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Total Concentrations of Virus and Bacteria in Indoor and Outdoor Air

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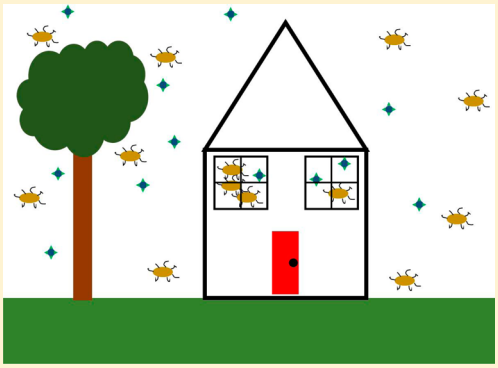
Supporting Information

ABSTRACT: Viruses play important roles in microbial ecology and some infectious diseases, but relatively little is known about the concentrations, sources, transformation, and fate of viruses in the atmosphere. We have measured total airborne concentrations of virus-like and bacterium-like particles (VLPs between 0.02 and 0.5 μm in size and BLPs between 0.5 and 5 μm) in nine locations: a classroom, a daycare center, a dining facility, a health center, three houses, an office, and outdoors. Indoor concentrations of both VLPs and BLPs were $\sim 10^5$ particles m^{-3}, and the virus:bacteria ratio was 0.9 $\pm$ 0.1 (mean $\pm$ standard deviation across different locations). There were no significant differences in concentration between different indoor environments. VLP and BLP concentrations in outdoor air were 2.6 and 1.6 times higher, respectively, than in indoor air. At the single outdoor site, the virus:bacteria ratio was 1.4.



INTRODUCTION

Airborne viruses, bacteria, and fungi, known as bioaerosols, are of interest because some can cause human, plant, and animal diseases,[1−3] while others are beneficial to human health and the environment.[4−6] Advances in high-throughput sequencing are allowing unprecedented characterization of microbiological communities, but one aspect missing from such studies is the total number of microbes present. From scientific, public policy, and public health points of view, it is important to understand not only what types of microbes are present in air but also how many there are.

There have been many studies that have examined the concentrations of airborne bacteria and fungi in different environments, both indoors and outdoors. Concentrations of bacteria and fungi of approximately 10^2−10^6 colony-forming units (CFU) m^{-3} and 10^2−10^3 spores m^{-3}, respectively, are typical.[7−12] In contrast, studies examining airborne virus concentrations have been limited because of technical challenges and underappreciated importance.[13] Griffin et al.[14] collected airborne viruses and bacteria in African desert dust transported to the Caribbean and found that concentrations were similar for the two types of bioaerosols, ranging from 10^4 to 10^5 particles m^{-3}. Whon et al.[15] reported total concentrations of airborne virus and bacteria of 10^5−10^7 particles m^{-3} in Korea. To the best of our knowledge, these are the only studies in the literature reporting total viral bioaerosol concentrations, and both examined outdoor air only. Humans spend >90% of their time indoors,[16] so there is a gap in knowledge regarding the concentrations to which we are exposed a majority of the time.

Historically, the virus:bacteria ratio (VBR) has been used to describe the relative abundance of viruses compared to that of bacteria, and it can vary dramatically depending on the specific environment being studied. VBR values have been obtained for many different environments, including the Arctic Ocean, the Pacific Ocean, lakes, agricultural soil, forest soil, and the human gut.[17−23] Values range from 0.2 in the human gut to 2750 in agricultural soil.[20−23] The VBR is important because interactions between viruses and bacteria are relevant to both human health and ecology.[24−26] For example, studies have shown an association between viruses and bacteria that cause respiratory infections in children with asthma; additionally, *Pneumococcus* bacteria and influenza virus have been shown to interact with each other.[25,26]

The majority of previous work on indoor bioaerosols has focused on understanding the concentrations and community structure of bacteria.[27,28] The few studies of viral bioaerosols have focused on specific viruses, such as influenza A.[29] Quantifying total virus concentrations is more challenging than quantifying total bacterial concentrations[13] because unlike bacteria and fungi, viruses lack a conserved common gene that can be used for quantification through quantitative polymerase chain reaction.[30,31] Additionally, viruses are obligate parasites and thus cannot be quantified on a growth plate exposed to air, a method that can be used for some bacteria and fungi. Studies examining total virus concentrations have relied on fluorescence microscopy, a simple yet effective method for quantification of virus-like particles.[14,32,33]

The specific objectives of this study were to determine concentrations of virus-like particles (VLPs) and bacterium-like particle (BLPs) and VBR values in both indoor and outdoor air. We collected samples in a classroom, a daycare center, a dining facility, a health center, three houses, an office, and outdoors.

