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Epidemiologic Investigation of Highly Pathogenic H5N2 Avian Influenza Among Upper Midwest U.S. Turkey Farms, 2015

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SUMMARY. In 2015, an outbreak of H5N2 highly pathogenic avian influenza (HPAI) occurred in the United States, severely impacting the turkey industry in the upper midwestern United States. Industry, government, and academic partners worked together to conduct a case-control investigation of the outbreak on turkey farms in the Upper Midwest. Case farms were confirmed to have HPAI-infected flocks, and control farms were farms with noninfected turkey flocks at a similar stage of production. Both case and control farms were affiliated with a large integrated turkey company. A questionnaire administered to farm managers and supervisors assessed farm biosecurity, litter handling, dead bird disposal, farm visitor and worker practices, and presence of wild birds on operations during the 2 wk prior to HPAI confirmation on case premises and the corresponding time frame for control premises. Sixty-three farms, including 37 case farms and 26 control farms were included in the analysis. We identified several factors significantly associated with the odds of H5N2 case farm status and that may have contributed to H5N2 transmission to and from operations. Factors associated with increased risk included close proximity to other turkey operations, soil disruption (e.g., tilling) in a nearby field within 14 days prior to the outbreak, and rendering of dead birds. Observation of wild mammals near turkey barns was associated with reduced risk. When analyses focused on farms identified with H5N2 infection before April 22 (Period 1), associations with H5N2-positive farm status included soil disruption in a nearby field within 14 days prior to the outbreak and a high level of visitor biosecurity. High level of worker biosecurity had a protective effect. During the study period after April 22 (Period 2), factors associated with HPAI-positive farm status included nonasphalt roads leading to the farm and use of a vehicle wash station or spray area. Presence of wild birds near dead bird disposal areas was associated with reduced risk. Study results indicated that the initial introduction and spread of H5N2 virus likely occurred by both environmental and between-farm pathways. Transmission dynamics appeared to change with progression of the outbreak. Despite enhanced biosecurity protocols, H5N2 transmission continued, highlighting the need to review geographic/topologic factors such as farm proximity and potential dust or air transmission associated with soil disruption. It is likely that biosecurity improvements will reduce the extent and speed of spread of future outbreaks, but our results suggest that environmental factors may also play a significant role in farms becoming infected with HPAI.

RESUMEN. Investigación epidemiológica de la influenza aviar altamente patógena H5N2 en granjas de pavos en la parte norte del medio oeste de los Estados Unidos en el año 2015.

En el año 2015, se produjo un brote de influenza aviar altamente patógena H5N2 en los Estados Unidos, lo que afectó severamente a la industria del pavo en el medio oeste de este país. La industria, el gobierno y el sector académico trabajaron juntos para llevar a cabo un estudio de casos y controles con relación al brote en granjas de pavos en la parte norte del medio oeste de los Estados Unidos. Se confirmó que las granjas de los casos tenían parvadas infectadas por la influenza aviar y las granjas de control eran granjas con parvadas de pavos no infectadas en una etapa similar de producción. Tanto las granjas de casos como las controles estaban afiliadas a una gran empresa integrada de pavos. Se administró un cuestionario a los gerentes y supervisores de la granja que evaluó la bioseguridad de la granja, el manejo de la cama, la eliminación de la mortalidad, las prácticas de los visitantes y trabajadores y la presencia de aves silvestres en las operaciones durante las dos semanas antes de la confirmación de la influenza aviar de alta patogenicidad en las instalaciones incluidas como casos y durante el correspondiente marco de tiempo en las instalaciones controles. Se incluyeron en el análisis 63 granjas, incluyendo 37 granjas de casos y 26 granjas controles. Se identificaron varios factores significativamente asociados con las probabilidades del estado de la granja como caso con H5N2 y que pudieron haber contribuido a la transmisión del virus H5N2 desde y hacia las operaciones. Los factores asociados con el riesgo aumentado incluyeron proximidad a otras operaciones de pavos, la alteración del suelo (por ejemplo, cultivo) en un campo cercano dentro de los 14 días previos al brote, y el procesamiento de aves muertas. La observación de mamíferos salvajes cerca de las granjas de pavos se asoció con un riesgo menor. Cuando los análisis se centraron en las explotaciones identificadas con la infección por H5N2 antes del 22 de abril (período 1), las asociaciones con el estado de la granja H5N2 positiva incluyeron alteraciones del suelo en un campo cercano dentro de los 14 días previos al brote y un alto nivel de bioseguridad para los visitantes. El alto nivel de bioseguridad de los trabajadores tuvo un efecto protector. Durante el período de estudio después del 22 de abril (período 2), los factores asociados con el estado de la granja positiva para HPAI incluyeron caminos no asfaltados que conducían a la granja y el uso de una estación de lavado de vehículos o área de aspersión. La presencia de aves silvestres cerca de áreas de desecho de aves muertas se asoció con un menor riesgo. Los resultados del estudio indicaron que la introducción inicial y la propagación del virus H5N2 probablemente ocurrieron tanto en el ambiente como entre las granjas. La dinámica de transmisión parecía cambiar con la progresión del brote. A pesar de la mejora de los protocolos de bioseguridad, la transmisión de H5N2 continuó, destacando la necesidad de revisar los factores geográficos/topológicos como la proximidad de las granjas y el potencial de polvo o la transmisión aérea asociada con la

