Detection of Airborne Rhinovirus and Its Relation to Outdoor Air Supply in Office Environments

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Rhinoviruses are major causes of morbidity in patients with respiratory diseases; however, their modes of transmission are controversial. We investigated detection of airborne rhinovirus in office environments by polymerase chain reaction technology and related detection to outdoor air supply rates. We sampled air from 9 A.M. to 5 P.M. each workday, with each sample run for 1 work week. We directly extracted RNA from the filters for nested reverse transcriptase–polymerase chain reaction analysis of rhinovirus. Nasal lavage samples from building occupants with upper respiratory infections were also collected. Indoor carbon dioxide (CO_2) concentrations were recorded every 10 minutes as a surrogate for outdoor air supply. To increase the range of CO_2 concentrations, we adjusted the outdoor air supply rates every 3 months. Generalized additive models demonstrated an association between the probability of detecting airborne rhinovirus and a weekly average CO_2 concentration greater than approximately 100 ppm, after controlling for covariates. In addition, one rhinovirus from a nasal lavage contained an identical nucleic acid sequence similar to that in the building air collected during the same week. These results suggest that occupants in buildings with low outdoor air supply may have an increased risk of exposure to infectious droplet nuclei emanating from a fellow building occupant.

Keywords: rhinovirus; air sampling; ventilation; office buildings

Viral respiratory infections are the most common infectious disease in the United States and are associated with $25 billion in direct and indirect costs, excessive and inappropriate antibiotic prescribing, and 20 million days of lost work each year (1). Despite this, in the past 25 years, only a handful of studies have considered the link between ventilation of buildings and respiratory illness in their occupants (2–7). In a study of military barracks, Brundage and colleagues (2) detected significantly higher rates of febrile respiratory illness among soldiers housed in modern barracks with low outdoor air ventilation rates as compared with soldiers housed in older barracks with higher ventilation rates. In a study conducted during the 1991 Gulf War, Richards and colleagues (7) concluded that upper respiratory tract infections were more frequent in troops housed in air-conditioned barracks when compared with those housed in tents. These studies collectively suggest that increased ventilation of buildings could reduce the burden of respiratory viral infections among the occupants and that airborne transmission is an important mode of transmission.

Several studies have been conducted investigating the air quality on aircraft and upper respiratory symptoms of passengers. Case studies of outbreaks of airborne infection on aircraft have been described for influenza and tuberculosis (8, 9). More recently, a study found no difference in self-reported upper respiratory symptoms of passengers in planes that did not recirculate air compared with passengers in planes that recirculated approximately 50% of the cabin air (10). Unfortunately, this study did not record air quality measurements or evaluate the filtration efficiencies of the air-handling units in the planes that recirculated air. If the filtration efficiencies were high, the delivery rate of uncontaminated air may have been similar on both types of aircrafts and no difference would be expected.

A study of office workers suggested that workers who share offices were at increased risk of upper respiratory tract infections compared with workers in private offices, although this study failed to prove that the infections were transmitted as airborne infections (4). In an older study, first published in 1960, Gwaltney and colleagues conducted an extensive study at a large insurance company. Their results suggested that working adults were responsible for introducing 32% of the upper respiratory tract infections into their families (11–13), but again, the mode of transmission at work was not described.

Although rhinoviruses have been associated with approximately 40 to 65% of "common colds" throughout the year (14, 15) and up to 80 to 92% of colds during outbreaks (16), their method of transmission is controversial. Gwaltney and Hendley (17) conducted an experiment indicating that rhinovirus was not transmitted via airborne droplet nuclei. Several other experimental studies, however, appear to demonstrate that airborne transmission of rhinovirus is possible (18–20). In this study, we report an investigation of rhinoviruses in office buildings using molecular techniques to detect and identify rhinovirus in building air and in nasal mucus of building occupants. We relate the frequency of detection to the concentration of exhaled breath in building air using carbon dioxide (CO_2) as a marker for exhaled breath and outdoor air supply rate. In addition, we demonstrate the ability to detect identical rhinovirus in an occupant's nasal mucus sample and the corresponding air sample taken during the occupant's illness.

METHODS

Study Design

We studied three office buildings in suburban Boston, Massachusetts over a 20-month period. All offices were of an open-plan design, with large open areas containing cubicles, surrounded by private offices on

the exterior walls. All three office buildings were mechanically ventilated without humidification (Table 1). Head counts were performed on randomly selected days once or twice per week to record actual occupancy. The outdoor air-supply dampers were alternately adjusted approximately every 3 months to reduce or increase the outdoor air supply and consequently increase the range of CO_2 concentrations in the study buildings. All ventilation levels and CO_2 concentrations were within the American Society of Heating, Refrigerating, and Air-Conditioning Engineers guidelines (21). The study design was reviewed and approved by the Harvard School of Public Health Human Subjects Committee.

