (including other producers, meter readers, package delivery (UPS), repair person, wildlife services, and service personnel)	☐1 Yes ☐3 No	☐1 Yes ☐3 No
r. Other nonbusiness visitors (including neighbors, friends, and school field trips)	☐1 Yes ☐3 No	☐1 Yes ☐3 No
46. Where specifically in this barn did increased mortality or clinical signs first appear (e.g., near entry, near vents, back of barn. Diagram may help)?		NA
47. Was there a pattern of spread in the barn? If "Yes," describe.	☐1 Yes ☐3 No If "Yes," describe:_____ _____ _____	NA
48. What was the *first* indication of infection within the barn?		NA
a. Surveillance testing	☐1 Yes ☐3 No	
b. Increased mortality	☐1 Yes ☐3 No	
c. Clinical signs	☐1 Yes ☐3 No If "Yes," (specify: _____ _____ _____)	

COMMENT SECTION:

Please use this section for anything else that you would like to add. For example, how do you think HPAI is spreading within your geographic area?

CHECKLIST

INSTRUCTIONS
This section refers to data that can be acquired through other sources.

1. Please verify grayed areas from the questionnaire.

2. If possible, attach a diagram, farm map or photographs showing orientation of barn(s) including barn numbers, water location, feed storage, rendering bin, litter storage, ventilation, and windbreaks.
3. For the first infected barn, attach a diagram including proximity of initial infection to vents, doors, personnel entrances, manure storage, and other potential contributing factors.

4. How many commercial poultry farms (of any production type) are located:
    a. Within 1 mile of this farm? ..... _____ #
    b. Within 3 miles of this farm? ..... _____ #

5. How far (in yards or in miles) is the nearest backyard flock to this farm?_____ yards
    OR _____ miles

6. How far (in yards or in miles) is the nearest HPAI positive premises to this farm? ……_____ yards
    OR _____ miles

7. Inquire about truck routing. Are feed trucks, egg trucks, and live haul trucks routed in particular way? E.g., to avoid driving past a known positive farm, to avoid delivering to a known positive farm, or to visit known positive farms last? Please explain.

8. Collect mortality sheets from both case and control barns.

9. Collect ventilation control records from both case and control barns for the past 14 days.

10. Which feed mill supplies feed to this farm? _____

APPENDIX C. AEROSOL TRANSMISSION OF AVIAN INFLUENZA VIRUS IN PAST OUTBREAKS – NEW

A review of past outbreak experiences indicates that the majority of local area spread of avian influenza virus between farms can be attributed to the movement of people and equipment. Local area spread refers to mechanisms where the transmission likelihood increases with proximity to infected farms. In most outbreaks, the limited role of local area spread through mechanisms not involving movements of people and equipment indicates a limited role of aerosol spread.

Aerosol spread has been implicated in a few HPAI outbreaks. Ypma et al. (2012) estimated the contribution of a possible wind-mediated mechanism to the total amount of spread during the 2003 H7N7 HPAI virus outbreak in the Netherlands to be approximately 18% (Ypma et al., 2012). This estimate was based on the observed correlation between the wind direction and the direction of the spread of disease, estimated through phylogenetic and epidemiological data. The statistical analysis also accounted for the possibility that the direction of spread coincided with the wind direction by chance.

Aerosol transmission between poultry barns that were in close proximity was suspected as a possible means of spread in the 2004 H7N3 HPAI virus outbreak in British Columbia. In this outbreak, there were anecdotal reports that some of the infected farms were in close proximity and downwind of other infected flocks. Some anecdotal reports of aerosol transmission were associated with depopulation methods used early in the outbreak (IICA, 2005). Although suspected, there is no conclusive evidence that aerosol transmission played a major role in this outbreak.

Activities that can generate avian influenza virus infected dust or aerosols

Activities that can generate avian influenza virus infected dust or aerosols in very close proximity to susceptible poultry have also been implicated as a transmission mechanism.

Live haul

Trucking poultry actively infected with avian influenza virus along a public roadway resulted in aerosol spread to flocks located within 200 meters (219 yards) of the road (Dave Halvorson, personal communication; Brugh and Johnson, 1986).

Depopulation

Depopulation activities up to 366 meters (400 yards) upwind from a susceptible flock can represent a risk for aerosol transmission (Dave Halvorson, personal communication). In an H7N2 LPAI virus outbreak in Pennsylvania, aerosols generated by stirring up organic materials during depopulation was considered to be a potential mechanism of spread to farms within 1.61 to 2.01 kilometers (1 to 1.25 miles; Henzler et al., 2003). Depopulation methods used early in the 2004 H7N3 HPAI virus outbreak in Canada such as grinding carcasses outside the barn, and bringing birds outside the barn for depopulation were implicated in spread of HPAI virus to neighboring farms (IICA, 2005).