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alteración del suelo. Es probable que las mejoras en la bioseguridad reduzcan el alcance y la velocidad de propagación de futuros brotes, pero nuestros resultados sugieren que los factores ambientales también pueden desempeñar un papel importante en las granjas infectadas por la influenza aviar de alta patogenicidad.

Key words: highly pathogenic avian influenza, H5N2, epidemiologic study, risk factors, environment, farm biosecurity, turkeys

Abbreviations: AF = attributable fraction; AI = avian influenza; CI = confidence interval; HPAI = highly pathogenic avian influenza; IQR = interquartile range; OR = odds ratio; rRT-PCR = real-time reverse transcriptase–polymerase chain reaction; USDA-APHIS = U.S. Department of Agriculture–Animal and Plant Health Inspection Service

An unprecedented outbreak of highly pathogenic H5N2 avian influenza (HPAI) occurred in Upper Midwest U.S. poultry farms during early March–mid-June 2015. In Minnesota, 108 operations in 23 counties were reportedly affected by the virus (10). The epidemiologic peak of the HPAI epidemic in this region occurred on April 22, 2015, and the last known HPAI turkey case in Minnesota occurred on June 5, 2015 (Fig. 1). By July 30, 2015, all control zones (10-km areas around infected premises, within which targeted diagnostic testing, movement controls, and biosecurity requirements were implemented) had been released (10). The University of Minnesota Extension estimated that lost turkey and egg production and processing depressed Minnesota's economic output by an estimated \$647.2 million (19), including \$171.7 million of lost wages, salaries, and benefits. In addition, 2,500 jobs were temporarily affected. Because of the severe economic losses involved with HPAI and concern that HPAI is still circulating in migratory birds, poultry producers remain concerned regarding the potential for similar future outbreaks.

Wild aquatic birds are the primary reservoirs for avian influenza viruses (16). Previous work has demonstrated that there are many diverse subtypes isolated from migrating waterfowl in the North American Mississippi Migratory Flyway with dynamic exchange of gene segments among wild birds along the migratory flyway (4). Previously reported routes of introduction of avian influenza onto turkey farms include 1) direct exposure to infected birds, 2) exposure to equipment or materials contaminated with virus-containing feces or respiratory secretions, 3) movement of virus by human shoes or clothing, 4) virus-contaminated water, and 5) airborne movement of virus (18). Historically, the most important sources of horizontal transmission have been movement of contaminated poultry manure, movement of infected live or dead poultry, unwashed eggs, and contaminated people and equipment (5).

To identify risk factors for HPAI infection on Upper Midwest U.S. turkey farms during the 2015 outbreak, a case-control study of turkey farms was conducted. This case-control study involved turkey farms in Minnesota and Wisconsin affiliated with a large integrated turkey company.

MATERIALS AND METHODS

Case definition and farms. Case farms were considered positive for H5N2 HPAI if Eurasian-lineage H5 avian influenza was detected by real-time reverse transcriptase–polymerase chain reaction (rRT-PCR) by the U.S. Department of Agriculture–Animal and Plant Health Inspection Service (USDA-APHIS) and were identified by a turkey company within the company's production system in the following categories: company-owned farms, leased farms, contract farms, and affiliated independent farms. For each case farm, the 14-day period of time prior to confirmation of infection was identified as the time period for investigation.

The turkey company provided a blinded list of eligible company-affiliated control farms (e.g., no confirmed HPAI-infected turkeys during the study period), which were randomly selected by study investigators. Initially, one control farm was selected for each case farm based on the following criteria: having turkeys of the same production stage (growing, brooding, breeder) for at least 9 days of the case farm's 14-day reference period and located within 32 km of the case farm. However, over the course of the outbreak, control farms within 32 km of a case farm became unavailable in some regions. As a result, the matching strategy changed during enrollment so that control farms were no longer matched to case farms based on proximity.

