Investigation of the effects of an ultraviolet germicidal irradiation system on concentrations of aerosolized surrogates for common veterinary pathogens

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OBJECTIVE
To determine whether exposure to UV germicidal irradiation (UVGI) reduces concentrations of viable aerosolized microorganisms (attenuated strains of common veterinary pathogens) in a simulated heating, ventilation, and air conditioning (HVAC) system.

SAMPLE
42 air samples seeded with bacteriophage MS2 or attenuated strains of *Bordetella bronchiseptica*, feline calicivirus, feline herpesvirus-1, canine parvovirus, or canine distemper virus (6/microorganism) or with no microorganisms added (6).

PROCEDURES
A simulated HVAC unit was built that included a nebulizer to aerosolize microorganisms suspended in phosphate-buffered water, a fan to produce airflow, 2 UVGI bulb systems, and an impinger for air sampling. Ten-minute trials (3 with UVGI, 3 without UVGI, and 1 negative control) were conducted for each microorganism. Impingers collected microorganisms into phosphate-buffered water for subsequent quantification with culture-based assays. Results for samples yielding no target microorganisms were recorded as the assay's lower limit of detection. Statistical analysis was not performed.

RESULTS
The UVGI treatment resulted in subjectively lower concentrations of viable MS2, *B bronchiseptica*, and canine distemper virus (arithmetic mean ± SD $\log_{10}$ microorganism reduction, 2.57 ± 0.47, ≥ 3.45 ± 0.24, and ≥ 1.50 ± 0.25, respectively) collected from air. Feline herpesvirus-1 was detected in only 1 sample without and no samples with UVGI treatment. Feline calicivirus and canine parvovirus were not detectable in any collected samples.

CONCLUSIONS AND CLINICAL RELEVANCE
Results for some surrogates of veterinary pathogens suggested a potential benefit to supplementing manual disinfection practices with UVGI-based air cleaning systems in animal care environments. Further research is needed to investigate the utility of UVGI in operating HVAC systems. (*Am J Vet Res* 2020;81:506–513)

Each year, an estimated 8 to 10 million animals are housed in animal shelters, veterinary clinics, and boarding facilities.[1] The proximity of animals to each other in dense animal housing environments increases the likelihood of exposure to contaminated fomites and aerosolized pathogens.[1] In addition to potential pathogen exposure, the physical and social confinement of animals in shelters has been linked to markers of increased stress (eg, decreased ratios of urinary adrenaline to creatinine concentrations and noradrenaline to creatinine concentrations) which, in turn, have been linked to immunologic susceptibility to infection and illness.[2,3] Pathogens of concern to veterinarians include sapovirus, feline noroviruses, canine circoviruses, canine influenza viruses, feline calicivirus, and feline panleukopenia virus.[1,4,5] Canine distemper virus, feline herpesvirus-1, and feline calicivirus have been identified as some of the most common causative agents of disease among animals in shelter settings.[1] Respiratory pathways of infection are of particular concern in environments housing animals.[6] The presence of many animals in a single confined environment is a risk factor for respiratory disease outbreaks, most commonly in shelters and kennels; however, these conditions and the potential for similar challenges may also exist in veterinary clinics.[3] Research has also shown that veterinary settings can have pathogen transmission issues similar to those in human healthcare settings, such as the need for prevention and control of nosocomial infections,[2] but there has been less

ABBREVIATIONS
HVAC Heating, ventilation, and air conditioning
PFU Plaque-forming unit
UVGI UV germicidal irradiation

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research into methods of minimizing illness risks in animal care environments.

Animals in clinical and shelter settings have contact with diverse surfaces, including floors, walls, and crates, that may be contaminated by pathogens.[7,8] Some recommendations for maintaining healthy environments include thoughtful floorplans, separating newly admitted and sick animals from the general population, monitoring and maintaining the health and vaccination status of all animals, and following specific hygiene and disinfection protocols.[7–9] Heating, ventilation, and air conditioning systems are commonly used in health-care facilities for temperature control and air distribution; however, the efficacy of these systems depends on proper design and maintenance.[10]

Minimizing the spread of pathogens in animal care environments is necessary for effective infection prevention and to reduce reliance on antimicrobials and ultimately decrease antimicrobial resistance prevalence. The purpose of the study reported here was to evaluate the use of a UVGI system to reduce concentrations of viable aerosolized microorganisms (bacteriophage MS2 and attenuated vaccine-derived strains of *Bordetella bronchiseptica*, feline calicivirus, feline herpesvirus-1, canine parvovirus, and canine distemper virus) in a custom-made simulated HVAC unit. The study was intended to contribute to a broader goal of improving infection prevention in indoor animal care environments through operational and management actions targeting the aerosolization route of pathogen exposure.