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Using fluorescence microscopy, we quantified the number of VLPs and BLPs collected and calculated particle concentrations in the air. We hypothesized that VLP concentrations would be significantly higher than BLP concentrations in the air, because of the smaller size of VLPs and their ability to remain airborne longer, leading to a VBR of >1. Our results provide novel information about total concentrations of virus and bacteria in air.

MATERIALS AND METHODS

Air Sampling Sites and Collection. We collected air samples during September and October 2014 at nine locations in Blacksburg, VA. We collected samples in triplicate from a classroom, a daycare center, a dining facility, a health center, three single-family houses, an office, and outdoors. In the houses, the mechanical heating, ventilation, and air conditioning (HVAC) systems were not running during the sampling period. The outdoor sampling site was in the middle of a university campus, surrounded by grass, paved paths, and buildings. For each sample, a pump (SKC AirChek 2000) pulled air through a 0.2 μm pore size, 25 mm diameter Anodisc filter mounted in a stainless steel filter holder (Advantec) at a flow rate of ~0.9 L min^{-1} for ~120 min for a total sample volume of ~100 L. We used a primary flow calibrator (A. P. Buck mini-Buck) to measure the flow rate at the start and end of each sampling period and multiplied the average flow rate during the period by the sampling duration to calculate the sample volume. Immediately following sampling, we removed filters from the filter holder, placed them in a sterile Petri dish, and refrigerated them until further analysis. We included three unexposed samples from the same batch of filters as controls. Table S1 of the Supporting Information lists individual sample dates, times, and flow rates.

Particle Detection. The quantification methodology for viral aerosols followed a previously published approach.[14,32,33] Briefly, we treated each filter with a fluorescent dye that stained DNA and RNA (SYBRGold). We placed the filter exposed-side up in a 100 μL working solution of the dye (97.5 μL of deionized water and 2.5 μL of 1:10 diluted SYBRGold) and incubated it in the dark for ~20 min. The dye wicked through the bottom of the filter and stained any nucleic acid on the top side. We removed the filter from the staining drop, blotted away excess dye, positioned the filter on a glass slide, and gently placed a coverslip containing 25 μL of mounting solution [50% 1× PBS/50% glycerol and 1% (w/v) absorbic acid] over the filter. We analyzed samples within 1 h of slide preparation using an epifluorescence microscope (Leica CTR-6000). We imaged 25 fields per slide, which ensured a large enough sample size before photobleaching of the stain began to occur; the fields traversed an S-shaped pattern across the filter.

Particle Quantification. Using the ImageJ image processing program,[34] we counted the total number of VLPs and BLPs collected on each filter based on size. We counted fluorescent particles between 0.02 and 0.50 μm as VLPs and those between 0.50 and 5.00 μm as BLPs.[14,15,32,33] VLPs appeared as pinpricks, whereas BLPs were larger fluorescent signals, consistent with previous studies.[32,33] We automated counting through batch processing in ImageJ and subtracted the average particle count obtained from the control filters to account for preexisting particles on an unexposed filter. To calculate airborne concentrations, we extrapolated results from the 25 images to estimate the total number of VLPs and BLPs on each filter and divided by the volume of air sampled.

Statistical Analysis. Because microbial counts are typically log-normally distributed, we applied a $\log_{10}$ transformation to the VLP and BLP concentrations. We used one-way analysis of variance to test for significant differences ($p < 0.05$) between VLP and BLP concentrations. For pairwise comparisons between all the different sampling sites, we performed a Tukey's HSD test.

RESULTS AND DISCUSSION

The average concentrations of VLPs were $(4.7 \pm 2.5) \times 10^5$ and $(1.2 \pm 0.7) \times 10^6$ VLPs m^{-3} across all samples collected in indoor and outdoor environments, respectively (Table 1). The corresponding geometric means and geometric standard deviations ($\log_{10}$ transformation) were 5.6 ± 0.3 indoors and 6.0 ± 0.3 outdoors. The average concentrations of BLPs were $(5.4 \pm 2.6) \times 10^5$ and $(8.4 \pm 4.4) \times 10^5$ BLPs m^{-3} across all samples collected in indoor and outdoor environments, respectively (Table 1). The corresponding geometric means and geometric standard deviations were 5.7 ± 0.2 indoors and 5.9 ± 0.2 outdoors. The virus:bacteria ratio (VBR) for indoor environments averaged 0.9 ± 0.1 and ranged between 0.7 and 1.1 (Table 1), indicating that more BLPs than VLPs were typically present in indoor environments. The VBR for the outdoor sample was 1.4, indicating that ~40% more viruses than bacteria were present in outdoor air at our specific sampling location and time (Table 1).

