



HAL
open science

The fate of mengovirus on fiberglass filter of air handling units

Victor Bandaly, Aurélie Joubert, Pierre Le Cann, Yves Andres

► **To cite this version:**

Victor Bandaly, Aurélie Joubert, Pierre Le Cann, Yves Andres. The fate of mengovirus on fiberglass filter of air handling units. *Food and Environmental Virology*, 2017, 9 (4), pp.464-472. 10.1007/s12560-017-9310-8 . hal-01636427

HAL Id: hal-01636427

<https://univ-rennes.hal.science/hal-01636427>

Submitted on 16 Nov 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The fate of mengovirus on fiberglass filter of air handling units

Victor Bandaly^{1,2,3,*}, Aurélie JOUBERT¹, Pierre LE CANN^{2,3} and Yves ANDRES¹

¹ GEPEA- CNRS UMR 6144, IMT Atlantique, Nantes, 44000, France

² EHESP, Rennes, 35000, France

³ IRSET- INSERM UMR 1085, Rennes, 35000, France

*Corresponding email: victor.bandaly@mines-nantes.fr, tel. +33251858289

ORCID iD: 0000-0002-3358-2989

Keywords: Indoor air quality, fiberglass filter, AHU, Mengovirus

Abstract

One of the most important topics that occupy public health problems is the air quality. That is the reason why mechanical ventilation and air handling units (AHU) were imposed by the different governments in the collective or individual buildings. Many buildings create an artificial climate using heating, ventilation and air-conditioning (HVAC) systems. Among the existing aerosols in the indoor air, we can distinguish the bioaerosol with biological nature such as bacteria, viruses, fungi. Respiratory viral infections are a major public health issue because they are usually highly infective. We spend about 90% of our time in closed environments such as homes, workplaces or transport. Some studies have shown that AHU contribute to the spread and transport of viral particles within buildings. The aim of this work is to study the characterization of viral bioaerosols in indoor environments and to understand the fate of mengovirus eukaryote RNA virus on glass fiber filter F7 used in AHU. In this study, a set-up close to reality of AHU system was used. The mengovirus aerosolized was characterized and measured with the Electrical Low Pressure Impact (ELPI) and the Scanner Mobility Particle Size (SMPS) and detected with RT-qPCR. The results about quantification and the level of infectivity of mengovirus on the filter and in the biosampler showed that mengovirus can pass through the filter and remain infectious upstream and downstream the system. Regarding the virus effectiveness on the filter under a constant air flow, and mengovirus was remained infectious during 10 hours after aerosolization.

Acknowledgments

This study was supported by the IMT-Atlantique Bretagne-Pays de Loire and the Ecole des Hautes Etudes de Santé Publique (EHESP). The authors are thankful for the mengovirus eukaryote RNA virus provided by the Prof. Albert Bosch from the Enteric Virus Laboratory, Universitat de Barcelona, Spain and grateful for the Buffalo Monkey African green kidney cell (BGM) provided by the Inovalys laboratory, Nantes, France.

1 Introduction

Many buildings, especially office buildings, create an artificial climate by using heating, ventilation and air-conditioning (HVAC) systems. These systems manage and deliver fresh air with a well controlled temperature (T) and relative humidity (RH) and a reduction in the concentration of particles from the outside air (Seppänen and Fisk, 2004). Good maintenance of air handling units (AHUs) leads to health benefits and better productivity. Some individualized biological elements like spores, bacterial cells and viruses are present in the indoor air and included in the term “bioaerosol” (Douwes et al. 2003). Particles can be classified on a scale of 0.005 μm to 100 μm . Closed environments are 5-10 times more polluted than outside (Jones 1999). A few studies have provided the number of hours that people spend in closed environments like offices, homes, shops, gyms, etc. In 2014, Export Enterprises SA published the number of hours spent indoors per day in different countries in the world. They estimated an average of 8.12 h per day, equivalent to 34% of the day (Export Enterprises SA, 2014). According to neuroscience research, people sleep for 29 to 33% of their day (Aritake et al. 2004; Siegel 2003) while another sociological study revealed that people spend 14% of their day at home (Turcotte 2005).

Respiratory viral infections are one of today’s most important public health topics due to their high contagiousness. Most of them are easily transmitted by direct contact, droplets or aerosolization. These infections can evolve from an ordinary viral infection to an outbreak (Tellier 2006). All particles smaller than 30 μm in size can invade a room very rapidly (Morawska 2006). It is estimated that 7 mg of particles less than 2 μm in size can be emitted by coughing (Zhu, Kato, and Yang 2006). The study of Fabian et al. (2011) demonstrated that a person infected with influenza can exhale 3 to 20 influenza viruses per min with just normal breathing. Another study of Coxsackie virus explained that 160 viral particles are emitted during sneezing and coughing (Couch et al. 1966; Downie et al. 1965). On average, 480 liters of air are breathed by an adult in one hour (Hermann and Cier 1973). The studies of Couch et al. (1966) and Downie et al. (1965) observed that infected people can generate 0.1 to 10 viruses. $\text{L}^{-1} \cdot \text{h}^{-1}$ and proved in their study that 2 infected people simply breathing (without coughing or sneezing) and occupying an office of 40 m^3 for 4 hours can contaminate indoor air with 0.24 virus $\text{L}^{-1} \cdot \text{m}^{-3}$ (with no renewal of air).

An air handling unit is composed of several sections of mixing, heating, cooling, humidification and filtration. Three main filter types are distinguished according to the efficiency of filtration: G1 to G4 (low efficiency), M5 to M6 ($40 \leq$ average efficiency < 80), F7 to F9 ($80 \leq$ average efficiency < 95) and E10 to E12/H13 to H14/U15 to U17 (very high efficiency > 95) (EN779:2012, EN1822:2009). Air filters consist of clusters of fibers (mats, felts, papers, glass). The efficiency of the air filter is based on 4 mechanisms (sieving, inertia, interception and diffusion) (Bailly 2001).