Materials and Methods

Simulated HVAC system

A simulated HVAC unit was designed in the Mel and Enid Zuckerman College of Public Health Laboratories at the University of Arizona for use in the study. The controlled system consisted of a sheet-metal air duct 2.44 m long × 0.46 m high × 0.46 m wide that was connected to a sheet-metal flange at 1 end to facilitate attachment of a fan[a] to produce laminar airflow **(Figure 1)**. The fan was operated at the manufacturer's setting of 556 cubic feet/min, equivalent to a mean ± SD of 183.6 ± 17.6 feet/min of airflow in the simulated HVAC system. Filters were in place at the inlet and outlet of the simulated HVAC chamber. Air speed was monitored at regular intervals in a grid pattern throughout the length and width of the duct with an anemometer.[b] The mean velocity weighted over the inner surface area of the duct was multiplied by the duct cross-section area to determine the airflow.[11] The research team aimed to create laminar airflow within the simulated HVAC system by calculating the Reynolds number, taking cross-sectional measurements of air speed throughout the system at the time of setup, and making modifications as needed to confirm that no more than minimal air speed fluctuations were observed throughout the system. The Reynolds number was calculated on the basis of properties of the fluid (sterile PBS solution[c]) in which the microorganisms were suspended and aerosolized as follows:

$$V_m D/v = \rho V_m D/\mu$$

where D is the characteristic length of the geometry (ie, the product of HVAC duct cross-sectional area [height × width] multiplied by duct length), V_m is the mean flow velocity (in meters per second; measured by anemometer[b]), and ν is the kinematic viscosity of the fluid (in square meters per second) and equal to μ/ρ, where μ represents dynamic viscosity (kilograms per second per meter) and ρ represents density (kilograms per cubic meter). A Reynolds number ≤ 2,300 is classified as indicating laminar flow.[12]

The UVGI system[d] installed was a series of 4 UVGI bulbs consisting of 2 sets of 2 bulbs[e] placed 34.3 cm apart in the simulated HVAC system. Each bulb emitted germicidal UV light at 253.7 nm, 60 W, and a power of 60 μW/cm^2.[13] All trials were completed within the useful lifetime of the UVGI bulbs (9,000 hours).[13]

Microorganism selection and aerosolization for testing

Concentrated bacteriophage MS2 (a nonpathogenic bacterial virus commonly used as a surrogate for enteric and respiratory viral pathogens)[f] and live, attenuated, vaccine-derived strains of the common animal pathogens *B bronchiseptica*,[g] feline herpesvirus-1,[h] feline calicivirus,[h] canine parvovirus,[i] and canine distemper virus[i] were aerosolized during separate trials into the simulated HVAC system by nebulization, collected from the air with impingers, and quantified. The tested microorganism details, including starting concentration (as determined by standard laboratory cultivation and serial dilution methods) added to the nebulizer, are provided **(Appendix)**. Attenuated strains represented the same microorganisms found

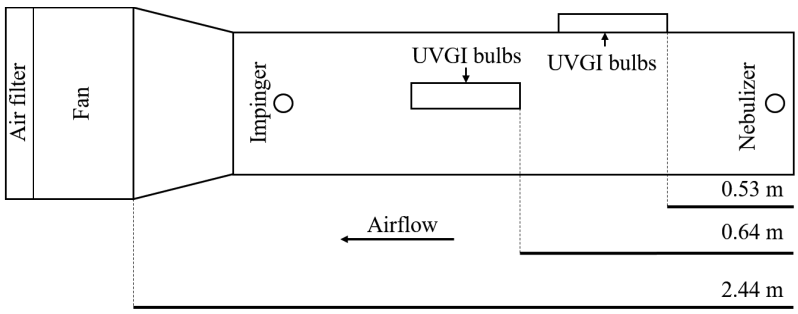

Figure 1—Schematic drawing depicting the configuration of a custom-made simulated HVAC system created for use in a study to investigate whether exposure to UVGI reduces concentrations of viable aerosolized microorganisms used as surrogates for common veterinary pathogens within such a system.

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in naturally occurring diseases except that the microorganisms had been modified to reduce virulence to make the target safer for laboratory experiments or vaccinations without causing illness.

