Risk Analysis, Vol. 29, No. 9, 2009 DOI: 10.1111/j.1539-6924.2009.01253.x

Relative Contributions of Four Exposure Pathways to Influenza Infection Risk

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The relative contribution of four influenza virus exposure pathways—(1) virus-contaminated hand contact with facial membranes, (2) inhalation of respirable cough particles, (3) inhalation of inspirable cough particles, and (4) spray of cough droplets onto facial membranes—must be quantified to determine the potential efficacy of nonpharmaceutical interventions of transmission. We used a mathematical model to estimate the relative contributions of the four pathways to infection risk in the context of a person attending a bed-ridden family member ill with influenza. Considering the uncertainties in the sparse human subject influenza dose-response data, we assumed alternative ratios of 3,200:1 and 1:1 for the infectivity of inhaled respirable virus to intranasally instilled virus. For the 3,200:1 ratio, pathways (1), (2), and (4) contribute substantially to influenza risk: at a virus saliva concentration of 10^6 mL^{-1}, pathways (1), (2), (3), and (4) contribute, respectively, 31%, 17%, 0.52%, and 52% of the infection risk. With increasing virus concentrations, pathway (2) increases in importance, while pathway (4) decreases in importance. In contrast, for the 1:1 infectivity ratio, pathway (1) is the most important overall: at a virus saliva concentration of 10^6 mL^{-1}, pathways (1), (2), (3), and (4) contribute, respectively, 93%, 0.037%, 3.3%, and 3.7% of the infection risk. With increasing virus concentrations, pathway (3) increases in importance, while pathway (4) decreases in importance. Given the sparse knowledge concerning influenza dose and infectivity via different exposure pathways, nonpharmaceutical interventions for influenza should simultaneously address potential exposure via hand contact to the face, inhalation, and droplet spray.

KEY WORDS: Influenza; microbial risk assessment; transmission pathway

1. INTRODUCTION

Concern about an influenza pandemic has led to considerations of pharmaceutical and nonpharmaceutical interventions—social distancing, cough etiquette, hand washing, and the use of personal protective equipment (PPE) such as respirators and gloves—among the general public and high-risk subgroups.[1] Recommendations regarding the use of respirators are controversial. Currently, the U.S. Department of Health and Human Services (DHHS) recommends surgical masks only for health-care personnel in close contact with a symptomatic patient and recommends N95 filtering facepiece respirators in the event of pandemic influenza with high transmissibility.[1] However, respiratory protection for the general public in the event of pandemic influenza has been advocated in a *New York Times* editorial,[2] and an Institute of Medicine committee was formed in 2006 to consider appropriate PPE for health-care

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workers.[3] The efficacy of nonpharmaceutical measures, however, depends on the dominant pathway(s) of transmission.

Possible exposure pathways for infection by influenza virus are (1) finger contact to virus-contaminated surfaces (commonly termed "fomites") and subsequent finger contact to the eyes, nostrils, and lips; (2) inhalation of virus carried in airborne cough particles that have aerodynamic diameter, d_a, less than 10 μm (termed respirable particles); (3) inhalation of virus carried in airborne cough particles that have 10 $\mu m < d_a < 100$ μm (termed inspirable, but nonrespirable, particles); and (4) droplet spray, the direct projection of virus carried in cough particles (generally, $d_a > 100$ μm) onto facial target membranes. Respirable particles deposit throughout the respiratory tract, including the alveolar region, whereas inspirable particles deposit in the head airways and tracheobronchial regions only.[4] Unlike pathways (1) and (2), pathways (3) and (4) require "close contact," usually defined as being within 3 ft of the infector.

With regard to nonpharmaceutical interventions, appropriate controls for pathway (1) include frequent hand washing to remove/inactivate virus, periodic cleaning of surfaces with virucidal chemicals, wearing disposable gloves for specific tasks, and decreasing the touch rate to the eyes, nostrils, and lips. Appropriate controls for pathway (2) include wearing respiratory protection (at a minimum, an N95 filtering facepiece respirator), increasing the viral particle removal rate from room air by mechanical dilution ventilation and in-room filtration devices, and possibly inactivating virus by increasing relative humidity or using upper room air ultraviolet irradiation. Other than maintaining physical distance, the only appropriate control for pathway (3) is wearing respiratory protection (at a minimum, an N95 filtering facepiece respirator). Appropriate controls for pathway (4) include wearing a fluid-resistant mask that covers the nose and mouth (e.g., a surgical mask) and wearing eye goggles or a face shield. In general, occupational hygienists do not consider surgical masks to afford adequate respiratory protection, especially against the inhalation of respirable virus particles. Wearing a surgical mask or respirator can be considered a pathway (1) control to the extent that its use reduces touching facial membranes. Recommendations from the DHHS are based on the perception that the dominant modes of influenza transmission are pathways (1) and (4).[1]

Experiments with human subjects have demonstrated influenza infection via inhalation[5–7] and via intranasal instillation.[8,9] However, debate continues about the dominant transmission pathway in natural settings because the available circumstantial evidence is inconclusive. Documentation of secondary influenza only among persons physically inside the infectors' rooms in outbreaks in hospitals and long-term care settings has been interpreted as both evidence *for* hand contact and droplet spray transmission and evidence *against* inhalation transmission.[10] Other outbreaks, such as the one on a grounded airplane in which influenza was transmitted to 71% of passengers and crew having no direct contact with the infector (who "lay across two seats coughing, too ill to move about"), suggest exposure by respirable particle inhalation.[11]

That receptors for human-origin influenza A are located throughout the respiratory tract (from the nasal mucosa to the bronchioles) suggests that all exposure pathways may be relevant.[12,13] However, the apparent absence of receptors in oral and ocular tissues implies that virus deposited in these regions must be transported to nasopharyngeal tissues to initiate infection. Receptors for avian-origin influenza A are localized in the terminal bronchioles, alveoli, corneal and ocular conjunctive tissue, and occasionally in the nasal mucosa; these locations suggest that respirable particle inhalation may be the more important pathway.[13,14] Concordance of the receptor locations with viral binding sites has been confirmed in human bronchiole and alveolar tissues.[13,15]

The goal of our theoretical analysis was to examine the competing ideas that influenza transmission involves a single dominant exposure pathway versus two or more significant exposure pathways. Our method was to estimate the relative contribution to infection risk of each of the four exposure pathways across a broad range of viral concentration in an infector's emitted cough aerosol fluid and for two very different assumptions about the infectivity of a virus dose when delivered by alternative exposure routes. For our analysis, we posed the scenario of a susceptible person attending a bed-ridden family member ill with influenza in a residential bedroom. We consider this to be a common scenario and analogous to hospital venues in which healthcare workers attend influenza patients. On the other hand, our stationary infector/residential scenario is not meant to capture the interactions of a mobile infector with susceptible persons in community settings such as a classroom, auditorium, or shopping mall. We used a mathematical model[16] to estimate the viral doses to the lower and upper respiratory tract during a short (15 minutes) visit to the infector's room.

