Absolute humidity modulates influenza survival, transmission, and seasonality

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Edited by Burton H. Singer, Princeton University, Princeton, NJ, and approved January 7, 2009 (received for review July 16, 2008)

Influenza A incidence peaks during winter in temperate regions. The basis for this pronounced seasonality is not understood, nor is it well documented how influenza A transmission principally occurs. Previous studies indicate that relative humidity (RH) affects both influenza virus transmission (IVT) and influenza virus survival (IVS). Here, we reanalyze these data to explore the effects of *absolute* humidity on IVT and IVS. We find that absolute humidity (AH) constrains both transmission efficiency and IVS much more significantly than RH. In the studies presented, 50% of IVT variability and 90% of IVS variability are explained by AH, whereas, respectively, only 12% and 36% are explained by RH. In temperate regions, both outdoor and indoor AH possess a strong seasonal cycle that minimizes in winter. This seasonal cycle is consistent with a wintertime increase in IVS and IVT and may explain the seasonality of influenza. Thus, differences in AH provide a single, coherent, more physically sound explanation for the observed variability of IVS, IVT and influenza seasonality in temperate regions. This hypothesis can be further tested through future, additional laboratory, epidemiological and modeling studies.

virus survival | vapor pressure | droplet nuclei | aerosol

There are 4 suspected modes of influenza virus transmission (IVT) (1): (*i*) transmission through direct physical contact with an infected individual, (*ii*) transmission via intermediate, often inanimate, objects (i.e., fomites), (*iii*) transmission via droplets expelled from infected individuals (e.g., by sneezing or coughing) that deposit on nasal or oral mucosa of a susceptible individual, and (*iv*) airborne transmission via expelled particles <2.5 μm in radius, which are referred to as droplet nuclei and remain suspended in air as aerosols for extended periods of time. The relative importance of these 4 transmission modes remains a subject of much debate (1–7).

A recent laboratory experiment examining influenza A transmission among guinea pig hosts suggests that airborne transmission may indeed be an important mode of IVT (8). In this study, groups of 4 infected and 4 susceptible hosts were maintained in separate cages at different temperature and relative humidity (RH) conditions. Transmission rates, measured as the percentage of susceptible hosts infected, were found to increase at lower RH. Two hypotheses were proposed to explain this relationship (8): (*i*) virus-laden droplet nuclei are more efficiently produced at lower RH because of increased evaporation of expelled droplet particles, such that more virus remains airborne longer; (*ii*) influenza virus survival (IVS) increases as RH decreases, such that the airborne virus remains viable longer at lower RH.

Heretofore, analyses of the effects of humidity on both IVS and IVT have focused on RH as the metric for air moisture. RH is the ratio of the actual water vapor pressure of the air to the equilibrium, or saturation, vapor pressure of the air. Because saturation vapor pressure increases exponentially as temperature increases, RH varies as a function of both the temperature and actual water vapor content of air. For instance, if the water vapor content of a volume of air remains constant, but the temperature of the air is increased, the RH will decrease. As a consequence, air with RH of 50% at 20 °C has much more water vapor than air with 50% RH at 5 °C.

RH is a meaningful physical quantity and for certain organisms may affect biological response; however, measures of absolute humidity (AH), i.e., the actual water vapor content of air irrespective of temperature, can be of greater biological significance for many organisms. Several such measures of AH are routinely used in meteorology, including specific humidity, mixing ratio, and vapor pressure (VP). VP can be calculated from just the temperature and RH, and is the measure of AH used for this analysis.

Results

Previous work has dismissed AH as a factor controlling IVS (9–10), although no physical, physiological, or statistical justification for this discounting has been provided. In addition, the effects of AH on IVT have not been previously studied. Using the data from the laboratory guinea pig study (8) described above, we calculated VP conditions for each experiment. We then linearly regressed the observed IVT rates on temperature, RH, and VP, in turn (Fig. 1 *Left*). The linear regression models for temperature and RH are marginally statistically significant ($P = 0.048$ and $P = 0.059$, respectively), whereas the regression upon VP provides a much more statistically powerful model ($P = 0.00027$). This finding suggests that VP exerts a much stronger control on airborne IVT rates than either temperature or RH.

