Interface focus

J. R. Soc. Interface (2009) **6**, S783–S790
doi:10.1098/rsif.2009.0302.focus
Published online 22 September 2009

Review

Aerosol transmission of influenza A virus: a review of new studies

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Over the past few years, prompted by pandemic preparedness initiatives, the debate over the modes of transmission of influenza has been rekindled and several reviews have appeared. Arguments supporting an important role for aerosol transmission that were reviewed included prolonged survival of the virus in aerosol suspensions, demonstration of the low infectious dose required for aerosol transmission in human volunteers, and clinical and epidemiological observations were disentanglements of large droplets and aerosol transmission was possible. Since these reviews were published, several new studies have been done and generated new data. These include direct demonstration of the presence of influenza viruses in aerosolized droplets from the tidal breathing of infected persons and in the air of an emergency department; the establishment of the guinea pig model for influenza transmission, where it was shown that aerosol transmission is important and probably modulated by temperature and humidity; the demonstration of some genetic determinants of airborne transmission of influenza viruses as assessed using the ferret model; and mathematical modelling studies that strongly support the aerosol route. These recent results and their implication for infection control of influenza are discussed in this review.

Keywords: aerosol; transmission; influenza A

1. INTRODUCTION

Concerns about an influenza pandemic have been recently rekindled by the emergence in southeast Asia of highly pathogenic strains of avian influenza A (H5N1) with pandemic potential. These concerns have been vindicated by the emergence of a pandemic caused by a new influenza A(H1N1) virus of swine origin, the full impact of which remains unclear at the time of the writing of this article.

In turn, these concerns have generated a renewed interest in the study of the transmission modes of influenza not only for a better understanding of the pathogenesis of the disease but also for the rational design of infection-control strategies. Three modes of transmission have been postulated, which are not mutually exclusive: aerosol transmission, transmission by large droplets and self-inoculation of the nasal mucosa by contaminated hands. The mode of transmission that, arguably, has the greatest impact for infection control is aerosol transmission since it requires specialized personal protective equipment (PPE), e.g. N95 respirators, and procedures.

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One contribution of 10 to a Theme Supplement 'Airborne transmission of disease in hospitals'.

There is considerable support in the scientific literature for a contribution of aerosol transmission to the spread of influenza A, which has been reviewed elsewhere (Tellier 2006). Briefly, supportive evidences include the prolonged persistence of infectivity in aerosolized influenza A virus at low humidity, the transmission to volunteers of influenza by aerosols, reproducing the full spectrum of disease, at doses much smaller than the doses required by intranasal drop inoculation (which mimics large droplet transmission), and the interruption of transmission of influenza by blocking the aerosol route through UV irradiation of upper room air. In addition, whereas intranasal zanamivir prophylaxis protected against intranasal drop inoculation in the laboratory, it is ineffective against natural transmission; in contrast, zanamivir prophylaxis by inhalation was effective.

Over the past few years many new studies have been published which lend further support to the hypothesis that aerosol transmission plays an important role in the spread of influenza. These new studies and their implications are reviewed here.

2. DEFINITION OF AEROSOLS

Fundamentally, aerosols are suspensions in air (or in a gas) of solid or liquid particles small enough that they

Received 20 July 2009
Accepted 24 August 2009

will remain airborne for a prolonged period of time because of their low settling velocity. The settling velocity in still air can be calculated using Stokes' law (Hinds 1999); for example, a 3 m fall takes 4 min for a 20 μm particle (aerodynamic diameter), 17 min for 10 μm and 67 min for 5 μm.

When studying bioaerosols generated by human subjects, it is important to distinguish between the initial diameter of particles and the diameter after evaporation of water in ambient air; the resulting desiccated particles are termed 'droplet nuclei'; for particles with an initial diameter <20 μm the evaporation occurs in <1 s (Nicas *et al.* 2005; Xie *et al.* 2007) and the diameter shrinks to a little less than half the initial diameter (Nicas *et al.* 2005).

Another important consideration for the pathogenesis of infectious diseases acquired by aerosols is the penetration of the respiratory tract. Particles of 5 μm or less have a significant penetration into the respiratory tract all the way to the alveolar region (30% penetration for 5 μm particles); penetration into the alveolar region rapidly diminishes beyond 5 μm, but significant penetration into the tracheobronchial region still occurs for particles in the 5–10 μm range (for 10 μm, 50% penetration) but diminishes rapidly after that. At 20 μm and beyond there is essentially no penetration below the trachea (Hinds 1999). Of course, penetration is not the same thing as deposition and only a fraction of the penetrating particles will be deposited, the remainder being exhaled back (Yu & Diu 1983). All these considerations likely explain why aerosols are defined differently by various authors. There is essential agreement that particles with an aerodynamic diameter of 5 μm or less are aerosols, whereas particles >20 μm would be large droplets. Some authors define aerosols as ≤ 10 μm or even ≤ 20 μm (Knight 1973; Treanor 2005); particles between 5 and 15 to 20 μm have also been termed 'intermediate' (Couch *et al.* 1966; all values refer to the aerodynamic diameter; for bioaerosols, they refer to the aerodynamic diameter after evaporation). When reviewing the literature, it is therefore important to verify the size of the particles being studied and the authors' definitions.

