



# High infectivity and pathogenicity of influenza A virus via aerosol and droplet transmission

Peter F.M. Teunis<sup>a,b,\*</sup>, Nicole Brienen<sup>a</sup>, Mirjam E.E. Kretzschmar<sup>a,c</sup>

<sup>a</sup> Centre for Infectious Disease Control, Epidemiology and Surveillance Unit, RIVM, Anthonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, Netherlands

<sup>b</sup> Hubert Department of Global Health, Rollins School of Public Health, Emory University, 1518 Clifton Road, Atlanta, GA 30322, USA

<sup>c</sup> Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht University, Universiteitsweg 100, 3584 CG Utrecht, Netherlands

## ARTICLE INFO

### Article history:

Received 18 August 2010

Accepted 3 October 2010

### Keywords:

Influenza A virus

Dose response

Droplet exposure

Aerosol exposure

Infection risk

## ABSTRACT

Influenza virus may be transmitted through the respiratory route by inhalation of an aerosol of non-sedimenting droplets, or by deposition of sedimenting droplets in the upper respiratory tract. Whichever of these is the predominant route for infection with influenza virus has been subject of continuing debate, resulting in detailed studies of aerosol versus droplet exposure. A decisive knowledge gap preventing a satisfying conclusion is absence of a well defined human dose response model for influenza virus.

This study uses a hierarchical approach generalizing over twelve human challenge studies collected in a literature search. Distinction is made between aerosol and intranasal inoculation. The results indicate high infectivity via either route, but intranasal inoculation leads to about 20 times lower infectivity than when the virus is delivered in an inhalable aerosol.

A scenario study characterizing exposure to airborne virus near a coughing infected person in a room with little ventilation demonstrates that with these dose response models the probabilities of infection by either aerosol or sedimenting droplets are approximately equal. Droplet transmission results in a slightly higher illness risk due to the higher doses involved.

Establishing a dose response model for influenza provides a firm basis for studies of interventions reducing exposure to different classes of infectious particles. More studies are needed to clarify the role of different modes of transmission in other settings.

© 2010 Elsevier B.V. All rights reserved.

## Introduction

Transmission of influenza is thought to occur through contact with small infectious particles. Infectious virus present in or on the mucosae of the upper respiratory tract is expelled through coughing or sneezing, or even through normal exhalation, producing small droplets that may contain various amounts of virus (Fabian et al., 2008; Blachere et al., 2009). Droplets that are small enough may evaporate rapidly, leaving a microscopic particle that can remain suspended in air for an indefinite time (Riley, 1974). While part of the produced infectious particles may be small enough for a non-sedimenting aerosol, the remainder of the expelled droplets is bigger and tends to be removed from the air by sedimentation (Duguid, 1946). Virus present on surfaces (skin or inanimate) may be transferred to mucosa by hand and still cause infection (Ryan et al., 2001). Virus may thus infect by different routes. The relative

importance of these routes for transmission has been debated intensively but it still remains unclear if any route is dominant (Tellier, 2006; Weber and Stilianakis, 2008).

The different modes of transmission of respiratory infections may be studied by quantitative modelling of production of droplets containing virus and their transport to mucosal surfaces in a susceptible host (Xie et al., 2007; Atkinson and Wein, 2008; Nicas and Jones, 2009). Although such studies describe exposure to respiratory virus with considerable sophistication, one essential stage in the infection chain, the dose response relation for infection, has remained relatively obscure. Infectivity estimates are based on small data sets containing few observations and biological variation (heterogeneity) in infectivity is ignored.

The present paper attempts to fill this gap by using a hierarchical approach to dose response modelling, based on data from several human challenge studies reported in scientific journals. This allows us to provide a quantitative description of the infectivity of influenza A virus in humans, either by aerosol inoculation or by intranasal droplet inoculation, including its heterogeneity among hosts and virus isolates. Based on these dose response models, improved estimates of the risk of infection (and of acute respiratory symptoms) can be

\* Corresponding author. Centre for Infectious Disease Control, Epidemiology and Surveillance Unit, RIVM, PO Box 1, 3720BA Bilthoven, The Netherlands. Fax: +31 30 274 4409.

E-mail address: [peter.teunis@rivm.nl](mailto:peter.teunis@rivm.nl) (P.F.M. Teunis).

**Table 1**  
Wild-type influenza virus challenge studies with aerosol inoculation.

Reference	Virus type	Dose (TCID <sub>50</sub> )	Exposed	Infected	Ill
Henle et al. (1946)	A (F-12)	0.6 × 10 <sup>10a</sup>	4	— <sup>b</sup>	4
		0.6 × 10 <sup>10a</sup>	4	— <sup>b</sup>	4
		0.6 × 10 <sup>9a</sup>	4	— <sup>b</sup>	1
	A (F-99)	0.6 × 10 <sup>8.5a</sup>	4	— <sup>b</sup>	1
		0.6 × 10 <sup>8.5a</sup>	6	— <sup>b</sup>	5
		0.6 × 10 <sup>8.5a</sup>	4	— <sup>b</sup>	4
	A (PR-8)	0.6 × 10 <sup>7.5a</sup>	6	— <sup>b</sup>	2
		0.6 × 10 <sup>8.2a</sup>	33	— <sup>b</sup>	27
		0.6 × 10 <sup>8.2a</sup>	30	— <sup>c</sup>	12
Jao et al. (1965)	A2 (Elisberg)	30	30	— <sup>c</sup>	12
Alford et al. (1966)	A2/Bethesda/10/63	126	3	0	0 <sup>d</sup>
		78	3	0	0 <sup>d</sup>
		59	3	1	0
		1	1	1	1
		2	4	1	0
		5	9	4	3

<sup>a</sup> Not tissue culture but ID<sub>50</sub> in chick embryos.  
<sup>b</sup> Not studied.  
<sup>c</sup> Virus excretion and seroconversion studied but not reported.  
<sup>d</sup> These subjects were presumably immune, as they had high antibody levels to the virus.

calculated for aerosol and for droplet transmission. For a given exposure scenario the relative strengths of either transmission mode can then be estimated.

The improved dose response information contributes to quantitative estimates of the infectious droplet transmission process by including variation in host susceptibility as well as variation in infectivity among different virus isolates.

**Dose response assessment**

A literature study of human challenge experiments with influenza virus has produced two sets of studies, with virus delivered either via aerosol inhalation or via intranasal droplet inoculation. Aerosol inoculation may allow the virus to reach smaller bronchiae where receptor densities are high (Hatch, 1961) and infection may be more likely. Alternatively, deposition of a small droplet of virus suspension onto the nasal mucosa may serve as a model for transmission via droplets of sedimenting sizes (Brankston et al., 2007).

To analyze these dose response data, a hierarchical model is used, extending the hit theory model for microbial infection (Haas, 1983; Teunis and Havelaar, 2000) to a multilevel framework (Teunis et al., 2008b).

*Dose response model*

When exposed to a sample taken from a well mixed microbial suspension the probability of exposure to one or more infectious virus particles is

$$\text{Prob}_{\text{exp}}(cV) = 1 - e^{-cV} \tag{1}$$

assuming a volume *V* was inoculated from a suspension of Poisson distributed particles with concentration *c*.