Microorganisms were aerosolized in the simulated HVAC chamber with a positive pressure pump[j] operated at 12.7 L/min with an ultimate pressure of 110 torr (14,665.5 Pa). The pump was calibrated[k] before and after each sample testing day. The pump was attached to a Collison nebulizer.[l] Microorganisms were tested individually, and 10 mL of concentrated microorganism solution was added to the nebulizer for each trial. Pathogen surrogates were tested in the following order for a total of 6 trials/microorganism: MS2, *B bronchiseptica*, feline calicivirus, feline herpesvirus-1, canine parvovirus, and canine distemper virus. Air temperature and relative humidity were not controlled during experiments; these environmental conditions were measured inside the chamber by use of a hygrometer and were recorded once for each trial. Between trials, the nebulizer was rinsed 3 times with 70% ethanol and 3 times with deionized water and allowed to dry.

Air sample collection

Air samples were collected with sterile impingers according to methods specified by the American Conference of Governmental Industrial Hygienists.[14] Glass impingers[m] were sterilized by use of an autoclave and aseptically filled with 3 mL of PBS solution just prior to sample collection. Impingers were wrapped in aluminum foil to protect collected microorganisms from being affected by UV light throughout the experimental trial. Impinger flow rates were calibrated[k] before and after each experiment. Impingers concentrated aerosolized microorganisms into the PBS solution by means of a vacuum pressure pump calibrated to 3.8 to 4.2 L/min. Each impinger was modified by adding clear vinyl tubing (0.6 cm internal diameter) at a 90° angle to the intake valve to collect air in a direction parallel to the airflow. All plastic tubing was disinfected prior to use in experiments by immersion in a 10% bleach solution for 15 to 20 minutes. Tubing was then rinsed with sterile 10% sodium thiosulfate solution and allowed to air dry. Residual nonviable microorganisms could not impact subsequent results because the enumeration methods assessed only viable microorganisms. Impingers (1/trial) were placed in the outflow section of the chamber at a distance of 58.4 cm from the nearest UVGI device. The research team aimed to achieve isokinetic sampling by optimizing impinger and nebulizer flow rates. Isokinetic sampling conditions were calculated as follows[2]:

$$Q_i/Q_d = (D_i/D_d)$$

where Q_i is the impinger flow rate (in liters per minute), Q_d is the duct flow rate (in cubic feet per minute), D_i is the impinger inlet diameter (in meters), and D_d is the duct cross-sectional area (in meters).

The 6 trials performed for each microorganism comprised 3 tests of seeded air with no UVGI treatment (in which all bulbs were turned off [inactive]) and 3 tests of seeded air with UVGI treatment (in which all bulbs were activated for the entirety of each test). All trials with and without UVGI treatment lasted for 10 minutes. The fan was operated continuously during each trial to simulate real-world HVAC airflow. Air was sampled with the impingers, and the airflow was seeded with 1 microorganism/trial through nebulization. Negative control trials (1/set of microorganism experiments) were also performed in which the entire system was operating as described but no microorganisms were seeded into the air and the UVGI system was not activated. Negative control samples were processed in the same manner as all other samples to ensure no residual microorganisms persisted between trials.

Between trials, the simulated HVAC system was allowed to run for 30 minutes with UVGI activated and airflow as described to sterilize the chamber between uses. Before each set of trials for a given microorganism, a negative control was run wherein an air sample was collected with the system fully functional but with no microorganisms being aerosolized into the system. The surfaces inside the system were not sampled for residual contamination between trials.

Sample processing and microorganism enumeration

After every trial, the impinger was vortexed at medium speed for 10 seconds. The impingers and tubing were then rinsed with 1 mL of sterile PBS solution. The total volume (approx 4 mL) was collected and aseptically transferred into a sterile 15-mL conical tube in preparation for microbial culture assays. All samples were processed at the University of Arizona Environment, Exposure Science and Risk Assessment Center laboratory or the University of Arizona Water and Energy Sustainable Technology Center.

Enumeration of MS2 was performed by 10-fold serial dilution of the sample solution and duplicate plating with a double agar overlay comprised of a 1.5% tryptic soy agar base and a 0.7% tryptic soy agar overlay.[n] A log-phase host culture of *Escherichia coli*[o] was suspended in the overlay concurrently with the sample. Plates were incubated at 37°C for 24 hours. Visible PFUs in the bacterial lawn were counted, and MS2 concentrations were reported as PFUs/mL. The vaccine strain of *B bronchiseptica* was enumerated by 10-fold serial dilution of sample solution and duplicate plating of each dilution onto Bordet-Gengou agar[p] supplemented with sheep's blood[q] at a 15% concentration. Plates were incubated at 37°C for ≤ 7 days and were observed for growth daily with final counts confirmed and recorded on day 7, with results reported as CFUs/mL.