We also repeated the regression analysis, this time including the results from 4 additional guinea pig experiments reported in a subsequent study by the same group (11). These additional IVT experiments were performed at high temperature (30 °C) but differing RH levels. The additional data improve the linear regression model for temperature ($P = 0.0013$) but reduce the relationship with RH to nonsignificant levels ($P = 0.098$). However, the statistical significance of the association with VP remains strongest ($P = 0.00011$); in addition, there is some indication that the relationship with VP is nonlinear (Fig. 1*F*).

Possible mechanisms underlying the strong association between VP and IVT rates are the same as for RH and include: (*i*) increased production of virus-laden droplet nuclei in low-VP conditions; (*ii*) increased IVS in low-VP conditions. We begin by examining the first mechanism.

Influenza-laden droplets expelled from infected hosts undergo 2 processes upon release into the atmosphere: sedimentation and evaporation. The sedimentation rates of small particles, such as virus-laden droplets, are determined by 2 opposing forces: gravitational acceleration, which brings the particle toward the surface of the earth, and Stokes drag force, a friction,

Author contributions: J.S. designed research; J.S. performed research; J.S. analyzed data; and J.S. and M.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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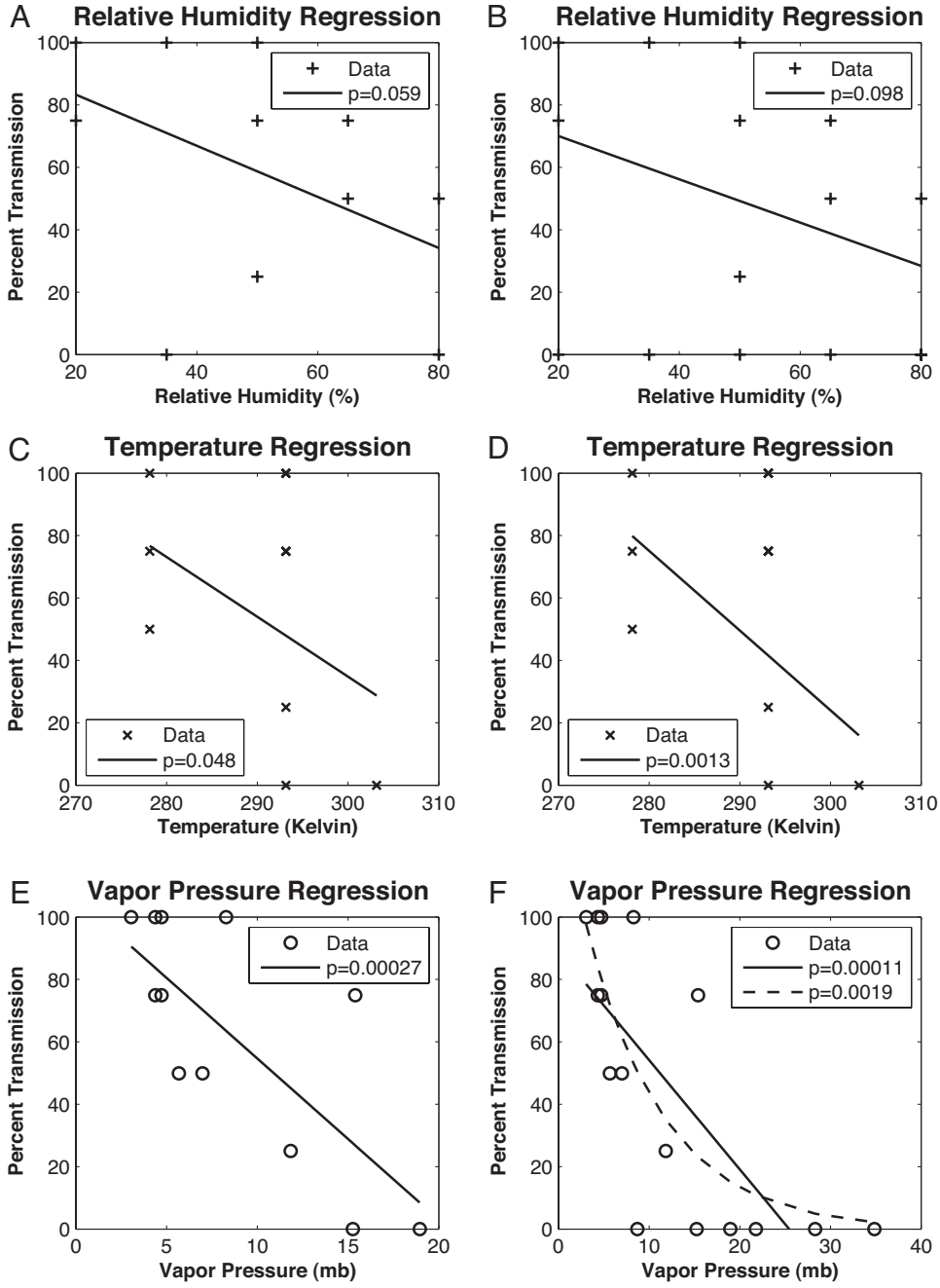