The issue of long-range transmission has been contentious. Because aerosols settle very slowly in still air, they are easily carried over long distance by turbulences and air currents, and this may potentially cause long-distance infections. Certainly, the demonstration of long-range infection implies aerosol transmission. The converse, however, is not necessarily true; aerosol particles are rapidly diluted, and are removed by ventilation; the infectious risk is critically affected by parameters such as the infectious dose, the amount of infectious particles aerosolized at the source, and the rate of biological decay of the infectious agent. For influenza, a quick 'back of the envelope' type of calculation suggests that even for patients with a high viral load the amount of viruses aerosolized in a single sneeze is in fact quite small and would be rapidly diluted as the aerosol disperses; yet, because the infectious dose by aerosol is so small, the infectious risk in proximity of the patient would be significant (Tellier 2007). It should also be pointed out that a low frequency of long-range transmission can be extremely difficult to demonstrate (or rule out), especially if the disease considered is widely prevalent in the community (as is typically the case with influenza). For example, long-range transmission of smallpox could be unequivocally demonstrated only during a hospital outbreak at a time when the disease had long been eradicated from the community (Gellfand & Posch 1971).

3. DIRECT DETECTION OF INFLUENZA VIRUSES IN BIOAEROSOLS FROM PATIENTS

Generation of a large number of aerosol particles by coughing or sneezing has been documented for a long time (reviewed in Nicas *et al.* 2005). A recent study (Yang *et al.* 2007) confirmed yet again the generation of a large number of aerosol particles, and showed a significant heterogeneity between individuals in the amount and size distribution of aerosols. Recently, the technique of Schlieren photography has been used to visualized the aerosols emitted by coughing (Tang & Settles 2008). It is often less appreciated that exhalation during normal breathing also produces aerosol particles. A recent study (Edwards *et al.* 2004) has confirmed the production of aerosol size particles by normal breathing, and confirmed that the size of the majority of the particles exhaled by mouth is 1 μm or less. This is explained by the fact that aerosol particles from normal breathing are generated in the lower respiratory tract (LRT), and the larger particles tend to be retained through impaction or deposition. Another remarkable finding is that there is considerable heterogeneity in aerosol production between individuals, consistently over time (Edwards *et al.* 2004). Although coughing and sneezing produce more aerosols per breathing manoeuvre than normal breathing, since normal breathing is continuous it would account for a significant fraction of the bioaerosols produced over the course of a day (Fiegel *et al.* 2006).

Direct measurement of influenza viruses in aerosols produced by cough or sneezing has not been reported in the literature. This is in part because of the significant experimental difficulties in working with bioaerosols, including the low concentration of particles and, in the case of influenza virus, the relative insensitivity of virus detection by culture methods, the lability of the viruses and potential inactivation caused by the aerosol-sampling methodology. It should be noted however that in the laboratory setting, infectivity measurements of aerosolized influenza viruses could be reliably predicted from the infectious titre of the viral culture fluid used to generate aerosols, over several orders of magnitude (Alford *et al.* 1966). The development of sensitive detection assays based on reverse transcriptase–polymerase chain reaction (RT–PCR) now permits much more sensitive detection of viral particles. Using this methodology, Fabian and colleagues (Fabian *et al.* 2008) have recently directly detected influenza virus RNA in aerosol particles generated by normal breathing in patients with influenza and collected through an oronasal facemask. Patients were

selected on the basis of symptoms and a positive rapid detection test for influenza (given the relative insensitivity of these tests, in all likelihood these patients had a high viral load). Out of 12 patients subjected to analysis, four had detectable influenza virus RNA in exhaled breath (three with influenza A, one with influenza B); as measured by quantitative RT–PCR, one patient with influenza A exhaled 20 RNA copies per minute, the three others exhaled less than 3.2 RNA copies per minute. Evaluation of the size distribution of the particles showed that 87 per cent of the exhaled particles had a diameter of less than 1 μm, with less than 0.1 per cent larger than 5 μm (Fabian *et al.* 2008).

An important limitation of such a study is that RT–PCR cannot establish the infectivity of the viral particles detected; one must make inferences based on other studies that looked at the biological decay of aerosolized influenza viruses (e.g. Hemmes *et al.* 1960,1962). Based on their RT–PCR assay and the influenza virus stock they used for calibration, Fabian *et al.* (2008) established a ratio of 300 copies per tissue culture infectious 50 per cent ($TCID_{50}$), which is well within previously published estimates of 100–350 or 650 (van Elden *et al.* 2001; Wei *et al.* 2007). This would translate, for the highest producer in the study, to a rate of 4 $TCID_{50}\,h^{-1}$ in exhaled breath; the human infection dose 50 per cent (HID_{50}) by aerosol inoculation (using particles of 1–3 μm) has previously been measured as between 0.6 and 3 $TCID_{50}$ (Alford *et al.* 1966).

Another significant recent observation on the occurrence of naturally produced influenza bioaerosols is the report of Blachere and colleagues (Blachere *et al.* 2009) of the detection of aerosolized influenza viruses in a hospital emergency department. Aerosol samples were collected over the course of 4–5 h, during the month of February, using aerosol sampling that allowed for size fractionation. Samples were analysed with a quantitative RT–PCR method. The authors reported the detection of influenza A RNA in 14 samples; the largest amounts of RNA recovered were equivalent to 15 532 $TCID_{50}$ in the fraction of particles greater than 4 μm, and 13 426 $TCID_{50}$ in the fraction of particles in the 1–4 μm range (Blachere *et al.* 2009). The description of the method for quantitative measurements in this study is imprecise, with the unfortunate 'assumption that 1 $TCID_{50}$ is equivalent to one viral RNA copy'. However, a careful reading of the previous study by this group, which describes in detail their quantitative RT–PCR assay, reveals that the quantitative PCR was in fact calibrated in $TCID_{50}$ by using serial dilution of a virus preparation quantitated in $TCID_{50}\,ml^{-1}$ (Blachere *et al.* 2007). The viral preparation used was the live attenuated vaccine, Flumist, containing $10^{6.5}$–$10^{7.5}\,TCID_{50}\,ml^{-1}$; the authors assumed $10^{7}\,TCID_{50}\,ml^{-1}$ in their calculation and therefore the amounts reported in Blachere *et al.* (2009) may have to be adjusted (upward or downward, by a factor of at most $10^{0.5}$ or approx. 3.16-fold), but even so remain impressive when compared with the HID_{50} by aerosol (Alford *et al.* 1966). As was the case with the study of Fabian *et al.* (2008) above, detection by RT–PCR alone does not imply infectivity, but it is noteworthy that on the days where samples were obtained, the relative humidity (RH) was 30 ± 3.3 per cent (Blachere *et al.* 2009), well under the 40 per cent threshold below which aerosolized influenza virus infectivity decays slowly (Hemmes *et al.* 1960).

