Use of a Human Influenza Challenge Model to Assess Person-to-Person Transmission: Proof-of-Concept Study

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Background. Influenza transmission in humans remains poorly understood. In particular, the relative contribution of contact, large droplet, and aerosol transmission is unknown. The aims of this proof-of-concept study were to determine whether an experimentally induced influenza infection is transmissible between humans and whether this would form a viable platform for future studies.

Methods. In a quarantine facility, healthy volunteers ("donors") were inoculated with A/Wisconsin/67/2005 (H3N2) influenza virus via intranasal drops. On study days 2 and 3 "recipient" volunteers were exposed to donors under close living conditions. Volunteers socialized for 30 hours during a 2-day period. Infection was confirmed by ≥ 1 positive results from polymerase chain reaction, virus culture, or serology.

Results. After inoculation, 4 of 9 donors developed symptoms consistent an influenza-like illness (ILI) and 7 of 9 were proven to be influenza-infected. After exposure, 4 of 15 recipients developed symptoms of ILI and 3 of 15 were proven to be infected. Serum collected within 2 days of study initiation indicated that 1 donor and 3 recipients were seropositive at study initiation. After adjustment for preexposure immunity, the overall secondary attack rate was 25% (3 of 12).

Conclusions. Experimental human exposure studies offer an attractive potential method for answering outstanding questions related to influenza transmission and the evaluation of interventions to reduce it.

Limited understanding of influenza transmission has been a frequent obstacle during the development of pandemic influenza infection prevention and mitigation strategies. The science is hotly debated, especially the

relative importance of transmission via large droplets or aerosols [1, 2]. In the aftermath of the 2009 A (H1N1) pandemic, clarification of the relative importance of different modes of transmission is critical for the refinement of evidence-based infection control advice for healthcare settings, schools, workplaces, and homes; the European Centre for Disease Prevention and Control, World Health Organization, and US Institute of Medicine have all prioritized understanding of the modes of influenza transmission as a critical need for future pandemic planning [3–5].

Experimental human challenge studies present an attractive way to study influenza transmission; serologically susceptible subjects can be infected at specific times, and subject behavior and environmental conditions can be controlled. The first successful influenza challenge study took place in 1936 when volunteers

were infected with atomized suspensions of infected mouse lung [6]. In present-day influenza challenge studies, susceptible healthy adults are selected by serum antibody levels and infected intranasally with a well-characterized pool of wild-type influenza virus (the aerosol route of inoculation is not used because there is a concern that infections induced in this way may be more severe). Under these conditions, the majority of subjects will be infected and develop a mild illness accompanied by recovery of virus from the nasopharynx. This model has been used to evaluate antiviral agents, including neuraminidase inhibitors, and influenza vaccines [7].

Although previous human influenza challenge studies contribute to an understanding of influenza transmission by demonstrating the potency of aerosol inoculation compared with instillation of nasal drops [8–10], this system has not been used thus far to initiate infection in volunteers and then study the generation of secondary infections in other deliberately exposed volunteers (although such work has been performed to study transmission of a number of other respiratory viruses [11–13]). If this approach were successfully demonstrated, it would be possible to consider future challenge-based intervention studies aimed at interrupting specific transmission modalities to better understand the relative contribution of different transmission modes or to evaluate the effectiveness of specific interventions such as facemasks or respirators for reducing transmission. We describe a proof-of-concept study to assess the feasibility of using a human challenge model to study influenza transmission.

METHODS

The study took place over 13 days in May and June 2009 and was conducted with written informed consent from participants and in accordance with the principles of the Declaration of Helsinki and UK regulatory and ethical requirements. The primary objective was to determine whether A/Wisconsin/67/2005 (H3N2) influenza virus (A/WI) infection, induced by means of intranasal droplet challenge, was transmissible between humans resulting in secondary infections. Secondary objectives were to confirm the safety of transmitted infection, to determine the suitability of the model for future studies of influenza transmission (estimation of secondary attack rate [SAR]), and to assess the deposition of influenza virus in the environment.

