United States
Department of
Agriculture

Animal and Plant
Health Inspection
Service

Veterinary Services

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

Contents

EXECUTIVE SUMMARY - UPDATED

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 the report released by APHIS on June 5, 2015.

With the data from both 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 edition of the report 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.

In a case series investigating 81 turkey farms across the Midwestern United States, we found 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.

Fomites, such as equipment, are probably playing a role in this outbreak. In the majority of cases in this study, feed trucks, live haul loaders, pre-loaders, and other items were shared by multiple farms. While equipment sharing makes economical and logistical sense, it also increases the risk of lateral spread of HPAI between farms. Wild birds, another possible route of disease transmission, were observed inside barns on 35 percent of the farms, with the frequency ranging from daily to occasionally.

While most of the 81 farms surveyed had biosecurity protocols in place, 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 a 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 preliminary analysis. Factors associated with an increased risk of becoming infected with HPAI included being located within one of the 10-kilometer control zones; using rendering of dead birds as a disposal method; sharing of company trucks, trailers, bird removal and egg removal vehicles; sharing of equipment between farms like egg rack, pallets and flats; and visits by company service personnel who entered barns. Factors associated with a lowered risk of infection included being more than 100 yards from a public gravel or dirt road, having wash stations for vehicles on the farm, and being more than 100 miles from the egg processing facility used by the farm.

Also in this edition are preliminary results of a study of wildlife near affected and unaffected premises. Testing is ongoing on the over 2,600 samples collected.

APHIS will continue to investigate how the HPAI virus is introduced and spread 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 and the June 5 report, which included wind and airborne virus studies as possible causes of viral spread as well as a genetic analysis of the viruses detected in the United States, 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 in an effort to provide producers, industry, and other stakeholders tangible and effective ways to mitigate initial introduction of HPAI viruses into commercial poultry operations and transmission of virus between operations.

I. FIELD-BASED OBSERVATIONAL STUDIES

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

Background and Summary

This case series describes 81 turkey farms in IA, MN, ND, SD, and WI 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

A survey instrument (Appendix I) continues to be administered by State and Federal animal health officials in multiple States affected by highly pathogenic avian influenza virus strain H5N2 (HPAI-H5N2). 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-Veterinary Services (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

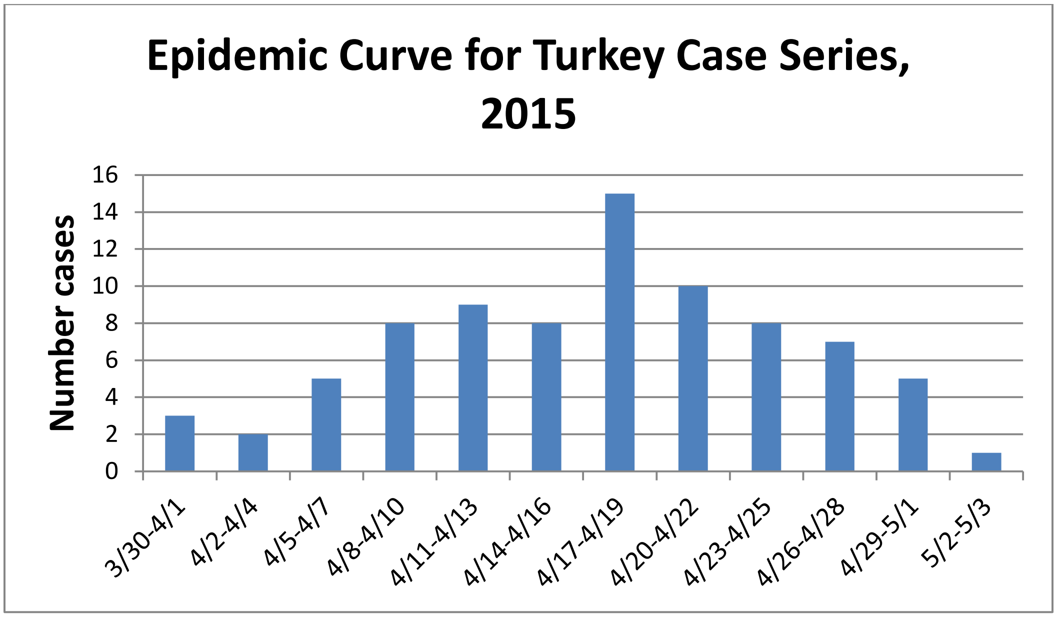

Figure 1. Epidemic curve for turkey case series farms

Bird Age

None of the case farms (that provided bird age information) had birds less 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*

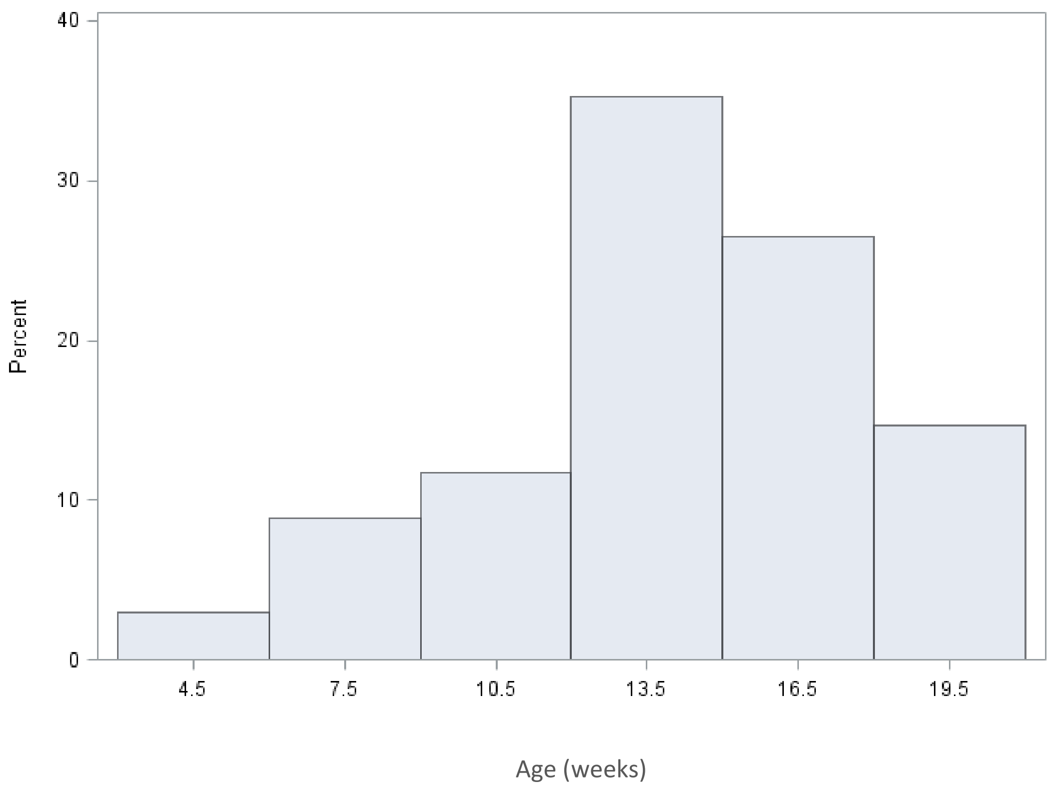

Figure 2. Histogram of bird age at time of outbreak on tom farms

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
	68	Poultry trailers (h616)	31

Equipment	Number Respondents	Level or Response	Percent Farms
	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 NAHMS 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 time frame (Table 14). 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 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.

References

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B. Case-Control Study: Layers – Farm and Barn Level Results - NEW

Background

A case-control study for HPAI was conducted among layer and pullet operations in Iowa to investigate potential risk factors for initial introduction of HPAI virus onto poultry farms and the transmission of HPAI virus between farms.

Data Collection and Management

The study included all detected case farms as of May 15, 2015, in Iowa or Nebraska. Control farms were recruited from the surrounding geographic area for each case farm. Contact information for case farms was obtained from VS-Emergency Management Response System. A 28-page questionnaire was sent to each participating farm with a follow-up interview conducted in person or via telephone. The questionnaire focused on the 2-week period leading up to the detection of disease on a case farm (either via clinical signs/increased mortality or through active surveillance). This 2-week period was defined as the reference period. Participants with control farms responded for the reference period of the matched case survey.

All interviews were conducted by USDA and Iowa State University epidemiologists. Surveys were reviewed before data entry and responses validated for error detection prior to analysis with SAS software.

Statistical Methods

Two univariate case-control analyses were performed for this preliminary report. The first was a farm-level comparison of case farms versus control farms; the second was a barn-level study, comparing case barns on case farms with control barns on control farms.

The percent case farms/barns and percent control farms/barns having each characteristic were calculated. A univariate analysis was performed to identify variables potentially associated with presence of HPAI infection at the farm or barn level at the time of data collection. A χ-square test was used to evaluate the statistical significance of the association between an independent variable and the farm's or barn's infection status.

For categorical predictor variables, when any cell had an expected value <5, the Fisher's exact test *p*-value is reported. Odds ratios are reported for predictor variables with two levels, with at least one observation in each cell. Relative risk (RR) estimates are reported for predictor variables with cells containing 0 observations. (When the control group contained a count of 0 in a cell for either risk level of a factor, "RR-1" is the relative risk of being a case farm/barn; likewise, when the case group contained a count of 0 in a cell, "RR-2" is the RR of being a control farm/barn.) Variables with a *p*-value ≤ 0.20 will be considered for entry into multivariable models. Therefore, odds ratios (and relative risk) are reported for variables with $p \leq 0.20$ and those with $p \leq 0.10$ are in boldface. Matched case-control statistical analyses were not performed.

