Distribution of Airborne Influenza Virus and Respiratory Syncytial Virus in an Urgent Care Medical Clinic

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Background. Considerable controversy exists with regard to whether influenza virus and respiratory syncytial virus (RSV) are spread by the inhalation of infectious airborne particles and about the importance of this route, compared with droplet or contact transmission.

Methods. Airborne particles were collected in an urgent care clinic with use of stationary and personal aerosol samplers. The amounts of airborne influenza A, influenza B, and RSV RNA were determined using real-time quantitative polymerase chain reaction. Health care workers and patients participating in the study were tested for influenza.

Results. Seventeen percent of the stationary samplers contained influenza A RNA, 1% contained influenza B RNA, and 32% contained RSV RNA. Nineteen percent of the personal samplers contained influenza A RNA, none contained influenza B RNA, and 38% contained RSV RNA. The number of samplers containing influenza RNA correlated well with the number and location of patients with influenza ($r = 0.77$). Forty-two percent of the influenza A RNA was in particles ≤ 4.1 μm in aerodynamic diameter, and 9% of the RSV RNA was in particles ≤ 4.1 μm.

Conclusions. Airborne particles containing influenza and RSV RNA were detected throughout a health care facility. The particles were small enough to remain airborne for an extended time and to be inhaled deeply into the respiratory tract. These results support the possibility that influenza and RSV can be transmitted by the airborne route and suggest that further investigation of the potential of these particles to transmit infection is warranted.

Influenza and respiratory syncytial virus (RSV) are common highly transmissible respiratory viruses. Influenza results in an estimated 36,000 deaths in the United States each year [1] and is of great concern because of the potential for a severe influenza pandemic. RSV is a common respiratory illness among young children, with a very high attack rate, and is also important among the elderly and adults at high risk [1, 2].

The transmission of influenza among individuals is not well understood. Influenza is thought to spread via infectious secretions transferred by touch, by large ballistic drops, and possibly through the inhalation of small aerosol particles [3]. However, the relative importance of these routes of transmission is controversial. Several studies have concluded that airborne transmission of influenza is a key pathway (reviewed in [4–6]). Other investigators maintain that airborne particles are not a significant means of infection (reviewed in [7]).

When infected individuals cough, sneeze, speak, and breathe, they produce a cloud of potentially infectious particles. Using polymerase chain reaction (PCR), Fabian et al [8] showed that 60% of patients with influ-

enza A and 14% of patients with influenza B had detectable levels of viral RNA in their exhaled breath. Alford et al [9] showed that humans can contract influenza by inhaling an experimental infectious aerosol. Studies in ferrets demonstrated airborne animal-to-animal influenza transmission [10]; similar results were seen in mice, although at much shorter distances [11]. Several studies using guinea pigs have shown that airborne transmission of influenza readily occurs and that the efficiency of transmission depends on the environmental humidity and temperature, as well as the viral strain [12–14]. Some observational and epidemiological studies suggest that influenza may spread from person to person by infectious aerosols [15, 16], but this interpretation is disputed [7].

RSV is usually thought to spread by direct and indirect contact with respiratory secretions and not by inhalation of airborne particles. A study of RSV transmission between infants and health care workers in a pediatric ward concluded that RSV spreads by direct inoculation with large droplets or by self-inoculation after touching contaminated surfaces [17]. On the other hand, a hospital study found airborne RSV RNA in 63% of rooms with RSV-infected patients and as far as 7 m from the patient's bedside [18]. Aside from these few reports, very little is known about the amount of airborne RSV in health care environments or the possibility of airborne RSV transmission.

Previously, we performed a study in a hospital emergency department and found airborne influenza virus RNA in the waiting areas and in personal samplers worn by health care workers. Half of the airborne virus RNA was in particles in the respirable size range [19]. The purpose of the present work was to study the amount and size of airborne particles containing influenza and RSV RNA in an urgent care clinic and to examine the relationship between airborne influenza RNA detection and the number and location of patients with influenza.