CO_2 Measurements

As exhaled air is the only indoor source of CO_2 in office buildings, the ventilation measure used was the CO_2 differential above the background in each building. Methods used to measure the CO_2 differential are described elsewhere (22). The weekly CO_2 differential was averaged across all monitors in the study area to obtain a weekly CO_2 differential measurement for each study area. We calculated the average fraction of exhaled breath by dividing the weekly CO_2 differential by 38,000 ppm, the concentration contained in exhaled breath, and used it to compute the volume of exhaled breath sampled by each filter.

Air Sample Collection and Analysis

Weekly air samples were collected in two centralized locations in each study area from 9 A.M. to 5 P.M. on workdays at 4 L/minute with closed-faced cassettes on 37-mm Teflon filters (Teflo 2.0 μm pore; Pall Gelman, Ann Arbor, MI). The filters were placed approximately 1 m from and horizontal to the floor (23). The closed-faced cassettes prevented direct contact with occupants and limited the chance that large droplets would be collected. We prepared weekly field and laboratory blanks and outdoor background samples. Filters were stored at −80°C until analysis.

We extracted filters in 560 μl of AVL buffer containing carrier RNA (QIAamp Viral RNA Mini Kit; Qiagen Inc., Valencia, CA) and 140 μl phosphate-buffered saline (PBS) for 20 minutes at 360 rpm on an orbital tabletop shaker (VWR Scientific Products, Thorofare, NJ). We extracted the RNA from the eluted liquid with QIAamp Viral RNA Mini Kit following manufacturer's protocol. We analyzed the air samples for picornaviruses (rhinoviruses and enteroviruses) using a nested reverse transcriptase–polymerase chain reaction assay that was modified from a published protocol (24, 25) using newly developed primer OL26-Z (5′-CTT CTG TTT CCC CG-3′).

Nasal Mucus Sample Collection and Analysis

Occupants complaining of an upper respiratory tract infection were encouraged to volunteer a nasal mucus sample. Lavages were collected using a standard protocol (26). To increase participation, we also collected nasal mucus by asking volunteers to blow their noses into a paper tissue. The tissue was eluted with 0.25 ml of antibiotic antimycotic solution and 5 ml of PBS. Samples were transported on dry ice and stored at −80°C.

Each volunteer was asked to rate the symptoms on a scale of 0 to 3, and a total symptom score was tabulated. Clinical colds were diagnosed if the total score was 14 or more (27, 28).

Nasal mucus samples were analyzed by polymerase chain reaction for picornaviruses, coronaviruses 229E and OC43, respiratory syncytial viruses A and B, influenza viruses A and B, parainfluenza viruses 1 to 3, adenoviruses, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* (29).

Polymerase Chain Reaction Product Cloning, Single-stranded Conformational Polymorphism Assay, and Sequencing

As there are over 100 rhinoviruses and over 70 enteroviruses, to determine if picornaviruses in nasal samples were identical to those in the air filters, amplified nested reverse transcriptase–polymerase chain reaction products from mucus and air samples were ligated into pCR2.1 vectors and transformed into One Shot competent cells according to manufacturer's protocol (Original TA Cloning Kit; Invitrogen Corp., Carlsbad, CA).

Single-stranded conformational polymorphism assays (SSCP) were performed on 10 to 12 clones picked from each sample according to a published protocol using primers JWA-1b and OL26-Z to identify viruses with identical SSCP fingerprints (30). Sequences of the several picornavirus clones with unique SSCP patterns were determined by purifying the 289-bp polymerase chain reaction products using the QIAquick Gel Extraction Kit (Qiagen Corp.) using manufacturer's protocol followed by fluorescence sequencing. The sequence data for the clones were matched against published sequences to determine the picornavirus species.

Data Analysis

Because the probability of detecting rhinovirus may not be linear, we used generalized additive models with logit links to model the probability of detecting airborne rhinovirus dependent on the volume of exhaled breath sampled by each filter. Generalized additive models permit the outcome to rely nonparametrically on smooth functions of some or all the predictors. We controlled for nonlinear trends in seasonality and indoor relative humidity using smoothing functions and used the smoothing spline for function estimation. We controlled for interbuilding differences with indicator variables.

