Environmental Role in Influenza Virus Outbreaks

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Abstract

The environmental drivers of influenza outbreaks are largely unknown. Despite more than 50 years of research, there are conflicting lines of evidence on the role of the environment in influenza A virus (IAV) survival, stability, and transmissibility. With the increasing and looming threat of pandemic influenza, it is important to understand these factors for early intervention and long-term control strategies. The factors that dictate the severity and spread of influenza would include the virus, natural and acquired hosts, virus-host interactions, environmental persistence, virus stability and transmissibility, and anthropogenic interventions. Virus persistence in different environments is subject to minor variations in temperature, humidity, pH, salinity, air pollution, and solar radiations. Seasonality of influenza is largely dictated by temperature and humidity, with cool-dry conditions enhancing IAV survival and transmissibility in temperate climates in high latitudes, whereas humid-rainy conditions favor outbreaks in low latitudes, as seen in tropical and subtropical zones. In mid-latitudes, semiannual outbreaks result from alternating cool-dry and humid-rainy conditions. The mechanism of virus survival in the cool-dry or humid-rainy conditions is largely determined by the presence of salts and proteins in the respiratory droplets. Social determinants of heath, including health equity, vaccine acceptance, and age-related illness, may play a role in influenza occurrence and spread.

The interpandemic global burden of influenza is estimated at 1 billion cases of clinical influenza, 3–5 million cases of severe illness, and 300,000–500,000 deaths annually (1). Influenza, caused by three types of influenza viruses (A, B, and C), is an acute respiratory disease in humans and animals. It usually manifests as regular seasonal epidemics and occasional severe pandemics in humans and as epizootics and panzootics in animals. Influenza pandemics owing to influenza A virus (IAV) continuously threaten existing public health and veterinary infrastructure. Several factors have been identified for the severity and spread of influenza globally, including the virus, natural and acquired hosts and host factors, and the environmental and anthropogenic factors that affect these three elements. Here, we attempt to review the contribution of the environment to the survival and spread of IAV, although it is not possible to segregate these intricately interlinked factors.

Influenza viruses have a negative sense RNA genome and belong to the family Orthomyxoviridae. The eight segments of the viral genome of IAV encode 16 protein products, including hemagglutinin (HA), neuraminidase (NA), nucleoprotein, M1, nonstructural protein (NS) 1, polymerase acidic protein (PA), polymerase basic protein (PB) 1, and PB2, that are directly translated, as well as some alternatively spliced proteins (NS2, M2, and M3), some by ribosomal frame shift (PB1-F2, N40, and PA-X) and some by different in-frame translation initiation codons (PA-N115 and PA-N182) (2, 3). In nature, 18 HA and 11 NA subtypes are found (4); H1-16 subtypes with varying combinations of N1-9 circulate subclinically by fecal-oral transmission in aquatic waterfowl, with periodic spillover to other species. The H17N10 and H18N11 subtypes are found exclusively in bats (5, 6).

Influenza viruses are air- and waterborne pathogens with the capacity to infect a wide variety of hosts and undergo genetic reassortment with seasonal patterns; this, along with rapid globalization, potentiates influenza as a repeated threat to public health. Thus, understanding factors (i.e., biotic- viral determinants, host factors, and abiotic-environmental factors) affecting viral persistence and hence transmission would enable us to deal with influenza more effectively.

INFLUENZA VIRUS IN THE ENVIRONMENT

Globally diverse IAVs are widely distributed in wild aquatic birds and other shore birds, most particularly Anseriformes (e.g., ducks) and Charadriiformes (e.g., gulls) (7, 8). The natural history of avian influenza virus (AIV) over the past 140 years has been well documented (9–12). A total of 16 HA and 9 NA antigenic subtypes have been found in dabbing ducks. It is suggested that most if not all IAV have an avian host somewhere in the past, including host-adapted viruses to humans, equines, and swine, with the exception of H17 and H18 in bats (13). Influenza prevalence among mallards is seasonally dependent, with peak season during the autumn migration (14), and is driven by host density (i.e., number of naïve juvenile birds) during breeding and/or migratory periods (14, 15), as well as other abiotic factors that affect viral survival in the environment (16). Phylogenetically, AIV HA show high subtype diversity and little internal genetic diversity (17). Extensive diversity is also observed in NA and NS genes, whereas five remaining gene segments (PB2, PB1, PA, NP, and M) are highly conserved. IAV in wild-type birds may exist as functional gene segments, which are interchangeable and form transient genome constellations, without the strong selective pressure to be maintained as linked genomes (18). Interestingly, evolutionary divergence has been observed with highly diverged AIVs in spatially separated regions of the world, from Asia to Antarctica (19–22). The so-called evolutionary sinks, in which AIVs are seeded into distinct geographic regions of the world and then become established to evolve independently, suggest specific AIV reservoirs dependent on geographic separation and availability of wild birds/animals that can support AIV replication.

Primary introduction of low-pathogenicity influenza virus into acquired species such as domestic poultry is a result of wild aquatic and shore bird activity. Evidence indicates that prevalence of low-pathogenic influenza outbreaks in poultry farms is correlated with the migratory season (23, 24), stages of flyways routes (25, 26), farm conditions (e.g., turkeys in range, ducks on fattening fields) (27, 28), and probable waterfowl contact (24). Other means, such as through pigs and humans, have also been implicated in introducing low-pathogenic influenza virus into the domestic poultry population (29, 30). Secondary spread within the poultry population is due largely to mechanical transfer of infected feces either by personnel movement (e.g., caretakers, farm owners, and staff) or by fomites (i.e., delivery trucks, inseminators) (31–34). Owing to such dynamic transmission, some of the low-pathogenic strains, such as H9N2, have become endemic in domestic poultry populations (35). Occurrences of H9N2 incidence have been reported in various parts of the world, including Europe (36, 37), Africa (38), the United States (39), several parts of the Middle East (40), and Asian countries (41–43). Similarly, low-pathogenic H7N2 has been endemic in the domestic poultry population of the United States (44). Field-based poultry, such as quail and pheasants, carry receptors for both avian- and mammalian-adapted IAV in their upper respiratory tract and potentially could serve as reservoirs and mixing vessels (45). Pheasants have also been shown to shed some subtypes, such as H10 AIV, for extended periods of time (46).

High-pathogenic (HP) AIV strains arise owing to antigenic drift/shift within low-pathogenic strains. Most HP strains belong to H5 and H7 subtypes (7). The end of the past decade saw the emergence of HP H5N1 viral strains; the progenitor strain was believed to be from an endemic H5N1 strain that originated from commercial geese in the Guangdong province of China in 1996 (47), and by approximately 2003–2004, the H5N1 strain spread across Asia (48). By 2005–2006, cases of the HP H5N1 were found in Europe (49, 50) and African countries (50, 51). Both wild birds and transmission by personnel have been implicated for the widespread nature of this epizoonosis (52, 53).

The mechanism by which influenza virus crosses species barriers remains elusive. Pigs are major carriers and act as mixing vessels (15). Since the late 1970s, avian-like swine H1N1 has been detected in circulation among European swine populations (54). The recent 2009 H1N1 pandemic is a triple assortment between avian, swine, and human influenza viruses (55). Epizoonosis of avian-derived IAV in various mammals has been reported. H3N8 affected equines from China in 1989 (17), H7N7 affected harbor seals from the United States in 1980, and H10N4 was reported in domestic mink in Sweden during 1984 (56). Most avian-like influenza viral subtypes do not infect humans, with the exception of H5N1, H7N7, H7N2, H7N3, and H9N2. Most of the human infections occur owing to direct contact with infected birds. The largest documented zoonosis caused by avian-like influenza in humans was due to H5N1 in Europe, Asia, and Africa (57). Smaller epizoonotic outbreaks have been reported from the Netherlands with H7N7 (58) and from Canada with H7N3 (59), and a few cases of human infection have been attributed to low-pathogenic H9N2 (60). Studies have pointed to the contribution of continuous spillover of H5N1 from wild birds to domestic poultry as a major factor, which leads to maintenance of this subtype among the human population (17, 57).

Similarly, mammalian-to-mammalian host switch has been documented. Epizoonosis of equine influenza H3N8 has been reported from humans (61), dogs (62), and pigs (63). Several instances of interspecies zoonotic transmission events have occurred from swine to humans, including asymptomatic infections to the recent swine-origin H1N1 pandemic of 2009 (64–68). During 1976, an outbreak of classical swine H1N1 infection was reported in Fort Dix, New Jersey, and human-to-human transmission was also reported in this outbreak. However, most swine-adapted influenza strains do not result in a stable host switch and emergence of a pandemic

influenza viral strain (69). In recent years, there have been several outbreaks of influenza in humans owing to swine-origin IAV, including the variant H3N2 virus and other subtypes (66–68).

Among other mammals, cats were considered to be resistant to IAV infection and disease. In recent years, cats were shown to be naturally susceptible to IAV, including H5N1 and 2009 pandemic H1N1 viruses (70, 71). Domestic and wild felids have been shown to be susceptible to natural and experimental infection with IAV, exhibiting a plethora of clinical signs ranging from systemic disease to subclinical infection with seroconversion (72–76). Reports of infection with other subtypes of IAV in cats and other felids are minimal, but experimental evidence indicates that cats are more susceptible to IAV, including low-pathogenic aquatic waterfowl-origin viruses (77). Infection with IAV in other mammals is also rarely reported. Bovine are susceptible to infection with IAV, and multiple subtypes have been isolated from cattle (78). Indirect serological evidence indicates that respiratory disease and reduction in milk yield could be induced by IAV in cattle (79). Influenza C viruses (ICV) were reported only in humans, pigs, and dogs (80, 81). However, antibodies to IAV, influenza B viruses, and ICV have been recorded in cattle (82). Recently, an ICV-like virus was reported from swine with influenza-like illness (83). Subsequently, three bovine viruses genetically similar to ICV-like swine virus were isolated and, based on serology and genome characteristics, these viruses from swine and cattle are proposed to be included in a new genus, influenza D virus (84).

BIOTIC AND ABIOTIC FACTORS AFFECTING IAV PERSISTENCE IN THE ENVIRONMENT

Waterfowl, including Anseriformes and Charadriiformes, act as major carriers of 16 HA subtypes of IAV in the wild (15, 85, 86). Transmission and persistence of AIV among wild birds are waterborne transmission processes that are regulated by host density (87) and other abiotic factors (16). AIV remains infectious for months in low-temperature water and for over a week at 22°C (88, 89). Multiple lines of evidence point to the persistence of AIV in water (16, 90–97). Survivability of both low-pathogenic and HP influenza virus is influenced by physicochemical factors, such as pH, salinity, and temperature (90, 98, 99). The loss of influenza-virus infectivity over time in various water samples has been investigated (92, 100). The viral infectivity and persistence are dependent on both viral strain and physicochemical characteristics of water (92). A recent study on the effects of physicochemical variables in surface water samples collected from 38 different waterfowl habitats distributed across the United States showed that influenza virus persisted for a longer period at low temperature (<17°C), neutral-basic pH (7.0–8.5), and low ammonia concentration (< 0.5 mg/L) (100). The results were comparable to a previous in vitro study, which showed IAV survivability is more stable in water at lower temperature, slightly basic pH, and lower salinity (101, 102). However, the factors controlling the environmental persistence and transmission of AIV via aquatic habitats are poorly understood. Several studies point to the seasonal variation of IAV prevalence in waterfowl, which is probably driven by the influx and aggregation of juvenile birds during breeding and migration and favorable environmental conditions, including optimum pH, temperature, and salinity of water, that promote survival outside the host (16). Current evidence supports the idea that even minor fluctuations in temperature, pH, and salinity in aquatic habitats may enhance or diminish persistence and infectivity of AIV (97), but how these variables affect individual AIV subtypes is unknown. Besides, there is a lack of field validation of experimental results, as several variables may affect persistence and infectivity of AIV in water bodies.

Influenza survivability in mammals and domestic birds has been attributed to viral reassortment, which indirectly influences the replication fitness and persistence among the population (103, 104). However, reassortment does not contribute to viral-replication fitness in wild birds and

viral persistence in water (105). AIV has cryostability in frozen environmental waters (106) and persists in aquatic flora and fauna (107, 108). The migratory water birds may interlink various water bodies at various geographic locations through their flyways, and water bodies in arctic and subarctic regions remain frozen for up to 4–10 months annually. Consequently, virus shed by the migratory birds in these water bodies can remain entrapped in ice during the winter months, which has potential implications in the ecophylogenetics and epidemiology of influenza virus among wild waterfowl (106, 109).