Questionnaire. To identify potential risk factors, a questionnaire was developed by the coauthors in collaboration with company personnel, USDA-APHIS Veterinary Services animal health officials, and University of Minnesota faculty and staff. The questionnaire was designed to capture information describing the farm premises and potential exposures related to farm biosecurity and management practices. Similar questionnaires have been used elsewhere to evaluate other outbreaks of avian influenza (9,20). Data collected from turkey operations include turkey age, crop management practices on nearby fields, bodies of water on or nearby farm property, observation of waterfowl and other wild birds on the farm before the outbreak, proximity of the flock to other commercial poultry operations and to HPAI-positive farms, farm biosecurity practices, frequency and number of employees and visitors to the farm, and other on-farm environmental and management practices.

Farm managers and supervisors completed the questionnaire, and follow-up interviews and review of questionnaire responses were conducted by trained study personnel. Farm managers and supervisors were instructed to respond to questions in the context of the 14-day period before infection on the farm was confirmed (for case farms) or a designated matching 14-day period for control farms. Surveys were reviewed for accuracy by interviewers prior to data entry. Survey data were double-entered into a Microsoft Access database and validated for error detection prior to analysis.

Exposures of interest. In the analysis, geographic proximity of poultry farms was dichotomized as three or more poultry farms within 4.8 km (3 miles) of the study farm, and two or fewer poultry farms within 4.8 km. A cumulative score for worker biosecurity was created by summing the number of worker biosecurity protocols that were "always" or "nearly always" used by farms. These included the following: 1) showering required before entering barn, 2) assignment of different personnel to different barns, 3) use of dry footbaths, 4) use of disposable coveralls, 5) requiring a change of clothes upon barn entry, 6) requiring a change of shoes or shoe covers upon barn entry, and 7) requiring footwear to be scrubbed with a bucket and brush. This cumulative biosecurity score was dichotomized into two or fewer worker biosecurity protocols and three or more protocols in place for employees. Similarly, a cumulative score for visitor biosecurity was created by summing the number of visitor biosecurity protocols "always" or "nearly always" used for each farm, and dichotomized at the median of two or fewer *vs.* three or more protocols for visitors. The visitor biosecurity protocols included 1) requiring a change of clothes upon barn entry, 2) requiring a change of shoes or shoe covers upon barn entry, 3) use of a mask, 4) use of hand sanitizer or gloves, 5) not allowing multiple farm visits in the same day, and 6) other control measures.

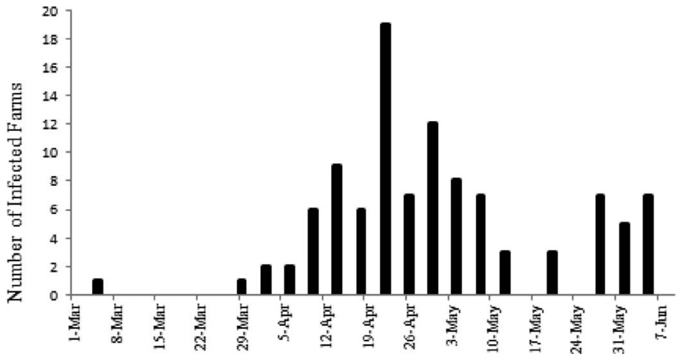

Fig. 1. Minnesota poultry farms affected with highly pathogenic H5N2 avian influenza.

To compare air movement within barns, farms reported the percentage of time that curtains were open over the 14-day time period as reported from the barn manager and farm supervisor. Barns with curtains opened greater than 10% of the time were compared to barns opened less than 10% of the time. These cut-offs were based on company recommendations during the outbreak to provide adequate airflow yet potentially reduce the risk of debris carrying influenza virus being introduced into the barn.

Statistical analysis. Unmatched univariable analyses were performed for all variables in the questionnaire. Association between each variable and case farm status was assessed using chi-square tests of association, Fisher's exact test, Student's *t*-test, and Wilcoxon rank sum, as appropriate.

As the study outbreak progressed, case farms became geographically clustered, which prevented enrollment of control farms located in proximity to each case farm. In order to ensure a similar geographic risk of HPAI exposure among case and control farms, analysis was limited to farms that were within 32 km (20 miles) of a HPAI-positive turkey farm, based on the distance to HPAI-positive turkey farms reported by the farmers and the turkey company. This restriction resulted in the loss of some farms for analysis, but allowed for more accurate estimates and better control for selection bias, and helped alleviate unmeasured confounding by regional differences.

Variables were considered for inclusion in unmatched multivariable logistic regression models based on statistical association from univariable analyses ($P < 0.20$) and biologic plausibility for risk of HPAI infection. Multivariable models were built via one of two processes: 1) backward selection, in which all eligible variables were included in an initial model, and variables with P-values > 0.1 were progressively removed; 2) forward selection, in which variables were progressively added to models, with variables with P-values > 0.1 removed after each addition. Parameters were considered significant in final multivariable models if $P < 0.1$. This cut-off value was chosen to provide statistical power to detect potentially causal associations, though the sample size was limited by the outbreak magnitude.

