



Short report

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

J.R. Brown^a, J.W. Tang^{b,*}, L. Pankhurst^c, N. Klein^d, V. Gant^e, K.M. Lai^f,
J. McCauley^g, J. Breuer^d

^a Great Ormond Street Hospital, London, UK

^b University Hospitals Leicester, Leicester, UK

^c University of Oxford, John Radcliffe Hospital, Oxford, UK

^d University College London, London, UK

^e University College London Hospitals, London, UK

^f Hong Kong Baptist University, Hong Kong, China

^g The Francis Crick Institute, Mill Hill Laboratory, London, UK

ARTICLE INFO

Article history:

Received 8 June 2015

Accepted 4 August 2015

Available online 24 August 2015

Keywords:

Airborne
Air-sampling
Infection
Influenza
Nebulizer
Transmission

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.

© 2015 The Authors. Published by Elsevier Ltd on behalf of the Healthcare Infection Society. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).



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

* Corresponding author. Address: Clinical Microbiology, University Hospitals of Leicester NHS Trust, Level 5 Sandringham Building, Leicester Royal Infirmary, Infirmary Square, Leicester LE1 5WW, UK. Tel.: +44 (0)116 258 6516; fax: +44 (0)116 255 1949.

E-mail address: julian.tang@uhl-tr.nhs.uk (J.W. Tang).

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 m³), 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 3.48×10⁷ pfu/mL (or 7.21×10⁹ 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 6.44×10⁶ pfu/m³ and 1.34×10⁹ RNA copies/m³.

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

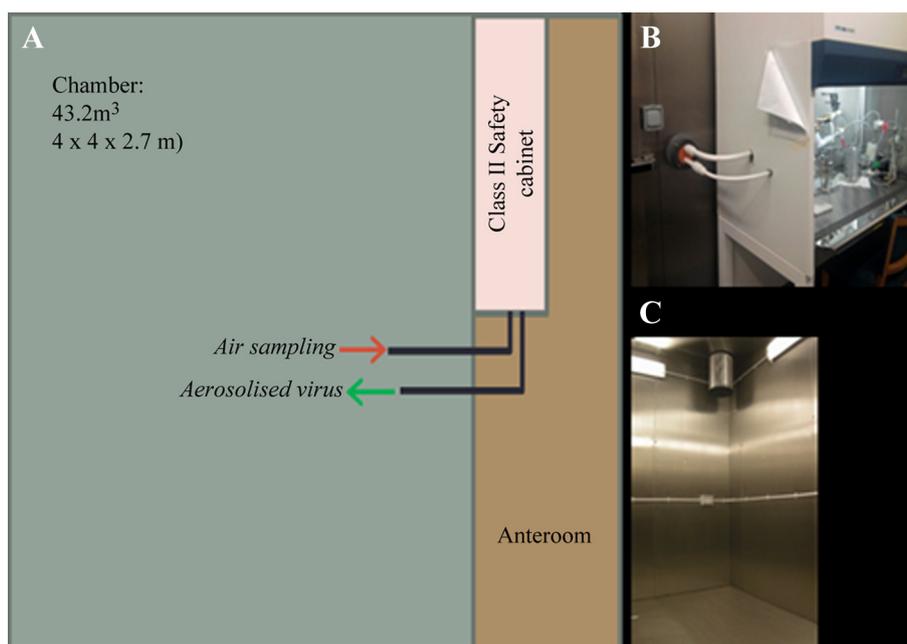


Figure 1. Experimental chamber and anteroom (A) containing the BSC II cabinet. (B) Collison nebulizer source 'aerosolized virus' outlet (green arrow) and the 'air-sampling' inlet to the SKC BioSampler (red arrow). (C) Interior of the stainless steel experimental chamber. (For interpretation of the references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

the nebulizer had been turned on. It is estimated that 0.6 m³ of air is sampled during each 30 min period.

Air-sampling was done using an SKC BioSampler impinger situated in the BSC II cabinet, through which air was drawn at 20 L/min through 20 mL of a collection fluid that had been optimized for viral survival (Dulbecco's Modified Eagle Medium, supplemented with 0.1% bovine serum albumin, 10 µg/mL vancomycin, 10 µg/mL gentamycin, and 0.25 µg/mL amphotericin B).

Samples were stored at 4°C during each experiment, then quantified by plaque assay and qRT–PCR methods to give pfu/m³ and RNA copies/m³. Plaque assays were done within 1 h of experiment completion; preliminary experiments showed the virus in solution to be stable at 4°C for up to 4 h (practical details available upon request).

Results

Sampling directly from the nebulizer solution at the end of the experimental run time (after nebulization) showed no loss of viable virus nor viral RNA, suggesting virus survival within the nebulizer chamber (reservoir) is not affected by the mechanical action of nebulization.

The difference between the RNA and pfu quantification is remarkably similar between the different collection time-periods, being of the order of 10⁴–10⁵ difference for each of the 30 min sampling periods, with the RNA loads being in the range 10⁷–10⁹ and the corresponding pfu values being in the range 10²–10⁴. There is a trend to this difference increasing at the later sampling periods (90–120 min) due to a more rapid fall in pfu (i.e. virus viability) (Figure 2).

