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Investigation into the Airborne Dissemination of H5N2 Highly Pathogenic Avian Influenza Virus During the 2015 Spring Outbreaks in the Midwestern United States

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SUMMARY. We investigated the plausibility of aerosol transmission of H5N2 highly pathogenic avian influenza (HPAI) virus during the 2015 spring outbreaks that occurred in the U.S. midwest. Air samples were collected inside and outside of infected turkey and layer facilities. Samples were tested to assess HPAI virus concentration (RNA copies/m^3 of air), virus viability, and virus distribution by particle size. HPAI virus RNA was detected inside and up to 1000 m from infected facilities. HPAI virus was isolated from air samples collected inside, immediately outside, up to 70 m from infected facilities, and in aerosol particles larger than 2.1 μm. Direct exposure to exhausted aerosols proved to be a significant source of environmental contamination. These findings demonstrate HPAI virus aerosolization from infected flocks, and that both the transport of infectious aerosolized particles and the deposition of particles on surfaces around infected premises represent a potential risk for the spread of HPAI.

RESUMEN. Investigación sobre la diseminación por el aire del virus de la influenza aviar de alta patogenicidad H5N2 durante los brotes de la primavera en el año 2015 en la región del medio oeste de los Estados Unidos.

Se investigó la posibilidad de la transmisión por el aire del virus de la influenza aviar altamente patógena H5N2 (con las siglas en inglés HPAI) durante los brotes de primavera en el año 2015 que ocurrieron en la región del medio oeste de los Estados Unidos. Se recolectaron muestras de aire de los interiores y exteriores de las instalaciones de pavos y de gallinas de postura infectadas. Las muestras se analizaron para evaluar la concentración del virus de influenza aviar altamente patógeno (número de copias de ARN viral por metro cúbico de aire), la viabilidad del virus y la distribución de virus por el tamaño de las partículas. Se detectó ARN del virus de influenza aviar altamente patógeno en el interior de las instalaciones infectadas y hasta 1000 m de dichas instalaciones. El virus de la influenza aviar altamente patógeno se aisló de muestras de aire recogidas en el interior, en el exterior y hasta 70 m de las instalaciones infectadas y en partículas de aerosol de más de 2.1 μm. La exposición directa a los aerosoles demostró ser una fuente importante de contaminación ambiental. Estos hallazgos demuestran la aerosolización del virus de la influenza aviar de alta patogenicidad de las parvadas infectadas y que tanto el transporte de las partículas en aerosol infecciosas como el depósito de partículas en superficies alrededor de los locales infectados representan un riesgo potencial para la propagación de la influenza aviar de alta patogenicidad.

Key words: avian influenza, HPAI, airborne transmission, particle size

Abbreviations: ACI = Andersen cascade impactor; AI = avian influenza; AIV = avian influenza virus; APHIS = Animal and Plant Health Inspection Services; CC = cyclonic collector; Ct = cycle threshold; ECE = embryonated chicken eggs; GM = geometric mean; GSD = geometric standard deviation; MEM = minimum essential media; OIE = World Organisation for Animal Health; qRT-PCR = quantitative real-time reverse-transcription PCR; rRT-PCR = real-time reverse-transcription PCR; TCI = Tisch cascade impactor; USDA = U.S. Department of Agriculture

A novel H5N2 strain of highly pathogenic avian influenza (HPAI) virus (HPAIV) was first detected in the United States, in December 2014 (11). Subsequently, this virus caused unprecedented losses to the U.S. poultry industry, with 219 detections reported and over 48 million birds dying or destroyed as of June 17, 2015 (21). Specifically in Minnesota, HPAI virus spread rapidly, affecting 108 farms and over 9 million birds distributed among 23 counties. Similar situations were observed in other states, and because of the rapid regional spread and the clustering of cases in specific areas, we investigated the potential for aerosol spread of HPAI virus to contribute to local spread.

