Measurement of Airborne Influenza Virus in a Hospital Emergency Department

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Size-fractionated aerosol particles were collected in a hospital emergency department to test for airborne influenza virus. Using real-time polymerase chain reaction, we confirmed the presence of airborne influenza virus and found that 53% of detectable influenza virus particles were within the respirable aerosol fraction. Our results provide evidence that influenza virus may spread through the airborne route.

Influenza is a highly contagious respiratory illness that results in >250,000 deaths annually worldwide [1]. Currently, influenza virus is known to be spread from person to person by at least 2 mechanisms: direct and indirect transfer of respiratory secretions and contact with large droplets that settle onto fomites [2]. In addition, influenza virus may also be transmitted by inhalation of small airborne particles [3], but this potential route is not well characterized and remains controversial.

Coughing, sneezing, talking, and breathing generate a cloud of airborne particles with diameters that can range from a few millimeters to <1 μm. Large droplets (diameter, >50 μm) settle on the ground almost immediately, and intermediate-sized droplets (diameter, 10–50 μm) settle within several minutes. Small particles (diameter, <10 μm), including droplet nuclei from evaporated larger particles, can remain airborne for hours and are easily inhaled deeply into the respiratory tract [4]. Alford et al. [5] revealed that humans could contract influenza by inhaling an experimental small-particle aerosol containing low levels of influenza virus. Studies involving mice, ferrets, and guinea pigs have demonstrated airborne animal-to-animal transmission [6, 7, 8]. Observational and epidemiological studies suggest that airborne influenza transmission occurs among people [9, 10], although these studies have been unable to clearly delineate a causal relationship.

The purpose of the present study was to measure the amount and size of airborne particles containing influenza virus in a health care facility. Size-fractionated aerosol samples were collected in a hospital emergency department during the February 2008 influenza season and were analyzed using real-time PCR. The results demonstrate that influenza virus was present in airborne particles in the respirable size range.

Materials and methods. Aerosol samples were collected using a modified National Institute for Occupational Safety and Health 2-stage cyclone aerosol sampler [11]. The first stage of the sampler was enlarged to have a 3-mm inlet, a 6-mm outlet, and a disposable 15-mL collection tube (35-2096; Falcon). The second stage had a 1.3-mm inlet, a 2.5-mm outlet, and a disposable 1.5-mL tube (02-681-339; Fisher Scientific). Then, the samples were passed through a 37-mm polytetrafluoroethylene filter with 2-μm pores (225-27-07; SKC). At 3.5 L/min, the first stage collected particles with a diameter >4 μm, the second stage collected particles with a diameter of 1–4 μm, and the filter collected particles with a diameter <1 μm. The sampler conforms to the American Conference of Governmental Industrial Hygienists/International Organization for Standardization criteria for respirable particle sampling. To eliminate carry-over contamination, samplers were washed with isopropanol and air dried after each sampling day.

Samples were collected in the emergency department at the West Virginia University Hospital (Morgantown) during February 2008. Collection of samples was conducted on 6 afternoons; a total of 74 stationary aerosol samplers and 7 personal aerosol samplers were used. Two stationary samplers were mounted 91 cm and 183 cm above the floor on each tripod in the general waiting room (1 tripod on the first day and 3 tripods on subsequent days). Tripods with 2 samplers were also placed in the children's waiting room and in 2 randomly selected examination rooms. One stationary sampler was placed ~135 cm above the floor in the reception and triage room. Stationary samplers were operated for 4–5 h. Personal aerosol samplers were worn by 7 physicians for 3–4 h, and each phy-

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The findings and conclusions in this report have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.

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Table 1. Clinical investigation of airborne influenza in a hospital emergency department.

Day	No. of patients reporting influenza-like symptoms	Total no. of stationary samplers	Total no. of personal samplers	Samplers showing results positive for influenza virus	No. of $TCID_{50}$-equivalent RNA particles detected in the sampler			
					First stage	Second stage	Filter	Total
1	4	9	4	Waiting room (lower sampler)	460	0	0	460
				Waiting room (upper sampler)	0	13,426	2852	16,278
				Reception and triage room	0	1941	0	1941
				Personal sampler (physician 1)	3160	0	0	3160
				Personal sampler (physician 2)	309	0	0	309
				Personal sampler (physician 3)	0	4623	0	4623
2	0	13	0	Waiting room, (upper sampler)	1114	0	0	1114
3	5	13	1	None	…	…	…	…
4	3	13	0	Children's waiting room (lower sampler)	4025	11,040	0	15,065
				Children's waiting room (upper sampler)	5762	<100	0	5762
				Waiting room (lower sampler)	15,532	0	0	15,532
				Waiting room (lower sampler)	0	1367	0	1367

NOTE. $TCID_{50}$, median tissue culture infective dose.

sician underwent a QuickVue Influenza test (Quidel) to rule out influenza virus particles being collected from the physician.

The mean indoor temperature (±SD) was 23.5°C ± 1.4°C, with a mean relative humidity (±SD) of 30.0% ± 3.3% and a mean air pressure (±SD) of 97,200 ± 700 Pa. Design room air–change rates were 8–12 air changes/h in the waiting rooms and in the reception and triage room and 3–15 air changes/h in the examination rooms.