Discussion

These results suggest that detecting influenza RNA alone may greatly overestimate the amount of viable virus available to cause infection. For a healthy individual, the levels of viable virus (~3000 virions) at 60–90 min were still sufficient to cause infection (assuming that 1 pfu corresponds to one virus/virus genome, where 3 TCID₅₀ is equivalent to 3000 viral genomes) (Figure 2).⁶

Several processes may contribute to loss of virus viability: the nebulization process, time spent airborne (increasing the

chance of surface impaction damage, contact with toxic airborne pollutants, desiccation or degradation), or the air-sampling process. In addition, air-sampling can reduce virus viability through creating sudden changes in the viral particles' microenvironment, and/or induced shear stress.⁷ However, the amount of viable virus loss in artificially produced aerosols, such as those produced in this experiment, compared to naturally produced aerosols from natural influenza-infected volunteers remains unknown.

Two recent studies, using naturally influenza-infected human volunteers, found that whereas diagnostic swabs from the respiratory tract showed relatively high influenza viral RNA loads of up to 10⁵–10⁸ RNA copies/mL, there were much lower levels (up to 10³–10⁴-fold less) of viable virus, recovered by air-sampling, after exhaling or coughing.^{8,9} Although the suspension times were much less for these human influenza experiments (where infected patients coughed directly into conical air-sampling receptors), the amount of viable virus loss appears similar to that seen in this study. However, for human volunteers, other additional mucosal/salivary immunological factors may affect the amount of virus expelled in such exhaled or coughed aerosols.¹⁰

These results indicate that influenza virus does not survive well in the airborne state, at least under these experimental conditions. Although detectable viral RNA remains relatively stable, the numbers of viable virus appear to fall significantly, with a 10⁴–10⁵ loss within 30–60 min after aerosolization, with further loss up to 120 min post nebulization. Further, larger experiments may examine at what time-point the pfu count drops to zero (undetectable). However, it is clear from the experiments described here that when performing environmental air-sampling for influenza (and possibly other enveloped RNA viruses), the detection of RNA by PCR, which represents non-viable as well as viable virus, should be interpreted with caution unless combined with viral culture methods.

Conflict of interest statement

None declared.

Funding sources

J. McCauley was supported by the UK Medical Research Council (MRC U117512723) during this study. J. Brown was supported by an educational grant from Medixair, Brandenburg UK, during this study.

References

- Seto WH. Airborne transmission and precautions: facts and myths. *J Hosp Infect* 2015;**89**:225–228.
- Blachere FM, Lindsley WG, Pearce TA, et al. Measurement of airborne influenza virus in a hospital emergency department. *Clin Infect Dis* 2009;**48**:438–440.
- Lindsley WG, Noti JD, Blachere FM, et al. Viable influenza A virus in airborne particles from human coughs. *J Occup Environ Hyg* 2015;**12**:107–113.
- Harper GJ. Airborne micro-organisms: survival tests with four viruses. *J Hyg* 1961;**59**:479–486.
- Lai KM, Burge HA, First MW. Size and UV germicidal irradiation susceptibility of *Serratia marcescens* when aerosolized from different suspending media. *Appl Environ Microbiol* 2004;**70**:2021–2027.

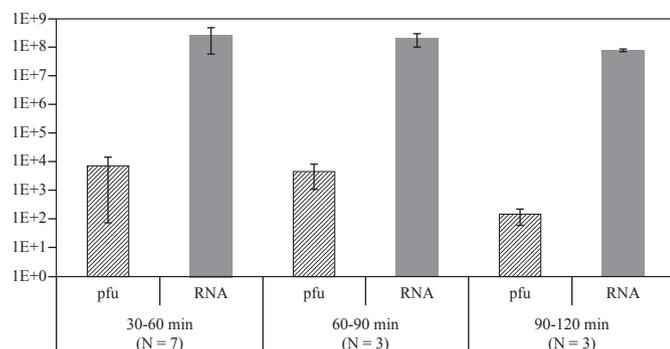


Figure 2. Quantification of viable virus (plaque-forming units, pfu/m³) and viral RNA (copies/m³) recovered from air-sampling after various suspension times, post-nebulization. Error bars represent mean ± 1 standard deviation.

6. Nikitin N, Petrova E, Trifonova E, Karpova O. Influenza virus aerosols in the air and their infectiousness. *Adv Virol* 2014;**2014**:859090.
7. Verreault D, Moineau S, Duchaine C. Methods for sampling of airborne viruses. *Microbiol Mol Biol Rev* 2008;**72**:413–444.
8. Milton DK, Fabian MP, Cowling BJ, Grantham ML, McDevitt JJ. Influenza virus aerosols in human exhaled breath: particle size, culturability, and effect of surgical masks. *PLoS Pathogens* 2013;**9**:e1003205.
9. Hatagishi E, Okamoto M, Ohmiya S, et al. Establishment and clinical applications of a portable system for capturing influenza viruses released through coughing. *PLoS One* 2014;**9**:e103560.
10. Tang JW, Gao CX, Cowling BJ, et al. Absence of detectable influenza RNA transmitted via aerosol during various human respiratory activities – experiments from Singapore and Hong Kong. *PLoS One* 2014;**9**:e107338.