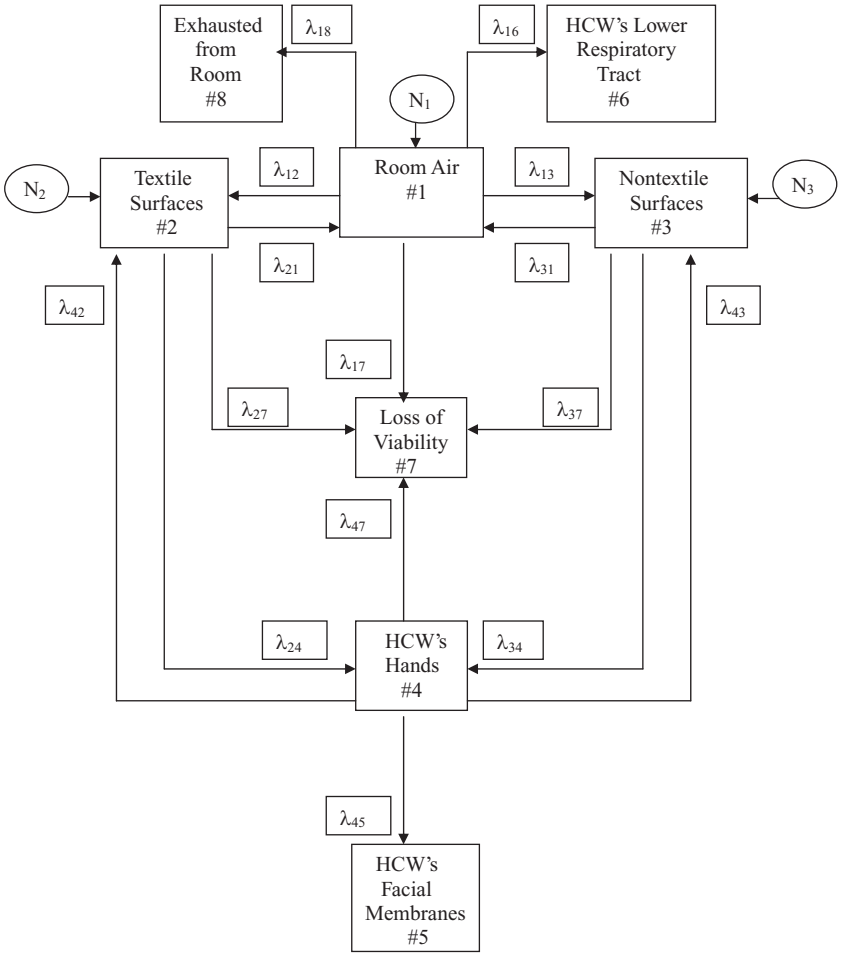

Fig. 1. The exposure pathway model considers eight physical elements in the residential bedroom. Virus moves between the states according to rate constants λ_{ij} in the direction of the arrows. N_i indicates the initial virus load in the environment resulting from virus-laden cough particles emitted by a bed-ridden infector prior to the start of exposure.

Considering the uncertainties in the sparse human subject influenza dose-response data and in our estimates of virus infectivity, we assumed alternative ratios of 3,200:1 and 1:1 for the infectivity of virus deposited in the lower respiratory tract versus the upper respiratory tract. Given the 3,200:1 ratio, we conclude that hand contact, respirable virus inhalation, and droplet spray could all contribute substantially to transmission. However, given the 1:1 ratio, we conclude that hand contact would be the dominant transmission pathway, with some contribution from droplet spray and respirable virus inhalation.

2. METHODS

2.1. The Exposure Pathway Model

A Markov chain was used to describe the movement of virus between select physical elements (states) in a residential bedroom with a bed-ridden infector (Fig. 1). The states represent: (1) room air, (2) textile surfaces near the infector, (3) nontextile surfaces near the infector, (4) the attender's hands, (5) the attender's facial membranes, (6) the attender's lower respiratory tract, (7) virus rendered noninfectious by environmental degradation, and (8) room exhaust airflow. Virus can exchange between state 1 and states 2 and 3 because of particle settling from air and resuspension into air. Virus can exchange between state 4 and states 2 and 3 via hand contact with surfaces. Virus transferred to states 5, 6, and 8 cannot leave these states. Virus transfers from states 1–4 to state 7 when infectivity is lost.

Virus is emitted into room air in cough particles. When the attender first enters the room, there is an initial load of virus in the air (N_1), on textile surfaces (N_2), and on nontextile surfaces (N_3). These N_i values are equated with their respective theoretical steady-state loads, which depend on the specified rate of coughing, the virus concentration in saliva, the particle size count distribution per cough, and the loss rate from each state. The quantity N_1

corresponds to virus carried in respirable particles; these small particles settle slowly and disperse throughout room air.[4] The quantities N_2 and N_3 correspond predominantly to virus carried in inspirable particles ($10\ \mu m < d_a < 100\ \mu m$) and noninspirable particles ($d_a > 100\ \mu m$); these large particles deposit relatively rapidly onto surfaces near the emission point.[4]

The first-order rates of transfer between states are denoted λ_{ij} (min^{-1}), where ij signifies movement from state i to state j. For simulation, the exponential probabilities of an infective virus moving from state i to state j in the time step $\Delta t = 1 \times 10^{-4}$ minutes were computed using the λ_{ij}, and entered into the (i,j) cell of an 8×8 matrix **P**.[16] After n time steps, the probability that an infective virus initially in state $i = \{1, 2, 3\}$ was in state $j = \{5, 6\}$ is the (i,j) entry of the matrix **P** multiplied by itself n times, designated $P^{(n)}{}_{ij}$. The expected dose of infective virus to the facial target membranes via hand contact, $E[D_5]$, and to the respiratory tract via respirable particle inhalation, $E[D_6]$, because of the initial virus loads are computed as follows:

$$E[D_5] = N_1 P_{15}^{(n)} + N_2 P_{25}^{(n)} + N_3 P_{35}^{(n)} \text{ and} \qquad (1)$$

$$E[D_6] = N_1 P_{16}^{(n)} + N_2 P_{26}^{(n)} + N_3 P_{36}^{(n)}. \qquad (2)$$

The relatively high frequency of hand contact and respirable particle inhalation enables these exposure events to be treated as continuous via the model. The relatively low frequency of close-contact droplet spray and inspirable particle inhalation events, however, are better treated as episodic. For these pathways, the unconditional infection risk because of an event is the product of the infection risk conditioned on event occurrence and the probability of event occurrence.[16] The details of assigning the N_i and λ_{ij} values are developed in the context of our scenario. The overall infection risk combines the risks from the four pathways by an inclusion-exclusion method.