Fig. 1. IVT response to RH, temperature, and VP. (*Left*) Regression of guinea pig IVT data (8) ($n = 20$) on RH (*A*), temperature (*C*), and VP (*E*). (*Right*) Regression of larger guinea pig IVT dataset (8, 11) ($n = 24$) on RH (*B*), temperature (*D*), and VP (*F*). Significance of each model fit was assessed by using the *t* statistic for which the *P* value is shown in the legend. Symbols are the data; the black lines are linear regression model solutions. The dashed line plots the regression of log(percent transmission).

which provides an acceleration directed opposite to the particle motion, away from the surface (12). Gravitational acceleration is nearly constant, but Stokes drag increases with droplet velocity. Consequently, a droplet moving toward the surface will experience gravitational acceleration and speed up to the point at which its increased velocity engenders an equivalent acceleration directed away from the surface because of Stokes drag force. At that point, with the drag and gravitational forces in balance, the droplet neither speeds up nor slows down but proceeds at a constant terminal velocity. Smaller droplets have slower terminal velocities and, very small droplets, such as droplet nuclei, because of near-surface turbulence and air currents, may stay aloft for days.

Surface air is typically subsaturated, such that droplets expelled into this air also undergo evaporation. As water from a falling droplet evaporates, droplet size diminishes, and thus droplet terminal velocity is reduced. Whether an expelled virus-laden droplet reaches the surface or remains airborne as a droplet nucleus will depend on both sedimentation and evaporation rates. To explore this behavior, we modeled the evolution of different-size droplets as they fell and evaporated in various temperature and humidity conditions. Model simulations indi-