Feline calicivirus and feline herpesvirus-1 concentrations were quantified by culture with the Crandell-Rees feline kidney host cell line.[r] Canine parvovi-

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rus and canine distemper virus concentrations were quantified by culture with A-72 dog tumor cells[s] and African green monkey kidney cells,[t] respectively. After collection from impingers, virus samples were passed through a 0.22-µm filter pretreated with 3% beef extract and diluted 10-fold with minimal essential medium containing no fetal bovine serum. All dilutions were plated in quadruplicate onto host cell monolayers at 80% to 90% confluency and then incubated for 60 minutes at 37°C in an orbital rotator to facilitate virus-host cell adsorption. Maintenance medium (minimal essential medium supplemented with fetal bovine serum to a concentration of 2%) was added to the cell cultures, and they were incubated for 7 days at 37°C in a 5% CO_2 atmosphere. Assay cultures were examined every 24 to 48 hours by use of an inverted light microscope to assess cytotoxicity and viral cytopathic effects. The $TCID_{50}/m^3$ for feline herpesvirus-1, feline calicivirus, canine parvovirus, and canine distemper virus was reported at the 50% end point (ie, the dilution at which contents of 50% of the inoculated cell culture flasks had viral cytopathic effects observed).

Data analysis

Log_{10} reductions in microbial concentrations attributable to UVGI treatment were calculated by comparing concentrations of microorganisms collected from air with and without UVGI treatment into sample solution as follows:

$$\log_{10}(C_i) - \log_{10}(C_f)$$

where C_i is the microbial concentration measured in 1 mL of PBS solution after sampling of seeded air without UVGI treatment and C_f is the microbial concentration measured in 1 mL of PBS solution after sampling of seeded air with UVGI treatment. The same calculation was performed to assess $\log_{10}$ reductions in the starting concentration of microorganisms attributable to sample manipulations independent of UVGI treatment, where C_i is the impinger-collected microbial concentration in 1 mL of PBS solution during sampling of microorganism-seeded aerosol without UVGI treatment and C_f is the impinger-collected microbial concentration in 1 mL of PBS solution during sampling of microorganism-seeded aerosol with UVGI treatment. When a microorganism was not detectable in any sample, the C_f was set to the lower limit of detection for that assay. The lower limits of detection were 1 PFUs/mL for MS2, 10 CFUs/mL for *B bronchiseptica*, and 3.16 $TCID_{50}$/mL for feline calicivirus, feline herpesvirus-1, canine parvovirus, and canine distemper virus.

Assay results were mathematically converted to the number of microorganisms per cubic meter of air sampled. The vacuum pump processed 4 L of air/min for 10 minutes, translating to 0.04 m^3/trial. Given that a total of 4 mL of solution was processed for each trial, the contents in 1 mL of sample solution at the end of a trial was considered representative of the contents of 0.01 m^3 of chamber air. Log_{10} reductions calculated for collected 1-mL samples are reported as unitless values by convention. Statistical analyses were not performed.

Results

Environmental conditions and flow rate determinations

During the study, air temperatures in the chamber ranged from 18.7°C to 26.7°C, and relative humidity ranged from 12% to 50%. The mean ± SD airflow during sampling was 413.1 ± 39.7 cubic feet/min (equivalent to 11.7 ± 1.1 m^3/min), resulting in passage of 117.0 ± 11.2 m^3 of air through the chamber during each 10-minute run. The PBS solution density (1.0062 kg/m^3), mean PBS solution flow velocity (0.93 m/s), duct measurement (0.46 × 0.46 × 2.44 m), and PBS solution viscosity (0.001043 kg/s/m) yielded a calculated Reynolds number of 694, indicating laminar flow. The duct flow rate (556 cubic feet/min), impinger inlet diameter (0.0064 m), and duct cross-sectional area (0.4572 m) yielded a calculated optimal impinger airflow rate of 3.08 L/min. Owing to physical limitations, pumps producing airflow through the impingers were set to sample at the lowest possible flow rate (3.8 to 4.2 L/min).

Protocol and UVGI effects on aerosolized microorganisms

Seven samples (3 seeded air with no UVGI treatment, 3 seeded air with UVGI treatment, and 1 negative control) were collected for each of 6 trials (1 trial/target microorganism) for a total of 42 samples. Feline calicivirus and canine parvovirus were not detected in samples from seeded air with UVGI treatment, seeded air without UVGI treatment, or negative control samples (air samples with no seeding or UVGI treatment).

