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Influence of Suspending Liquid, Impactor Type, and Substrate on Size-Selective Sampling of MS2 and Adenovirus Aerosols

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Size-selective sampling methods for detecting viral aerosols are needed to assess the risk of airborne transmission of disease. Andersen and MOUDI nonviable cascade impactors were used to separate test aerosols containing MS2 bacteriophage or adenovirus into size fractions spanning much of the human respirable range. Culture-based methods and a fluorescent tracer dye allowed quantification of the viral particles that remained infective after being aerosolized into a test apparatus and collected by the impactors. In addition, various suspension fluids and impaction surfaces were evaluated for their effect on virus viability. Both the Andersen and MOUDI impactors were able to sample live viruses from test aerosols, although the relative recovery rate of MS2 was higher than adenovirus ($P < 0.001$). The MS2 and adenovirus aerosols were sensitive to different test factors. MS2 recovery was dependent on the suspension fluid ($P < 0.0001$) and RH ($P = 0.001$), whereas adenovirus recovery was dependent on aerodynamic particle size ($P < 0.001$). Relative recovery of adenovirus was highest in the 0.56–1.9 μm diameter range. The results confirm that nonviable cascade impactors are capable of size-separating and detecting aerosolized viruses in the human respirable range, and that MS2 and adenovirus can retain viability after nebulization under experimental conditions. The findings cast doubt, however, on the suitability of MS2 as a general surrogate for human and animal viruses.

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1. BACKGROUND

Although viruses may be expelled from humans in a large range of droplet sizes, whether viruses preferentially associate or remain viable with particular particle sizes is unknown. Characterizing airborne viruses in the respirable size range (<10 μm) is an important prerequisite for making decisions about worker protection, pandemic preparedness, and agricultural practice. For example, if viable viruses are commonly found in particles smaller than 1 μm in diameter, then health care workers treating a patient harboring a serious viral infection might be strongly advised to wear respiratory protection. Furthermore, improved ventilation systems and higher efficiency filters in the air handling units of those systems would be warranted. Knowing whether viruses are present in particle sizes that can lodge deep in the lungs versus in the upper respiratory tract, coupled with information about viral tropism, can help experts assess the risk of disease following exposure to infectious aerosols.

Tidal breathing, coughing, and sneezing generate particles in a large range of sizes, from droplets that settle quickly, to fine aerosols that are capable of remaining in the air for long periods of time (Duguid 1946; Nicas et al. 2005). Most of the droplets produced from talking and coughing have diameters of 1–24 μm (Chao et al. 2009). Recently, Chiang et al. (2010) confirmed that the vast majority of respiratory particles expelled during coughing, breathing, and talking are <10 μm in diameter and that less than 1% of particles from coughs are >10 μm in diameter. Moreover, most of the water portion of droplets smaller than about 25 μm in diameter evaporates almost instantaneously, leaving particles that can remain suspended in the air (Musher 2003). Therefore, most of the particles generated during coughing and sneezing are capable of remaining airborne

long enough to spread widely and potentially cause human or animal exposure.

A variety of chemicals may be added to aqueous suspensions of viruses to maintain their viability once aerosolized (Ferris and Donaldson 1980), implying that some of the chemicals in human and animal body fluids may promote virus viability while airborne. Under experimental conditions, the addition of protein and organic matter to aqueous suspensions of viruses generally prolongs the viability of viral aerosols generated from those suspensions (Benbough 1969; Sobsey and Meschke 2003). In addition to the effects salts and proteins exert on virus survival, the nonvolatile fraction of body fluids may form a solid or gooey matrix around viruses once the water evaporates, perhaps imparting physical protection to viruses from desiccation.

Methods exist for sampling viral aerosols, but relatively limited research has been performed to determine the best methods for viable virus recovery (Verrault et al. 2008). Cascade impactors are capable of capturing size-separated samples on flat plates. These impaction plates may be modified with a coating of an adhesive or grease to reduce particle bounce (Kirychuk et al. 2009). Alternately, when particle bounce is less of a concern, but when biological activity may be affected by impaction, materials that preserve infectivity may be used in conjunction with impaction plates. Gelatin filters can be used with air samplers to collect particles and then dissolved directly into water or culture media for quantification (Verrault et al. 2008). Whether gelatin filters can improve viable recovery of viral aerosols when used as impaction surfaces is unknown.

Adenoviruses are frequently associated with upper respiratory infections in children, and epidemics of adenovirus-associated acute respiratory disease (AdARD) have been repeatedly reported at US Department of Defense Training Centers (Hendrix et al. 1999; Gray et al. 2000; Metzgar et al. 2007). Adenovirus infections are believed to be spread by respiratory droplets and fomite contamination, but detailed studies describing transmission modes of adenoviruses are still needed to design effective control measures in populations such as military recruits and school-aged children. Human adenovirus serotype-1 (HAdV-1) was selected for use in this study. Adenoviruses are medium sized (90–100 nm), nonenveloped, icosahedral viruses containing double-stranded DNA, and require BSL-2 containment.