Lysis/Binding Solution (Ambion) was added directly to the 2 aerosol sampler tubes. Back-up filters were transferred to 50-mL polypropylene conical tubes (Becton Dickinson) containing the Lysis/Binding Solution. Samples were spiked with XenoRNA-01 (Ambion) as an internal control. RNA was extracted from all samples with use of the MagMAX-96 Viral RNA Isolation Kit (Ambion). Isolation of viral RNA from a dose of FluMist vaccine (MedImmune Vaccine) was performed as described elsewhere [12].

Complementary DNA (cDNA) was generated by reverse transcription of the isolated RNA with use of the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Samples were amplified in an Eppendorf Mastercycler under the following conditions: 25°C for 10 min, 37°C for 120 min, and 85°C for 5 s. A negative control (without a template) was run for each day that samples were obtained.

Real-time PCR detection of a conserved region of the matrix gene (M1) of influenza A virus and the XenoRNA-01 internal control was performed using the AIV-M Primer Probe Mix and Detection Enhancer from the AgPath-ID AIV-Matrix Gene Reagent Kit (Ambion). Primers for the homologous region in the influenza B matrix gene were synthesized by the Applied Biosystems Custom Oligo Synthesis Service.

Real-time PCR for influenza was run using TaqMan chemistry (Applied Biosystems) on a 7500 Real-Time PCR System (Applied Biosystems) at the following conditions: 50°C for 2 min, 95°C for 10 min, 65 cycles at 95°C for 15 s, and 60°C for 1 min. XenoRNA-01 reactions were run similarly, except a total of 50 cycles was used. With the assumption that 1 median tissue culture infective dose ($TCID_{50}$) unit is equivalent to 1 viral RNA copy, the relative number of $TCID_{50}$ viral particles was calculated by regression analysis for comparison with 10-fold serial dilutions of FluMist cDNA isolated from $10^{6.5–7.5}$ $TCID_{50}$ live-attenuated influenza virus [12]. A negative control without a template was included in all real-time PCR assays, and all reactions were run in duplicate.

PCR samples that tested positive for the influenza A matrix gene were column purified using the QIAquick PCR Purification Kit according to the manufacturer's instruction (Qiagen). Samples were submitted to the Sequencing and Synthesis Facility at the University of Georgia Office of Research Services (Athens, GA). All sequencing reactions were run using the Matrix-specific primer: 5′-TGCAAAAACATCTTCAAGTCTCTG-3′ (Applied Biosystems). Basic Local Alignment Search Tool (BLAST) was used to confirm the identity of the amplified viral M1 RNA.

Results. Aerosolized influenza A virus was detected on 3 separate days in 11 samplers (table 1). On 2 days (days 5 and 6), the internal controls were negative; therefore, the data were not used. On days with positive internal controls, influenza-positive samples were found in 8 stationary samplers located in the waiting rooms and triage room and in 3 personal samplers worn by emergency department physicians. No influenza virus was detected in stationary samplers located in examination rooms. Because of limited amounts of cDNA, testing for influenza B virus was performed only on the samples collected

from the emergency department on day 1; the results of these tests were negative. Eighty-four percent of all tests positive for influenza at the West Virginia University Hospital laboratory during February 2008 were positive for influenza A (data not shown).

Forty-six percent of the influenza virus particles were found in the first stage of the samplers, which collected particles with a diameter >4 μm. However, 49% of the isolates were collected in the second stage, which collects particles with a diameter of 1–4-μm, and 4% were collected on the back-up filter, which collects particles with a diameter <1 μm. These findings indicate that >50% of the total viral particles were found in the respiratory aerosol fraction. All 14 influenza A virus–positive PCR samples were sequenced and were confirmed to be an amplification product of the influenza A matrix gene sequence (data not shown).

Discussion. In our study, we revealed, to our knowledge for the first time, the presence of airborne influenza virus particles in a health care environment. Patients acutely infected with influenza have elevated viral titers in their respiratory secretions [13], and the mean duration of influenza viral shedding is ~5 days [2]. Because a large number of exhaled respiratory secretions are <10 μm in diameter, the potential transmission of influenza virus through the airborne route cannot be overlooked. In our study, more than one-half of the viral particles detected by PCR were within the respirable aerosol fraction (diameter, <4 μm), and these results support the hypothesis that influenza virus can be transmitted through the airborne route.

The detection of airborne viral particles is difficult. Because of greater sensitivity and specificity, real-time PCR was used in conjunction with a novel cyclone aerosol sampler [11]. Potential pitfalls of PCR include the inability to determine viability of the organism, false-positive reactions, and interferences. Airborne contaminates have been shown to interfere with PCR in aerosol samples [14]. In our study, all aerosol samples were spiked with a control RNA before processing. The internal control failed to generate a detectable signal for samples obtained from the emergency department on days 5 and 6, which suggests the presence of an interfering substance, poor RNA isolation, or degradation of the RNA sample on those days. To rule out false-positive results, all positive results were confirmed by subsequent sequence analysis. Future studies will examine various methods for enhancing viral RNA isolation and diminishing inhibitors of reverse transcription and/or real-time PCRs.

In conclusion, health care facilities may contain detectable amounts of airborne influenza virus during the influenza season. A number of factors, including temperature, humidity, and severity of the influenza season, could influence the concentration of viral particles in an aerosol sample. Future studies will be needed to address the viability and infectivity of these viral aerosols and, ultimately, will shed light on the relative importance of airborne transmission of influenza.

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