Unlike in domestic poultry, pigs, and humans, influenza is subclinical in most species of aquatic birds. Migratory patterns, and the ability of IAV to undergo antigenic shift and drift in waterfowl, provide a classic reservoir host niche. The ecology and evolution of IAV in this niche are subject to alteration by migratory behavior and anthropogenic environmental changes, including land use, agricultural practices, globalization, and climate change (14). The role of migratory birds in the transmission of influenza is heavily debated (110, 111). The long-term persistence of the influenza virus gene pool in North American wild birds might be independent of the migratory flyways as migration between populations throughout North America occurs (112). For example, the AIV gene pool in the Charadriformes of Delaware Bay was not represented in the Anseriformes of North America, whereas the AIV genetic diversity in Anseriformes in Alberta significantly contributed to the gene pool in Anseriformes in North America (112). Analyses of host-pathogen models using attributes of within-season transmission dynamics, between-season migration and reproduction, and environmental variation show that environmental transmission provides a persistence mechanism within small avian communities (113). However, note that wild birds are capable of being infected with and transferring HP H5N1 AIV over long distances (114). The HP avian influenza (HPAI) H5N1 virus was also pathogenic to wild birds. However, available evidence suggests that migratory wild birds are not capable of sustaining H5N1 HPAI viruses for more than a few years (110), and in countries where H5N1 becomes endemic, backyard waterfowl may serve as reservoirs (115).

PERSISTENCE OF INFLUENZA A VIRUS IN AIR

The diversity of viruses circulating in a given local/regional population contributes to the possibilities for emergence of new IAVs owing to viral reassortment. Unlike in avian hosts, IAV usually spreads by airborne or contact routes in other species. Multiscale analysis of factors influencing virus persistence in the environment and within a host has predicted that virus transmission is predominantly regulated by temperature-dependent decay, whereas virus load, virulence, and host immune response impart a negligible influence (116).

Airborne transmission of IAV is the major route of transmission in mammalian hosts. Coughing, sneezing, talking, exhaled breath, showering, tap water use, sewage aerosolization, wet cleaning of indoor surfaces, and agricultural spraying produce droplets ranging in size from <1 to 2,000 μm (117, 118). After expulsion, the evaporation rate of these droplets is dependent upon temperature and relative humidity (RH). Evaporation ceases when the aerosol's surface vapor pressure attains equilibrium with the RH (119). Rate of evaporation in turn affects droplet size and pathogen viability (118).

Droplet size is determined by temperature, RH, and composition of the droplet (117, 118). It is generalized that 10-μm particles account for 99.9% of droplet volume, and particles 4–6 μm in size are usually respired (117, 120). Droplets of >20 μm in size settle owing to gravity (117). Fate of droplet dispersion/settling can be predicted based on their size, Brownian motion, gravity, turbulent diffusion, and other physical factors (117). In general, under standard atmospheric conditions, droplets of sizes <100 μm evaporate before reaching the ground, and the droplet

residue remains suspended in air for a prolonged period of time (117). Therefore, under given environmental conditions, the droplet size can determine the airborne and/or contact transmission rate of influenza viruses.

Both the aerosol size and settling rate influence the rate of inhalation and where within the respiratory system the droplets will deposit. The settling velocity of a droplet is proportional to its diameter squared (118). Therefore, smaller aerosols can remain suspended in air for longer periods of time, whereas larger droplets settle quicker (104). Inhalability of droplets of size >50 μm is determined to be below 30% (121). Droplets 6.1 μm, 2.7 μm, 1.4 μm, and 4.7 μm in size may deposit efficiently in the head airways (87.4%), the tracheobronchial region (6.1%), the alveolar region (12.8%), and the whole respiratory tract (94.8%), respectively (120, 122). Recently, several studies have measured the influenza RNA content in various droplet sizes. Presence of influenza viral RNA has been reported predominantly from droplets of sizes <1 μm (123–125). In one study, 64% of virus-laden samples were found in particles less than 2.5 μm with enough virus to infect if inhaled for 1 h at minimum (124).

INACTIVATION OF VIRUS IN AIR

Survival capabilities of influenza virus in aerosols have been studied intensively (124, 126–128). Maximal survival time in droplets has been found to vary between 1 and 24 h depending on the RH and influenza strain (124, 127). Virus viability is also influenced by factors such as ultraviolet (UV) radiation, salt concentration, porous/nonporous surface, and open air factors (104). Ability of the UV rays from sunlight to inactivate influenza virus varies (from <2.3–9.4 $\log_{10}$/day), depending on the geographical location and season (129, 130). Open-air factors are composed of resultant variations in air environment that arise owing to interaction of, e.g., pollution, ozone, and electromagnetic radiations, at a given temperature and RH as compared with the indoor/built-in environment. Some of these open-air factors, such as pollutants and ozone, have been reported to inactivate IAV (104, 131). The exact mechanisms by which influenza viruses are inactivated in an aerosol environment remain to be demonstrated experimentally. However, several mechanisms of inactivation have been hypothesized. These include (*a*) RNA damage owing to UV, (*b*) loss of lipid bilayer structural stability owing to temperature and/or water content of the droplet (i.e., RH and absolute humidity), and (*c*) loss of glycoprotein structural conformation owing to increased temperature (104). The relative inactivation rate of IAV in an aerosol environment may depend on the size and composition of the respiratory droplet (132).

ENVIRONMENTAL FACTORS RELATED TO INFLUENZA VIRUS TRANSMISSION AND STABILITY

Studies on the persistence of IAV in the environment outside the host are very limited (88, 104), and there is a complete lack of information on IAV genomic stability in the environment. The survival of different subtypes of IAV in aqueous environments (16, 90, 97) and on surfaces (133, 134) is well documented. Few studies suggest that susceptibility of virus in water and on surfaces to a given temperature was not due to genomic degradation (102, 135). For example, using lentivirus pseudotyped cleavable HA, it was reported that high temperature and salinity had a negative effect on virus survival (98, 101, 102), and the nature of the HA plays a role in the virus stability in the aqueous environment (102, 136). Molecular instability of HA in excess salinity or high temperature may affect the tertiary structure (137). However, these studies used lentiviral pseudotyped HA in single cycle infectivity assays, and the stability of HA was correlated to infectivity. It remains to be seen whether the infectivity of different subtypes of IAV would follow the same pattern under

varying environments (air and water), including temperature; salinity; relative/absolute humidity; and physical factors such as UV rays, pH, mechanical forces, and the presence or absence of inactivating chemicals. Although we have demonstrated that the relationship between influenza virus infectivity and relative humidity was dependent on droplet composition (118), the molecular stability of HA was not determined in our study. It should also be borne in mind that physical factors such as temperature may affect viral polymerase activity and alter infectivity (138, 139), and uncleaved HA are more stable in the environment than cleaved HA. This explains why duck influenza viruses with uncleaved HA spread better in aqueous environments than HPAI H5N1 with cleaved HA (140). Low pH at 37°C in the absence of target cells can inactivate IAV (141). Low-pH inactivation is due to HA conformational changes that affect viral fusion to target cells (142, 143). Similarly, the inactivation of IAV by UV light is dependent on the distance from the source and the shallowness of the exposed surface (144), necessitating the presence of virus on surfaces and in air. It is important, therefore, to understand the environmental factors that dictate the stability and persistence of IAV to develop mitigation strategies.

SURVIVAL OF INFLUENZA A VIRUS IN BUILT-IN ENVIRONMENTS

Many of the studies that described outbreaks of IAV in relation to the built-in environment in hospital wards were inconclusive and did not take into account the ventilation rates inside the buildings (145, 146). A one-dimensional spatial model has been developed to evaluate the spatial dynamics of airborne droplet transmission and the influence of airflow on disease spread in ventilated and unventilated environments. It predicted that smaller droplets (0.4 μm) are weak disease vectors owing to their small viral load (147). Although viral RNA can be found in <1-μm droplets, the amount of virus in smaller droplets may be insufficient and may require a longer exposure time for infection to set in. The droplet diffusion rate in an unventilated environment, based on a typical Brownian diffusion timescale, was estimated to be 10^9–10^{13} days for 4-μm droplets and 10^8–10^{12} days for 0.4-μm droplets, for coverage of 10^1–10^3 m. For a short-term spread of infection, diffusion is an insufficient mechanism to transport droplets throughout the domain (147). Human movement has been attributed to be the main cause for disease spread in a homogeneous setup (147). Conversely, in a heterogeneous scenario, where infected individuals recover before coming in contact with susceptible populations, the rate of secondary outbreaks was influenced mainly by ventilation rate and the subsequent transport of the droplet (147). Other studies on avian influenza models in the wild have predicted the dynamics of HPAI outbreaks to be influenced by the presence of increased migratory bird populations and high-density poultry production (148). A recent systematic review found strong and sufficient evidence on the association between ventilation, air movements in buildings, and the transmission/spread of infectious diseases, such as measles, tuberculosis, chicken pox, influenza, smallpox, and SARS (149).

TRANSMISSION OF INFLUENZA VIRUSES

The three proposed modes of influenza transmission that are not mutually exclusive include contact (direct and indirect), large respiratory droplets, and small droplet nuclei (aerosols) (103, 104). Understanding each of these modes of transmission is of great importance, as it influences the choice of infection control measures in health-care settings and animal agriculture. As of now, the relative roles of each of these modes of transmission have not been established.

Large droplets (≥5 μm in diameter) are generated during coughing, sneezing, breathing, and talking by the infected individual. These droplets can be propelled over a distance of 1 m by air currents and deposited on the nasal or oral mucosa of a new susceptible host or in their immediate

environment (103, 150). Owing to their larger size, these droplets do not remain suspended in air (150). Infectious viral RNA can be found in both large particles (>5 μm) and small particles (<5 μm) during tidal breathing (151–153). Influenza aerosols are smaller droplet nuclei (<5 μm) that will remain suspended for prolonged periods of time and desiccate quickly (104, 150). Infectious aerosols can also be produced during tidal breathing, with concentrations of particles varying from 1 to >10,000 particles per liter, with the majority measuring <0.3 μm in diameter.

Analysis of indoor air in a day-care center revealed that 64% of the influenza viral genome copies were associated with fine particles <2.5 μm in size and a concentration of $1.6 \pm 1.2 \times 10^5$ copies of viral genome per m^{-3} air per hour (124). Noninvasive ventilation and chest physiotherapy produced droplets in a size range >10 μm, whereas aerosols were produced by a nebulizer, hence suggesting a possibility of IAV transmission during these procedures at health-care set up (154). A more recent study evaluated the infectivity and load of virus aerosol in the exhaled breath of an infected individual and the effect of surgical masks in curtailing viral shedding from the exhaled breath (153). The results showed that fine particles (size <5 μm) contained 8.8-fold more viral copies than coarse particles, and the presence of a surgical mask produced an overall 3.4-fold reduction in viral aerosol shedding (153). Another study determined that approximately 35% of the influenza RNA was contained in particles of >4 μm, whereas 23% was in particles of 1–4 μm and 42% in particles of <1 μm particles created during human coughing (123). A recent modeling study based on data from two randomized controlled trials of surgical masks and hand hygiene, conducted in Hong Kong and Bangkok, also indicated that aerosol transmission accounted for half of all transmission events in households (155). These studies point to the fact that airborne transmission of influenza is more probable, especially in close range. Therefore, strategic control measures must be planned for the needs of a given environment. Given the basic reproductive number of ~1.5 for IAV, public health measures to reduce the overall transmission by approximately one-third are likely to be successful. Interestingly, in human challenge studies, low doses of aerosolized IAV are more likely to induce typical influenza-like disease with fever and cough than is contact or droplet transmission (156, 157), suggesting smaller aerosolized particles induce a vigorous response in the conducting airways.

Influenza is also transmitted via direct and indirect contact. The direct contact mode of transmission refers to transmission of IAV through direct contact with the infected host, whereas indirect-contact transmission is a passive mode of transmission involving an intermediate object rather than direct person-to-person contact. This mode of influenza transmission involves a susceptible host coming in contact with a contaminated nonporous surface or environment. Large respiratory droplets (>5 μm) are more likely to be involved in this type of transmission. All three modes of transmission have been confirmed in animal studies with ferrets and guinea pigs (150, 158). In light of recent studies in animal models, aerosols, and modeling based on clinical intervention strategies, the importance of contact and large droplet transmission modes for larger outbreaks is seriously questioned (152, 153, 155, 158).

To develop efficient control measures, a thorough understanding of how influenza virus spreads between farms is imperative. Only limited studies exist on farm-to-farm transmission modes of influenza viruses. Outbreaks of IAV in farms are usually attributed to short inter-farm buffer distance; critical farm density; local reproduction number; improper disposal of carcasses and other organic wastes into environment; entry of feral birds into the shed; and cross-contamination through farm workers, equipment, sharing of egg trays, and vaccination crews (44, 159–163). A case-control study on transmission of equine influenza during an outbreak in Australia has also attributed a fomite mode of transmission (164). Studies on swine influenza have also proposed that farm management conditions, both housing system–level and farm-level, could potentially influence the disease spread among pigs (165). However, climatic conditions of the

farm did not contribute to the infection spread rate among the swine population (165). On the contrary, a study by Bos et al. (166) noted that none of the risk factors, including housing system, bird density, or species, contributed to HPAI transmission rate within the flocks.