Because risk factors during infectious disease outbreaks may change over the course of the outbreak, including changes to primary sources of virus and practices within farms, separate univariable and multivariable analyses were performed for farms stratified into two time periods using the date of confirmation of infection for each case farm (or the reference date for control farms). Period 1 cases occurred before April 22, 2015, the approximate peak of the Minnesota epidemic, and Period 2 cases occurred on or after that date.

For each variable result as part of the multivariable model for the full period, average attributable fractions were calculated according the methods described and the Stata code included in Rückinger *et al.* (14). This analysis was not conducted for the individual time periods because the data in each time period were sparse and yielded unstable estimates.

RESULTS

Farm demographics. Data were collected from a total of 83 turkey farms in Minnesota and Wisconsin, including 43 case farms with known outbreaks of H5N2 HPAI and 40 control farms. One farm was interviewed as a control farm, but later became a case farm in the outbreak; this farm was excluded from analysis. Excluding case or control farms located farther than 32 km from a HPAI-positive turkey farm, the final analysis dataset included 63 turkey farms (37 case farms and 26 control farms). Of case farms, 21 (57%) were company farms, seven (19%) independent farms, five (14%) contract farms, and four (11%) lease farms. All of the control farms were either company ($n = 22$; 85%) or contract ($n = 4$, 16%) operations. The majority ($n = 61$, 97%) of farms were meat production operations, and two farms were breeder operations. The median size of case and control farms was 56,930 (range: 7,200–315,000) and 51,847 (range: 7,200–328,148) birds per farm, respectively. The mean number of days between the first observation of clinical signs or increased mortality and the interview date was 31 days for case farms (minimum 16, maximum 63) and the corresponding time for control farms was 36 days (minimum 5, maximum 82).

General univariable observations. A comprehensive list of univariable results is provided in the Supplemental Appendix. Selected factors relating to previously identified risks for transmission of HPAI are reported below.

Farm management, wild birds and animals. Use of rendering, the process of converting waste animal tissue to value-added products, as a bird disposal practice was more common on case farms (81%) than on control farms (57%) ($P = 0.04$). Observing waterfowl (89% of case farms) or gulls (60% of case farms) on or near the farm but outside of barns in the 14-day reference period was not related to overall case status ($P = 0.29$ and $P = 0.46$, respectively). Seven case farms (19%) and no control farms reported that bulk stored litter was accessible to wild birds prior to use ($P = 0.04$), and six case farms (16%) and no control farms reported their bulk stored litter was accessible to wild animals prior to use ($P = 0.04$).

Wild birds and mammals were more commonly observed around dead bird collection areas on control farms than case farms (wild birds: 77% of control farms *vs.* 46% of case farms, $P = 0.01$; wild mammals: 31% of control farms *vs.* 5% of case farms, $P = 0.01$). Eight case farms (22%) reported poor barn maintenance compared to one (4%) control farm ($P = 0.01$).

Farm workers and visitors. The number of farm employees was similar for both case and control farms (case farms, median = 2, interquartile range [IQR] = 1–4 compared to control farms, median = 2.5, IQR = 2–4; $P = 0.63$). Always or nearly always using disposable coveralls and scrubbing footwear were more common among control farms (disposable coveralls: 22% of case farms *vs.* 39% of control farms, $P = 0.15$; scrubbing footwear: 22% of case farms *vs.* 42% of control farms, $P = 0.08$). Farms often had more than one type of footbath used for barns, and liquid footbaths were used on all but one farm. In addition, control farms more commonly "always or nearly always" used dry footbaths when entering barns than case farms did (dry footbaths: 3% of case farms *vs.* 27% of control farms, $P = 0.01$). A greater percentage (73%) of control farm workers were more likely to always or nearly always use three or more worker biosecurity protocols (i.e., biosecurity score) than case farms (41%, $P = 0.01$).

Visits from common farm visitors (e.g., flock service personnel, feed delivery personnel), having a worker employed at another farm, or having a worker employed by another poultry operation were not related to case status. We observed that case farms "always or nearly always" followed visitor biosecurity protocols to a greater degree than control farms. However, these factors were generally not associated with case farm status when analyzed individually. Specifically, having visitors change outer clothing ($P = 0.06$), change footwear or wear foot covers ($P = 0.14$), and use hand sanitizer or wear gloves ($P = 0.15$) were not statistically more common among control farms than case farms. Case farms were more likely to follow three or more of the visitor biosecurity protocols (87%) than control farms (65%; $P = 0.05$). However, there were few recorded visitors from outside the farm for both case and control farms and, if visits occurred, they had limited access to the turkey barns.