Results

Respondents representing 26 case farms participated in the study, with a set of 33 controls selected within a defined time period relative to case farms. Interviews were conducted from May 14 to June 3, 2015.

Farm Level

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 9% of control farms (OR=10.0, p=.0005). Corn on a nearby field was a risk factor (OR=3.6, p=.04). Farm location ≥100 yards from a public gravel or dirt road was associated with lower risk (i.e., "protective").

APHIS gathered data on a wide range of farm characteristics (Tables 16-23). Rendering dead birds was a risk factor: 42% of case farms (compared to 25% of control farms) used rendering to dispose of dead birds. Forty-two percent (42%) of case farms (compared to 12% of control farms) reported that the renderer comes to the farm. Additionally, 31% of case farms (and only 3% of control farms) reported that rendering trucks came near the barns. In the 14-day period prior to HPAI virus detection, respondents at 27% of case farms (and only 9% of control farms) reported their renderer coming to the farm.

Although a similar percentage of case and control farms reported that garbage trucks come to the farm, 61% of case farms (compared to 27% of control farms) reported that the garbage trucks come near the barns.

Visits in the 14-day period prior to detection by a company service person were associated with farm status: 50% of case farms (compared to 18% of control farms) had a company service person visit (OR=4.5, p=.009). Additionally, 42% of case farms (compared to 15% of control farms) reported that the service person entered the barn.

Sharing vehicles with other farms was associated with farm status; in particular, sharing company trucks/trailer, bird-removal, and egg-removal vehicles was associated with a higher risk. Sharing equipment with other farms was also associated with higher risk; in particular, sharing egg racks or pallets, egg flats, pressure sprayer/washer and skid steer loader.

Presence of wild birds on or around the farm was inconsistent as to association with farm status. For example, in many cases, control farms were more likely to see birds daily and also more likely to never see birds, while case farms saw birds less than daily. Beetle, fly, and rodent control all were negatively associated with farm status (i.e., were protective). Other protective factors include hand washing, use of hand sanitizer or gloves, use of a visitor log, requiring visitors to not visit multiple farms on the same day, and being ≥100 miles from the egg processing plant.

Barn Level

Several factors were statistically significant (at the $p \leq 0.05$ level) in the case-control barn level analysis. Operational characteristics such as disinfection in addition to cleaning were associated with a lower risk of infection when hard surface entry pads were present (OR=0.29, p=0.04). Having a shower for employees to use was associated with a higher risk of barn infection (OR=3.64, p=0.02); however, when case and control barns were compared with requirements for employees to shower, the significance of the association declined. Having a company service person enter a barn was also associated with a higher risk (OR=3.30, p=0.04).

Many factors were less strongly associated with infection risk, i.e., those with *p*-values ranging 0.05-0.20. Disposing of dead birds at a greater distance (>30 yards from the barn) was considered protective. Having a catch crew in a barn was associated with lower risk. Vaccination in the past 14 days was mildly protective, as was having a vaccination crew in the barn. The type of footbath showed a mixture of associations: control barns (21%) were more likely than case barns (3.8%) to report not having a footbath, and the percentages of case and control barns using dry or liquid footbaths were quite similar.

Structural facility factors were also considered. Case farms tended to have a higher percentage of conventional cage housing type compared with enriched cages or cage free. Barns more than 10 years old appeared to be at slightly lower risk. Barn maintenance (well, moderate, or poor) showed mixed results, with slightly higher percentages of case farms being maintained well or poor, compared with moderately maintained barns. Ventilation type also had mixed results, with ceiling/eaves inlet associated with lower percentages of case farms, and tunnel inlets the highest percentage of case farms. Having locks on doors tended to be protective, and having a changing area tended to be associated with higher risk.

Interpretation

The univariate results contained herein are the first step in the analysis of case-control data. Significant independent variables at this level of analysis serve as candidates for further study in more complex models that may more specifically identify risk or protective factors. They may also be considered as candidates for generating hypotheses for further study or discussion.

Limitations and Next Steps

The analysis presented herein is a preliminary screening of all variables. With this large number of variables, many may appear significant by chance alone, and some may be significant in the opposite direction of biologic sense. In addition, a small number of new completed surveys have been received since this analysis was prepared, and those will be included in multivariable modeling. A p-value ≤ 0.20 is the criteria for selecting variables for introduction into candidate multivariable models. Because the sample size is relatively small, the ability to detect statistical significance within the multivariable model at the $p \leq 0.05$ level will be limited, and we anticipate that few independent variables will remain in the model. Increasing the p-value cutoff to 0.10 for statistical significance may allow us to consider other important risk factors in the presence of smaller sample sizes.

In Tables 15-24, **bolded variables** have $p<0.10$.

Farm Level Univariate Analysis

Table 15. Percent case farms and percent control farms by premises characteristics*

Factor	Level*	Percent case farms	Percent control farms	OR	P-value
In an existing control zone		**50**	**9**	**10.0**	**.0005**
NPIP		92	100	.42(RR)	.19
Housing	**Cage-free not organic**	**0**	**16**	**2.0(RR)**	**.06**
Distance to closest water	>=500 yds	54	33	2.3	.11
Number of wild water fowl	None	45	42		.18
	Tens	45	58		
	Hundreds	9	0		
	Thousands	0	0		
Water body types within 350 yards	Pond	15	34	.35	.10
Closest field where crops are harvested	<350 yards	46	58		.38
Crop type	**Corn**	**85**	**61**	**3.6**	**.15/.04**
(2nd p-value and OR for corn –YN)	Soybeans	15	27		
	Alfalfa/grass	0	6		
	Other	0	6		
Waterfowl type in field	Ducks	15	6		.39
	Geese	35	24		.40
	Shorebirds	**15**	**0**	**2.5(RR)**	**.03**
	Other	8	0	2.4(RR)	.19
Water treatments	**Acidifiers**	**27**	**6**	**5.7**	**.04**
Public gravel or dirt road	>=100 yards	35	55	.44	.13

**yes/no variables reference level=no*

Table 16. Percent case farms and percent control farms by biosecurity characteristics

Factor	Level*	Percent case farms	Percent control farms	OR	P-value
Vehicles:					
Garbage/dumpster	**Come to perimeter**	**0**	**36**	**4.3****	**.008****
	Enter farm but not near barns	**23**	**24**		
	Come near barns	**61**	**27**		
	Do not come	**15**	**12**		
Renderer	**Come to perimeter**	**0**	**3**	**14.2****	**.007****
	Enter farm but not near barns	**12**	**6**		
	Come near barns	**31**	**3**		
	Do not come	**58**	**88**		
*** p-value and OR for "comes near barn" Yes/No*					
Perimeter security fence		15	3	5.8	.16
Frequency vegetation mowed	**4+ times per month**	**88**	**66**	**4.0**	**.04**
Wash station used for vehicles	**Located on farm**	**52**	**79**	**.29**	**.03**
	Interior cleaned	**8**	**27**	**.23**	**.09**
Rat/mouse bait stations	Checked 4+/month	72	58		.20
	Checked < 4/month	24	42		
	none	4	0		
Beetle control	Any	15	31	.40	.16
Fly control	Any	69	85	.40	.15
	Larvacide (spot)	19	6	3.7	.12
	Larvacide (feed)	11	3		.20
	Space spray/fogger	35	55	.44	.13
	Biologic predators	15	24		.40
Rodent severity	**High**	**0**	**0**	**7.2**	**.007/.003**
(2nd p-value and OR for any–YN)	**Moderate**	**4**	**6**		
	Low	**85**	**45**		

Factor	Level*	Percent case farms	Percent control farms	OR	P-value
	None	**12**	**48**		
Rodent index	**Low**	**77**	**94**		**.02**
	Moderate	**12**	**0**		
	High	**0**	**6**		
	Not monitored	**12**	**0**		
Wild mammals seen		**15**	**42**	**.25**	**.03**
Access to poultry feed:					
Wild birds	Always/nearly always	0	3		.19
	Most of the time	12	0		
	Sometimes	31	36		
	Never	58	61		
Feed treatment	Formaldehyde	44	25	2.4	.13

Table 17. Percent case farms and percent control farms by wild bird characteristics

Factor	Level*	Percent case farms	Percent control farms	OR	P-value
Bird type on adjacent habitat:					
Gulls	Daily	0	3		.17
	Less than daily	38	18		
	Never	61	79		
Wild turkeys, pheasants, quail	Daily	0	15		.10
	Less than daily	58	55		
	Never	42	30		
Raptors	Daily	0	15		.11
	Less than daily	50	45		
	Never	50	39		
Wild waterfowl use this area at other times of year		**38**	**63**		**.07**
Bird type outside barn:					
Blackbirds/crows	Daily	38	55		.12
	Less than daily	50	24		
	Never	12	21		

Factor	Level*	Percent case farms	Percent control farms	OR	P-value
Wild turkeys, pheasants, quail	Daily	0	0	.36	.11
	Less than daily	15	33		
	Never	85	67		
Bird type in barns:					
Large birds (pigeons, crows)	Daily	0	0	2.4(RR)	.19
	Less than daily	8	0		
	Never	92	100		