Filters in ventilation systems could be a source of microbial air pollution in the closed environment (Bluyssen et al. 2003). Some studies have shown that ventilation systems and AHUs spread and transport viral particles inside buildings (Ezratty and Squinazi 2008; Fabian et al. 2008; Tang et al. 2006). In a meta-analysis, 22 studies out of 40 proved the direct contribution of ventilation and airflow to spreading indoor infectious airborne viruses in office buildings during the SARS outbreak and other viral outbreaks (Li et al. 2007). Some recent studies have detected airborne bacteria and some specific viruses on HVAC filters in a daycare center (Prussin et al. 2016) and large public buildings (Goyal et al. 2011). Myatt et al. (2004) analyzed 181 filters of AHUs used for 47 h and showed that 58 filters were contaminated with 59% picornavirus, 24% corona virus and

17% parainfluenza virus. The temperature and the relative humidity (% RH) in the AHU can have an effect on the persistence of viral particles; as temperature and humidity decrease, the persistence of influenza viruses increases. For example, with a temperature of 20-25°C and 20% RH, the persistence of the influenza virus is higher than at 50% RH. The persistence decreases when humidity increases to 80% (Harper 1961; Lowen et al. 2007; Schaffer, Soergel, and Straube 1976). The mengovirus exhibits rapid inactivation at RH between 40 and 60% (Songer 1967).

There are few studies concerning the behavior of viral aerosols in AHUs. This study aimed to develop and standardize an experimental set-up closer to reality to study the spread of viruses in indoor air. It investigates what happens to mengovirus after aerosolization and its fate downstream of the fiberglass filter in closed environments used in air handling units. The performance of the filter in the AHU is assessed in order to reduce the risk of viral transmission in closed environments and have a good indoor air quality and a good life quality.

2 Materials and methods

Viral and cell models

The infectious mengovirus strain MC0 (ATCC VR-1957) was used throughout this study. Mengovirus is an RNA animal virus of the Picornaviridae family. This respiratory virus presents a similar structure to the rhinovirus responsible for the common cold with a size of 27 nm. Renal epithelial cells, type BGM (Buffalo Green Monkey kidney), enabled the replication of mengovirus. BGM cells were grown in Minimum Essential Medium Eagle (MEME) (Sigma, M2279, Missouri, United States) supplemented with 10% fetal calf serum (Sigma F2442), 1% non-essential amino acid (100×) (Sigma, M7145 Missouri, United States) and 1% antibiotic-antimycotic (100X) (Sigma, A5955, Missouri, United States) at 37°C under an atmosphere enriched with 5% CO₂.

Characteristics of the filter medium

A high efficiency filter classified F7 according to the standard EN 779 (2012) was chosen for this study (Camfil Farr, Stockholm, Sweden) because it is commonly used in AHU systems in commercial buildings. This filter is made of fiberglass felt with a developed surface area of $3500 \pm 500 \text{ cm}^2$. The F7 is a highly ventilated multi-layered medium. The filtration velocity is $0.2 \text{ m}\cdot\text{s}^{-1}$ and the median fiber diameter is $1 \pm 0.2 \mu\text{m}$. In this study, a circular filter of 17.72 cm^2 was used in each output (González et al. 2016).

Experimental set-up (Forthomme et al. 2012) (Fig 1)

The experimental design consists of a vertical column made of polymethyl methacrylate where the viruses will be generated and homogenized in the upward air flow. This generation is carried out by the medical nebulizer (Omron C29 - CompAir Pro, Hoofddorp, The Netherlands) which ensures an air flow of $4.5 \text{ L}\cdot\text{min}^{-1}$ supplied by compressed air. At the end of the cylinder, four circular outputs of diameter 4 cm, which accommodate the filters, are arranged perpendicular to the flow. Three of the 4 outputs are equipped with the filter to be studied. Downstream of the filters, the BioSampler (SKC Inc., Pennsylvania, USA) samples the particles passing through the filters. The sampling rate is $13 \text{ L}\cdot\text{min}^{-1}$ at the entry of each BioSampler (SKC Inc., Pennsylvania, USA). Two volumetric pumps are set downstream of the device to ensure the flow of air treated through the filters. The frontal filtration velocity at each filter is $0.16 \text{ m}\cdot\text{s}^{-1}$ and is representative of the velocity

observable in the AHUs on a pleated F7 filter. The device is positioned under a laminar flow hood with clean and sterile air.

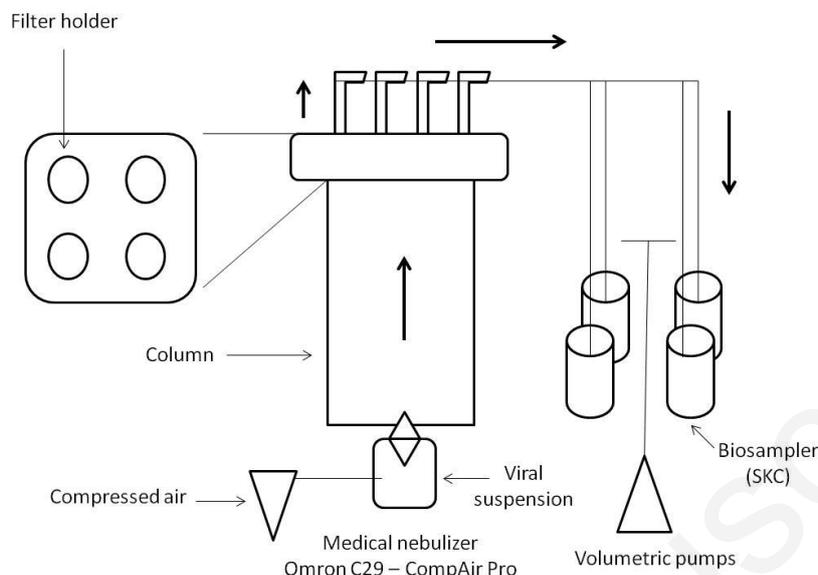


Fig 1: Description of the experimental set-up (adapted from Forthomme et al. (2012))

Characteristics and efficiency of the BioSampler

The BioSampler (SKC Inc., Pennsylvania, USA) is based on the principle of particle sampling. With a curved inlet tube, it simulates the entry of air through the nasal passage by separating the respirable particles from the non-respirable particles. Air samples are collected on 20 mL of MEME medium at 13 L min⁻¹ for 15 or 20 minutes because the liquids are evaporable (Faure 2010). The BioSampler shows an efficiency greater than 50% for particles larger than 0.03 μm, more than 80% for particles with a size between 0.5 and 1 μm and more than 95% for particles larger than 1 μm. In this study with a virus size of 27 nm, the efficiency of the BioSampler is up to 78%. The collecting liquid can be analyzed.