The use and sharing of vehicles (e.g., feed trucks, poult trailers) or equipment (e.g., lawn mowers, live haul loaders, pressure sprayers) in the 14 days before the outbreak was also not associated with increased risk of H5N2 infection. Use of a vehicle wash station or spray area was more common on case farms (87% of cases *vs.* 65% of controls, $P = 0.05$).

Proximity to other farms and environmental factors. There were more poultry barns within a 4.8-km radius of case farms than control farms (case farms = median of 3 farms (IQR = 2–4) compared to control farms = median of 1 farm (IQR = 0–2; $P < 0.01$)). In addition, case farms were, on average, closer to a confirmed HPAI farm compared to controls (case farms median of 2.4 km (IQR = 0.8–5.4) compared to control farms' median of 8.4 km (IQR = 1.7–25; $P = 0.01$). Having a nearby field actively worked in the 14 days before the outbreak occurred more frequently near case (57%) than control farms (39%; $P = 0.15$). For case and control farms reporting use of curtain-sided ventilation, the odds of H5N2 infection increased with the greater percentage of time that curtains were left open (>10% of the time compared to <10%; $P = 0.03$). We also found that cases had curtains open for a median of 14 days compared to a median of 4 days for controls ($P = 0.06$).

Period 1. During this period before April 22, control farms were more likely than case farms to always or nearly always use three or more worker biosecurity protocols than case farms ($P = 0.05$; data not shown). On the other hand, case farms were more likely to follow three or more of the visitor biosecurity protocols than control farms ($P = 0.04$). The use of bird delivery vehicles was significantly more common on control farms during Period 1 ($P < 0.01$). Wild mammals near dead bird collection areas was more commonly observed on control farms ($P = 0.01$).

Period 2. During the period after April 22, factors associated with H5N2 infection included presence of wild birds, use of wash stations, and proximity to gravel roads (data not shown). Wild birds near the dead bird collection area was more commonly observed on control farms ($P = 0.01$). The use of a vehicle wash station or spray area was more common on case farms ($P = 0.03$). H5N2 infection was more commonly observed on case farms that had gravel road access ($P = 0.16$).

Multivariable analyses. We conducted a multivariable logistic regression model for the entire epidemic period using backward selection. Controlling for other model parameters, case farms had a higher odds of having three or more farms within a 4.8-km radius than did controls (odds ratio [OR] = 46, 95% confidence interval [CI] = 6.0–358; Table 1). Other factors associated with increased odds of H5N2 HPAI infection included active crop-related work (tilling or discing) in the field closest to the turkey barns within 14 days prior to the outbreak (OR = 6.5, 95% CI = 1.4–30.8), and use of rendering for dead birds (OR = 9.8, 95% CI = 1.5–66). Observing wild mammals near case barns was marginally associated with reduced odds of HPAI infection (OR = 0.06, 95% CI = 0.02–1.06). Close proximity to poultry farms had the highest attributable fraction (0.43), followed by rendering of dead birds (0.35) and actively working fields closest to turkey barns (0.20). The presence of wild mammals near barns had a negative attributable fraction, indicating it was associated with slightly reduced odds of case farm status (–0.05).

During Period 1, controlling for other model parameters, active work in a nearby field within 14 days prior to the outbreak (OR = 13.88, 95% CI = 1.04–185) and a high level of visitor biosecurity (OR = 7.92, 95% CI = 0.88–71.41) were associated with increased odds of case farm status, and a high level of worker biosecurity (OR = 0.07, 95% CI = 0.01–0.96) exhibited a protective effect. Unlike control farms, only case farms were in close proximity to three or more farms; therefore the proximity variable was not included in the Period 1 multivariable regression model.

For Period 2, close proximity to three or more poultry farms was identified as the only independent predictor of case status (OR = 12.1, 95% CI = 2.4–61.2) in the multivariable regression model. When this variable was excluded from analysis, nonasphalt road for vehicles coming onto the farm (OR = 10.05, 95% CI = 0.65–156) and use of a vehicle wash station or spray area (OR = 12.4, 95% CI = 0.94–164) were associated with increased odds of H5N2 infection. Observation of wild birds near dead bird disposal areas was associated with reduced odds of case farm status (OR = 0.12, 95% CI = 0.02–0.72).

DISCUSSION

This study provides unique insight into a large multifarm outbreak of HPAI. However, study results do not indicate a clear single causal pathway. Influenza virus introduction may have occurred through environmental and geographic factors (i.e., tillage of nearby fields and proximity to poultry farms) and perpetuated by biosecurity factors (i.e., wash station practices and worker biosecurity) and geographic factors (i.e., proximity to other poultry farms). The findings from this investigation raise new questions requiring further research and corroborate some previously identified risk factors (e.g., rendering of dead birds) (5,9). Study findings also point to changing effects of specific risk factors during the outbreak (Period 1 compared to Period 2), consistent with findings from phylogenetic analysis of the H5N2 virus in the midwestern United States (20). This analysis showed multiple virus clusters in this region from February to April 20, 2015, consistent with several largely independent point-source introductions of virus with limited evidence of lateral spread. In contrast, H5N2 viruses identified from March 26 to May 14, 2015, showed a phylogenetic pattern consistent with concurrent point introductions and lateral spread.