Table 18. Percent case farms and percent control farms by farm help/worker characteristics

Factor	Level*	Percent case farms	Percent control farms	OR	P-value
Shower	Always/nearly always	38	15		.11
	Most of the time	4	0		
	Sometimes	4	3		
	Never	54	82		
Wash hands	Always/nearly always	58	70		.11
	Most of the time	15	18		
	Sometimes	0	6		
	Never	27	6		
Change of clothing (washable)	**Always/nearly always**	**77**	**45**		**.08**
	Most of the time	**4**	**3**		
	Sometimes	**0**	**3**		
	Never	**19**	**48**		

Table 19. Percent case farms and percent control farms by visitor characteristics

Factor	Level*	Percent case farms	Percent control farms	OR**	P-value**
Company service person	**Entered poultry barn**	**42**	**15**	**4.5**	**.03/.009**
	Did not enter barn	**8**	**3**		
	Did not visit	**50**	**82**		
Litter services (delivery/pickup)	Entered poultry barn	0	3		.18/.12
	Did not enter barn	0	9		
	Did not visit	100	88		
Renderer	**Entered poultry barn**	**4**	**0**	**3.7**	**.16/.09**
	Did not enter barn	**23**	**9**		
	Did not visit	**73**	**91**		
Visitor log used		**81**	**97**	**.13**	**.08**
Visitor requirements:					
Mask	**Always/nearly always**	**50**	**41**		**.06**
	Sometimes	**17**	**45**		
	Never	**33**	**14**		
Hand sanitizer or gloves	**Always/nearly always**	**75**	**83**		**.07**
	Sometimes	**0**	**10**		
	Never	**25**	**7**		
Not visit multiple farms same day	Always/nearly always	63	86		.16
	Sometimes	29	11		
	Never	8	4		

***second p-value for visitor yes-no*

Table 20. Percent Case Farms and Percent Control Farms by Vehicle and Equipment Characteristics

Factor	Level*	Percent case farms	Percent control farms	OR**	P-value**
Vehicle present in past 14 days:					
Company truck/trailer	**Shared – not disinfected**	**12**	**6**	**2.6**	**.14/.08**
	Shared – disinfected	**46**	**28**		
	Not shared	**23**	**53**		
	Not present	**19**	**13**		
Bird removal	**Shared – not disinfected**	**0**	**0**	**0.3**	**.21/.08**
	Shared – disinfected	**12**	**30**		
	Not shared	**12**	**12**		
	Not present	**77**	**58**		
Egg removal	**Shared – not disinfected**	**24**	**0**		**.04/.24**
	Shared – disinfected	**32**	**40**		
	Not shared	**8**	**13**		
	Not present	**36**	**47**		
Equipment present in past 14 days:					
Lawn mowers	**Shared – not disinfected**	**12**	**6**		**.06/.43**
	Shared – disinfected	**8**	**22**		
	Not shared	**46**	**63**		
	Not present	**35**	**9**		
Egg racks or pallets	Shared – not disinfected	8	0	3.5	.26/.16
	Shared – disinfected	20	10		
	Not shared	20	30		
	Not present	52	60		
Egg flats	Shared – not disinfected	4	0	2.5	.48/.18
	Shared – disinfected	24	13		
	Not shared	20	27		
	Not present	52	60		
Pressure sprayer/washer	Shared – not disinfected	0	0		.11/1.0

Factor	Level*	Percent case farms	Percent control farms	OR**	P-value**
	Shared – disinfected	8	9		
	Not shared	23	48		
	Not present	69	42		
Skid steer loader	**Shared – not disinfected**	**0**	**0**	**2.4(RR)**	**.02/.08**
	Shared - disinfected	**12**	**0**		
	Not shared	**38**	**70**		
	Not present	**50**	**30**		
Manure handling	**Shared – not disinfected**	**0**	**0**		**.08/.32**
	Shared - disinfected	**12**	**3**		
	Not shared	**12**	**34**		
	Not present	**77**	**63**		

*** second p-value and OR are for shared – yes/no*

Table 21. Percent case farms and percent control farms by egg handling characteristics (excludes pullet farms)

Factor	Level*	Percent case farms	Percent control farms	OR	P-value
100+ miles to processing plant (n=21)		**45**	**90**	**.09**	**.06**

Table 22. Percent Case Farms and Percent Control Farms by Litter and Manure Handling Characteristics

Factor	Level*	Percent case farms	Percent control farms	OR	P-value
Litter bedding used		0	13	1.9(RR)	.12
Manure stored on farm	Any	46	63		.20
	Open structure	8	37	.14	.10

Table 23. Percent Case Farms and Percent Control Farms by Dead Bird Disposal Characteristics

Factor	Level*	Percent case farms	Percent control farms	OR	P-value
Disposal methods	Render	42	25	3.2	.16

Barn Level Univariate Analysis

Table 24. Percent case barns (on case farms) and percent control barns (on control farms) by barn level factors

Factor	Level	Percent case barns (n=26)	Percent control barns (control farms) (n=33)	OR (95% CI)	P-value
Vaccinated past 14 d		11.5	29.0	0.32 (0.08,1.33)	0.19
Housing	Conventional cage	100	87.9		0.18
	Enriched cage	0	3.0		
	Cage free	0	9.1		
Barn 10+ years old		69.2	84.8	0.40 (0.11, 1.42)	0.15
Barn maintenance	Well	80.8	66.7		0.17
	Moderate	15.4	33.3		
	Poor	3.8	0		
Ventilation	Curtain	0	0		0.17
	Sidewall inlet	38.5	24.2		
	Ceiling or eaves inlet	46.2	69.7		
	Tunnel	15.4	6.1		
Hard surface entry pad	**No hard surface entry**	**19.2**	**18.2**		
	Yes – cleaned and disinfected (for those with hard surface entry pad)	**36.4**	**66.7**	**0.29 (0.08, 0.96)**	**0.04**
	Yes – cleaned (for those with hard surface entry pad)	**63.6**	**33.3**		
Locks on doors		**88.5**	**100**	**0.41 (RR-1)**	**0.08**
Changing area		76.9	57.6	2.45 (0.78,7.71)	0.12
Shower		**57.7**	**27.3**	**3.64 (1.22, 10.84)**	**0.02**
Footbath	Dry	50.0	48.5		0.14
	Liquid	26.9	24.2		
	Other (mostly both dry and liquid)	19.2	6.1		
	None	3.8	21.2		

Factor	Level	Percent case barns (n=26)	Percent control barns (control farms) (n=33)	OR (95% CI)	P-value
Dead bird disposal >= 30 yds		**38.5**	**63.6**	**0.36 (0.12, 1.03)**	**0.054**
People entered barn:					
Company service person		**42.3**	**18.2**	**3.30 (1.02,10.72)**	**0.04**
Pullet delivery		0	12.1	1.9 (RR-2)	0.12
Vaccination crew		3.8	18.2	0.18 (0.02,1.61)	0.12
Catch crew		**0**	**15.2**	**1.93 (RR-2)**	**0.06**

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

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 25) 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 26). 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 27).

Table 25. 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 26. 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.

Topical Area	Themes
	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 27. 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	Wild Bird Attractants	Perceived 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/