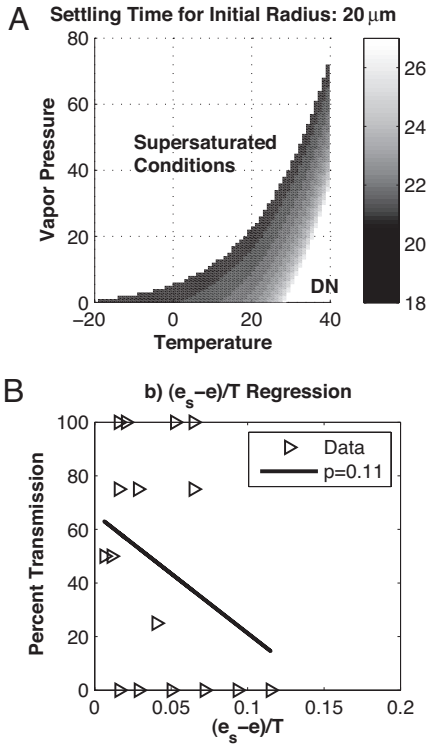

Fig. 2. Modeled droplet nuclei formation and IVT response to evaporation rates. (*A*) Time in seconds for a spherical droplet of pure water and initial radius of 20 μm to fall 1 m in different temperature and vapor pressure conditions. Conditions represented by the white area in the upper left of the plot were not modeled, because these represent supersaturated humidity levels. Droplets in the lower right area of the plot (labeled DN) evaporated to $r \leq 2\mu$m and were assumed to remain aloft as droplet nuclei. (*B*) Regression of IVT data (8, 11) ($n = 24$) on the ratio of vapor pressure deficit to temperature [$(e_s - e)/T$]. This regression model is not statistically significant ($P = 0.11$).

cate that droplet nuclei are indeed produced in typically observed surface air conditions through evaporation and that warmer temperatures and lower VP increase this production of droplet nuclei (Fig. 2*A*).

This evaporation of spherical droplets proceeds as:

$$\frac{dr}{dt} \propto \frac{e_s - e}{T}, \qquad [1]$$

where r is the droplet radius, t is time, e is vapor pressure, e_s is saturation vapor pressure, and T is temperature. The quantity $e_s - e$ is referred to as the vapor pressure deficit. Our model findings and Eq. **1** suggest that if the production of droplet nuclei via evaporation is the means through which changes in VP affect IVT, then a relationship between IVT and $(e_s - e)/T$ should also exist. Specifically, our model results demonstrate that increased evaporation produces smaller aerosols that stay airborne longer (Fig. 2*A*), and Eq. **1** shows that the rate of droplet evaporation is proportional to the ratio of vapor pressure deficit to temperature, i.e., $(e_s - e)/T$. Consequently, changes in $(e_s - e)/T$ modulate droplet nucleus production, and, if droplet nucleus abundance is important for airborne IVT, should also affect observed rates of IVT. However, when the guinea pig transmission data are linearly regressed upon $(e_s - e)/T$, a clear, statistically significant relationship fails to emerge (Fig. 2*B*). This evidence suggests that increased production of airborne droplet nuclei in low-VP conditions is not the means by which AH modulates IVT.

The alternate hypothesis is that IVS is affected by VP conditions. A number of laboratory studies have examined the effects of RH on airborne IVS (9–10, 13–19). Harper (15) provides one of the most detailed studies and is the only analysis to test IVS over a wide range of both RH and temperature conditions. For this study, aerosols were generated with an atomizer and stored in a rotating stainless steel drum from which samples were drawn and assayed for IVS at 5 and 30 min and 1, 4, 6, and 23 h after generation. These experiments were repeated at different RH and temperature conditions. The author found that IVS increased at lower temperatures and lower RH, but did not explore the effect of AH on IVS.

We calculated VP from the RH and temperature data reported by Harper (15), and compared IVS with all 3 meteorological variables. Scatter plots show evidence of some relationship between IVS and both RH and temperature (Fig. 3 *A* and *C*); however, a clearer, nonlinear relationship appears in the plot of IVS and VP (Fig. 3*E*). These relationships are confirmed by linear regression of both IVS and log(IVS) on each meteorological variable. For example, regressions of the log transform of IVS 1 h after aerosol generation reveal a statistically significant relationship with RH and temperature (Fig. 3*B*, $P = 0.054$; Fig. 3*D*, $P = 0.024$), but a much stronger, statistically significant relationship with VP (Fig. 3*F*, $P < 0.0001$) in which VP accounts for 90% of IVS variance. This finding suggests that VP strongly modulates airborne IVS, and that this modulation of IVS is the means through which VP affects airborne IVT.

A number of other studies of IVS and RH present more limited data (13, 16–18), which we also analyzed for the effect of VP on IVS. These data were found to be consistent with the relationship shown in Fig. 3 *C* and *F*. In fact, all of the laboratory studies of airborne IVS find, for a given temperature, the highest IVS rates at low RH (9–10, 13–19), as in Fig. 3*A*, and consistent with our findings for VP (Fig. 3*E*). Two of these studies (14, 19) find some evidence of lowest IVS at midrange RH of 40–60%, rather than high RH, but neither presents their data in a form that permits our analysis and comparison with VP as shown here.