Close proximity to other poultry farms was identified as a significant factor associated with case farm status during Period 1, Period 2, and the overall time period. Generally close proximity may allow interfarm traffic, including sharing common equipment or work staff. This was not documented in the study findings. However, geographic factors may explain part of this risk. The

Table 1. Factors associated with case farm status (from multivariable analyses).

Multivariable model	No. of controls (%)	No. of cases (%)	Variables	*P*-values	Odds ratio (95% CI)	Average AF[C]
Full period	10 (38.5)	21 (56.8)	Tilled in last 14 days	0.02	6.46 (1.36–30.78)	0.2
	9 (34.6)	4 (11.1)	Wild mammals near barns	0.06	0.14 (0.02–1.06)	−0.05
	15 (57.7)	30 (81.1)	Render dead birds	0.02	9.80 (1.46–65.96)	0.35
	3 (11.5)	23 (62.2)	Close proximity to poultry farms	<0.01	46.14 (5.96–357.55)	0.43
Period 1 (before April 22)[A]	4 (40.0)	15 (83.3)	High visitor biosecurity	0.07	7.92 (0.88–71.41)	
	7 (70.0)	5 (27.8)	High worker biosecurity	0.05	0.07 (0.01–0.96)	
	3 (30.0)	12 (66.7)	Tilled in last 14 days	0.05	13.88 (1.04–184.85)	
Period 2 (after April 22)[B]	12 (75.0)	17 (94.4)	Nonasphalt roads	0.1	10.05 (0.65–156.49)	
	10 (62.5)	18 (94.7)	Use of vehicle wash/spray stations	0.06	12.40 (0.94–163.52)	
	12 (75.0)	6 (31.6)	Wild birds near dead bird disposal	0.02	0.12 (0.02–0.72)	

[A]Close proximity to other poultry farms could not be included in the early model because no control farms were in a high-farm-concentration area. Therefore, close proximity to other poultry farms alone may be a comparable or better predictor of being a case in the early period than the set of variables together in the multivariable model shown. However, that cannot be determined with the given data.

[B]The model shown was the result of not including close proximity to other poultry farms in the multivariable model selection process. When this variable was included, the model reduced to only including this variable. Therefore, similar to the early model, close proximity to other poultry farms alone may be a comparable or better predictor of being a case than the set of variables together in the late period multivariable model shown here.

[C]AF = attributable fraction.

geographic landscape in the outbreak area is generally flat cropland with very few trees, allowing potential spread of airborne virus and debris movement (i.e., feathers, dust) between operations. Proximity to other poultry operations was observed as a risk from previous genetic and epidemiologic modeling analyses (8,23), and one recent study (12) reported influenza A may be viable in air and on surfaces up to a week after reported onset of illness within swine production facilities.

Farms with turkey barns located near fields actively worked (tilling or discing) with soil disruption in the previous 14 days had higher odds of infection. This association was identified during Period 1, early in the spring season. The 2015 spring in the Upper Midwest was abnormally dry and windy, potentially creating conditions that allowed for the spread of airborne particles contaminated with influenza virus over distances (Southwest Research and Outreach Center, Lamberton, Minnesota, pers. comm. from J. Spohr; West Central Research and Outreach Center, University of Minnesota, 46352 State Hwy 329, Morris, MN, and from review of Willmar Airport Records). In addition to soil disruption, proximity to nonasphalt roads used by vehicles entering the farm later in the outbreak further supports potential airborne or particulate transmission as a route of exposure, especially during the height of the outbreak. This could include vehicle traffic moving between farms (2,5).

There may be benefit to windbreaks or other structures to redirect prevailing winds flowing from one farm to another; providing buffer areas around farms or greater separation between tilled ground and turkey barns may also be beneficial. Unfortunately, the limited curtain and ventilation data from case and control farms did not allow assessment of the value of various barn ventilation systems to prevent potential infections. It is possible that modestly restricting air flow into turkey barns could prevent potential HPAI introductions to susceptible turkeys. It is important to consider adequate air flow for poultry welfare yet assess the value of restricted air movement especially during high-risk periods such as during outbreaks. The poultry industry should consider placement of future farms in areas with fewer other poultry operations.