A recent study assessed the airborne transmission of swine influenza virus in farms by evaluating airborne IAV using RT-PCR (167). Viral RNA was detected in barn indoor air, exhaust air, and air samples collected between 1.5 and 2.1 km away from the farm. Therefore, it is speculated that IAV infectious aerosols originated in pig farms could be transported downwind (167). Similar studies conducted in Brazil have also identified that 9% of asymptomatic piglet tracheal samples showed IAV positivity in RT-PCR (168). Hence, continuous exposure of farm animals or humans to the aerosols generated in-farm could potentiate zoonotic IAV transmission to human caretakers.

It is important to differentiate airborne transmission to wind-related transmission of IAV. Aerosol transmission of IAV is dependent on the size of the infectious droplets, whereas wind-related transmission on the contrary points to the direction of spread of IAV in the direction of wind. There are difficulties in defining windborne transmission as a mechanism. For example, even without a causal association, a correlation can be found if the index farm is located west of a densely populated farm area and if there were westerly winds (169). Geographic information systems (GIS) can be used to understand parameters involved in windborne spread and subsequent exposure to the virus (170). Large quantities of particulate matter are generated in a farm as a result of routine activity (171). Sedlmaier et al. (172) showed influenza virus remained viable in particulate matter originated from chicken fecal samples, and the virus viability in chicken feces was dependent on both temperature (20° C) and high RH. A consistent trend was observed between new infected premises and predominant wind patterns in equine influenza outbreaks (173), whereas the rate of airborne transmission among chickens is low or unlikely (160, 174). A recent modeling study to understand the dispersal pattern of avian influenza between farms (175) took into account the quantity of viable virus and reproduction rates, along with abiotic factors, such as wind speed, settling velocity, and diffusion for model prediction. It was predicted that windborne dispersion of AIV could play a significant role in shorter-distance transmission rate, but this alone cannot be sufficient to support long-range transmission. The animal-to-animal transmission rate under field conditions is dependent on infected animal density, and the contact transmission mode was found to be more efficient than airborne transmission in the field (160).

Secondly, most windborne transmission estimates fail to take into account the possibility of transmission through insects (176) and free-flying birds (177) that carry the virus from an infected farm in the direction of the wind. Taking these factors into consideration, Ypma et al. (169) conservatively estimated the contribution of a possible wind-mediated transmission to 18% during an avian influenza H7N7 outbreak in 2003. Adegboye & Kotze (178) recently analyzed H5N1 outbreaks in Nigeria using a point process model and predicted that the spread and transmission of H5N1 are dependent on geographical heterogeneity, seasonal effects, temperature, wind, and proximity to the first outbreak.

SEASONALITY OF INFLUENZA A VIRUS

There are distinct transmission patterns of influenza around the world. Peak seasonal influenza occurs in temperate regions during late winter and early spring (179, 180) and in tropical and subtropical regions during the rainy season each year (181, 182), and a biannual incidence is suggested to be the norm (183). Several factors, both biotic and abiotic, are ascribed to the seasonality of influenza. These include host immune status; host behavior (staying indoors during winter and overcrowding in public places, including schools); and temperature, absolute and

relative humidity, direction of air movement in upper atmosphere, and UV exposure (184, 185). Several studies have shown that temperature and humidity play a major role in IAV transmission. Using a mouse model, Schulman & Kilbourne (186) reported that, apart from host age and virus virulence, environmental factors such as temperature and humidity contributed to IAV transmission. Lowen et al. (187) further demonstrated, using the guinea pig model, that airborne transmission efficiency of influenza virus was dependent on RH at 20°C and independent of RH at 30°C. At lower RH (i.e., 20–35%), the transmission efficiency was higher compared to at higher RH (50–80%) (188). Transmission efficiency was at the lowest at mid-range RH (i.e., 40–60%). Their elegant studies using the guinea pig model suggested that IAV transmission is efficient at 5°C with dry conditions and blocked at 30°C (187–189). The IAV survival trend showed an asymmetrical V-shaped curve at various RH at 20°C (190). In England, the 2009 pandemic flu strain caused three waves of epidemics during the period from 2009 to 2011. The third wave occurred during the period from November 2010 to February 2011. In 2013, Dorigatti et al. (191) pointed out that this third wave of flu epidemic in England was possibly due to cold weather, which along with virus fitness and waning preexisting immunity in the population increased the transmission rates.

RH is defined as the ratio of partial pressure of the water vapor in air to the saturated vapor pressure of water at a given temperature. As saturation vapor pressure is exponentially related to temperature, RH varies depending on both temperature and water vapor content in air. Hence, both temperature and RH have an effect on evaporation, in turn affecting droplet size (192). Persistence of viral infectivity in aerosol is prolonged at lower humidity, which in turn results in a lower viral dose requirement for transmission (156, 188). Recent studies have shown that both size distribution and the dynamic of influenza virus emitted from coughing are influenced by RH (128, 193). Results of these studies suggested that virus inactivation is linearly associated with increasing RH, whereas at lower RH there is increased virus survivability. These studies also postulate that settling is an important mode of removal for larger droplets, whereas ventilation and inactivation are important for removal of influenza virus associated with aerosols.

Absolute humidity refers to the actual water vapor content in air, irrespective of the temperature. In an epidemiological study, absolute humidity was strongly associated with influenza transmission efficiency as compared with RH (194). In temperate regions, low absolute humidity strongly correlates with flu epidemic onset (194). The correlation between longitudinal weather and influenza mortality data, observed by using a flexible regression model during the period from January 1973 to December 2002 in urban US counties, suggested that half of the average difference in the seasonal influenza mortality could be attributed mainly to absolute humidity alone, whereas temperature imparts only a modest influence (195). In tropical countries, such as Singapore, there is a negative correlation between upper and lower respiratory tract infection and RH (196).

The mechanism of IAV survival under differing RH and thereby seasonality was explained recently. Virus survival is proposed to be dependent on the salt and protein concentration of the droplet medium (132). It is further proposed that at high RH, virus survives in the moist environment of the droplet under physiological concentrations of salt; at intermediate RH, salt concentration increases because of evaporation that inactivates the virus; and at low RH, salts crystallize out of solution and leave the virus intact (132). It is also suggested that under cool, dry indoor conditions, such as winter in temperate countries, low RH may allow IAV aerosols to persist longer in air owing to their smaller size, thereby permitting effective IAV transmission, whereas in tropical countries, the low temperature and near-saturation RH during rainy seasons afford transmission through large droplets and/or aerosols. A recent modeling study based on data from hand hygiene and facemask efficacy studies in subtropical Hong Kong and Bangkok indicated that aerosol spread remained the dominant mode of IAV transmission (155). Taken

together, these studies suggest that IAV transmission is dependent on temperature and humidity and that virus viability in aerosols is determined by the RH and the salt and protein concentrations of the droplet.

OTHER ENVIRONMENTAL FACTORS

Many meteorological studies attempting to link climatic conditions to influenza seasonality have been performed. Apart from temperature and humidity, air pollution, UV radiation, and precipitation affect transmissibility of influenza virus. An association between rainfall and influenza transmission has been reported in India (197) and Bangladesh (198), whereas no such trend was found in Singapore, Hong Kong, Ulaanbaatar, Vancouver, Brisbane, Melbourne, and Sydney (199). In a study in Egypt, rainfall was negatively correlated with human influenza incidents (200). Some of the drawbacks in Indian, Bangladeshi, and Egyptian studies are lack of statistical significance, limitations in study design, and small sample numbers, respectively. Another study suggested that rainfall might increase the exposure to acute respiratory infections in tropical regions owing to increased crowding (201). A recent study has shown that influenza virus is susceptible to UV exposure that is negatively correlated to RH (202).

Air pollution represents one major concern in urban environments. In a seven-year study (2001 to 2008) conducted in Brisbane, the interaction effects between ozone levels, particulate matter, and nitrogen oxide level were compared with temperature during pediatric influenza. The study found significant interaction between particulate matter and mean temperature in pediatric influenza, whereas the ozone level–influenza incidence relationship was independent of temperature (203). Sloan et al. (131) recently pointed out that correlation between air pollution and infectious disease varies depending on the city, region, and pollutant under investigation. The environmental drivers of IAV survival and transmissibility are provided in **Table 1**.

To date, the most comprehensive modeling study on climatic variability and seasonality of influenza sampled 78 sites globally and determined that there were two types of environmental conditions associated with influenza seasonality and epidemics: cold-dry and humid-rainy conditions (183). Although this model predicted influenza seasonality in high versus low latitudes reasonably well, it performed poorly in mid-latitude sites where large seasonal swings in climate were evident, suggesting that the semiannual outbreaks in these sites are probably due to cool-dry versus humid-rainy conditions predisposing to influenza outbreaks (183). This study also proposed that using specific humidity to determine transmission has a low predictive power at low- and mid-altitude sites and therefore should be considered inconsistent. A comprehensive outline of various biotic and abiotic factors affecting transmission and outbreak of influenza is shown in **Figure 1**.

EFFECTS OF VIRUS EVOLUTION AND INTERSPECIES SPREAD IN TRANSMISSION

IAV remains a strong candidate for possible pandemics owing to its ability to infect a wide range of hosts (humans, animals, and avian species) and its ability to undergo mutations and reassortment (**Figure 2**). Genetic drift and reassortment are two mechanisms for the generation of genetic variability of influenza viruses and have been reviewed thoroughly (204–206). Genetic drift occurs owing to the accumulation of nucleotide mutations in the viral genome. Most of these nucleotide substitutions are silent mutations so that viral replication fitness is not compromised, i.e., negative selection (204). However, certain situations, such as host switch, could induce a selective pressure on the virus and thus result in increased heterogeneity among the viral progeny, i.e., positive

Table 1 Environmental drivers of influenza outbreaks

Driver	Effect on virus stability and/or transmission	Reference
Droplet size		
Large droplets (>5 um)	Travel less than 1 m and deposit on surfaces, enabling transmission by droplet and contact modes	117, 118
Smaller droplets (<5 um)	Remain suspended in air for longer periods, enabling aerosol transmission	117–120
Deposition	Larger droplets deposit in the upper respiratory tract, whereas smaller droplets evaporate quickly, forming droplet nuclei that are inhaled deeply into the lungs	104, 117, 120, 122
Persistence in water	Minor fluctuations in temperature, pH, and salinity enhance or reduce stability and transmission	100–102
Migratory behavior	Sustain viruses in small avian communities but fail to sustain HPAI	113–114
Persistence in air		
Temperature	Cooler temperatures enhance virus survival and transmission	124, 127, 131
Humidity	Low RH and high RH (cool-dry or humid-rainy conditions) facilitate virus survival, and intermediate RH decreases virus stability; absolute humidity and specific humidity have inconsistent roles in transmission	123–125, 132
Other environmental factors	Solar radiation kills influenza A virus but is negatively correlated to RH Air pollution: Particulate matter and mean temperature correlate with pediatric influenza Ozone levels and influenza survival independent of temperature Windborne transmission: virus particles in droplets or fomites carried in the direction of wind	104, 124, 127, 131, 169, 175, 178

selection (for example, antigenic drift in HA). Substitutions that result in immune escape variants potentiate enhanced viral replication and transmission fitness (204, 206).

Amino acid changes in HA protein, such as Q222L, G224S, E186D, K189R, S223N, and N182K, have been shown to modulate virus-receptor interaction in H9N2 and H5N1 strains (207–211), whereas Q226L mutation resulted in enhanced replication and transmission of an H9N2 strain (212). In vitro experiments using H5N1 have shown that none of these mutations contribute to airborne transmission (209, 213). Apart from the HA gene, mutations in other viral proteins contribute to changes in viral fitness and transmission, including viral polymerase protein PB1 (206, 214, 215) and PB2 (216, 217). The roles of other viral proteins—PA, NP, NA, M, and NS—in determining host range and transmissibility remain to be elucidated. Therefore, transmission efficacy of a given influenza strain depends on receptor specificity, viral fitness, amount of virus shed, duration of shedding, and virus stability in the environment. Increased transmission to a susceptible host may be due to a longer duration of virus shedding when there is high viral titer in the source host (205).