MATERIALS AND METHODS

Aerosol samples were obtained at the West Virginia University Urgent Care Clinic (Morgantown) for 11 days for 4–5 h each day during February 2009. Stationary National Institute for Occupational Safety and Health 2-stage cyclone aerosol samplers [19] were mounted in pairs on tripods, with 1 sampler 152 cm above the floor ("upper") and 1 sampler 102 cm above the floor ("lower"). Three tripods were placed in the patient waiting room, 1 was placed in each of 6 examination rooms and in 2 procedure rooms, and 1 was placed next to the patient scale. Aerosol samplers were also worn as personal samplers by 2 health care workers each day.

Health care workers who wore personal aerosol samplers were screened for influenza-like symptoms, elevated oral temperature, and influenza with use of a rapid influenza test (QuickVue Influenza test; Quidel). Sixty-seven percent of the full-time staff and residents at the clinic received influenza vaccine before the study. All adult patients and visitors who presented at the clinic with respiratory symptoms were asked to volunteer for influenza testing. If the result of the rapid test was negative, the result was verified using PCR. All study participants provided informed consent and were notified of their test results. Influenza testing was not performed for persons aged <18 years as part of the study; however, if the physician ordered an influenza test, the results were provided to us. No testing was done for RSV. All procedures involving humans were reviewed and approved by the National Institute for Occupational Safety and Health and the West Virginia University Institutional Review Boards.

After collection, aerosol samples were suspended in lysis/binding solution concentrate (Ambion) and stored at −20°C overnight. After thawing, carrier RNA (Ambion) was added to enhance RNA extraction, and XenoRNA (Applied Biosystems) was added as a quantitative PCR internal control. Total RNA was extracted as reported elsewhere [19].

To detect influenza and RSV RNA, the AgPath-ID One-Step Reverse-Transcription PCR kit (Applied Biosystems) was used. Primers and probes for influenza were from the Centers for Disease Control and Prevention real-time reverse-transcription PCR assay for detection and characterization of influenza (provided by S. Lindstrom, Centers for Disease Control and Prevention; protocols are available from the Centers for Disease Control and Prevention upon request). Primers and the probe

Table 1. Number and Locations of Aerosol Samplers Positive for Each Type of Viral RNA

Samplers	No. (%) of samplers					
	Personal samplers	All stationary samplers	Examination rooms	Waiting room	Procedure rooms	Scale in corridor
Total deployed	21	264	132	66	44	22
Positive for influenza A	4 (19.1)	46 (17.4)	29 (22.0)	10 (15.2)	6 (13.6)	1 (4.6)
Positive for influenza B	0 (0)	3 (1.1)	2 (1.5)	0 (0)	0 (0)	1 (4.6)
Positive for RSV	8 (38.1)	84 (31.8)	48 (36.4)	23 (34.9)	6 (13.6)	7 (31.8)

NOTE. A sampler was positive if viral RNA was detected in at least 1 sampler stage (ie, in the first or second tube or on the filter). RSV, respiratory syncytial virus.
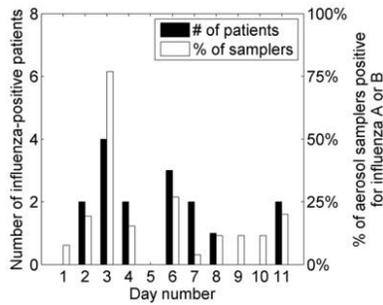
Figure 1. Number of patients with clinically confirmed influenza and percentage of aerosol samplers positive for influenza A or B, by sampling day.

for RSV were from Kuypers et al [20]. To determine the relative quantity of viral RNA, reference standards were isolated from FluMist influenza vaccine (MedImmune) and RSV-A RNA (VR-1540; ATCC). The FluMist vaccine and RSV-A stocks have infectivities of ~4.2 50% tissue culture infective dose per pg RNA and 0.060 50% tissue culture infective dose per pg RNA, respectively. The concentration of both standards was determined using absorbance spectroscopy (Nanodrop; Thermo Scientific) and was initially diluted to 24 ng/μL. Standard curves were generated from 10-fold serial dilutions and were analyzed in parallel with the collected aerosol samples. Real-time PCR detection of the XenoRNA internal control was performed using the XenoRNA Control TaqMan Gene Expression Assay from the TaqMan Cells-to-CT Control Kit (Applied Biosystems). The internal controls were amplified in all samples. For the first-stage samples from day 7, the internal controls had higher threshold cycle values than were seen in other samples, which may indicate that some interference occurred with the cDNA transcription and/or quantitative PCR analysis. Because the effect of any interference would be to reduce the estimate of the amount of influenza in the samplers, no attempt was made to adjust for this. A negative control without template was included in all real-time PCR reactions.