Migratory waterfowl have been implicated in the introduction and movement of the novel H5N2 HPAI virus (11), which is a reassortant virus between Eurasian H5 clade 2.3.4.4 and North American strains, and evidence for both point source introduction and farm-to-farm spread were identified during the spring outbreaks in the midwestern United States (20). Several routes of AIV transmission are considered important during AI epidemics, including movement of birds, visitors, fomites, and materials (18). Aerosol transmission of AIV has been suspected (12,19,23), and considered highly probable in a few cases, as evidenced by the sporadic detection of virus in the air (15). Aerosol transmission of influenza virus in pigs and people is better understood, since the virus is shed through the respiratory tract of mammals. However, aerosol transmission of AIV, often predominantly shed in feces of natural waterfowl hosts, is more contentious. Transmission of AIV by the fecal–oral route when infected feces or contaminated materials are ingested by susceptible hosts is better understood (14). However, there is increasing evidence that pathogens shed in

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feces may also be transmitted through the air (1,4) and more information is needed to determine the potential contribution of the airborne route in the spread of systemic pathogens with an important enteric component. Aerosolized pathogens are associated with particles of diverse nature including fecal material, dust, debris, water, respiratory fluids, and bedding material. The composition and size distribution of these particles will determine the sites of deposition in exposed hosts, and influence the duration that infectious agents remain suspended in the air, the distance across which they can be transported, and the survivability and infectivity of the pathogens (3,17,22,24). Thus the likelihood of aerosol transmission depends on both the pathogen characteristics and the physico-chemical environment at the host–pathogen interface (10). Furthermore, the infectious dose for AIV varies and is strain, route, and host dependent; for lineage H5N1 viruses the respiratory route requires fewer viruses than the enteric route (9). Understanding the dissemination of H5N2 HPAI virus from affected birds is necessary to assess the plausibility of aerosol transmission among flocks, particularly in areas of high poultry density. Furthermore, understanding the likelihood of HPAI virus aerosol transmission in environments where birds and people interact is crucial, since certain strains of HPAI virus have a high case-fatality rate in infected humans (8,9).

We report the detection, quantification, and viability of H5N2 virus in air samples collected inside and outside of housing of actively infected H5N2 HPAI poultry flocks, provide information on virus-associated particle sizes relevant to transmission risk, show evidence of environmental contamination from airborne particle deposition, and provide first evidence of risk of aerosol spread of HPAI. This study was designed and performed as part of a rapid outbreak response in collaboration with members of United States Department of Agriculture/APHIS.

MATERIALS AND METHODS

Ethics statement. Procedures and protocols used in this study were approved by the University of Minnesota Institutional Biosafety Committee. Permission to sample the air and the environment of the affected flocks was obtained by USDA/APHIS veterinarians as part of the regulatory response to the outbreak.

Flock selection/description. Selection of flocks was done in collaboration with USDA/APHIS veterinarians working with poultry systems recently diagnosed with HPAI. Three turkey flocks located in Minnesota, two layer flocks in Iowa, and one in Nebraska were selected. Selected flocks had a confirmed diagnosis of HPAI virus at the time of sampling, and researchers conducted testing within 2 and 11 days of the confirmation. One flock had initiated depopulation at the time of sampling; the others had active infections. Characteristics of flocks are summarized in Table 1.

Sampling procedures. To measure concentration of aerosolized virus, samples were collected inside and outside of affected flocks at approximately 5-, 70–150-, and 500–1000-m distances with the use of three distinct air samplers: 1) liquid cyclone collector (CC, Midwest Micro-tek, Brookings, SD) (6), 2) Andersen cascade impactor (ACI; Thermo Electron Corporation, Waltham, MA) (2), and 3) a high-volume Tisch cascade impactor (TCI; Model 230, Tisch Environmental, Inc., OH)]. Outside samples at 70–150 and 500–1000 m were collected downwind from the infected facilities, and wind parameters were measured with an anemometer (Kestrel, 3000. Nielsen-Kellerman, Boothwyn, PA) at the time of sampling. Both the ACI and the TCI are size-differentiating air samplers that capture each sampled particle into one of several size intervals.

Sample collection using the cyclonic air collector (processing 200 L of air per minute) was carried out for 30 min inside and outside the facilities. Ten milliliters of minimum essential media (MEM) supplemented with 4% of bovine albumin serum were used as collection media. After collection, an average of 4 ml of sample was recovered, divided into two aliquots, and stored at −80 C. The collector was then disinfected with 70% ethanol, rinsed with distilled water, and dried with paper towels. After disinfection, the collection vessel and the turbine were swabbed as controls, and samples stored at −80 C until analysis.