Discussion

In temperate regions, influenza incidence is characterized by a strong peak during winter months (20), which is sometimes attributed to a wintertime decrease in indoor RH (Fig. 4*A*). Such assertions, however, do not square with the fact that outdoor RH peaks in winter (Fig. 4*B*). In contrast, both indoor and outdoor AH have a pronounced seasonal cycle marked by a prominent wintertime low (Fig. 4). Thus, the evidence presented here indicates that both IVT and IVS are much more tightly constrained by AH than RH (Figs. 1 and 3) and that seasonal changes in AH match seasonal patterns of influenza incidence better than seasonal changes in RH do (Fig. 4).

Recent theoretical work has shown that slight seasonal changes in transmission efficiency can produce the strong seasonal cycle in influenza incidence through dynamical resonance (21). The findings presented here indicate that the seasonal cycle of AH may be the factor producing this resonance. Specifically, low wintertime AH levels, both indoor and outdoor, increase influenza IVS and thus increase IVT efficiency.

These findings also suggest that humidification of indoor air, particularly in places where transmission to those at high risk for complications, such as nursing homes and emergency rooms, may help decrease the spread and the toll of influenza during influenza season. Previous studies using RH (8–10, 13–19, 22) also supported similar air humidification practices; however, these assertions were perhaps undermined by the marginal association of RH with IVS and IVT. Our findings, which attribute a much stronger effect of AH on both IVT and IVS (Figs. 1 and 3), suggest that humidification measures may be warranted.