Bacteriophage MS2 is frequently used in aerosol experiments as a surrogate for human and animal viruses (Rengasamy et al. 2010; Woo et al. 2010). MS2 is nonpathogenic, can be prepared at high titers ideal for detection in many aerosol experiments, and responds to antimicrobial agents in ways similar to human viruses. MS2 was selected as a test virus to examine whether it behaved like animal viruses in environmental aerosols. MS2 is a small (27–34 nm), icosahedral, RNA bacteriophage of *Escherichia coli*, and requires BSL-1 containment.

The primary objectives of this study were to develop methods for the size-selective sampling of viral aerosols and to determine the best method for maximizing recovery of viable viruses. The recovery of two test viruses was measured using two types of impactors capable of separating particles by size onto impaction surfaces. In addition, the effects of the suspension fluid and impactor substrate on recovery were examined.

2. MATERIALS AND METHODS

2.1. Test Viruses and Propagation

Stocks of HAdV-1 (VR-1, ATCC, Manassas, VA) were propagated in 24-h-old A-549 human lung carcinoma epithelial cells (CCL-185, ATCC). The cells were grown in Eagle's minimum essential medium (MEM) (Mediatech, Herndon, VA) supplemented with 150 IU/mL penicillin, 150 μg/mL streptomycin, 50 μg/mL neomycin, 1 μg/mL fungizone, and 8% fetal calf serum. After inoculation, the virus was allowed to adsorb to the cells at 37°C for 1 h, followed by the addition of MEM without fetal calf serum and incubation at 37°C with 5% CO_2 and humidity control. After the appearance of virus-induced cytopathic effects (CPE), generally 4 days postinfection, the cells underwent three cycles of freezing and thawing (−80°C/25°C) and cell lysates were harvested. Cell debris was removed by centrifugation at 4000 *g* for 20 min. The supernant was then aliquoted into 3 mL vials followed by storage at −80°C until use.

MS2 (15597-B1, ATCC) was propagated in the *E. coli* C-3000 host strain (15597, ATCC). Top agar tubes were prepared and held at 48°C. Then, 0.1 mL MS2 and 1 mL of a log phase culture of *E. coli* C-3000 were inoculated into each tube. After rotating to mix, the tubes were poured on trypticase soy agar (TSA) plates. The top agar was allowed to solidify, then inverted and incubated at 37°C for 24 h. After plaques were confluent, 5 mL tryptic soy broth (TSB) was added to each plate. After 2 h at room temperature, the solution was aspirated, lightly centrifuged, and sterile-filtered. The resulting solution was aliquoted into 3 mL vials, followed by storage at −80°C until use.

2.2. Test Aerosol Apparatus and Procedures

Experiments were carried out in a test apparatus with ventilated secondary containment appropriate for handling BSL-1 and BSL-2 organisms (Figure 1). Suspensions of HAdV-1 and MS2 with a fluorescent tracer dye (fluorescein sodium salt, Fluka, Buchs, Switzerland) were aerosolized at 20 psi from a 6-jet Collison-type nebulizer (BGI Inc., Waltham, MA) into filtered, humidity-conditioned air flowing through the test apparatus at 92 L/min. An Andersen 8-stage nonviable cascade impactor (ACI) with 80 mm aluminum impaction plates (Thermo Scientific, Franklin, MA) and an 8-stage nonrotating micro-orifice uniform deposit impactor (MOUDI) with 47 mm aluminum foil substrates (Model 100, MSP Corp., Shoreview, MN) drew air from the test duct at 28.3 L/min and 30 L/min, respectively. Stage 7, the lowest stage of the ACI, was not used. A 15-min test length was selected to maximize recovery of viable HAdV-1 and MS2. Relative humidity (RH) was maintained