The ACI sampled air from the inside and immediately outside (5 m) of the facilities at 28.3 L/min for 1 hr, and separated particles into eight size intervals (stages) based on their aerodynamic diameter: 0.4–0.7, 0.7–1.1, 1.1–2.1, 2.1–3.3, 3.3–4.7, 4.7–5.8, 5.8–9.0, and >9.0 μm. Particles smaller than 0.4 μm were captured by a backup filter. Samples from stages of this device were eluted from every aluminum plate with the use of a cell scraper and 1 ml of MEM (2). All samples were transferred into 1.5-ml sterile plastic tubes, placed on ice, and stored at −80 C until testing. The ACI was then disassembled and plates and stages were scrubbed with alkyl dimethyl benzyl ammonium chloride soap (Lysol, Reckitt Benckiser), rinsed, and disinfected again with 70% ethanol prior to being dried with paper towels. After disinfection, a random number of collection plates and individual ACI stages were swabbed, and samples stored at −80 C.

The TCI device sampled air from inside and outside facilities, separating particles into four stages with aerodynamic particle diameter cut points of 0.95, 1.5, 3, and 7.2 μm. Particles were captured with the use of slotted glass fiber collection substrates (Model P/N TE-230-GF, New Star Environmental, Inc. Roswell, GA) and particles smaller than 0.95 μm were captured by a backup filter. After each sampling period, the substrates and filter were removed with the use of sterile gloves, folded, placed into individual labeled petri dishes, and refrigerated at 4 C until sample elution and processing. Between sampling events, all stages were disinfected with 70% alcohol, air dried, and finally, rinsed and dried with paper towels. After disinfection and drying, all stages were swabbed, and samples stored at −80 C. In order to extract the virus from substrates and filters, samples were removed from the petri dishes, cut individually in 1-cm^2 squares with sterile scissors, and eluted in 50-ml Falcon tubes with 20 ml of the eluent solution (3% beef extract 0.05 M glycine solution at pH 9.1). As part of the elution process, each tube was inverted 10 times to mix the contents. The mixing step was followed by a 5-min set period; another mixing step of 10 inversions; and finally each tube was vortexed for a 5-min period at 1,500 rpm. Volumes eluted from filters were measured, transferred to a 15-ml Falcon tube and the pH adjusted to 7. All samples were transferred into 1.5-ml sterile plastic tubes, and stored at −80 C until testing. For each sampling event, there was one sample assayed from the CC, eight stages from the ACI, four stages from the TCI, and two backup filter samples.

Relative humidity (%), temperature (C), and wind speed (m/s) were recorded (Kestrel 3,000, Nielsen-Kellerman, Boothwyn, PA) during each sampling event to monitor weather conditions.

Airborne particle deposition. To assess airborne particle deposition, environmental samples were collected outside the premises from surfaces in locations at high risk of direct exposure to the air exhausted from layer flocks. Surface samples of an area of approximately 20×20 cm^2 were collected aseptically with disposable gloves with gauze dipped into sterile media as previously described (5). After collection, samples were eluted from the gauze in 50-ml Falcon tubes and transported to the laboratory on ice. All samples were then transferred into 1.5-ml sterile plastic tubes, and stored at −80 C until testing. Surfaces tested included farm fixtures (e.g., silos, walls, fans, door handles) and temporary fomites exposed to exhausted air for approximately 2 hr (e.g., sampling equipment, plastic containers).

Table 1. Attributes of farms studied and environmental conditions at sampling events.