Validation of the system by fluorescein

In order to monitor the amount of particles generated at each of the four filter gates, a fluorescein (CAS number: 518-47-8, Merck KGaA, Darmstadt, Germany) solution was generated by the medical nebulizer through the filter device, with or without a filter, at a rate of 4.5 L.min⁻¹. After 25 min of aerosolization, the absorbance of the collection liquids contained in the BioSamplers and the liquid extracted from the filters was measured at the wavelength $\lambda = 470$ nm corresponding to fluorescein. It was thus possible to deduce the fluorescein mass retained in the system from a calibration curve (absorbance of fluorescein as a function of the fluorescein concentration in deionized water). This validation was carried out in triplicate. On average, up to 0.58 mg of fluorescein was collected in the system (BioSampler, filter and tube) from an initial output of 0.76 mg leading to a set-up efficiency of 76.3%. Table 1 shows an example of the system validation by the mass balance of fluorescein.

	Mass of fluorescein recovered (mg)
Fluorescein mass distributed between outputs	0.1902
Output 1 (filter + BioSampler)	0.1681
Output 2 (filter + BioSampler)	0.1884

Output 3 (filter + BioSampler)	0.1249
Output 4 (tube + BioSampler)	0.0991
Total fluorescein recovered in the system	0.5805
Initial fluorescein mass	0.7609
Mass balance %	76.3

Table 1: An example of the system validation by the mass balance of fluorescein

Preparation of aerosolized virus suspension

After multiplication of the mengovirus in the BGM cells and taking into account the fragility of the virus, the virus suspension to be aerosolized was obtained directly from the cell culture medium. The culture was centrifuged for 10 minutes at 1750 xg to remove cell debris and the supernatant containing the virus was recovered.

Aerosol characterization

The aerosol size was analyzed by the Electrical Low Pressure Impactor (ELPI™, Dekati, Kangasala, Finland) and the Scanning Mobility Particle Sizer (SMPS™, TSI, Marseille, France). The ELPI™ can characterize the aerodynamic size of particles from 7 nm to 4 µm and consists of several removable trays to extract the deposit. The SMPS™ can characterize the electrical mobility size of particles from 10 nm to 1 µm.

Extraction, primers, probes, and molecular assays

Mengovirus RNA was extracted using a NucliSens® easyMAG™ Platform (Biomérieux, Marcy l'Etoile, France). The reverse transcription was done on the mengovirus ARN and then was quantified using a standardized real-time TaqMan PCR carried out by the Brilliant II QRT-PCR Master Mix kit (Agilent Technologies, California, USA) with the reverse primer Mengo209 (5'-GAAGTAACATATAGACAGACGCACAC-3'), the forward primer Mengo110 (5'-GCGGGTCCTGCCGAAAGT-3'), and the FAM-MGB probe Mengo147 (5'-ATCACATTACTGGCCGAAGC-3') (Pintó, Costafreda, and Bosch 2009). The QRT-PCR program was as follows: 30 min at 50°C for RT activation, 10 min at 95°C for enzyme activation, 40 cycles of 15 s at 95°C for denaturation, and 1 min at 60°C for annealing-extension. It was carried out on a PCR platform Agilent MXPRO3005P (Agilent Technologies, California, USA). The RNA extract of mengovirus was used as PCR positive control. Two PCR negative controls were adopted for this study: the extraction of the medium (MEME) without virus and the water used in the QRT-PCR mix. The virus was quantified by the standard curve, based on the calibration of mengovirus initial solution.

Determination of infectivity by tissue culture infective dose test (TCID₅₀).

The TCID₅₀ test was used to determine the infectivity of mengovirus upstream or downstream of the system. This test was carried out on the BGM cells seeded in 96-well plates, infected with serial dilutions of the sample and then incubated for 30 h at 37°C with 5% CO₂. The plates were examined under a microscope to count the positive wells (with a cytopathic effect). Infectious titers were then calculated using the Spearman-Kärber method (Ramakrishnan 2016).

Extracting viruses from the filter

The filters were rinsed in 20 ml of the preheated culture medium (MEME) with stirring for 30 min at 250 rpm in two stages. The medium was recovered and treated with chloroform to remove any other microorganisms that may be parasitic. An extraction of the virgin filter showed no filter effect on BGM cells

when using the TCID₅₀ test. Mengovirus was quantified per genomic unit (GU) on a filter to test the loss of quantity by using this extraction method. From 2.82×10^4 GU.cm⁻² of mengovirus theoretically deposited on the filter, 2.08×10^4 GU.cm⁻² was detected by RT-qPCR. Thus, a loss of 0.13 log of virus was calculated for this extraction method, giving a yield of 74%.

3 Results

Study of temperature and relative humidity in the pilot

The medium without virus was aerosolized for 25 min in the experimental set-up. The temperature and relative humidity were measured at the level of the filters. The temperature remained constant at $16^\circ\text{C} \pm 2^\circ\text{C}$. After 3 min of aerosolization, the RH% increased from 37% to 80% and reached a max of 89% in 14 min. Then, the RH decreased to 59% in 20 min and to 52% after 25 min of aerosolization (Fig 2).

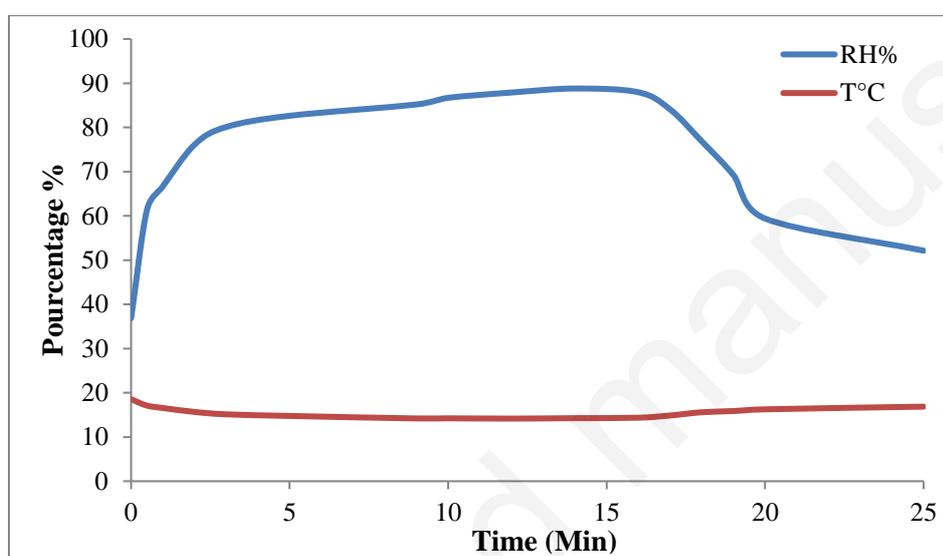


Fig: 2 Temperature and relative humidity measured in the system close to the filters during aerosolization