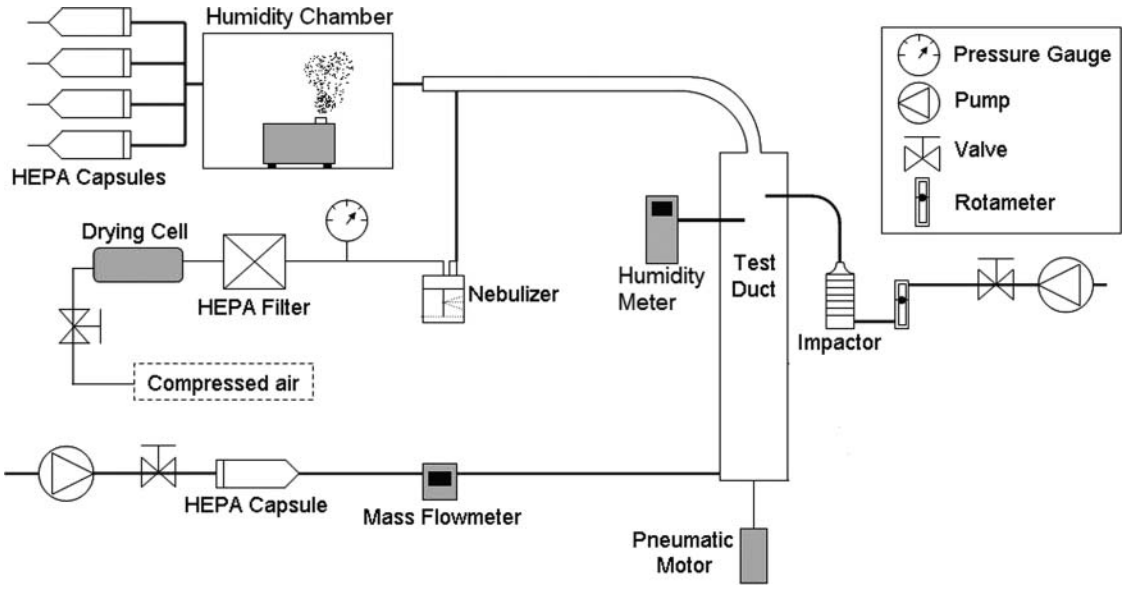

FIG. 1. Schematic diagram of the test apparatus.

within a range of 45–55% and continually monitored along with temperature, which ranged from 19 to 26°C in the test apparatus.

Pumps and humidity controllers were turned on 30 min prior to beginning experiments to allow airflow and RH to stabilize. All nebulizers and collection surfaces were steam-sterilized in an autoclave before use. Nebulizer suspensions were mixed in a biosafety cabinet and carried to the test apparatus, where the nebulizer was clamped into place and attached with tubing. Collected viral samples were transported within 2 h to the virology laboratory on ice. Fluorescence samples were stored at 4°C for later analysis.

On each day of testing, HAdV-1 or MS2 stock was thawed at ambient temperature immediately preceding experiments. 1 mL of thawed stock was diluted in 48 mL of suspension fluid and decanted into a nebulizer. The suspension fluids tested were deionized, filtered (DIF) water, appropriate maintenance media (MEM for HAdV-1, TSB for MS2), and artificial saliva consisting of maintenance media with 7.6% w/v glycerol. After addition of 100 μL antifoam Y-204 (Sigma Chemical Co., St. Louis, MO, USA) and 1 mL tracer dye (25 μg/mL), the solution was gently mixed, then nebulized. The HAdV-1 concentration in the 50 mL suspensions ranged from 3.16×10^4 to 1.78×10^6 $TCID_{50}$/mL (50% tissue-culture infecting dose per milliliter) during this study. The MS2 nebulizer suspension concentrations ranged from 5.60×10^8 to 1.3×10^9 PFU/mL (plaque-forming units per milliliter).

Glycerol was used as an additive to simulate saliva, as indicated by Wan et al. (2009). Glycerol is a feasible nonvolatile suspension additive because it mimics the carbohydrate-rich ends of respiratory mucins that are in contact with viruses in respiratory droplets. It can also be adjusted to a concentration that models the nonvolatile content of saliva. In preliminary tests, aerosols generated with glycerol were stable in size and output.

Two sets of tests were conducted with each virus. The first set evaluated the effect of impactor type (ACI, MOUDI) and suspension fluid (water, maintenance medium, maintenance medium with glycerol) on the recovery of viruses from size-separated aerosol particles. All combinations of the two impactors and three nebulizer suspensions were tested in triplicate for a total of eighteen tests with each virus. During the course of the tests with MS2, it was discovered that the RH controller used was miscalibrated, resulting in the initial 8 tests of the series being conducted at 35% (±5%) relative humidity, rather than the 50% (±5%) planned. The problem was corrected for the remaining 10 tests in the series, and RH was factored into the data analysis. Because the test order was completely randomized, it is unlikely that the humidity level biased the results.

Using the ACI and maintenance medium nebulizer suspension, a second set of 6 tests was performed with each virus to evaluate the ability of gelatin filters used as impaction substrates to improve recovery. For 3 of the tests, sterile 80 mm gelatin filters (Sartorius, Germany) were fitted on top of the ACI collection stages by spotting ~25 mg silicone gel (Chemplex 710 Silicone Compound, Fuchs Lubritech, USA) at 3 equidistant points around the edge of the filter. For the top two ACI stages, a precision knife was used to cut out an inner circle on the filter to accommodate the collection surface of the stage. The other 3 tests were performed with no filters.

2.3. Sample Collection

Samples (1 mL) were collected from the HAdV-1 and MS2 nebulizer suspensions before and after each test, on forty-eight

different occasions. An elution buffer of 3% beef extract in 0.05M glycine, adjusted to pH 9.1, was used to collect samples from ACI and MOUDI stages as well as to dissolve gelatin filters. After each ACI or MOUDI test, the impactor was carefully disassembled in a certified biosafety cabinet, where deposited viruses from all samples were eluted. The impaction plates or foil substrates were transferred with forceps into sterile petri dishes. A sterile cell scraper was used to collect impacted particles from each stage using 1 mL elution buffer for ACI plates and 0.5 mL for MOUDI substrates. After the elution buffer was pipetted onto each stage, particles were scraped into the fluid with circular motions covering the entire stage until all particle deposits were suspended in the buffer. Sample solutions were then collected by aspirating with a pipette and transferring into 1.5 mL collection tubes. For the gelatin filter tests, plain impactor samples were eluted with 1 mL elution buffer as in ACI/MOUDI tests. Sterile forceps were used to carefully fold and transfer gelatin filters to 50 mL centrifuge tubes. After 5 mL elution buffer was added, the tubes were vortexed at medium speed for 5 s to dissolve the filters.