4. OBSERVATIONS FROM ANIMAL INFECTIONS WITH INFLUENZA

One of the most interesting recent developments in the field has been the establishment of the guinea pig model for influenza transmission by the group of Palese and co-workers (Lowen *et al.* 2006). It was shown that guinea pigs are readily infected by human strains of influenza A virus, without prior viral adaptation, that the virus replicates in both the upper and lower RT, and that the virus is readily transmitted between guinea pigs. The infection can be asymptomatic or not, depending largely on the strain of guinea pig used; Hartley strain guinea pigs were asymptomatic, whereas strain 13 animals displayed weight loss, hair loss, lethargy and hypotermia (Lowen *et al.* 2006). However, in another study by the same group, strain 13 animals were also asymptomatic (Mubareka *et al.* 2009). Importantly, coughing and sneezing do not occur.

In their initial study Lowen *et al.* demonstrated several instances of influenza transmission between guinea pigs, including when the source animal and the contact were in different cages separated by 91 cm (Lowen *et al.* 2006). It may be argued that transmission over such a distance rules out large droplet transmission and therefore would have to be ascribed to aerosol transmission. In many infection control guidelines for influenza, it is stated that large droplets do not travel farther than 3 ft ('the three-feet rule'), although this was not rigorously demonstrated. Xie *et al.* recently performed an analysis of dispersion of respiratory droplets (Xie *et al.* 2007); in their model, they assumed that respiratory droplets behave like droplets of 0.9 per cent NaCl in water. Respiratory droplets initially all move forward with the exhaled air jet; very large droplets leave the jet quickly and fall on the ground; 'intermediate size' large droplets leaving the jet desiccate in ambient air (partially or completely, depending on their size); and small droplets completely desiccate within the jet. They calculated that, using a horizontal jet emitted at a height of 2 m, at an RH of 50 per cent, the horizontal distance travelled by particles of initial diameter of 30–50 μm is less than a metre if the initial velocity of the jet is 1 $m\,s^{-1}$ (typical of normal breathing), and more than 6 m if the initial jet velocity is 50 $m\,s^{-1}$ (typical of sneezing); larger particles travel less far because they fall on the ground quickly, and smaller particles desiccate into aerosol-size particles. Increase in RH decreases the horizontal distance travelled by non-aerosol droplets, and conversely (Xie *et al.* 2007). In the guinea pig experiments described above, obviously the height at which the jet was emitted was considerably lower than 2 m, and they do not sneeze or cough when infected with influenza; it would therefore appear that 91 cm is too far to be explained by

large droplets transmission. Two missing measurements prevent a definitive conclusion: the RH for those experiments was not specified; it was noted that transmission followed the airflow direction but the velocity of the airflow was not stated (Lowen *et al.* 2006). Again using the guinea pig model, the same group described recently a stronger experimental evidence for aerosol transmission when they documented instances of transmission with the cage of the contact animal above the cage of the source animal at a distance of 80 or 107 cm (Mubareka *et al.* 2009). Another important observation in this study is that different influenza strains differ considerably in their capacity for aerosol transmission.

Palese and colleagues also used their guinea pig model to study the effects of temperature and RH on transmission in two additional studies (Lowen *et al.* 2007, 2008) where they compared contact transmission and airborne transmission. Of note in these two studies airborne transmission was assessed with the source and contact guinea pigs in two different cages side by side; this short distance makes it impossible to rule out a contribution from large droplets. Indeed, the authors explicitly stated that they used the term 'aerosol' to encompass both large droplets and droplet nuclei. They showed that airborne transmission (large droplets and/or droplet nuclei) was enhanced at low temperature (5°C) and that high temperature (30°C) interrupted airborne transmission at all values of RH. At 20°C, transmission was highly efficient at an RH of 20 and 35 per cent, low at 50 per cent, efficient again at 65 per cent and absent at 80 per cent. The authors tentatively attributed the effect of low temperature to the increased viral load observed in the animals at this temperature (Lowen *et al.* 2007), but proposed no explanation for the effect of high temperature, which interestingly enough did not interfere with contact transmission between animals in the same cage (Lowen *et al.* 2008). As the authors noted, the effect of RH is reminiscent on studies of infectivity decay in influenza virus aerosols. Hemmes and colleagues, using aerosols in the 5–6 μm range, showed that the biological decay of aerosolized influenza is low at RH < 40 per cent, but rapidly increases with RH (Hemmes *et al.* 1960, 1962). However, Schaffer *et al.* did observe a biphasic effect of RH, with the greatest stability at an RH of 20 per cent, the greatest decay at 50–60 per cent, and a return to moderate stability at greater RH; unfortunately, they did not state the size of the particles in their aerosols (Schaffer *et al.* 1976).