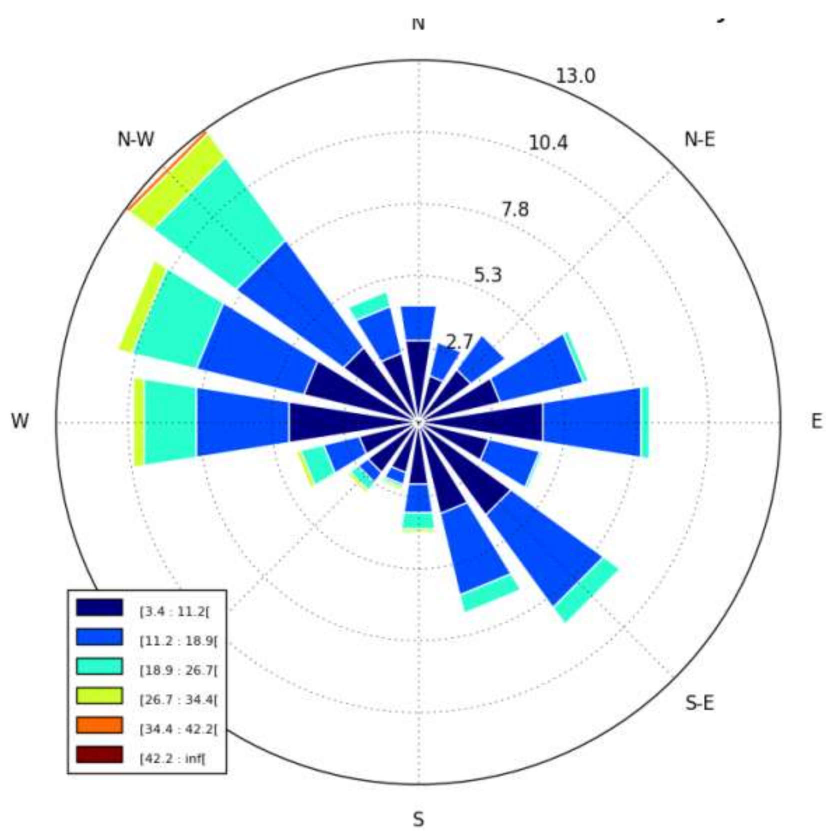

Figure 3. Wind Rose Minnesota: Combined BDH D39 LJF PEX

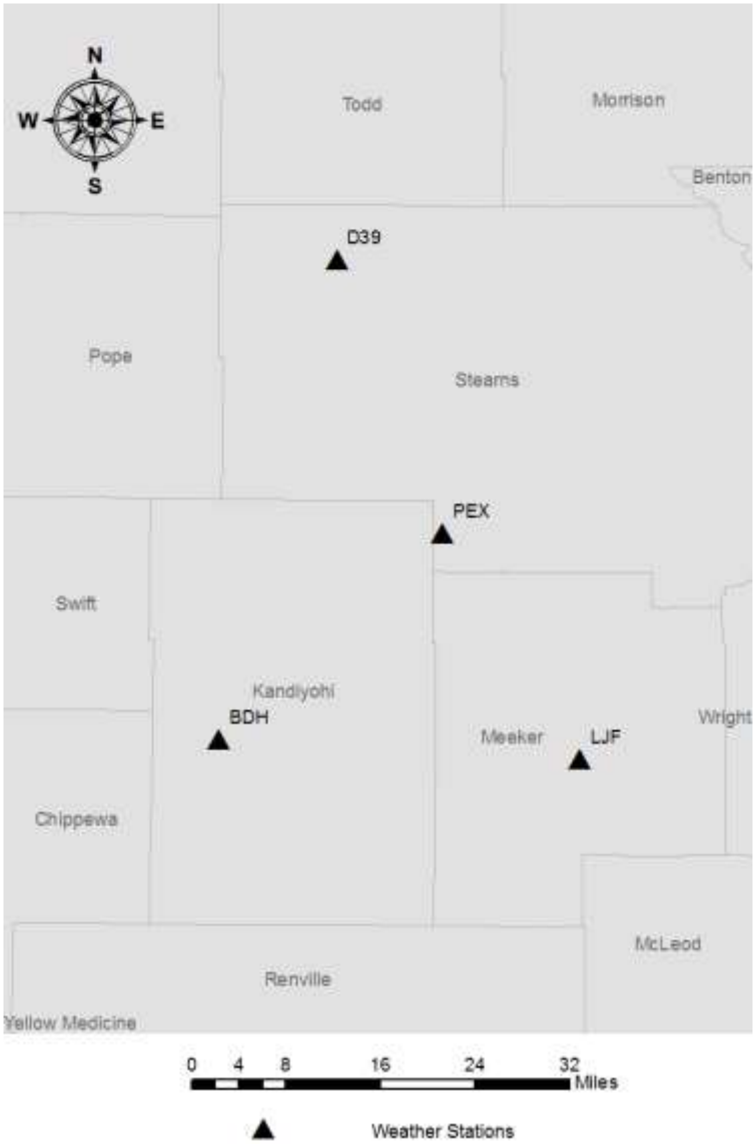

Figure 4. Location of weather stations used to create wind rose and resulting wind rose integrating data from all four stations.

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 premise status represents the date premises were confirmed positive by NVSL and these dates were used for ClusterSeer analysis. The selected set of 35 cases were exported from ArcGIS as a text file and then prepared for input to ClusterSeer.

Results

Based on the ClusterSeer directional test, subsequent cases typically occurred in the southwest direction (221.288 degrees) to previous cases (Figure 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.

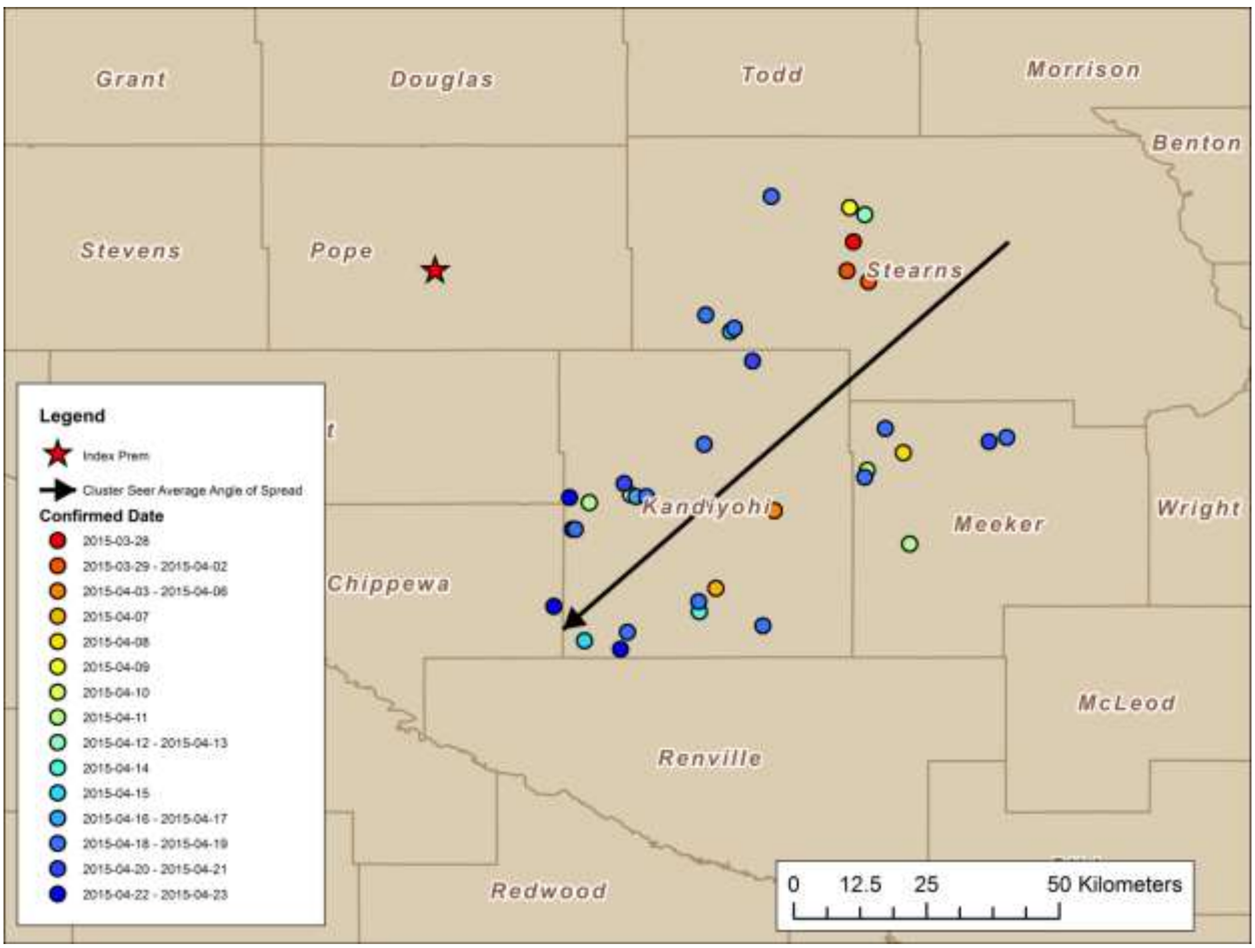

Figure 5. Positive premises used in ClusterSeer analysis and direction of spread as reported by ClusterSeer.

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.