Reassortment occurs when the host is coinfected with two or more influenza strains, as well as the resulting exchange of one or more gene segments between different influenza viral strains. Owing to the segmented genome of the influenza virus, reassortment of genetic material is more efficient in this group of viruses. Reassortments are of more biological importance as they lead to novel influenza viral strains and rapid viral adaptation to the changing environment, i.e., host

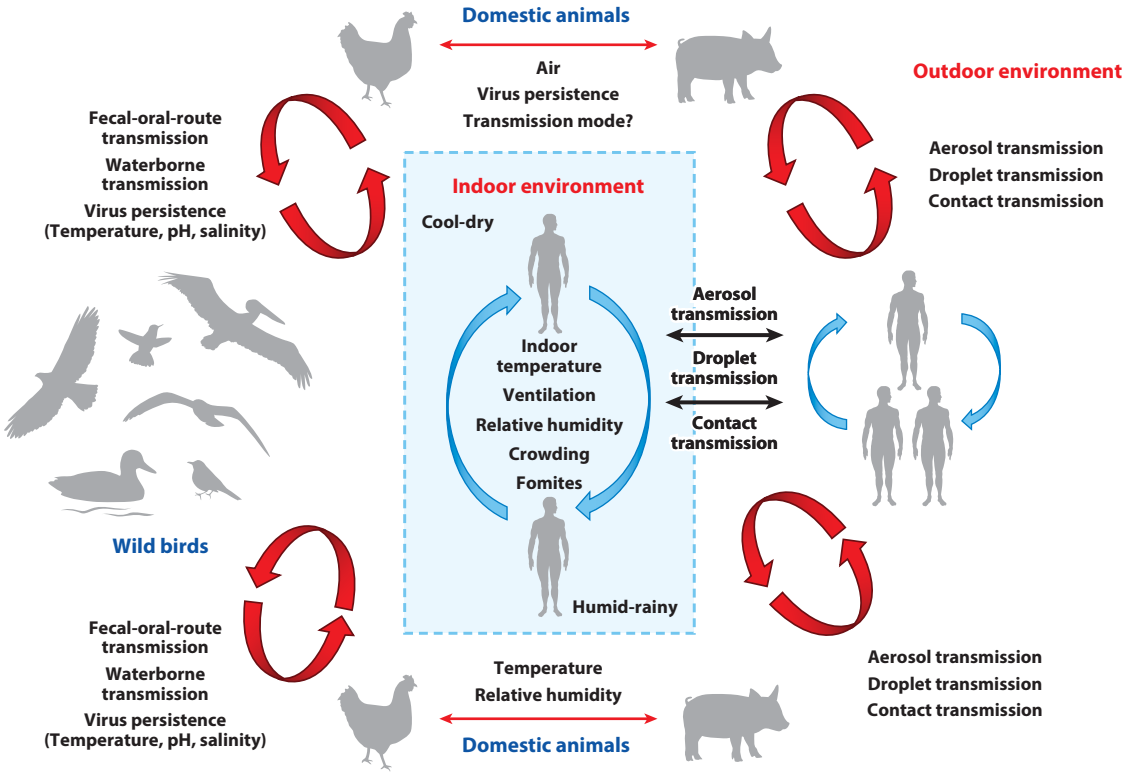

Figure 1

Environmental drivers of influenza A virus persistence, transmission, and host switch. The role of environment, including virus persistence in water and air, modes of transmission, and the relative roles of temperature and humidity in maintaining virus in natural and acquired hosts, is described schematically.

change (204). In addition, reassortments are the major contributors of emergence of pandemic strains. The recent 2009 H1N1 pandemic was a result of reassortment between a swine triple-reassortant virus and European avian-like influenza viruses (206).

Mechanisms by which influenza virus crosses the species barrier remain an enigma. Several factors, such as the cell receptor, replication fitness, the counteracting host's immune response, and persistent viral shedding, have been postulated to contribute to the zoonotic potential of influenza virus (218). Phylogenetic studies have shown that most of the influenza virus infections in mammals (including humans) have avian origins (17).

At the turn of twentieth century, the 1918 influenza pandemic strain was associated with swine influenza. In the 1930s, a classical swine influenza strain, likely derived from the 1918 strain, remained in circulation among pig populations in the United States and worldwide (17). During the 1970s, a novel H1N1 lineage emerged in Europe as a combination of avian and swine influenza. From 1998, triple reassortant influenza strains of H3N2, H1N2, and H1N1 emerged and began to circulate among the swine population in the United States and worldwide. During the past decade, avian-to-human transmissions of H5, H7, and H9 virus subtypes have occurred (218), and cases have been reported in Europe, Africa, and the Middle East (17, 219). There is very little evidence showing direct avian-to-human transmission of low-pathogenic influenza virus.

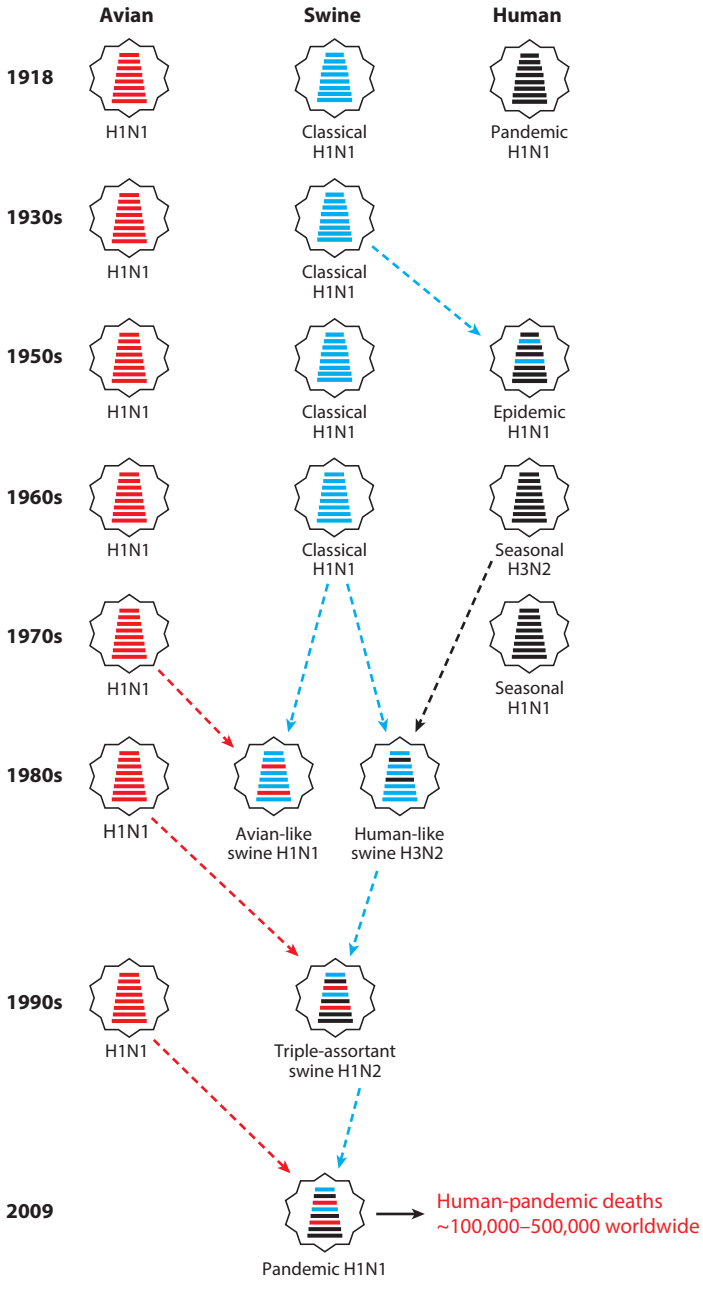

Figure 2

Emergence of pandemic influenza A virus strains by reassortment of genomes since the first pandemic of 1918.

Because pigs support both avian and human influenza strains, they are known to be mixing vessels (15). This characteristic is attributed to the presence of both α2,3 and α2,6 sialic acid linkages on the glycocalyx of epithelial cells lining the pig trachea. Recent studies have also shown

that the sialic acid receptor pattern in the pig respiratory tract is similar to that in the human respiratory tract (220, 221). Lu et al. (222) showed that general patterns of reassortment among five internal segments (PB1, PB2, M, PA, and NP) remain similar, with the exception of the NS gene, which presented more divergent phylogeny. This study also pointed out the presence of significant variation in reassortment rates between subtypes, depending on host species. They further postulate that wild bird populations, rather than domestic poultry, are the major source of new reassortants (222). Therefore, factors other than receptor affinity should also be considered when evaluating influenza zoonosis.

METHODS OF STUDYING TRANSMISSION USING ANIMAL MODELS, AND THEIR RELATIVE MERITS AND DEMERITS

In recent years, several reviews have analyzed influenza transmission and the factors influencing it (56, 103, 104, 150). In vitro studies have shown that clinical influenza could be produced in mice, ferrets, ponies, squirrel monkeys, and humans exposed to an aerosolized suspension of IAV. Comparisons of intranasal inoculation with inhalation of aerosolized virus studies were done in both humans and mice from the 1940s to the 1970s. In mouse models, intranasal inoculation with a small quantity of virus was sufficient to cause high morbidity (223), and it also resulted in increased viral replication in the lower respiratory tract (i.e., lungs) as compared with the upper respiratory tract (i.e., trachea) (224). However, in humans a small quantity of virus could induce disease even when delivered in an aerosol form (156). Another study showed that experimentally inoculated IAV resulted in attenuated disease as compared with the naturally acquired disease (157). A major drawback of these studies is that the level of inhaled virus remains unquantified.

An earlier study, in the 1940s, used a ferret model to show that influenza transmission can occur between the source animal and exposed animal even when they are separated by up to 2.5 m, and this was dependent on the direction of air flow from the source to the exposed animal (225). A more recent study showed that AIVs were incapable of transmission between ferrets, either by direct contact or via airborne (droplet and/or aerosol) transmission. When researchers substituted the HA and NA genes from an avian strain to the 1918 pandemic strain, they achieved direct contact transmission between ferrets, and addition of PB1-F2 of the 1918 pandemic strain resulted in airborne transmission (217). This change in transmission ability is presumably attributed to the higher replication rate of the influenza in the upper respiratory tract (217, 226).

Palese's group conducted a series of studies on transmission and factors that affect transmission in guinea pigs. In their first study, they showed that guinea pigs were readily infected by human strains of IAV without any prior viral adaptation, that the virus replicated in both the upper and lower respiratory tracts, and that the virus was readily transmitted between guinea pigs (158). In their later studies, they also showed that a mutation in the PB2 gene (216) influenced transmission ability of the influenza virus strain via the airborne route. Further studies also showed that temperature affects viral replication in infected animals (188), whereas RH influences viral survivability in the environment (187). They also provided stronger experimental evidence for aerosol transmission by placing the cage of the contact animal above the cage of the source animal at a distance of 80 or 107 cm (227). The limitation of these studies is that both the source and contact guinea pigs were kept in two different cages side by side or one over the other; hence, the level of contribution of the droplet and droplet nuclei mode of transmission remains obscure. Other factors that contribute to disease transmission between guinea pigs are the strain of virus used and the strain of host (228). Mathematical modeling has shown that association between viral replication in epithelial cells, human immune response, and viral titers plays an important role in

affecting viral dynamics and hence infection rate (229). Therefore, influenza transmissibility in animals or humans varies according to viral strain, host susceptibility, and environmental factors.

SOCIOECONOMIC DRIVERS IN THE SPATIOTEMPORAL SPREAD OF OUTBREAKS

Indoor transmission of influenza within a small group is influenced by social contacts and socioeconomic conditions. Thus, understanding spatiotemporal dynamics will aid us in evaluating the spread of a given disease/pathogen within a population. Human behavioral studies have shown increased coincidence between cold climate and increased indoor crowding/dwelling and the beginning of school year, and these factors plausibly enhance the disease incidence of seasonal influenza at the local level (131). The differences in childhood and adult influenza cases are also attributed to the fact that children are more socially connected owing to the school system and hence are more susceptible to the first season of a new influenza (131). In another scenario, infants younger than six months of age have a higher incidence of influenza-associated hospitalizations (230–232), suggesting the need to prevent influenza in this age group, for which vaccines are not currently licensed for use by maternal immunization. Other factors, including smoking and lower vaccination coverage, may also contribute to seasonal influenza spread. At the other end of the spectrum, over 90% of influenza-related deaths occur in adults aged 65 years or older (233). A recent Cochrane review showed that the effectiveness of vaccination in these age groups is modest (234). Social determinants of heath, including health equity, vaccine acceptance, and age-related illness, may play a role in influenza occurrence and spread (235, 236). Other social factors that influence the magnitude of pandemic or seasonal influenza spread are air travel (237) and population density (131). Air transportation of livestock offers the potential for intercontinental mixing of potentially zoonotic pathogens; hence, airports that serve as major hubs could be targets for disease surveillance, and rapid deployment of control measures could be implemented (238). Recent research has focused on data mining on social signals from search engine query volume and social media chatter to detect temporal trends of influenza activity spatiotemporally (239, 240).

