

# Roles of Humidity and Temperature in Shaping Influenza Seasonality

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**Experimental studies in guinea pigs demonstrated that influenza virus transmission is strongly modulated by temperature and humidity. A number of epidemiological studies have followed up on these findings and revealed robust associations between influenza incidence in temperate regions and local conditions of humidity and temperature, offering a long-awaited explanation for the wintertime seasonality of influenza in these locales. Despite recent progress, important questions remain as to the mechanism(s) by which humidity and/or temperature affects transmission.**

In temperate regions of the globe, influenza epidemics show a marked wintertime seasonality, with circulation detected over a 2- to 3-month period between November and March in the Northern Hemisphere and between May and September in the Southern Hemisphere (1). In tropical and subtropical regions, patterns of influenza outbreaks are more diverse. While annual epidemics coinciding with the rainy season have been observed in many (sub)tropical locations, biannual incidence is the norm in some regions, and influenza activity occurs throughout the year in others (1).

The seasonal recurrence of influenza epidemics in temperate regions has been well characterized for decades. The factors driving this seasonality have, however, been poorly understood until recently. In efforts to account for the striking regularity of wintertime epidemics, a number of theories have been put forward over the years. These include fluctuations in host immune competence mediated by seasonal factors, such as melatonin and vitamin D levels, seasonal changes in host behavior, such as school attendance or crowding indoors during inclement weather, and environmental factors, including temperature, humidity, UV irradiation, and the direction of air movement in the upper atmosphere (2, 3). While correlations exist to support many of these putative mechanisms for influenza seasonality, experimental data establishing a causal link between any of these factors and influenza virus spread were sparse until 2007.

**Influenza virus transmission is dependent on humidity and temperature.** Using the then newly developed guinea pig model of influenza virus transmission (4), we tested directly the impact of ambient temperature and relative humidity (RH) on the efficiency of viral spread between hosts. When inoculated and exposed guinea pigs were housed in separate cages, transmission was found to be dependent on both temperature and RH (5, 6). Transmission was highly efficient at 5°C but was blocked or inefficient at 30°C. Dry conditions (20% and 35% RH) were also found to be more favorable for spread than either intermediate (50% RH) or humid (80% RH) conditions (Fig. 1A). These results were obtained initially using a seasonal human strain, A/Panama/2007/1999 (H3N2), and were subsequently confirmed with a 2009 pandemic isolate, A/Netherlands/602/2009 (H1N1). Transmission at low (5°C) versus intermediate (20°C) temperatures was also tested with two influenza B viruses and found to be more efficient under colder conditions (7). Thus, transmission of human influenza viruses by a respiratory droplet or aerosol route in the guinea pig model proceeds most readily under cold, dry conditions. These findings suggested two means by which environmental factors may drive the wintertime

seasonality of influenza in the Northern Hemisphere. Average daily temperatures outdoors are lower in winter. Conversely, average outdoor RH is higher in winter than in summer, essentially excluding outdoor RH as a possible driver of influenza outbreaks. However, because indoor air is commonly heated, indoor RH is lowest during the winter. Thus, exposure to cold air outside or dry air inside during the wintertime may increase influenza virus transmission and potentially trigger a flu season.

When similar experiments were performed with guinea pigs housed together in the same cage, little to no effect of humidity or temperature was seen (7, 8). Transmission among caged guinea pigs can occur through direct contact between guinea pigs, indirect contact via contaminated fomites, or the transfer of respiratory droplets over a short range. Our data suggest that one or all of these modes of transmission are less sensitive to environmental conditions than is respiratory droplet spread over a greater distance.

It should be noted that, while the guinea pig is an excellent model to study influenza virus transmission, influenza disease in this host is mild and does not fully recapitulate the disease signs seen in humans. Differences in the responses of human and guinea pig hosts to infection may impact transmission. Nevertheless, the utility of the guinea pig model is evidenced by the high efficiency with which human influenza viruses transmit among guinea pigs, coupled with reduced or no detectable transmission of strains adapted to swine, avian, or laboratory hosts.