RESULTS

A total of 264 stationary aerosol samplers and 21 personal aerosol samplers were deployed during the 11 days of the study. The number and locations of aerosol samplers positive for viral RNA are shown in Table 1. During the study period, 38 adult patients and 3 adult visitors presented at the clinic with respiratory symptoms. Of these, 39 agreed to participate in the study and were tested for influenza. Two participants were positive for influenza by rapid test (the test did not distinguish between influenza A and B), and 9 of the rapid test–negative participants were positive for influenza A by subsequent PCR analysis. Twenty-nine children with respiratory illness also presented at the clinic during this time. Ten children were tested

for influenza with use of the rapid test, with 5 positive results. PCR results of 2 of the negative rapid test results were also negative.

Figure 1 compares the number of patients clinically confirmed to have influenza with the number of influenza-positive aerosol samplers for each day during which samples were obtained. All patients entered the clinic through the waiting area and passed by the patient scale in the corridor. However, because patients typically only entered 1 examination room (sometimes followed by a procedure room), it was possible to track the influenza-positive patients to individual rooms and compare this with the number of influenza-positive aerosol samplers. Stationary samplers were placed in 6 examination and 2 procedure rooms during each of 11 days for a total of 88 room sampling sessions. Influenza-positive patients were present during 16 of these sessions (in some instances, 2 patients occupied a room at the same time or sequentially during the session). In 13 of these 16 sessions, one or both aerosol samplers were positive for influenza A, and both samplers were negative for influenza A and B during the other 3 sessions. Conversely, in 13 of the remaining 72 sessions, one or both samplers were positive even though no patients in these rooms were verified to have influenza during sampling (11 room sessions were positive for influenza A and 2 for influenza B). In 59 sessions, samplers were negative for influenza and no confirmed influenza-positive patients occupied these rooms during sampling.

The 2-stage cyclone aerosol sampler separates the aerosol particles into 3 size fractions, as shown in Figure 2. In the stationary samplers, 43% of influenza A strains were in particles $\leqslant$4.1 μm in aerodynamic diameter, and none of the influenza B strains were contained in particles in this size range. In the personal samplers, 48% of influenza A strains were in particles $\leqslant$4.9 μm in aerodynamic diameter. Influenza B was not found in any of the personal samplers. Nine percent of RSV strains
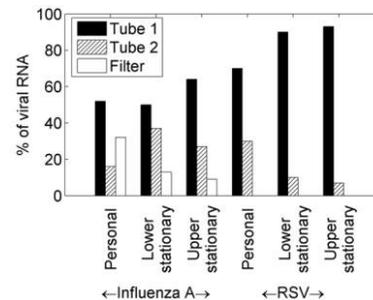

Figure 2. Distribution of viral RNA within samplers. Aerosol size ranges for stationary samplers were: tube 1, particles $\geqslant$4.1 μm aerodynamic diameter; tube 2, 1–4.1 μm; filter, $\leqslant$1 μm. Because they operated at a lower flow rate, the collection size fractions for the personal samplers were slightly larger: tube 1 contained particles $\geqslant$4.9 μm; tube 2, 1.7–4.9 μm; filter, $\leqslant$1.7 μm.
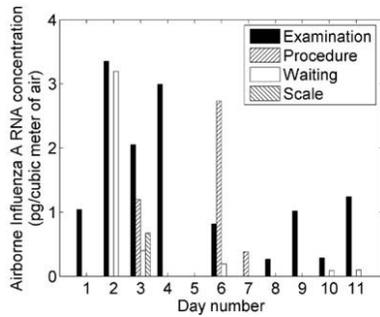
Figure 3. Airborne concentration of influenza A RNA in influenza A–positive clinic locations, by day of sampling. For instances in which >1 examination room or procedure room was positive for influenza A on a particular day, the geometric mean concentration is shown.

were in particles ≤4.1 μm, and virtually none of the RSV strains in the personal or stationary samplers was found in the smallest particles (≤1.7 and 1 μm, respectively). Fifty-one percent of the influenza A RNA and 55% of the RSV RNA from the stationary locations was found in the upper samplers, with the remainder in the lower samplers.