In case each particle is equally infectious, the dose response relation for infection is (Riley and O’Grady, 1961)

$$\text{Prob}_{\text{inf}}(cV|p_m) = 1 - e^{-p_m cV} \tag{2}$$

where any infectious virus survives the host barriers to infection with probability *p<sub>m</sub>* (Teunis and Havelaar, 2000). Biological variation in

**Table 2**  
Wild-type influenza virus challenge studies with nasal inoculation.

Reference	Virus type	Dose (TCID <sub>50</sub> )	Exposed	Infected	Ill
Henle et al. (1946)	A (F-12)	10 <sup>10a</sup>	4	— <sup>b</sup>	1
	A (F-99)	10 <sup>8.5a</sup>	6	— <sup>b</sup>	0
Murphy et al. (1973)	A/Bethesda/88 (H3N2)	10 <sup>4.5</sup>	7	7	7 <sup>c</sup>
Murphy et al. (1980)	A/Hong Kong/77 (H1N1)	10 <sup>4.2</sup>	6	6	5
	A/Udorn/72 (H3N2)	10 <sup>4.0</sup>	6	5	5
	A/Alaska/77 (H3N2)	10 <sup>4.2</sup>	8	8	4
Clements et al. (1983)	A/Alaska/6/77 (H3N2)	10 <sup>4.2</sup>	8	8	4
Clements et al. (1984b)	A/Washington/897/80 (H3N2)	10 <sup>6.0</sup>	24	23	11
	A/Washington/897/80 (H3N2) <sup>d</sup>	10 <sup>6.0</sup>	24	23	11
Murphy et al. (1984)	A/California/10/78 (H1N1)	10 <sup>4.0</sup>	9	9	5
Murphy et al. (1985)	A/Washington/897/80 (H3N2) <sup>d</sup>	10 <sup>6.0</sup>	24	23	11
Clements et al. (1986)	A/Washington/897/80 (H3N2) <sup>d</sup>	10 <sup>6.0</sup>	24	23	11
Snyder et al. (1986)	A/California/10/78 (H1N1)	10 <sup>4.5</sup>	14	13	6
	A/Korea/1/82 (H3N2)	10 <sup>6.2</sup>	14	14	7
Sears et al. (1988)	A/Texas/1/85 (H1N1)	10 <sup>6.4</sup>	28	26	12
	A/Bethesda/1/85 (H3N2)	10 <sup>7.0</sup>	10	10	3
Youngner et al. (1994)	A/Kawasaki/9/86 (H1N1)	10 <sup>7.0</sup>	14	14	6

<sup>a</sup> Not tissue culture but ID<sub>50</sub> in chick embryos.  
<sup>b</sup> Not studied.  
<sup>c</sup> Of these subjects, 6 had severe symptoms with fever, 1 had mild symptoms without fever.  
<sup>d</sup> Same as Clements et al. (1984b).

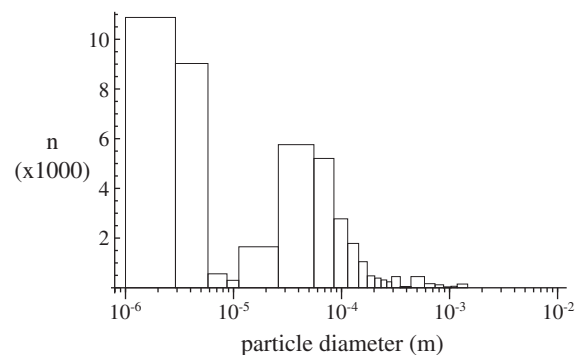
host susceptibility and virus infectivity may be expressed as (random) variation in *p<sub>m</sub>*. The resulting (marginal) dose response model

$$\text{Prob}_{\text{inf}}(cV|\alpha, \beta) = 1 - {}_1F_1(\alpha, \alpha + \beta; -cV) \tag{3}$$

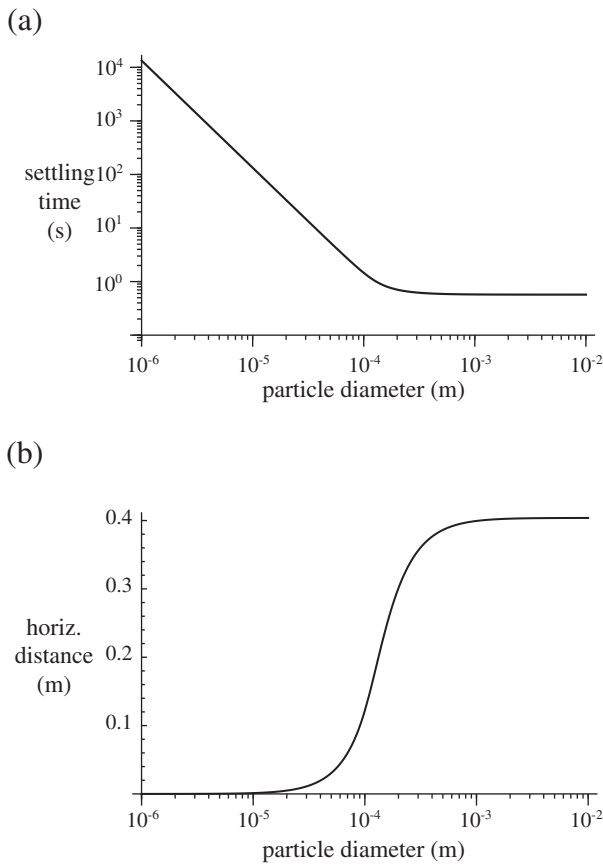
where  ${}_1F_1$  is a (Kummer) confluent hypergeometric function and  $\alpha$  and  $\beta$  the parameters of a beta distribution describing the variation in *p<sub>m</sub>*, is the beta-Poisson model for microbial infection (Haas, 1983; Teunis and Havelaar, 2000).

A person infected with influenza virus may develop symptoms of acute respiratory illness with probability again depending on the inoculated dose. A conditional dose response model for illness in infected subjects is defined as

$$\text{Prob}_{\text{ill|inf}}(cV|\eta, r) = 1 - (1 + \eta cV)^{-r} \tag{4}$$



**Fig. 1.** Counts of particles of various diameters in air expelled by (90) coughs (Loudon and Roberts, 1967).



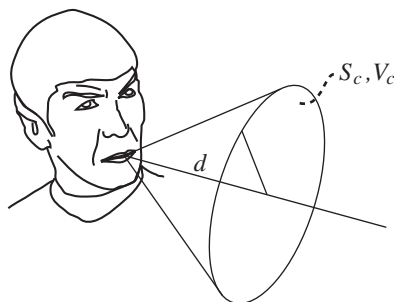
**Fig. 2.** (a) Average settling time (in s) for a particle produced at body height (1.6 m) to reach the floor, as a function of particle diameter. (b) Horizontal distance (in m) travelled when a particle is expelled with velocity 1 m/s and falls 0.8 m (half body height).

assuming a hazard function of developing symptoms that depends on the duration of infection (parameters  $\eta$  and  $r$  describe a (gamma) distribution for the dose dependent duration of infection). Details can be found in [Teunis et al. \(1999\)](#).