Screening
Volunteers were screened to establish antibody susceptibility to the challenge virus before undergoing further study-specific screening against inclusion and exclusion criteria. In brief, volunteers needed to be healthy with no uncontrolled acute or chronic medical condition, be between the ages of 18 and 45, not live with a person at high risk of influenza complications on discharge, and not have had a seasonal influenza vaccine in the

last 3 years (see Supplementary Data for full criteria). Blood samples from volunteers were collected immediately before quarantine entry for repeat antibody testing, although results were not available until after the study. A hemagglutination inhibition (HI) titer of ≤ 10 and/or a microneutralization (MN) titer of <80 were taken to indicate susceptibility to infection.

Study Design and Conduct
Sixty volunteers underwent study-specific screening; 24 were eligible and were randomly allocated to 1 of 2 groups ("donors" or "recipients") before the start of the study. Nine donors entered the quarantine unit on day –2 and were inoculated intranasally with A/WI virus on the morning of day 0. Fifteen recipients entered the quarantine unit on day 0 but were segregated from donors. Volunteers were housed in pairs. On day 2, 36 hours after inoculation, 6 donors (selected on the basis of the highest symptom scores) and 15 recipients were placed into shared accommodation and took part in 3 separate exposure events (EEs), each comprising 2 donors and 5 recipients; for a total period of 30 hours over study days 2 (10.00–24.00) and 3 (09.00–01.00) (Figure 1). Volunteers played games, watched television, and ate meals together (Figure 2). Donors and recipients were separated overnight and at the end of the EE. The EE rooms measured 42.3 m³ (4.6 m × 4.0 m × 2.3 m) in size. Room temperature ranged between 22°C and 26°C, and the humidity ranged between 38% and 53%. Windows were kept closed, but recycling of air (not exchange) by an air conditioner allowed for volunteer comfort.

Follow-up in the quarantine unit continued until day 6 for donors and day 10 for recipients. Recipients who developed symptoms were separated from roommates who did not have symptoms to prevent recipient-recipient transmission. Further follow-up of all volunteers took place at study day 28 ± 3.

Challenge Virus and Rescue Medication
The challenge influenza virus A/WI (produced according to good manufacturing practices) was used in this study. For inoculation, individuals were positioned supine with the chin up while a 0.5-mL solution containing approximately 5.5 $\log_{10}$ median tissue culture infective dose (TCID$_{50}$)/mL of virus was instilled into each nostril.

To minimize the possibility of postexperiment transmission, all volunteers were given a 5-day course of oseltamivir (donors from day 4 and recipients from day 8) and had a negative rapid antigen test before discharge. Analgesics and antipyretics were available at the discretion of the study physician.

Clinical Assessments and Sample Collections
Volunteers recorded symptoms twice daily, and vital signs were monitored 4 times a day; a daily physical examination, electrocardiogram (ECG), and spirometry were also
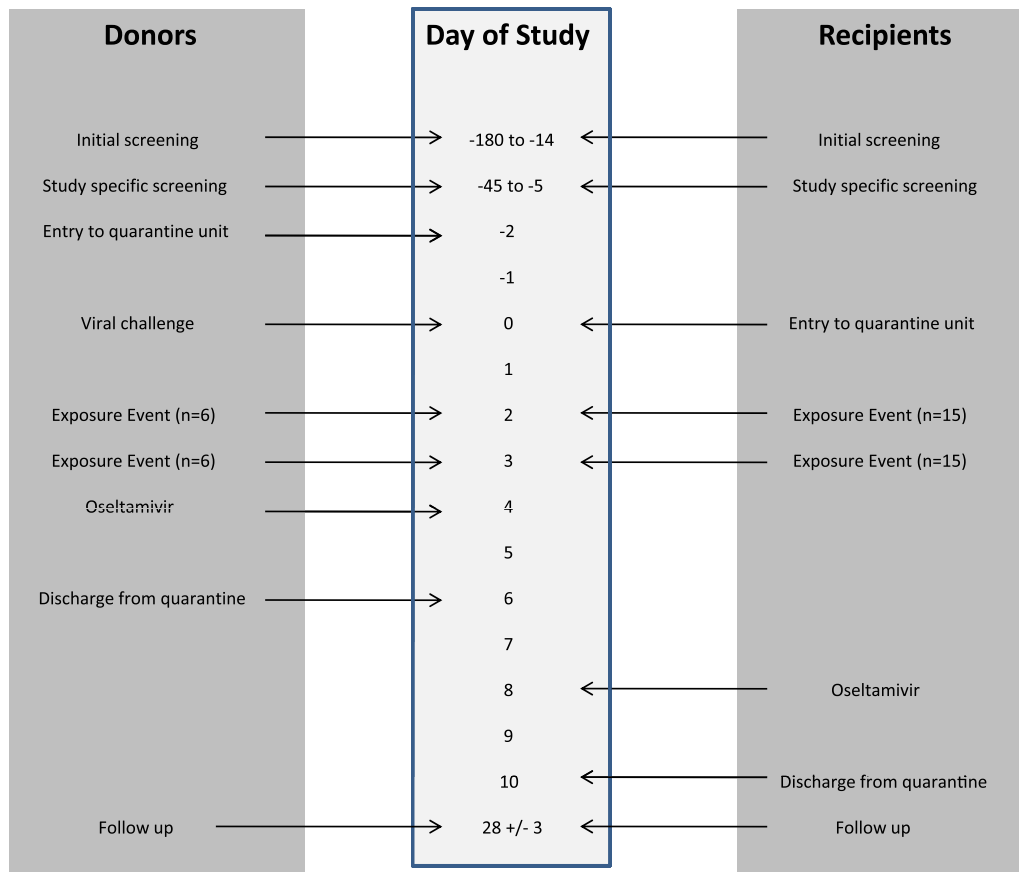
Figure 1. Study timeline.