Figure 3 shows the concentration of airborne influenza A RNA for each day in the locations where it was detected. For the influenza A-positive locations, the geometric mean concentration (±standard deviation [SD]) was 1.2 ± 4.4 pg RNA/m³ in the examination rooms, 1.1 ± 3.0 pg RNA/m³ in the procedure rooms, 0.3 ± 4.3 pg RNA/m³ in the waiting room, and 0.7 pg RNA/m³ next to the patient scale (no SD because only 1 day was positive). The airborne concentrations in the influenza A-positive examination rooms ranged from 0.1 to 7.3 pg RNA/m³ of air, except for 1 room with a concentration of 75.4 pg RNA/m³. In the examination and procedure rooms in which influenza A was detected and patients with laboratory-confirmed influenza were present, the mean airborne concentration (±SD) was 2.1 ± 4.7 pg/m³. In examination and procedure rooms not occupied by a patient with confirmed influenza but in which influenza A was detected, the mean airborne concentration (±SD) was 0.6 ± 2.5 pg/m³.

Similarly, Figure 4 shows the concentration of RSV RNA by day at the locations in which it was detected. The geometric mean concentration (±SD) in the RSV-positive locations was 0.052 ± 4.1 pg RNA/m³ in the examination rooms, 0.044 ± 2.6 pg RNA/m³ in the procedure rooms, 0.031 ± 5.5 pg RNA/m³ in the waiting room, and 0.042 ± 2.8 pg RNA/m³ next to the patient scale. The airborne concentrations in the RSV-positive examination rooms ranged from 0.01 to 0.53 pg RNA/m³, except for 1 room with a concentration of 9.92 pg RNA/m³. In the waiting room, the concentration ranged from 0.004 to 0.14 pg RNA/m³, except for 1 day when the concentration was 0.87 pg RNA/m³.

Influenza virus was detected in 4 personal aerosol samplers. For workers wearing samplers positive for influenza, the amount of airborne influenza in their work environment was 0.6–3.9 pg influenza A RNA/m³ air, with a geometric mean exposure (±SD) of 1.6 ± 2.3 pg influenza A RNA/m³ air. Airborne RSV RNA was detected in 8 personal aerosol samplers. For these samplers, the amount of airborne material in the work environment ranged from 1.4×10^{-4} to 0.21 pg/m³, with a geometric mean (±SD) of 0.028 ± 11.0 pg/m³.

During the study, the mean temperature (±SD) was 22.8 ± 0.9°C), with a relative humidity of 18.2 ± 5.8%). The air exchange rate in an examination room was 6.3 air changes/h, and the air exchange rate in the waiting room was 24 air changes/h.

DISCUSSION

In our study, airborne influenza RNA was detected on 10 of 11 days, and airborne RSV RNA was detected every day. During the peak day with 4 patients with confirmed influenza, 79% of the stationary samplers and 1 of 2 personal samplers (50%) collected influenza A RNA. These results show that airborne influenza A virus RNA was widespread in this clinic, especially during the periods of heaviest patient loads. Much less airborne influenza B RNA was detected; this was expected because influenza A was more prevalent in the community during the study period. Airborne RSV RNA was more widespread than was influenza; it was found in 32% of stationary samplers and 38% of personal samplers overall and in 71% of the stationary samplers and both personal samplers (100%) during the day with the highest number of RSV-positive samplers.

The number of influenza-positive samplers correlated well with the number of influenza-positive patients ($r = 0.77$). The correlation was probably reduced by undetected cases of influenza in the clinic, because not all patients and visitors were tested for influenza, influenza virus shedding begins before
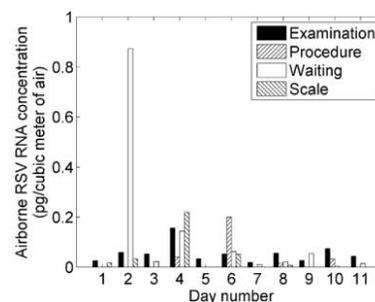

Figure 4. Airborne concentration of respiratory syncytial virus (RSV) RNA in RSV-positive clinic locations, by day of sampling. For instances in which >1 examination room or procedure room was positive for RSV on a particular day, the geometric mean concentration is shown.

symptoms appear, and asymptomatic cases of influenza are thought to be fairly common [21]. In the examination and procedure rooms, airborne influenza A RNA was detected during 81% of the room sampling sessions in rooms that contained patients with influenza. Influenza A or B was also detected at lower concentrations in 18% of rooms that did not have confirmed influenza cases, which again is most likely attributable to undetected influenza cases.

