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Ionizing air affects influenza virus infectivity and prevents airborne-transmission

Authors : Marie Hagbom, Johan Nordgren, Rolf Nybom, Kjell-Olof Hedlund, Hans Wigzell, and Lennart Svensson

Abstract

By the use of a modified ionizer device we describe effective prevention of airborne transmitted influenza A (strain Panama 99) virus infection between animals and inactivation of virus (>97%). Active ionizer prevented 100% (4/4) of guinea pigs from infection. Moreover, the device effectively captured airborne transmitted calicivirus, rotavirus and influenza virus, with recovery rates up to 21% after 40 min in a 19 m3 room. The ionizer generates negative ions, rendering airborne particles/aerosol droplets negatively charged and electrostatically attracts them to a positively charged collector plate. Trapped viruses are then identified by reverse transcription quantitative real-time PCR. The device enables unique possibilities for rapid and simple removal of virus from air and offers possibilities to simultaneously identify and prevent airborne transmission of viruses.

There is an urgent need for simple, portable and sensitive devices to collect, eliminate and identify viruses from air, to rapidly detect and prevent outbreaks and spread of infectious diseases1. Each year, infectious diseases cause millions of deaths around the world and many of the most common infectious pathogens are spread by droplets or aerosols caused by cough, sneeze, vomiting etc.

Knowledge of aerosol transmission mechanisms are limited for most pathogens, although spread by air is an important transmission route for many pathogens including viruses.

Today no simple validated technology exists which can rapidly and easily collect viruses from air and identify them. The problem is not the analyzing technique, since molecular biological methods such as real-time PCR enable a sensitive detection system of most pathogens. The difficulty is to develop an effective sampling method to rapidly collect small airborne particles including viruses from large volumes of air.

Furthermore, the sampling method should be robust with easy handling to enable a wide distribution and application in many types of environment. At present, the most commonly used techniques aimed to collect pathogens from air are airflow and liquid models. These systems are complex, and their efficiency has not been thoroughly evaluated.

Spread of infectious diseases in hospitals can be most significant. In many situations there is a need for a pathogen- and particle-free environment, e.g. in operation wards, environments for immunosuppressed patients as well as for patients with serious allergies. This makes it desirable to have a method not only for collection and identification, but also for eliminating virus and other pathogens from air. Ozone gas has been shown to inactivate norovirus and may be used in empty rooms to decontaminate surfaces, however in rooms with patients ozone should not been used due to its toxicity. Generation of negative ions has previously been shown to reduce transmission of Newcastle disease virus and several kind of bacteria in animal experimental set-ups.

The ionizing device used in this study operates at 12 V and generates negative ionizations in an electric field, which collide with and charge the aerosol particles. Those are then captured by a positively charged collector plate. For safety reasons, the collector plate has a very low current, less than 80μA, however the ionizer accelerates a voltage of more than 200,000 eV, which enables high production of several billion electrons per second. Moreover, this device does not produce detectable levels of ozone and can thus be safely used in all environments.

This technique is known to effectively collect and eliminate cat-allergens from air26. Aerosolized rotavirus, calicivirus and influenza virus particles exposed to the ionizing device were attracted to the collector plate and subsequently identified by electron microscopy and reverse transcription quantitative real-time PCR techniques. Most importantly, we demonstrate that this technology can be used to prevent airborne-transmitted influenza virus infections.

Results

Visualization and efficiency of aerosol sampling as determined by electron microscopy

To develop and validate the ionizing technique for collection and identification of viral pathogens, we used several viruses of clinical importance; calicivirus, rotavirus and influenza virus (H3N2, strain Salomon Island) as well as latex particles. Canine calicivirus (CaCV, strain 48) was used as a surrogate for human norovirus, the

aetiological agent behind the “winter vomiting disease”, causing outbreaks of great clinical and economic importance. Rhesus rotavirus was used as a surrogate marker for human rotavirus.

The device consists of a small portable 12 volt operated ionizer, with a collector plate of positive charge attached to the ionizer, attracting negative particles from the air by electrostatic attraction. To determine optimal time collection parameters, latex particles with sizes ranging from <1 to >10 μm were nebulized into a room of 19 m3.

Testing revealed that 40–60 min was required to eliminate >90% of free latex particles in the air as determined by real-time particle counting (PortaCount Plus).

The particle counter can detect particles with size greater than 0.02 μM. Visualization by scanning electron microscopy (SEM) on grids from active- and inactive ionizer collector plates showed that accumulation of latex particles was dramatically enhanced on active ionizer collector plates compared to the inactive.

Next, high numbers of rotavirus and formalin-inactivated influenza virus were aerosolized under the same conditions. While, after 40 min the inactive collector plates contained few (<5) rotavirus and influenza virus, the active collector contained >50 virus particles, as determined by transmission electron microscopy (TEM).

Ionizing air and electrostatic attraction collects aerosol-distributed viruses as determined by RT-qPCR

We next determined the capacity of RT-qPCR technology to quantitate the capacity of the ionizer technique to collect and concentrate viruses. Three independent experiments with each of the three viruses were carried out using the same virus concentrations in each experiment.