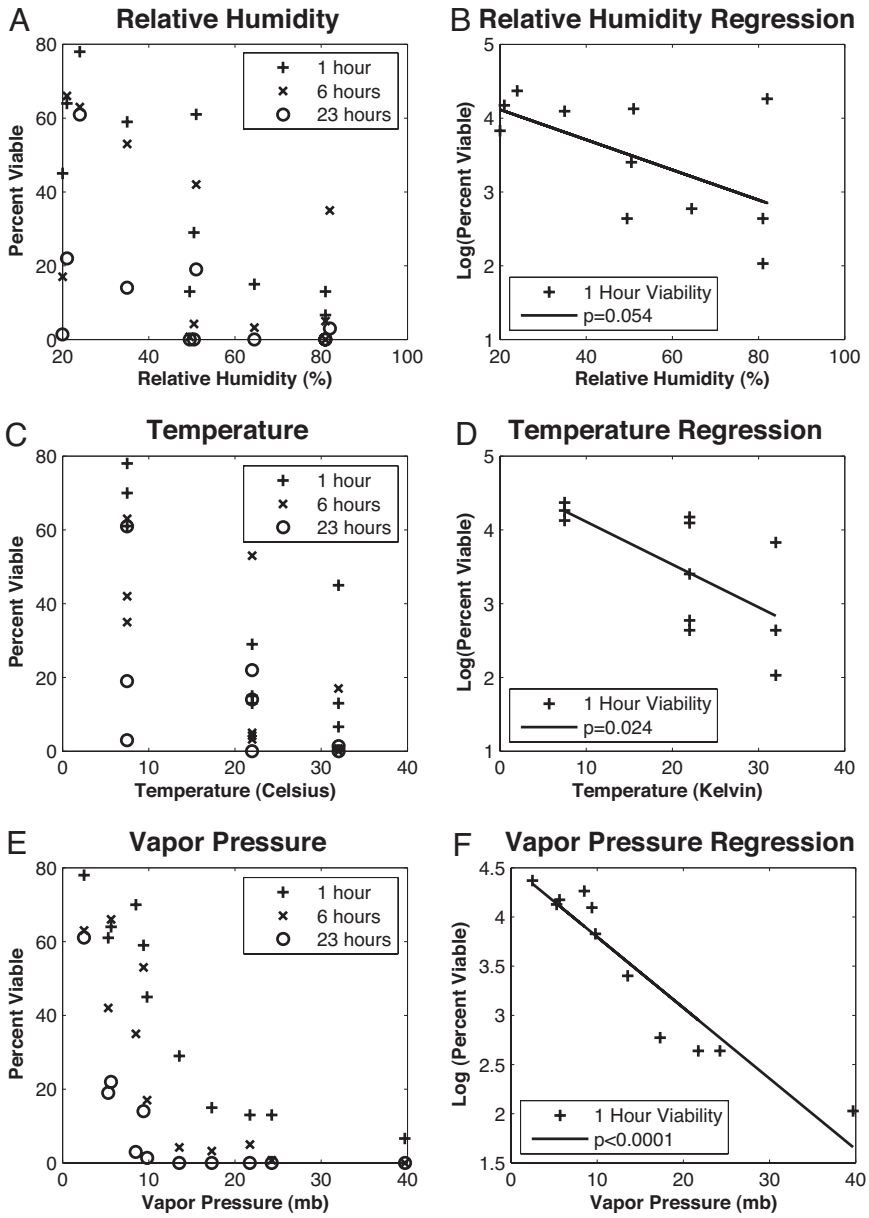

Fig. 3. IVS response to RH, temperature, and VP. (*Left*) Scatter plots of IVS data (15) ($n = 11$) at 1, 6, and 23 h after aerosol generation plotted versus RH (*A*), temperature (*C*), and VP (*E*). (*Right*) Linear regression of log(percent viable) at 1 h on RH (*B*), temperature (*D*), and VP (*F*). Significance of each model fit was assessed by using the *t* statistic for which the *P* value is shown in the legend. Symbols are the data; the black lines are regression model solutions.

The precise mechanisms through which AH affects IVS are not understood. The findings presented here indicate that IVS responds to the amount of water vapor in the surrounding air (i.e., AH), and not how close that air is to saturation (i.e., RH); however, it is unclear why the stability of an influenza virus encased within a droplet nucleus would be sensitive to atmospheric AH conditions. It has been hypothesized that high atmospheric humidity levels lead to surface inactivation of lipid-containing viruses, such as influenza (23). An exact determination of how IVS is affected by AH, and whether there exists any change in the size distribution of expelled particles, including coughed or sneezed droplets, under different environmental conditions, is needed.

The relationship between AH and IVS is strongly nonlinear (Fig. 3*F*), and there is the appearance of a similar nonlinear relationship between AH and IVT (Fig. 1*F*). A new, more comprehensive series of laboratory investigations in varying AH conditions is needed to validate these findings, determine whether the relationship with IVT is linear or nonlinear, and identify how IVT varies as a function of IVS.

The transmission efficiency findings shown in Fig. 1 are based on laboratory data using a guinea pig host animal model (8, 11). Although evidence indicates the guinea pig is a good model for influenza transmission to humans (24), the degree to which AH constrains IVS rates and airborne IVT efficiency to humans in the real world also needs to be further investigated.

The effects of AH on other modes of IVT should also be explored, including the effect on IVS for droplets settled on surfaces. A prior study indicates that IVS on fomites increases in lower RH (25); the effects of AH need to be examined as well. In addition, epidemiological models should be used to explore the effects of AH on influenza seasonality. Models in which IVT