Changes in mean ± SD $\log_{10}$ concentrations of MS2, *B bronchiseptica*, feline herpesvirus-1, and canine distemper virus after UVGI treatment are summarized **(Figure 2)**. Negative control samples tested negative by culture for the target microorganism in each assay, indicating that if any viable aerosolized microorganisms were present, the numbers were less than the lower limit of detection for the assay.

The initial concentration of MS2 in the nebulizer prior to aerosolization was 1.21×10^{10} PFUs/mL. After aerosolization and 10 minutes of airflow without UVGI exposure, the mean ± SD concentration measured in samples collected from seeded air was $4.66 \times 10^5 \pm 6.96 \times 10^5$ PFUs/mL, equivalent to $4.66 \times 10^7 \pm 6.96 \times 10^7$ PFUs/m^3 in chamber air. This reflected a mean ± SD $\log_{10}$ reduction of 4.32 ± 0.08 attributable to air exchange rates alone. Under the same conditions with UVGI treatment, the mean ± SD measured MS2 concentration in collected samples was $3.23 \times 10^3 \pm 5.37 \times 10^3$ PFUs/mL, equivalent to $3.23 \times 10^5 \pm 5.37 \times 10^5$ PFUs/m^3 in air, representing a mean ± SD

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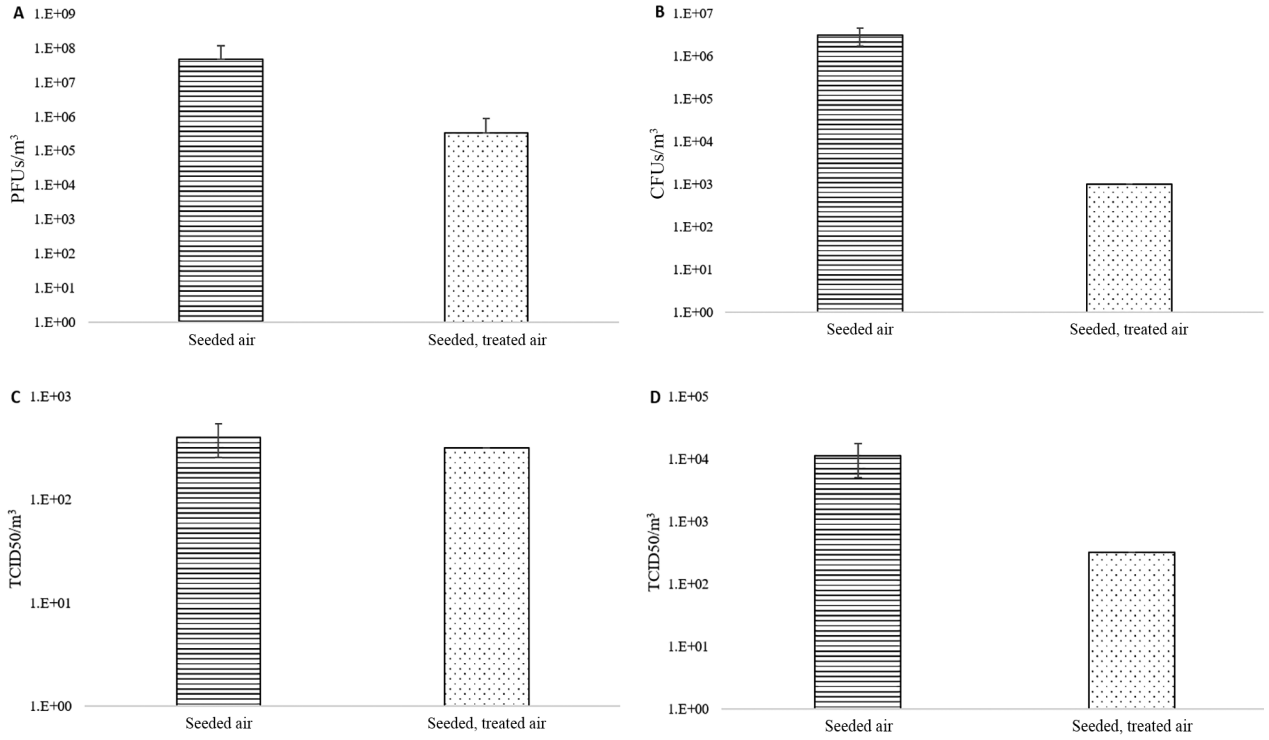