2.4. Virus Titration

For HAdV-1 samples, serial 10-fold dilutions (10^0 to 10^{-6}) of the nebulizer suspensions, the impactor-stage eluates, and the dissolved gelatin filter samples were prepared in MEM with 8% fetal bovine serum, followed by inoculation in 24-h-old A-549 cells grown in 96-well plates using four wells per dilution. After 5 days of incubation at 37°C with 5% CO_2 and humidity control, inoculated cells were examined microscopically for the appearance of virus-specific CPE. Viral titers were calculated by the Karber method (Karber 1931), and results were expressed as $TCID_{50}$/mL. The lower limit of detection for this method was 10 $TCID_{50}$/mL. If there were no viral particles detected in a particular sample, a value of 1 $TCID_{50}$/mL was used to allow statistical analysis. Because logarithms of the titers were used for the data analyses, a value of 0.1 times the limit of detection was selected to avoid zero values.

MS2 samples were enumerated using a double agar layer (DAL) procedure. Top agar tubes (0.7% TSA) were prepared and held in a 48°C water bath. Serial 10-fold dilutions (10^0 to 10^{-10}) of the nebulizer suspensions, the impactor-stage eluates, and the dissolved gelatin filter samples were prepared in TSB. MS2 sample dilutions were added to prepared top agar tubes along with log-phase *E. coli* (13706, ATCC), gently mixed, and poured onto 1.5% TSA bottom agar plates. After 18 h of incubation at 37°C, DAL plates were examined for the appearance of plaques, and results were expressed as PFU/mL. The lower limit of detection for this method was 10 PFU/mL. For any sample where there was no virus detected, a value of 1 PFU/mL was used for statistical analysis.

2.5. Fluorometric Quantification

Fluorescein dye was used to track the physical collection efficiency of the sampling methods, as recommended by Ijaz et al. (1987) and Agranovski et al. (2005). The fluorescein levels in the nebulizer suspensions, the impactor-stage eluates, and the dissolved gelatin filter samples were assessed using a spectrofluorometer (Model RF-5201PC, Shimadzu Scientific Instruments, Columbia, MD). Samples were prepared in fluorescence cuvettes with DIF water and fluorescence intensity was measured at $\lambda = 515$ nm after excitation at $\lambda = 485$ nm. Results were expressed as fluorescence intensity per milliliter. To calibrate the instrument readings, standardized concentrations of fluorescein were prepared in each of the different nebulizer fluids with an amount of elution buffer equivalent to the amount in the test samples. For each fluid, a curve was created to calibrate the raw readings from the spectrofluorometer. The lower limit of detection for this method was 0.1 fluorescence units (FU) per sample. For any samples where there was no fluorescence detected, a value of 0.01 FU/sample was used for statistical analysis.

2.6. Data Analysis

To assess the effect of nebulization on MS2 and HAdV-1 while controlling for any concentration effects due to evaporation, the titers and fluorescence intensity of the nebulizer suspensions after each 15-min test were compared to the titers and fluorescence intensity before nebulization using the quantity γ:

$$\gamma = \frac{\left(\frac{C}{FI}\right)_a}{\left(\frac{C}{FI}\right)_b} \quad [1]$$

in which C_a is the virus titer after nebulization, C_b is the titer before nebulization, FI_a is the fluorescence intensity after nebulization, and FI_b is the fluorescence intensity before nebulization. When the ratio of the titer to the fluorescence intensity is the same after nebulization as before, γ will equal 1. Pairs of virus titers and fluorescence samples were compared: 24 pairs of titers for HAdV-1 and 20 pairs for MS2. One titer pair and 3 fluorescence pairs for MS2 were not complete and could not be used in the evaluation. Geometric means and 95% confidence intervals were calculated from these data. SAS 9.1 software (SAS Institute) was used to perform *t*-tests to determine if the mean of γ was different from 1.

The recovery of viable viruses from particles in the test impactor relative to the recovery of fluorescein in the same sample was the primary experimental outcome. The detected value of fluorescence intensity (*FI*) per volume of liquid was combined with quantities of virus ($TCID_{50}$/mL or PFU/mL) measured in the nebulizer suspension and in air samples to calculate relative virus recovery:

$$R_{\text{rel}} = \frac{\left(\frac{\text{Virus/stage}}{FI/\text{stage}}\right)_{\text{sample}}}{\left(\frac{\text{Virus/mL}}{FI/\text{mL}}\right)_{\text{nebulizer}}} \quad [2]$$

in which R_{rel} is the relative recovery rate of virus from the sample. If all of the virus in a sample is recovered live without sampling and analytical error, $R_{rel} = 1$. When a conventional analytical technique that quantifies live virus is used, the value of R_{rel} tells us the fraction of nebulized virus that remains live after we sample and analyze it. The geometric mean and standard deviation of R_{rel} were calculated from the three replicates for each combination of test parameters.

