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# Preventing Zoonotic Influenza

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.76966>

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## Abstract

The public health risk of influenza at the human-animal interface is dicey, due in part to continuous evolution of the virus. Influenza virus consist of 7 genera of which only influenza A is at present zoonotic, where subtypes H5, H7 and H9 of avian origin and subtype H1 and H3 of swine origin are important. The most devastating influenza pandemic in history was suspected to have emerged from avian reservoir and manifested in 1918. The first recognized direct human transmission of highly pathogenic avian influenza (HPAI) H5N1 occurred in 1997 in Hong Kong. Subsequently, many cases of varying severity have been described in people who were exposed to poultry. More recently in 2009, triple reassortant influenza A of swine origin (A/H1N1pdm09) caused the first pandemic of the twenty-first century and since 2013, H7N9 though initially benign in birds, caused fatal infection in humans who had contact with poultry. These public health threats from animal influenza virus are aggravated by increase co-mingling in shared human-animal environment. Therefore, the challenge of emerging zoonotic influenza viruses on human host immunity, efficacy of vaccines and antiviral resistance require continuous risk assessment of virological and clinical changes that have impact on control measures including advances in vaccines and chemotherapeutics.

**Keywords:** influenza viruses, zoonotic transmission, reassortment, immunity and vaccines, antiviral resistance

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## 1. Historical perspective on zoonotic influenza

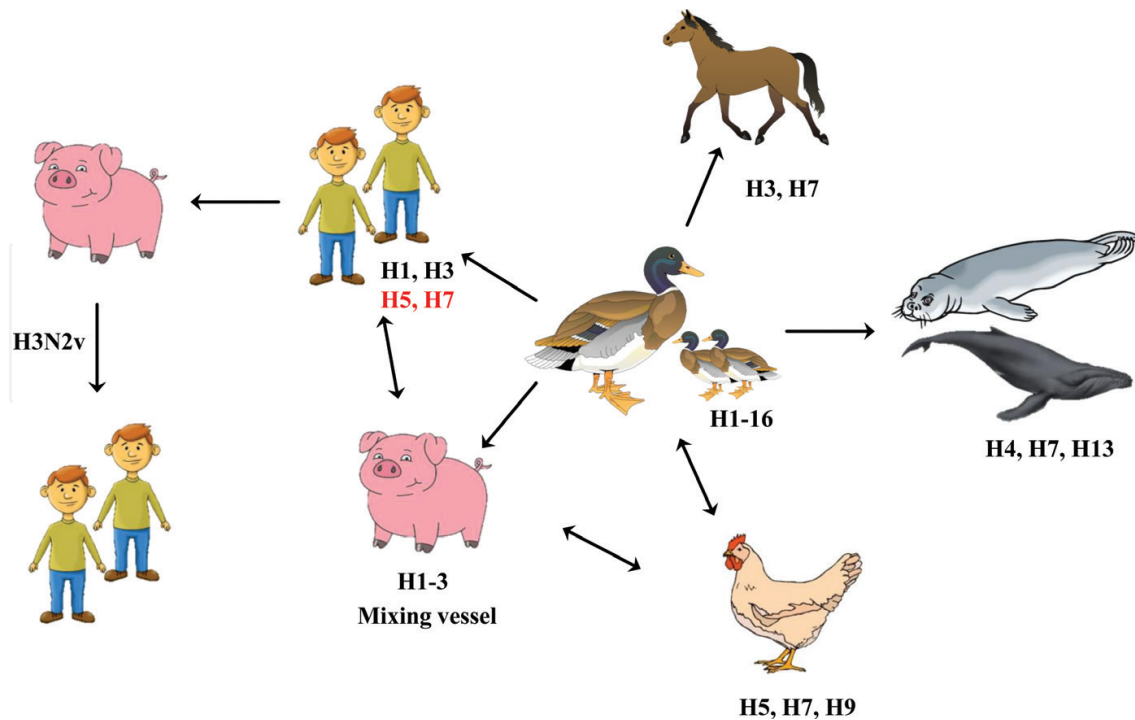
The family of influenza virus, known as *Orthomyxoviridae*, consists of 7 genera viz.: Influenza A, B, C and D. Others are *Thogotovirus*, *Quaranjavirus* and *Isavirus* which are associated with arthropods and fish [1]. Only 3, influenza A, B and C so far have been described in humans, while only Influenza A is commonly transmitted from animals to human and vice versa [2, 3]. Influenza A virus (IAV) is further divided into subtypes based on the Hemagglutinin surface

glycoproteins (HA1-18) and Neuraminidase (NA1-11), HA1-16 and NA1-9 are those that are up till date identified to occur naturally in avian host, mostly waterfowls where they exist in benign form (low pathogenic) [4, 5]. Two additional subtypes (HA 17 and 18, NA 10 and 11) were identified in bats [6]. Genetic mutations and reassortment may occur spontaneously or over a long period in reservoir hosts. These result in the emergence of novel subtypes, reassortants, strains or variants from the Low Pathogenic Avian Influenza (LPAI) precursors. These phenomena that have been described as antigenic shift and drift also contribute to the evolution, adaptation and inter-species transmission of influenza viruses and provide opportunities for gain of function in nature including molecular and or biological properties that may enhance zoonotic transmission. Sometimes a strain may arise in animals with adaptations of fitness to cause fatal infection or increase transmission and potentials to cause pandemics in human population [7, 8].