Part of the debate about whether respiratory viruses can be transmitted by air depends on the size of the aerosol particles produced by infected individuals. In our study, 42% of the influenza A RNA in the stationary samplers was contained in particles with an aerodynamic diameter <4.1 μm, and 11% had aerodynamic diameters <1 μm. In the personal samplers, 48% of the influenza A RNA was in particles <4.9 μm, and 32% was in particles <1.7 μm. These results suggest that particles containing influenza RNA are present that are small enough to remain airborne for a long time and disperse throughout a room occupied by a patient with influenza. A 4-μm particle takes 33 min to settle 1 m in still air, and a 1-μm particle takes 8 h; in addition, room air mixing and turbulence can keep these particles airborne even longer. It is worth noting that the stationary samplers in the examination rooms, where most of the airborne influenza RNA was detected, were located 1.8 m (6 ft) from the patient examination table next to the wall on the opposite side of the room. The influenza RNA was also distributed fairly evenly between the upper and lower samplers, suggesting that the particles were not settling appreciably and that the room air currents were dominating their movement. Thus, the influenza virus particles detected in this study were clearly able to travel beyond the often-cited 3–6-ft limit for droplet transmission of influenza, although whether these particles can cause infection is not yet known.

In addition to remaining airborne for an extended time, the aerosol fraction that is <4 μm (the "respirable fraction" [22]) is small enough to be drawn down into the alveolar region of the lungs. Human experiments have suggested that a considerably smaller amount of influenza is needed to initiate an infection in the alveolar region, compared with intranasal inoculation [23]. For this reason, even though these particles are small, their potential for the spread of illness should not be dismissed.

In contrast to influenza, only 8% of the RSV RNA in the stationary samplers was <4.1 μm, and 30% of the RSV RNA in the personal samplers was <4.9 μm. This suggests that the RSV-laden viral particles are generally larger than those containing influenza, although the collected particles were still small enough to remain airborne and be drawn into the samplers.

The airborne concentrations of influenza A and RSV RNA

were highest in the examination rooms. This was expected, because patients tended to be moved relatively quickly from the waiting area and spent most of their time in the examination rooms with the door closed. In addition, the examination rooms were much smaller than the waiting area and had a lower air exchange rate (6 air changes/h, which is the current standard for medical examination rooms [24]). By comparison, the waiting room had an air exchange rate of 24 air changes/h, which is exceptionally high. In all rooms, the air inlets and outlets were in the ceiling, which may have reduced air mixing and increased the persistence of airborne material in some locations.

Finally, some limitations of our study must be acknowledged. Our detection methods determined the amount of viral RNA present in the clinical aerosol samples but did not measure the viability or infectivity of the airborne viral particles or other factors that may affect transmission. Thus, the risk of infection of health care personnel and patients as the result of exposure to these particles remains to be determined. Viral culture of samples would be an important step to add to subsequent studies. Second, although the majority of the adult patients with symptoms of upper respiratory illness were tested for influenza, most of the children were not. It is also possible that some asymptomatic individuals were shedding influenza virus into the environment, especially because influenza viral shedding typically peaks 24 h before symptoms do [21]. It is therefore likely that the numbers of people with influenza in the clinic were undercounted in our study.

In conclusion, our results show that airborne influenza and RSV RNA can be found throughout a typical health care clinic during influenza season. Especially in the case of influenza, much of the viral material was contained in particles small enough to remain airborne for an extended time, to disperse widely throughout the environment, and to be inhaled deeply into the respiratory tract. These results support the possibility that airborne transmission may occur during peak periods of seasonal influenza or an influenza pandemic and indicate that additional research is needed to understand the potential for the transmission of influenza by aerosols. RSV tends to be present in lower concentrations but more widely distributed, which suggests that the possibility of airborne transmission of RSV also should be investigated further.

Potential conflicts of interest. All authors: no conflicts.

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