2.2. Dose-Response Assessment

We used a model that assumes that a single virus can infect the host with probability α. For a noninteger expected dose, $E[D]$, the dose-response function is:[17]

$$R = 1 - \exp(-\alpha \times E[D]). \qquad (3)$$

Table I. Occurrence of Fever ≥37.5°C in Adult Male Volunteers Subsequent to Nasopharyngeal Inoculation of Influenza A Strains Passaged in Eggs (E) and/or Tissue Culture (TC), with Subscripts Denoting the Number of Passages (Alford[8])

Virus Strain	Passage History	Dose ($TCID_{50}$)	No. Exposed	No. with Fever
A2/Japan/305/37	E_3	158	1	0
	TC_3	790	1	1
	TC_{18}	790	4	1
A2/Japan/170/62	E_4	1,600	3	0
A2/Japan/305/57	TC_{18}	1,975	1	1
A2/Japan/305/57	TC_{21}	10,000	2	2
	TC_3	10,000	1	1
A2/Japan/170/62	E_3, TC_1	10,000	9	0
A2/Bethesda/10/63	TC_4	80,000	10	10

The sparse human data for influenza A infectivity preclude the use of two-parameter models that account for variable host susceptibility. Equation (3), however, is consistent with observed dose-infection response data for a variety of pathogenic viruses.[17] The ID_{50} (the virus dose that successfully infects 50% of individuals receiving it) is related to α by Equation (3): $\alpha = \ln(2) \div ID_{50}$, where $ID_{50} \geq \ln(2)$.

Equation (3) was fit to observed infection response data using the method of maximum likelihood. The 95% confidence interval (CI) for the parameter α was estimated using a nonparametric percentile bootstrap with 100,000 samples. Model fit was assessed using a permutation test with 200,000 permutations of the Boolean responses, where the null hypothesis is that the observed response is not a function of dose, and the alternative hypothesis is the dose-response function.[18] The p-value is the proportion of permutations that yield a log-likelihood larger than that observed with the original data. Statistical significance at level 0.05 is defined as $p > 0.95$.

We used data for dose-response assessment when they met three criteria: (1) doses were quantified, (2) at least three doses were used, and (3) at least three different response proportions were observed. One human study of influenza A nasopharyngeal infectivity met the data criteria—fever subsequent to intranasal instillation of virus from one of three strains onto the naso- and oropharynx was reported (Table I).[8] Preexposure serum antibody titers were not reported. One human study of influenza A aerosol infectivity met the data criteria—clinical influenza subsequent to inhaling A2/Bethesda/10/63 in particles with $1\ \mu m < d_a < 3\ \mu m$ was reported (Table II).[5]

Table II. Occurrence of Typical Clinical Influenza and Antibody Titers in Human Volunteers Subsequent to the Inhalation of Influenza A2/Bethesda/10/63 in a Small-Particle Aerosol (Alford *et al.*[5])

Inhaled Virus ($TCID_{50}$)	Volunteer No.	Antibody Titer		Clinical Influenza
		Before	After	
1	10	<5	80	Yes
2	11	<5	<5	No
2	12	<5	<5	No
2	13	<5	<5	No
2	14	<5	320	Yes
5	20	5	5,120	Yes
5	21	<5	<5	No
5	22	<5	640	Yes
5	23	<5	<5	No

We included only subjects with preexposure serum antibody titers ≤ 5, because higher levels of serum antibody are indicative of immunity. We used the intranasal instillation study to estimate the dose-response parameter for exposure to the head airways and mucous membranes, α_{URT} (which we loosely term the upper respiratory tract). We used the respirable particle inhalation study to estimate the dose-response parameter for exposure to the respiratory tract distal to the head airways, α_{LRT} (which we loosely term the lower respiratory tract).

2.3. A Common Scenario

The scenario involves a susceptible person (the attender) who cares for a bed-ridden family member (the infector) ill with influenza A in a residential bedroom. The room has volume $V = 64$ m^3 (15 ft $\times$ 15 ft $\times$ 10 ft) and receives 0.5 air change per hour ($\lambda_{18} = 8.3 \times 10^{-3}$ min^{-1}); the latter is the median air exchange rate of U.S. homes.[19] The assigned surface area immediately near the infector is 10^4 cm^2, 90% of which is textile (e.g., bedding) and 10% of which is nontextile (e.g., bed stand). The attender visits the infector for 15 minutes.

2.3.1. Virus Emission Rates

Virus-containing particles are emitted by the infector in coughs that occur 12 times per hour, the approximate 40th percentile of cough rates seen in pneumonia patients.[20] The number and size distribution of cough particles adhere to values presented in a previous analysis, and an estimated 0.044 mL of fluid (saliva) is emitted per cough.[21] The infective virus concentration in saliva, C_{saliva} (mL^{-1}), likely results from drainage from the nasal passages. Peak influenza A virus concentrations in nasal washes among a small panel of subjects were found to range from 6×10^2 $TCID_{50}$ mL^{-1} to 2×10^7 $TCID_{50}$ mL^{-1}.[22] The $TCID_{50}$ is the virus dose that successfully infects 50% of the tissue culture assays receiving it. We consider C_{saliva} in the broad range of 10^4–10^8 mL^{-1}. Thus, the assumed virus emission rate is: $E = (0.2\ \text{min}^{-1}) \times (0.44\ \text{mL}) \times (C_{saliva}\ \text{mL}^{-1})$ virus min^{-1}. More than 99.9% of the emitted virus are in nonrespirable particles, which settle within several feet of the infector. Because the infector is bed-ridden, approximately 100% of the emitted virus settles on surfaces near the bed. On the basis of the relative area of textile and nontextile surfaces, we assume that 90% of the emitted virus settles on the infector's bed linens and clothing (state 2) and 10% settles onto nontextile surfaces near the bed (state 3). We assume that only 1×10^{-4}% of the virus emitted in a cough is associated with respirable particles that disperse in room air (state 1).