Aquatic birds are the most important group of animals in the ecology and epidemiology of influenza virus. Almost all naturally circulating subtypes of influenza virus in birds and mammals (including human) can be traced to avian descendants including earlier description in literature by Perroncito in 1878 [4, 9]. The first pandemic of influenza virus that occurred in 1918 (Spanish flu) was caused by an avian influenza virus, as revealed by sero-archeology and molecular characterization [10, 11]. The 1918 influenza pandemic killed over 50 million people and about one third (500 million persons) of the world's population had clinically apparent illnesses. The Case-fatality rate was greater than 2.5% in comparison to less than 0.1% in other influenza pandemics. Nearly half of influenza-related deaths were observed in young adults between the ages of 20–40 years, an indication that the virus was newly introduced possibly from animal reservoir to naive human population [12].

The causative virus of the 1918 pandemic, following human transmission, was concurrently transmitted to pigs in America, Europe and China. This was to play out again in 2009 when A/H1N1pdm09 virus was also transmitted via anthropogenic means to swine. In both scenarios, the causative virus was eventually isolated in pigs [2, 13]. More epidemics and pandemics arising from descendants of the 1918 virus were subsequently recorded in 1957 Asian flu (H2N2), 1968 Hong Kong flu (H3N2) and the more recent H1N1 2009 influenza pandemic that originated in Mexico (Mexican flu). The common precursor of these viruses appeared to be an avian influenza virus that entered the human population directly or indirectly through intermediate hosts probably at some points involving pigs as enunciated by Nelson et al. [14] and in **Figure 1**. Exceptionally, the 1918 pandemic virus appeared to have been wholly derived from avian-like influenza virus from an unknown source [15]. Thus zoonotic influenza transmission seems to be the foundation of influenza virus infection in human including previous pandemics, contemporary and more recent transmissions and fatal human infections caused by avian H5, H7 and H9 in many countries [16, 17].

In the last 100 years, influenza virus in human are generally manifested as seasonal, zoonotic and pandemic with clinico-pathological manifestation that vary from mild, severe to fatal. However, the most threatening influenza infections are those caused by zoonotic and/or pandemic strains following their introduction usually from animal reservoir into human population that has little or no pre-existing specific or cross protective immunity [18]. The burden of



**Figure 1.** Illustration of cross-species transmission of avian, swine and human influenza viruses. Waterfowls as natural reservoirs, pigs serve as ‘mixing vessels’, viruses from humans seed pH1N1 in swine populations, reassortment between pH1N1 viruses and co-circulating triple reassortant H3N2 viruses in pigs generate novel reassortant H3N2v.

zoonotic and potentially pandemic influenza A virus infections has therefore attracted global concern since the identification of avian and swine influenza viruses that can (with or without biological or molecular adaptations) be transmitted directly, and cause severe disease in humans and other mammals. This was notable with the advent of A/Goose/Guangdong/96 lineage of H5N1, which had infected 860 people and killed 454 (52% case-fatality rate) up till December 2017 [19]. Continuous circulation of H5N1 in birds and zoonotic transmission to human may cause influenza virus to acquire adaptive genetic features for efficient human to human transmission through mutations (insertions/deletions), reassortment or emergence of immune or antiviral resistant strains. Those may likely be precursors of emerging influenza virus with pandemic potential. Global surveillance for influenza diversity in animals and human may therefore greatly improve our ability for early detection, to identify and anticipate which strains are more likely to evolve and be better prepared [18].

## 2. Human infections with avian influenza virus

The first human transmission of Highly Pathogenic Avian Influenza (HPAI) subtype H5N1 occurred in 1997 in Hong Kong. It became a global public health concern, knowing that pandemic influenza viruses in the past originated from animals [20]. The H5N1 was thus considered a potential pandemic threat [21]. The HPAI H5N1 lineage (A/Goose/Guangdong/1/96) was initially isolated from a goose farm in Guangdong Province, China in 1996. In the following

year, outbreaks of highly pathogenic H5N1 were reported in poultry at farms and live bird markets in Hong Kong. Subsequently, contact with poultry and exposure to infected live and/or dead birds became the medium for human exposure and in Hong Kong there were altogether, 18 cases (6 fatal) reported in the first known instance of human infection with this virus [22].

Early symptoms of influenza H5N1 virus usually develop 2 to 4 days after exposure to sick poultry and most patients infected with influenza H5N1 virus presents symptoms of fever, cough, shortness of breath and radiological evidence of pneumonia [21]. The number of human H5N1 cases reported globally was heightened in 2003 and since then, the virus has maintained a steady infection, morbidity and mortality at the animal-human interface. Those primarily at risk are cohorts of poultry farmers, handlers and operators in live bird markets and their immediate family members or contact. Though human to human transmission of H5N1 is not yet efficient, evolving nature of influenza virus in the environment is a reminder of the risk of emergence of a strain adapted for that possibility.