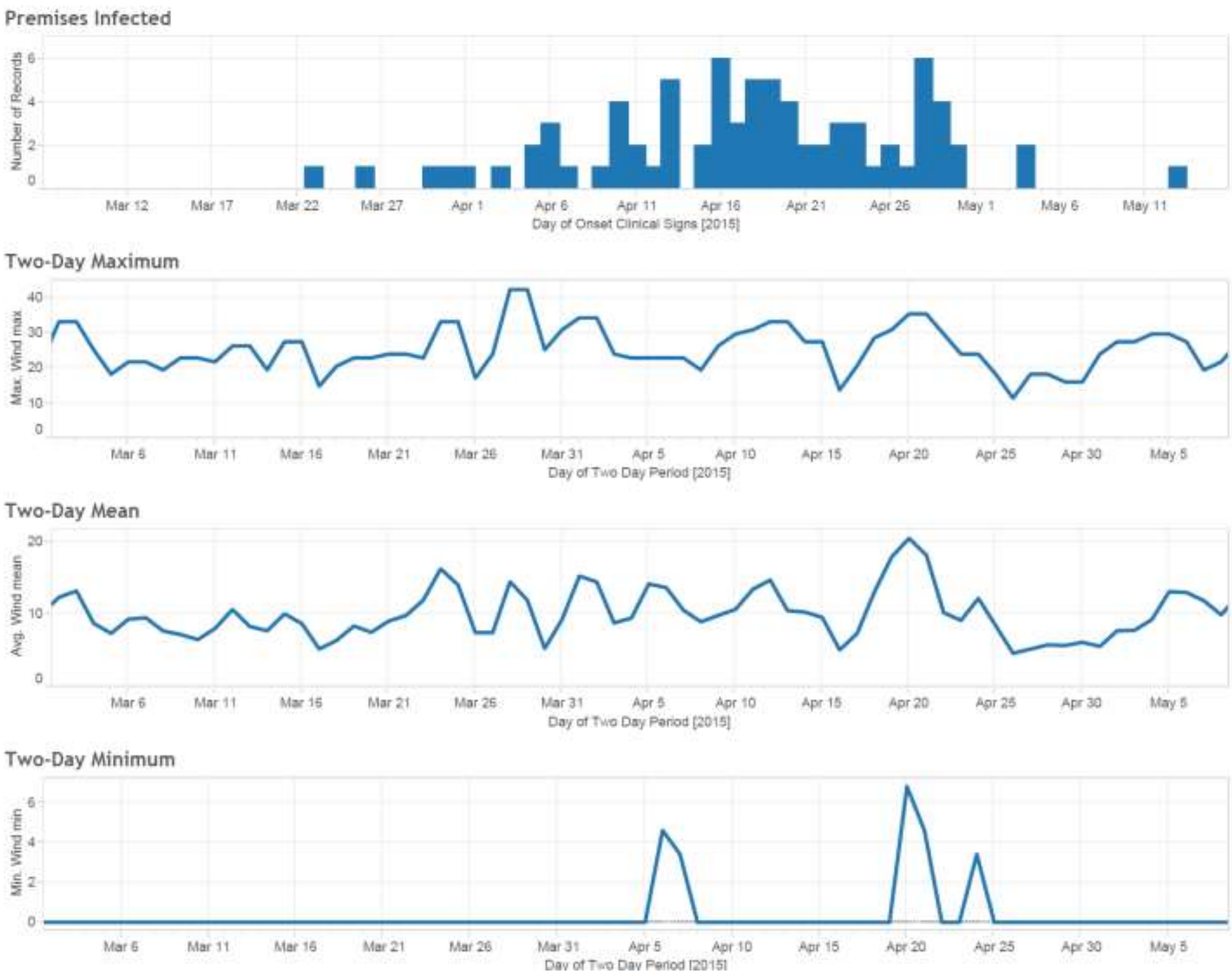

Figure 6. Associations between wind speed and clusters of HPAI cases in Kandiyohi and Stearns Counties, Minnesota

Limitations

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

II. ON-FARM SAMPLING

A. Detection of HPAI Virus in Air at Affected Premises

Objective

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

Materials and methods

Affected Flocks

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

Sampling Procedures

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

Environmental samples were also collected from surfaces in locations at high risk of direct exposure to the air exhausted from layer flocks. 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. Ct values < 35 were considered positive, 35-40 suspect, and >40 negative. To assess the infectivity of RT-PCR positive and suspect air samples, virus isolation in embryonated eggs was attempted at the National Veterinary Services Laboratories 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 28). 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 29. 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 7). 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 30). In the latter flock, Ct values ranging between 29 and 32 indicated relatively high amounts of HPAI RNA on the surfaces of farm fixtures and temporary fomites exposed for 60 minutes.

Conclusions

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

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

Acknowledgements

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

Table 28. 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 29. 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 30. 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*				

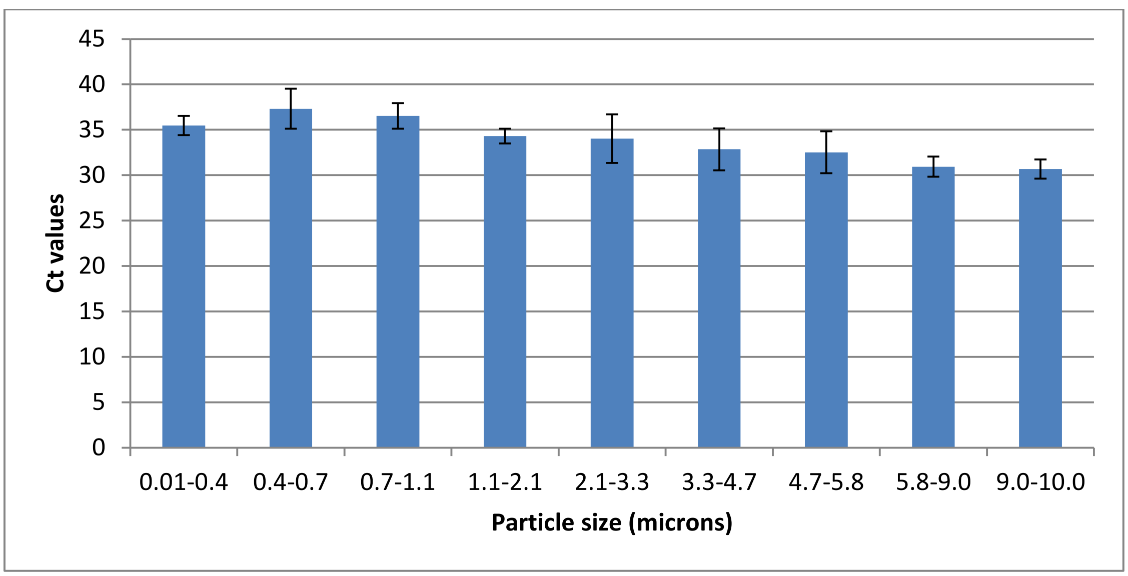

Figure 7. Average RT-PCR cycle threshold (Ct) values by particle size of air samples collected inside and immediately outside of turkey and layer flocks using the Anderson Cascade Impactor. Ct <35: positive; Ct 35-40: suspect; Ct >40 negative.

B. Sampling for HPAI Virus in Synanthropic Wildlife at Affected and Unaffected Premises - NEW

Objective

In order 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 31). 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 31. 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, IAVs, (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), a blood sample and lung tissue were also collected. For mammals, an oral swab, nasal swab/wash, and external swab were collected. For targeted species (e.g., mice), a blood sample and lung and/or trachea tissue samples were also collected. Further, any observed aberrant tissue was also collected (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. Samples were shipped overnight on ice to testing laboratories within 24 hours during the week or stored in a refrigerator and then shipped overnight on ice.

Laboratory Procedures

Laboratory testing is still ongoing. Swabs, washes, and tissue samples are being tested for influenza A virus (IAV) matrix gene RNA via RT-PCR. Avian oral and cloacal swabs are being tested at the Avian Veterinary Diagnostic Laboratory at Colorado State University. All other samples tested via matrix RT-PCR are being tested at the National Wildlife Research Center Virology Laboratory. The National Animal Health Laboratory Network (NAHLN) protocol considers any sample with a cycle threshold (Ct) value <40 as positive. Sample testing as positive for matrix gene RNA are being submitted to the USDA's NVSL in Ames, Iowa, to be screened against H5 primers. Any H5 positives will be tested for infectivity by virus isolation (VI) in embryonated chicken eggs. The pathogenicity of any positive VI sample will be characterized by cleavage site analysis. All serum samples will be submitted to NVSL for hemagglutinin inhibition assay testing against the Eurasian H5 icA as the antigen. Any confirmed positive samples will also be screened for N2 via neuraminidase inhibition assay.

Results

Across the 10 sampled farms (5 infected, 5 uninfected), we collected 2,627 samples from 426 individuals (Table 32). 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 33). On infected farms, we sampled 220 individual birds across 17 species and on uninfected farms we sampled 199 individuals across 18 species (Table 34). House sparrows, European starlings, Rock pigeons, swallow, and American robins were the most commonly sampled bird species.

Table 32. 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 33. 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 34. 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

Laboratory testing is ongoing. None of the serum samples have been tested for IAV exposure to date, but preliminary results are expected within 4 weeks. All RT-PCR results are preliminary and have not been confirmed. To date, 77% of the avian samples from infected farms have been tested for IAV matrix gene RNA. None of the avian tissues from infected farms have been tested. Of the 517 screened oral, cloacal, and external swabs, one oral swab, one cloacal swab, and one external swab (from three individual birds) from infected premises have tested positive. The oral and cloacal swabs were tested against H5 primers and were negative. The external swab has been submitted for further testing.

All oral and cloacal swabs from birds sampled on uninfected farms have been screened for IAV matrix gene RNA and were negative. One hundred eighty-three of 197 external swabs have been tested. One sample tested positive and has been submitted for further testing; 28/155 tissue samples have been tested and were negative.

For mammals on infected farms, the 26 external swabs that were collected have been tested for matrix RNA and were negative. 18/206 tissue samples, 163/188 nasal samples, 162/188 oral swabs, and 28/155 tissue samples have been tested and were negative.

For mammals on uninfected farms, 7/7 external swabs, 38/39 nasal samples, 36/38 oral swabs, and 38/39 tissue samples were tested for matrix RNA and were negative.

Interpretation and Limitations

These results are preliminary, and limitations should be recognized. Sampling at infected farms occurred 2-4 weeks after clinical signs appeared in poultry and depopulation was complete or ongoing on each of the infected farms. Therefore, the likelihood of detecting viral RNA is lower than if premises had been sampled while poultry were actively infected. Consequently, negative results should be interpreted with caution and do not necessarily imply that the sampled wildlife species do not pose a biosecurity threat. A second caveat is that the RT-PCR assay for the H5 subtype is not as sensitive as the matrix gene assay such that a negative H5 test for samples positive for matrix RNA does not conclusively exclude H5 as the infection subtype. Stronger inferences should be possible once laboratory analyses are complete and confirmed.

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

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

The 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. The whole genome sequence is used to monitor the virus evolution and assess risk to veterinary or public health based upon presence/absence of specific amino acid substitutions or protein motifs.