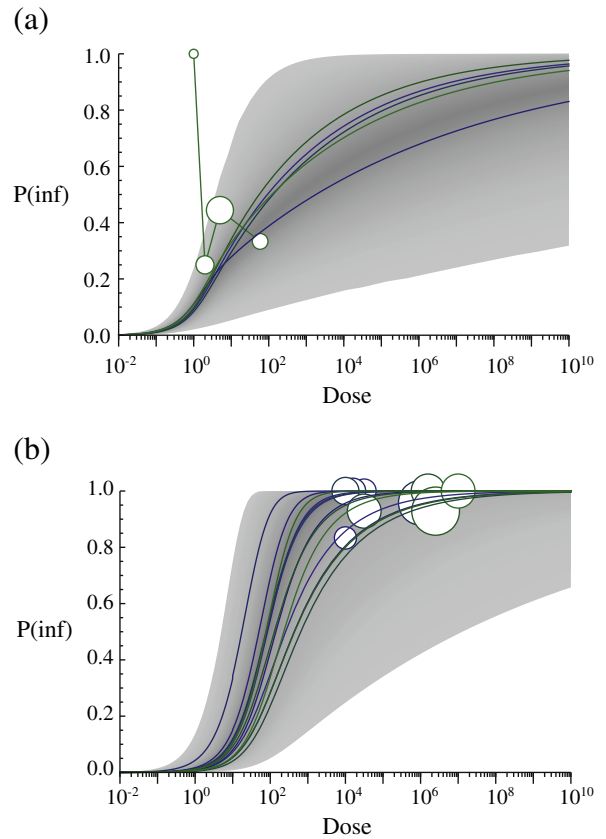
As subject status is binary (infected or not, symptomatic or not) the model may be analyzed with a binomial likelihood function ([Teunis and Havelaar, 2000](#)) that can be extended to a two-level framework ([Teunis et al., 2002, 2008b](#)). Additional information on statistical analysis is provided in an online appendix (supporting information).

*Dose response data*

Three studies administered the virus through inhalation of a standardized aerosol of influenza A virus isolated from patients (5



**Fig. 3.** Conical region where sedimenting droplets ( $>10 \mu\text{m}$ ) may occur after expulsion through coughing or sneezing. The horizontal distance  $d$  (and the circular area  $S_c$  and the corresponding volume  $V_c$ ) depends on the initial velocity and the particle size.



**Fig. 4.** Dose response for infection by wild type influenza A virus, via aerosol or intranasal droplet inoculation. ‘Best fit’ dose response relations and density graph of predicted infection risk as a function of dose (margins span 99% interval). Also shown is a bubble chart of observed fractions (symbol size proportional to numbers exposed).

different isolates, shown in [Table 1](#)). Twelve papers reported on influenza A virus challenge through intranasal droplet inoculation, three of which appeared to re-report results from an earlier study, leaving nine studies with 14 different isolates ([Table 2](#)). Note that the oldest study ([Henle et al., 1946](#)) only documented illness responses: numbers of infected subjects (excreting virus) were not reported. Because illness is conditional on infection these data still provide information about the infectivity of the virus.

In most studies the virus dose was expressed in TCID<sub>50</sub> units. This is the median 50% tissue culture infectious dose (TCID<sub>50</sub>). Assuming perfect susceptibility 1 TCID<sub>50</sub> would correspond to  $\log_2 \approx 0.69$  infectious virus particles because the dose response for a perfectly susceptible host system is  $P_{\text{inf}}(D) = 1 - e^{-D}$ , hence  $1 - e^{-\text{TCID}_{50}} = 0.5$ . This is quite close to 1 and therefore we feel safe in assuming that 1 TCID<sub>50</sub> approximately equals 1 infectious virus particle ([Blachere et al., 2009](#)). In one of the studies the dose was expressed as 50% infectious dose in chick embryo culture ([Henle et al., 1946](#)). Chick embryos are also a highly sensitive medium ([Hirst, 1942](#)) and it does not seem very likely that the chick embryo assay is less susceptible than the tissue culture assay by more than an order of magnitude ([Donald and Isaacs, 1954](#)). Therefore, in the following analysis it is assumed that  $1 \text{ EID}_{50} = 1 \text{ TCID}_{50} = 1$  virus particle.

**Exposure**

Droplets are generated during breathing, coughing or sneezing as expelled air strikes surfaces covered with mucus in the upper respiratory tract. Various accounts have been published of the diameters of the fluid particles produced during either of these activities, with

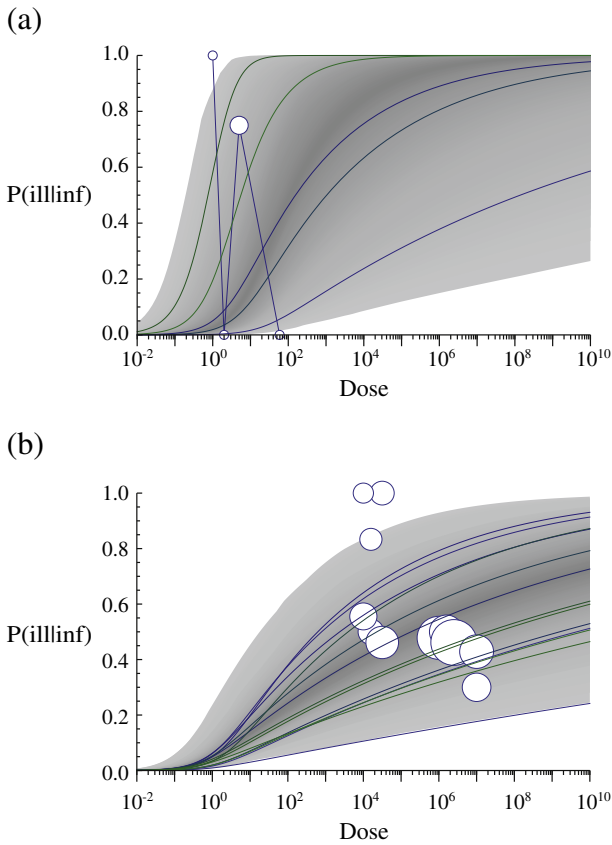


Fig. 5. Dose response for illness given infection by wild type influenza A virus, via aerosol or intranasal droplet inoculation. ‘Best fit’ dose response relations and density graph of predicted conditional illness risk as a function of dose. Also shown is a bubble chart of observed fractions (symbol size proportional to numbers infected).

comparable outcomes (Duguid, 1946; Loudon and Roberts, 1967; Xie et al., 2009). A review of airborne infectious particle emission (Nicas et al., 2005) describes three different studies reporting particle size distributions. In order to not unduly complicate the following account of airborne exposure the sizes recorded in one study (Loudon and Roberts, 1967) will be used, because that study provides a detailed account of the particle size distribution, combining small non-sedimenting particles and large size particles that sediment rapidly. The reported particle diameters range from 1 μm to more than 1.5 mm, and the frequencies counted in air expelled with 90 coughs are given.