The HPAI (H5, H7) viruses circulating in terrestrial poultry (Chicken and turkeys) and are transmitted to human, normally emerge from the low pathogenic precursors in waterfowls. This arises by mutations in the gene and occurrence of multiple basic amino acids in the connecting peptide between the HA1 and HA2 domains of the HA0 precursor protein [23]. Trypsin-like proteases found in the respiratory and gastrointestinal tracts may be responsible for this limited enzymatic cleavage hence pathology are usually restricted to these systems. However, when multiple basic amino acids are introduced by insertion or deletion in the HA cleavage site, the HA0 precursor becomes cleavable by a wide range of ubiquitous proteases found in many host tissues [24]. Consequently, the virus is able to replicate in almost all the tissues/organs beside the respiratory and gastrointestinal tracts such as brain (nervous) and the cardiovascular (hematopoietic) system, resulting in fulminant and disseminated disease with high mortality index particularly in turkey and chicken [25].

In a peculiar incident, in February and March 2013, three patients were hospitalized with severe lower respiratory tract disease of unknown cause in China. The causative virus was later identified as novel avian-origin reassortant influenza A (H7N9), and phylogenetic analysis of all genes of the isolate showed that each gene segment was of avian origin [17]. The HA cleavage site possessed only a single basic amino acid R (arginine) as against polybasic, indicating tendency to be of low pathogenicity in poultry. On the contrary, cases in human host were severe, with patients developing severe pneumonia, acute respiratory distress, and eventually death. All the three patients had pre-existing medical conditions, but more importantly two of them had a history of direct contact with poultry [17]. The switch in virulence and pathogenicity were associated with certain mutations in the reassorted virus that may have contributed to severity of human infection and death. Similarly, waves of H5N8 outbreaks, first detected in domestic birds in China in 2010, which later spread from 2014 through 2016 in Europe and North America was heightened in the winter of 2016 and affected a wide range of domestic and wild birds but no human infection was recorded. Experimental studies even showed low virulence in ferret hence risks to human were considered low [26], even though the contemporary HPAI viruses of subtypes H5N2, H5N5, H5N6 and H5N8, all contain genes from 1997 A/Goose/Guangdong H5N1 lineage with acquired internal genes

from LPAIs. Interestingly, the H5N8 (clade 2.3.4.4) though virulent in poultry has remained of low susceptibility in human, but another newly emerged H5N6 first identified in a peafowl arising from reassortment of H5 clade 2.3.4.4 has shown virulence in human and has killed 7 people among 17 that were infected since 2016 [27]. Repeated cases in human have raised concerns that subtype H5N6 virus also has the potential for crossover human infection which if sustained, may also be a candidate for influenza pandemic [28]. The notification of the first human case of novel subtype H7N4 to the Centre for Health Protection in Hong Kong on the 14th of February 2018 is a reminder that avian influenza is continually evolving bird-human transmission [29]. This H7N4 event and previous H7N9 detection first in human before cases in poultry were noticed also shows that humans are fast becoming sentinel for influenza surveillance at the human-animal interface.

### **3. The pandemic H1N1pdm09, zoonosis and reverse zoonosis**

Triple reassortant influenza A/H1N1pdm09 originating in swine caused the 1st pandemic of the twenty-first century in 2009. This was at the time of HPAI H5N1 epizootics in Asia, Europe and Africa. The strain and the region thought to be probable epicenter of future pandemic was H5N1 and Asia. The severity and spread of HPAI H5N1 in poultry and subsequent transmission to human, lent credence to scientific speculations that the zoonotic virus might have been a pandemic strain. Unpredictably, while attention was on HPAI H5N1, a pandemic H1N1 influenza virus emerged in Mexico, although the virus was believed to have been circulating in pigs many years before its first detection in human [16, 30]. The 2009 influenza pandemic spread to more than 214 countries and an estimated 151,700–575,400 respiratory and cardiovascular deaths were associated with the infection worldwide [31]. Lessons learnt include the realization that a zoonotic and pandemic virus may emerge from an animal reservoir in an unexpected location and spread rapidly throughout the world within a short time [32]. Also important is the realization that the 2009 H1N1 pandemic virus was subsequently transmitted from human to pigs in a phenomenon that has been variously described as reverse zoonosis reported in more than 20 countries in America, Europe, Asia and Africa. Interestingly, the swine influenza sequence data available in public gene bank showed that humans transmit far more influenza A virus to swine than pigs have ever transmitted to humans, at least in terms of viruses that are transmitted onward in the new host as against dead end or accidental hosts [14]. The implication is that endemic human-like influenza virus that is enzootic in pigs will continuously pose public health risk in the generation of Influenza variants (combination of human and swine influenza viruses). This has also been reported to cause human infections in people exposed to pigs especially in America [14].

Virus strains or variants resulting from reassortment of swine influenza A(H3N2) and influenza A/H1N1pdm09 and similar viruses have been detected in swine in many countries. It is therefore of concern that emerging influenza variants could efficiently be transmitted among humans. Over 300 human cases of A(H3N2)v have been described between 2011 and 2012 in the United State alone beside clusters of human-human transmission further demonstrating that variant influenza viruses also pose a public health threat at the human-animal interface. Animals and humans may infect each other in intensive farms, abattoirs and agricultural fairs

when in close proximity [33, 34]. Our ability to predict and prevent outbreaks of zoonotic pathogens like influenza therefore requires an understanding of their ecology and evolution in reservoir hosts [35]. This is important because Influenza A viruses from animals including reassortant, novel and variants are considered of significant threat in the emergence of the next pandemic due to the abundance of permanent animal reservoirs harboring viruses that are now frequently spilling over into human.