SAS 9.1 software was utilized for all statistical analyses of log-scale values of R_{rel}. After significant main effects were determined using one-way analysis of variance (ANOVA), two-way ANOVA tests were used to refine the models and to evaluate significant interactions between the main effects of impactor, particle size, and nebulizer fluid. Scheffé's method was used to determine the levels of the factors where significant differences were found within ANOVA models. Least-squares means and standard errors of the means for significant factors were calculated.

3. RESULTS

3.1. Virus Stability

Values of γ calculated from Equation (1) and MS2 and HAdV-1 titers before and after nebulization are summarized in Table 1. The geometric mean of γ was not significantly different from 1 for either MS2 or HAdV-1. The values of γ for MS2 varied among fluids from 0.77 for TSB to 0.89 for TSB with 7.6% glycerol. The values of γ for HAdV-1 varied among fluids, ranging from 0.76 for MEM to 1.23 for MEM with 7.6% glycerol. Variations in γ among fluid types were not significant for either MS2 or HAdV-1 ($P = 0.81$).

3.2 Bacteriophage MS2

There were significant differences in R_{rel} for MS2 that were dependent on the nebulization fluid ($P < 0.0001$) and RH ($P = 0.001$). RH did not change the effects of impactor or fluid. Rather, it was a significant independent factor. As presented in Figure 2, no significant differences were detected in R_{rel} that were dependent on aerodynamic particle size interval ($P = 0.98$).

Figure 3 shows that the ACI yielded higher R_{rel} of MS2 than the MOUDI, although the results were not statistically significant ($P = 0.22$). Overall, the relative recovery of MS2 in the ACI was approximately twice as high as in the MOUDI.

Figure 4 summarizes the effects of RH and nebulizer fluid. The tests at 50% RH yielded approximately 3.3 times higher R_{rel} than the 35% RH tests. The interaction of humidity with nebulizer fluid was the most pronounced. At 35% RH, R_{rel} of MS2 in TSB suspensions was much higher than R_{rel} of MS2 in DIF water or TSB with 7.6% w/v glycerol. However, at 50% RH, R_{rel} of MS2 in TSB and DIF water suspensions was similar. For the different suspension fluids at both 35% and 50% RH, a similar pattern was seen: TSB was the best fluid for maximizing MS2 infectivity, followed by DIF water and TSB with 7.6% glycerol. The presence of glycerol, added to mimic the nonvolatile content of saliva, had no beneficial effect on the recovery of MS2.

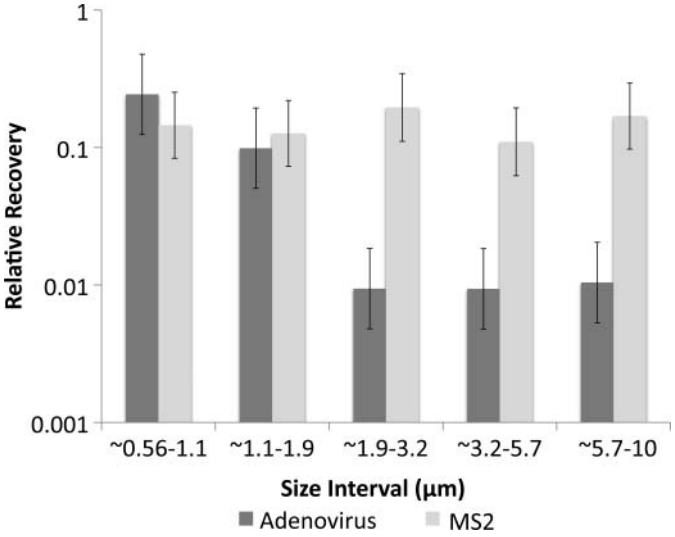

FIG. 2. Relative recovery of bacteriophage MS2 and human adenovirus serotype-1 (HAdV-1) as a function of aerodynamic particle size. For each virus and size interval, $n = 18$. Values are least-square geometric means with error bars representing ± the standard error of the mean.

The use of gelatin filters had no significant effect on R_{rel} ($P = 0.17$). Collection of MS2 from gelatin filters resulted in lower R_{rel} than when untreated aluminum collection plates were used, as shown in Figure 5.

3.3. Human Adenovirus Serotype-1

Figures 3 and 6 indicate that nebulization fluid ($P = 0.16$) and impactor type ($P = 0.34$) did not have statistically significant