2.3.2. Virus Inactivation Rates

Graphically presented data for influenza A Brazil/11/78-like (H1N1) suggests inactivation rates $\lambda_{27} = 1.6 \times 10^{-2}$ min^{-1} (for virus on pajamas) and $\lambda_{37} = 2.0 \times 10^{-3}$ min^{-1} (for virus on stainless steel).[23] Tabled data for influenza A virus transferred to the fingers from paper tissues (a surrogate textile surface) and from stainless steel (a nontextile surface) indicate inactivation rates on the hands of, respectively, 0.92 min^{-1} and 1.47 min^{-1};[23] we assumed an average value such that $\lambda_{47} = 1.2$ min^{-1}. We note that the latter inactivation rate is approximately 50-fold the inactivation rate reported for rotavirus on the hands[24] and about 670-fold the inactivation rate reported for hepatitis A virus on the hands.[25] In 15–40% relative humidity, the inactivation rate for aerosolized influenza A strain PR-8 virus is $\lambda_{17} = 7.3 \times 10^{-3}$ min^{-1}.[26]

2.3.3. Initial Virus Loads

Prior to the attender entering the room, the number of infective virus in each of states 1–3 corresponds to its theoretical steady-state values, equal to the rate of virus delivery to the state divided by the sum of the first-order loss rates from the state. Given a quiescent infector, the pertinent loss mechanisms from state 1 (air) are inactivation due to environmental stress, exhaust airflow, and deposition of respirable particles. For simplicity, the deposition rate of respirable particles was

calculated using the terminal settling velocity of particles with $d_a = 3$ μm, for which $\lambda_{12} = 0.0049$ min^{-1} and $\lambda_{13} = 0.00054$ min^{-1}. These rate constants overestimate deposition onto surfaces near the infector *per se*, but the contribution to N_2 and N_3 of virus associated with respirable particles is negligible. The only loss mechanism from states 2 and 3 (surfaces) is inactivation. The initial infective virus loads are $N_1 = (1.0 \times 10^{-6} \times \mathrm{E}) \div (\lambda_{12} + \lambda_{13} + \lambda_{17} + \lambda_{18})$, $N_2 = (0.9 \times \mathrm{E}) \div \lambda_{27}$, and $N_3 = (0.1 \times \mathrm{E}) \div \lambda_{37}$.

2.3.4. Virus Transfer Rates

Published data exhibit substantial variability in the percentage of virus that can be transferred from a surface to a fingertip during a touch. Tabled data for influenza A virus transferred to the fingers from paper tissues and from stainless steel suggest that the virus transfer efficiency per touch is, respectively, 0.25% and 7.9%.[23] This transfer efficiency is in line with that measured with bacteriophages and other viruses. For example, the transfer of bacteriophage PRD-1 from a seeded dish cloth or faucet handle to fingers had efficiencies of 0.03% and 33%, respectively.[27] Fifteen minutes after seeding bacteriophage ϕX174 onto a door handle, 0.4% of the phage transferred to the palm during a touch. [28] Sixty minutes subsequent to the seeding of hepatitis A virus onto a metal disk, 15% of virus transferred to a finger pad during a touch.[25] However, in the same context, only 1.8% of rotavirus transferred to a finger pad during a touch.[24] And 30–39 minutes subsequent to seeding rhinovirus onto a metal disk, 0.58% of the virus transferred to a finger pad during a touch.[29]

For this scenario, we relied on the transfer efficiencies reported for influenza A virus. We assumed that upon room entry, the attender touches contaminated textile surfaces once a minute and contaminated nontextile surfaces once every two minutes with the five fingertips (10 cm^2) of one hand. Thus, the assigned transfer rate from textiles to the hand is $\lambda_{24} = (10\ \mathrm{cm}^2 \div 9{,}000\ \mathrm{cm}^2) \times (1\ \mathrm{min}^{-1}) \times 0.0025 = 2.8 \times 10^{-6}$ min^{-1}. The transfer rate from a nonporous surface to a fingertip is $\lambda_{34} = (10\ \mathrm{cm}^2 \div 1{,}000\ \mathrm{cm}^2) \times (0.5\ \mathrm{min}^{-1}) \times 0.079 = 4 \times 10^{-4}$ min^{-1}. For the absent data on the transfer efficiency of influenza A virus from the fingertips to textile and nontextile surfaces, we assumed these values were 0.25% and 7.9%, respectively, or the same as the surface-to-finger transfer efficiencies. Therefore, the assigned transfer rate from the fingertips to textile surfaces is $\lambda_{42} = (1\ \mathrm{min}^{-1}) \times 0.0025 = 2.5 \times 10^{-3}$ min^{-1}, and the transfer rate from the fingertips to nontextile surfaces is $\lambda_{43} = (0.5\ \mathrm{min}^{-1}) \times 0.079 = 0.04$ min^{-1}.

The combined rate of nose-picking and eye-rubbing episodes has been reported to be 0.70 h^{-1}.[30] However, in 30 person-hours of observation, we found the mean rate of all finger contacts with the eyes, nostrils, and lips to be 15 h^{-1}.[31] Here, we posit the hand contact rate with facial target membranes to be 0.083 min^{-1} (5 h^{-1}). We assume that a touch involves one fingertip on the same hand that contacts room surfaces, so one-fifth of the contaminated fingertip surface area contacts a facial target membrane per touch. The transfer efficiency of virus from a fingertip to the lips is 35% per touch.[27] We assume that this same transfer percentage applies to the eyes and nostrils. Thus, the transfer rate from the hands to the attender's facial target membranes is $\lambda_{45} = (2\ \mathrm{cm}^2 \div 10\ \mathrm{cm}^2) \times (0.083\ \mathrm{min}^{-1}) \times 0.35 = 5.8 \times 10^{-3}$ min^{-1}. The attender inhales at the rate of 0.020 m^3 min^{-1}, as does an individual performing light work,[32] for which $\lambda_{16} = (0.020\ \mathrm{m}^3\ \mathrm{min}^{-1}) \div (64\ \mathrm{m}^3) = 3.1 \times 10^{-4}$ min^{-1}. Pathogens emitted in coughs are associated with aqueous particles containing salts and proteins,[21] which makes it likely that particle residues will adhere to surfaces and not be resuspended by casual contact; thus, we assume that $\lambda_{21} = \lambda_{31} = 0$.