#### 4. Mutation, reassortment and variants influenza virus

Over the past 100 years, the IAVs have caused several pandemics including the one that has been described as “the greatest medical holocaust in history” [36]. Mutation and reassortment are two well established factors that have contributed in zoonotic influenza viruses gaining the ability to adapt to humans, leading to pandemics and thereafter sustained human-to-human transmissions. The accumulation of mutations and genome reassortments have been the driving force for most of the IAV adaptability in humans as the IAV RNA genome replication lacks the exonuclease proofreading capability, thus giving rise to high nucleotide mutation rates [37]. Antigenic drift and shift are the two major phenomena in influenza viruses that lead to antigenically variant influenza viruses [1, 38, 39]. The antigenic drift refers to point mutations in the HA and/or NA while the antigenic shift leads to the formation of a new virus subtype with a novel combination of HA and NA from different subtypes. While the antigenic drift is responsible for yearly epidemics, the antigenic shift has been responsible for some of the devastating pandemics in influenza history claiming many lives, including the 1918-Spanish flu. A list of zoonotic influenza outbreaks have been summarized in **Table 1**.

The human influenza viruses have limited subtypes of HA and NA (H1, H2, H3 and N1, N2) whereas the avian influenza viruses infecting the poultry may harbor almost all the subtypes of HA and NA [40], thus giving rise to multiple recombination of HA and NA in avian species. Since 1996, the HPAI-H5N1 virus have claimed several lives resulting in high mortality rate while the recently identified LPAI-H7N9 in East China region had a mortality rate of 40% [41]. The H7N9 virus isolates have the capability of binding to both avian and human influenza virus receptors due to presence of a leucine at amino acid position 217 [42]. A relatively limited number of mutations in the zoonotic IAV genome can lead to production of new viral progenies with capability of efficient transmission among mammals and studies have also demonstrated that amino acid substitutions in the HA protein can change the preference of binding receptors of influenza viruses. For example, the G186 V mutation in HA protein of H7N9 virus has been identified as the potential adaptation of the virus to human-type receptors [43]. A recent study conducted on a non-laboratory-adapted virus A/Vietnam/1203/2004 (H5N1) with an HA2-K58I point mutation (K to I at position 58) showed that a decrease in the HA activation pH (pH 5.5) influenced the viral properties as compared to the wild type virus (HA activation pH 6.0) in mammalian hosts [44]. The mutation increased the viral load in ferret’s nasal cavity while it reduced the viral load in lungs thus supporting the fact that a single mutation could lead to an increased viral growth in mammalian upper respiratory tract [44].

Year (Country)	Influenza subtype	Confirmed cases	Adaptation in segment
1997/2003-present (Asia, Europe and Africa)	H5N1	660	N224 K (HA) N158D (HA) T160A (HA) E627K (PB2)
2003 (USA)	H7N2	1	Not determined
2003 (Hong Kong)	H9N2	1	Q226L (HA) G228S (HA) T212A (HA)
2003 (The Netherlands)	H7N7	89	E627K (PB2)
2004 (Egypt)	H10N7	2	Not determined
2004 (Canada)	H7N3	2	Not determined
2007 (UK)	H7N2	4	Not determined
2008–2009 (Hong Kong)	H9N2	2	Not determined
2012 (Mexico)	H7N3	2	Not determined
2013 (China)	H10N8	3	Not determined
2013 (China, Taiwan, Hong Kong)	H7N9	137	Q226L (HA) E627K (PB2)
2013 (Taiwan)	H6N1	1	P186L (HA)
Since 2014 (China)	H5N6	16	G540A (NS)
2018 (China)	H7N4	1	Not determined

**Table 1.** Zoonotic influenza A viruses and identified adaptations (reviewed in [53] with modification).

Several studies in ferrets have shown that the viruses such as H5N1 [45], H7N9 [46] and H7N1 [47] could transmit through respiratory droplets after acquiring mutations in their genomes. Another study on A/Anhui/1/13 (H7N9) virus showed that substitutions at G219S and K58I resulted in high affinity for  $\alpha$ 2,6-linked sialic acid receptor and acid and temperature stability [48]. The increased polymerase activity due to mutation in the viral PB2 has also been linked to enhanced viral replication. The PB2 subunit from all avian viruses generally contains polymerases with glutamic acid at amino acid position 627 (E627) while the PB2 from human viral isolates almost exclusively have lysine at 627 (K627). Mehle et al. have shown that E627K mutation of PB2 conferred a high level of polymerase activity in human and porcine cells thus increasing the viral replication [49]. Another study showed that a basic amino acid at position 591 of the PB2 subunit compensated for the lack of PB2-627 K in HPAI-H5N1 and pandemic H1N1viruses markedly increased the replication of these viruses in mammalian species [50]. The PB2 gene mutation in duck H7N9 also enhanced the polymerase activity and thus viral replication in human cells [51]. The H1N1 influenza virus that caused the 1918 pandemic and



the H5N1 avian influenza virus isolated in 1997 (Hong Kong) both harbors the N66S mutation in PB1-F2 which drastically enhanced the pathogenicity of these viruses [52].