Aerosol characterization

Particle size distributions of the mengovirus suspension generated by the medical nebulizer were measured with the ELPITM and the SMPSTM and are presented in figures 3 and 4. The polydisperse particle size distribution measured by the ELPITM presents a median aerodynamic diameter ($d_{ae,50}$) of 80 nm (Fig 3). The particle distribution measured by the SMPSTM presents a median electric mobility size ($d_{el,50}$) of 76 nm (Fig 4). The particles collected on the different stages of the ELPITM were extracted in the MEME. A RT-qPCR was carried out on each extract to detect and quantify the virus. Mengovirus was detected on all the collected fractions above 92.8 nm except for the fraction at 2390 nm. The virus was quantified based on the concentration of nebulized virus in the initial solution (Fig 5). Following the calibration of mengovirus detection by RT-qPCR, the limit detection of this virus was defined to be less than 10 viral particles per liter of air. In the figures, the error deviation of 1109 samples was used (1 measure per second during 18 min).

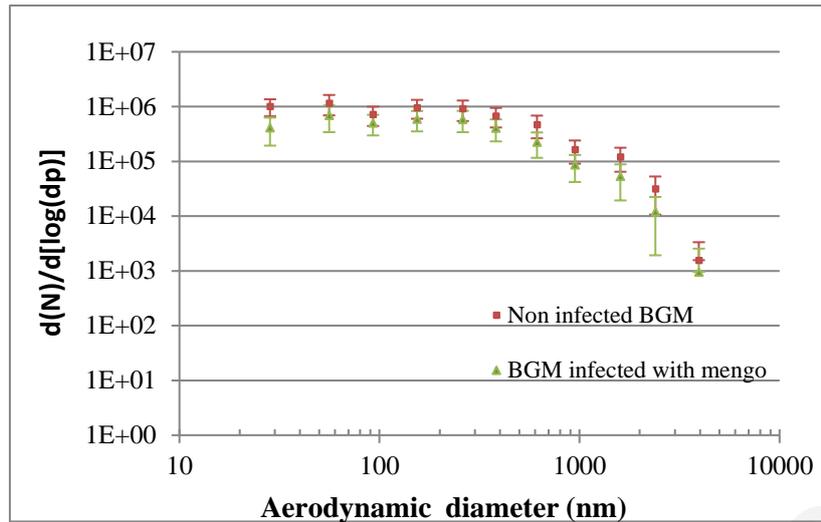


Fig 3: Particle size distribution of the mengovirus suspension generated by the medical nebulizer measured with the ELPI™ (d(N) = number of particles; dp = particle diameter)

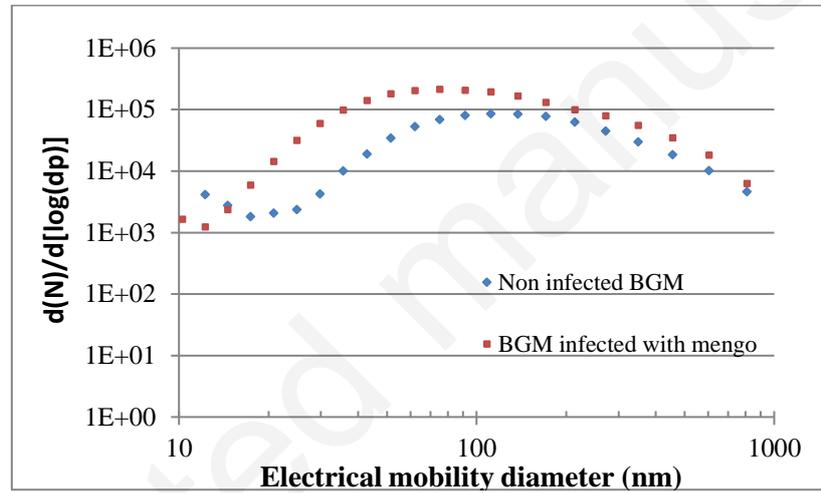


Fig 4: Particle size distribution of the mengovirus suspension generated by the medical nebulizer measured with the SMPS™ (d(N) = number of particles; dp = particle diameter)

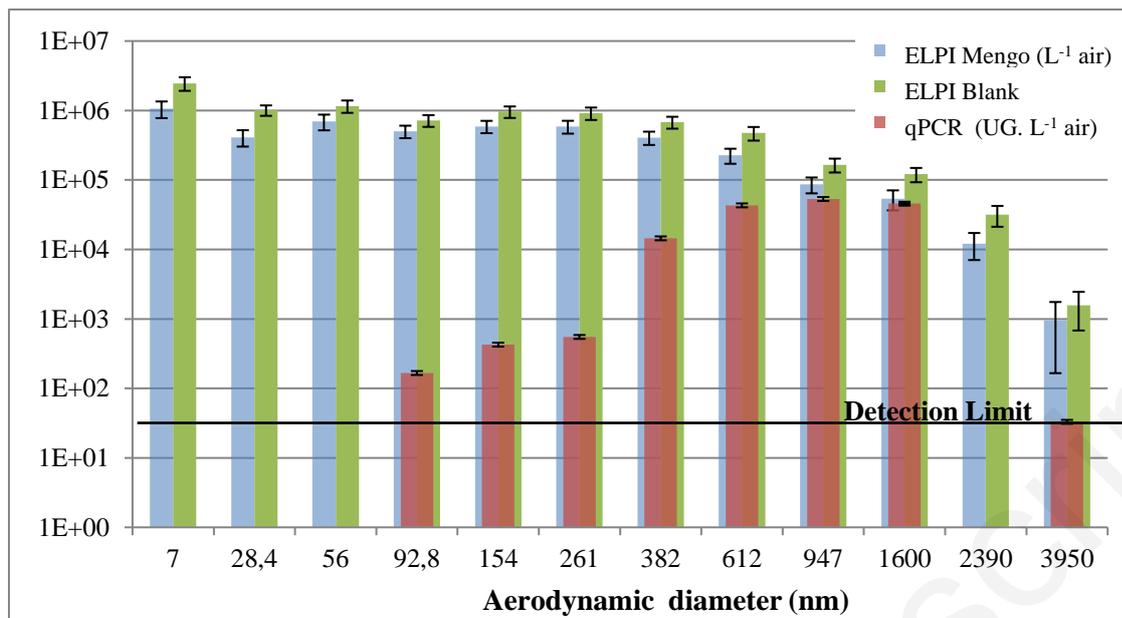


Fig 5: Detection and quantification of mengovirus on each stage of the ELPI™

Detection and quantification of mengovirus in the experimental set-up (upstream, downstream and on the filters)