**A role for absolute humidity.** The two physical properties identified, RH and temperature, are inextricably linked. RH is a measure of the water content in a gas, relative to the maximum capacity of that gas to hold water vapor. The maximum capacity, in turn, varies with temperature. While RH is the most relevant parameter when considering, for example, the rate with which water will evaporate from a respiratory droplet, it is a somewhat complex means of describing the water content of air. Temperature is removed from the equation when one considers absolute humidity (AH), the mass of water per unit volume of a gas.

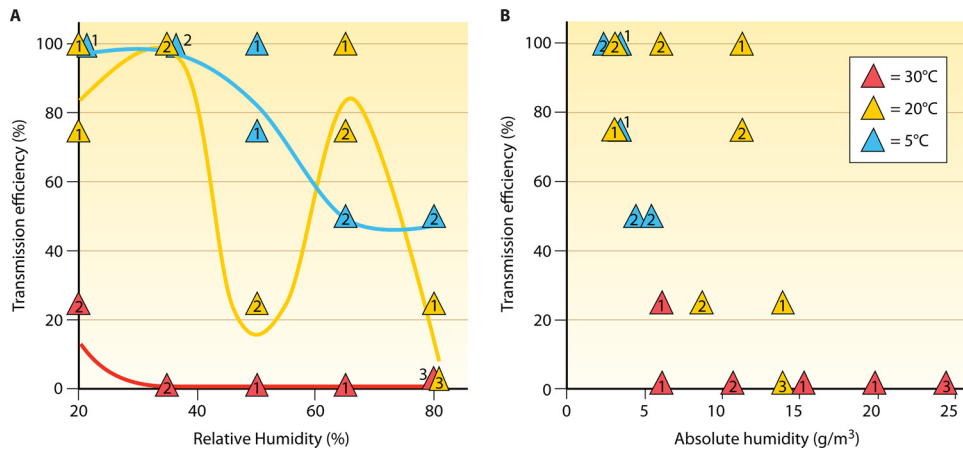
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**FIG 1** The efficiency of respiratory droplet transmission in guinea pigs varies with humidity and temperature. Transmission efficiency, calculated as the percentage of exposed guinea pigs that contracted infection, is plotted against relative humidity (A) and absolute humidity (B). In each case, data points obtained at 5°C are blue, those obtained at 20°C are yellow, and those obtained at 30°C are red. Numbers within or adjacent to the symbols indicate the number of replicate experiments represented. Trend lines shown in panel A were drawn freehand. Data are reported in references 5, 6, and 8, with the exception of the results of the experiment at 5°C and 20% RH, which is unpublished. The data shown include results obtained with both the A/Panama/2007/1999 (H3N2) and A/Netherlands/602/2009 (H1N1) viruses.

The observed dependence of influenza virus transmission on both RH and temperature raised the question of whether transmission efficiency was in fact modulated by absolute humidity. Reanalysis of our data in a manner similar to that shown in Fig. 1B indicated a statistically significant relationship between AH and transmission (9). Nevertheless, several of our transmission results are not explained by this relationship. For example, where AH was equal to  $\sim 6 \text{ g/m}^3$  and  $\sim 11 \text{ g/m}^3$ , the results of 5 and 6 experiments, respectively, spanned the full spectrum from 0/4 to 4/4 contacts infected (Fig. 1B). While the number of replicates under each set of RH and temperature conditions is limited, we saw consistent results between experiments and between the viruses tested (Fig. 1A) (5, 6). For these reasons, we suggest that the outlying data points on the plot of transmission versus AH are meaningful and may point to the existence of more than one mechanism by which humidity and temperature affect transmission. Additional experimentation is needed to fully resolve whether transmission varies solely with AH or is modulated independently by temperature and humidity.