Using the ferret model, Van Hoeven and colleagues showed that not all influenza strains are capable of 'airborne transmission', by which they meant large droplets and/or aerosols, as their experimental set-up did not allow for the distinction (Van Hoeven *et al.* 2009); it should be noted, however, that aerosol transmission of influenza between ferrets was demonstrated a long time ago (Andrewes & Glover 1941). An avian influenza strain was shown to be incapable of transmission between ferrets, by either direct contact (co-caged animals) or large droplets and/or aerosols. By reverse genetics, the HA and NA genes from the 1918 A(H1N1) pandemic strain were substituted to those of the original virus (thereby changing the sialic acid receptor affinity), which resulted in direct contact transmission between ferrets, but still not by large droplets/aerosols. The latter was finally achieved by the subsequent addition of the *PB2* gene from the 1918 A(H1N1) virus. The impact of the *PB2* gene is presumably mediated through higher replication rate in the cells of the respiratory tract. Using the guinea pig model, Steel and colleagues also showed how mutations in the *PB2* gene allow for efficient transmission, including through the large droplet and/or aerosol route (Steel *et al.* 2009). Another example of strain differences in their capacity for large droplets and/or aerosol transmission is provided by the study of Bouvier *et al.* using the guinea pig model. They showed that oseltamivir resistance in an A(H3N2) strain modifies its transmission efficacy. The wild-type transmitted efficiently by both contact and large droplets/aerosols, but introduction of a single or two amino acids mutation conferring oseltamivir resistance significantly decreased or completely interrupted, respectively, droplet transmission while contact transmission was unaffected (Bouvier *et al.* 2008). In a similar manner, Sorrell *et al.* have shown that re-assortant influenza viruses carrying the surface proteins of avian A(H9N2) on a backbone of human A(H3N2) (which therefore included a *PB2* gene well adapted to mammalian hosts) could be rapidly adapted to ferrets. The authors observed ferret-to-ferret transmission by direct contact, and between ferrets in adjacent cages, thereby demonstrating transmission by aerosol and/or large droplets (Sorrell *et al.* 2009).

Also using the ferret model, Munster *et al.* compared experimental infections with the seasonal A(H1N1) and the swine origin A(H1N1) currently responsible for an ongoing pandemic; from the point of view of transmission, again using an experimental set-up with adjacent cages, they showed that both strains were equally proficient in aerosol and/or large droplet transmission (Munster *et al.* 2009). In contrast however, Maines *et al.* (2009), who also compared seasonal A(H1N1) with the swine origin A(H1N1), found that the latter was less proficient at aerosol/large droplet transmission (six of nine contacts infected, compared with three of three for seasonal A(H1N1); Maines *et al.* 2009).

As a last example involving animal transmission, a recent outbreak of equine influenza A(H3) in Queensland, Australia offered a unique opportunity to study the spread of the virus in a region of the world where it was previously absent (Davis *et al.* 2009). The cluster of equine influenza started at a quarantine facility and eventually involved 437 other premises, 81 per cent of which were not contiguous to a premise with cases. Distances between non-contiguous premises were often several kilometres (most within 5 km but there was one instance of a 13 km separation). Strict quarantines and a standstill were put in place at the onset of the outbreak, precluding transfer of horses between premises in the region. Transfer of fomites between premises was pre-empted by extensive media and publicity campaigns; surveillance reports showed that compliance was high. The authors further note that the observed geographic spread was consistent with the predominant direction of wind patterns at

the time (Davis *et al.* 2009). All in all, an outbreak with several instances of transmission consistent with and suggestive of aerosol transmission which, as the authors note in their discussion, has been implicated before in equine influenza transmission. In fact, experimental transmission of equine influenza to horses has been shown to be more reliably achieved by aerosol inoculation than by intranasal instillation (Mumford *et al.* 1990).

5. MATHEMATICAL MODELLING

As mathematical models for infectious diseases become increasingly sophisticated, it is only natural that they would be brought to bear on the problem of influenza transmission.

Recognizing that aerosol, large droplets and self-inoculation by contaminated hands are not mutually exclusive, Nicas and Sun built a Markov chain model incorporating these three modes of transmission and identifying several parameters that would need to be measured experimentally in order to use the model for a specific pathogen. For purposes of illustration they ascribed values to several parameters for a hypothetical pathogen and showed that for that pathogen, if a healthcare worker spent 15 min at the bedside of a patient without coughing or sneezing events, the probability of infection by self-inoculation with contaminated hands was 0.029 and by aerosols in the room air was 8.3×10^{-6} (taking 5 μm particles as 'representative'). Coughing within 0.6 m of the healthcare worker is associated with a probability of infection of 0.14 by large droplets; for a 15 min stay in the room, estimating the probability of coughing and of the proximity at any given time, the probability of transmission by large droplets originating in a cough is 0.021. A similar treatment for aerosols shows, for a 15 min visit, a probability of 4.5×10^{-4} (Nicas & Sun 2006). Of course, these probabilities depend critically on the values ascribed to the parameters; for example, the infectivity parameter α, calculated from the HID_{50}, is assumed to be the same for all routes. But as the authors themselves point out, influenza A virus is a counterexample since the HID_{50} by aerosol is about 100 times less than by large droplets, as reviewed (Tellier 2006).