RESULTS

Building 1 was studied from March 2000 to May 2001, Building 2 from November 2000 to June 2001, and Building 3 from March 2000 to October 2000. We collected 212 filters with an average sampling time of 47 hours. Filters that were tampered or had other sampling problems (9) and filters collected when CO_2 data were unavailable (22) were not analyzed. Of the 181 filters analyzed, 58 (32%) were positive for picornavirus (Table 1).

Figure 1 shows the weekly indoor CO_2 differential measurement for the three study buildings and shows that there were large differences within and between the buildings in terms of CO_2 content above the background. To investigate whether building ventilation was related to virus recovery from filters, we modeled the relationship between the frequency of detecting airborne rhinovirus and the amount of exhaled breath sampled with generalized additive models and a smoothing spline curve. Our initial model indicated that the relationship was not linear but rather consisted of two linear segments with an inflection at 30 L of exhaled breath (*see* Figure E1 in the online supplement). Therefore, we modeled exhaled breath as a two-piece linear function (*see* Figure E2 in the online supplement) with smooth functions for seasonality and indoor relative humidity. Exhaled breath samples greater than 30 L demonstrated a significant positive relationship with the detection of airborne rhinovirus ($p = 0.039$), whereas exhaled breath less than 30 L was not significant (Table 2). Inflection points of 20 and 40 L were also

## TABLE 1. BUILDING CHARACTERISTICS AND AIR FILTER VIRUS DETECTION RESULTS

	Building 1	Building 2	Building 3
Year built	1966	1963*	1965[†]
Type of HVAC	VAV	CAV	CAV
Number of HVAC units serving study area	1	1	4
Floor area, m²	2,979	2,508	1,477
Approximate study area population[‡]	69	60	71
Number of filters collected	97	42	42
Positive filters	38	9	11
Percentage of filters positive for picornaviruses	39	21	26

Definition of abbreviations: CAV = constant air volume; HVAC = heating ventilation and air conditioning; VAV = variable air volume.
* Converted to office space in 1988.
[†] Converted to office space in 1980.
[‡] Determined by random headcounts.
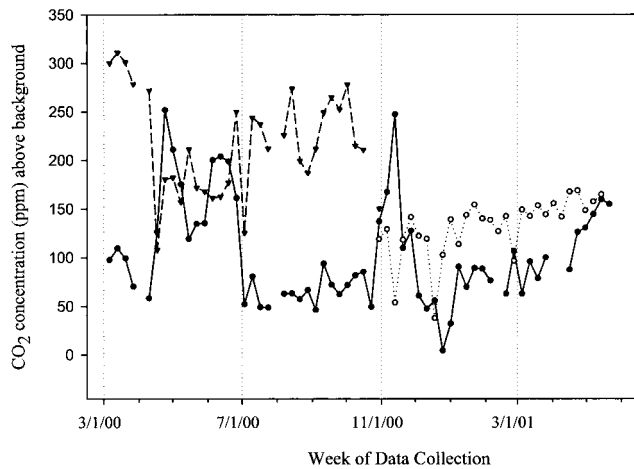
Figure 1. Variability in building ventilation within and between buildings as measured by weekly workday indoor carbon dioxide (CO_2) differential above the background. Average CO_2 concentrations between 9 A.M. and 5 P.M. minus the average concentration between 1 A.M. and 3 A.M. (equivalent to outdoor CO_2) for the three buildings studied (Building 1, *solid line with filled circle*; Building 2, *dotted line with open circle*; Building 3, *dashed line with inverted triangle*).

analyzed; these points resulted in significant positive relationships with rhinovirus detection above the inflection. However, the 30-L inflection point provided a better fit. Using the average sampling time (47 hours), the 30-L inflection point is equivalent to an average CO_2 concentration differential of 100 ppm. Thus, above this concentration, we detected a significant positive relationship between the occurrence of airborne rhinovirus and building ventilation, as measured by the average indoor CO_2 concentration differential.

The reverse transcriptase–polymerase chain reaction panel was conducted on 29 cases who identified themselves and were confirmed by a symptom score of at least 14 as having a cold. Of the 29 cases, 17 (59%) had respiratory microorganisms detected. Picornaviruses were the most commonly detected organism (10/17, 59% of positive samples), followed by coronaviruses (4/17, 24%), *M. pneumoniae* (3), *C. pneumoniae* (2), and parainfluenza viruses (1). Influenza viruses, adenoviruses, and respiratory syncytial viruses were not detected in any of the nasal mucus samples.