performed. Venous blood was collected for serology and to examine a panel of safety parameters. Respiratory tract samples were obtained by nasal wash and throat swab and kept on wet ice for transport to the laboratory. Influenza-like illness (ILI) was defined as an illness lasting ≥24 hours with either (1) fever >37.9°C plus at least 1 respiratory symptom or (2) ≥2 symptoms, at least 1 of which must be respiratory.

Environmental Sampling

Swabs were taken from a variety of surfaces and commonly touched objects (eg, computer keyboard, door handle) on study days 2 and 3. Cotton swabs (FB57835; Fisherbrand) were moistened with viral transport medium (VTM) and then rubbed across an area of 2×2 cm^2 in 6 different directions with even pressure applied.

Air particles were collected using a National Institute for Occupational Safety and Health (NIOSH) 2-stage cyclone bio-aerosol sampler, which has been described and validated for use with influenza [14]. The sampler separates air particles into 3 size fractions: >4 μm (stage 1 tube), 1–4 μm (stage 2 tube), and <1 μm (filter paper). Three samplers were used during the

EE on study days 2 and 3: (1) 1 carried by a donor, (2) 1 carried by a recipient, and (3) 1 freestanding in the room at a height of 120 cm. The samplers ran for 3 hours at a flow rate of 3.5 L/minute. Before sampling, 750 μL and 250 μL of VTM were added to stage 1 and 2 tubes, respectively. After sampling, the volume of VTM in both tubes was reconstituted to 750 μL and the filter paper was immersed in a 15-mL tube containing 750 μL of VTM.

Samples were placed on dry ice, transported to the laboratory, and stored at −70°C.