2.3.5. Respirable Particle Inhalation and Hand Exposure

At this point, all the λ_{ij} values have been assigned and are summarized in Table III. Given the transition probability matrix, **P**, and $n = 150{,}000$ time steps (equal to 15 minutes), Equation (1) gives the expected dose to facial target membranes via hand contact, $\mathrm{E}[D_5]$, and Equation (2) gives the expected dose to the lower respiratory tract via respirable particle inhalation, $\mathrm{E}[D_6]$, if there was no additional coughing during the room visit. The infection risk for the hand contact pathway would be $R_{\mathrm{hands}} = 1 - \exp(-\alpha_{\mathrm{URT}} \times \mathrm{E}[D_5])$. The infection risk for the respirable particle inhalation pathway would be $R_{\mathrm{respirable}} = 1 - \exp(-\alpha_{\mathrm{LRT}} \times \mathrm{E}[D_6])$.

However, given a cough rate of 12 h^{-1}, the infector is expected to cough three times during the room visit, at 3.75, 7.5, and 11.25 minutes after room entry. The virus emission from these coughs was incorporated into the model as follows. The theoretical steady-state N_i values were assumed at time zero (room entry). The model was run for 3.75 minutes ($n = 37{,}500$), and $\mathrm{E}[D_5]_0$ and $\mathrm{E}[D_6]_0$ values were computed using Equations (1) and (2), where

Table III. Model Inputs for the Representative Scenario Involving an Attender Visit to an Infector's Room

State 1:	$\lambda_{14} = \lambda_{15} = 0, \lambda_{12} = 4.9 \times 10^{-3}$ min^{-1}, $\lambda_{13} = 5.4 \times 10^{-4}$ min^{-1}, $\lambda_{16} = 3.1 \times 10^{-4}$ min^{-1}, $\lambda_{17} = 7.3 \times 10^{-3}$ min^{-1}, $\lambda_{18} = 8.3 \times 10^{-3}$ min^{-1}
State 2:	$\lambda_{21} = \lambda_{23} = \lambda_{25} = \lambda_{26} = \lambda_{28} = 0, \lambda_{24} = 1.1 \times 10^{-6}$ min^{-1}, $\lambda_{27} = 1.6 \times 10^{-2}$ min^{-1}
State 3:	$\lambda_{31} = \lambda_{32} = \lambda_{35} = \lambda_{36} = \lambda_{38} = 0, \lambda_{34} = 2.5 \times 10^{-5}$ min^{-1}, $\lambda_{37} = 2.0 \times 10^{-3}$ min^{-1}
State 4:	$\lambda_{41} = \lambda_{46} = \lambda_{48} = 0, \lambda_{42} = 1 \times 10^{-3}$ min^{-1}, $\lambda_{43} = 2.5 \times 10^{-3}$ min^{-1}, $\lambda_{45} = 5.8 \times 10^{-3}$ min^{-1}, $\lambda_{47} = 9.3 \times 10^{-2}$ min^{-1}
State 5:	$\lambda_{51} = \lambda_{52} = \lambda_{53} = \lambda_{54} = \lambda_{56} = \lambda_{57} = \lambda_{58} = 0$
State 6:	$\lambda_{61} = \lambda_{62} = \lambda_{63} = \lambda_{64} = \lambda_{65} = \lambda_{67} = \lambda_{68} = 0$
State 7:	$\lambda_{71} = \lambda_{72} = \lambda_{73} = \lambda_{74} = \lambda_{75} = \lambda_{76} = \lambda_{78} = 0$
State 8:	$\lambda_{81} = \lambda_{82} = \lambda_{83} = \lambda_{84} = \lambda_{85} = \lambda_{86} = \lambda_{87} = 0$
Initial pathogen loads:	$N_1 = 4.8 \times 10^{-5} \times \mathrm{E}, N_2 = 56 \times \mathrm{E}, N_3 = 50 \times \mathrm{E}$

The λ_{ij} are the rates of virus transfer from state i to state j and have units min^{-1}; their estimation is defined in the text. The N_i are the initial number of virus in state i at the time the attender enters the room.

the subscript indicates the cough number. At time 3.75 minutes, a cough occurred and the infectious virus emitted (0.44 mL saliva $\times$ C_{saliva} mL^{-1}) were apportioned to state 1 (1×10^{-4}%), state 2 (90%), and state 3 (10%) and added to the virus remaining in these states from the initial virus load, giving new values for the N_i. The model was run for 3.75 minutes, when new $\mathrm{E}[D_5]_1$ and $\mathrm{E}[D_6]_1$ values were computed using Equations (1) and (2). This procedure was repeated for each remaining cough, giving $\mathrm{E}[D_5]_2$, $\mathrm{E}[D_6]_2$, $\mathrm{E}[D_5]_3$, and $\mathrm{E}[D_6]_3$. The total dose is the sum of the doses resulting from all the coughs: $\mathrm{E}[D_5] = \mathrm{E}[D_5]_0 + \mathrm{E}[D_5]_1 + \mathrm{E}[D_5]_2 + \mathrm{E}[D_5]_3$, and $\mathrm{E}[D_6] = \mathrm{E}[D_6]_0 + \mathrm{E}[D_6]_1 + \mathrm{E}[D_6]_2 + \mathrm{E}[D_6]_3$.

2.3.6. Droplet Spray Exposure

To estimate droplet spray exposure, we assume that the attender faces the infector at 0.6 m during a cough and that emitted particles with $d_a > 100$ μm travel at least 0.6 m while spreading as a three-dimensional cone with a 60° angle, as measured in the plane.[16] Thus, at 0.6 m from the infector, the attender's face is within a circle of particle spread with surface area 0.38 m^2. The projected surface area of the membranes of the eyes, nostrils, and lips is approximately 15 cm^2. If a cough particle can randomly strike any position in the circle, the probability that it strikes a membrane target is 3.9×10^{-3}.

The conditional infection risk because of droplet spray from one cough, $R_{1,\text{spray}}$, is the product of the probability that, given close contact, one or more particles of different sizes (carrying different numbers of virus) contact a target membrane and cause infection.[16] The numerical method for estimating this probability has been described elsewhere.[16] The unconditional infection risk is the product of the conditional risk and the probability that the attender is in close contact at the time the infector coughs. We assume that the latter probability is low, say, 0.05. The infection risk resulting from the three coughs expected during the attender's 15-minute visit is $R_{\text{spray}} = 1 - [1 - (0.05 \times R_{1,\text{spray}})]^3$.