Based on the RT-qPCR measurement, the total initial output of mengovirus aerosolized and the viruses recovered in the system (filters and BioSampler) were measured. Comparing the quantity of mengovirus downstream in the absence of a filter and viruses recovered on the filter, the filter F7 shows an efficiency between 83.8 and 98.5%. With reference to the efficiency curves of the four filtration phenomena (Bailly 2001) and the size of the particles, the experimental set-up used with the virus presents results coherent with filtration performance.

The average quantification of 4 experiments showed that from 6.68×10^7 GU.L⁻¹ air of mengovirus aerosolized for 25 minutes, 9.20×10^6 GU.cm⁻² was collected on 3 filters and 1.19×10^6 GU of mengovirus per liter of air passed through the filters. The output without a filter (BioSampler 4) was 5.33×10^6 GU.L⁻¹ air (Table 2). Comparing the initial concentration aerosolized and the concentration of virus collected downstream of the system without a filter, there was a loss of 1 log of virus in the system. Referring to the fluorescein validation, this loss is attributed to the presence of viruses on the column and pipes, which are difficult to recover. A few microliters of the viral suspension remained in the Omron containing 1.26×10^{11} GU of mengovirus. Comparing the concentration of the virus in the rest of the Omron to the initial solution, no concentration effect was observed during aerosolization (data not shown).

Average	Quantity (GU.L ⁻¹)	Quantity (GU.cm ⁻²)
Initial viral suspension	6.68×10^7	
Filter 1		3.48×10^6
Filter 2		3.02×10^6
Filter 3		2.71×10^6
BioSampler 1	4.61×10^5	
BioSampler 2	2.43×10^5	
BioSampler 3	4.90×10^5	

BioSampler 4	5.33 x 10 ⁶	
---------------------	------------------------	--

Table 2: Average quantification of the initial aerosolized viral suspension, samples from BioSamplers and the virus extracted from filters by RT-qPCR measurement (GU.L⁻¹ air)

Infectivity of mengovirus in the experimental set-up (upstream, downstream and on the filters)

According to the infectivity levels of 4 x 10⁸ TCID.L⁻¹ air aerosolized upstream of the system and 8.48 x 10² TCID.L⁻¹ air downstream of the system in the absence of a filter, a loss of 5.6 log of infectivity of mengovirus could be estimated (Table 3). This result demonstrates an aerosolization effect on the infectivity of the virus. The TCID₅₀ test showed that mengovirus remained infectious in the air passed through filters (downstream of the system) with 9.81 x 10¹ TCID₅₀.L⁻¹ air whereas 9.09 x 10² TCID₅₀.cm⁻² was recovered on the 3 filters. The infectivity of the mengovirus remaining in the Omron was tested. Comparing the infectivity of the virus in the rest of the suspension of the Omron and the initial one, the virus did not lose infectivity during aerosolization (data not shown).

Average	Quantity (TCID.L ⁻¹)	Quantity (TCID.cm ⁻²)
Initial viral suspension	4 x 10 ⁸	
Filter 1		3.26 x 10 ²
Filter 2		4.23 x 10 ²
Filter 3		1.59 x 10 ²
BioSampler 1	3.77 x 10 ¹	
BioSampler 2	2.13 x 10 ¹	
BioSampler 3	3.91 x 10 ¹	
BioSampler 4	8.48 x 10 ²	

Table 3: Average infectivity detected in the initial aerosolized viral suspension, samples from BioSamplers and the virus extracted from filters by RT-qPCR measurement

Quantification versus infectivity of mengovirus (Fig 6)

Quantification and infectivity were compared on filters and in BioSamplers using the Plaque Forming Unit (PFU) (1 TCID is equal to 0.69 PFU). The results measured from the output without filter (BioSampler 4) showed 5.33 x 10⁶ GU.L⁻¹ air of which 1.23 x 10³ PFU.L⁻¹ was infectious. Thus, 4 logs of virus lost infectivity during the 25 minutes of aerosolization. Concerning the comparison between the infectivity and quantification of mengovirus on the filters, 3.07 x 10⁶ GU.cm⁻² was detected of which 4.39 x 10² PFU.cm⁻² remained infectious whereas 4 logs of virus lost infectivity. In the BioSamplers, 4.74 x 10¹ PFU.L⁻¹ of 3.98 x 10⁵ GU.L⁻¹ air remained infectious whereas 4 logs of virus lost infectivity. In the figure, the highest and the lower values of 3 different essays were shown.

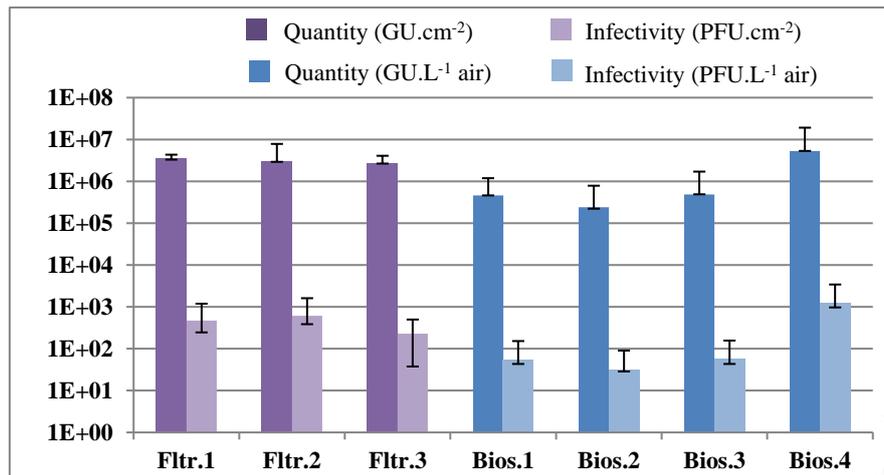


Fig 6: Comparison of infectivity detected and quantification of mengovirus downstream of the system and on the filters