Genetic reassortment in influenza viruses is yet another vital event that leads to sudden outbreaks of influenza. Influenza viruses have a segmented genome and thus, simultaneous infection with other IAVs results in reassortment event leading to formation of new viral progenies containing gene segments of mixed parental origin. Several pandemics have emerged in the past [54] and appears to be more frequent now than previously thought [55]. The reassortment event can be a result of errors during the replication of viral RNA polymerase, the host environment, the immune or evolutionary pressure [56]. The pandemics of 1957 and 1968 were caused by reassortant viral strains [57]. The HA, NA, and PB1 genes of the H2N2 1957 pandemic strain and the HA and PB1 fragments of the H3N2 1968 pandemic strain were both derived from avian influenza virus strains [57]. The HPAI subtype H5N1 isolated from geese in Guangdong province in 1996 evolved to produce H5N8 clades 2.3.4.4 Gs/GD HPAIV. A recent study on the evolution and pathogenicity of H5N2 avian influenza viruses isolated in H5N1 endemic areas in China revealed that these viral isolates were derived from reassortment events in which few isolates had the HA and NS derived from H5N1 while few had the HA derived from endemic H9N2 viruses [58]. A similar study from South Korea reported the emergence of novel reassortant H5N8 viruses in 2014 in ducks raised in breeder farms [59]. Since its first appearance, lineage of the HPAI H5N1 continues to circulate with lots of diversification of the HA gene into multiple genetic clades. The H5 clade 2.3.4.4 of the H5N8 subtype was subsequently detected in several countries of Europe by the end of 2014 and in summer of 2016, it was detected again in wild aquatic birds sampled in western Siberia [60]. A recent study has also shown that the reassortment event between the Gs/GD lineage H5N8 virus and North American origin viruses further resulted in the emergence of H5N1 and H5N2 viruses in the US [61].

Experimental observations have further revealed that reassortment between zoonotic and seasonal IAVs can result in production of airborne-transmissible viruses in mammals [62–65]. A study showed that a reassortant virus, comprising of the H5 hemagglutinin having 4 mutations from H5N1 avian virus and remaining seven segments from the 2009 pandemic H1N1 virus lead to reassortant H5 HA/H1N1 virus that gained the capacity of droplet transmission in ferret model [62]. Another experiment further showed that the avian H5N1 subtype viruses do have the potential to attain mammalian transmissibility by genetic reassortment [63]. The authors utilized reverse genetics to create several reassortant viruses between duck H5N1 (HA gene) and human-infective H1N1 virus to show that the new reassortant viruses could efficiently infect and sustained droplet transmission in guinea pigs without mortality [63]. Similar study reported that the avian-human H9N2 reassortant virus harboring the surface proteins of avian H9N2 in a human H3N2 backbone gained the ability of transmission through the respiratory droplets and caused clinical infection in ferrets similar to human influenza infections [64]. A recent study performed in a novel transfection-based inoculation system generated a reassortant H9N1 virus by transfecting the plasmids containing genes from H9N2 virus and pandemic H1N1 (pH1N1) virus into HEK 293 T cells. The resulting transfections gave rise to two reassortant viruses (H9N1) that had the capability of droplet-transmissibility [65].

According to the Centers for Disease Control and Prevention (CDC), when an influenza virus that normally circulates in swine (not in humans) is detected in humans, it is referred to as variant influenza viruses. The human infections with H1N1, H3N2 and H1N2 variant viruses have been reported from United States [13, 65]. Although the variant influenza viruses rarely show sustained human-to-human transmission, yet there have been few strains that overcame this barrier. All the cases reported in US were of swine origin rather than avian origin. In 2009, triple reassortant variant influenza virus was detected throughout the world and caused the first pandemic of twenty-first century. This variant virus had genes from avian, human, and swine influenza viruses claiming more than 12,500 lives in the US alone and about 575,400 globally [31, 66]. Later H3N2 variant viruses which had similarity with triple-reassortant viruses were detected in US swine but had acquired the matrix gene from highly transmissible influenza A H1N1-2009 viruses which contributed in efficiency of transmission of the variant virus [67]. A recent study has also identified two distinct variants of H3N2 influenza virus that grows in cell culture [68]. Both the variants differed in just one single mutation at amino acid 151 of NA. The D151 viral variant could efficiently grow in cell culture while the G151 viral variant showed extremely poor growth in cell culture system [68]. More in-depth studies are still needed to better understand the viral properties of variant influenza viruses as they continue to pose threat to human lives.