Of the 10 nasal mucus samples positive for picornaviruses, 4 were collected the same week or the week after a positive air sample from the same building. These four nasal mucus and contemporaneous air samples were analyzed by SSCP. Of the contemporaneous nasal and air filter samples, one pair collected during the same week in the same building had SSCP band patterns that were an exact match. Sequence analysis confirmed

TABLE 2. GENERALIZED ADDITIVE MODEL OF THE PROBABILITY OF DETECTING AIRBORNE RHINOVIRUS*

Coefficients	Parameter Estimate	SE	*t* Value	p Value
Intercept	1.28227	1.03698	1.23654	
Exhaled breath < 30 L	−0.03815	0.03147	−1.21221	0.1127
Exhaled breath > 30 L	0.06794	0.03855	1.76246	0.0389
Building 2	−0.99838	0.58354	−1.71091	0.045
Building 3	−1.23973	0.54243	−2.28551	0.012

* Exhaled breath below 30 L and above 30 L modeled as independent linear functions controlled for nonlinear trends in seasonality and indoor relative humidity.

that the detected sequences were rhinovirus and furthermore that the one nasal sample/air filter pair with identical SSCP patterns was the same virus with an exact match in the amplified region of 289 bp (for figures of SSCP gels, *see* Figures E3a and E3b in the online supplement).

DISCUSSION

We detected airborne picornavirus in 32% of air sampling filters in office buildings using molecular methods. We show a significant positive relationship between the frequency of virus detection in air filters and degree of building ventilation with outdoor air as measured by average CO_2 concentrations greater than 100 ppm above the background. Thus, these data suggest that lower ventilation rates and resulting increased CO_2 concentrations are associated with increased risk of exposure to potentially infectious droplet nuclei. Whether these droplet nuclei are important vectors of infection remains controversial. We have recently shown (31) that during experimental studies of airborne rhinovirus transmission between volunteers (20), the rate of airborne infectious particle generation must have been very low. This implies (*see* online supplement) that the experiments by Gwaltney and Hendley (17) did not have sufficient power to detect airborne infection transmission. Thus, airborne transmission remains a potentially important mechanism of propagation for rhinoviral colds.

In addition to demonstrating the increased risk of exposure with reduced ventilation, we demonstrated detection of an identical rhinovirus in a nasal mucus sample from an occupant with an upper respiratory tract infection and from an air sample collected in the same building during the occupant's illness. We believe the rhinovirus collected from the air was either expelled from the occupant who gave the matching nasal mucus sample or from another occupant who was part of the same chain or network of infection transmission for several reasons. First, there are over 100 known human rhinovirus serotypes, and new serotypes are continuously emerging (32). Our SSCP method was capable of detecting genetic variations as small as a single base pair, and identity was confirmed by sequence analysis. The homology in published sequences for the 47 rhinovirus serotypes for which we have data over the 289-bp region used in this study is between 63 and 87%, indicating the extreme improbability of detecting identical sequences in unrelated rhinoviruses. Finally, we were able to demonstrate extensive heterogeneity among different rhinoviruses present in air and nasal mucus. Although this study does not provide definitive proof that rhinovirus is transmitted through the aerosol route and is modulated by outdoor air supply rates, it does provide support for this hypothesis.

Our current results may help explain previous results from analysis of sick leave at Polaroid Corp. (6) in which lower levels of outdoor air supply in office buildings were associated with increased sick leave. The association between ventilation and absences occurred at ventilation levels that, although lower than the current study, have not often been associated with building-related complaints (33).

The burden of disease attributable to viral respiratory infections is enormous with an estimated 500 million episodes of viral respiratory infection occurring in the United States each year (1), resulting in 20 million days of lost work among adults and 22 million days of missed school among children (1). Schools are the major source of community outbreaks of respiratory infections (34). However, there have been few studies examining school ventilation (35–37), and none that has examined the association between ventilation and risk of infection transmission. In a study of 385 classrooms in 60 elementary schools in Texas,

86% of the classrooms had in excess of 1,000 ppm of CO_2, and the median CO_2 concentration was 1,671.5 ppm, values indicating that exhaled breath concentrations in schools are 5 to 11 times greater than the average in our study (38). There is recent evidence that improved ventilation and reduced CO_2 concentration is associated with reduced school absence and improved asthma control (39, 40). Given our results in well ventilated offices, the importance of rhinovirus infection in asthma exacerbation (24), and the generally poor ventilation in schools (36, 38), further studies of airborne transmission of rhinovirus in schools are needed.

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