2.3.7. Inspirable Particle Exposure

The method for estimating the inhaled dose of inspirable particles, $D_{1,\text{inspirable}}$, conditioned on the attender facing the infector at 0.6 m when the infector coughs, has been described elsewhere and is based on the volume of the three-dimensional cone of emission, the carriage of 0.36% of emitted virus in inspirable particles, and the inhalation of only 50% of the inspirable particles contained in the volume of one breath.[16,21] If the attender takes one breath after the cough before moving away, the infection risk because of one cough is $R_{1,\text{inspirable}} = 1 - \exp(-\alpha_{\text{head}} \times D_{1,\text{inspirable}})$. Given the 0.05 probability of the attender facing the infector during a cough, and given three coughs during the room visit, the infection risk because of inspirable virus inhalation is $R_{\text{inspirable}} = 1 - [1 - (0.05 \times R_{1,\text{inspirable}})]^3$.

2.3.8. Overall Infection Risk

The overall infection risk, R, is computed by an inclusion-exclusion method:

$$\begin{aligned} R = {} & R_{\text{hands}} + R_{\text{respirable}} + R_{\text{spray}} + R_{\text{inspirable}} \\ & - R_{\text{hands}} R_{\text{respirable}} - R_{\text{hands}} R_{\text{spray}} - R_{\text{hands}} R_{\text{inspirable}} \\ & - R_{\text{respirable}} R_{\text{spray}} - R_{\text{respirable}} R_{\text{inspirable}} \\ & - R_{\text{spray}} R_{\text{inspirable}} + R_{\text{hands}} R_{\text{respirable}} R_{\text{spray}} \\ & + R_{\text{hands}} R_{\text{respirable}} R_{\text{inspirable}} \\ & + R_{\text{hands}} R_{\text{spray}} R_{\text{inspirable}} + R_{\text{respirable}} R_{\text{spray}} R_{\text{inspirable}} \\ & - R_{\text{hands}} R_{\text{respirable}} R_{\text{spray}} R_{\text{inspirable}}. \end{aligned}$$

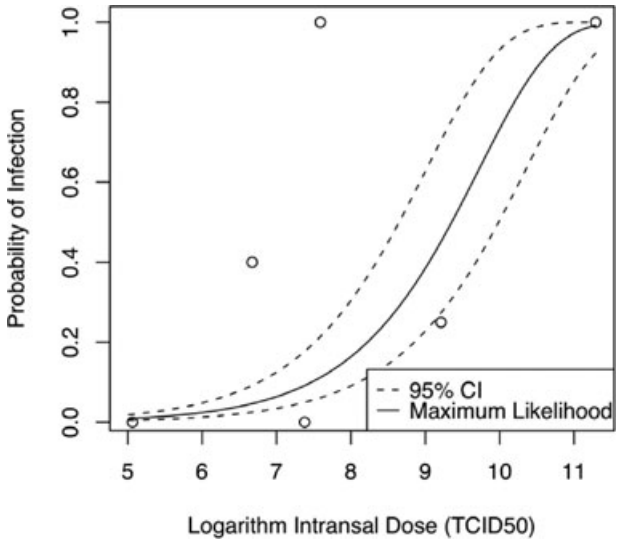

Fig. 2. Infectivity of influenza A virus strains in humans exposed to virus by inoculation onto the naso- and oropharynx. The proportion of subjects infected observed by the original investigators are indicated by circles.[5] The maximum likelihood dose-response function with $\alpha_{URT} = 5.7 \times 10^{-5}$ is indicated by the solid line, and the 95% CI (3.2×10^{-5}–1.2×10^{-4}) is indicated by dashed lines.

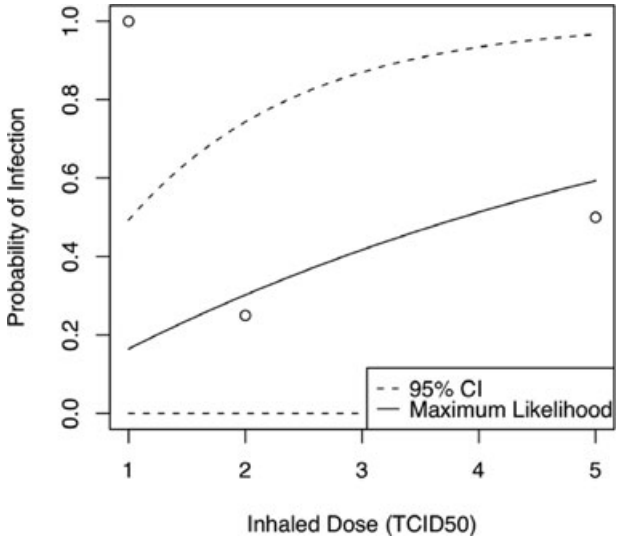

Fig. 3. Infectivity of influenza A2/Bethesda/10/63 virus aerosol in humans. The proportion of subjects infected observed by the original investigators are indicated by circles.[8] The maximum likelihood dose-response function with $\alpha_{LRT} = 0.18$ is indicated by the solid line, and the 95% CI (0–0.68) is indicated by dashed lines.

3. RESULTS

Analysis of the dose-response data for human subjects exposed to influenza A virus by intranasal instillation (Table I) found the log-likelihood function to be maximized at −8.94 by the parameter value $\alpha = 5.7 \times 10^{-5}$ (95% CI: 3.2×10^{-5}–1.2×10^{-4}). Thus, for deposition in the upper respiratory tract (strictly, the oro- and nasopharyngeal region), $\alpha_{URT} = 5.7 \times 10^{-5}$ for doses reported in $TCID_{50}$; the corresponding $ID_{50} = 12{,}000$ $TCID_{50}$. However, the permutation test p-value was only 0.002, whereas the criterion for statistical significance at level 0.05 for the permutation test is $p > 0.95$. Thus, we cannot reject the null hypothesis that infection response is unrelated to dose, a circumstance consistent with the poor fit evident in Fig. 2.