Laboratory Methods

Influenza serology at screening (day −200 to −14), quarantine entry (donors = day −2, recipients = day 0), and day 28 was performed by HI assay (Retroscreen Virology [RVL]). Serology was also performed on quarantine entry and day 28 by HI and MN assays (CDC Laboratories) [15, 16]. Data from quarantine entry were not available until after completion of the quarantine event. Serological results presented are those of the CDC; RVL results were comparable. Culture of nasal wash and throat swab specimens was performed by RVL. Influenza antigen rapid tests
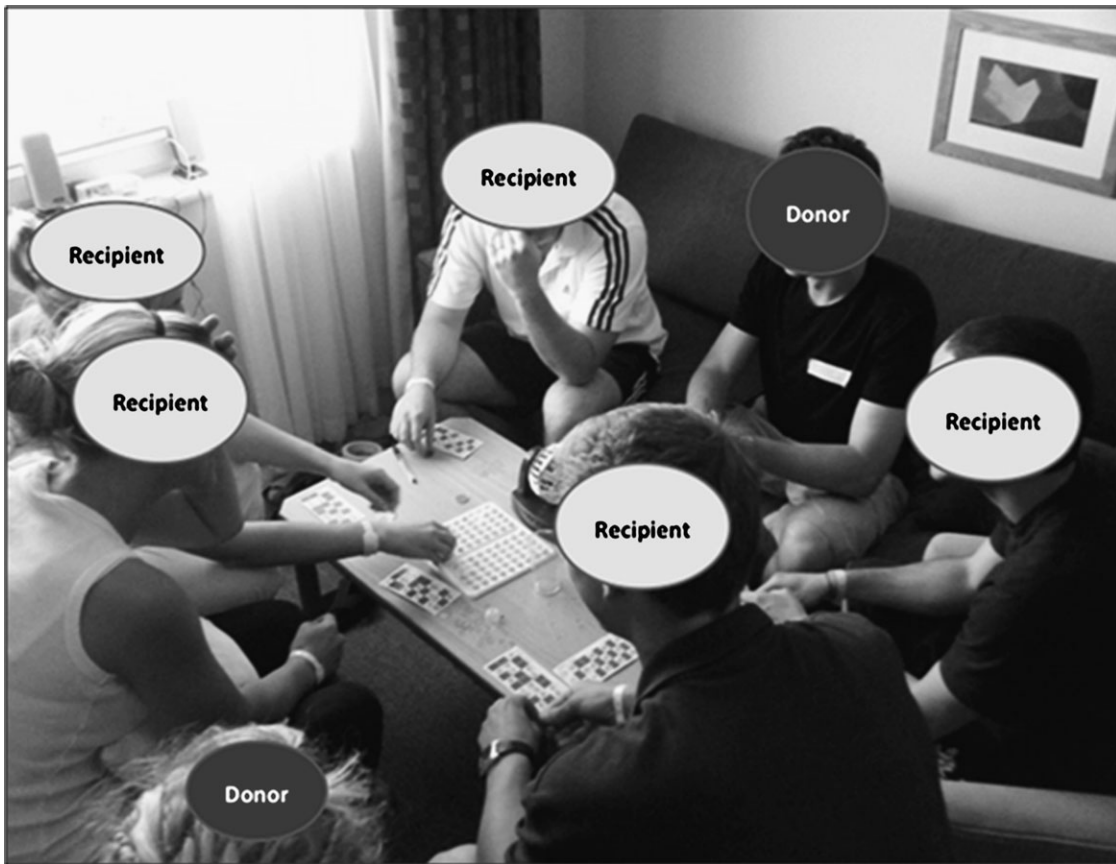
Figure 2. Volunteers interacting (playing bingo) during the exposure event.

were performed on fresh nasal wash specimens using a Quidel QuickVue Influenza A+B test. Polymerase chain reaction (PCR) analysis was performed at Lab 21 Healthcare Laboratories (Cambridge, UK) for influenza A [17] and HPA Laboratories (Addenbrooke's Hospital, Cambridge, UK) for influenza A and respiratory virus panel. (For further detail, see Supplementary Data.)

Outcome Measures

A laboratory-confirmed case was defined on the basis of a ≥4-fold rise in either HI or MN titers between the day −2 or day 0 serum specimen and the day 28 serum specimen, or a positive test by either viral culture or PCR.

Statistical Methods

Sample size was based on the premise of attaining an SAR of ≥25%, defined in advance as the level of SAR that would be consistent with the viability of future transmission studies. Assuming a 25% attack rate and that the infection of each recipient is independent of that of any other recipient (ie, no correlation of infection risk by EE group), using 6 donors and 15 recipients (in 3 groups of 2:5) gives a 76% chance of observing ≥3 cases.

If no cases were detected, the upper 95% confidence bound on the attack rate would be 22%.

RESULTS

Twenty-four volunteers (median age, 27) entered the quarantine unit. The virus inocula used to infect the donors had a geometric mean titer of 5.64 $\log_{10}$ $TCID_{50}$/mL (Supplementary Data).

Exposure Event

On day 2, 3 donors who showed symptoms consistent with ILI (D01, D02, and D03) and 1 donor who had respiratory symptoms but did not fulfill the definition of ILI (D06) were selected for the EE. In addition, 2 further asymptomatic donors were randomly selected to take part (D04 and D08). By day 3, 1 of the 3 reserves (D05) had become symptomatic with ILI; thus, D08 was randomly withdrawn in favor of D05 (Supplementary Data Table 2).