Farm ID	U.S. state	Species/type	Flock size	Number of barns	Positive confirmation date (dd/mm/yy)	Sampling date (dd/mm/yy)	Barn ID sampled	Sampling location/ distance (meters)	Average mortality[A] (%)	Average relative humidity (%)	Average Temp (C°)	Average wind velocity (meters/second)
1	MN	Turkey/layer	28,000	4	20/04/15	24/04/15	1	Inside	0.4	50.6	18.7	NA
1	MN	Turkey/layer	28,000	4	20/04/15	24/04/15	2	Inside	70–80	66.6	16.7	NA
1	MN	Turkey/layer	28,000	4	20/04/15	24/04/15	2	5	70–80	NR	NR	NR
2	MN	Turkey/grow	70,000	7	25/04/15	27/04/15	3	Inside	30–40	40	23.3	NA
2	MN	Turkey/grow	70,000	7	25/04/15	27/04/15	3	5	30–40	32.5	19.7	NR
2	MN	Turkey/grow	70,000	7	25/04/15	27/04/15	3	70–150	30–40	37	17.8	NR
3	MN	Turkey/breeder	4205	2	26/04/15	28/04/15	1	Inside	30	92.8	13	NA
3	MN	Turkey/breeder	4205	2	26/04/15	28/04/15	1	5	30	94.7	10.4	3.5
3	MN	Turkey/breeder	4205	2	26/04/15	28/04/15	1	70–150	30	72	12.9	2.4
4[B]	IA	Chickens/layers	575,000	6	NR[C]	12/05/15	2	5	10	36	19.1	1.0
4[B]	IA	Chickens/layers	575,000	6	NR	12/05/15	2	70–150	10	42.5	17.9	1.4
4[B]	IA	Chickens/layers	575,000	6	NR	12/05/15	NA[D]	500–1000	NA	47.6	13.7	2.8
5	NE	Chickens/layers	1.7M	18	05/11/15	05/22/15	5	70–150	37	69.2	17.3	6
5	NE	Chickens/layers	1.7M	18	05/11/15	05/22/15	NA	500–1000	NA	55.2	22.8	3.9
6[B]	NE	Chickens/layers	1.8M	15	05/13/15	05/23/15	21	Inside	14	81.4	21.8	NA
6[B]	NE	Chickens/layers	1.8M	15	05/13/15	05/23/15	21	5	14	82.4	19.3	1
6[B]	NE	Chickens/layers	1.8M	15	05/13/15	05/23/15	NA	500–1000	NA	92.2	13.4	2.8

[A]Average total mortality as reported at the time of sampling.
[B]Environmental samples from surfaces were collected from Farms 4 and 6.
[C]NR—not registered.
[D]NA—not applicable.

Laboratory tests. Air samples were screened for influenza A by real-time reverse-transcription polymerase chain reaction (rRT-PCR) targeting the matrix gene at the University of Minnesota Veterinary Diagnostic Laboratory (16). Positive samples were tested by subtype specific H5 and N2 rRT-PCRs (testing conducted by an approved National Animal Health Laboratory Network laboratory as described in U.S. Department of Agriculture [USDA] WI-AV-0033.05 Real-Time RT-PCR Assay for the Detection of Influenza A subtype H5 of Americas and Eurasian lineage [H5 AM/EA 2014]). Cycle threshold (Ct) values <35 were considered positive, 35–40 suspect, and >40 negative. Positive and suspect samples were also tested with the use of a quantitative RT-PCR (qRT-PCR) targeting the matrix gene (7) in order to estimate the number of viral copies per cubic meter of air.

To assess the infectivity of the air samples, virus isolation in embryonated chicken eggs (ECE) was attempted from all rRT-PCR positive and suspect air samples at the National Veterinary Services Laboratory, Ames, IA or the UMN Veterinary Diagnostic Laboratory following published procedures. Briefly, samples were inoculated in four 9–11-day-old ECE, incubated at 37 C for 4–5 days, and candled daily to observe embryo mortality. Allantoic fluid harvested from all live and dead eggs was tested for the presence of hemagglutinating virus and if positive further characterized by virus typing.

Select virus isolates were sequenced and confirmed as HPAI H5N2 virus strains per cleavage site analysis as defined by World Organisation for Animal Health [OIE] (sequence >99% similar to the index case A/Northern pintail/Washington/40964/2014, novel H5N2 HPAI virus) (13).

Statistical analysis. Data from the qRT-PCR, weather variables, sampling events, type of air collector, distance, and day of sampling were consolidated in a spreadsheet (Microsoft EXCEL; Microsoft Corporation, Redmond, WA) and organized for analysis. Means, standard deviations, and minimum and maximum values for quantitative variables, and frequency counts and percentages for qualitative variables, were calculated for descriptive analysis. Negative sampling events (considered those of which results from all three collectors were negative) were not included in the quantitative analysis. Negative air samples or negative stages of size-selective air samplers (multiple stages within the same sampling event) that were included in the analyses were designated a value of 100 RNA copies/ml according to the limit of detection of the qRT-PCR technique. For the frequency description of the data, a sampling event of size-selective air samplers was considered positive when at least one stage had Ct < 35, and suspect when at least one stage had Ct 35–40. Because of the number of variables in the study a multivariate modeling analysis was performed. The variable "replicate" (a group of sampling events with different air collectors at a certain location during the study) was considered as a repeated measure, and "farm" as a random variable. Under these premises, the association between total quantity of virus (RNA copies/m^3) and the explanatory variables (sampling distance, type of animal, and air sampler) was assessed for significance with the use of a mixed linear regression model in SAS 9.1 (SAS Institute, Cary, NC). *P* values of the multiple comparisons were adjusted by the Tukey–Kramer test.

