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Short report

Influenza virus survival in aerosols and estimates of viable virus loss resulting from aerosolization and air-sampling

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SUMMARY

Using a Collison nebulizer, aerosols of influenza (A/Udorn/307/72 H3N2) were generated within a controlled experimental chamber, from known starting virus concentrations. Air samples collected after variable suspension times were tested quantitatively using both plaque and polymerase chain reaction assays, to compare the proportion of viable virus against the amount of detectable viral RNA. These experiments showed that whereas influenza RNA copies were well preserved, the number of viable viruses decreased by a factor of 10^4–10^5. This suggests that air-sampling studies for assessing infection control risks that detect only influenza RNA may greatly overestimate the amount of viable virus available to cause infection.

Introduction

Assessing the potential for the airborne transmission of influenza has been hindered by differences in the experimental sampling and detection methods.[1] In recent years air-sampling detection and quantification of this virus has been mostly by the polymerase chain reaction (PCR), which only detects and quantifies the viral RNA.[2] However, this technique detects both

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viable and non-viable virus. Culture detects only viable virus, but is time-consuming and difficult to perform for airborne viruses.[3]

Here, we investigate the impact of aerosolization and air-sampling on viable and non-viable influenza to inform the assessment of airborne influenza transmission.

Methods

Experimental chamber

Experiments took place inside a stainless steel, controlled environmental chamber (4×4×2.7 m, 43.2 m^3), designed to mimic a single-bedded hospital isolation room. This was maintained at 25°C and 30% relative humidity as these conditions have been shown to be optimal for influenza airborne survival.[4] A biosafety class (BSC) II cabinet situated outside the chamber was connected to the interior by two pipes (polyvinyl chloride, 850 mm long, 19.9 mm inside diameter, wall thickness of 1.2 mm) to allow the injection of experimental aerosols and the extraction of air-samples (Figure 1).

Nebulization, air-sampling, virus detection, and quantification

A laboratory-adapted influenza A/H3N2 strain (A/Udorn/307/72 H3N2), passaged in eggs, was nebulized using a Collison jet nebulizer (set at 20 pounds per square inch, to nebulize 8 mL for 30 min), as previously described.[5]

The starting concentration of virus, pre-nebulization, was quantified in copies/mL using an in-house quantitative reverse–transcription real-time polymerase chain reaction (qRT–PCR) assay targeting a 202 nt region of the matrix (M) gene, using a dilution series of plasmid containing the target sequence as standards.

Briefly, PCR reactions consisted of 1× SuperScript III Platinum One-Step qRT–PCR mastermix (Invitrogen), 0.8 μM each primer (forward, 5′CTTCTAACCGAGGTCGAAACGTA; reverse, 5′GGTGACAGGATTGGTCTTGTCTTTA), 0.2 μM probe (5′FAM-TCAGGCCCCCTCAAAGCCGAG-BHQ1), 5 μL purified RNA and water to make the reaction volume up to 25 μL. Cycling on an ABI 7500 consisted of 50°C for 30 min and 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 60 s. Live virus was quantified in plaque-forming units (pfu) per millilitre with a viral plaque assay using Madin–Darby canine kidney (MDCK) cells kindly provided by the Worldwide Influenza Centre at the Francis Crick Institute.

The nebulizer source solution contained an initial mean starting influenza viral load of 3.48×10^7 pfu/mL (or 7.21×10^9 RNA copies/mL) for all experiments. Therefore, assuming that all 8 mL of this source solution was nebulized into the chamber and continuously well mixed with the ambient air contained therein, over the first 0–30 min, the volumetric airborne concentration of virus was predicted to be 6.44×10^6 pfu/m^3 and 1.34×10^9 RNA copies/m^3.

For these experiments, there were no mechanical air changes. A fan positioned on the chamber ceiling ran continuously during these experiments, ensuring that the air in the chamber, and therefore the airborne virus, was well mixed.

Experimental protocol

Experiments to ascertain airborne survival of influenza entailed switching on the nebulizer for 30 min to aerosolize the virus, then air-sampling from the chamber during the periods: 30–60 min ($N = 7$, where N is the number of experiments performed), 60–90 min ($N = 3$) and 90–120 min ($N = 3$) after

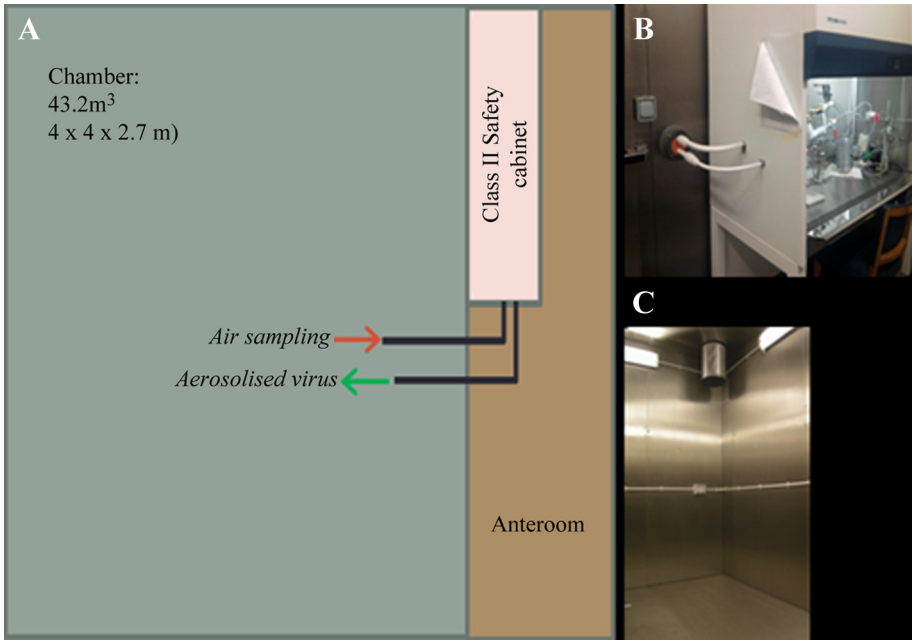

Figure 1. Experimental chamber and anteroom (A) containing the BSC II cabinet. (B) Collison nebulizer source 'aerosolized virus' outlet (green arrow) and the 'air-sampling' inlet to the SKC BioSampler (red arrow). (C) Interior of the stainless steel experimental chamber. (For interpretation of the references to colour in this figure legend, the reader is referred to the online version of this article.)