Donors

Seven of 9 donors were found to be infected (78%): 5 reported symptoms, 4 of whom had symptoms consistent with ILI. One

Table 1. Results of Donor Influenza Challenge

Volunteer Donor[a]	Results				Influenza Infection Status
	ILI (Day)	PCR	Culture ($TCID_{50}$/mL)	Serology (Day $-2 \rightarrow 28$)	
D01[B]	2–6	NW4-7	NW3 (3.5)	*HI 5 → 40	**ILI, infected**
				*MN 28 → 640	
D02[C]	2–5	TS3 & NW4-7	NW4 (3.0)	*HI 5 → 20	**ILI, infected**
				*MN 14 → 80	
D03[A]	2, 3, 5	NW3-4	–	*HI 40 → 80	**ILI, infected**
				*MN 160 → >1280	
D04[A]	–	–	–	HI 160 → 160	Immune preexposure
				MN >1280 → >1280	
D05[B]	3–5	TS3 & NW4-7	NW3 and 4 (4.75 and 3.75)	*HI 5 → 80	**ILI, infected**
				*MN 10 → 640	
D06[C]	–	–	–	HI 5 → 5	Symptomatic, not infected
				MN 40 → 80	
D07	–	NW3-5	NW4 (2.0)	HI 5 → 5	**Asymptomatic, infected**
				*MN 10 → 40	
D08[B]	–	NW 5–6	–	HI 5 → 5	**Asymptomatic, infected**
				*MN 20 → 80	
D09	–	TS3 & NW4	–	*HI 10 → 80	**Symptomatic, infected**
				*MN 80 → >1280	

Serology results are presented as geometric mean titers. Positive serological results are defined as a 4-fold rise in titer and are marked with *. Bold formatting indicates volunteers with confirmed influenza infections.

Abbreviations: –, negative; HI, hemagglutination inhibition; ILI, influenza-like illness—days when subject recorded symptoms consistent with ILI; MN, microneutralization; NW, nasal wash; PCR, polymerase chain reaction: numbers refer to day(s) when positive (eg, NW5-6, nasal wash positive day 5 and 6); $TCID_{50}$, median tissue culture infective dose; TS, throat swab.

[a] Donor used in the exposure event. Capital superscripted letters signify exposure event group.

donor (D05) had a recorded fever. Four donors were culture-positive from nasal wash, 7 were PCR-positive on nasal wash (3 were also positive on throat swab), and 7 seroconverted (Table 1 and Supplementary Table 1). One donor (D04) was found (retrospectively) to have high serum antibody titers (HI, 160; MN, 1280) on day −2 and was likely immune.

Recipients

After the EE, 10 recipients reported symptoms, of whom 4 (R04, R07, R08, and R15) had an illness consistent with ILI. Three (20%) recipients (R08, R12, and R15) had laboratory-confirmed influenza; R12 was confirmed serologically, and R08 and R15 were PCR-positive on a nasal wash from day 4 and day 6, respectively (see Table 2 and Supplementary Data Table 1). Three recipients had MN titers ≥80 on day −2 and were considered preimmune.

Attack rates in each of the 3 EE groups (A, B, and C) were 0%, 20%, and 40%, respectively. Excluding preimmune recipients gives attack rates of 0, 25%, and 50% per group and an overall attack rate of 25% (95% confidence interval, 6%–57%) (Supplementary Table 2).

Coinfection

A panel of respiratory virus PCRs performed on recipient nasal washes from days 4 to 7 detected rhinovirus from R15 (day 4)

and R04 (day 6). All donor nasal washes on day 3 were negative for noninfluenza viruses.

Safety Parameters

No adverse events or clinically significant changes in ECGs, spirometry, vital signs, or blood tests occurred. All volunteers completed the first 2 days of a 5-day course of oseltamivir and returned a negative influenza rapid antigen test before discharge. All donors were discharged on day 6 and all recipients were discharged on day 10. All subjects remained well through the day 28 follow-up visit.