## 5. Immunity and challenges of vaccination

The isolation of influenza A/H1N1 in 1933 quickly ushered the development of the first generation of live-attenuated influenza vaccines (LAIV). The initially developed inactivated influenza vaccine (IIV) only targeted a single influenza strain (influenza A). Then, in 1942, a vaccine targeting both influenza A and B viruses were produced soon after the discovery of influenza B. Subsequently, scientists discovered that influenza viruses mutated, leading to antigenic changes (antigenic drift and antigenic shift). Since 1973, the World Health Organization (WHO) has been providing yearly recommendations for the composition of the influenza vaccine, based on results of the virological surveillance conducted by the WHO's Global Influenza Surveillance and Response System (GISRS). Later in 1978, the trivalent influenza vaccine was developed that included two influenza A strains and one influenza B strain. Currently, two influenza B lineages are circulating (Yamagata and Victoria) therefore, since 2013, the WHO recommendations suggested a second B strain to be added to make a quadrivalent influenza vaccine (QIV) [69]. Influenza vaccines protect against infection and can reduce illness and severity of infection especially in groups at risk for flu complications such as children, the elderly, pregnant women, and individuals with underlying medical conditions like asthma, HIV/AIDS, and chronic heart or lung diseases [70]. Frequent influenza infections at the human-animal interface may also warrant occupational vaccination for veterinarians, researchers, health care providers, farmers and animal traders who are more likely to be exposed to zoonotic influenza virus [71].

For over half a century now, WHO has been collaborating with scientists, epidemiologists, and policymakers to create an integrated approach to manufacture, test, and approve

influenza vaccine research and development efforts, including their proper use and efficient distribution. Since the virus mutates frequently, WHO, GISRS network and collaborating centers predict the strains that are expected to circulate in the following season because of the time required to manufacture vaccines. This happens twice a year, one for the northern hemisphere and another for the southern hemisphere [70]. But the virus can mutate during the time it takes to develop the vaccine, resulting in a mismatch between circulating virus and the vaccine.

Although the effectiveness of the flu vaccine varies from year to year depending mainly on the match of the strain in the vaccine and the circulating strain, most provide modest to high protection against influenza [72]. The US-CDC has reported that flu vaccination reduces medical visits, flu illness, hospitalizations, and deaths [73]. Vaccination is still the most efficient way to prevent infection and severe outcomes caused by influenza viruses.

The WHO and CDC recommend yearly vaccination for nearly everyone over 6 months of age, especially those at higher risk of influenza complications and mortality [70, 73]. The European Centre for Disease Prevention and Control (ECDC) also recommends yearly vaccination of high risk groups: older adults and all persons (over 6 months of age) with chronic medical conditions including those with diseases of the respiratory system (e.g. asthma), cardiovascular system (e.g. coronary artery disease), endocrine system (e.g. diabetes), hepatic system (e.g. liver cirrhosis), renal system (e.g. chronic renal failure), neurological/neuromuscular conditions (e.g. parkinsonism), any condition compromising respiratory functions e.g. morbid obesity (BMI > 40), physical handicap in children and adults, and immunosuppression due to disease or treatment including due to hematological conditions and HIV infection [74].

Currently licensed flu vaccines include inactivated influenza vaccine (IIV), live attenuated influenza vaccine (LAIV), and recombinant HA vaccines [75]. These vaccines are either trivalent or quadrivalent with components representing influenza A and B viruses predicted to circulate in the next influenza season. The IIV is either a split virion or subunit vaccine containing 15 µg of each purified HA protein administered intramuscularly, or 9 µg of each purified HA protein administered intradermally [75]. A higher dose version with 60 µg of each HA antigen is available for older adults aged 65 years and above. The IIV induces a strain-specific serum IgG antibody response. A vaccine with an oil-in-water adjuvant MF59 also enhances the immunogenicity of IIV in the elderly [76].

The LAIV contains live viruses with temperature-sensitive and attenuating mutations [77] and is a combination of the same four strains as the QIV. The LAIV is administered intranasally as a spray. The mutations in the LAIV strains allow the viruses to replicate at the cooler temperature of the nasal cavity but prohibit replication at the temperature of the lower respiratory tract. The LAIV results in the production of strain-specific serum IgG as well as mucosal IgA and T cell responses [77]. The recombinant HA vaccine with HA proteins expressed in insect cells from baculovirus vectors is currently licensed only for adults aged 18 to 49 years and are recommended for individuals who are allergic to eggs [75]. The manufacturing process for the recombinant HA vaccine is shorter than the IIV and LAIV, which would be important in case of a pandemic. The 2009 pandemic showed the challenges in production and distribution of vaccines against a newly emerged virus within a short timeframe given

the production timeline for both IIV and LAIV [78]. Production of IIV and LAIV require the use of embryonated eggs. Disadvantages for egg-based flu vaccine production include being contraindicated in people with severe allergies to eggs, and in the event of a pandemic where the virus is pathogenic to poultry, embryonated eggs may be in short supply [69]. Currently, licensed influenza vaccines focus on the production of antibodies against the viral HA protein, which binds host receptors to mediate viral entry. Strain-specific antibodies produced against the HA neutralize the virus and prevent infection. However, the HA is under positive selection for antigenic escape from neutralization by pre-existing antibodies [70].