**Possible mechanisms linking temperature and humidity to transmission.** It remains unclear precisely why influenza virus transmission is most efficient under cold, dry conditions, but a number of possible mechanisms have some experimental support. Through nebulization of virus into a chamber followed by serial sampling and determination of infectious titers, influenza virus stability in an aerosol was shown to vary with RH (or AH, given that temperature was held constant) (10, 11). Influenza virus was consistently found to be stable at low RH and relatively unstable at intermediate RH (e.g., 50% RH). Studies differ in the results seen at higher humidities; stability was low in some reports (11) but high in others (10). A more recent examination of the problem reconciles the findings at high humidity by demonstrating a dependence on salt and protein concentrations within droplet media (12). The authors of that report furthermore proposed that the effect of RH on virus viability is mediated by salt concentration within droplets: at high RH, physiological concentrations are maintained and viruses are relatively stable, at intermediate RH,

evaporation leads to increased salt concentrations, resulting in virus inactivation, and at low RH ( $< 50\%$ ), salts crystallize out of solution, yielding low salt concentrations and high virion stability (12). Taken together, these reports suggest that improved stability of influenza viruses within aerosols may account for the enhanced transmission seen at low ambient humidity.

Influenza viruses are also known to be more stable in the cold; thus, robust transmission at 5°C and highly inefficient transmission at 30°C may be due to an increased virus half-life at lower temperatures. In considering the effects of temperature, it is important to note that guinea pigs shed higher titers of influenza A and B viruses when housed in the cold (5, 7). As discussed below, increased shedding may be due to an effect of cold conditions on the host. Alternatively, virus may be more stable within the nasal passages when the epithelial surface is cooled by colder ambient air (13). Increased virion stability at lower temperatures is likely due in part to decreased activities of proteases. In addition, changes in the physical properties of the virion envelope may contribute. At temperatures of  $< 41^\circ\text{C}$ , domains of ordered and disordered lipids were found to coexist within virion membranes. The fraction of lipids within ordered domains, or a gel phase, increased with decreasing temperature (14). Lipid ordering has not, as yet, been linked directly to infectivity, however.

In addition to having an effect on the virus, temperature and humidity may affect the host side of the host-pathogen equation by altering susceptibility to influenza virus infection or the course of disease following infection. Cooling of the nasal epithelium through inhalation of cold air has been shown to inhibit mucociliary clearance and may limit phagocytosis by innate immune cells resident in the upper airways (13). Similarly, inhalation of dry air for a 30-min period was found to slow mucociliary clearance significantly (15). Both cold air and dry air are thought to alter the rheological properties of mucus (13, 15). At lower temperatures, cellular metabolic functions are also slowed, which in turn may decrease the frequency of ciliary beats, reduce mucus secretion, and restrict phagocytosis (13).

Finally, environmental conditions may impact transmission

through effects on the vehicle itself, the respiratory droplet. The length of time a droplet remains airborne, and therefore available for inhalation, is dependent on its size: droplets of  $>20\ \mu\text{m}$  in diameter settle out of the air rapidly, whereas those of  $<5\ \mu\text{m}$  remain airborne for prolonged periods (16). Evaporation of water from respiratory droplets, which occurs more rapidly with declining RH, decreases droplet size and therefore increases the distance and time over which transmission can occur.

Thus, cold and/or dry conditions impact the stability of influenza virus particles, the innate defense of host nasal epithelia, and the production of infectious bioaerosols. Each of these effects contributes a plausible explanation for the impact of RH and temperature on respiratory droplet transmission, and more than one mechanism most likely contributes to the observed transmission outcomes.