There appears to be a bimodal distribution of small and large particles (Fig. 1) and a binary mixture of lognormal distributions provides a good fit of these observed particle sizes. Small particles have a mean size of 3.0 μm (99% range 1.27–6.25 μm). The average diameter of large particles is 111.4 μm (99% range 8.7–616.6 μm). A little less than half of the particles is in the small size class (48.3%). Note that, assuming spherical particles, this means that the total volume in the small particle class is about  $2.4 \times 10^{-6}$  of the total volume of all expelled particles.

Sedimentation of fluid particles

A very basic description of sedimentation of fluid particles can be given by considering only gravitational and frictional forces

$$\begin{cases} mx''(t) = -bx'(t) \\ my''(t) = -by'(t) - mg \end{cases} \begin{cases} x(0) = 0, x'(0) = a \\ y(0) = h, y'(0) = 0 \end{cases} \quad (5)$$

where  $x$  and  $y$  are horizontal and vertical distances,  $m$  is the mass of the fluid particle,  $g$  is the gravitational constant and  $b$  is a frictional

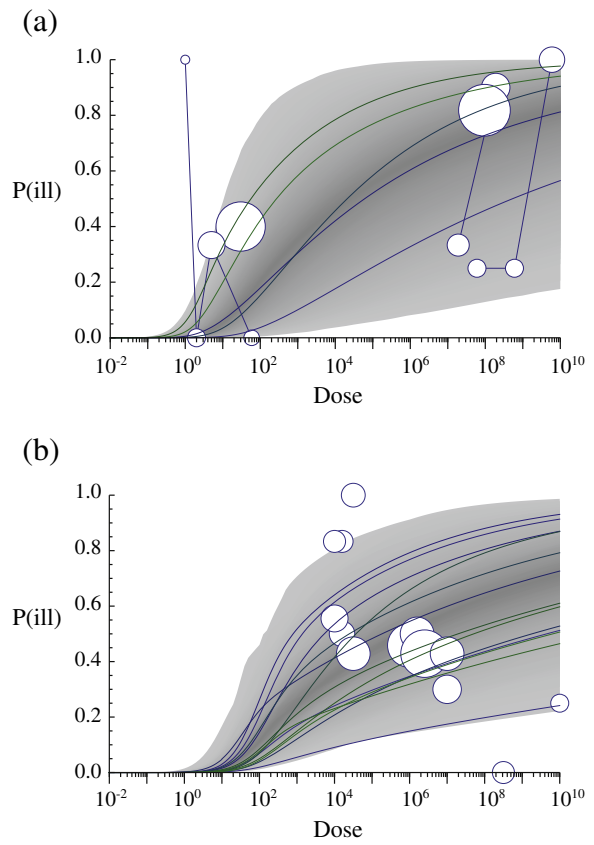


Fig. 6. Dose response for illness by wild type influenza A virus, via aerosol or intranasal droplet inoculation. ‘Best fit’ dose response relations and density graph of predicted illness risk as a function of dose. Also shown is a bubble chart of observed fractions (symbol size proportional to numbers exposed).

coefficient. Initial height above the floor is  $h$  (m) and particles are expelled with initial horizontal velocity  $a$  (m/s). For spherical particles

$$m = \frac{4}{3}\pi r^3 \rho(\text{kg}), \quad b = 6\pi\eta r(\text{kg s}^{-1}) \quad (6)$$

where  $\eta = 1.82 \times 10^{-5}$  (kg m<sup>-1</sup> s<sup>-1</sup>) and  $g = 9.81$  (m s<sup>-2</sup>). Eq. (5) can be solved to yield

$$x(t) = \frac{am}{b} \left(1 - e^{-\frac{bt}{m}}\right); \quad y(t) = h - \frac{mg}{b}t + \frac{m^2g}{b^2} \left(1 - e^{-\frac{bt}{m}}\right) \quad (7)$$

so that the time a particle is suspended can be estimated (Fig. 2a). For a given initial velocity the horizontal distance travelled appears to only depend on particle diameter in a fairly narrow range, from 40 μm to 1 mm (Fig. 2b).

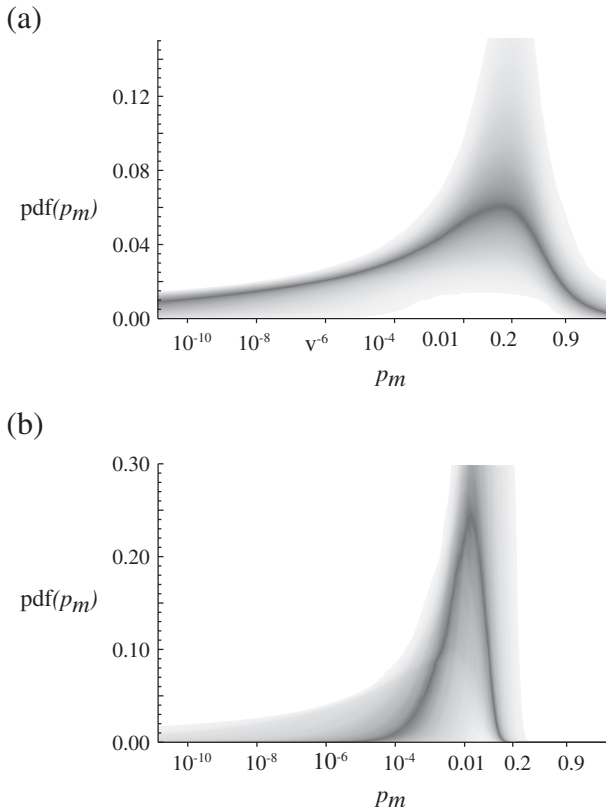
A sedimenting particle is assumed to be expelled in a random direction within a cone shaped region (Tang et al., 2009) of solid angle  $\alpha$  steradians (1 steradian corresponds to an apex angle of  $\approx 65.5^\circ$  in a cross-section of the cone). The surface area of the base of the cone (as a spherical cap) is

$$S_c = \alpha d^2 \quad (8)$$

when  $d$  is the horizontal distance travelled by the particle. See Fig. 3. The volume of the cone is approximately

$$V_c = \frac{\pi d^3}{3} \left(1 - \frac{\alpha}{2\pi}\right) + \frac{\alpha^2 d^3}{4\pi} \left(1 - \frac{\alpha}{6\pi}\right) \quad (9)$$

(Nicas and Sun, 2006; Atkinson and Wein, 2008).



**Fig. 7.** Distribution of single virus unit infectivity for wild type influenza A virus, via aerosol inoculation and via intranasal droplet inoculation. Density chart determined from (posterior) predictive distribution of the infectivity parameters.