It has been recently reported that various peridomestic mammalian species can shed and potentially spread H7N9 avian influenza virus (13). Farms that are close in proximity may have peridomestic mammals traveling between farms, highlighting the need for enhanced barn biosecurity control plans to account for this potential risk. From our analysis, reporting small mammals near turkey barns was associated with reduced odds of case farm status, and no H5N2-positive test results were identified from a USDA-APHIS study of 190 small mammals (185 house mice (*Mus musculus*), 3 deer mice (*Peromyscus maniculatus*), 2 Eastern cottontail (*Sylvilagus floridanus*)) sampled from infected farms in a neighboring state (Iowa) (20). Yet, this potential hypothesis needs further exploration.

While wild aquatic birds are considered a primary reservoir of avian influenza viruses, this study did not identify a direct association between the odds of infection and presence of wild aquatic or other wild birds. Further, there is neither supporting evidence that this HPAI H5N2 virus was present in the Upper Midwest in the autumn of 2014 nor evidence of widespread viral infection of returning migratory birds in the spring of 2015 (11). The USDA-APHIS reported over 7,000 birds tested in the US through wild bird surveillance between July 2014 and June 2015; HPAI H5 was confirmed in 65 birds, and species detected in the Central and Mississippi flyways included snow geese (*Chen caerulescens*), Canada geese (*Branta canadensis*), a ring-necked duck (*Aythya collaris*), a snowy owl (*Bubo scandiacus*), and a Cooper's hawk (*Accipiter cooperii*) (23). From Minnesota Department of Natural Resources testing of over 2,900 wild birds during the summer and fall of 2015 (6), two wild birds tested positive, while all tested wild aquatic birds tested in Minnesota were negative. Additional testing performed in wild aquatic birds sampled in 2014 and 2015 before and following the outbreak failed to detect HPAI viruses, though low-pathogenic avian influenza viruses were detected (7).

Also, this study found no association between the odds of infection and proximity to a backyard flock. During the outbreak, a single backyard poultry flock was identified with H5N2. During this time, the Minnesota Board of Animal Health tested additional backyard flocks in control zones (i.e., within 10 km of infected premises), and no HPAI virus was detected from these surveillance samples. Considering that wild aquatic birds are considered a maintenance host for avian influenza viruses and that backyard flocks have potential outdoor access and exposure to wild birds, it is noteworthy that only a single backyard flock tested positive during the outbreak. This may reflect timing and frequency of sample collection and other host-pathogen interactions. Recent USDA

Agriculture Research Service research has documented that turkeys are more susceptible to certain strains of H5N2 than other strains (15) and that some H5N2 strains are poorly adapted to chickens (1). This might suggest that some backyard chicken flocks may have been exposed to HPAI H5N2 infection during the outbreak, but were refractory to virus infection.

This study identified use of rendering to dispose of dead birds as a risk factor for HPAI infection. The practice of rendering of dead birds as a method of disposal has been identified as a risk factor for lateral spread in previous studies (9,20) and was also associated with H5N2 infection among case farms in this study. The removal of mortalities from a farm prior to an avian influenza detection or diagnosis can allow for the potential spread either by truck movement between farms or potential airborne movement of contaminated feathers/dander to other farms from trucks driving by susceptible farms (2). Rendering trucks need to be emptied and thoroughly disinfected when moving between farms. Rendering materials need to be securely contained and rendering (mortality) bins need to be placed away from the production barns. Veterinarians should maintain a high degree of suspicion for unusual mortalities and promptly test for the presence of virus on the farm prior to releasing mortalities for rendering. This practice could mitigate the risk associated with dead bird movement.

Most farm biosecurity practices (including visitors in the barn and/or on the farm, sharing vehicles or equipment with other farms, controlling vehicle traffic near barns, and pest control for barns) were not associated with HPAI infection. However, control farms more commonly reported always or nearly always following biosecurity protocols related to their workers (requiring designated farmworker personnel for different barns; requiring footwear to be scrubbed before workers enter barns). Adhering to more worker biosecurity protocols (*vs.* fewer) was significantly associated with reduced odds of HPAI infection during Period 1, suggesting that the combined impact of having increased worker biosecurity had a protective effect early in the outbreak. Effective biosecurity should be standard practice for poultry as well as other livestock operations (5,8,9,17).

Use of multiple visitor biosecurity practices was associated with increased odds of H5N2 infection in this study. This finding is counterintuitive, and indicated that case farms practiced more visitor biosecurity measures that controls. In reality, visitor access to farms and poultry barns was minimal and discouraged for both case and control farms, making the impact of this observation difficult to assess. Farm supervisors and managers should continue to review visitor protocols to prevent unauthorized access to farms and prevent interfarm transmission.