Figure 2—Mean ± SD concentrations of 4 microorganisms used as surrogates for common veterinary pathogens (bacteriophage MS2 [A] and vaccine-derived attenuated strains of *Bordetella bronchiseptica* [B], feline herpesvirus-1 [C], and canine distemper virus [D]) collected by use of impingers over a 10-minute period after aerosolization by use of a nebulizer in a custom-made simulated HVAC system without (horizontally striped bars) and with (dotted bars) UVGI treatment. Data represent results of culture-based assays for 3 trials/treatment type/microorganism; results for samples in which the target microorganism was not detected (2/3 samples for feline herpesvirus-1 without UVGI treatment; 3/3 samples each for *B bronchiseptica*, feline herpesvirus-1, and canine distemper virus with UVGI treatment) were recorded as the lower limit of detection for the assay used. Attenuated vaccine strains of feline calicivirus and canine parvovirus were also tested, but no microorganisms were detected in any samples collected from seeded HVAC chamber air with or without UVGI treatment (not shown).

$\log_{10}$ reduction of 2.57 ± 0.47 attributed to the UVGI treatment.

The initial concentration of *B bronchiseptica* in the nebulizer before aerosolization was 1.15×10^9 CFUs/mL. After aerosolization and 10 minutes of airflow without UVGI exposure, the mean ± SD concentration measured in samples from seeded air was $3.06 \times 10^4 \pm 1.40 \times 10^4$ CFUs/mL, equivalent to $3.06 \times 10^6 \pm 1.40 \times 10^6$ CFUs/m^3 in chamber air. This represented a mean ± SD $\log_{10}$ reduction of 4.62 ± 0.20 attributable to air exchange rates alone. After the described UVGI treatment, *B bronchiseptica* concentrations in all collected samples were less than or equal to the lower limit of detection for the assay (10 CFUs/mL). This represented a mean ± SD $\log_{10}$ reduction of ≥ 3.45 ± 0.24 attributed to the UVGI treatment.

The initial concentration of feline herpesvirus-1 in the nebulizer prior to aerosolization was 3.16×10^6 $TCID_{50}$/mL. After aerosolization and 10 minutes of airflow without UVGI exposure, feline herpesvirus-1 was above the lower limit of detection in 1 of 3 samples (concentration, 5.62 $TCID_{50}$/mL [equivalent to 5.62×10^2 $TCID_{50}$/m^3 in chamber air]). After the described UVGI treatment, feline herpesvirus-1 concentrations were less than the lower limit of detection (3.16 $TCID_{50}$/mL) in all collected samples. Therefore, a single $\log_{10}$ reduction value (0.25) was attributed to the UVGI treatment.

The initial concentration of canine distemper virus in the nebulizer before aerosolization was 2.40×10^7 $TCID_{50}$/mL. After aerosolization and 10 minutes of airflow without UVGI exposure, the mean ± SD concentration measured in samples from seeded air was $1.11 \times 10^2 \pm 6.17 \times 10^1$ $TCID_{50}$/mL, equivalent to $1.11 \times 10^4 \pm 6.17 \times 10^3$ $TCID_{50}$/m^3 in chamber air. This represented a mean ± SD $\log_{10}$ reduction of 5.38 ± 0.20 attributable to air exchange rates alone. After the described UVGI treatment, canine distemper virus concentrations were less than the lower limit of detection (3.16 $TCID_{50}$/mL) in all samples. This represented a mean ± SD $\log_{10}$ reduction of ≥ 1.50 ± 0.25 attributed to the UVGI treatment.

Discussion

The reduction of aerosolized animal pathogens in shelter and veterinary settings is an important step in mitigating the spread of disease for susceptible populations. The findings of the preliminary study reported here supported the usefulness of UVGI bulbs for reducing concentrations of viable aerosolized micro-