Similarly, Atkinson and Wein built a model of multiple modes of transmission, based on an infinite set of differential equations, and estimated parameters using the available literature and various hypotheses (Atkinson & Wein 2008). In a household transmission model, initially omitting transmission at close range, a comparison of transmission through hands contaminated by fomites and transmission by aerosols showed aerosol transmission to be dominant. The authors also presented a detailed analysis of the transmission of influenza by a cough or a sneeze at close range (60 cm) from an infected patient with a peak shedding of 2.88×10^7 $TCID_{50}$, with 99 per cent of shedding occurring in sneezes and 1 per cent in cough. The authors separately analysed the infection probability from inhalation of particles <20 μm, of particles between 20 and 200 μm, and from droplets landing directly on the mucosa because of their trajectory. A first analysis shows that for a cough, the probability of infection is 0.244 with the greatest contribution from particles of 20–200 μm (transmission fraction of 0.91); for a sneeze the probability would be 0.9999 with equal contribution from particles <20 μm, from particles between 20 and 200 μm, and from droplets landing directly (assuming that the contact person is breathing in at the time of the cough or sneeze). The authors proposed a different analysis where the distribution of particles in the cone-shaped cloud caused by coughing or sneezing is uneven because of the different stopping distances of different size particles. With this analysis, the probability for a cough is 0.011 and for a sneeze 0.981, all caused by direct deposition (Atkinson & Wein 2008). The stopping distance is the maximum distance travelled by an aerosol particle in still air for a given initial speed (Hinds 1999). The application of this concept in Atkinson & Wein (2008) appears incorrect, since following a cough or a sneeze particles are carried by a jet of air, and in fact bioaerosols from the respiratory tract are created by air jets over the airway lining fluid (Fiegel *et al.* 2006). The use of stopping distance for particles in a jet of air is more relevant when the jet changes direction, in the context of an analysis of impaction (Hinds 1999). For the horizontal distance travelled by particles expelled from the respiratory tract, the analysis of Xie *et al.* (2007), reviewed above, appears more convincing.

Both Nicas & Sun (2006) and Atkinson & Wein (2008) calculated the aerosolized infectious agents in coughs and sneezes from published studies of particles distribution in coughs and sneezes and from measured infectious titres in nasal washing. While this appears adequate for sneezing, it may be an underestimate for cough. The fluid aerosolized in a cough comes in part from the LRT; in a patient with an LRT infection with influenza, it may well be that the concentration of virus is greater in the fluid lining the LRT than the nasal mucosa; to my knowledge this has not been measured, but in humans and in mice the surface ratio of epithelial surfaces between the LRT and the nasal mucosa is roughly 1000, whereas the ratio of the volumes of fluid and mucous lining the epithelium in the LRT and nasal mucosa is approximately 9 (Ito *et al.* 2003).

In a recently published study, Nicas and Best provided a mathematical analysis of the risk of influenza infection by hand-to-face contact. This mode of infection, which has been documented for some respiratory viruses, has been deemed plausible since Bean *et al.* (1982) demonstrated the persistence of influenza virus infectivity on hard non-porous surfaces for 24–48 h, theoretically allowing for hand contamination and infection by self-inoculation. One problem with that proposed route of infection is that the same study also showed that on the hands, the infectivity titre of a suspension of influenza A viruses drops by two to three logs within 5 min (Bean *et al.* 1982); similarly, Schurmann and co-workers documented a decay of three logs on the hands within 12 min (Schurmann & Eggers 1983); thus the interval for self-inoculation is relatively short.

Nicas and Best made a systematic analysis of this possible mode of transmission, including a measurement of the frequency of hand-to-face contact by videotaping volunteers for several hours, and estimating other relevant parameters from the literature. As an example using influenza A virus, for a 30 min exposure in an environment with contaminated surfaces, followed by a 30 min decay period before hand-to-face contact, the probability of infection is estimated at 0.00 011 (Nicas & Best 2008). Of course, as the authors noted, the estimate of several parameters remains uncertain. For example, the infectivity parameter α was estimated from a study of experimental infection by nasal instillation in volunteers that was one of the first successful reports of reliable infection by nasal instillation, but which used doses in the range of 790–10 000 $TCID_{50}$, which are larger than the more recent estimates of HID_{50} by nasal instillation, namely 127 $TCID_{50}$ and 320 $TCID_{50}$ (Couch *et al.* 1971, 1974). Taking HID_{50} as 127 $TCID_{50}$, one can re-calculate $\alpha = 5.46 \times 10^{-3}$ and the probability of infection in the scenario above is 0.01, still rather low. Thus it appears unlikely that in most circumstances hand-to-face contact is a major route of transmission for influenza (Nicas & Best 2008).

Shaman and Kohn re-examined the experimental data of several studies to question whether RH is in fact a better parameter to predict infectivity persistence of aerosolized influenza than absolute humidity (Shaman & Kohn 2009). Hemmes *et al.* concluded that RH was a determining factor based on the sharp transition at about 40 per cent RH for the values of the death rate constant when plotted against RH (Hemmes *et al.* 1960, 1962). Analysing the data from experiments on transmission of influenza between guinea pigs (Lowen *et al.* 2007, 2008), Shaman and Kohn showed a better statistical correlation with vapour pressure compared with RH; a similar re-analysis of the data from Harper (1961) on infectivity decay in aerosolized influenza viruses also showed a better correlation using vapour pressure (Shaman & Kohn 2009). When considering aerosol transmission, the latter is the strongest argument put forth by Shaman and Kohn (even though Harper did not state the size of the aerosols studied) since, as noted by Lowen and colleagues, and as reviewed above, the experimental set-up in these guinea pig studies did not allow for the contributions of aerosol and large droplets to be disentangled. Other studies reviewed were found to be consistent with the notion that vapour pressure was a better parameter, although it is unclear what contributions the cited studies by Mitchell *et al.* could bring since they were all conducted at a temperature of 70°F and an RH of 75 per cent (Mitchell *et al.* 1968; Mitchell & Guerin 1972).

The questions raised in this study are however important from a fundamental point of view, as the biochemistry of the effect of humidity on aerosolized influenza viruses (or other enveloped viruses for that matter) remains incompletely understood. Furthermore, Shaman and Kohn, noting the agreement between several studies that low humidity is associated with prolonged infectivity persistence of aerosolized influenza virus but that the decay accelerates at higher humidity, suggest that humidification measures for infection control may be warranted (Shaman & Kohn 2009). Other engineering measures would be expected to interfere with aerosol transmission of influenza, starting of course with adequate ventilation and mechanical ventilation (Liao *et al.* 2005; Qian *et al.* 2006; Furuya 2007; Bolashikov & Melikov 2009; Gao *et al.* 2009). Recently, interest in upper room air UV irradiation has been rekindled (McDevitt *et al.* 2008); it is interesting to note that 50 per cent RH is considered optimal for this method, whereas RH above 75 per cent significantly decreases its performance (Bolashikov & Melikov 2009). Other methods of engineering control include induct UV irradiation, filtration and more speculative methods such as photocatalytic oxidation, desiccant rotor and plasmacluster ions (Bolashikov & Melikov 2009).