Vaccine-induced HAI antibody titer is currently accepted as the correlate of protection against influenza. An HAI titer of  $\geq 1:40$  in healthy adults is the titer at which approximately 50% of individuals are protected from infection. However, some studies have indicated that a higher HAI titer may be required in children and that T cells may be a better indicator for protection in the elderly [79, 80]. Also, serum HAI antibody titer is not a reliable correlate of protection for seasonal and pandemic LAIV vaccines. LAIV has been shown to be effective in the absence of a robust serum antibody response [77]. The HAI antibody titer also fails to take into account other aspects of immune memory against the virus, including the contribution of non-neutralizing antibodies and T cell responses to protection. The immune response to influenza is complicated, and there could be several correlates of protection apart from HAI antibodies. A more comprehensive immunological analysis and an integrative genomic analysis of the human immune response [81] using the different influenza vaccines could further define other correlates of protection to better interpret influenza vaccine efficacy [82].

Influenza A viruses (IAVs) infects human, swine, and domestic poultry; therefore; interspecies and intercontinental spread make IAV more complicated. Vaccination of domestic poultry (including chicken and turkey) is common against the HPAI, H5/H7 LPAI, and H9N2 LPAI worldwide. In the past, emergency vaccination against HPAI to control epizootics has occurred. Areas include Mexico (H5N1, 1995), Pakistan (H7N3, 1995–2004), Asia/Africa/Europe (H5N1, 1996–continuing), and North Korea (H7N7, 2005) to aid in stamping out programmes [83]. Poultry vaccines are manufactured inexpensively and are not filtered and purified like human vaccines and usually contain a whole virus, and not just HA antigen. Mineral oil, which induces a strong immune reaction and causes inflammation and abscesses, is added as an adjuvant to poultry vaccines.

Usage of vaccine to control swine influenza virus (SIV) varies by countries; some countries use vaccination strategies, while others do not. For examples, SIV vaccination is conducted extensively in Europe and North America. In Korea, on the other hand, vaccines for SIV control are rarely used despite availability in the market. Because of the genetic diversity of circulating SIV strains, most commercial vaccines consist of multiple strains of subtype H1N1, H1N2, and H3N2. Nevertheless, the rapid evolution of circulating viruses could surpass the updates of commercial vaccines. Combining the herd-specific autogenous vaccine with other commercialized vaccines occurs in some countries; about 20% of pig farms in the United States used autogenous vaccines in 2006. However, compared to avian influenza viruses, vaccines against SIVs have not been used extensively by swine veterinarians in many countries because other major pathogens including the porcine reproductive syndrome virus and porcine circovirus

are considered more important [83]. Nevertheless, successful application of influenza vaccines in animals may contribute in reducing zoonotic transmission.

## 6. Antiviral resistance mutants

Antiviral resistance in influenza viruses is a global concern and the number of resistant mutants is increasing year after year. The antiviral drugs have been formulated mainly against the M2 ion channel (amantadine and rimantadine) and the neuraminidase proteins (oseltamivir and zanamivir) of influenza viruses. These FDA approved drugs are currently used for prophylaxis and treatment of influenza A infections and are effective against the HPAI H5N1 viruses [84]. The effectiveness of these drugs ranges from 80 to 90% if the treatment had begun within 48 hours of infection [85]. The antiviral resistance in influenza may develop during disease treatment and occasionally spreads widely to replace the susceptible strains in the absence of drug pressure. An example of this event is the global spread of adamantane-resistant H3N2 viruses in the year 2003, oseltamivir-resistant seasonal H1N1 viruses since 2007 and more recently the adamantane-resistant pandemic A (H1N1) viruses in 2009. Such events show the highly unpredictable nature of influenza viruses and increase the challenge of its management. Sometimes a single reassortment event or mutations leads to emergence of variant influenza viruses such as the pandemic 2009 or seasonal A (H1N1) viruses that becomes completely unresponsive to most antiviral drugs. The amantadine resistance was soon observed after the discovery of the drug in early 1960s and studies subsequently reported that a single point mutation in the M2 protein lead to the emergence of high-level resistant mutant viruses showing resistance to both amantadine and rimantadine [86]. Other studies also suggested that resistance to M2 blockers (amantadine/rimantadine) can be achieved by only a few substitutions in the codon L26, L27, A30, A31 and G34 of the M2 gene [87] and these mutants retain the virulence and are transmissible between humans [88]. A study showed that adamantane resistance emerged in about 30% of patients post few days of treatment [89]. Another study has shown the synergistic antiviral effects of amantadine-oseltamivir combination chemotherapy [90]. The adamantanes were very effective for almost 4 decades after which the frequency of adamantine resistance among influenza A H3N2 viruses started to increase. The global resistance among H3N2 virus was as low as 0.8% between the periods 1991 to 1995. The adamantine resistance has now been reported for human H1N1, H3N2 and H5N1 avian influenza viruses. The frequency of resistance further increased to 28% during 2004–2005 and to 72% in 2005–2006 for H1N1 variant viruses [1]. The US reported around 92% resistance among H3N2 viruses by the year 2005. A recent study based on the frequency and distribution of M2 gene mutations in influenza virus variants that circulated between 1902 and 2013 showed that 45.2% of all resistant influenza A viruses (H1-H17) circulating globally had S31 N mutations [91].