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organisms in a simulated HVAC unit designed to mimic systems that might be used in such a setting. It has been established that UV disinfection is effective against many types of microorganisms on surfaces and in air.[15,16] Importantly, UV treatment effects can be impacted by many variables, including exposure duration, distance of the targets from the UV source, airflow rates, air temperature (eg, overcooling), and humidity, and investigation of the influences of temperature and humidity were beyond the scope of our study. However, a primary limitation of the present study was a loss of microorganisms to the system before UVGI treatment effects could be evaluated.[15,16] Overall, a mean ± SD 5.21 ± 0.10 $\log_{10}$ reduction in the concentrations of viable microorganisms (including samples below the limit of detection taken as the lower limit [n = 8]) was attributable to losses in the system even in the absence of UVGI treatment. The $\log_{10}$ reductions were calculated by use of the lower limits of detection for the relevant assay when no microorganisms were detected in samples after UVGI treatment, and as such, the actual reductions in concentrations of feline herpesvirus-1, *B bronchiseptica*, and canine distemper virus was potentially greater than that reported here because each had posttreatment concentrations below the lower limit of detection (3.16 $TCID_{50}$/mL, 10 CFUs/mL, and 3.16 $TCID_{50}$/mL, respectively). The inability to detect feline calicivirus or canine parvovirus during air sampling, coupled with the ability to detect other microorganisms under the same conditions, suggested microorganism-specific issues with collection and cultivation under the described conditions. Potential characteristics contributing to the inability to detect these microorganisms could have included morphological characteristics that interfere with aerosolization or contribute to inactivation of viruses during the aerosolization or sampling process. In addition, high-efficiency aerosolization and subsequent collection of microorganisms from air are inherently difficult. Results of 1 study[17] to investigate the aerosolization and sampling of bacterial species reveal recovery efficiencies of only 20%. In another study,[18] recovery efficiency for aerosolized feline calicivirus ranged from 0.37% to 38%. Microorganism concentration quantified from impingers may not be entirely representative of concentrations in the chamber air in the present study owing to the limitations involved with creating a simulated HVAC system. Although steps were taken to optimize the conditions, perfect laminar flow and isokinetic sampling may not have been achieved. Observed fluctuations in the airflow were minimal; however, it is possible that airflow was disrupted on a nondetectable scale when passing the UVGI bulbs or hardware inside the unit. Further, limitations in the sample setup prevented impingers from sampling at the optimized rate. The variability seen between trials for each microorganism may have been attributable to the disruptions in microbial transport through the air and subsequent recovery caused by these factors.

Attenuation is designed to reduce infectivity of a microorganism in a host. The use of attenuated strains may have reduced our ability to mirror true clinical scenarios of aerosolized pathogen transport, including factors such as persistence of microorganisms. However, attenuated strains of clinical pathogens are a safer tool for evaluation of this process and are expected to share more characteristics with the source pathogen than an alternative surrogate might. The authors believe these attenuated strains were used appropriately and represented the pathogenic strains of the same microorganisms. Some of the microorganisms investigated (feline herpesvirus-1, canine distemper virus, and feline calicivirus) were representative of pathogens that are not generally transmitted via the airborne route. These surrogates were selected for evaluation because of their potential to survive transport through air and deposition on various environmental surfaces. In animal care settings, exposure to microorganisms that are deposited on surfaces from the air can occur by contact with these surfaces or through ingestion.

Further limitations of the present study included the testing of a single UVGI product. Research into other products' ability to reduce concentrations of aerosolized microorganisms would contribute to understanding the potential usefulness of such systems in animal care center HVAC units. Additionally, conclusions regarding the potential efficacy of UVGI for reducing rates of disease associated with these pathogens could not be drawn. The infectious doses of common veterinary pathogens represented by surrogates used in the present study vary. If the infectious dose is very low, reductions in numbers of viable microorganisms such as those found in our study may not be beneficial unless implemented in combination with other microbial exposure reduction techniques.

Overall, the UVGI bulb system used in the present study showed a capacity for microorganism reduction when the microorganism was detectable in collected samples (feline herpesvirus-1, *B bronchiseptica*, MS2, and canine distemper virus) and under the conditions of the simulated HVAC unit. These results suggested that UVGI bulbs may be useful to reduce concentrations of aerosolized microorganisms capable of causing disease in the animals in these environments, although further research is needed to confirm these findings in working HVAC systems under various temperature and humidity conditions and to assess whether the differences found are consistent and clinically meaningful. These findings supported those in previous studies[19–21] of UVGI applicability in human health-care settings. Three separate studies[19–21] of different types of UVGI units were shown to reduce concentrations of viable airborne microorganisms or particles as well as infection rates in intensive care facilities. The established effectiveness of UVGI coupled with the findings in our study suggest the potential for these types of units to contribute to a meaningful impact on infection preven-

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tion in animal care settings. Ultraviolet technology may be combined with other methods of aerosolized microbial reduction or design enhancements, such as super-reflective interior coating of air ducts, to improve pathogen reduction.[22] In practice, combining disinfection methods has had mixed impacts on pathogen reduction in human health-care settings. Miller et al[23] showed that UV treatment can be effective for reducing surface concentrations of a spore-forming, fecal-oral pathogen (*Clostridium difficile*). However, results of a separate study[24] indicate that when UV treatment was used to enhance cleaning protocols, it did not reduce *C difficile* illness risk or did so in the targeted areas but not throughout the studied hospital. On the basis of these findings in human health-care settings, it is intriguing to consider tracking various infection rates in animal care facilities with and without UVGI air treatment.