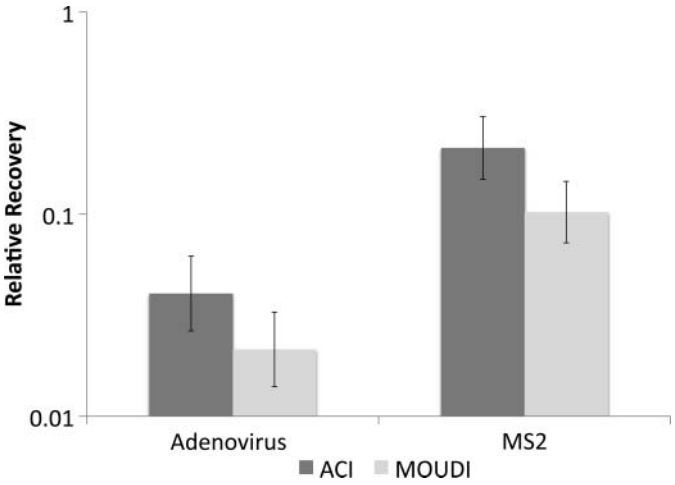

FIG. 3. Relative recovery of bacteriophage MS2 and human adenovirus serotype-1 from two test impactors, the micro-orifice uniform deposit impactor (MOUDI) and the Andersen 8-stage nonviable cascade impactor (ACI). For each combination of virus and impactor, $n = 9$. Values are least-square geometric means with error bars representing ± the standard error of the mean.

TABLE 1
Comparison of virus titers before and after nebulization. The term γ, which is the ratio of the titer after nebulization to the titer before nebulization, is calculated using Equation (1)

Virus	Suspension fluid	Number of tests	Geometric mean (range) of virus titer, $TCID_{50}$/mL, before nebulization	Geometric mean (range) of virus titer, $TCID_{50}$/mL, after nebulization	Geometric mean (lower and upper 95% confidence interval) of γ
MS-2	DIF water	5	2.35×10^8 (1.80×10^8–3.3×10^8)	2.37×10^8 (1.90×10^8–3.40×10^8)	0.87 (0.65, 1.16)
	TSB	9	3.83×10^8 (1.40×10^8–$5.60 \times 10^{8)}$	3.01×10^8 (5.60×10^7–1.30×10^9	0.77 (0.52, 1.16)
	TSB with 7.6% glycerol	6	3.07×10^8 (2.30×10^8–4.30×10^8)	3.14×10^8 (2.00×10^8–4.30×10^8)	0.89 (0.76, 1.05)
HAdV-1	DIF water	6	3.83×10^5 (1.00×10^5–1.00×10^6)	3.83×10^5 (3.16×10^4–1.00×10^6)	0.96 (0.18, 5.27)
	MEM	12	5.11×10^5 (1.00×10^5–1.78×10^6)	4.22×10^5 (1.78×10^5–1.00×10^6)	0.76 (0.38, 1.54)
	MEM with 7.6% glycerol	6	2.87×10^5 (3.16×10^4–1.00×10^6)	3.83×10^5 (1.78×10^5–1.00×10^6)	1.23 (0.54, 2.76)

Note. $TCID_{50}$ is 50% tissue culture infecting dose, PFU is plaque-forming units, DIF is deionized filtered, TSB is tryptic soy broth, and MEM is Eagle's minimum essential media.

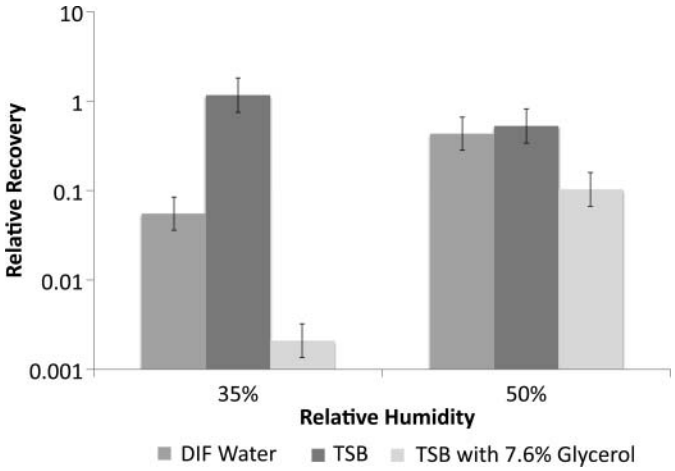

FIG. 4. Relative recovery of bacteriophage MS2 as a function of relative humidity and nebulizer suspension fluid. Eight trials were conducted at 35% relative humidity. Ten trials were conducted at 50% relative humidity. Values are least-square geometric means with error bars representing ± the standard error of the mean.

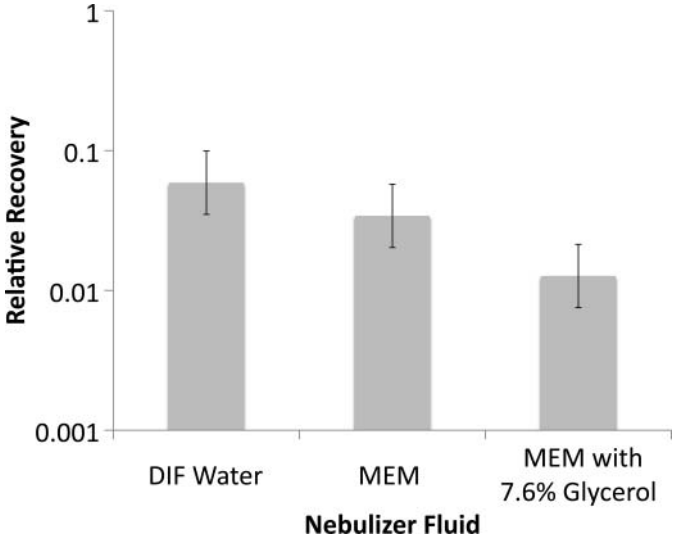

FIG. 6. Relative recovery of human adenovirus serotype-1 dependent on nebulizer suspension fluid. For each nebulizer fluid, $n = 6$. Values are least-square geometric means with error bars representing ± the standard error of the mean.