Environmental Sampling

Air sampling took place within EE group B on study days 2 and 3 and generated 18 samples. Samplers were carried by D01 and R10, and 1 sampler was freestanding. Only 1 sample was PCR-positive, from day 3 in the 1–4-μm size range. This sample was collected by a device carried by a donor (D01). Culture assays conducted on this and all other samples were negative.

Fomite swabbing was performed in 2 volunteer bedrooms (D01 and D02/D03) and the EE rooms of groups A and B on study days 2 and 3. Forty-eight samples were collected, and 9 (19%) collected from the following fomites were PCR-positive:

Table 2. Results of Recipient Influenza Challenge

Volunteer Recipient[a]	Results				Influenza Infection Status
	ILI (Day)	PCR	Culture	Serology (Day −2 → 28)	
R1[A]	–	–	–	HI 10 → 10 MN 80 → 80	Immune preexposure
R2[A]	–	–	–	HI 10 → 7 MN 57 → 57	Asymptomatic, not infected
R3[A]	–	–	–	HI 5 → 5 MN 40 → 40	Asymptomatic, not infected
R4[A]	5–6	–	–	HI 5 → 5 MN 40 → 40	Asymptomatic, not infected[#]
R5[A]	–	–	–	HI 5 → 5 MN 20 → 10	Asymptomatic, not infected
R6[B]	–	–	–	HI 5 → 5 MN 20 → 14	Asymptomatic, not infected
R7[B]	4–5	–	–	HI 5 → 10 MN 80 → 160	Immune pre-exposure
R8[B]	3	NW4	–	HI 5 → 5 MN 20 → 10	**ILI, infected**
R9[B]	–	–	–	HI 10 → 5 MN 40 → 40	Asymptomatic, not infected
R10[B]	–	–	–	HI 5 → 5 MN 20 → 40	Asymptomatic, not infected
R11[C]	–	–	–	HI 5 → 5 MN 20 → 40	Asymptomatic, not infected
R12[C]	–	–	–	*HI 5 → 20 *MN 40 → 160	**Asymptomatic, infected**
R13[C]	–	–	–	HI 40 → 40 MN 160 → 160	Immune preexposure
R14[C]	–	–	–	HI 5 → 5 MN 20 → 20	Asymptomatic, not infected
R15[C]	3–8	NW6	–	HI 5 → 5 MN 20 → 10	**ILI, infected[#]**

Serology results are presented as geometric mean titers. Positive serological results are defined as a 4-fold rise in titer and are marked with *. Bold formatting indicates volunteers with confirmed influenza infections.

Abbreviations: HI, hemagglutination inhibition; ILI, influenza-like illness—days when subject recorded symptoms consistent with ILI; MN, microneutralization; NW, nasal wash; PCR, polymerase chain reaction: numbers refer to day(s) when positive (eg, NW4, nasal wash positive on day 4).

[a] Capital superscripted letters signify exposure event group.

[#] Rhinovirus detected.

donor bedroom (D01), day 2: ceramic mug and computer touchpad; donor bedroom (D01), day 3: computer touchpad; donor bedroom (D02/03), day 3: bedside table, teaspoon, door handle, ceramic mug, and plastic bottle; exposure room (B), day 3: computer touchpad.

DISCUSSION

We report, for the first time, the successful and safe deployment of a human challenge model to demonstrate transmission of influenza infection from experimentally infected volunteers to other susceptible participants. The overall attack rate was 20%, but taking into account nonsusceptible (immune) recipients, the

adjusted attack rate was 25%. The human challenge model could offer opportunities to study the transmission of influenza and is an alternative to the study of naturally infected patients.

In our study, most susceptible donors (88%) developed infection, which is in keeping with data from previous challenge studies [7]. Five of 7 infected donors (63%) developed symptoms, which is similar to the overall rate (65%) observed in other H3N2 challenge studies, although a lower incidence of fever was observed (13% vs 41%) [7].