Manure handling

Spreading of non-composted contaminated litter on adjacent fields was suspected as a transmission mechanism during the 1983 H5N2 HPAI virus outbreak in Pennsylvania (Dave Halvorson, personal communication; Brugh and Johnson, 1986). Spreading of non-composted manure from infected farms approximately 2 kilometers (1.25 miles) from susceptible poultry was suspected to have

resulted in transmission in one instance during an H7N2 LPAI virus outbreak in Pennsylvania (Henzler et al., 2003).

Aerosol sampling studies on or near avian influenza virus infected farms

Only a couple of studies have reported air-sampling results from or around HPAI virus infected poultry houses during previous outbreaks. These studies demonstrate the effect of dilution on aerosol concentration with increasing distance from the generating source.

High volume air sampling was conducted in and near an infected layer flock where birds experienced high mortality during the H7N7 HPAI virus outbreak in Canada (Schofield et al., 2005). Inside the barn, a viral titer of 292 $TCID_{50}/m^3$ was detected in air samples.[4] Air sampling at a command post outside the barn showed a much lower viral load of 12.5 $TCID/m^3$ based on quantitative PCR. However, no viable virus was recovered. Low concentration and inactivation of virus by sunlight was hypothesized as a possible explanation for the apparent absence of viable virus in these samples.

In the 1983 H5N2 HPAI virus outbreak in Pennsylvania, 5 of 6 samples taken 3 to 6 meters downwind of affected flocks on 6 farms were virus-positive, whereas only 1 of 12 samples taken 45 to 85 meters downwind of affected flocks on 8 farms was virus-positive. The positive sample was taken 45 meters downwind (Brugh and Johnson, 1986).

The previous USDA epidemiology investigation report described the results of air and environmental sampling of three turkey flocks located in Minnesota and three layer flocks located in Iowa and Nebraska. Air samples were collected inside and immediately outside (5 meters) of affected barns, and at extended distances ranging from approximately 70 to 1,000 meters downwind from the barns. Five of the six flocks had at least one air sample test positive (Epidemiologic and Other Analyses of HPAI-Affected Poultry Flocks: July 15, 2015 Report, available at: http://www.aphis.usda.gov/animal_health/animal_dis_spec/poultry/downloads/Epidemiologic-Analysis-July-15-2015.pdf).

Experimental laboratory studies on avian influenza virus aerosol transmission

Several experimental studies indicate that airborne transmission of HPAI virus between turkeys and chickens in adjacent pens or cages is possible but inefficient. These studies also suggest that aerosols may not be the primary route of transmission within a flock.

In several studies, aerosol transmission of avian influenza virus was not observed between groups of inoculated and susceptible chickens housed in adjacent cages or chambers with direct airflow (Forman et al., 1986; Shortridge et al., 1998; Perdue et al., 2000; van der Goot et al., 2003).

A few studies showed inefficient transmission or low transmission of avian influenza virus between groups of inoculated and susceptible chickens housed in adjacent cages or chambers with direct airflow. LPAI Turkey/Wis/66 virus was transmitted via aerosol between groups of 400 turkeys in different compartments of a building (Homme et al., 1970). In this experiment, influenza virus was transmitted to one out of three exposed groups of turkeys in different compartments. Airborne transmission of H5N1 HPAI virus occurred inefficiently when one to two chickens were infected, but efficiently when four to eight chickens were infected (Tsukamoto et al., 2007).

Experimental studies indicate that variability between strains can impact transmissibility via aerosols. For example, Zhong et al. (2014) found different strains of H9N2 LPAI virus to have

[4] $TCID_{50}$ refers to the 50% tissue culture infectious dose. The MDCK cell line was used for the tissue culture.

markedly different aerosol transmissibility between chickens (Zhong et al., 2014). The study proposed that HA and PA genes are important in determining aerosol transmissibility. Several recent studies indicated efficient transmission of H5N1 HPAI virus and H9N2 LPAI virus to chickens by aerosols that were mechanically generated by nebulizing stock fluid containing virus to very small particle sizes (2 to 5 µm; Guan et al., 2013; Sergeev et al., 2013). Several studies have found that Influenza A viruses experience decreased survivability in aerosols at higher temperature and higher relative humidity (Tellier, 2006; Weber and Stilianakis, 2008).

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For more information, contact:

Brian J. McCluskey, DVM, MS, PhD, Dip. ACVPM
USDA, APHIS, Veterinary Services
Fort Collins, CO
970-494-7184 email: Brian.J.Mccluskey@aphis.usda.gov