Table 1. Airborne Virus-like (VLP) and Bacterium-like Particle (BLP) Concentrations (mean ± standard deviation) in Different Environments

location	VLP concentration[a] (particles/m^3) [$\log_{10}$-transformed)][b]	BLP concentration[a] (particles/m^3) [$\log_{10}$-transformed)][b]	virus:bacteria ratio
classroom	$(5.7 \pm 3.3) \times 10^5$ [5.7 ± 0.3]	$(6.5 \pm 3.4) \times 10^5$ [5.8 ± 0.3]	0.9
daycare center	$(4.5 \pm 2.0) \times 10^5$ [5.6 ± 0.2]	$(5.0 \pm 1.2) \times 10^5$ [5.7 ± 0.1]	0.9
dining facility	$(3.9 \pm 0.4) \times 10^5$ [5.6 ± 0.0]	$(4.3 \pm 0.8) \times 10^5$ [5.6 ± 0.1]	0.9
health center	$(2.9 \pm 2.3) \times 10^5$ [5.2 ± 0.7]	$(3.4 \pm 1.6) \times 10^5$ [5.5 ± 0.2]	0.9
house 1	$(5.9 \pm 3.9) \times 10^5$ [5.7 ± 0.4]	$(5.6 \pm 2.7) \times 10^5$ [5.7 ± 0.2]	1.1
house 2	$(5.2 \pm 1.5) \times 10^5$ [5.7 ± 0.1]	$(6.5 \pm 1.5) \times 10^5$ [5.8 ± 0.1]	0.8
house 3	$(4.6 \pm 4.2) \times 10^5$ [5.5 ± 0.4]	$(6.8 \pm 5.6) \times 10^5$ [5.7 ± 0.4]	0.7
office	$(4.9 \pm 2.5) \times 10^5$ [5.6 ± 0.3]	$(4.8 \pm 2.0) \times 10^5$ [5.7 ± 0.2]	1.0
outdoors	$(1.2 \pm 0.7) \times 10^6$ [6.0 ± 0.3]	$(8.4 \pm 4.4) \times 10^5$ [5.9 ± 0.2]	1.4

[a]Concentrations are based on three independent samples and have been corrected for the number of particles present on unexposed filters. [b]Mean and standard deviation of the $\log_{10}$-transformed data, or the geometric mean and geometric standard deviation.

VLP concentrations were not significantly different (p = 0.43) between sampling locations, and the same was true for BLP concentrations (p = 0.63). Table S2 of the Supporting Information shows pairwise comparisons of VLP and BLP counts between different sampling environments.

We are aware of only two studies that measured VLP concentrations in air, and those studies examined outdoor air[14,15] but not indoor air. The magnitudes of VLP, BLP, and VBR values in our outdoor samples are similar to the

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magnitudes of results of the previous studies. Whon et al.[15] examined outdoor air in Korea and found VLP and BLP concentrations of 1.7×10^6 to 4.0×10^7 and 8.6×10^5 to 1.1×10^7 particles m^{-3}, respectively, with an average VBR of 2.2, although the numbers are not directly comparable because Whon et al. used a different sample collection and preparation method. They excluded particles larger than 1 μm, collected bioaerosols into liquid first using an impinger, and then passed the liquid through an Anodisc filter. Griffin et al.[14] found VLP and BLP concentrations of 2.1×10^5 and 1.6×10^5 particles m^{-3}, respectively, in Caribbean air, which correspond to a VBR of 1.3. While Griffin et al.[14] collected air samples directly onto a 0.02 μm pore size filter, we used a 0.2 μm pore size filter with lower pressure drop due to concerns about noise from the sampling pump in indoor, occupied environments. On the basis of studies of membrane filters with pore sizes similar to or larger than 0.2 μm,[35−37] we expect the collection efficiency to be >99% for both VLPs and BLPs, so the use of filters with different pore sizes should not bias the comparison. The VBR of 1.4 in outdoor air in Blacksburg, VA, is 7% higher than in the Caribbean and 58% lower than in Korea.

In all the indoor environments examined in this study, the VBR is close to 1, contradicting our hypothesis it would be greater than 1 because of the smaller size of viruses and their ability to remain airborne longer than bacteria. In reality, the ratio also depends on the source strength of VLPs relative to BLPs. Gibbons et al.[38] report a VBR of approximately 1 on restroom surfaces, lower than expected, and speculate that bacteriophages are not able to replicate and spread because of microbial dormancy and the inability of lytic cycles to occur in this microenvironment.[39] It is possible that a similar phenomenon occurs in indoor air, as some fraction of surface bacteria responsible for replicating and releasing bacteriophages into the air may be dormant. Jones and Lennon[40] claim that the proportion of dormant bacteria may be as high as 40% in a nutrient-poor ecosystem, such as surfaces. It is likely that bacteriophages constitute a large fraction of the total VLP population, and thus, a decrease in bacteriophage production would cause a significant decrease in total VLP concentrations.[41,42] Finally, viruses might be attached to carrier particles or clumped together in the air, increasing the size and removal rate by settling in comparison to those of free viruses and thus leading to lower airborne VLP concentrations.[43]