6. DISCUSSION

Increasing evidences point towards a role for aerosol transmission in the spread of influenza, at least over short distance where exposure to both aerosol and large droplets occurs. In most settings where there is adequate ventilation, long-range transmission does not appear to occur frequently. This distinction of 'short-range aerosol transmission' is not merely academic; aerosolized particles would readily penetrate or circumvent ordinary surgical masks, and penetration of aerosolized influenza viruses into the LRT where they can initiate infection would account well for the association of aerosol transmission and severe disease.

The recent results reviewed here include direct detection of aerosolized influenza virus (albeit by RT–PCR) in patients' exhaled breath and in the ambient air of an emergency department room; the demonstration of aerosol transmission between guinea pigs of human influenza strain; and mathematical modelling studies predicting an important contribution of aerosol transmission, whereas a rigorous mathematical analysis of transmission by hand-to-face contact suggests that it plays but a minor role.

Studies using guinea pig models have further confirmed the effect of temperature and humidity on influenza transmission by respiratory droplets, although the experimental setting was such that the role of aerosol and large droplets could not be disentangled; the authors made a very good case that the relative contribution of different modes of transmission may be different depending on the setting (including temperature and humidity). These studies, and the physical properties of aerosols, suggest that in addition to PPE, several engineering control methods implemented in hospitals and other institutions could be beneficial.

Studies using the guinea pig model and the ferret model have demonstrated differences between strains in their capacity for droplets (aerosols and/or large droplets) transmission, including genetic characterization of some required mutations. This exciting development is expected, as our knowledge increases, to allow for

better prediction of the pandemic potential of new influenza strains.

The accumulating evidence for an important contribution of the aerosol route in the transmission of influenza implies that infection-control protocols must take it into account, and especially during a pandemic. As an additional consideration, it may well be that aerosol transmission is responsible for the most severe cases of disease involving viral infection of the LRT. Whereas engineering control methods are useful, and indeed necessary, to prevent long-range infections, they would be of little help to healthcare workers in close proximity of a patient to provide care. Precautions should include the use of an N95 respirator (or better) when appropriate, including in close proximity of an infected patient.

Finally, a note on the heterogeneity of patients' contagiousness. It was already well established that not all patients have the same viral load (Murphy *et al.* 1973); this heterogeneity is now compounded by the demonstration of heterogeneity between subjects of the size distribution and quantity of aerosol particles generated in coughing and sneezing, and during normal breathing. This heterogeneity must be remembered when establishing infection-control protocols, and taken into account for mathematical modelling (Lloyd-Smith *et al.* 2005; Pourbohloul *et al.* 2005).

The opinions expressed in this article are those of the author and do not necessarily reflect the opinions of the institutions with which he is affiliated.

REFERENCES

Alford, R. H., Kasel, J. A., Gerone, P. J. & Knight, V. 1966 Human influenza resulting from aerosol inhalation. *Proc. Soc. Exp. Biol. Med.* **122**, 800–804.

Andrewes, C. H. & Glover, R. E. 1941 Spread of infection from the respiratory tract of the ferret: I. Transmission of influenza A virus. *Br. J. Exp. Pathol.* **22**, 91–97.

Atkinson, M. P. & Wein, L. M. 2008 Quantifying the routes of transmission for pandemic influenza. *Bull. Math. Biol.* **70**, 820–867. (doi:10.1007/s11538-007-9281-2)

Bean, B., Moore, B. M., Sterner, B., Peterson, L. R., Gerding, D. N. & Balfour Jr, H. H. 1982 Survival of influenza viruses on environmental surfaces. *J. Infect. Dis.* **146**, 47–51.

Blachere, F. M., Lindsley, W. G., Slaven, J. E., Green, B. J., Anderson, S. E., Chen, B. T. & Beezhold, D. H. 2007 Bioaerosol sampling for the detection of aerosolized influenza virus. *Influenza Other Resp. Viruses* **1**, 113–120. (doi:10.1111/j.1750-2659.2007.00020.x)

Blachere, F. M. *et al.* 2009 Measurement of airborne influenza virus in a hospital emergency department. *Clin. Infect. Dis.* **48**, 438–440. (doi:10.1086/596478)

Bolashikov, Z. D. & Melikov, A. K. 2009 Methods for air cleaning and protection of building occupants from airborne pathogens. *Build. Environ.* **44**, 1378–1385. (doi:10.1016/j.buildenv.2008.09.001)

Bouvier, N. M., Lowen, A. C. & Palese, P. 2008 Oseltamivir-resistant influenza A viruses are transmitted efficiently among guinea pigs by direct contact but not by aerosol. *J. Virol.* **82**, 10052–10058. (doi:10.1128/JVI.01226-08)

Couch, R. B., Cate, T. R., Douglas Jr, R. G., Gerone, P. J. & Knight, V. 1966 Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. *Bacteriol. Rev.* **30**, 517–529.

Couch, R. B., Douglas Jr, R. G., Fedson, D. S. & Kasel, J. A. 1971 Correlated studies of a recombinant influenza-virus vaccine. 3. Protection against experimental influenza in man. *J. Infect. Dis.* **124**, 473–480.

Couch, R. B., Kasel, J. A., Gerin, J. L., Schulman, J. L. & Kilbourne, E. D. 1974 Induction of partial immunity to influenza by a neuraminidase-specific vaccine. *J. Infect. Dis.* **129**, 411–420.