effects on R_{rel}. As displayed in Figure 2, there were significant differences in R_{rel} that were based on aerodynamic particle size ($P < 0.001$). Higher R_{rel} values were detected with particles 0.56–1.9 μm compared to particles 1.9–10 μm. R_{rel} was significantly higher ($P < 0.001$) for MS2 versus HAdV-1, as shown in Figure 3.

The use of gelatin filters had a borderline significant effect on HAdV-1 R_{rel} ($P = 0.057$). Figure 5 illustrates that viral impaction onto filters improved the recovery of viable virus. Following the same trend seen in the ACI/MOUDI test series, the effect of aerodynamic particle size was significant ($P = 0.023$) for HAdV-1 in the gelatin filter tests.

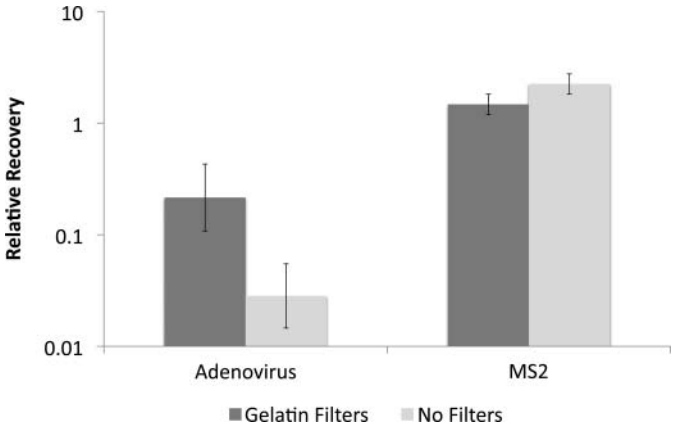

FIG. 5. Relative recovery of bacteriophage MS2 and human adenovirus serotype-1 from an Andersen 8-stage nonviable impactor, with gelatin filters on impaction plates versus without filters. All combinations of virus and impaction surface were conducted in triplicate. Values are least-square geometric means with error bars representing ± the standard error of the mean.

3.4. Comparison of Test Viruses

Figure 2 summarizes the R_{rel} of MS2 and HAdV-1 by particle size. MS2 was readily recovered from all particles sizes measured, from 0.56 to 10 μm, and infectivity was not associated with any particular size range. In contrast, R_{rel} of HAdV-1 was strongly associated with particle size. Infectivity was best preserved in the 0.56–1.9 μm size range. Almost no HAdV-1 was recovered between 1.9 and 10 μm. The trends seen in recovery of MS2 versus HAdV-1 were significantly different; MS2 R_{rel} was stable between 0.56 and 10 μm whereas HAdV-1 R_{rel} was similar to MS2 in the smallest size intervals, then significantly lower than MS2 in the larger size intervals. The apparent effect of size on R_{rel} of HAdV-1 in the impactor samples may have been confounded by the relatively low titer of HAdV-1 in the nebulizer suspension. There is some potential that a higher starting titer could increase R_{rel} for HAdV-1 in the 1.9–10 μm range.

Figure 3 summarizes the effect of the test impactor on the overall recovery of MS2 and HAdV-1. The ACI yielded higher R_{rel} for both HAdV-1 and MS2, although the difference in R_{rel} between the impactors was not statistically significant.

4. DISCUSSION

4.1. Virus Stability

Values of γ in Table 1 indicate that there was no significant change in the titer of either MS2 or HAdV-1 after nebulization, confirming that the concentration of virus particles in the nebulizer fluid was stable throughout the experiments and that the nebulizer fluids had no significant effect on the survival of HAdV-1 or MS2 during the nebulization process. It is possible that the virus suspensions could have been concentrated as a

result of evaporation during the test period, artificially raising the apparent postnebulization virus titer. However, the tracer dye in the suspension was measured and used to normalize the titer values included in the calculation of γ, and confirmed that any evaporation effect was small in comparison to the overall stability of the suspensions.

The stability of the virus suspensions implies that physical stress to the viruses was minimal as they passed through the nebulizer. Ijaz et al. (1987) and Kim et al. (2007) found similar results after 10–30 min nebulization periods of transmissible gastroenteritis virus, human coronavirus, poliovirus type-1, rotavirus, and rhinovirus. Kim et al. (2007) suggest that viruses have limited inertia in suspensions due to their small size and, therefore, experience little stress from acceleration, deceleration, or impaction during nebulization.

The comparison between aerosolizaton of viruses versus vegetative bacteria is noteworthy. Kim et al. (2008) were unable to recover *M. haemolytica* and *Y. ruckeri* after nebulization into the same test apparatus used in this study. Heidelberg et al. (1997) had similar results when aerosolizing Gram-negative bacteria. These data suggest that a greater fraction of viruses may possibly remain viable after aerosolization from natural sources, when compared to vegetative bacteria.