*Inhalation of aerosol*

If a person resides in a room with little ventilation where another infected person produces virus suspended in aerosol, the probability of inhaling a suspended particle (e.g. a droplet nucleus) is

$$P_{\text{inhal}}(\tau, Q_r, V_0) = 1 - e^{-\frac{Q_r \tau}{V_0}} \tag{10}$$

assuming perfect mixing, where  $V_0$  is the room volume ( $\text{m}^3$ ),  $Q_r$  is the respiration rate ( $\text{m}^3 \text{s}^{-1}$ ) and  $\tau$  is the residence time, i.e. the average time the particle remains in suspension (as in Fig. 2a). It will be assumed that the respiration rate is 50 l/min and the room volume is  $3 \times 4 \times 4 = 48 \text{ m}^3$ . When the receiving subject remains in the room for a defined period  $T$ , say 1 h, the probability of inhalation is determined by  $\text{Min}(\tau, T)$  instead of  $\tau$ .

*Droplet inoculation*

In the same situation as above: a closed room with a person coughing, and another person who may be close enough to be exposed, the probability of contact with sedimenting infectious droplets may be considered. Above (sedimentation of fluid particles) the volume of space was calculated where an expelled droplet may be found (Eq. (9), see Fig. 3).

Assuming that contact with such a droplet may occur in a rectangular volume where the receiving person can be (in a room of  $4 \times 4 \text{ m}^2$  a volume of approximately  $2 \times 16 = 32 \text{ m}^3$ ) the probability of contact is proportional to  $V_c/32$ . A small fraction of the exposed body surfaces is mucosa (Nicas and Sun (2006) assume  $15 \text{ cm}^2 = 15 \times 10^{-4} \text{ m}^2$ ) and the probability of a droplet hitting exposed mucosa is proportional to

$15 \times 10^{-4}/S_c$ . The probability of contact through a sedimenting infectious droplet then is

$$P_{\text{droplet}} = \frac{15 \times 10^{-4} V_c}{32 S_c} = Kd \tag{11}$$

with  $d$  again the horizontal distance travelled by the droplet, and

$$K = \frac{15 \times 10^{-4}}{16 \times 9.6} \left( \frac{\pi}{3} \left( 1 - \frac{\alpha}{2\pi} \right) + \frac{\alpha^2}{4\pi} \left( 1 - \frac{\alpha}{6\pi} \right) \right) \frac{1}{\alpha} \tag{12}$$

where the solid angle  $\alpha$  describes the dispersion in direction of sedimenting droplets.

*Simulation of exposure*

The following scenario was assumed: an infectious person produces droplets containing virus by coughing, with size distribution as in Fig. 1. The median horizontal velocity was assumed to be 2 m/s, its maximum (95 percentile) 12.5 m/s, and a gamma distribution was used to simulate its variation (parameters  $r = 0.65$ ,  $\lambda = 5.48$ ). Based on a hierarchical model analysis of nasal excretion data (Baccam et al., 2006) the concentration of virus was assumed to be lognormal with geometric mean  $10^8$  and 95% range  $10^5$ – $10^{12} \text{ (m}^{-3}\text{)}$ . At the time of coughing another person enters the room and remains there for 1 h, while there is neither little ventilation nor strong air movements.

The probabilities of exposure and infection (and acute symptoms of respiratory illness) were estimated for a single infectious particle (either sedimenting or non-sedimenting), and for a coughing attack consisting of a Poisson distributed number of coughs (15 coughs average) and negative binomially distributed numbers of particles per cough, average 466 (Loudon and Roberts, 1967), and dispersion parameter  $\rho = 10$  (Teunis et al., 2008b). The resulting distribution of numbers of particles is shown in Fig. 10a.

Virus inactivation due to aerosol formation and drying was not accounted for because it is likely that the periods required are longer than the 1 h scenario assumed here. A reduction in infectivity of less than 1 log unit has been reported after 6 h suspension in air room temperature (Harper, 1961), at high humidity survival may be lower (Hemmes et al., 1960).

**Results**

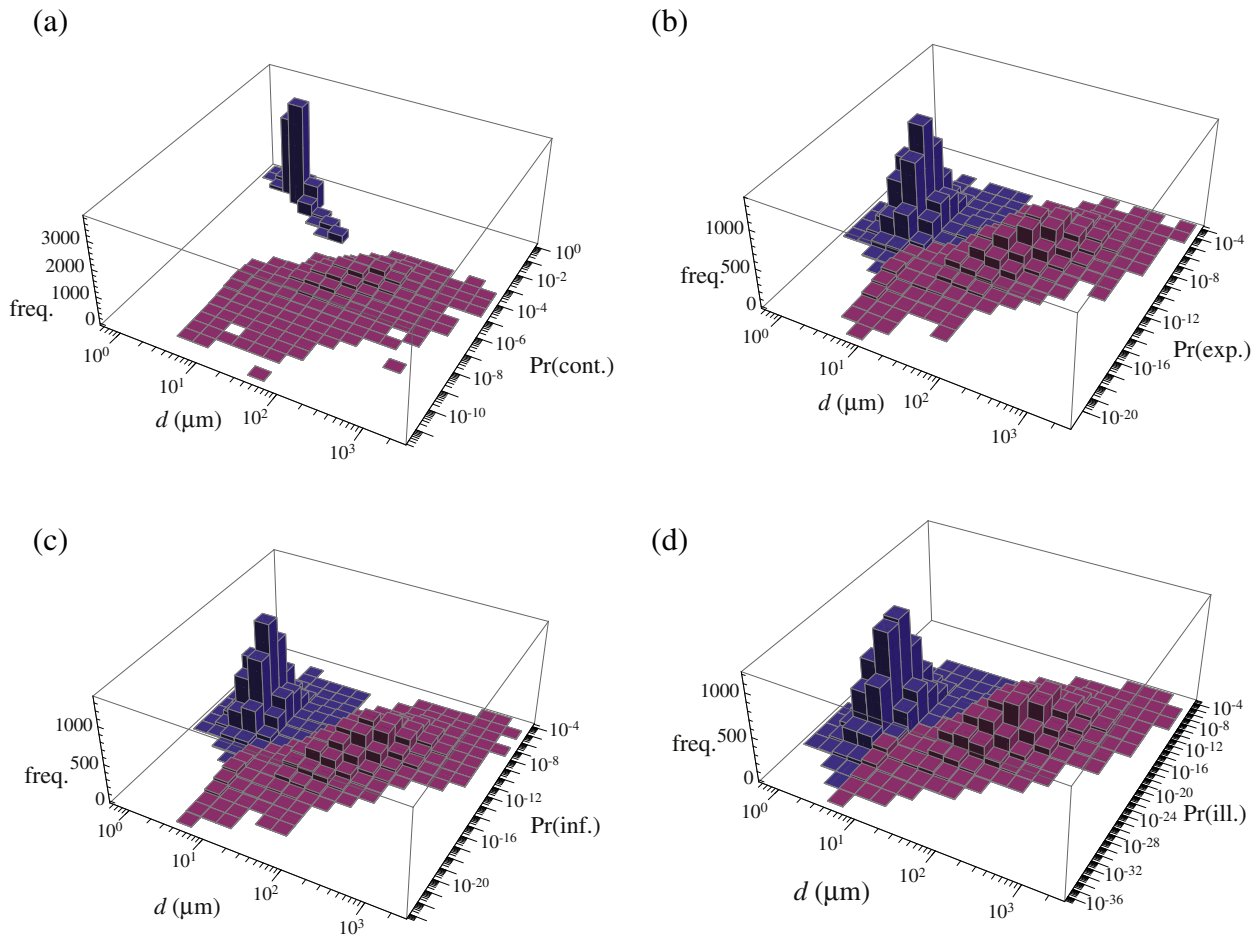
*Dose response assessment*

The dose response relations for infection, illness among infected, and illness are shown in Figs. 4–6. These graphs show ‘best fit’ dose response relations for all individual isolates, as well as the (posterior) density of the predicted probabilities (of infection, illness given infection, or illness unconditionally). The latter densities can be thought of as estimates of infection or illness risk, generalized from the complete set of included dose response relations. The outer margins correspond to a 99% predictive interval. See online supporting information for more explanation and additional results. Also shown are the observed fractions, as far as these can be calculated.

