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

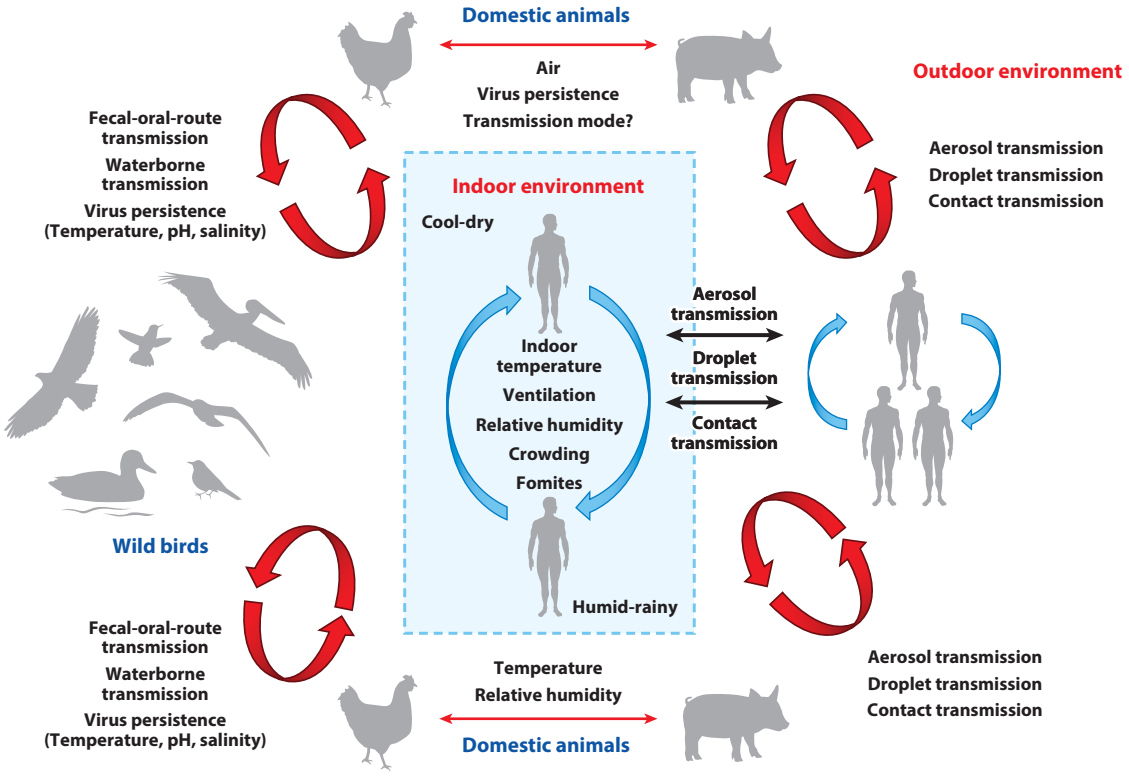

Figure 1

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.

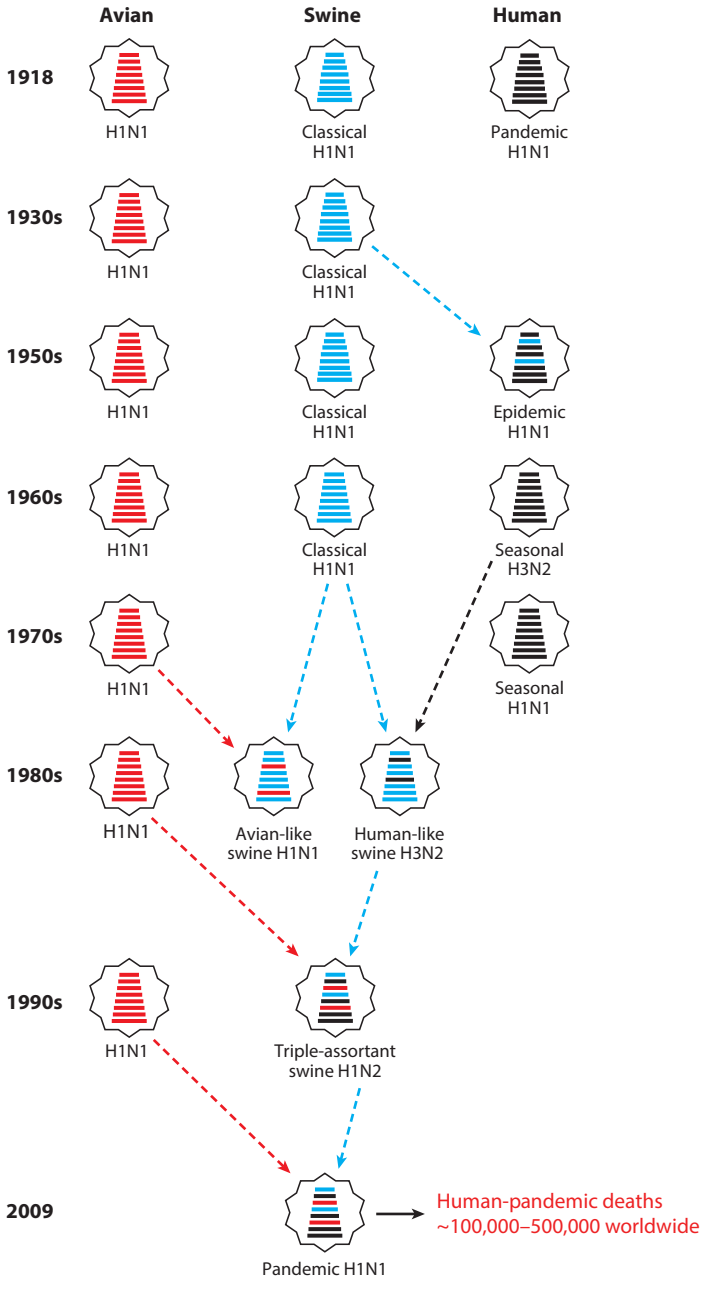

Figure 2

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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