CONCLUSIONS

The environment is a major driver in the evolution and transmissibility of IAV. Spatiotemporal separations of distinct geographic regions exist as evolutionary sinks where IAVs evolve and maintain independently in their natural reservoirs. Environmental misalignments or anthropogenic interventions may result in spillover of the viruses from these sinks, leading to epizoonoses. The infectivity, fitness, transmissibility, and persistence of IAV in the aquatic environment and natural avian reservoirs are subject to even minor variations in temperature, pH, and salinity, but how these affect individual IAV strains remains a question. Available evidence suggests bird migration may contribute to environmental transmission in small avian communities, but the failure to sustain HPAI viruses for longer periods brings into question the role of migratory birds in transmission and outbreaks of HPAI. The relative rate of inactivation of IAV in air may be dependent on the size and composition of droplets and droplet nuclei. Strong and sufficient evidence exists for the association between ventilation, air movements in buildings, and the transmission/spread of IAV. The seasonality of IAV is found to be dependent on temperature and RH based on in vitro and animal model studies. Cool, dry conditions with low RH in temperate regions (cool-dry) or near-saturation RH with low temperatures during rainy seasons (humid-rainy) in tropical/subtropical zones favor IAV survival and transmissibility. All three modes of transmission, including contact, large droplets, and aerosols, may play a role in transmission depending

on the environment. Irrespective of the climatic zone, aerosol transmission appears to be the most common mode of transmission during outbreaks. The role of absolute humidity in transmissibility remains a question. Social determinants of health, such as health disparity, vaccine acceptance or vaccination policies, increased international movement of people and animals, and age of the susceptible hosts, play a major role in influenza outbreaks in different regions of the world. Social networks have been shown to be reliable predictors of public health emergencies such as influenza before official confirmation of outbreaks are made available. Several unanswered questions remain regarding the role of environment in influenza outbreaks for the reason that many of the predisposing situations could not be mimicked experimentally, and conclusions must be drawn on indirect evidence with confounding variables. Detailed experimentation on the role of environment, virus-host interactions in evolution, fitness, stability, and transmission of IAV and socioeconomic drivers of influenza outbreaks are needed to predict future pandemics and to develop strategic interventions.

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LITERATURE CITED

1. World Health Organ. 2008. *Topics: Immunizations, Vaccines and Biologicals*. Geneva: World Health Organ. **http://www.who.int/immunization/topics/influenza/en/**
2. Medina RA, García-Sastre A. 2011. Influenza A viruses: new research developments. *Nat. Rev. Microbiol.* 9:590–603
3. Muramoto Y, Noda T, Kawakami E, Akkina R, Kawaoka Y. 2013. Identification of novel influenza A virus proteins translated from PA mRNA. *J. Virol.* 87:2455–62
4. Webster RG, Govorkova EA. 2014. Continuing challenges in influenza. *Ann. N.Y. Acad. Sci.* 1323:115–39
5. Crisci E, Mussa T, Fraile L, Montoya M. 2013. Review: influenza virus in pigs. *Mol. Immunol.* 55:200–11
6. Gerber M, Isel C, Moules V, Marquet R. 2014. Selective packaging of the influenza A genome and consequences for genetic reassortment. *Trends Microbiol.* 22:446–55
7. Alexander DJ. 2007. An overview of the epidemiology of avian influenza. *Vaccine* 25:5637–44
8. Easterday BC, Trainer DO, Tumova B, Pereira HG. 1968. Evidence of infection with influenza viruses in migratory waterfowl. *Nature* 219:523–24
9. Alexander DJ, Brown IH. 2009. History of highly pathogenic avian influenza. *Rev. Sci. Tech.* 28:19–38
10. Halvorson DA. 2009. Prevention and management of avian influenza outbreaks: experiences from the United States of America. *Rev. Sci. Tech.* 28:359–69
11. Morens DM, Taubenberger JK, Fauci AS. 2013. Pandemic influenza viruses—hoping for the road not taken. *N. Engl. J. Med.* 368:2345–48
12. Swayne DE. 2007. Understanding the complex pathobiology of high pathogenicity avian influenza viruses in birds. *Avian Dis.* 51:242–49
13. Simms L, Jeggo M. 2014. Avian influenza from an ecohealth perspective. *EcoHealth* 11:4–14

14. Vandegrift KJ, Sokolow SH, Daszak P, Kilpatrick AM. 2010. Ecology of avian influenza viruses in a changing world. *Ann. N. Y. Acad. Sci.* 1195:113–28
15. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. 1992. Evolution and ecology of influenza A viruses. *Microbiol. Rev.* 56:152–79
16. Stallknecht DE, Kearney MT, Shane SM, Zwank PJ. 1990. Effects of pH, temperature, and salinity on persistence of avian influenza viruses in water. *Avian Dis.* 34:412–18
17. Taubenberger JK, Kash JC. 2010. Influenza virus evolution, host adaptation, and pandemic formation. *Cell Host Microbe* 7:440–51
18. Dugan VG, Chen R, Spiro DJ, Sengamalay N, Zaborsky J, et al. 2008. The evolutionary genetics and emergence of avian influenza viruses in wild birds. *PLOS Pathog.* 4:e1000076
19. Vijaykrishna D, Deng YM, Su YC, Fourment M, Iannello P, et al. 2013. The recent establishment of North American H10 lineage influenza viruses in Australian wild waterfowl and the evolution of Australian avian influenza viruses. *J. Virol.* 87:10182–89
20. Hansbro PM, Warner S, Tracey JP, Arzey KE, Selleck P, et al. 2010. Surveillance and analysis of avian influenza viruses, Australia. *Emerg. Infect. Dis.* 16:1896–904
21. Bulach D, Halpin R, Spiro D, Pomeroy L, Janies D, Boyle DB. 2010. Molecular analysis of H7 avian influenza viruses from Australia and New Zealand: genetic diversity and relationships from 1976 to 2007. *J. Virol.* 84:9957–66
22. Hurt AC, Vijaykrishna D, Butler J, Baas C, Maurer-Stroh S, et al. 2014. Detection of evolutionarily distinct avian influenza A viruses in Antarctica. *mBio* 5:e01098-14
23. Halvorson D, Karunakaran D, Senne D, Kelleher C, Bailey C, et al. 1983. Epizootiology of avian influenza—simultaneous monitoring of sentinel ducks and turkeys in Minnesota. *Avian Dis.* 27:77–85
24. Halvorson DA, Kelleher CJ, Pomeroy BS, Sivanandan V, Abraham AS, et al. 1987. Surveillance procedures for avian influenza. *Proc. 2nd Int. Symp. Avian Influenza, Athens, GA, Sept. 3–5*, pp. 155–63. St. Joseph, MO: US Anim. Health Assoc.
25. Hinshaw VS, Webster RG, Turner B. 1980. The perpetuation of orthomyxoviruses and paramyxoviruses in Canadian waterfowl. *Can. J. Microbiol.* 26:622–29
26. Pomeroy BS. 1982. Avian influenza in the United States (1964–1981). *Proc. 1st Int. Symp. Avian Influenza, Beltsville, MD, April 22–24*, pp. 13–17. Jacksonville, FL: Am. Assoc. Avian Pathol.
27. Lang G. 1982. A review of influenza in Canadian domestic and wild birds. *Proc. 1st Int. Symp. Avian Influenza, Beltsville, MD, April 22–24*, pp. 21–27. Jacksonville, FL: Am. Assoc. Avian Pathol.
28. Pomeroy BS. 1987. Avian influenza—avian influenza in turkeys in the USA. *Proc. 2nd Int. Symp. Avian Influenza, Athens, GA, Sept. 3–5*, pp. 14–21. St. Joseph, MO: US Anim. Health Assoc.
29. Mohan R, Saif YM, Erickson GA, Gustafson GA, Easterday BC. 1981. Serologic and epidemiologic evidence of infection in turkeys with an agent related to the swine influenza virus. *Avian Dis.* 25:11–16
30. Wood GW, Banks J, Brown IH, Strong I, Alexander DJ. 1997. The nucleotide sequence of the HA1 of the haemagglutinin of an HI avian influenza virus isolate from turkeys in Germany provides additional evidence suggesting recent transmission from pigs. *Avian Pathol.* 26:347–55
31. Wells RJH. 1963. An outbreak of fowl plague in turkeys. *Vet. Rec.* 75:783–86
32. Homme PJ, Easterday BC, Anderson DP. 1970. Avian influenza virus infections. II. Experimental epizootiology of influenza A-turkey-Wisconsin-1966 virus in turkeys. *Avian Dis.* 14:240–47
33. Utterback W. 1984. Update on avian influenza through February 21, 1984 in Pennsylvania and Virginia. *Proc. 33rd West. Poult. Dis. Conf., Davis, CA, Feb. 27–28*, pp. 4–7. Davis: Univ. Calif.
34. Glass SE, Naqi SA, Grumbles LC. 1981. Isolation of avian influenza virus in Texas. *Avian Dis.* 25:545–49
35. Pepin KM, Lloyd-Smith JO, Webb CT, Holcomb K, Zhu H, et al. 2013. Minimizing the threat of pandemic emergence from avian influenza in poultry systems. *BMC Infect. Dis.* 13:592
36. Campbell G. 1998. Report of the Irish national reference laboratory for 1996 and 1997. *Proc. Joint 4th Annu. Meet. Natl. Newctle. Dis. Avian Influenza Lab. Ctries. Eur. Union, Brussels, Dec. 9–10*, p. 13. Brussels: Eur. Union
37. Werner O. 1998. Avian influenza—situation in Germany 1995–1997. *Proc. Joint 4th Annu. Meet. Natl. Newctle. Dis. Avian Influenza Lab. Ctries. Eur. Union, Brussels, Dec. 9–10*, pp. 9–10. Brussels: Eur. Union

38. Banks J, Speidel EC, Harris PA, Alexander DJ. 2000. Phylogenetic analysis of influenza A viruses of H9 haemagglutinin subtype. *Avian Pathol.* 29:353–59
39. Halvorson DA, Frame DD, Friendshuh AJ, Shaw DP. 1998. Outbreaks of low pathogenicity avian influenza in USA. *Proc. 4th Int. Symp. Avian Influenza, Athens, GA*, pp. 36–46. Jacksonville, FL: Am. Assoc. Avian Pathol.
40. Bashashati M, Vasfi Marandi M, Sabouri F. 2013. Genetic diversity of early (1998) and recent (2010) avian influenza H9N2 virus strains isolated from poultry in Iran. *Arch. Virol.* 158:2089–100
41. Alexander DJ. 2003. Report on avian influenza in the Eastern Hemisphere during 1997–2002. *Avian Dis.* 47:792–97
42. Shanmuganatham K, Feeroz MM, Jones-Engel L, Smith GJ, Fourment M, et al. 2013. Antigenic and molecular characterization of avian influenza A(H9N2) viruses, Bangladesh. *Emerg. Infect. Dis.* 19:1393
43. Lee DH, Song CS. 2013. H9N2 avian influenza virus in Korea: evolution and vaccination. *Clin. Exp. Vaccine Res.* 2:26–33
44. Senne DA, Suarez DL, Stallnecht DE, Pedersen JC, Panigrahy B. 2006. Ecology and epidemiology of avian influenza in North and South America. *Dev. Biol.* 124:37–44
45. Yu H, Zhou YJ, Li GX, Ma JH, Yan LP, et al. 2011. Genetic diversity of H9N2 influenza viruses from pigs in China: A potential threat to human health? *Vet. Microbiol.* 149:254–61
46. Humberd J, Boyd K, Webster RG. 2007. Emergence of influenza A virus variants after prolonged shedding from pheasants. *J. Virol.* 81:4044–51
47. Xu X, Subbarao K, Cox NJ, Guo Y. 1999. Genetic characterization of the pathogenic influenza A/Goose/Guangdong/1/96 (H5N1) virus: similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. *Virology* 261:15–19
48. Tiensin T, Chaitaweesub P, Songserm T, Chaisingh A, Hoonsuwan W, et al. 2005. Highly pathogenic avian influenza H5N1, Thailand, 2004. *Emerg. Infect. Dis.* 11:1664–72
49. Needham H, Influenza Proj. Team. 2007. H5N1 in wild and domestic birds in Europe—remaining vigilant in response to an ongoing public health threat. *Eurosurveillance* 12: E071206.1
50. Proença-Módena JL, Macedo IS, Arruda E. 2007. H5N1 avian influenza virus: an overview. *Braz. J. Infect. Dis.* 11:125–33
51. Neumann G, Macken CA, Karasin AI, Fouchier RA, Kawaoka Y. 2012. Egyptian H5N1 influenza viruses—cause for concern? *PLOS Pathog.* 8:e1002932
52. Steensels M, Van Borm S, Boschmans M, van den Berg T. 2007. Lethality and molecular characterization of an HPAI H5N1 virus isolated from eagles smuggled from Thailand into Europe. *Avian Dis.* 51:401–7
53. Van Borm S, Thomas I, Hanquet G, Lambrecht B, Boschmans M, et al. 2005. Highly pathogenic H5N1 influenza virus in smuggled Thai eagles, Belgium. *Emerg. Infect. Dis.* 11:702–5
54. Dunham EJ, Dugan VG, Kaser EK, Perkins SE, Brown IH, et al. 2009. Different evolutionary trajectories of European avian-like and classical swine H1N1 influenza A viruses. *J. Virol.* 83:5485–94
55. Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, et al. 2009. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science* 325:197–201
56. Vittecoq M, Thomas F, Renaud F, Gauthier-Clerc M. 2011. Avian influenza viruses: environmental influence reference module in earth systems and environmental sciences. In *Encyclopedia of Environmental Health*, ed. JO Nriagu, pp. 253–61. Amsterdam: Elsevier
57. Peiris JS, de Jong MD, Guan Y. 2007. Avian influenza virus (H5N1): a threat to human health. *Clin. Microbiol. Rev.* 20:243–67
58. Fouchier RA, Schneeberger PM, Rozendaal FW, Broekman JM, Kemink SA, et al. 2004. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *PNAS* 101:1356–61
59. Tweed SA, Skowronski DM, David ST, Larder A, Petric M, et al. 2004. Human illness from avian influenza H7N3, British Columbia. *Emerg. Infect. Dis.* 10:2196–99
60. Lin YP, Shaw M, Gregory V, Cameron K, Lim W, et al. 2000. Avian-to-human transmission of H9N2 subtype influenza A viruses: relationship between H9N2 and H5N1 human isolates. *PNAS* 97:9654–58
61. Taubenberger JK, Morens DM. 2009. Pandemic influenza—including a risk assessment of H5N1. *Rev. Sci. Tech.* 28:187–202