VLPs and BLPs in outdoor air likely contribute substantially to those found indoors, as our results show that concentrations are higher outdoors, and particulate matter (PM) has been shown to penetrate effectively from outdoor air to indoor environments.[44,45] In some cases, variation in outdoor PM explains the majority of variation in indoor PM.[45−48] Nazaroff[49] suggests that for a naturally ventilated building, the penetration efficiency of bioaerosols is close to 1, meaning that all bioaerosols flowing through leaks in the building envelope remain suspended, although they are subject to removal upon arrival indoors.

Although penetration of outdoor air appears to be the dominant factor affecting indoor VLP and BLP concentrations, indoor sources could also contribute to the bioaerosols observed indoors.[49] As humans carry 10^{12} microbes on their epidermis and 10^{14} microbes in their alimentary tract,[50] human occupancy is a factor in determining bioaerosol concentrations indoors.[28,51,52] The VBR is lower indoors than outdoors, suggesting enhanced sources of bacteria relative to viruses indoors or preferential removal of viruses as air penetrates indoors.[53] The removal efficiency of filters used in HVAC systems varies with particle size,[54] so the indoor VBR could be affected by the presence of an HVAC system. With current technology and methods, it has been difficult to quantify the contribution of human occupancy and other indoor sources versus that of outdoor air to total indoor bioaerosols. Recently, researchers have been able to measure emission rates of bacteria and fungi in occupied classrooms;[51,55] however, measuring emission rates of viruses remains challenging. This topic requires further study by microbiologists and building scientists.

VLP and BLP concentrations are higher in houses than in most of the public spaces monitored in this study. Both filtration by the HVAC system and a higher ventilation rate in public buildings[56] may contribute to this finding. During this study, residential HVAC systems were off, while public buildings still had their HVAC systems running. Previous studies have shown a correlation between low ventilation rates and an increased incidence of viral respiratory disease.[57−59] If reducing indoor exposure to VLPs and BLPs is of interest, we suggest simple building engineering modifications, such as sealing cracks in buildings to minimize outdoor air penetration, increasing ventilation rates, and using high-quality HVAC filters.

Inhalation is one route of exposure to VLPs and BLPs. For comparison, a recent study estimates that humans inhale between 60 and 60000 fungal spores daily,[60] depending on indoor mold levels. Exposure to fungal spores is associated with asthma, respiratory problems, and nasal congestion.[60−63] On the basis of the VLP concentrations measured in this study, we estimate that the total number of VLPs inhaled daily by humans is approximately 6×10^6 VLPs, where 5×10^6 VLPs are encountered indoors and 1×10^6 VLPs outdoors. We estimate the total number of BLPs inhaled daily by humans to be approximately 6×10^6 BLPs, where 5×10^6 BLPs are encountered indoors and 9×10^5 BLPs outdoors. These calculations are based on the assumptions that the average human spends 90% of their time indoors, has a respiratory rate of 15 breaths min^{-1}, and inhales 500 mL of air per breath.[16,64,65] Predicting the number of VLPs and BLPs actually deposited in the respiratory system would require knowledge of their size distribution in carrier aerosols. This is another topic for future research.

This is the first study to report VLP concentrations and VBR values for different indoor air environments. While this research relies on the same fluorescence-based method used in other studies,[14,32,33] it has limitations. Primarily, it does not allow confirmation of whether the particles are truly virus particles (hence the "VLP" term). It is possible that some VLPs and BLPs are actually free DNA or RNA associated with a particle. Additionally, viruses and bacteria could form aggregates, which we cannot differentiate from individual particles. For example, viruses might be attached to carrier particles or clumped together, which would cause misidentification as a bacterium.

This work establishes the foundation for more in-depth investigations of viral ecology in the atmosphere, an important and emerging field. Future studies could examine how bioaerosol concentrations vary diurnally, seasonally, and geographically and how bioaerosol viability is affected by environmental factors. Additionally, microbiologists and building scientists should collaborate to investigate how building characteristics (e.g., occupancy, air-exchange rate, rating of HVAC filter, etc.) influence indoor bioaerosol

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concentrations. Many important questions remain about the health and environmental effects of airborne microbes.

■ ASSOCIATED CONTENT

Supporting Information

Sampling parameters of individual field samples and pairwise comparisons of VLP and BLP counts between different sampling environments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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