Analysis of the dose-response data for human subjects exposed by inhalation of influenza A2/Bethesda/10/63 in respirable particles (Table II) found the log-likelihood function to be maximized at −3.74 by the parameter value $\alpha = 0.18$ (95% CI: 0, –0.68) (Fig. 3). Thus, for inhaled respirable particles, many of which deposit distal to the head airways region (which we loosely term the lower respiratory tract), $\alpha_{LRT} = 0.18$ for doses reported in $TCID_{50}$; the corresponding $ID_{50} = 3.7$ $TCID_{50}$. However, the permutation test p-value was only 0.42. Thus, we cannot reject the null hypothesis that infection response is unrelated to dose, a circumstance consistent with the poor fit evident in Fig. 3.

Although the fit of the exponential model (Equation (3)) to the data did not attain statistical significance in either study, we reject as biologically implausible the notion that infection risk is not a monotonically increasing function of dose. Because the exponential model is the simplest dose-infection response model, we rely on it for this analysis but acknowledge the great uncertainty in the estimates $\alpha_{LRT} = 0.18$ and $\alpha_{URT} = 5.7 \times 10^{-5}$, for which the ratio $\alpha_{LRT} : \alpha_{URT} = 3{,}200{:}1$. In part to account for model uncertainty, we also consider $\alpha_{URT} = 0.18$ such that the ratio $\alpha_{LRT}{:}\alpha_{URT} = 1{:}1$. Support for the use of the ratio $\alpha_{LRT}{:}\alpha_{URT} = 1{:}1$ is drawn from our analysis of data from a nasal instillation study of A/Panama/2007/99 (H3N2) in guinea pigs,[33] which yielded a parameter estimate $\alpha_{URT} = 0.16$ and was similar to our estimate of $\alpha_{LRT} = 0.18$ for human subjects. In addition, inherent strain differences and varied laboratory culture histories of the challenge viruses in the studies could explain the different infection responses for the two routes of exposure. Finally, preexposure antibody titers of the subjects in the human intranasal instillation study are unknown, and antibody titers are inversely related to the probability of developing clinical influenza.

Pathway	Percent Contribution for C_{saliva}				
	10^4 mL^{-1}	10^5 mL^{-1}	10^6 mL^{-1}	10^7 mL^{-1}	10^8 mL^{-1}
$\alpha_{LRT} : \alpha_{URT} = 3{,}200 : 1$					
(1) Hand contact	27	27	31	45	58
(2) Respirable particles	14	14	17	24	31
(3) Inspirable particles	0.45	0.45	0.52	0.74	0.99
(4) Droplet spray	58	58	52	30	11
Overall risk (R)	2.0×10^{-5}	2.0×10^{-4}	1.7×10^{-3}	1.1×10^{-2}	7.2×10^{-2}
$\alpha_{LRT} : \alpha_{URT} = 1 : 1$					
(1) Hand contact	73	90	93	85	82
(2) Respirable particles	0.012	0.017	0.037	0.30	2.8
(3) Inspirable particles	1.2	1.7	3.3	11	12
(4) Droplet spray	26	8.5	3.7	3.4	3.3
Overall risk (R)	1.6×10^{-3}	0.10	0.61	1	1

Table IV. Overall Infection Risk, R, and the Percentage of Risk Contributed by Each of the Four Exposure Pathways for Different Saliva Virus Concentrations, C_{saliva} (virus mL^{-1}), Given a Relative Infectivity of α_{LRT}:α_{URT} = 3,200:1 and α_{LRT}:α_{URT} = 1:1

We note two further issues regarding the α_{LRT} estimate. First, the dose inhaled, rather than the dose that deposited distal to the head airways region, was used to estimate $\alpha_{LRT} = 0.18$. This procedure imparts a negative bias on a per-virus deposited basis. To compensate, we considered inhaled, not deposited, dose in our exposure scenario. Second, although approximately 50% of inhaled particles with d_a in the 1- to 3-μm range deposit in the head airways region, we attributed all the infection response to virus depositing distal to that region. However, because α_{LRT} is three orders of magnitude greater than α_{URT}, any bias introduced is negligible.

Table IV shows the attender's overall infection risk and the percent apportionment of that risk among the four exposure routes for a range of C_{saliva} values, given α_{LRT}:α_{URT} ratios of 3,200:1 and 1:1. The four percentages do not sum exactly to 100% because of rounding off. The actual risk because of each pathway is approximately equal to the product of the overall risk and the percent contribution from the respective pathway.

When α_{LRT}:α_{URT} is 3,200:1, the hand contact, respirable particle inhalation, and droplet spray pathways all contribute substantially to the infection risk. For example, at a virus saliva concentration of 10^6 mL^{-1}, the hand contact, respirable particle inhalation, and droplet spray pathways contribute, respectively, 31%, 17%, and 52% of the infection risk. Respirable particle inhalation increases in importance with increased saliva virus concentration; its contribution to the infection risk increases from 14% at 10^4 mL^{-1} to 31% at 10^8 mL^{-1}. In contrast, inspirable particle inhalation contributes negligibly at all saliva virus concentrations considered.

When α_{LRT}:α_{URT} is 1:1, the hand contact pathway is the most important overall, with inspirable particle inhalation and droplet spray being significant but lesser contributors. For example, at a virus saliva concentration of 10^6 mL^{-1}, the hand contact, inspirable particle inhalation, and droplet spray pathways contribute, respectively, 93%, 3.3%, and 3.7% of the infection risk. Inspirable particle inhalation increases in importance, while droplet spray decreases in importance with increasing saliva virus concentration. Respirable particle inhalation contributes negligibly at all saliva virus concentrations considered.

For both α_{LRT}:α_{URT} ratios, the contributions of the respirable particle inhalation and inspirable particle inhalation pathways reflect the total number of virus emitted by the infector, so they increase with increasing C_{saliva} values. In contrast, the decline in the percent contribution of the droplet spray pathway at high C_{saliva} values for both α_{LRT}:α_{URT} ratios is due to the limited number of droplets that can strike the face, given an assumed fixed count and size distribution of cough particles emitted. Thus, the absolute risk because of the droplet spray pathway levels off at high C_{saliva} values, while the absolute risk because of the inhalation pathways increases.