62. Crawford PC, Dubovi EJ, Castleman WL, Stephenson I, Gibbs EP, et al. 2005. Transmission of equine influenza virus to dogs. *Science* 310:482–85
63. Tu J, Zhou H, Jiang T, Li C, Zhang A, et al. 2009. Isolation and molecular characterization of equine H3N8 influenza viruses from pigs in China. *Arch. Virol.* 154:887–90
64. Krueger WS, Gray GC. 2013. Swine influenza virus infections in man. *Curr. Top. Microbiol. Immunol.* 370:201–25
65. Naffakh N, van der Werf S. 2009. April 2009: an outbreak of swine-origin influenza A(H1N1) virus with evidence for human-to-human transmission. *Microbes Infect.* 11:725–28
66. Epperson S, Jhung M, Richards S, Quinlisk P, Ball L, et al. 2013. Human infections with influenza A(H3N2) variant virus in the United States, 2011–2012. *Clin. Infect. Dis.* 57(Suppl. 1):S4–S11
67. Jhung MA, Epperson S, Biggerstaff M, Allen D, Balish A, et al. 2013. Outbreak of variant influenza A(H3N2) virus in the United States. *Clin. Infect. Dis.* 57:1703–12
68. Bowman AS, Nelson SW, Page SL, Nolting JM, Killian ML, et al. 2014. Swine-to-human transmission of influenza A (H3N2) virus at agricultural fairs, Ohio, USA, 2012. *Emerg. Infect. Dis.* 20:1472–80
69. Van Reeth K, Nicoll A. 2009. A human case of swine influenza virus infection in Europe—implications for human health and research. *Eurosurveillance* 14:19124
70. Songserm T, Amonsin A, Jam-on R, Sae-Heng N, Meemak N, et al. 2006. Avian influenza H5N1 in naturally infected domestic cat. *Emerg. Infect. Dis.* 12:681–83
71. Sponseller BA, Strait E, Jergens A, Trujillo J, Harmon K, et al. 2010. Influenza A pandemic (H1N1) 2009 virus infection in domestic cat. *Emerg. Infect. Dis.* 16:534–37
72. Desvaux S, Marx N, Ong S, Gaidet N, Hunt M, et al. 2009. Highly pathogenic avian influenza virus (H5N1) outbreak in captive wild birds and cats, Cambodia. *Emerg. Infect. Dis.* 15:475–78
73. Keawcharoen J, Oraveerakul K, Kuiken T, Fouchier RA, Amonsin A, et al. 2004. Avian influenza H5N1 in tigers and leopards. *Emerg. Infect. Dis.* 10:2189–91
74. Leschnik M, Weikel J, Möstl K, Revilla-Fernández S, Wodak E, et al. 2007. Subclinical infection with avian influenza A (H5N1) virus in cats. *Emerg. Infect. Dis.* 13:243–47
75. Rimmelzwaan GF, van Riel D, Baars M, Bestebroer TM, van Amerongen G, et al. 2006. Influenza A virus (H5N1) infection in cats causes systemic disease with potential novel routes of virus spread within and between hosts. *Am. J. Pathol.* 168:176–83
76. van den Brand JM, Stittelaar KJ, van Amerongen G, van de Bildt MW, Leijten LM, et al. 2010. Experimental pandemic (H1N1) 2009 virus infection of cats. *Emerg. Infect. Dis.* 16:1745–47
77. Driskell EA, Jones CA, Berghaus RD, Stallknecht DE, Howerth EW, Tompkins SM. 2013. Domestic cats are susceptible to infection with low pathogenic avian influenza viruses from shorebirds. *Vet. Pathol.* 50:39–45
78. Lopez JW, Woods GT. 1984. Influenza virus in ruminants: a review. *Res. Commun. Chem. Pathol. Pharmacol.* 45:445–62
79. Crawshaw TR, Brown I. 1998. Bovine influenza. *Vet. Rec.* 143:372
80. Yuanji G, Desselberger U. 1984. Genome analysis of influenza C viruses isolated in 1981/82 from pigs in China. *J. Gen. Virol.* 65(Pt. 11):1857–72
81. Ohwada K, Kitame F, Homma M. 1986. Experimental infections of dogs with type C influenza virus. *Microbiol. Immunol.* 30:451–60
82. Kawano J, Onta T, Kida H, Yanagawa R. 1978. Distribution of antibodies in animals against influenza B and C viruses. *Jpn. J. Vet. Res.* 26:74–80
83. Hause BM, Ducatez M, Collin EA, Ran Z, Liu R, et al. 2013. Isolation of a novel swine influenza virus from Oklahoma in 2011 which is distantly related to human influenza C viruses. *PLOS Pathog.* 9:e1003176
84. Hause BM, Collin EA, Liu R, Huang B, Sheng Z, et al. 2014. Characterization of a novel influenza virus in cattle and swine: proposal for a new genus in the Orthomyxoviridae family. *mBio* 5:e00031–14
85. Olsen B, Munster VJ, Wallensten A, Waldenström J, Osterhaus ADME, Fouchier RAM. 2006. Global patterns of influenza A virus in wild birds. *Science* 312:384–88
86. Hurt AC, Hansbro PM, Selleck P, Olsen B, Minton C, et al. 2006. Isolation of avian influenza viruses from two different transhemispheric migratory shorebird species in Australia. *Arch. Virol.* 151:2301–9

87. Roche B, Lebarbenchon C, Gauthier-Clerc M, Chang CM, Thomas F, et al. 2009. Water-borne transmission drives avian influenza dynamics in wild birds: the case of the 2005–2006 epidemics in the Camargue area. *Infect. Genet. Evol.* 9:800–5
88. Hinshaw VS, Webster RG, Turner B. 1979. Water-bone transmission of influenza A viruses? *Intervirology* 11:66–68
89. Markwell DD, Shortridge KF. 1982. Possible waterborne transmission and maintenance of influenza viruses in domestic ducks. *Appl. Environ. Microbiol.* 43:110–15
90. Brown JD, Swayne DE, Cooper RJ, Burns RE, Stallknecht DE. 2007. Persistence of H5 and H7 avian influenza viruses in water. *Avian Dis.* 51:285–89
91. Ito T, Okazaki K, Kawaoka Y, Takada A, Webster RG, Kida H. 1995. Perpetuation of influenza A viruses in Alaskan waterfowl reservoirs. *Arch. Virol.* 140:1163–72
92. Keeler SP, Lebarbenchon C, Stallknecht DE. 2013. Strain-related variation in the persistence of influenza A virus in three types of water: distilled water, filtered surface water, and intact surface water. *Virol. J.* 10:13
93. Khalenkov A, Laver WG, Webster RG. 2008. Detection and isolation of H5N1 influenza virus from large volumes of natural water. *J. Virol. Methods* 149:180–83
94. Lang AS, Kelly A, Runstadler JA. 2008. Prevalence and diversity of avian influenza viruses in environmental reservoirs. *J. Gen. Virol.* 89:509–19
95. Nielsen AA, Jensen TH, Stockmarr A, Jorgensen PH. 2013. Persistence of low-pathogenic H5N7 and H7N1 avian influenza subtypes in filtered natural waters. *Vet. Microbiol.* 166:419–28
96. Shi J, Gao L, Zhu Y, Chen T, Liu Y, et al. 2014. Investigation of avian influenza infections in wild birds, poultry and humans in Eastern Dongting Lake, China. *PLOS ONE* 9:e95685
97. Stallknecht DE, Goekjian VH, Wilcox BR, Poulson RL, Brown JD. 2010. Avian influenza virus in aquatic habitats: What do we need to learn? *Avian Dis.* 54:461–65
98. Domanska-Blicharz K, Minta Z, Smietanka K, Marche S, van den Berg T. 2010. H5N1 high pathogenicity avian influenza virus survival in different types of water. *Avian Dis.* 54:734–37
99. Keeler SP, Berghaus RD, Stallknecht DE. 2012. Persistence of low pathogenic avian influenza viruses in filtered surface water from waterfowl habitats in Georgia, USA. *J. Wildl. Dis.* 48:999–1009
100. Keeler SP, Dalton MS, Cressler AM, Berghaus RD, Stallknecht DE. 2014. Abiotic factors affecting the persistence of avian influenza virus in surface waters of waterfowl habitats. *Appl. Environ. Microbiol.* 80:2910–17
101. Brown JD, Goekjian G, Poulson R, Valeika S, Stallknecht DE. 2009. Avian influenza virus in water: Infectivity is dependent on pH, salinity and temperature. *Vet. Microbiol.* 136:20–26
102. Dublineau A, Batejat C, Pinon A, Burguiere AM, Leclercq I, Manuguerra JC. 2011. Persistence of the 2009 pandemic influenza A (H1N1) virus in water and on non-porous surface. *PLOS ONE* 6:e28043
103. Brankston G, Gitterman L, Hirji Z, Lemieux C, Gardam M. 2007. Transmission of influenza A in human beings. *Lancet Infect. Dis.* 7:257–65
104. Weber TP, Stilianakis NI. 2008. Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. *J. Infect.* 57:361–73
105. Lebarbenchon C, Sreevatsan S, Lefevre T, Yang M, Ramakrishnan MA, et al. 2012. Reassortant influenza A viruses in wild duck populations: effects on viral shedding and persistence in water. *Proc. Biol. Sci.* 279:3967–75
106. Shoham D, Jahangir A, Ruenphet S, Takehara K. 2012. Persistence of avian influenza viruses in various artificially frozen environmental water types. *Influenza Res. Treat.* 2012:912326
107. Stumpf P, Failing K, Papp T, Nazir J, Böhm R, Marschang RE. 2010. Accumulation of a low pathogenic avian influenza virus in zebra mussels (*Dreissena polymorpha*). *Avian Dis.* 54:1183–90
108. Horm VS, Gutiérrez RA, Nicholls JM, Buchy P. 2012. Highly pathogenic influenza A(H5N1) virus survival in complex artificial aquatic biotopes. *PLOS ONE* 7:e34160
109. Farnsworth ML, Miller RS, Pedersen K, Lutman MW, Swafford SR, et al. 2012. Environmental and demographic determinants of avian influenza viruses in waterfowl across the contiguous United States. *PLOS ONE* 7:e32729
110. Feare CJ. 2010. Role of wild birds in the spread of highly pathogenic avian influenza virus H5N1 and implications for global surveillance. *Avian Dis.* 54:201–12