Although the majority of recipients experienced symptoms after EE, most were not clinically significant and may relate to the effect of quarantine itself (eg, nasal congestion due to confinement indoors). Three of 4 recipients who had an ILI had
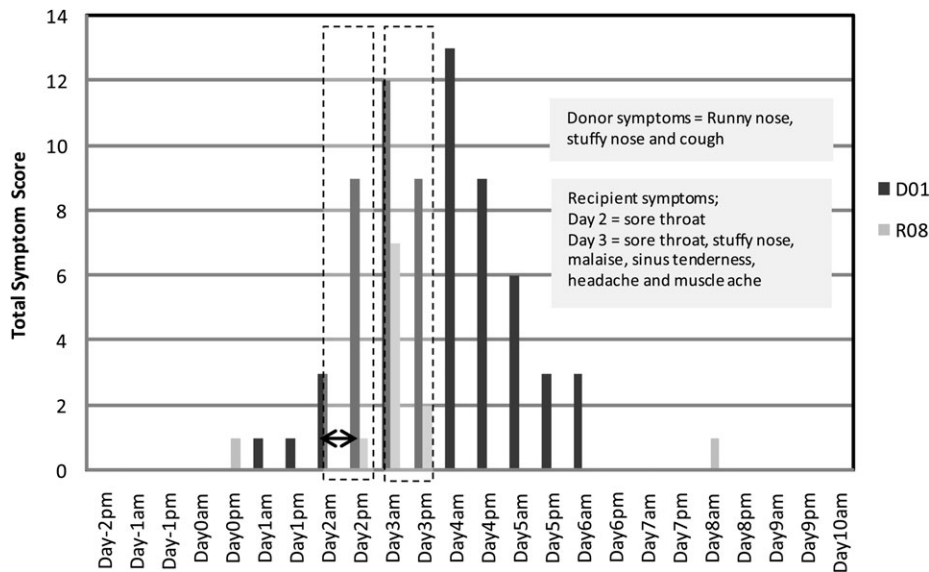
Figure 3. Timelines of symptoms in donor D01 and recipient R08. The arrow indicates the 12 hours it took for illness to appear in R08. The dashed areas represent the exposure event periods. R08's symptom on day 0 was earache and he also reported diarrhea; the volunteer was isolated outside of the quarantine unit until these symptoms had resolved.

virological evidence of infection (2 influenza, 1 rhinovirus). Although we could confirm influenza infection in 3 recipients, we recognize the lack of coherence across diagnostic modalities, particularly the fact that antibody responses to the transmitted infection were not strong. This likely reflects low viral loads and the mild nature of illness seen.

Antibody detection was undertaken by both HI and MN assays. Virus neutralization assays are largely regarded as being a more sensitive method to detect antibody responses to influenza [18]; however, in general, there are no established immune correlates of protection for neutralizing antibody. Virus neutralization assays likely detect a broader range of antibody than does the HI assay, including antibody directed against the stem region of the HA molecule [19, 20]. Whereas an MN titer of 80 indicates some level of preexisting antibody, it is not known whether this represents a threshold for clinical protection. For recipients R01 and R07, we have assumed that it did. However, 2 donors (D09 and D03) who had baseline MN titers of ≥1:80 were clearly infected. It is possible that the total inoculation dose of approximately 5.64 $\log_{10}$ $TCID_{50}$ per donor represents a substantially larger inoculum relative to the doses to which the recipients were exposed, and that this larger amount of virus was able to overcome existing levels of MN antibody.

Based on the times that symptoms began, it appears that R08 caught and incubated infection from D01 within 12 hours. This illness transmission timeline is depicted in Figure 3A. The incubation period in the recipient is more expedient than might have been expected but is consistent with previous data [21]. The finding of rhinovirus on a sample from R15 makes it difficult to interpret a similar timeline for transmission from D02 to R15 because rhinovirus may have been causing the early symptoms.

The detection of rhinovirus (by PCR) in 2 recipient volunteers (1 of whom was also infected with influenza during the EE) was unexpected. Screening by PCR for a panel of respiratory viruses in both volunteers and staff on entry to the quarantine unit may help avoid the occurrence of coinfections in future studies.

Samples taken from the environment around infected volunteers show that virus is deposited by experimentally infected individuals, validating the model for the investigation of the role of contact transmission. Evidence supporting the potential for bioaerosol transmission of influenza infection has been reviewed [22]; corroborative evidence comes from the detection of influenza virus (by PCR) in airborne respirable-sized particles [23, 24, 25] to which this study now adds.