Effect of time on the infectivity of mengovirus on the filters

In this part of the study, the effect of time on the infectivity of mengovirus on the filters was tested. The air flow in the system remained continuous during 50, 75, 360, 600 and 1440 minutes with 25 minutes of aerosolization of mengovirus. From an average of 4.43×10^8 PFU.L⁻¹ of initial solution of virus aerosolized, 3.43×10^2 PFU.cm⁻² of infectious mengovirus was detected after 25 minutes of air flow. The infectivity increased to 6.27×10^3 PFU.cm⁻² after 50 min, then remained constant until 360 min (6 hours) and then decreased to 1.82×10^1 PFU.cm⁻² for 600 min (10 h) of continuous air flow. The detection of infectivity at 600 min corresponded to the detection limit of the virus with the TCID₅₀ test. After 1440 min (24 h), no virus infectivity was detected. With a continuous air flow in the system, the persistence of mengovirus was assessed at different times and showed infectivity on the filter up to 10 hours after aerosolization (Fig 7). In the figure, the highest and the lower values of 3 different essays were shown.

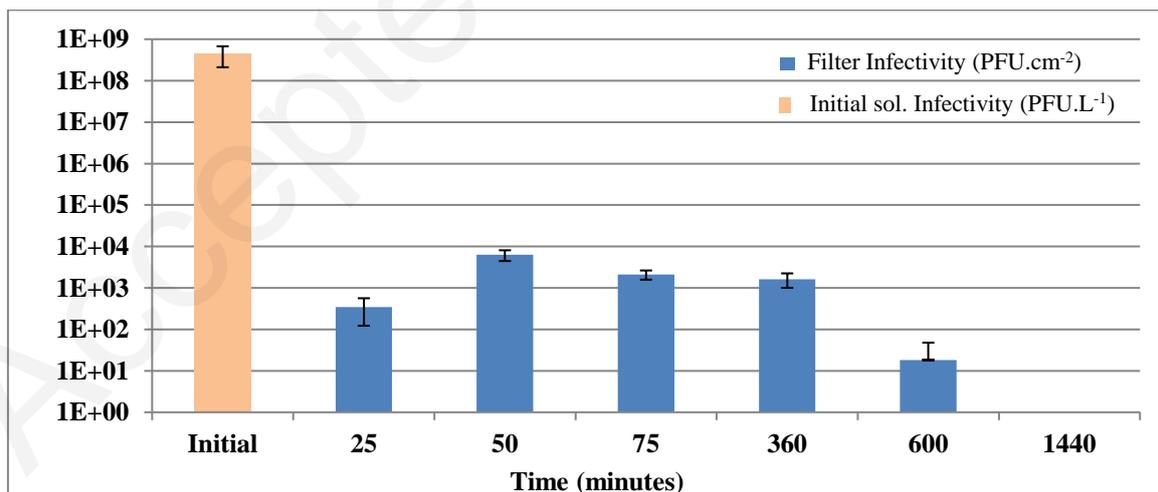


Fig 7: Effect of time of aerosolization on the infectivity of mengovirus on the filters

4 Discussion

In recent years, we have seen the benefits of efforts in the development of indoor air treatment techniques and evolving knowledge of the microbial ecology of bioaerosols, which may play an important role in

human health. The experimental set-up used in this study represents a model of the real-life functioning of an air handling unit. The pilot was validated using fluorescein and then confirmed with mengovirus showing an efficiency between 78.8 and 81%. The loss observed in the pilot validation is due to viruses that are retained on the walls of the pipes and the column (difficult to extract), the efficiency of the BioSampler collector (78%) and of the filter extraction (74%). The filter used in this study (F7-CompAir) is commonly used in AHUs and shows the same filtration speeds ($0.16 \text{ m}\cdot\text{s}^{-1}$) and an effectiveness of between 83.8 and 98.5%. This calculated effectiveness was confirmed in the study of Bailly (2001) on the efficiency of the air filter resulting from curves of the four filtration phenomena. The dynamics and characteristics of bioaerosols is an important topic. The size of mengovirus was determined by the study of Faulkner et al. (1961) as 27 nm. In this study, some hypotheses can be made: The nebulized mengovirus cannot be aerosolized as a single virus as no virus was detected on the ELPI™ stage with the specific size of the virus. The aerosol could be constituted of groups of viruses (more than 3 viruses). The virus could be associated with proteins or debris cells present in the centrifuged medium. This can be confirmed in real life as infectious respiratory viruses are expelled with cell debris and mucus.

Based on the quantification and detection of infectivity in the system by RT-qPCR and TCID₅₀ measurement, the study shows that viruses stopped by the filter and those that passed through the filters remained infectious. This loss of infectivity confirms the study of Songer (1967) who showed the rapid inactivation of the mengovirus due to the RH% between 40 and 60% measured in this study during the 25 minutes of aerosolization.

Goyal et al. (2011) did not detect any infectious virus in used ventilation filters from two large public buildings but they were able to detect viruses by PCR. Farnsworth et al. (2006) showed the difficulty of detecting the persistence of viruses in AHUs since most viruses present on AHU filters are deactivated within a day. The study of Vasickova et al. (2010) focused on the inactivation rate of viruses on different supports. The review of Gerba (2013) revealed different inactivation between viruses. They pointed out differences in inactivation between enveloped and non-enveloped respiratory viruses. For example, they found that the inactivation rate was $0.25 \log_{10} \cdot \text{h}^{-1}$ for rhinovirus and $0.625 \log_{10} \cdot \text{h}^{-1}$ for the respiratory syncytial virus (RSV). They concluded that non-enveloped respiratory viruses can survive longer than enveloped viruses. This shows the importance of using mengovirus in this study, which is a model of non-enveloped rhinoviruses. The WHO review about the potential transmission of Avian Influenza (H5N1) through Water and Sewage (2017b) reports that, in general, virus half-lives are of the order of hours. The literature states that the virus support, for example here the filter samples, can play a role in reducing infectivity. The WHO report on the stability and resistance of the SARS coronavirus (2017a) indicates that the persistence of this virus on wood and cotton cloth is only 12 hours. Other studies like Sizun, Yu, and Talbot (2000) have proved that human coronavirus can persist for only 3–6 hours on sterile sponges while the study of Hall, Douglas, and Geiman (1980) pointed out that RSV can persist for 2.5 hours on cloth gowns and 1 hour on paper towels.