111. Keawcharoen J, van Riel D, van Amerongen G, Bestebroer T, Beyer WE, et al. 2008. Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). *Emerg. Infect. Dis.* 14:600–7
112. Bahl J, Krauss S, Kuhnert D, Fourment M, Raven G, et al. 2013. Influenza A virus migration and persistence in North American wild birds. *PLOS Pathog.* 9:e1003570
113. Breban R, Drake JM, Stallknecht DE, Rohani P. 2009. The role of environmental transmission in recurrent avian influenza epidemics. *PLOS Comput. Biol.* 5:e1000346
114. Sims LD, Domenech J, Benigno C, Kahn S, Kamata A, et al. 2005. Origin and evolution of highly pathogenic H5N1 avian influenza in Asia. *Vet. Rec.* 157:159–64
115. El-Zoghby EF, Arafa AS, Hassan MK, Aly MM, Selim A, et al. 2012. Isolation of H9N2 avian influenza virus from bobwhite quail (*Colinus virginianus*) in Egypt. *Arch. Virol.* 157:1167–72
116. Handel A, Brown J, Stallknecht D, Rohani P. 2013. A multi-scale analysis of influenza A virus fitness trade-offs due to temperature-dependent virus persistence. *PLOS Comput. Biol.* 9:e1002989
117. Morawska L. 2006. Droplet fate in indoor environments, or can we prevent the spread of infection? *Indoor Air* 16:335–47
118. Yang W, Marr LC. 2012. Mechanisms by which ambient humidity may affect viruses in aerosols. *Appl. Environ. Microbiol.* 78:6781–88
119. Posada JA, Redrow J, Celik I. 2010. A mathematical model for predicting the viability of airborne viruses. *J. Virol. Methods* 164:88–95
120. Thomas RJ. 2013. Particle size and pathogenicity in the respiratory tract. *Virulence* 4:847–58
121. Dai YT, Juang YJ, Wu YY, Breysse PN, Hsu DJ. 2006. In vivo measurement of ultralarge aerosol particles in calm air by humans. *J. Aerosol Sci.* 37:967e73
122. Hinds WC. 1999. Respiratory deposition. In *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles*, pp. 233–59. New York: Wiley-Intersci. 2nd ed.
123. Lindsley WG, Blachere FM, Thewlis RE, Vishnu A, Davis KA, et al. 2010. Measurements of airborne influenza virus in aerosol particles from human coughs. *PLOS ONE* 5:e15100
124. Yang W, Elankumaran S, Marr LC. 2011. Concentrations and size distributions of airborne influenza A viruses measured indoors at a health centre, a day-care centre and on aeroplanes. *J. R. Soc. Interface* 8:1176–84
125. Lednicky JA, Loeb JC. 2013. Detection and isolation of airborne influenza A H3N2 virus using a Sioutas Personal Cascade Impactor Sampler. *Influenza Res. Treat.* 2013:656825
126. Mitchell CA, Guerin LF, Robillard J. 1968. Decay of influenza A viruses of human and avian origin. *Can. J. Comp. Med.* 32(4):544–46
127. Mitchell CA, Guerin LF. 1972. Influenza A of human, swine, equine and avian origin: comparison of survival in aerosol form. *Can. J. Comp. Med.* 36(1):9–11
128. Yang W, Marr LC. 2011. Dynamics of airborne influenza A viruses indoors and dependence on humidity. *PLOS ONE* 6:e21481
129. Sagripanti JL, Lytle CD. 2007. Inactivation of influenza virus by solar radiation. *Photochem. Photobiol.* 83:1278–82
130. Sutton D, Aldous EW, Warren CJ, Fuller CM, Alexander DJ, Brown IH. 2013. Inactivation of the infectivity of two highly pathogenic avian influenza viruses and a virulent Newcastle disease virus by ultraviolet radiation. *Avian Pathol.* 42:566–68
131. Sloan C, Moore ML, Hartert T. 2011. Impact of pollution, climate, and sociodemographic factors on spatiotemporal dynamics of seasonal respiratory viruses. *Clin. Transl. Sci.* 4:48–54
132. Yang W, Elankumaran S, Marr LC. 2012. Relationship between humidity and influenza A viability in droplets and implications for influenza's seasonality. *PLOS ONE* 7:e46789
133. Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, Balfour HH Jr. 1982. Survival of influenza viruses on environmental surfaces. *J. Infect. Dis.* 146:47–51
134. Shahid MA, Abubakar M, Hameed S, Hassan S. 2009. Avian influenza virus (H5N1): effects of physicochemical factors on its survival. *Virol. J.* 6:38
135. Shigematsu S, Dublineau A, Sawoo O, Batejat C, Matsuyama T, et al. 2014. Influenza A virus survival in water is influenced by the origin species of the host cell. *Influenza Other Respir. Viruses* 8:123–30

136. Sawoo O, Dublineau A, Batejat C, Zhou P, Manuguerra JC, Leclercq I. 2014. Cleavage of hemagglutinin-bearing lentiviral pseudotypes and their use in the study of influenza virus persistence. *PLOS ONE* 9:e106192
137. Rachakonda PS, Veit M, Korte T, Ludwig K, Bottcher C, et al. 2007. The relevance of salt bridges for the stability of the influenza virus hemagglutinin. *FASEB J.* 21:995–1002
138. Stanwick TL, Hallum JV. 1975. Comparison of RNA polymerase associated with Newcastle disease virus and a temperature-sensitive mutant of Newcastle disease virus isolated from persistently infected L cells. *J. Virol.* 17:68–73
139. Dalton RM, Mullin AE, Amorim MJ, Medcalf E, Tiley LS, Digard P. 2006. Temperature sensitive influenza A virus genome replication results from low thermal stability of polymerase-cRNA complexes. *Virol. J.* 3:58
140. Scholtissek C. 1985. Stability of infectious influenza A viruses at low pH and at elevated temperature. *Vaccine* 3:215–18
141. Puri A, Booy FP, Doms RW, White JM, Blumenthal R. 1990. Conformational changes and fusion activity of influenza virus hemagglutinin of the H2 and H3 subtypes: effects of acid pretreatment. *J. Virol.* 64:3824–32
142. White J, Kartenbeck J, Helenius A. 1982. Membrane fusion activity of influenza virus. *EMBO J.* 1:217–22
143. Mittal A, Shangguan T, Bentz J. 2002. Measuring pKa of activation and pKi of inactivation for influenza hemagglutinin from kinetics of membrane fusion of virions and of HA expressing cells. *Biophys. J.* 83:2652–66
144. Budowsky EI, Bresler SE, Friedman EA, Zheleznova NV. 1981. Principles of selective inactivation of viral genome. I. UV-induced inactivation of influenza virus. *Arch. Virol.* 68:239–47
145. Drinka PJ, Krause P, Schilling M, Miller BA, Shult P, Gravenstein S. 1996. Report of an outbreak: nursing home architecture and influenza-A attack rates. *J. Am. Geriatr. Soc.* 44:910–13
146. Drinka PJ, Krause P, Nest L, Tyndall D. 2004. Report of an outbreak: nursing home architecture and influenza-A attack rates: update. *J. Am. Geriatr. Soc.* 52:847–48
147. Robinson M, Stilianakis NI, Drossinos Y. 2012. Spatial dynamics of airborne infectious diseases. *J. Theor. Biol.* 297:116–26
148. Zhang L, Guo ZW, Bridge ES, Li YM, Xiao XM. 2013. Distribution and dynamics of risk factors associated with highly pathogenic avian influenza H5N1. *Epidemiol. Infect.* 141:2444–53
149. Li YLG, Tang JW, Yang X, Chao CY, Lin JZ, et al. 2007. Role of ventilation in airborne transmission of infectious agents in the built environment—a multidisciplinary systematic review. *Indoor Air* 17:2–18
150. Tellier R. 2009. Aerosol transmission of influenza A virus: a review of new studies. *J. R. Soc. Interface* 6(Suppl. 6):S783–90
151. Fabian P, McDevitt JJ, DeHaan WH, Fung RO, Cowling BJ, et al. 2008. Influenza virus in human exhaled breath: an observational study. *PLOS ONE* 3:e2691
152. Lindsley WG, Blachere FM, Davis KA, Pearce TA, Fisher MA, et al. 2010. Distribution of airborne influenza virus and respiratory syncytial virus in an urgent care medical clinic. *Clin. Infect. Dis.* 50:693–98
153. Milton DK, Fabian MP, Cowling BJ, Grantham ML, McDevitt JJ. 2013. Influenza virus aerosols in human exhaled breath: particle size, culturability, and effect of surgical masks. *PLOS Pathog.* 9:e1003205
154. Simonds AK, Hanak A, Chatwin M, Morrell M, Hall A, et al. 2010. Evaluation of droplet dispersion during non-invasive ventilation, oxygen therapy, nebuliser treatment and chest physiotherapy in clinical practice: implications for management of pandemic influenza and other airborne infections. *Health Technol. Assess.* 14:131–72
155. Cowling BJ. 2012. Airborne transmission of influenza: implications for control in healthcare and community settings. *Clin. Infect. Dis.* 54:1578–80
156. Alford RH, Kasel JA, Gerone PJ, Knight V. 1966. Human influenza resulting from aerosol inhalation. *Proc. Soc. Exp. Biol. Med.* 122:800–4
157. Little JW, Douglas RG Jr, Hall WJ, Roth FK. 1979. Attenuated influenza produced by experimental intranasal inoculation. *J. Med. Virol.* 3:177–88

158. Lowen AC, Mubareka S, Tumpey TM, García-Sastre A, Palese P. 2006. The guinea pig as a transmission model for human influenza viruses. *PNAS* 103:9988–92
159. Abbas T, Wilking H, Horeth-Bontgen D, Conraths FJ. 2012. Contact structure and potential risk factors for avian influenza transmission among open-sided chicken farms in Kamalia, an important poultry rearing area of Pakistan. *Berl. Munch Tierarztl Wochenschr.* 125:110–16
160. Tsukamoto K, Imada T, Tanimura N, Okamatsu M, Mase M, et al. 2007. Impact of different husbandry conditions on contact and airborne transmission of H5N1 highly pathogenic avian influenza virus to chickens. *Avian Dis.* 51:129–32
161. Boender GJ, Hagenaars TJ, Bouma A, Nodelijk G, Elbers AR, et al. 2007. Risk maps for the spread of highly pathogenic avian influenza in poultry. *PLOS Comput. Biol.* 3:e71
162. Thompson PN, Sinclair M, Ganzevoort B. 2008. Risk factors for seropositivity to H5 avian influenza virus in ostrich farms in the Western Cape Province, South Africa. *Prev. Vet. Med.* 86:139–52
163. te Beest DE, Stegeman JA, Mulder YM, van Boven M, Koopmans MP. 2011. Exposure of uninfected poultry farms to HPAI (H7N7) virus by professionals during outbreak control activities. *Zoonoses Public Health* 58:493–99
164. Firestone SM, Schemann KA, Toribio JA, Ward MP, Dhand NK. 2011. A case-control study of risk factors for equine influenza spread onto horse premises during the 2007 epidemic in Australia. *Prev. Vet. Med.* 100:53–63
165. Elbers AR, Tielen MJ, Cromwijk WA, Hunneman WA. 1992. Variation in seropositivity for some respiratory disease agents in finishing pigs: epidemiological studies on some health parameters and farm and management conditions in the herds. *Vet. Q.* 14:8–13
166. Bos ME, Nielen M, Koch G, Bouma A, De Jong MC, Stegeman A. 2009. Back-calculation method shows that within-flock transmission of highly pathogenic avian influenza (H7N7) virus in the Netherlands is not influenced by housing risk factors. *Prev. Vet. Med.* 88:278–85
167. Corzo CA, Culhane M, Dee S, Morrison RB, Torremorell M. 2013. Airborne detection and quantification of swine influenza A virus in air samples collected inside, outside and downwind from swine barns. *PLOS ONE* 8:e71444
168. Ribeiro Amorim A, Garcete Fornells LAM, da Costa Reis F, Rezende DJ, da Silva Mendes G, et al. 2013. Influenza A virus infection of healthy piglets in an abattoir in Brazil: animal-human interface and risk for interspecies transmission. *Mem. Inst. Oswaldo Cruz* 108:548–53
169. Ypma RJ, Jonges M, Bataille A, Stegeman A, Koch G, et al. 2013. Genetic data provide evidence for wind-mediated transmission of highly pathogenic avian influenza. *J. Infect. Dis.* 207:730–35
170. Beckett S, Garner MG. 2007. Simulating disease spread within a geographic information system environment. *Vet. Ital.* 43:595–604
171. Cambra-Lopez M, Aarnink AJ, Zhao Y, Calvet S, Torres AG. 2010. Airborne particulate matter from livestock production systems: a review of an air pollution problem. *Environ. Pollut.* 158:1–17
172. Sedlmaier N, Hoppenheidt K, Krist H, Lehmann S, Lang H, Buttner M. 2009. Generation of avian influenza virus (AIV) contaminated fecal fine particulate matter (PM(2.5)): genome and infectivity detection and calculation of immission. *Vet. Microbiol.* 139:156–64
173. Davis J, Garner MG, East IJ. 2009. Analysis of local spread of equine influenza in the Park Ridge region of Queensland. *Transbound. Emerg. Dis.* 56:31–38
174. Spekreijse D, Bouma A, Koch G, Stegeman JA. 2011. Airborne transmission of a highly pathogenic avian influenza virus strain H5N1 between groups of chickens quantified in an experimental setting. *Vet. Microbiol.* 152:88–95
175. Ssematimba A, Hagenaars TJ, de Jong MCM. 2012. Modelling the wind-borne spread of highly pathogenic avian influenza virus between farms. *PLOS ONE* 7:e31114
176. Sawabe K, Hoshino K, Isawa H, Sasaki T, Hayashi T, et al. 2006. Detection and isolation of highly pathogenic H5N1 avian influenza A viruses from blow flies collected in the vicinity of an infected poultry farm in Kyoto, Japan, 2004. *Am. J. Trop. Med. Hyg.* 75:327–32
177. Able KP. 1974. Environmental influences on the orientation of free-flying nocturnal bird migrants. *Anim. Behav.* 22:224–38