RESULTS

Air sampling results. At least one air sample tested positive in five of the six flocks investigated. A total of 382 samples were tested by qRT-PCR as part of 138 air sampling events analyzed for the study. Thirty-three percent (45/138) of all air sampling events tested positive, 38% (52/138) suspect, and 30% (41/138) negative. From those, 36% (50/138) and 64% (88/138) were collected in turkey and layer flocks, respectively (Table 2). A total of 67% (26/39) sampling events collected inside and 45% (18/40) collected at 5 m were positive. Sampling at distances from 70 m and for up to 1000 m approximately resulted in 2% positives and 58% suspects (Table 2).

Table 2. Number (%) of air sampling events tested using reverse transcription PCR (RT-PCR) for H5N2 avian influenza virus detected inside and outside infected turkey and layer flocks.

RT-PCR[A]	Inside ($n = 39$)	5 m ($n = 40$)	70–150 m ($n = 29$)	500–1000 m ($n = 30$)	Total ($n = 138$)
Turkeys					
Positive	14 (56)	7 (50)	0 (0)	NA	21 (42)
Suspect	7 (26)	7 (50)	5 (56)	NA	19 (38)
Negative	6 (22)	0 (0)	4 (44)	NA	10 (20)
Layers					
Positive	12 (100)	11 (42)	1 (5)	0 (0)	24 (27)
Suspect	0 (0)	4 (15)	18 (90)	11 (37)	33 (38)
Negative	0 (0)	11 (42)	1 (5)	19 (65)	31 (35)
Total					
Positive	26 (67)	18 (45)	1 (3.5)	0 (0)	45 (33)
Suspect	7 (18)	11 (27.5)	23 (79)	11 (37)	52 (38)
Negative	6 (15)	11 (27.5)	5 (17.5)	19 (63)	41 (30)

[A]Ct values: positive, <35; suspect, 35 to <40; negative, >40.

Quantity of virus estimated by qRT-PCR (expressed as geometric mean of estimated RNA copies/m^3 [geometric SD]) ranged between 3.4×10^2 (5.1, at 70–150 m) and 2.5×10^6 (4.7, inside) and between 1.8×10^1 (at 500–1000 m) and 2.5×10^5 (4.8, inside) in turkey and layer flocks, respectively (Table 3). Samples collected inside and immediately outside had similar concentrations of virus (with the exception of the cyclonic collector samples in layer premises [$P = 0.0163$]). There was a significant decrease in virus concentration in air samples as distance increased, the difference being 1.5×10^2 (3.5, $P = 0.0025$) and of 1.6×10^2 (3.1, $P = 0.0002$) RNA copies/m^3 (geometric SD) between 0 and 70–150 m in turkeys and layers, respectively. However, no difference in the average quantity at the furthest compared to the closest distance was evident with the TCI sampler (Table 3).

AIV H5N2 clade 2.3.4.4 was isolated from 38% (18/48) of a subset of positive and suspect air samples collected inside, 52% (14/27) collected at 5 m outside and in one out of four samples collected at 70–150 m. Overall there were 14 sampling events (52%) from inside that yielded viable virus—10 (67%) at 5 m and 1 (25%) at 70 m. Most isolates were obtained from air samples with Ct ranges between 27.3 and 34.2, apart from one sample collected at 70–150 m with a Ct value of 37.5, and from which virus was recovered after second-passage egg inoculation. Viable virus was only recovered from stages collecting particles of diameter greater than 2.1 microns.

Particle size distribution. AIV H5N2 RNA was detected inside and immediately outside of the positive flocks in particles of all size ranges tested (Table 4). For the purposes of modeling the distribution of particles, Farm 4 was not included in the analysis, because only suspect and negative results were obtained. Overall there were significantly higher concentrations of airborne H5N2 virus associated with particles >9 μm and >3.3 μm in aerodynamic diameter compared to the smallest size ranges inside turkey and layer barns, respectively ($P < 0.05$; Table 4). A higher concentration of virus was also observed inside layer premises compared to immediately outside.