The dose response relation for infection is completely determined by the infectivity of a single infectious unit ( $p_m$  in the model described above). Its distribution can also be determined, as shown in Fig. 7, for aerosol and intranasal droplet inoculation.

Aerosol inoculation of influenza A virus (Fig. 4a) results in high infectivity, mainly because of the responses to low doses (Jao et al., 1965; Alford et al., 1966).

Aerosol inoculation is about 20 times as efficient as intranasal droplets in causing infection, but with greater variability (Fig. 7).



**Fig. 8.** Probabilities of contact with a fluid particle (a), exposure to infectious virus (b), infection (c), and symptoms of acute respiratory illness (d), as a function of the diameter of the expelled particle. Histograms for the two different transmission routes, aerosol inhalation and droplet inoculation, are shown in blue and red, respectively.

### Simulated risk

Using the scenario outlined above a Monte Carlo simulation of the risks of exposure (i.e. inhalation or mucosal contact with at least one infectious virus particle) and infection can be simulated. The conditional dose response relations for acute illness among infected subjects may be used to also estimate illness risks.

The probability of contact with an expelled fluid particle as a function of its diameter is shown in Fig. 8a, for sedimenting and non-sedimenting particles. Also shown are the probabilities of exposure to virus, infection, and acute respiratory symptoms (Figs. 8b–d).

Fig. 9 shows risks associated with the presence of a single infectious particle, either non-sedimenting (aerosol) or sedimenting (droplet), with diameter drawn at random from the distribution defined by Loudon and Roberts (1967). The probability of exposure due to either transmission route is approximately equal, as is the infection risk. The probability of acute respiratory symptoms is higher with droplets, because the dose involved is likely to be higher. Note that the distribution of risk is highly skewed, with mean risks near the 95 percentile or even above that level.

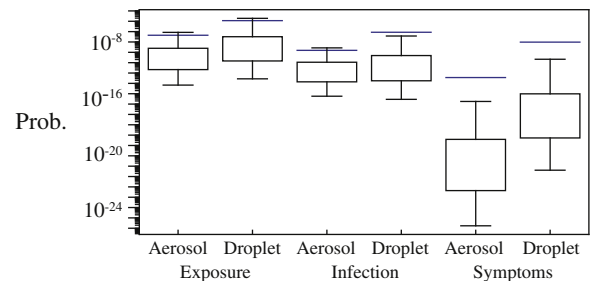
The simulated risks associated with the production of a greater number of infectious particles is shown in Fig. 10, for the numbers of particles corresponding to a coughing attack.

### Discussion

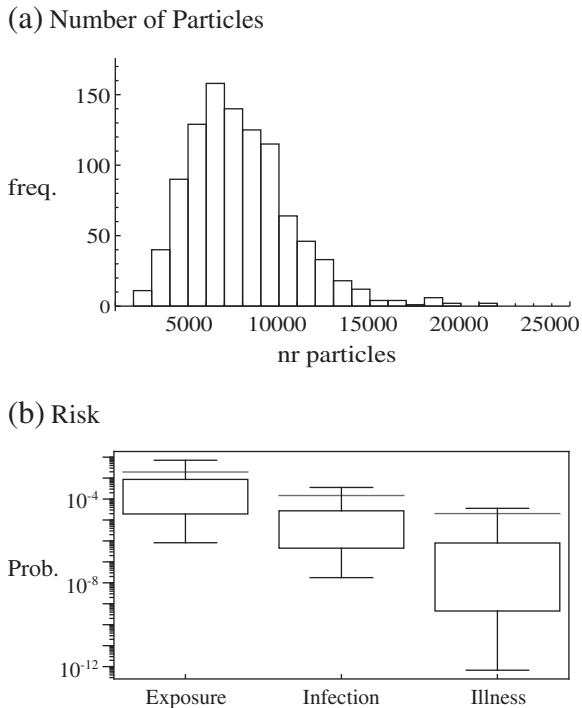
Previous studies on exposure issues in transmission of influenza have considered epidemic dynamics (Atkinson and Wein, 2008; Chen

et al., 2009; Li et al., 2009) or not, dealing only with transmission mechanisms (Nicas and Sun, 2006; Nicas and Jones, 2009). All of these studies have ignored heterogeneity, both in virus infectivity (and pathogenicity) and in susceptibility of the human hosts. Use of a hierarchical framework has not only allowed us to use a two-parameter model that includes a (beta) distribution characterizing heterogeneity at the level of the single challenge study, but also to characterize the variation among studies, representing different virus isolates.

It should be noted that volunteers in human challenge studies usually are young adults in good general health, selected to not



**Fig. 9.** Box plots of simulated risk of exposure to infectious virus, infection, and acute respiratory illness, when in the given scenario a single infectious particle is produced, either non-sedimenting (aerosol) or sedimenting (droplet). Boxes indicate quartiles, whiskers 95% ranges, and the horizontal lines indicate mean risks.



**Fig. 10.** Numbers of particles expelled in a coughing attack (a) and box plots of simulated risk of exposure to infectious virus, infection, and acute respiratory illness (b). Boxes indicate quartiles, whiskers 95% ranges, and the horizontal lines indicate mean risks.

develop severe illness. Although often the immune status of the volunteers is not known, especially in the older studies, the high observed infection and illness rates suggest low levels of immunity to infection or illness. In immune subjects the probability of illness (and possibly infection) is lower than in those with no immunity, given equal exposure. Therefore, when repeated exposure to similar virus strains is likely, the health risks may be lower than estimated here.

The dose response relation for illness among infected subjects implies that low dose exposure may lead to infection, due to the high infectivity of the virus, but of those infected only a small proportion may become ill. Exposure to high doses of virus results in most of the infected subjects also becoming ill. A shedding event releasing high numbers of viruses in the environment may therefore lead to clusters of cases that can be detected in disease surveillance. Where low numbers of viruses are present transmission may be mostly asymptomatic, and the odd person developing symptoms cannot easily be linked to other cases infected by the same source. When exposure to airborne virus is reduced, for instance by population-wide use of face masks, the relative decrease in numbers of illnesses is expected to be greater than the relative decrease in transmission, by numbers infected.