178. Adegboye OA, Kotze D. 2014. Epidemiological analysis of spatially misaligned data: a case of highly pathogenic avian influenza virus outbreak in Nigeria. *Epidemiol. Infect.* 142:940–49
179. Viboud C, Alonso WJ, Simonsen L. 2006. Influenza in tropical regions. *PLOS Med.* 3:e89
180. Alonso WJ, Viboud C, Simonsen L, Hirano EW, Daufenbach LZ, Miller MA. 2007. Seasonality of influenza in Brazil: a traveling wave from the Amazon to the subtropics. *Am. J. Epidemiol.* 165:1434–42
181. Dosseh A, Ndiaye K, Spiegel A, Sagna M, Mathiot C. 2000. Epidemiological and virological influenza survey in Dakar, Senegal: 1996–1998. *Am. J. Trop. Med. Hyg.* 62:639–43
182. Moura FE, Perdigão AC, Siqueira MM. 2009. Seasonality of influenza in the tropics: a distinct pattern in northeastern Brazil. *Am. J. Trop. Med. Hygiene* 81:180–83
183. Tamerius JD, Shaman J, Alonso WJ, Bloom-Feshbach K, Uejio CK, et al. 2013. Environmental predictors of seasonal influenza epidemics across temperate and tropical climates. *PLOS Pathog.* 9:e1003194
184. Lofgren E, Fefferman NH, Naumov YN, Gorski J, Naumova EN. 2007. Influenza seasonality: underlying causes and modeling theories. *J. Virol.* 81:5429–36
185. Tamerius J, Nelson MI, Zhou SZ, Viboud C, Miller MA, Alonso WJ. 2011. Global influenza seasonality: reconciling patterns across temperate and tropical regions. *Environ. Health Perspect.* 119:439–45
186. Schulman JL, Kilbourne ED. 1963. Experimental transmission of influenza virus infection in mice. II. Some factors affecting the incidence of transmitted infection. *J. Exp. Med.* 118:267–75
187. Lowen AC, Steel J, Mubareka S, Palese P. 2008. High temperature (30°C) blocks aerosol but not contact transmission of influenza virus. *J. Virol.* 82:5650–52
188. Lowen AC, Mubareka S, Steel J, Palese P. 2007. Influenza virus transmission is dependent on relative humidity and temperature. *PLOS Pathog.* 3:1470–76
189. Steel J, Palese P, Lowen AC. 2011. Transmission of a 2009 pandemic influenza virus shows a sensitivity to temperature and humidity similar to that of an H3N2 seasonal strain. *J. Virol.* 85:1400–2
190. Tang JW. 2009. The effect of environmental parameters on the survival of airborne infectious agents. *J. R. Soc. Interface* 6(Suppl. 6):S737–46
191. Dorigatti I, Cauchemez S, Ferguson NM. 2013. Increased transmissibility explains the third wave of infection by the 2009 H1N1 pandemic virus in England. *PNAS* 110:13422–27
192. Pica N, Bouvier NM. 2012. Environmental factors affecting the transmission of respiratory viruses. *Curr. Opin. Virol.* 2:90–95
193. Noti JD, Blachere FM, McMillen CM, Lindsley WG, Kashon ML, et al. 2013. High humidity leads to loss of infectious influenza virus from simulated coughs. *PLOS ONE* 8:e57485
194. Shaman J, Kohn M. 2009. Absolute humidity modulates influenza survival, transmission, and seasonality. *PNAS* 106:3243–48
195. Barreca AI, Shimshack JP. 2012. Absolute humidity, temperature, and influenza mortality: 30 years of county-level evidence from the United States. *Am. J. Epidemiol.* 176(Suppl. 7):S114–22
196. Loh TP, Lai FY, Tan ES, Thoon KC, Tee NW, et al. 2011. Correlations between clinical illness, respiratory virus infections and climate factors in a tropical paediatric population. *Epidemiol. Infect.* 139:1884–94
197. Agrawal AS, Sarkar M, Chakrabarti S, Rajendran K, Kaur H, et al. 2009. Comparative evaluation of real-time PCR and conventional RT-PCR during a 2 year surveillance for influenza and respiratory syncytial virus among children with acute respiratory infections in Kolkata, India, reveals a distinct seasonality of infection. *J. Med. Microbiol.* 58:1616–22
198. Abdullah Brooks W, Terebuh P, Bridges C, Klimov A, Goswami D, et al. 2007. Influenza A and B infection in children in urban slum, Bangladesh. *Emerg. Infect. Dis.* 13:1507–8
199. Tang JW, Lai FY, Nymadawa P, Deng YM, Ratnamohan M, et al. 2010. Comparison of the incidence of influenza in relation to climate factors during 2000–2007 in five countries. *J. Med. Virol.* 82:1958–65
200. Murray EJ, Morse SS. 2011. Seasonal oscillation of human infection with influenza A/H5N1 in Egypt and Indonesia. *PLOS ONE* 6:e24042
201. Murray EL, Klein M, Brondi L, McGowan JE Jr, van Mels C, et al. 2012. Rainfall, household crowding, and acute respiratory infections in the tropics. *Epidemiol. Infect.* 140:78–86
202. McDevitt JJ, Rudnick SN, Radonovich LJ. 2012. Aerosol susceptibility of influenza virus to UV-C light. *Appl. Environ. Microbiol.* 78:1666–69

203. Xu Z, Hu W, Williams G, Clements AC, Kan H, Tong S. 2013. Air pollution, temperature and pediatric influenza in Brisbane, Australia. *Environ. Int.* 59:384–88
204. Zell R, Scholtissek C, Ludwig S. 2013. Genetics, evolution, and the zoonotic capacity of European Swine influenza viruses. *Curr. Top. Microbiol. Immunol.* 370:29–55
205. Sorrell EM, Schrauwen EJ, Linster M, De Graaf M, Herfst S, Fouchier RA. 2011. Predicting 'airborne' influenza viruses: (Trans-)mission impossible? *Curr. Opin. Virol.* 1:635–42
206. Tscherne DM, García-Sastre A. 2011. Virulence determinants of pandemic influenza viruses. *J. Clin. Investig.* 121:6–13
207. Stevens J, Blixt O, Chen LM, Donis RO, Paulson JC, Wilson IA. 2008. Recent avian H5N1 viruses exhibit increased propensity for acquiring human receptor specificity. *J. Mol. Biol.* 381:1382–94
208. Stevens J, Blixt O, Tumpey TM, Taubenberger JK, Paulson JC, Wilson IA. 2006. Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. *Science* 312:404–10
209. Maines TR, Chen LM, Van Hoeven N, Tumpey TM, Blixt O, et al. 2011. Effect of receptor binding domain mutations on receptor binding and transmissibility of avian influenza H5N1 viruses. *Virology* 413:139–47
210. Matrosovich MN, Krauss S, Webster RG. 2001. H9N2 influenza A viruses from poultry in Asia have human virus-like receptor specificity. *Virology* 281:156–62
211. Chutinimitkul S, van Riel D, Munster VJ, van den Brand JM, Rimmelzwaan GF, et al. 2010. In vitro assessment of attachment pattern and replication efficiency of H5N1 influenza A viruses with altered receptor specificity. *J. Virol.* 84:6825–33
212. Wan H, Sorrell EM, Song H, Hossain MJ, Ramirez-Nieto G, et al. 2008. Replication and transmission of H9N2 influenza viruses in ferrets: evaluation of pandemic potential. *PLOS ONE* 3:e2923
213. Maines TR, Chen LM, Matsuoka Y, Chen H, Rowe T, et al. 2006. Lack of transmission of H5N1 avian-human reassortant influenza viruses in a ferret model. *PNAS* 103:12121–26
214. Gao Y, Zhang Y, Shinya K, Deng G, Jiang Y, et al. 2009. Identification of amino acids in HA and PB2 critical for the transmission of H5N1 avian influenza viruses in a mammalian host. *PLOS Pathog.* 5:e1000709
215. Yamada S, Hatta M, Staker BL, Watanabe S, Imai M, et al. 2010. Biological and structural characterization of a host-adapting amino acid in influenza virus. *PLOS Pathog.* 6:e1001034
216. Steel J, Lowen AC, Mubareka S, Palese P. 2009. Transmission of influenza virus in a mammalian host is increased by PB2 amino acids 627K or 627E/701N. *PLOS Pathog.* 5:e1000252
217. Van Hoeven N, Pappas C, Belser JA, Maines TR, Zeng H, et al. 2009. Human HA and polymerase subunit PB2 proteins confer transmission of an avian influenza virus through the air. *PNAS* 106:3366–71
218. Capua I, Munoz O. 2013. Emergence of influenza viruses with zoonotic potential: open issues which need to be addressed. A review. *Vet. Microbiol.* 165:7–12
219. Brown IH, Banks J, Manvell RJ, Essen SC, Shell W, et al. 2006. Recent epidemiology and ecology of influenza A viruses in avian species in Europe and the Middle East. *Dev. Biol.* 124:45–50
220. Van Poucke SG, Nicholls JM, Nauwynck HJ, Van Reeth K. 2010. Replication of avian, human and swine influenza viruses in porcine respiratory explants and association with sialic acid distribution. *Virol. J.* 7:38
221. Nicholls JM, Chan MC, Chan WY, Wong HK, Cheung CY, et al. 2007. Tropism of avian influenza A (H5N1) in the upper and lower respiratory tract. *Nat. Med.* 13:147–49
222. Lu L, Lycett SJ, Leigh Brown AJ. 2014. Reassortment patterns of avian influenza virus internal segments among different subtypes. *BMC Evol. Biol.* 14:16
223. Loosli CG, Robertson OH, Puck TT. 1943. The production of experimental influenza in mice by inhalation of atmospheres containing influenza virus dispersed as fine droplets. *J. Infect. Dis.* 72:142–53
224. Frankova V. 1975. Inhalatory infection of mice with influenza A0/PR8 virus. I. The site of primary virus replication and its spread in the respiratory tract. *Acta Virol.* 19:29–34
225. Andrewes CH, Glover RE. 1941. Spread of infection from the respiratory tract of the ferret: I. Transmission of influenza A virus. *Br. J. Exp. Pathol.* 22:91–97
226. Sorrell EM, Wan H, Araya Y, Song H, Perez DR. 2009. Minimal molecular constraints for respiratory droplet transmission of an avian-human H9N2 influenza A virus. *PNAS* 106:7565–70

227. Mubareka S, Lowen AC, Steel J, Coates AL, García-Sastre A, Palese P. 2009. Transmission of influenza virus via aerosols and fomites in the guinea pig model. *J. Infect. Dis.* 199:858–65
228. Bouvier NM, Lowen AC, Palese P. 2008. Oseltamivir-resistant influenza A viruses are transmitted efficiently among guinea pigs by direct contact but not by aerosol. *J. Virol.* 82:10052–58
229. Chen SC, You SH, Liu CY, Chio CP, Liao CM. 2012. Using experimental human influenza infections to validate a viral dynamic model and the implications for prediction. *Epidemiol. Infect.* 140:1557–68
230. Bender JM, Ampofo K, Gesteland P, Sheng X, Korgenski K, et al. 2010. Influenza virus infection in infants less than three months of age. *Pediatr. Infect. Dis. J.* 29:6–9
231. Chiu SS, Lau YL, Chan KH, Wong WH, Peiris JS. 2002. Influenza-related hospitalizations among children in Hong Kong. *N. Engl. J. Med.* 347:2097–103
232. Nelson EAS, Ip M, Tam JS, Mounts AW, Chau SL, et al. 2014. Burden of influenza infection in hospitalised children below 6 months of age and above in Hong Kong from 2005 to 2011. *Vaccine* 32:6692–98
233. Simonsen L, Taylor RJ, Viboud C, Miller MA, Jackson LA. 2007. Mortality benefits of influenza vaccination in elderly people: an ongoing controversy. *Lancet Infect. Dis.* 7:658–66
234. Jefferson T, Di Pietrantonj C, Al-Ansary LA, Ferroni E, Thorning S, Thomas RE. 2010. Vaccines for preventing influenza in the elderly. *Cochrane Database Syst. Rev.* 2010:CD004876
235. Damiani G, Federico B, Visca M, Agostini F, Ricciardi W. 2007. The impact of socioeconomic level on influenza vaccination among Italian adults and elderly: a cross-sectional study. *Prev. Med.* 45:373–79
236. Nagata JM, Hernández-Ramos I, Kurup AS, Albrecht D, Vivas-Torrealba C, Franco-Paredes C. 2013. Social determinants of health and seasonal influenza vaccination in adults ≥65 years: a systematic review of qualitative and quantitative data. *BMC Public Health* 13:388
237. Colizza V, Barrat A, Barthelemy M, Valleron AJ, Vespignani A. 2007. Modeling the worldwide spread of pandemic influenza: baseline case and containment interventions. *PLOS Med.* 4:e13
238. Hosseini P, Sokolow SH, Vandegrift KJ, Kilpatrick AM, Daszak P. 2010. Predictive power of air travel and socio-economic data for early pandemic spread. *PLOS ONE* 5:e12763
239. Ginsberg J, Mohebbi MH, Patel RS, Brammer L, Smolinski MS, Brilliant L. 2009. Detecting influenza epidemics using search engine query data. *Nature* 457:1012–14
240. Yuan Q, Nsoesie EO, Lv B, Peng G, Chunara R, Brownstein JS. 2013. Monitoring influenza epidemics in China with search query from Baidu. *PLOS ONE* 8:e64323