Prevention of disease and the transmission of infectious agents within animal populations will have broader disease control and economic benefits.[25] Although beyond the scope of the present study, the cost for disease prevention is likely to be far less than the economic burden of disease treatment, as the global cost of zoonotic diseases is estimated to exceed $200 billion.[22] Overall, future studies are needed to assess the efficacy of UVGI bulbs to reduce the transmission of pathogenic bacteria and viruses in shelter or veterinary settings and the associated adverse health outcomes in animals. Given the limitations of the present study, more research is needed to draw any definitive conclusions regarding UVGI use in HVAC systems of animal care facilities, but the results supported the possibility that applying UVGI treatment within an HVAC system may reduce viable, aerosolized microorganism concentrations in air passing through it and that this type of engineering control might be a useful adjunct to other methods of disinfection in animal care settings.

Acknowledgments

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Footnotes

a. Model AFS-1000B Jet Air Filtration System, Jet Tools North America, La Vergne, Tenn.
b. VelociCalc Air Velocity Meter 9535, TSI Inc, Santa Rosa, Calif.
c. Sigma-Aldrich Corp, St Louis, Mo.
d. UV Air Disinfector, PAH-N12, PetAirapy LLC, Saint Charles, Ill.
e. Product PL-L TUV60W, Philips, Andover, Mass.
f. Product 15597-B, American Type Culture Collection, Manassas, Va.
g. Intra-trac3, Merck & Co Inc, Kenilworth, NJ.
h. Felocell3, Zoetis Services LLC, Parsippany, NJ.
i. Canine 1-DAPPv, Merck & Co Inc, Kenilworth, NJ.
j. Model 2010B-01, Gardner Denver Welch Vacuum Technology Inc, Sheboygan, Wis.
k. Defender 510 Calibrator, Mesa Laboratories, Butler, NJ.
l. BGI Inc, Waltham, Mass.
m. Glass Midget Impinger, SKC Inc, Eighty Four, Pa.
n. VWR International, Radnor, Pa.
o. Product 15595, American Type Culture Collection, Manassas, Va.
p. Remel, Lenexa, Kan.
q. Quad Five, Ryegate, Mont.
r. ATCC CCL-94, American Type Culture Collection, Manassas, Va.
s. ATCC CRL-1542, American Type Culture Collection, Manassas, Va.
t. Vero cell line, ATCC CCL-81, American Type Culture Collection, Manassas, Va.

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Appendix

Details of microorganisms used in a study to investigate whether exposure to UVGI reduces concentrations of viable aerosolized microorganisms selected as surrogates for common veterinary pathogens in a simulated HVAC system.

Pathogen or disease	**Surrogate microorganism**	**Concentration in PBS solution prior to aerosolization**
Enteric and respiratory viral pathogens	Bacteriophage MS2[e]	1.21×10^{10} PFUs/mL
Kennel cough	Attenuated strain of *Bordetella bronchiseptica*[f]*	1.15×10^{9} CFUs/mL
Feline calicivirus	Attenuated vaccine strain of feline calicivirus[g]†	1.78×10^{6} $TCID_{50}$/mL
Feline herpesvirus-1	Attenuated vaccine strain of feline herpesvirus-1[g]†	3.16×10^{6} $TCID_{50}$/mL
Canine parvovirus	Attenuated vaccine strain of canine parvovirus[h]‡	5.62×10^{5} $TCID_{50}$/mL
Canine distemper virus	Attenuated vaccine strain of canine distemper virus[h]‡	2.40×10^{7} $TCID_{50}$/mL

*The aerosolized vaccine suspension contained modified-live virus and avirulent live culture of canine adenovirus type 2, parainfluenza, and *B bronchiseptica*; samples were tested for *B bronchiseptica* only. †The aerosolized vaccine suspension contained modified-live feline herpesvirus-1, feline calicivirus, and feline parvovirus; samples were tested for feline herpesvirus and feline calicivirus only. ‡The aerosolized vaccine suspension contained attenuated strains of canine distemper virus, adenovirus type 2, parainfluenza, and parvovirus; samples were tested for canine parvovirus and distemper virus only.

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