The quantitative characterization of influenza virus infectivity (and pathogenicity) provides a stronger basis for prospective studies of the effects of interventions, in particular those interventions that reduce exposure, for instance, the effect of face mask use on spread of pandemic influenza (Brienen et al., 2010).

In calculating the virus content of differently sized particles the virus concentration was assumed constant so that, given the virus concentration, the number of viruses in a particle of any size depends only on its volume. Sequestration of virus into fluid particles may however not be independent of particle size, and if this were the case the relative contributions of variously sized particles to exposure, infections and symptoms as shown in Fig. 7 may change.

It is worth noting here that virus in suspension may often be aggregated, causing the virus to be present in clumps of variable numbers

of single viruses or virions, instead of a fully dispersed suspension of virions (Wei et al., 2007). If the inoculum should contain aggregates the effect on the dose response relation would be an increase in apparent heterogeneity (compared to a monodisperse suspension of the same virus): any suspended particle then may consist of 1 or more virions, each of variable infectivity (Teunis et al., 2008a).

In freshly shed influenza virus most particles may be infectious: particle counts and TCID<sub>50</sub> do not differ greatly (Wei et al., 2007). Even the EID<sub>50</sub>, the 50% infectious dose in chick embryo culture has been estimated to correspond to less than 10 particles, also supporting the assumption that TCID<sub>50</sub> and EID<sub>50</sub> are approximately equal. However, when the virus has been exposed to environmental conditions the fraction infectious particles may decrease rapidly (Horsfall, 1954, 1955; Choppin and Tamm, 1960). Such loss of infectivity may not be important in the scenario considered here, but must be taken into account when considering exposure to virus in natural conditions.

The estimated probabilities of exposure and infection are within the same order of magnitude, indicating that one cannot readily discard either route as unimportant for transmission. The advantage of sedimenting droplets carrying a higher virus load is compensated by their smaller chance of contact combined with the lower infectivity of upper respiratory tract inoculation. Similarly, the more efficient inoculation of small aerosol particles is compensated by their smaller virus content. For example, outdoor aerosol transmission is not likely due to dilution and dispersion by ambient wind speeds and turbulence, whereas in closed environments, particularly with low ventilation, aerosol transmission is more likely.

Despite equal infection risks, the corresponding risks of acute respiratory illness are somewhat higher for droplets, due to the higher dose that is involved with larger particles.

Influenza virus may also be transmitted through hand contact with contaminated surfaces. Surface-to-hand-to-mucosa contacts were not considered in this study because the aim was to compare aerosol and droplet transmission in the absence of human behavioural factors, as these are poorly understood and the proximity of infectious and susceptible subjects cannot be easily quantified.

To improve the estimates of transmission of respiratory virus, further studies of exposure are needed, to determine how efficiently airborne virus may be transferred in the presence of ventilation, the relation between human contact behaviour and droplet infection, and most importantly, the role of contaminated surfaces in transmission of influenza.

Supplementary materials related to this article can be found online at doi:10.1016/j.epidem.2010.10.001.

## Acknowledgments

The constructive and important comments of two anonymous reviewers are gratefully acknowledged.

## References

- Alford, R.H., Kasel, J.A., Gerone, P.J., Knight, V., 1966. Human influenza resulting from aerosol inhalation. *Proc. R. Soc. Exp. Biol. Med.* 122 (3), 800–804.
- Atkinson, M.P., Wein, L.M., 2008. Quantifying the routes of transmission for pandemic influenza. *Bull. Math. Biol.* 70 (3), 820–867.
- Baccam, P., Beauchemin, C., Macken, C.A., Hayden, F.G., Perelson, A.S., 2006. Kinetics of influenza A virus infection in humans. *J. Virol.* 80 (15), 7590–7599.
- Blachere, F.M., Lindsley, W.G., Pearce, T.A., Anderson, S.E., Fisher, M., Khakoo, R., Meade, B.J., Lander, O., Davis, S., Thewlis, R.E., Celik, I., Chen, B.T., Beezhold, D.H., 2009. Measurement of airborne influenza virus in a hospital emergency department. *Clin. Infect. Dis.* 48 (4), 438–440.
- Brankston, G., Gitterman, L., Hirji, Z., Lemieux, C., Gardam, M., 2007. Transmission of influenza A in human beings. *Lancet Infect. Dis.* 7 (4), 257–265.
- Brienen, N.C.J., Timen, A., Wallinga, J., van Steenbergen, J.E., Teunis, P.F.M., 2010. The effect of mask use on the spread of influenza during a pandemic. *Risk Anal.* 30 (8), 1210–1218.