Davis, J., Garner, M. G. & East, I. J. 2009 Analysis of local spread of equine influenza in the Park Ridge region of Queensland. *Transbound. Emerg. Dis.* **56**, 31–38. (doi:10.1111/j.1865-1682.2008.01060.x)

Edwards, D. A., Man, J. C., Brand, P., Katstra, J. P., Sommerer, K., Stone, H. A., Nardell, E. & Scheuch, G. 2004 Inhaling to mitigate exhaled bioaerosols. *Proc. Natl Acad. Sci. USA* **101**, 17383–17388. (doi:10.1073/pnas.0408159101)

Fabian, P., McDevitt, J. J., DeHaan, W. H., Fung, R. O., Cowling, B. J., Chan, K. H., Leung, G. M. & Milton, D. K. 2008 Influenza virus in human exhaled breath: an observational study. *PLoS ONE* **3**, e2691. (doi:10.1371/journal.pone.0002691)

Fiegel, J., Clarke, R. & Edwards, D. A. 2006 Airborne infectious disease and the suppression of pulmonary bioaerosols. *Drug Discov. Today* **11**, 51–57. (doi:10.1016/S1359-6446(05)03687-1)

Furuya, H. 2007 Risk of transmission of airborne infection during train commute based on mathematical model. *Environ. Health Prev. Med.* **12**, 78–83. (doi:10.1007/BF02898153)

Gao, X., Li, Y. & Leung, G. M. 2009 Ventilation control of indoor transmission of airborne diseases in an urban community. *Indoor Built Environ.* **18**, 205–218.

Gellfand, H. M. & Posch, J. 1971 The recent outbreak of smallpox in Meschede, West Germany. *Am. J. Epidemiol.* **93**, 234–237.

Harper, G. J. 1961 Airborne micro-organisms: survival tests with four viruses. *J. Hyg.* (*Lond.*) **59**, 479–486.

Hemmes, J. H., Winkler, K. C. & Kool, S. M. 1960 Virus survival as a seasonal factor in influenza and poliomyelitis. *Nature* **188**, 430–431. (doi:10.1038/188430a0)

Hemmes, J. H., Winkler, K. & Kool, S. M. 1962 Virus survival as a seasonal factor in influenza and poliomyelitis. *Antonie Van Leeuwenhoek* **28**, 221–233. (doi:10.1007/BF02538737)

Hinds, W. C. 1999 *Aerosol technology*, 2nd edn. New York, NY: John Wiley and Sons, Inc.

Ito, R. *et al.* 2003 Roles of anti-hemagglutinin IgA and IgG antibodies in different sites of the respiratory tract of vaccinated mice in preventing lethal influenza pneumonia. *Vaccine* **21**, 2362–2371. (doi:10.1016/S0264-410X(03)00078-1)

Knight, V. 1973 Airborne transmission and pulmonary deposition of respiratory viruses. In *Airborne transmission and airborne infections. VIth Int. Symp. on Aerobiology* (eds J. F. Hers & K. C. Winkles), pp. 175–182. New York, NY: Wiley.

Liao, C. M., Chang, C. F. & Liang, H. M. 2005 A probabilistic transmission dynamic model to assess indoor airborne infection risks. *Risk Anal.* **25**, 1097–1107. (doi:10.1111/j.1539-6924.2005.00663.x)

Lloyd-Smith, J. O., Schreiber, S. J., Kopp, P. E. & Getz, W. M. 2005 Superspreading and the effect of individual variation on disease emergence. *Nature* **438**, 355–359. (doi:10.1038/nature04153)

Lowen, A. C., Mubareka, S., Tumpey, T. M., Garcia-Sastre, A. & Palese, P. 2006 The guinea pig as a transmission model for human influenza viruses. *Proc. Natl Acad. Sci. USA* **103**, 9988–9992. (doi:10.1073/pnas.0604157103)

Lowen, A. C., Mubareka, S., Steel, J. & Palese, P. 2007 Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathog.* **3**, 1470–1476. (doi:10.1371/journal.ppat.0030151)

Lowen, A. C., Steel, J., Mubareka, S. & Palese, P. 2008 High temperature (30°C) blocks aerosol but not contact transmission of influenza virus. *J. Virol.* **82**, 5650–5652. (doi:10.1128/JVI.00325-08)

Maines, T. R. *et al.* 2009 Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. *Science* **325**, 484–487. (doi:10.1126/science.1177238)

McDevitt, J. J., Milton, D. K., Rudnick, S. N. & First, M. W. 2008 Inactivation of poxviruses by upper-room UVC light in a simulated hospital room environment. *PLoS ONE* **3**, e3186. (doi:10.1371/journal.pone.0003186)

Mitchell, C. A. & Guerin, L. F. 1972 Influenza A of human, swine, equine and avian origin: comparison of survival in aerosol form. *Can. J. Comp. Med.* **36**, 9–11.

Mitchell, C. A., Guerin, L. F. & Robillard, J. 1968 Decay of influenza A viruses of human and avian origin. *Can. J. Comp. Med.* **32**, 544–546.

Mubareka, S., Lowen, A. C., Steel, J., Coates, A. L., Garcia-Sastre, A. & Palese, P. 2009 Transmission of influenza virus via aerosols and fomites in the guinea pig model. *J. Infect. Dis.* **199**, 858–865. (doi:10.1086/597073)

Mumford, J. A., Hannant, D. & Jessett, D. M. 1990 Experimental infection of ponies with equine influenza (H3N8) viruses by intranasal inoculation or exposure to aerosols. *Equine Vet. J.* **22**, 93–98.