Table 3. Quantity (geometric mean of RNA copies/m^3 of air (geometric standard deviation) or GM [GSD]) of H5N2 avian influenza virus in suspect and positive sampling events collected at different distances using the cyclonic air collector (CC), and two sizes of selective air samplers: an Andersen cascade impactor (ACI) and a high-volume Tisch cascade impactor (TCI), in turkey and layer premises.[A]

Animal	Air sampler	Inside			5 m			70–150 m			500–1000 m		
		N[B]	n[C]	GM (GSD)	N	n	GM (GSD)	N	n	GM (GSD)	N	n	GM (GSD)
Turkeys	ACI	6	4	2.5×10^5 (2.6)a			NT[D]			NT			NT
	CC	12	9	1.5×10^4 (4.5)b	9	9	4.9×10^3 (4.5)a	6	3	3.4×10^2 (5.1)a			NT
	TCI	9	7	2.3×10^5 (4.4)a,c	5	5	1.3×10^5 (4.6)b	3	2	8.7×10^3 (5.6)a			NT
	Subtotal	27	20	1.2×10^5 (3.8)	14	14	5.1×10^4 (4.5)	9	5	5.3×10^3 (5.1)			NT
Layers	ACI	2	2	3.1×10^5 (2.7)a	6	3	2.7×10^4 (6.0)a			NT			NT
	CC	8	8	2×10^4 (3.7)a	16	16	1.5×10^3 (3.3)b	16	16	7.7×10^2 (3.3)a	24	9	1.8×10^1 (3.6)a
	TCI	2	2	3.6×10^4 (4.8)a	4	4	6.2×10^4 (3.9)a	4	4	4.4×10^4 (3.9)b	6	2	2.5×10^5 (4.9)b
	Sub-total	12	12	2.8×10^4 (3.7)	26	23	3.2×10^4 (4.4)	20	20	8.8×10^3 (3.6)	30	11	3.4×10^3 (3.9)

[A]Distinct on-line letters indicate differences in virus concentration among the different samplers for each animal species and distance (Tukey's test, $P < 0.05$).
[B]N = total sampling events.
[C]n = positive or suspect sampling events (Ct < 40).
[D]NT = not tested.

Environmental sampling results. Two out of the six facilities visited were sampled for surface environmental contamination. A total of 7 out of 20 (35%) and 8 out of 20 (40%) of all samples tested RT-PCR positive and suspect, respectively. We detected greater surface contamination in Farm 6 compared to Farm 5 (chi square 11.9192, $P = 0.0026$). Ct values in Farm 6 ranged between 29 and 32, indicative of relatively high amounts of H5N2 RNA on the surfaces of farm fixtures and temporarily exposed fomites. None of the samples positive by molecular assays were positive by virus isolation.

DISCUSSION

The rapid spread of H5N2 HPAI virus during the outbreaks of 2015 in the Mississippi flyway of the United States raised questions about the possibility of the aerosol route as a possible means of spread of HPAI. We detected viral RNA and isolated H5N2 HPAI virus from air samples collected inside and outside of affected poultry facilities. Viable H5N2 virus was associated with inhalable particles of sizes relevant to both human and animal health, and the aerosol route served as a source of environmental contamination to the surroundings of the affected premises. We provide evidence that H5N2 virus can be aerosolized from infected flocks and that the transport of infectious airborne particles and the deposition of the particles on surfaces around infected premises represent potential mechanisms for the spread of HPAI. Information on the risk of aerosol exposure to people in terms of viral load, viability, and particle size is necessary to design adequate prevention programs to protect personnel handling birds.

H5N2 virus was isolated mostly from samples collected inside and immediately outside infected premises. This is as anticipated, and likely reflects decreasing virus concentration by dilution as a function of distance from the source. The stage of clinical progression of outbreaks, the percentage of actively infected birds at the time of sampling, and timing of sampling in relation to flock depopulation and environmental conditions for the outside measurements would be expected to influence the differences in positive yields observed between flocks. Interestingly, one air sample at 70 m was isolation positive despite the high cycle threshold PCR value of 37.5, suggesting that relatively small amounts of virus can remain viable during translocation to further distances. In addition, failure to detect viable virus may not necessarily indicate absence of viable virus, because the air

Table 4. Modeled distribution of the quantity [geometric mean of RNA copies/m^3 of air (geometric standard deviation)] of H5N2 avian influenza virus by particle size measured in air samples collected with the Andersen cascade impactor inside and outside up to 5 m from turkey and poultry layer facilities.[A]