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

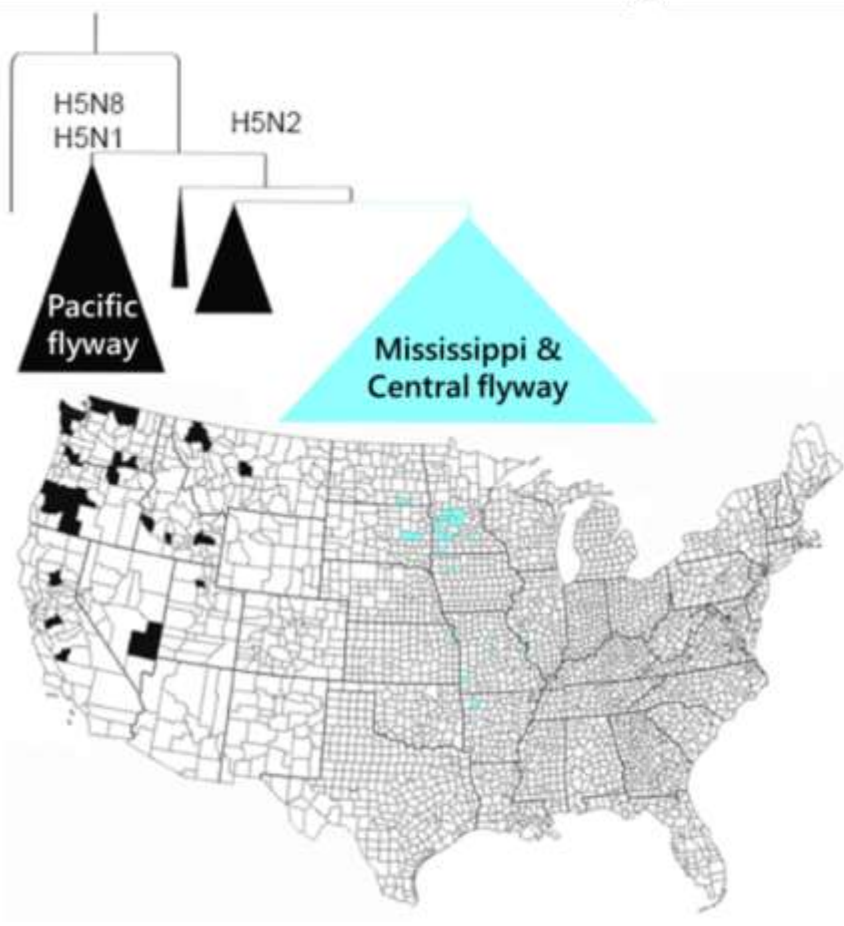

Figure 8. Phylogeny of the PB2, HA, and matrix genes of the H5Nx viruses and geographic distribution by subtype

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

Summary of H5Nx molecular analysis

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

This analysis includes viruses detected through early April 2015 from 16 states (n=92 viruses; H5N8=20, H5N2=68, H5N1=4; 13 from backyard, 36 from commercial, and 43 from wild and captive wild birds). While these viruses remain highly similar overall, analytical tools that identify amino acid substitutions along the HA1 protein, the neuraminidase (NA) gene and internal protein genes can improve our understanding of the virologic, antigenic, and epidemiologic features of the virus (refer to section on Diagnostics and Characterization for H5Nx viruses). The findings, depicted in Table 35, are summarized here:

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

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

Molecular epidemiology

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

Stearns County Minnesota Cluster

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

Minnesota/South Dakota Cluster

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

Public health aspects

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

Poultry vaccine strain selection considerations

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

Table 35. 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	MN	MN	ND	ND	WI	SD	MN	ND	SD	state
35	K		R	R	R																																		Substitutions with potential virologic significance
73	V												I																										
83	A																										T												
88	D																																			G	G		
114	T	N																																					
122	W					L																																	
136	P							Q																		S	S	S	S	S	S								Antigenic site A
141	S									P	P	P			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P				Antigenic site A
162	I																																					V	
194	P													S																									
218	Q								R																														
223	S									G																													Receptor binding; Antigenic site D
234	K																											R	R	R	R								
235	P						Q																																
257	V								I																														
294	I							V																															
309	N																															K	K	K	K				
312	V																																		I				
322	L														Q																								
# Sub	Ref	1	1	1	1	1	1	2	2	2	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	2	3	3	3	3	3	2	2	2	3	1	1	1	vs. A/gyrfalcon/Washington/41088-6/2014_H5N8

Diagnostics and characterization for H5Nx viruses

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

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

[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

segments of isolates were amplified using gene-specific and universal primers for each segment. The cDNA was purified and cDNA libraries were prepared for the Ion Torrent using the IonXpress Plus Fragment Library Kit (Life Technologies) with Ion Xpress barcode adapters. Prepared libraries were quantitated using the Bioanalyzer DNA 1000 Kit. Quantitated libraries were diluted and pooled for library amplification using the Ion One Touch 2 and ES systems. Following enrichment, DNA was loaded onto an Ion 314 or Ion 316 chip and sequenced using the Ion PGM 200 v2 Sequencing Kit.

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

APPENDIX A. HPAI INVESTIGATION – QUESTIONNAIRE

(Version 1.0 – March 2015)

Animal and Plant Health Inspection Service

Veterinary Services

HPAI Investigation - Questionnaire

INSTRUCTIONS

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

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

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

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

Date: ________________________

Interviewer name/organization: ____

Interviewee name/organization: ____

A. PREMISES INFORMATION

Farm name: ____

Farm address: ____

Farm (premises) ID: ____________ County: ____________________________

Township: _____________ Range: __________ Section: ____________

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

B. PREMISES CONTACT INFORMATION

1. Contact name: ____

   Phone: __________________ Cell phone: ______________ Email: ___________________________

2. Contact name: ____

   Phone: __________________ Cell phone: ______________ Email: ___________________________

3. Contact name: ____

   Phone: __________________ Cell phone: ______________ Email: ___________________________

4. Flock Veterinarian: ____

   Phone: _________________ Cell phone: _____________ Email: _____________________________

C. PREMISES DESCRIPTION

1. Poultry type: ☐1 Broiler ☐2 Layer ☐3 Turkey ☐4 Other (specify: ____________________)

2. Production type: ☐1 Meat ☐2 Egg ☐3 Breeding ☐4 Other (specify: ___________________)

3. Age: ☐1 Multiple age ☐2 Single age

4. Sex: ☐1 Hen ☐2 Tom ☐3 Both

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

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

   ☐ Brood

   ☐ Grow

   ☐ Other (specify: _______________________)

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

   ☐ Breeder

   ☐ Commercial

7. If brooder and grower houses are present on the same premises, are there multiple stages of management (brooding and growing), in the same house?...... ☐1 Yes ☐3 No

8. Farm capacity .... _____ # birds

   Number of barns .... _____ # barns

   Barn capacity .... _____ # birds

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

   ☐1 Curtain sided

   ☐2 Environmental control

   ☐3 Side doors

   ☐4 Other (specify: ____________________)

10. Are cool cell pads used? .... ☐1 Yes ☐3 No

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

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

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

☐ Pond

☐ Lake

☐ Stream

☐ River

☐ Other (specify: ________________________)

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

a.	Beef cattle	☐$_1$ Yes	☐$_3$ No
b.	Dairy cattle	☐$_1$ Yes	☐$_3$ No
c.	Horses..	☐$_1$ Yes	☐$_3$ No
d.	Sheep...	☐$_1$ Yes	☐$_3$ No
e.	Goats	☐$_1$ Yes	☐$_3$ No
f.	Pigs	☐$_1$ Yes	☐$_3$ No
g.	Dogs...	☐$_1$ Yes	☐$_3$ No
h.	Cats..	☐$_1$ Yes	☐$_3$ No
i.	Poultry or domesticated waterfowl	☐$_1$ Yes	☐$_3$ No
j.	Other (specify: _____________________________)	☐$_1$ Yes	☐$_3$ No

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

☐$_1$ Municipal

☐$_2$ Well

☐$_3$ Surface water (e.g., pond)

☐$_4$ Other (specify: __________________________)

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

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

D. FARM BIOSECURITY

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

What disinfectant is used? ___________________________

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

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

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

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

    c. Fly control used .... ☐1 Yes ☐3 No

       If Yes, type and frequency: ___________________________________

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

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

       If Yes, type, number, and frequency: ____

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

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

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

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

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

E. FARM HELP/WORKERS

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

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

   a. Family.. _____ #

   b. Nonfamily.. _____ #

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

   ☐1 Entire farm

   ☐2 Specific barns/areas

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

   If Yes, location: _________________________

5. Are workers employed by other poultry operations?.. ☐$_1$ Yes ☐$_3$ No

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

7. Are family members employed by other poultry operations or processing plants? ☐$_1$ Yes ☐$_3$ No

   If Yes, poultry operation or processing plant: ____

8. Do part-time/weekend help and other extended family members on holidays and vacations? .... ☐$_1$ Yes ☐$_3$ No

9. Are workers (full & part-time) restricted from being in contact with backyard poultry?.. ☐$_1$ Yes ☐$_3$ No

   How is this communicated? ____

F. FARM EQUIPMENT

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

1. Company vehicles/trailers:

   Farm specific? .... ☐$_1$ Yes ☐$_3$ No

   If No, by whom is equipment jointly used: ____

   Dates: _____________________________________

2. Feed trucks (excess feed):

   Farm specific? .... ☐$_1$ Yes ☐$_3$ No

   If No, by whom is equipment jointly used: ____

   Dates: _____________________________________

3. Gates/panels:

   Farm specific? .... ☐$_1$ Yes ☐$_3$ No

   If No, by whom is equipment jointly used: ____

   Dates: _____________________________________

4. Lawn mowers:

   Farm specific? .... ☐$_1$ Yes ☐$_3$ No

   If No, by whom is equipment jointly used: ____

   Dates: _____________________________________

5. Live haul loaders:

   Farm specific? .... ☐$_1$ Yes ☐$_3$ No

   If No, by whom is equipment jointly used: ____

   Dates: _______________________________________

6. Poult trailers: Farm specific?

   Farm specific? .... ☐$_1$ Yes ☐$_3$ No

   If No, by whom is equipment jointly used: ____

   Dates: _______________________________________

7. Pre-loaders:

   Farm specific? .... ☐$_1$ Yes ☐$_3$ No

   If No, by whom is equipment jointly used: ____

   Dates: _______________________________________

   Describe pre-loader cleaning and disinfection procedures:

   ____

   ____

8. Pressure sprayers/washers:

   Farm specific? .... ☐$_1$ Yes ☐$_3$ No

   If No, by whom is equipment jointly used: ____

   Dates: _______________________________________

9. Skid-steer loaders:

   Farm specific? .... ☐$_1$ Yes ☐$_3$ No

   If No, by whom is equipment jointly used: ____

   Dates: _______________________________________

10. Tillers:

    Farm specific? .... ☐$_1$ Yes ☐$_3$ No

    If No, by whom is equipment jointly used: ____

    Dates: _______________________________________

11. Trucks:

    Farm specific? .... ☐1 Yes ☐3 No

    If No, by whom is equipment jointly used: ____

    Dates: ___________________________________

12. Other equipment: ____

    Farm specific? .... ☐1 Yes ☐3 No

    If No, by whom is equipment jointly used: ____

    Dates: ___________________________________

G. LITTER HANDLING

1. Litter type: ____________________________

2. Supplier/source: _________________________

3. Is a litter shed present? .... ☐1 Yes ☐3 No

4. Do you do partial cleanouts?.. ☐1 Yes ☐3 No

   If Yes, give dates of last partial cleanout: ______________________________________

5. Date of last cleanout: .... _________ date

   Frequency of cleanout:... _____ times/month

6. Who does the cleanout?

   ☐1 Grower

   ☐2 Contractor

   If contractor, name and location____

7. Litter is disposed of:

   ☐1 On farm

   ☐2 Taken off site

   If taken offsite, name and location: ____

H. DEAD BIRD DISPOSAL

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

2. How is daily mortality handled?
   a. On-farm: Burial pit/incinerator/composted/other (specify: ___________________________)
   b. Off-farm: Landfill/rendering/other (specify: ____)
   c. Off-farm disposal performed by: Owner/employee/other (specify: _____________________)
   d. If burial or compost pits are used, are carcasses covered with soil on a daily basis? .... ☐1 Yes ☐3 No

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

   ____

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

4. What is the pickup schedule? ____

   5. Does the carcass bin have a cover? .... ☐1 Yes ☐3 No
      Is it routinely kept closed? .... ☐1 Yes ☐3 No

I. FARM VISITORS

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

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

3. Do you provide any outer clothing to visitors entering the farm? .... ☐1 Yes ☐3 No
   If Yes, identify items of clothing provided: _______________________________

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

Service		**Dates**	**Name**	**Contact?**
Service person	☐Yes ☐No	______________	________________________	☐1 Yes ☐3 No
Vaccination crew	☐Yes ☐No	______________	________________________	☐1 Yes ☐3 No
Moving crew (moving from brood to grow, or pullet house to layer house)	☐Yes ☐No	______________	________________________	☐1 Yes ☐3 No
Processing plant load out				

☐Yes ☐No ______________ ________________________ ☐$_1$ Yes ☐$_3$ No

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

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

Truck #/#'s ____

Trailer #/#'s ____

What plant did flock go to? ____

Load-out crew (flock previous to positive flock)

☐Yes ☐No _______________ ________________________ ☐$_1$ Yes ☐$_3$ No

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

Truck #/#'s ____

Trailer #/#'s ____

What plant did flock go to? ____

Poult delivery ☐Yes ☐No _______________ ________________________ ☐$_1$ Yes ☐$_3$ No

Rendering pickup ☐Yes ☐No _______________ ________________________ ☐$_1$ Yes ☐$_3$ No

Litter services ☐Yes ☐No _______________ ________________________ ☐$_1$ Yes ☐$_3$ No

Cleanout services ☐Yes ☐No _______________ ________________________ ☐$_1$ Yes ☐$_3$ No

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

☐Yes ☐No _________________ ________________________ ☐$_1$ Yes ☐$_3$ No

Feed delivery ☐Yes ☐No ________________ ________________________ ☐$_1$ Yes ☐$_3$ No

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

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

J. WILD BIRDS

1. Do you see wild birds around your farm? .... ☐1 Yes ☐3 No

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

   ☐ Waterfowl

   ☐ Gulls

   ☐ Small perching birds (sparrows, starlings, swallows)

   ☐ Other water birds (egrets, cormorants)

   ☐ Other ____________________

2. Do you see birds all year round? .... ☐1 Yes ☐3 No

   If Yes, what type of birds? __________________________________

3. Is there seasonality to the presence of some types of birds? .... ☐1 Yes ☐3 No

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

   ____

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

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

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

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

K. NARRATIVE/COMMENTS

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

North

APPENDIX B. HPAI CASE CONTROL QUESTIONNAIRE - LAYERS (NEW)

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

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Animal and Plant
Health Inspection
Service

Veterinary Services

HPAI Case-Control Questionnaire

National Animal Health
Monitoring System

2150 Centre Ave., Bldg B
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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. .... Mash e343 ☐1 Yes ☐3 No

b. .... Pellets e344 ☐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)? e346 ☐1 Yes ☐3 No

b. .... Antimicrobial (e.g., ionophores)? e347 ☐1 Yes ☐3 No

c. Other (specify: ______________________________________)? e348/348oth ☐1 Yes ☐3 No

23. .... Is the feed heat treated? e349

.... ☐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	e401
b. Gulls	☐1	☐2	☐3	e402
c. Small perching birds (e.g., sparrows, starlings, swallows)	☐1	☐2	☐3	e403
d. Blackbirds and crows	☐1	☐2	☐3	e404
e. Other water birds (e.g., egrets, cormorants)	☐1	☐2	☐3	e405
f. Wild turkeys, pheasants, quail	☐1	☐2	☐3	e406
g. Raptors (e.g., eagles, hawks, owls)	☐1	☐2	☐3	e407
h. Pigeons and doves	☐1	☐2	☐3	e408
i. Other (specify: ______________________________)	☐1	☐2	☐3	e409/e409oth

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	e411
b. Gulls	☐1	☐2	☐3	e412
c. Small perching birds (e.g., sparrows, starlings, swallows)	☐1	☐2	☐3	e413
d. Blackbirds and crows	☐1	☐2	☐3	e414
e. Other water birds (e.g., egrets, cormorants)	☐1	☐2	☐3	e415
f. Wild turkeys, pheasants, quail	☐1	☐2	☐3	e416
g. Raptors (e.g., eagles, hawks, owls)	☐1	☐2	☐3	e417
h. Pigeons and doves	☐1	☐2	☐3	e418
i. Other (specify: ______________________________)	☐1	☐2	☐3	e419/e419oth

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	e420
b. Small birds (e.g., finches, sparrows, starlings)	☐1	☐2	☐3	e421
c. Other (specify: ______________________________)	☐1	☐2	☐3	e422/e422oth

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	e423
b. Small birds (e.g., finches, sparrows, starlings)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e424
c. Other (specify: ______________________________)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e425/e425oth

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	e502
b. Shower	☐1	☐2	☐3	☐4	e503
c. Wash hands before entering and/or before leaving the barn	☐1	☐2	☐3	☐4	e504
d. Different personnel for different houses	☐1	☐2	☐3	☐4	e505
e. Wear disposable coveralls	☐1	☐2	☐3	☐4	e506
f. Change of clothing (washable)	☐1	☐2	☐3	☐4	e507
g. Change of shoes or use of shoe covers	☐1	☐2	☐3	☐4	e508
h. Foot bath (liquid)	☐1	☐2	☐3	☐4	e509
i. Foot bath (dry)	☐1	☐2	☐3	☐4	e510
j. Scrub footwear (bucket and brush)	☐1	☐2	☐3	☐4	e511

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): e514

   ____

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	e601/e619/e637
b. Extension agent or university veterinarian	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e602/e620/e638
c. Private or company veterinarian	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e603/e621/e639
d. Company service person	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e604/e622/e640
e. Nutritionist or feed company consultant	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e605/e623/e641
f. Pullet delivery	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e606/e624/e642
g. Vaccination crew	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e607/e625/e643
h. Catch crew	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e608/e626/e644
i. Feed delivery personnel	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e609/e627/e645
j. Egg truck personnel	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e610/e628/e646
k. Litter services (delivery, pick-up)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e611/e629/e647
l. Customer (private	☐1 Yes	_____ #	☐1 Yes ☐3	e612/e630/e648

individual)	☐3 No	visits	No	
m. Wholesaler, buyer, or dealer	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e613/e631/e649
n. Renderer	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e614/e632/e650
o Occasional worker (e.g., family member, part time help over holiday)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e615/e633/e651
p. Construction workers	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e616/e634/e652
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	e617/e635/e653
r. Other nonbusiness visitors (including neighbors, friends, and school field trips)	☐1 Yes ☐3 No	_____ # visits	☐1 Yes ☐3 No	e618/e636/e654