Munster, V. J. *et al.* 2009 Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. *Science* **325**, 481–483. (doi:10.1126/science.1177127)

Murphy, B. R., Chalhub, E. G., Nusinoff, S. R., Kasel, J. & Chanock, R. M. 1973 Temperature-sensitive mutants of influenza virus. 3. Further characterization of the ts-1(E) influenza A recombinant (H3N2) virus in man. *J. Infect. Dis.* **128**, 479–487.

Nicas, M. & Best, D. 2008 A study quantifying the hand-to-face contact rate and its potential application to predicting respiratory tract infection. *J. Occup. Environ. Hyg.* **5**, 347–352. (doi:10.1080/15459620802003896)

Nicas, M. & Sun, G. 2006 An integrated model of infection risk in a health-care environment. *Risk Anal.* **26**, 1085–1096. (doi:10.1111/j.1539-6924.2006.00802.x)

Nicas, M., Nazaroff, W. W. & Hubbard, A. 2005 Toward understanding the risk of secondary airborne infection: emission of respirable pathogens. *J. Occup. Environ. Hyg.* **2**, 143–154. (doi:10.1080/15459620590918466)

Pourbohloul, B., Meyers, L. A., Skowronski, D. M., Krajden, M., Patrick, D. M. & Brunham, R. C. 2005 Modeling control strategies of respiratory pathogens. *Emerg. Infect. Dis.* **11**, 1249–1256.

Qian, H., Li, Y., Nielsen, P. V., Hyldgaard, C. E., Wong, T. W. & Chwang, A. T. 2006 Dispersion of exhaled droplet nuclei in a two-bed hospital ward with three different ventilation systems. *Indoor Air* **16**, 111–128. (doi:10.1111/j.1600-0668.2005.00407.x)

Schaffer, F. L., Soergel, M. E. & Straube, D. C. 1976 Survival of airborne influenza virus: effects of propagating host, relative humidity, and composition of spray fluids. *Arch. Virol.* **51**, 263–273. (doi:10.1007/BF01317930)

Schurmann, W. & Eggers, H. J. 1983 Antiviral activity of an alcoholic hand disinfectant. Comparison of the *in vitro* suspension test with *in vivo* experiments on hands, and on individual fingertips. *Antiviral Res.* **3**, 25–41. (doi:10.1016/0166-3542(83)90012-8)

Shaman, J. & Kohn, M. 2009 Absolute humidity modulates influenza survival, transmission, and seasonality. *Proc. Natl Acad. Sci. USA* **106**, 3243–3248. (doi:10.1073/pnas.0806852106)

Sorrell, E. M., Wan, H., Araya, Y., Song, H. & Perez, D. R. 2009 Minimal molecular constraints for respiratory droplet transmission of an avian-human H9N2 influenza A virus. *Proc. Natl Acad. Sci. USA* **106**, 7565–7570. (doi:10.1073/pnas.0900877106)

Steel, J., Lowen, A. C., Mubareka, S. & Palese, P. 2009 Transmission of influenza virus in a mammalian host is increased by PB2 amino acids 627K or 627E/701N. *PLoS Pathog.* **5**, e1000252. (doi:10.1371/journal.ppat.1000252)

Tang, J. W. & Settles, G. S. 2008 Images in clinical medicine. Coughing and aerosols. *N. Engl. J. Med.* **359**, e19. (doi:10.1056/NEJMicm072576)

Tellier, R. 2006 Review of aerosol transmission of influenza A virus. *Emerg. Infect. Dis.* **12**, 1657–1662.

Tellier, R. 2007 Transmission of influenza A in human beings. *Lancet Infect. Dis.* **7**, 759–760; author reply 761–763. (doi:10.1016/S1473-3099(07)70269-4)

Treanor, J. J. 2005 Influenza virus. In *Mandell, Douglas and Bennett's principles and practice of infectious diseases*, vol. 2 (eds G. L. Mandell, J. E. Bennett & R. Dolin), pp. 2060–2085, 6th edn. New York, NY: Elsevier Churchill Livingstone.

van Elden, L. J., Nijhuis, M., Schipper, P., Schuurman, R. & van Loon, A. M. 2001 Simultaneous detection of influenza viruses A and B using real-time quantitative PCR. *J. Clin. Microbiol.* **39**, 196–200. (doi:10.1128/JCM.39.1.196-200.2001)

Van Hoeven, N., Pappas, C., Belser, J. A., Maines, T. R., Zeng, H., Garcia-Sastre, A., Sasisekharan, R., Katz, J. M. & Tumpey, T. M. 2009 Human HA and polymerase subunit PB2 proteins confer transmission of an avian influenza virus through the air. *Proc. Natl Acad. Sci. USA* **106**, 3366–3371. (doi:10.1073/pnas.0813172106)

Wei, Z. *et al.* 2007 Biophysical characterization of influenza virus subpopulations using field flow fractionation and multiangle light scattering: correlation of particle counts, size distribution and infectivity. *J. Virol. Methods* **144**, 122–132. (doi:10.1016/j.jviromet.2007.04.008)

Xie, X., Li, Y., Chwang, A. T., Ho, P. L. & Seto, W. H. 2007 How far droplets can move in indoor environments—revisiting the Wells evaporation–falling curve. *Indoor Air* **17**, 211–225. (doi:10.1111/j.1600-0668.2007.00469.x)

Yang, S., Lee, G. W., Chen, C. M., Wu, C. C. & Yu, K. P. 2007 The size and concentration of droplets generated by coughing in human subjects. *J. Aerosol Med.* **20**, 484–494. (doi:10.1089/jam.2007.0610)

Yu, C. P. & Diu, C. K. 1983 Total and regional deposition of inhaled aerosols in humans. *J. Aerosol Sci.* **14**, 599–609. (doi:10.1016/0021-8502(83)90065-4)