Thus, time has an effect on the infectivity of the virus; this study showed that, with a continuous air flow in the system, no infectious virus was recovered after 10 hours of aerosolization.

5 Conclusion

The gap between what we know and what we would like to know in this research field remains quite large as recent studies have focused on bioaerosols like bacteria and fungi. This study thus presents new information about the survival of viruses in indoor environments and their fate in air handling units. In further

studies, we need to investigate how to reduce the risk of viral transmission in closed spaces using different parameters to improve AHUs.

Accepted manuscript

6 References

- Aritake, S., M. Uchiyama, H. Tagaya, H. Suzuki, K. Kuriyama, A. Ozaki, X. Tan, et al. 2004. "Time Estimation during Nocturnal Sleep in Human Subjects." *Neuroscience Research* 49 (4): 387–93. doi:10.1016/j.neures.2004.04.006.
- Bailly, André. 2001. "Traitement de L'air et Climatisation - Généralités." April 10. <http://www.techniques-ingenieur.fr/base-documentaire/construction-et-travaux-publics-th3/le-chauffage-la-climatisation-et-l-eau-chaude-sanitaire-42582210/traitement-de-l-air-et-climatisation-be9270/>.
- Bluyssen, Philomena M, Christian Cox, Olli Seppänen, Eduardo de Oliveira Fernandes, Geo Clausen, Birgit Müller, and Claude-Alain Roulet. 2003. "Why, When and How Do HVAC-Systems Pollute the Indoor Environment and What to Do about It? The European AIRLESS Project." *Building and Environment* 38 (2): 209–25. doi:10.1016/S0360-1323(02)00058-6.
- Couch, R. B., T. R. Cate, R. G. Jr Douglas, P. J. Gerone, and V. Knight. 1966. "Effect of Route of Inoculation on Experimental Respiratory Viral Disease in Volunteers and Evidence for Airborne Transmission." *Bacteriological Reviews* 30 (3): 517–29.
- Douwes, J., P. Thorne, N. Pearce, and D. Heederik. 2003. "Bioaerosol Health Effects and Exposure Assessment: Progress and Prospects." *Annals of Occupational Hygiene* 47 (3): 187–200. doi:10.1093/annhyg/meg032.
- Downie, A. W., M. Meiklejohn, L. St Vincent, A. R. Rao, B. V. Sundara Babu, and C. H. Kempe. 1965. "The Recovery of Smallpox Virus from Patients and Their Environment in a Smallpox Hospital." *Bulletin of the World Health Organization* 33 (5): 615–22.
- Ezratty, Véronique, and Fabien Squinazi. 2008. "Pandemic Influenza Virus inside Buildings: Is There a Risk of Transmission by Ventilation or Air Conditioning Systems?" *Environnement, Risques & Santé* 7 (4): 255–63. doi:10.1684/ers.2008.0157.
- Fabian, Patricia, James J. McDevitt, Wesley H. DeHaan, Rita O. P. Fung, Benjamin J. Cowling, Kwok Hung Chan, Gabriel M. Leung, and Donald K. Milton. 2008. "Influenza Virus in Human Exhaled Breath: An Observational Study." *Plos One* 3 (7): e2691. doi:10.1371/journal.pone.0002691.
- Fabian, Patricia, Donald Milton, Matthew Angel, Daniel Perez, and James McDevitt. 2011. "Influenza Virus Aerosols in Human Exhaled Breath: Particle Size, Culturability, and Effect of Surgical Masks." *Epidemiology* 22 (1): S51–S51. doi:10.1097/01.ede.0000391818.35353.36.
- Farnsworth, James E., Sagar M. Goyal, Seung Won Kim, Thomas H. Kuehn, Peter C. Raynor, M. A. Ramakrishnan, Senthilvelan Anantharaman, and Weihua Tang. 2006. "Development of a Method for Bacteria and Virus Recovery from Heating, Ventilation, and Air Conditioning (HVAC) Filters." *Journal of Environmental Monitoring: JEM* 8 (10): 1006–13.
- Faulkner, P., Em Martin, S. Sved, Rc Valentine, and Ts Work. 1961. "Studies on Protein and Nucleic Acid Metabolism in Virus-Infected Mammalian Cells. 2. The Isolation, Crystallization and Chemical Characterization of Mouse Encephalomyocarditis Virus." *Biochemical Journal* 80 (3): 597–605. doi:10.1042/bj0800597.
- Faure, Marie. 2010. "PURIFICATION DE L'AIR AMBIANT PAR L'ACTION BACTERICIDE DE LA PHOTOCATALYSE." Lorraine: Ecole doctorale RP2E.
- Forthomme, A., Y. Andrès, A. Joubert, X. Simon, P. Duquenne, D. Bemer, and L. Le Coq. 2012. "Evolution of Microbial Aerosol Behaviour in Heating, Ventilating and Air-Conditioning Systems – Quantification of Staphylococcus Epidermidis and Penicillium Oxalicum Viability." *Environmental Technology* 33 (19): 2217–21. doi:10.1080/09593330.2012.689370.
- Gerba, C. P. 2013. "Occurrence and Transmission of Food- and Waterborne Viruses by Fomites," May. doi:10.1533/9780857098870.3.205.
- González, Luisa F., Aurélie Joubert, Yves Andrès, Myriam Liard, Christophe Renner, and Laurence Le Coq. 2016. "Filtration Performances of HVAC Filters for PM10 and Microbial Aerosols— Influence of Management in a Lab-Scale Air Handling Unit." *Aerosol Science and Technology* 50 (6): 555–67. doi:10.1080/02786826.2016.1167833.
- Goyal, Sagar M., Senthilvelan Anantharaman, M. A. Ramakrishnan, Suchitra Sajja, Seung Won Kim, Nicholas J. Stanley, James E. Farnsworth, Thomas H. Kuehn, and Peter C. Raynor. 2011. "Detection of Viruses in Used Ventilation Filters from Two Large Public Buildings." *American Journal of Infection Control* 39 (7): e30-38. doi:10.1016/j.ajic.2010.10.036.
- Hall, C. B., R. G. Douglas, and J. M. Geiman. 1980. "Possible Transmission by Fomites of Respiratory Syncytial Virus." *The Journal of Infectious Diseases* 141 (1): 98–102.
- Harper, G. J. 1961. "Airborne Micro-Organisms: Survival Tests with Four Viruses." *The Journal of Hygiene* 59 (December): 479–86.
- Hermann, Henri, and Jean-François Cier. 1973. *Précis de physiologie , 1: Bio-énergétique et rations alimentaires, sang, lymphe, compartiments liquidiens de l'organisme, circulation du sang, respiration.*