- Chen, S.C., Chio, C.P., Jou, L.J., Liao, C.M., 2009. Viral kinetics and exhaled droplet size affect indoor transmission dynamics of influenza infection. *Indoor Air* 19 (5), 401–413.
- Choppin, P.W., Tamm, I., 1960. Studies of two kinds of virus particles which comprise influenza A2 virus strains: I. characterization of stable homogeneous substrains in reactions with specific antibody, mucoprotein inhibitors, and erythrocytes. *J. Exp. Med.* 112, 895–920.
- Clements, M.L., O'Donnell, S., Levine, M.M., Chanock, R.M., Murphy, B.R., 1983. Dose response of A/Alaska/6/77 (H3N2) cold-adapted reassortant vaccine virus in adult volunteers: role of local antibody in resistance to infection with vaccine virus. *Infect. Immun.* 40 (3), 1044–1051.
- Clements, M.L., Betts, R.F., Maassab, H.F., Murphy, B.R., 1984a. Dose response of influenza A/Washington/897/80 (H3N2) cold-adapted reassortant virus in adult volunteers. *J. Infect. Dis.* 149 (5), 814–815.
- Clements, M.L., Betts, R.F., Murphy, B.R., 1984b. Advantage of live attenuated cold-adapted influenza A virus over inactivated vaccine for A/Washington/80 (H3N2) wild-type virus infection. *Lancet* 1 (8379), 705–708.
- Clements, M.L., Snyder, M.H., Buckler-White, A.J., Tierney, E.L., London, W.T., Murphy, B.R., 1986. Evaluation of avian–human reassortant influenza A/Washington/897/80 × A/Pintail/11/9/79 virus in monkeys and adult volunteers. *J. Clin. Microbiol.* 24 (1), 47–51.
- Donald, H.B., Isaacs, A., 1954. Counts of influenza virus particles. *J. Gen. Microbiol.* 10, 457–464.
- Duguid, J.P., 1946. The size and the duration of air-carriage of respiratory droplets and droplet nuclei. *J. Hyg. Camb.* 44 (6), 471–479.
- Fabian, P., Mcdevitt, J.J., Dehaan, W.H., Fung, R.O.P., Cowling, B.J., Hung Chan, K., Leung, G.M., Milton, D.K., 2008. Influenza virus in human exhaled breath: an observational study. *PLoS ONE* 3 (7), e2691.
- Haas, C.N., 1983. Estimation of risk due to low doses of microorganisms: a comparison of alternative methodologies. *Am. J. Epidemiol.* 118 (4), 573–582.
- Harper, G.J., 1961. Airborne micro-organisms: survival tests with four viruses. *J. Hyg.* 59, 479–486.
- Hatch, T.F., 1961. Distribution and deposition of inhaled particles in respiratory tract. *Bacteriol. Rev.* 25, 237–240.
- Hemmes, J.H., Winkler, K.C., Kool, S.M., 1960. Virus survival as a seasonal factor in influenza and poliomyelitis. *Nature* 188, 430–431.
- Henle, W., Henle, G., Stokes, J., Maris, E.P., 1946. Experimental exposure of human subjects to viruses of influenza. *J. Immunol.* 52, 145–165.
- Hirst, G.K., 1942. In vivo titrations of influenza virus and of neutralizing antibodies in chick embryos. *J. Immunol.* 45, 285–292.
- Horsfall, F.L., 1954. On the reproduction of influenza virus; quantitative studies with procedures which enumerate infective and hemagglutinating virus particles. *J. Exp. Med.* 100 (2), 135–161.
- Horsfall, F.L., 1955. Reproduction of influenza viruses; quantitative investigations with particle enumeration procedures on the dynamics of influenza A and B virus reproduction. *J. Exp. Med.* 102 (4), 441–473.
- Jao, R.L., Wheelock, E.F., Jackson, G.G., 1965. Interferon study in volunteers infected with Asian influenza. *J. Clin. Invest.* 44 (6), 1062 Abstract, 57th Annual Meeting of the American Society for Clinical Investigation, Atlantic City, N.J., May 3, 1965.
- Li, S., Eisenberg, J.N.S., Spicknall, I.H., Koopman, J.S., 2009. Dynamics and control of infections transmitted from person to person through the environment. *Am. J. Epidemiol.* 170 (2), 257–265.
- Loudon, R.G., Roberts, R.M., 1967. Droplet expulsion from the respiratory tract. *Am. Rev. Respir. Dis.* 95 (3), 435–442.
- Murphy, B.R., Chalhub, E.G., Nusinoff, S.R., Kasel, J., Chanock, R.M., 1973. Temperature-sensitive mutants of influenza virus. III. Further characterization of the ts-1[E] influenza A recombinant (H3N2) virus in man. *J. Infect. Dis.* 128 (4), 479–487.
- Murphy, B.R., Sly, D.L., Hosier, N.T., London, W.T., Chanock, R.M., 1980. Evaluation of three strains of influenza A virus in humans and in owl, cebus, and squirrel monkeys. *Infect. Immun.* 28 (3), 688–691.
- Murphy, B.R., Clements, M.L., Madore, H.P., Steinberg, J., O'Donnell, S., Betts, R., Demico, D., Reichman, R.C., Dolin, R., Maassab, H.F., 1984. Dose response of cold-adapted, reassortant influenza A/California/10/78 virus (H1N1) in adult volunteers. *J. Infect. Dis.* 149 (5), 816.
- Murphy, B.R., Clements, M.L., Tierney, E.L., Black, R.E., Steinberg, J., Chanock, R.M., 1985. Dose response of influenza A/Washington/897/80 (H3N2) avian–human reassortant virus in adult volunteers. *J. Infect. Dis.* 152 (1), 225–229.
- Nicas, M., Jones, R.M., 2009. Relative contributions of four exposure pathways to influenza infection risk. *Risk Anal.* 29 (9), 1292–1303.
- Nicas, M., Sun, G., 2006. An integrated model of infection risk in a health-care environment. *Risk Anal.* 26 (4), 1085–1096.
- 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.
- Riley, R.L., 1974. Airborne infection. *Am. J. Med.* 57 (3), 466–475.
- Riley, R.L., O'Grady, F., 1961. Airborne Infection: Transmission and Control. The Macmillan Company, New York.
- Ryan, M.A., Christian, R.S., Wohlrabe, J., 2001. Handwashing and respiratory illness among young adults in military training. *Am. J. Prev. Med.* 21 (2), 79–83.
- Sears, S.D., Clements, M.L., Betts, R.F., Maassab, H.F., Murphy, B.R., Snyder, M.H., 1988. Comparison of live, attenuated H1N1 and H3N2 cold-adapted and avian–human influenza A reassortant viruses and inactivated virus vaccine in adults. *J. Infect. Dis.* 158 (6), 1209–1219.
- Snyder, M.H., Clements, M.L., Betts, R.F., Dolin, R., Buckler-White, A.J., Tierney, E.L., Murphy, B.R., 1986. Evaluation of live avian–human reassortant influenza A H3N2 and H1N1 virus vaccines in seronegative adult volunteers. *J. Clin. Microbiol.* 23 (5), 852–857.
- Tang, J.W., Liebner, T.J., Craven, B.A., Settles, G.S., 2009. A schlieren optical study of the human cough with and without wearing masks for aerosol infection control. *J. R. Soc. Interface* 6 (Supplement 6), S727–S736.
- Tellier, R., 2006. Review of aerosol transmission of influenza A virus. *Emerg. Infect. Dis.* 12 (11), 1657–1662.
- Teunis, P.F.M., Havelaar, A.H., 2000. The beta Poisson model is not a single hit model. *Risk Anal.* 20 (4), 511–518.
- Teunis, P.F.M., Nagelkerke, N.J.D., Haas, C.N., 1999. Dose response models for infectious gastroenteritis. *Risk Anal.* 19 (6), 1251–1260.
- Teunis, P.F.M., Chappell, C.L., Okhuysen, P.C., 2002. *Cryptosporidium* dose response studies: variation between isolates. *Risk Anal.* 22 (1), 175–183.
- Teunis, P.F., Moe, C.L., Liu, P., Miller, S.E., Lindesmith, L., Baric, R.S., Le Pendu, J., Calderon, R.L., 2008a. Norwalk virus: how infectious is it? *J. Med. Virol.* 80 (8), 1468–1476.
- Teunis, P.F.M., Ogden, I.D., Strachan, N.J.C., 2008b. Hierarchical dose response of *E. coli* O157:H7 from human outbreaks incorporating heterogeneity in exposure. *Epidemiol. Infect.* 136 (6), 761–770.
- Weber, T.P., Stilianakis, N.I., 2008. Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. *J. Infect.* 57 (5), 361–373.
- Wei, Z., McEvoy, M., Razinkov, V., Polozova, A., Li, E., Casas-Finet, J., Tous, G.I., Balu, P., Pan, A.A., Mehta, H., Schenerman, M.A., 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. Meth.* 144 (1–2), 122–132.
- Xie, X., Li, Y., Chwang, A.T.Y., 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.
- Xie, X., Li, Y., Sun, H., Liu, L., 2009. Exhaled droplets due to talking and coughing. *J. R. Soc. Interface* 6 (Supplement 6), S703–S714.
- Youngner, J.S., Treanor, J.J., Betts, R.F., Whitaker-Dowling, P., 1994. Effect of simultaneous administration of cold-adapted and wild-type influenza A viruses on experimental wild-type influenza infection in humans. *J. Clin. Microbiol.* 32 (3), 750–754.