Although the hand contact route tends to dominate when $\alpha_{LRT} = \alpha_{URT}$, the importance of inhalation exposure cannot be dismissed if an infector emits large numbers of virus via coughing. A person with $C_{saliva} = 10^8$ mL^{-1} might be termed a "supershedder," and if the hand contact and droplet spray routes were eliminated, the inhalation infection risk to the attender would be 0.17, given $\alpha_{LRT} = \alpha_{URT}$ (for which $R_{respirable} = 0.034$ and $R_{inspirable} = 0.14$). In addition, infection risk increases with repeated

exposure. If only respirable particle exposure were to occur, and if $R_{\text{respirable}} = 0.034$ for a 15-minute period, three hours of such exposure (twelve 15-minute periods) would yield a cumulative risk of $1 - (1 - 0.034)^{12} = 0.34$, which is plausible in light of the grounded airplane incident.[11]

Our analysis to this point has implicitly assumed that all virus delivered to the eyes, nostrils, and lips reach target receptor sites in the upper respiratory tract. However, we are not aware of biological data concerning the fraction, ε, of virus deposited on these facial membranes that reaches target receptors and this information deficit creates further uncertainty in estimating the relative contribution of the four exposure pathways. If $\varepsilon < 1$, the internal dose because of the hand contact and droplet spray pathways decreases, whereas the internal dose because of the inhalation pathways remains unchanged and inhalation plays a more important role in the infection risk. For example, consider that only 10% of externally deposited virus reaches target receptors ($\varepsilon = 0.1$). If α_{LRT}:$\alpha_{\text{URT}} = 3{,}200{:}1$, and if the virus saliva concentration is 10^6 mL^{-1}, the hand contact, respirable particle inhalation, and droplet spray pathways contribute, respectively, 12%, 62%, and 25% of the infection risk; the corresponding percentages for $\varepsilon = 1$ are 31%, 17%, and 52%. If α_{LRT}:$\alpha_{\text{URT}} = 1{:}1$, and if the virus saliva concentration is 10^6 mL^{-1}, the hand contact, inspirable particle inhalation, and droplet spray pathways contribute, respectively, 79%, 13%, and 7.55% of the infection risk; the corresponding percentages for $\varepsilon = 1$ are 93%, 3.3%, and 3.7%. Lower ε values are associated with a more dramatic reapportionment. For example, for $\varepsilon = 0.01$ and α_{LRT}: $\alpha_{\text{URT}} = 3{,}200{:}1$, respirable particle inhalation contributes over 90% of infection risk across all the virus saliva concentrations considered.

4. DISCUSSION

On the basis of the available data and our modeling analysis, we judge that influenza A transmission in natural settings may involve multiple exposure pathways, although the relative contribution of each pathway is situation-specific and depends on a set of factors that will be unknown *a priori*. As a result, we conclude that nonpharmaceutical interventions for a pandemic virus must account for all routes of exposure—inhalation, hand contact, and droplet spray. Our conclusion differs markedly from the opinions recently offered by other investigators.

In 2007, Brankston *et al*. argued that epidemiological evidence showed a general requirement for close contact with an infector, and they interpreted such a requirement as supporting transmission by droplet spray and perhaps by direct hand contact with the infector.[10] They also argued that the lack of documentation for influenza transmission over "long distances" disproved a significant role for respirable particle inhalation; as a contrast, they cited *Mycobacterium tuberculosis* as an airborne pathogen known to cause infection over long distances. Although Brankston *et al*. did not quantify the term "long distance," they appeared to define it as a location outside an infector's room or several meters distant from an infector's bed within a room. With regard to inhalation exposure outside an infector's room, the airborne respirable virus concentration decreases tremendously as virus particles leave the room to simultaneously mix into larger exterior room air spaces and be further diluted by air supplied to those spaces. If the infectivity of influenza virus proves to be substantially lower than that of *M. tuberculosis* (for which the α parameter is assumed equal to 1), the failure to document secondary influenza infections among those not entering an infector's room is hardly surprising. The fact that influenza is not a reportable illness also impairs secondary case ascertainment. Moreover, Brankston *et al*. discounted the confounding of droplet spray exposure by inhalation exposure. That is, the close contact that permits droplet spray exposure also permits airborne exposure to inspirable virus particles and promotes higher inhalation exposure intensity to respirable virus particles because of a near-field effect.[34]

In 2008, Atkinson and Wein argued on the basis of a theoretical analysis that respirable particle inhalation is the dominant transmission route.[35] Although these investigators modeled multiple exposure pathways in a residential scenario analogous to our Markov model, several of their input values appear to have differed substantially from ours. Atkinson and Wein used a "composite" contact transmission parameter that was the product of four factors: (1) the surface area of the hands, (2) the hand-to-surface touch rate, (3) the virus transfer efficiency from surface to the hands (termed the "hand absorption fraction from surfaces"), and (4) the virus transfer efficiency from the hands to facial membranes (termed the "self-inoculation fraction"). They acknowledged that assigning the latter three values was difficult and, in the alternative, estimated the "composite" parameter by a "bounding

procedure" in an analysis of secondary attack rate data for rhinovirus. Their procedure led to assigning a composite parameter of 5×10^{-6} m^2 h^{-1} for contact transmission in the infector's bedroom. We did not use a similar "composite" parameter but our values for analogous factors (1)–(4) combined across textile and nontextile surfaces yields the product 8.8×10^{-4} m^2 h^{-1}, which is 176-fold greater than 5×10^{-6} m^2 h^{-1}. Assuming a 176-fold lower rate of virus delivery by hand contact to facial target membranes likely contributed to Atkinson and Wein finding a negligible role for the hand contact exposure route.

Our examination of exposure pathways has highlighted four important areas of sparse information and/or nonreplicated experimental data: (1) the relative infectivity of influenza virus at different sites with virus receptors (e.g., the nasopharyngeal region vs. the alveolar region); (2) the inactivation rate of influenza virus on environmental surfaces, including the hands; (3) the surface-to-hand and hand-to-surface transfer efficiencies of influenza A virus; and (4) the fraction of influenza virus depositing on facial membranes (eyes, nostrils, and lips) that reaches internal sites with virus receptors. More reliable information concerning these areas would lead to a less uncertain apportionment of influenza infection risk among the four exposure pathways.

ACKNOWLEDGMENTS

Mark Nicas was supported by the National Institute for Occupational Safety and Health (NIOSH) Contract 211-2006-M-16790, "Microbial Aerosol and Surface Contact Exposure and Risk Assessment Modeling" and by the National Science Foundation (NSF) Grant "Health Protective Textiles: Bridging the Disposable/Reusable Divide." Rachael M. Jones was supported by the NIOSH Contract 211-2006-M-16255, "Viral Aerosol Risk Assessment." The opinions expressed are solely those of the authors and do not necessarily reflect the views of the funding agencies.

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