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	e656
b. Foot covers or change of footwear	☐1	☐2	☐3	e657
c. Mask	☐1	☐2	☐3	e658
d. Hand sanitizing or gloves	☐1	☐2	☐3	e659
e. Not visit multiple farms in the same day	☐1	☐2	☐3	e660
f. Other (specify:_________________________)	☐1	☐2	☐3	e661/e661oth

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?	If "Yes," 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		e662/e671/ e680/e689
b. Feed trucks	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No		e663/e672/ e681/e690
c. Pullet delivery vehicles (i.e., placing pullets)	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No		e664/e673/ e682/e691
d. Bird removal vehicles	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No		e665/e674/ e683/e692
e. Egg delivery vehicles	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No		e666/e675/ e684/e693
f. Egg removal vehicles	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No		e667/e676/ e685/e694
g. Manure/litter hauling	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No		e668/e677/ e686/e695
h. ATV/4-wheeler	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No		e669/e678/ e687/e696
i. Other (specify: ________________)	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No		e670/e670oth e679/e688/e697

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?	If "Yes," 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		e698/e708/ e718/e728
b. Lawn mowers	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No		e699/e709/ e719/e729
c. Live haul loaders	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No		e700/e710/ e720/e730
d. Egg racks or pallets	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No		e701/e711/ e721/e731
e. Egg flats	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No		e702/e712/ e722/e732

f. Pressure sprayers/washers	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No		e703/e713/ e723/e733
g. Skid-steer loaders	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No		e704/e714/ e724/e734
h. Litter handling	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No		e705/e715/ e725/e735
i. Manure handling	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No		e706/e716/ e726/e736
j. Other (specify: ________________)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	☐1 Yes ☐3 No		e707 e717/ e727/e737/ e707oth

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: e903
☐1 Company personnel?
☐2 Litter provider?

☐3 Other (specify: ________________________________)? e903oth

4. What is the source (i.e., company name) of the litter? e904

____

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?
____ e1014

5. What do workers do after handling the carcass bin before returning to the live poultry area? e1015
____
____
____

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? _______________________________e1019

K. WEATHER CONDITIONS

1. In the past 14 days, how would you describe the wind? e1101

☐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?			e1201/ e1300
2. What type(s) of poultry are present in this barn?			e1202/ e1302
a. Pullet	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1203/ e1303
b. Layer	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1204/ e1304
c. Breeder	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1205/ e1305
d. Other	☐1 Yes ☐3 No If "Yes," specify: ______________	☐1 Yes ☐3 No If "Yes," specify: ______________	e1206/ e1206oth e1306/ e1306oth
3. How many birds were placed in this barn?	_____ # birds	_____ # birds	e1207/ e1307
4. What was the date of placement in this barn?	______ mm/dd/yy	______ mm/dd/yy	e1208/ e1308
5. How old were birds when placed in this barn?	_____ weeks	_____ weeks	e1209/ e1309
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	e1210/ e1310
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: _________________)	e1211/ e1311
8. Has this flock been molted?	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1212/ e1312

	CASE BARN	CONTROL BARN

9. Did birds in this barn have outside access?	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1213/ e1313
10. What was the bird density in the barn?	______ sq in/bird	______ sq in/bird	e1214/ e1314
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: ______________ _ ______________ _	e1215/ e1215sp e1315/ e1315spe
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	e1216/ e1316
13. Was this flock vaccinated in the past 14 days?	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1217/ e1317
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.**	e1218/ e1318
15. Are cages curtain backed?	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1219/ e1319
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	e1220/ e1320
17. How old is this barn structure?	_____ years	_____ years	e1221/ e1321
18. How long has it been since the last remodel of the barn structure?	_____ years	_____ years	e1222/ e1322
	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	e1223/ e1323
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	e1224/ e1324
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	e1225/ e1325
22. Where are fans located?	☐$_1$ Sidewall ☐$_2$ End of barn ☐$_3$ Both	☐$_1$ Sidewall ☐$_2$ End of barn ☐$_3$ Both	e1226/ e1326

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: _______________ _	e1227/ 1227spe e1327/ e1327spe
	CASE BARN	**CONTROL BARN**	
24. Describe ventilation protocol for the past 14 days.			e1228/ e1328
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	e1229/ e1329
26. Does this barn have a hard surface entry pad (e.g., concrete, asphalt)?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1230/ e1330
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: _______________ __	e1231/ e1231spe e1331/ e1331spe
b. Is disinfectant used?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1232/ e1332
27. Does this barn have:			
a. Locks on the doors?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1233/ e1333
b. A service room that personnel must enter through that separates "outside area" from "inside area"?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1234/ e1334

c. Changing area for employees	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1235/ e1335
d. A shower for employees?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1236/ e1336
e. Cool cell pads?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1237/ e1337
f. Misters?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1238/ e1338
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.***	e1239/ e1239oth e1339/ e1339oth
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	e1240t/ e1240u e1340t/ e1340u
30. What disinfectant is used in the footbaths?	specify: _______________	specify: _______________ _	e1241/ e1341
31. Does this barn have drop boards?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1242/ e1342
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.***	e1243/ e1343
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:* _______________ __	e1244/ e1244oth e1344/ e1344oth

34. Is the litter bagged (i.e., bailed) or bulk (i.e., load from shavings mill)?	☐$_1$ Bag ☐$_3$ Bulk	☐$_1$ Bag ☐$_3$ Bulk	e1245/ e1345
35. Who are the supplier(s)/source(s) of litter?			e1246/ e1346
36. Was litter "tilled" since it was placed in the barn?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1247/ e1347
If "Yes," when was it tilled?	______ mm/dd/yy	______ mm/dd/yy	e1248/ e1348
37. How many times was litter added to the barn in the past 14 days?	_______ times	_______ times	e1249/ e1349
38. When was the last full clean out of litter or manure?	______ mm/dd/yy	______ mm/dd/yy	e1250/ e1350
39. Were birds present during the last full cleanout?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1251/ e1351
40. Who performed the last full cleanout? *[Enter Code 1 or 2.]* 1. Producer 2. Contractor	_____ code	_____ code	e1252/ e1352
If contractor, specify name and location.	specify: n________________ l_________________	specify: n_______________ l________________	e1253n/ e1253l e1353n/ e1353l

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	e1254/ e1354
b. Small birds (e.g., finches, sparrows, starlings)	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1255/ e1355
42. What is the distance (in yards) of the closest body of water to this barn?	_____ yards	_____ yards	e1256/ e1356
43. Were wild waterfowl observed on this body of water in the past 14 days?	☐$_1$ Yes ☐$_3$ No	☐$_1$ Yes ☐$_3$ No	e1257/ e1357
44. How far is this barn (in yards) from:			

a. Dead bird disposal/holding area including carcass bin for rendering	____ yards	____ yards	e1258/ e1358
b. Nearest road	____ yards	____ yards	e1259/ e1359
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	e1260/ e1360
b. Extension agent or university veterinarian	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1261/ e1361
c. Private or company veterinarian	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1262/ e1362
d. Company service person	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1263/ e1363
e. Nutritionist or feed company consultant	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1264/ e1364
f. Pullet delivery	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1265/ e1365
g. Vaccination crew	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1266/ e1366
h. Catch crew	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1267/ e1367
i. Feed delivery personnel	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1268/ e1368
j. Egg truck personnel	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1269/ e1369
k. Litter services (delivery, pick-up)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1270/ e1370
l. Customer (private individual)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1271/ e1371
m. Wholesaler, buyer, or dealer	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1272/ e1372
n. Renderer	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1273/ e1373
o Occasional worker (e.g., family member, part time help over holiday)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1274/ e1374
p. Construction workers	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1275/ e1375

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	e1276/ e1376
r. Other nonbusiness visitors (including neighbors, friends, and school field trips)	☐1 Yes ☐3 No	☐1 Yes ☐3 No	e1277/ e1377
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	e1278
47. Was there a pattern of spread in the barn? If "Yes," describe.	☐1 Yes ☐3 No If "Yes," describe:______ _______________ _____	NA	e1279/ e1279d
48. What was the *first* indication of infection within the barn?		NA	
a. Surveillance testing	☐1 Yes ☐3 No		e1280
b. Increased mortality	☐1 Yes ☐3 No		e1281
c. Clinical signs	☐1 Yes ☐3 No If "Yes," (specify: _______________ _______________ _____)		e1282/ e1282spe

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? ....e1401 _______ #
   b. Within 3 miles of this farm? ....e1402 _______ #

5. How far (in yards or in miles) is the nearest backyard flock to this farm? ..e1403y/e1403m _______ yards OR _____ miles

6. How far (in yards or in miles) is the nearest HPAI positive premises to this farm? .... e1404y/e1404m _______ 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? ….. e1405 ____

USDA APHIS Veterinary Services

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