Although common vehicles entering the farm were not associated with case farm status, the use of a vehicle wash stations or spray areas was associated with H5N2 infection late in the outbreak. It is possible that this finding reflects improper and/or incomplete methods of disinfection, such as inadequate disinfectant application (i.e., amount, contact time, method of delivery) or a focus on washing tires rather than the entire vehicle undercarriage. Vehicle disinfection was problematic during the 1997–98 H7N2 outbreak in Pennsylvania (3), showing that high levels of environmental contamination of H5N2 HPAI could overwhelm biosecurity strategies employed (8). Further work is needed to provide best-practices guidelines for wash stations in controlling the spread of HPAI between farms. This would include a review of available disinfectants and the appropriate application to assure complete coverage and efficacy. Because influenza A viruses persist for longer in wet environments than in dry environments, it is possible that wash areas could create a reservoir for HPAI H5N2 on a farm if not properly disinfected. In addition, efforts must be made to ensure that cleaning and disinfection is always a two-step process. Disinfectants are not effective in the presence of organic debris such as dirt and fecal material. Vehicles, boots, footbaths, and any other items that come into contact with a disinfectant must first be cleaned to remove all organic debris.

The findings from this study should be viewed as hypothesis generation rather than confirmation of causative outbreak-associated factors. As such, it is important to understand factors associated with infection to develop appropriate prevention strategies to reduce future widespread transmission events from this disease. Collecting critical study information from a relatively large number of turkey farms during the course of such an outbreak was challenging, and was made possible through collaboration and support from a large integrated turkey company. Data collection methods were designed to collect data from both case and uninfected control farms through off-farm visits with farm supervisors and barn managers while minimizing risks to control farms.

These findings were derived from an observational case-control study conducted during an outbreak, and analyses involved multiple comparisons. Thus, the potential for spurious findings was present, and the presence of unmeasured confounders could influence study results. Only a few of the several hundred farm-level factors examined in this study were statistically associated with HPAI H5N2 infection. This could be a result of a small sample size ($n = 63$) and companywide consistency in biosecurity practices, as well as changes in practices during the course of the outbreak. Most farms were part of the same parent company with a certain level of uniformity with regard to biosecurity measures and farm practices and were part of a company network (with measurable and unmeasurable interconnections); biosecurity risks from this study might not be generalizable to other farms and/or companies in the region or country. There was potential for recall bias in this study, because farmers might have been interviewed at different intervals from the 14-day reference period to the time the interview was conducted. In some instances, this could reflect up to 8 wk from the reference date. The recorded observations were based on farm supervisor and barn manager reports; actual worker practices may differ. This effect was likely minimal because the biosecurity practices generally reflect company policy. Finally, as a result of selecting controls based on production stage, case and control farms also were similar with regard to bird age. This prevented assessment of age as an independent risk factor or effect modifier of other risk factors.

During the course of the outbreak, transmission routes and dynamics may have changed. Different risk factors identified in Period 1 *vs.* Period 2 of the outbreak may reflect these differences in exposures over time. After HPAI was identified in North American in November 2014, the company changed several biosecurity procedures to reduce risk of pathogen introduction (e.g., limiting movements of people and turkeys, changes in litter management). In addition, the timely depopulation of turkeys on infected operations became more challenging at the peak of the outbreak, potentially creating differential risks to nearby farms over time. Immediate depopulation of infected flocks is viewed as a critical control measure in preventing interfarm spread (8).

Another important factor (and potential confounder) in this study was the effect of geographic region. Farms in close proximity to each other are likely to share topographic features and certain farm practices. To control for potential differences in risk by location, the study design initially matched on farm location. However, due to lack of uninfected farms within certain outbreak-affected regions as the outbreak progressed, the study design was modified to select control farms not matched by region. Since region was demonstrated in preliminary analyses to be a significant factor associated with case farm status, some farms were later excluded ($n = 20$ farms) in order to restrict analysis to farms located within a certain distance from an affected farm. The final models reflect this analysis, though this exclusion negatively impacted the power of the analysis to detect associations.

In summary, potential mitigation strategies to reduce the risk of future HPAI outbreaks will involve enhanced biosecurity precautions but also will need to consider other environmental factors. The magnitude and speed of spread of this virus in a naïve host population highlights the need for prompt action (i.e., prompt depopulation), thoughtful planning, and continued regulatory and industry cooperation. A continued emphasis on human and vehicle biosecurity is prudent. System-wide biosecurity training, an assessment of physical structures (e.g., barns, feed storage units), and external on-farm biosecurity assessments are recommended to improve turkey farm biosecurity and farmer awareness. It is likely that biosecurity improvements along with preparedness activities will reduce the extent and speed of spread of future outbreaks. Our study results also suggest that environmental factors may also play a significant role in farms becoming infected with HPAI, requiring turkey producers to evaluate new strategies to reduce the risk of future introductions.

A supplemental appendix associated with this article can be found at http://dx.doi.org/10.1637/AVIANDISEASESJOURNAL-11543-112816-s1.

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