**Meteorological predictors of influenza virus outbreaks.** To test whether the observed impact of environmental factors on influenza virus transmission does in fact drive the seasonal periodicity of influenza, a number of epidemiological studies comparing influenza incidence to climatic conditions have been performed. The association between the month (or months) of peak influenza activity and a number of climatic variables was recently assessed for 78 localities around the globe. Analysis of average monthly temperature, RH, precipitation, solar radiation, and specific humidity (a measure of AH) revealed that, at high latitudes, influenza peaks coincided with months of lower temperature, lower solar radiation, and lower specific humidity. In contrast, peak influenza activity in localities within  $10^\circ$  of the equator correlated with months of high specific humidity and precipitation. At intermediate latitudes ( $12.5$  to  $25^\circ\text{N/S}$ ), no significant association was observed. The authors concluded that, across temperate and tropical climates, two distinct types of climatic conditions are associated with influenza epidemics: cold/dry and humid/rainy (1). Similarly, using data specific to the United States, the date of onset of influenza epidemics was strongly associated with periods of anomalously low absolute humidity and temperature conditions in the weeks prior to the epidemics (17). A mathematical model incorporating observed AH data, as a modulator of influenza virus transmission rates, was furthermore successful in simulating observed influenza-related death rates for individual states (17). AH also correlated with influenza activity in the Netherlands and Japan (18, 19). Importantly, AH and temperature conditions are highly correlated in meteorological data; thus, it is difficult to distinguish the two in terms of their association with influenza outbreaks. In sum, strong correlations exist between influenza activity and low AH and temperature conditions in temperate regions of the world. The picture is very different in the tropics, suggesting that distinct mechanisms underlie influenza seasonality in temperate versus tropical locales.

Low relative humidity was not found to be associated with influenza outbreaks in human populations (1, 17, 18). This is not surprising, however, since outdoor RH is maximal in the winter. The studies cited employed data on local weather conditions in their analyses; indoor RH, which is typically low in heated buildings, was not evaluated. Thus, further epidemiological studies are needed to determine whether influenza seasons are significantly correlated to indoor RH as well as outdoor temperature and AH. Given that indoor heating, and therefore RH, are linked to low outdoor temperatures, an association between low RH indoors and influenza activity is not improbable.

Despite the association of both AH and temperature with epidemics, AH has been put forward as the most likely driver of influenza seasonality in temperate climates (17). The rationale behind this suggestion is that temperature is highly regulated indoors during the winter, where individuals spend most of their time. Levels of AH, by contrast, are similar in indoor and outdoor environments. It is important to note, however, that a very small seasonal change in transmission rate is thought to be sufficient to drive large changes in influenza incidence, due to amplification through dynamical resonance (20). Thus, relatively brief exposure, or extended exposure of relatively few individuals, to cold temperatures may have a large impact on viral circulation.

**Conclusions.** Epidemiological analyses, spurred by experimental data on influenza virus transmission and stability, have identified absolute humidity and temperature as climatic predictors of influenza epidemics in temperate regions of the world. Transmission experiments using the guinea pig model indicate that the association between these environmental factors and influenza seasonality is due to their impact on the efficiency of respiratory droplet transmission. The mechanism(s) by which temperature and humidity alter transmission outcomes remains unclear but may include multiple effects acting at the level of the host, the virus, and the respiratory droplet. Further experimentation is needed to understand which of the potential mechanisms is at play. The relationship between salt concentrations and virus viability warrants further examination, and relatively simple analyses performed with bulk fluids rather than small droplets may be informative. Although the dilution of exhaled aerosols in the air is often a technical limitation, sampling of air surrounding infected hosts could be used to evaluate whether increased respiratory droplet production, increased infectivity of virus in droplets, or decreased droplet size are observed when infected animals are exposed to cold or dry conditions. Transmission experiments in which AH is held constant but RH and temperature are varied may provide valuable data for determining conclusively which of these factors affects transmission. Finally, if an appropriate caging system can be devised, testing transmission between recipient animals housed in warm or humid air and donor animals housed under standard conditions—or vice versa—would indicate at which stage(s) unfavorable environmental conditions block transmission. Despite recent advances, the mechanisms underlying influenza seasonality still provide fertile ground for further discovery.

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