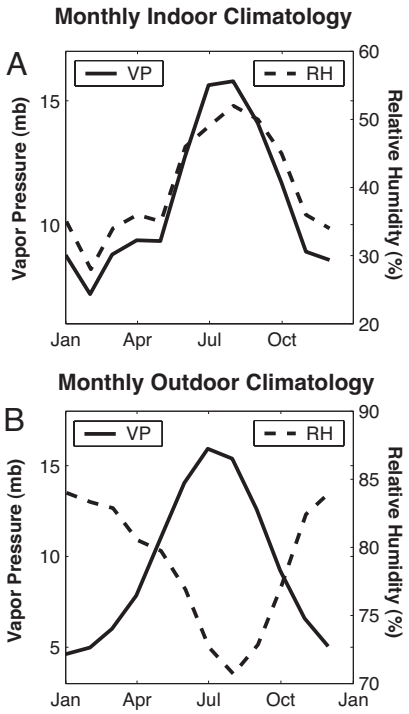

Fig. 4. Seasonal variations of VP and RH. (*A*) Plot of the monthly seasonal cycle of indoor VP in millibars and indoor RH in percent. Data are living room measurements taken during 1993–1995 in 8 dwellings in Gothenburg Sweden (31). (*B*) Plot of the monthly seasonal cycle of outdoor VP in millibars and outdoor RH in percent at the surface averaged for 130–70°W and 30–60°N. Data are National Center for Environmental Prediction–National Center for Atmospheric Research (NCEP–NCAR) reanalysis for 1968–1996 (32) and were provided by the National Oceanic and Atmospheric Agency/Oceanic and Atmospheric Research/Earth System Research Laboratory, Physical Science Division, Boulder, CO, from their web site at www.cdc.noaa.gov/.

rates are modulated by observed AH conditions can be constructed, and simulations run that attempt to replicate the seasonal cycle of influenza in both temperate and tropical regions. Such models might also be used to assess whether AH variability influences the annual geographical spread of influenza throughout the world.

Absolute humidity is predicted to increase in a warming world (26–27). The findings presented here indicate that such changes would decrease IVS and IVT rates. This potential outcome, as well as any effects on the seasonality of influenza incidence, needs to be explored carefully through additional laboratory and modeling experiments.

Methods

Vapor pressure was calculated from RH and temperature data using the Clausius–Clapeyron relation (12). Specifically, saturation vapor pressure was first calculated from the temperature data as:

$$e_s(T) = e_s(T_0) \times exp\left(\frac{L}{R_v}\left(\frac{1}{T_0} - \frac{1}{T}\right)\right),$$

where $e_s(T)$ is the saturation vapor pressure at temperature T, $e_s(T_0)$ is saturation vapor pressure at a reference temperature, T_0, L is the latent heat of evaporation for water, and R_v is the gas constant for water vapor. The reference temperature and saturation vapor pressure used were $T_0 = 273.15$ K and $e_s(T_0) = 6.11$ mb. VP, here denoted e, was then calculated by using the saturation vapor pressure and RH:

$$e = e_s(T) \times \frac{RH}{100}.$$

Spherical droplet settling was modeled through a coupling of equations for evaporation and sedimentation (12). The change in droplet radius because of evaporation was estimated as:

$$\frac{dr}{dt} = \frac{D(e - e_s)}{\rho_w R_v T r}, \qquad [2]$$

where r is the droplet radius, t is time, D is the diffusion coefficient of water vapor, e is vapor pressure, e_s is saturation vapor pressure, ρ_w is the density of liquid water, R_v is the gas constant for water vapor, and T is temperature. Droplet sedimentation was estimated as:

$$v = \frac{dz}{dt} = \frac{2\rho_w g r^2}{9\eta}, \qquad [3]$$

where v is droplet velocity, z is the height in meters, g is gravitational acceleration, and η is surface air viscosity.

The evolution of droplets was modeled as a coupling of Eqs. **2** and **3** for a range of temperature and VP conditions. Different initial droplet sizes, derived from studies of particle expulsion during coughing and sneezing (28–30), were modeled. Initial droplet height was 1 m, and sedimentation and evaporation were simulated for 1,000 s. Simulations were aborted if the droplet reached the ground, or if the droplet evaporated to $r \leq 2$ μm at which point it was assumed to remain aloft as a droplet nucleus.

Eqs. **2** and **3** describe the evolution of pure water droplets and represent an upper limit on droplet nucleus production. In fact, the presence of solutes and glycoproteins in expelled droplets will slow evaporation rates and, by virtue of their volume within the droplet, limit droplet size diminution. However, the model identifies conditions favorable to increased evaporation and droplet nucleus production and shows that droplet nucleus production may change substantially in a realistic range of temperature and humidity conditions.

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