There are inevitable limitations to this proof-of-concept study. First, there is an incomplete dataset for viral shedding because samples were not collected until 3 days after exposure in donors and 2 days in recipients. The primary outcome of the study was to confirm transmission; therefore, we did not want to compromise transmission by performing sampling that may have interfered with the establishment of infection in volunteers. Although there are no data to support such concerns, this was considered a prudent measure for an initial proof-of-concept study. As a result, it is possible that further positive samples may have been missed. Second, the involvement in the quarantine phase of serologically immune volunteers was unforeseen. All

volunteers were negative at initial screening using the HI assay; for practical reasons, this was performed up to 180 days in advance of the study. As a result, volunteers may have developed immunity between screening and quarantine (86–143 days between these time points in the 4 immune volunteers). In addition, the MN assay was not performed at screening. Third, we set an automatic start for the EEs, 44 hours after inoculation of donors irrespective of symptom onset. That decision was based on previous studies with the A/WI (H3N2) challenge virus, which have shown maximal virus shedding on day 2 and maximal symptoms on day 3 (Rob Lambkin-Williams, personal communication). In the present study, maximal symptoms also occurred on day 3, but asymptomatic donors needed to be randomly selected to take part in the EE on day 2 because not enough were showing symptoms at this time. This led to donors taking part who were subsequently found not to be infected. Finally, the fact that the study was conducted in early summer may not have provided optimal conditions for influenza challenge as has been shown in previous studies [26].

Potential exists to adapt the model to achieve a higher SAR in recipients. For example:

- Increase the number of donor volunteers either to ensure that a minimum number with significant symptoms can be used in the EE or to increase the ratio of donors/recipients in an EE
- Confirm infection and select donors with the highest viral loads by using an influenza rapid test and/or PCR before the EE
- Begin the EE on day 1 to allow an opportunity for presymptomatic donor transmission
- Perform more detailed immunological screening to assess susceptibility to infection (eg, MN assay) and confirmation of susceptibility just before study start
- Conduct the study under environmental conditions (ie, temperature and humidity) that are favorable to virus survival and transmission

A major reservation about human challenge studies is whether experimental infection can be used as a surrogate for natural (wild-type) influenza infection. First, symptom severity with challenge studies involving nasal inoculation is generally perceived to be lower than with wild-type infection, which in turn might affect the amount of virus shedding and reduce SARs. However, it is important to note that comparisons are often made with patients who seek medical services; the clinical profiles of community cases (patients who do not seek medical care) are probably more similar to those encountered during challenge studies [27, 28]. There is some evidence that inoculation by inhalation produces more severe symptoms [2], but this currently poses ethical constraints. Second, it has been questioned whether the profile of virus shedding in challenge models is comparable with wild-type infection; recent data on viral shedding collected from community influenza cases in Hong Kong appear similar to those seen in experimental

challenge studies [28]. Finally, questions can be raised about whether the relative contribution of droplet, aerosol, and contact transmission might be different between experimental and wild-type transmission and what impact environmental factors have in the different settings. There is no evidence for or against these issues at present, although any potential differences could be overcome by carefully controlling the exposure conditions in experimental settings to mimic close household contact, as we attempted to do.

CONCLUSIONS

Studying influenza transmission is difficult: seasonality, unpredictable attack rates, numbers of participants required, and confounding variables all present considerable obstacles to studying transmission of wild-type infections. Human influenza challenge studies could offer a promising approach to gain insights into both the mechanisms of influenza transmission and its prevention, so long as a reliable model of transmission can be developed. We demonstrate a successful proof of concept for such an approach and propose considerations for optimization of the model.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Pfizer to conduct (unrelated) research and attend and present at scientific meetings. J. N. V.-T. has received funding to attend influenza-related meetings and ad hoc lectures and received consultancy fees from several influenza antiviral drug and vaccine manufacturers (including Glaxo-SmithKline), ceasing in September 2010; he has performed ad hoc consultancy for Retroscreen Virology Ltd, and his unit is currently in receipt of research funds from GSK, AstraZeneca, and F. Hoffmann-La Roche; he is a former employee of SmithKline Beecham plc (now a part of GSK), Roche Products Ltd, and Sanofi Pasteur MSD, all such appointments prior to 2005. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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