4.2. Relative Humidity

MS2 R_{rel} was higher at 50% RH than at 35% RH. Viral aerosols are known to be sensitive to relative humidity, often exhibiting an optimal RH level for maximum infectivity. Similar to our findings with MS2, the T3 coliphage reaches maximum infectivity at high (>70%) RH levels (Verrault, et al. 2008). Our findings were in contrast to Dubovi and Akers (1970), who found that MS2 recovery decreased markedly above 30% RH. While the ideal RH for viruses does not follow a strict pattern, low RH tends to preserve enveloped viruses, while high RH tends to preserve nonenveloped viruses like MS2, perhaps due to structural damage to the virion (Akers and Hatch 1968).

To the best of our knowledge, there are no published studies characterizing the half-life of either MS2 or adenovirus in experimental aerosols or on environmental surfaces. However, Sattar et al. (1987) reported the half-life of human rhinovirus dried on environmental surfaces to range between 5 min and 14 h, depending on suspending medium and RH. Although the aerosolized particles in our study were only traveling in the test apparatus for approximately 1 min before being sampled, impacted particles were exposed to the RH conditions for the entire length they were in the samplers, up to nearly 15 min.

4.3. Gelatin Filter Tests

Gelatin filters were used as a collection medium for the viruses to determine if collection onto a gelatin surface resulted in higher R_{rel} versus plain metal impaction plates. Gelatin filters can be dissolved into collection media directly instead of scraping the impacted particles into an elution fluid. It was thought that gelatin filters might reduce viral infectivity losses during impaction or collection. For MS2, collection from gelatin filters actually reduced the overall R_{rel}. However, when gelatin filters were used with HAdV-1, there was a marked increase in R_{rel}. The ability to collect a sample that can be dissolved directly into fluid is appealing, but gelatin filters as collection media may provide benefits only to selected viruses. In addition, gelatin filters are brittle and can be difficult to work with. Gelatin media (6–12%, in phosphate buffered saline) may have potential for increasing R_{rel}. However, obstacles exist to using this type of media for long-term sampling (Dahlgren et al. 1961). Opportunities exist for development of collection media appropriate for long-term size-selective sampling of viruses.

4.4. Utility of MS2 as a Human Virus Surrogate

The findings bring into question the utility of MS2 as a general surrogate for human viruses. The factors that affected R_{rel} of MS2 were the opposite of the factors that affected HAdV-1 R_{rel}. This may be a by-product of the different ecological lifestyles of the viruses; HAdV-1 is a respiratory virus of humans that has had adaptive pressure to survive aerosol transmission while MS2 is a virus of *E. coli*, with minimal adaptive pressure to survive in aerosols. Alternately, differing inactivation factors may be a result of the differing structures of the viruses; DNA versus RNA, or large versus small. It is not surprising that fluid composition plays a greater role in preserving MS2 infectivity, given that much transmission between MS2 and its target organism likely happens in liquid matrices.

Culturing viruses is generally difficult and costly compared to bacterial cultures. While MS2 does provide a convenient model for viral behavior with the benefit of bacterial methodology, caution should be used when extrapolating MS2 results to viruses as a whole. In certain instances, the factors found to be significant for MS2 aerosols may be insignificant for some human viruses, as demonstrated in this study.

4.5. Limitations

Although the suspension liquid with glycerol was not beneficial to the recovery of MS2 or HAdV-1 in this study, other models for mimicking saliva may provide different results. For example, mucin-based or biologically active saliva formulations may provide additional protection to aerosolized viruses. Nonetheless, the glycerol-based suspension, as tested, did accurately model the nonvolatile content of saliva and might have provided similar physical protection from dessication. This study was also limited by the two test viruses selected. In future studies, it would be beneficial to replicate this work with a selection of respiratory viruses including both DNA and RNA viruses as well as enveloped and nonenveloped viruses. MS2 may not be a good surrogate for adenoviruses, but could still be useful in comparison to other viruses of interest, or for aerosol testing which is more closely focused on questions different from viability.

5. CONCLUSIONS

This research evaluated nonviable impactors as instruments for collecting size-selective viral aerosols. Relative recovery levels for MS2 and HAdV-1 were in the low to moderate range, but viable virus samples were readily collected from air particle sizes of 0.56–10 μm in diameter. MS2 was recovered uniformly from the entire range of sizes evaluated with the impactors. In contrast, HAdV-1 had increased recovery levels from particles 0.56–1.9 μm in diameter when compared to particles 1.9–10 μm in diameter. The data indicated that appropriate maintenance media for each test virus was generally the best choice for protecting viability in aerosols. This study also confirmed that relative humidity is an important factor in viral aerosol inactivation and found mixed benefits for collecting viruses onto a gelatin filter versus a foil or aluminum substrate. Finally, factors influencing the viability of MS2 were different from those influencing HAdV-1. When used as a general surrogate for human viruses, MS2 results should be interpreted cautiously.

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