Particle size range (μm)	Turkeys (sampling inside)	Layers (sampling inside)	Layers (sampling at 5 m)
0.01–0.4	8.7×10^2 (9.0)a	4.1×10^4 (1.8)a	5.1×10^2 (5.1)a
0.4–0.7	3.7×10^2 (3.7)a	1.2×10^3 (1.2×10^1)a	8.9×10^2 (3.1)a
0.7–1.1	4.2×10^3 (3.7)a,b	2×10^4 (1.2)a	4.5×10^1 (4.5)a
1.1–2.1	2.2×10^4 (1.9)b,c	1.9×10^4 (1.8)a	1.6×10^3 (4.0)a
2.1–3.3	1.1×10^5 (1.2)b,c	9.6×10^4 (1.3)a, b	3.1×10^3 (5.6)a
3.3–4.7	2.2×10^5 (1.1)b,c	2.5×10^5 (1.3)b	9.6×10^3 (1×10^1)a
4.7–5.8	4.5×10^5 (1.1)b,c	4.3×10^5 (1.5)b	1.1×10^4 (1.1×10^1)a
5.8–9.0	2.1×10^5 (1.1)b,c	5.5×10^5 (1.5)b	9.2×10^3 (1.0×10^1)a
>9.0	1.3×10^6 (1.1)c	1.4×10^6 (2.6)b	2.1×10^5 (1.7)a
Total	2.6×10^5 (2.6)	3.1×10^5 (2.7)	2.7×10^4 (6.0)

[A]Different on-line letters in rows of the same column indicate statistically significant differences (Tukey's test, $P < 0.05$).

sampling process could contribute to the inactivation of the virus. Overall, more than 75% of sampling events inside and immediately outside barns, and 60% at distances as far as 1000 m yielded detectable viral genetic material, suggesting some risk for aerosol spread. However, conclusions on likelihood of HPAI virus aerosol transmission should be made with supporting epidemiological evidence.

Determining the particle size distribution for the H5N2 virus has important implications for the control of animal and human disease, and the use of droplet and airborne infection control measures. Infectious particles with aerodynamic diameter smaller than 10 μm have more serious health implications than larger particles, as they are inhaled into the lower respiratory tract. H5N2 was detected in all particle size ranges measured, although isolation was particle-size dependent and limited to particles larger than 2.1 μm, suggesting a high risk of occupational exposure if proper personal protection measures are not taken. Similar results on virus viability and particle-size distribution have been shown for swine influenza virus (1).

Deposition of airborne particles on surfaces in environments surrounding infected premises is also potentially significant. We tested farm fixtures and study equipment that had been directly exposed to exhausted air for 2 hr. Although the results from farm fixtures were mostly PCR negative (likely because of desiccation of the genetic material), the study materials harbored high levels of viral genetic material, indicative of rapid contamination of the surrounding environments and fomites located temporarily near the farm. Although viable virus was not demonstrated in the surface samples, likely because of the direct exposure of sunlight and the warm environmental conditions at the time of sampling, we speculate that exhausted air could potentially be a relevant source of surface environmental contamination responsible for the spread of virus between barns or farms. Additionally, it is possible that footwear and hands, as well as fomites such as vehicles, feed trucks, outside equipment, and other materials that may be stored outside and shared between farms, may be contaminated as a result of airborne particle deposition and could contribute to the regional dissemination of viruses. In addition, infectious particles on surfaces could be accessible to birds and ingested, representing a potential source of infection to birds.

Our results also indicate that there is an immediate need to develop strategies and technologies to contain bioaerosols to limit the spread of epidemics in food animal production systems. Air sanitation technologies such as exhaust air filtration, or turning off fans in the case of mechanically ventilated facilities, as well as preventing vehicle movements from infected sites and completely stopping the sharing of equipment, materials, and personnel between sites should be considered in order to minimize the risk of area spread. Other considerations, such as the well-being of the animals and economics of the measures, will need to be taken into consideration, but overall our data point to the need for proactive bioaerosol containment measures that are rapidly deployable in the face of an outbreak.

In summary, our study provides evidence for the role of bioaerosols as a means of both airborne transport and environmental deposition of avian influenza virus particles during HPAI outbreaks. Data from this study should be used in conjunction with epidemiological findings to assess the risk of the airborne route in the spread of HPAI virus to neighboring farms fully.

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