Although several steps are involved from collection to detection the system was robust as to reproducibility. The RT-qPCR data shows that the active collector is concentrating and collecting virus 1500–3000 times more efficient as compared to the inactive collector.

When different dilutions of virus was used for aerosol production the proportion of aerosolized virus collected on the active collector was normally in the range of 0.1–0.6% for CaCV, rotavirus and influenza virus. A reproducible finding with regard to CaCV was a significant increase in relative recovery at the lowest concentrations increasing to 10–20% of the total amount of virus aerosolized.

Ionizing air reduces calicivirus and rotavirus infectivity

Next we determined if collected viruses retained their infectivity after being exposed to negative ions and/or after being exposed to the positively charged collector plate. Five mL of cell culture medium (Eagles Minimal Essential Media (Eagles MEM)) containing 1 × 106 peroxidase forming units of rotavirus respectively of CaCV were aerosolized and collected during 40 min to an active collector plate, containing 1 mL of Eagles MEM. CaCV in cell culture medium was also directly exposed to an active and inactive collector plate, without being aerosolized. Viral infectivity was determined essentially as described and the ratio between viral genome copy numbers versus infectivity was compared between aerosolized virus, virus exposed to active- and inactive collector plates and the viral stocks. CaCV exposed to an active collector plate, without being aerosolized, showed a slight reduction in infectivity (~40%) in comparison to virus that have been trapped on an inactive collector plate. In contrast, the infectivity of aerosolized viruses was greatly reduced by >97%, indicating that ionization of the aerosol accounts for the vast majority of infectivity reduction, and not the exposure to the charged collector plate.

Further support that ionizing was the mechanism by which viruses lost infectivity comes from experiments were rotavirus was nebulized without ionizing and allowed to be trapped to an inactive collector plate. Collectors were located at 30 cm from the nebulizer. The result concluded that the genome copy versus infectivity ratio was unchanged from that of the viral stock, thus suggesting that inactivation of virus is associated with ionized air.

Ionizing air and electrostatic attraction prevents airborne-transmitted influenza A/Panama virus infection between guinea pigs

Next we took advantage of an established influenza guinea pig model to study if ionizing air and electrostatic attraction could prevent airborne aerosol and droplet transmitted influenza A/Panama (Pan/99) virus infection between guinea pigs. The airborne/droplet transmission model was established essentially as described using two separate cages with the ionizer placed between the cages. Four guinea pigs were infected by intranasal route as described with 5 ×103 pfu of Pan/9931 and placed in cage “A”. At 30 hours post infection (h p.i.) 4 uninfected guinea pigs were placed in cage “B” 15 cm from the cage with infected animals, with no physical contact. The ionizer was placed between cages “A” and “B”. Two identical experiments were performed, one with active ionizer placed between the cages and one with an inactive ionizer.

Uninfected animals in cage “B” were exposed for 24 hours with airflow from cage “A” hosting the 4 infected guinea pigs and then placed in individually ventilated cages for the next 21 days, to ensure that the only time-point for being infected was the 24 hours when they were exposed to air from infected animals in cage “A”. RT-qPCR of lung- and trachea biopsies examined at 54 h p.i. from the nasally experimentally infected animals, revealed that 3 out of 4 guinea pigs in both experiments were positive for influenza.

We assessed transmission of infection from animals in cage “A” to exposed uninfected animals in cage “B” by development of an immune response 21 days post exposure. The results illustrate that when the ionizer was inactive, 3 of the 4 uninfected but exposed animals developed a serum IgG influenza-specific immune responses. In contrast, none of the 4 animals in cage “B” developed an immune response to influenza virus when the ionizer was active). Furthermore, influenza virus RNA could be detected by RT-qPCR, albeit at low concentration, on the collector plate from the active ionizer but not with the inactive ionizer, showing that the ionizing device indeed collected virus excreted from the infected animals in cage “A”.

Discussion

We describe a simple ionizing device operating at 12 volt that can prevent spread of airborne transmitted viral infections between animals in a controlled setting, whilst simultaneously collecting virus from air for rapid identification. Coupled with sensitive RT-qPCR assays, this sampling method enabled fast detection and highly sensitive quantification of several human clinically important viruses such as influenza virus, rotavirus and calicivirus. The device consists of a small portable ionizer, where a sampling cup of positive charge is attached to the ionizer attracting negative particles from the air. Important advantages with this novel ionizing device is the simple handling, high robustness as well as the wide applicability to airborne pathogens.

The observation that significantly higher numbers of rotavirus and CaCV particles were detected on the active ionizer compared to the inactive ionizer (~1500–3000 times), led to the conclusion that this technique can actively and efficiently collect viral particles from air. Similarly, visualization of latex particles by SEM revealed that latex particles of all sizes investigated were concentrated on the active collector. It is interesting to note that a broad range of particles sizes, from 35 nm to 10 µm was concentrated, suggesting a wide application range of the technology.