- 3^e éd. révisée. 1 vols. Précis de physiologie. Paris: Masson. <http://catalogue.bnf.fr/ark:/12148/cb351094909>.
- Jones, AP. 1999. "Indoor Air Quality and Health." *ATMOSPHERIC ENVIRONMENT* 33 (28): 4535–64. doi:10.1016/S1352-2310(99)00272-1.
- Li, Y., G. M. Leung, J. W. Tang, X. Yang, C. Y. H. Chao, J. Z. Lin, J. W. Lu, et al. 2007. "Role of Ventilation in Airborne Transmission of Infectious Agents in the Built Environment - a Multidisciplinary Systematic Review." *Indoor Air* 17 (1): 2–18. doi:10.1111/j.1600-0668.2006.00445.x.
- Lowen, Anice C., Samira Mubareka, John Steel, and Peter Palese. 2007. "Influenza Virus Transmission Is Dependent on Relative Humidity and Temperature." *Plos Pathogens* 3 (10): 1470–76. doi:10.1371/journal.ppat.0030151.
- Morawska, L. 2006. "Droplet Fate in Indoor Environments, or Can We Prevent the Spread of Infection?" *Indoor Air* 16 (5): 335–47. doi:10.1111/j.1600-0668.2006.00432.x.
- Myatt, T. A., S. L. Johnston, Z. F. Zuo, M. Wand, T. Kebabdz, S. Rudnick, and D. K. Milton. 2004. "Detection of Airborne Rhinovirus and Its Relation to Outdoor Air Supply in Office Environments." *American Journal of Respiratory and Critical Care Medicine* 169 (11): 1187–90. doi:10.1164/rccm.200306-760OC.
- "Norme de Filtration EN779:2012." 2017. Accessed April 10. http://www.camfil.fr/Technique-de-filtration/Specifications-industrielles/EN779_2012/.
- "Norme Européenne EN1822 Par Camfil." 2017. Accessed April 10. <http://www.camfil.fr/Technique-de-filtration/Specifications-industrielles/EN-1822/>.
- Pintó, Rosa M., M. Isabel Costafreda, and Albert Bosch. 2009. "Risk Assessment in Shellfish-Borne Outbreaks of Hepatitis A." *Applied and Environmental Microbiology* 75 (23): 7350–55. doi:10.1128/AEM.01177-09.
- Prussin, Aaron J., Amit Vikram, Kyle J. Bibby, and Linsey C. Marr. 2016. "Seasonal Dynamics of the Airborne Bacterial Community and Selected Viruses in a Children's Daycare Center." *PloS One* 11 (3): e0151004. doi:10.1371/journal.pone.0151004.
- Ramakrishnan, Muthannan Andavar. 2016. "Determination of 50% Endpoint Titer Using a Simple Formula." *World Journal of Virology* 5 (2): 85–86. doi:10.5501/wjv.v5.i2.85.
- Schaffer, Fl, Me Soergel, and Dc Straube. 1976. "Survival of Airborne Influenza-Virus - Effects of Propagating Host, Relative Humidity, and Composition of Spray Fluids." *Archives of Virology* 51 (4): 263–73. doi:10.1007/BF01317930.
- Seppänen, O. A., and W. J. Fisk. 2004. "Summary of Human Responses to Ventilation." *Indoor Air* 14 (August): 102–18. doi:10.1111/j.1600-0668.2004.00279.x.
- Siegel, J. M. 2003. "Why We Sleep." *Scientific American* 289 (5): 92–97.
- Sizun, J., M. W. Yu, and P. J. Talbot. 2000. "Survival of Human Coronaviruses 229E and OC43 in Suspension and after Drying On surfaces: A Possible Source Of hospital-Acquired Infections." *The Journal of Hospital Infection* 46 (1): 55–60. doi:10.1053/jhin.2000.0795.
- Songer, J. R. 1967. "Influence of Relative Humidity on the Survival of Some Airborne Viruses." *Applied Microbiology* 15 (1): 35–42.
- Tang, J. W., Y. Li, I. Eames, P. K. S. Chan, and G. L. Ridgway. 2006. "Factors Involved in the Aerosol Transmission of Infection and Control of Ventilation in Healthcare Premises." *Journal of Hospital Infection* 64 (2): 100–114. doi:10.1016/j.jhin.2006.05.022.
- Tellier, Raymond. 2006. "Review of Aerosol Transmission of Influenza A Virus." *Emerging Infectious Diseases* 12 (11): 1657–62. doi:10.3201/eid1211.060426.
- Turcotte, Martin. 2005. "Time Spent with Family during a Typical Workday, 1986 to 2005." *Canadian Social Trends*. <http://www.statcan.gc.ca/pub/11-008-x/2006007/9574-eng.htm>.
- Vasickova, P., I. Pavlik, M. Verani, and A. Carducci. 2010. "Issues Concerning Survival of Viruses on Surfaces." *Food and Environmental Virology* 2 (1): 24–34. doi:10.1007/s12560-010-9025-6.
- "WHO | First Data on Stability and Resistance of SARS Coronavirus Compiled by Members of WHO Laboratory Network." 2017. WHO. Accessed March 27. http://www.who.int/csr/sars/survival_2003_05_04/en/.
- "WHO | Review of Latest Available Evidence on Potential Transmission of Avian Influenza (H5N1) through Water and Sewage and Ways to Reduce the Risks to Human Health." 2017. WHO. Accessed March 27. http://www.who.int/water_sanitation_health/publications/potential-transmission-of-avian-influenza/en/.
- Zhu, Shengwei, Shinsuke Kato, and Jeong-Hoon Yang. 2006. "Study on Transport Characteristics of Saliva Droplets Produced by Coughing in a Calm Indoor Environment." *Building and Environment* 41 (12): 1691–1702. doi:10.1016/j.buildenv.2005.06.024.