Similarly the NA mutations causing resistance to neuraminidase inhibitors (NAI) has lots of variations. The most common mutation observed is the H275Y that confers high resistance

to oseltamivir [92]. A study showed that the amino acid changes at residue 223 (I → R/V) conferred reduced inhibition to oseltamivir and zanamivir [93]. The N2 subtype has been associated with oseltamivir resistance due to mutation at E119V and R292K. The R292K has also been linked to zanamivir resistance [94]. Studies have demonstrated that the most frequent mutation conferring the oseltamivir resistance in NA of the H1N1 and H5N1 subtypes was H274Y, while the E119V and R292K mutations were more common among the H3N2 and H7N9 subtypes [95]. Another study showed that R292K mutation in NA protein in the H7N9 virus strains were detected in patients after drug treatment. This substitution promoted resistance against oseltamivir [96]. Similarly oseltamivir resistance was associated with the H274Y NA mutation in H5N1 influenza viruses detected in patients during treatment or prophylaxis [97]. Few other studies have reported that the Egyptian H5N1avian influenza isolates from humans had N294S NA mutation [98]. Boltz et al. reported that H5N1 viruses of clade 2.3.2 isolated from the Republic of Laos in 2006–2008 had V116A, I222L, and S246 N mutations in NA [99]. The ongoing concerns about influenza A viruses and increasing antiviral resistance needs immediate attention, better antiviral surveillance for better management and control of future influenza pandemics.

## 7. Infection control, advances in vaccines and therapeutics

Generally, people infected with the flu are advised to stay home and rest, both to recover and to avoid infecting others. In severe cases, or for individuals at high risk of complications, physicians may prescribe antiviral medication. The antiviral drugs currently available against influenza viruses are adamantane derivatives (amantadine and rimantadine) and neuraminidase (NA) inhibitors (zanamivir, oseltamivir and peramivir). A viral infection can be inhibited at several crucial steps, such as entry, signaling, assembly, and egress [1].

Oseltamivir, works by blocking neuraminidase that enables newly made influenza virus to escape from an infected cell. Zanamivir (inhaled), peramivir (intravenous), and inavir (inhaled) operate in a similar way. Baloxavir, discovered in Osaka, received preliminary approval in Japan in January 2018 and will be filed for regulatory review in the US and Europe thereafter. Baloxavir requires a single dose, unlike oseltamivir which is taken twice a day for 5 days [100].

Efforts to improve currently available vaccines have been explored over the last 2 decades such as: increasing the antigen dose, intradermal route of administration to activate other arms of the immune system, and adding immunostimulating compounds such as adjuvants [78]. The main areas of research and development in flu vaccines involve:

1. Creation of vaccines with protective immunity lasting more than one season,
2. Shortening of the production time to allow a virological assessment nearer the upcoming influenza season. Cell-culture-based vaccines (e.g., Optaflu, Flucelvax, Preflucel, and Celvapan) are also being used to overcome this issue [101].

3. Development of a universal vaccine that protects against influenza regardless of what influenza viruses are circulating. These includes vaccine targeting the HA stalk domain [102, 103], and the use of influenza-virus-like particles as vaccines [104].

In addition to antiviral drugs and vaccines, several novel therapeutic alternatives may prove to be beneficial in the near future. The long-acting inhaled neuraminidase inhibitor CS-8958 (also known as R-118958) has shown promising results in murine models of influenza treatment while a polymerase inhibitor, T-705 (Toyama Chemical), that inhibits viral RNA polymerase has been found to be effective against all three influenza virus types (A, B and C) and to some extent against other RNA viruses, including hemorrhagic fever viruses. The drug, DAS181, a fusion construct that includes the sialidase from *Actinomyces viscosus*, affects the viral attachment process during the early stages of influenza replication. Another study demonstrated the antiviral properties of chlorogenic acid (CHA) and its inhibitory effect on A/PuertoRico/8/1934 (H1N1) and oseltamivir-resistant strains in the late stage of the infectious cycle. Other novel antiviral drugs under clinical development include AVI-7100, a 20-mer phosphorodiamidate morpholino oligomer (PMO) IV formulation that hinders translation and splicing of mRNA from the matrix gene. EV-077, a dual thromboxane receptor antagonist and thromboxane synthase inhibitor, prevents virus replication by inhibiting prostanooids associated with influenza infections. Aureonitol, a compound obtained from fungi, has shown inhibitory effects against both influenza A and B virus replication by impairing virus adsorption. Monoclonal antibodies, CR6261 and CR8020, bind to the conserved stalk region of HA and inhibit the entry and fusion stages. A broad spectrum human monoclonal antibody (mAb- MEDI8852), which unlike other stem-reactive antibodies, used a rare heavy chain VH (VH6-1) gene, was found to be effective in mice and ferrets and better than oseltamivir [1]. These novel approaches will potentially become effective tools for managing seasonal, zoonotic and pandemic influenza virus infections.

## 8. Conclusions

Influenza viruses have a silent reservoir in the aquatic avian species and continuously pose threat to human population. The avian, swine and other zoonotic influenza infections may range from a mild upper respiratory tract infection to a more severe pneumonia, acute respiratory distress syndrome and even death. Humans can be infected with a wide range of avian [subtypes A(H5N1), A(H7N9), and A(H9N2)] and swine [subtypes A(H1N1), A(H1N2) and A(H3N2)] influenza viruses. Although sustained human to human transmission is lacking, these viruses can be transmitted when there is a direct contact with infected animals or contaminated environments. The virus shows a tremendous potential to mutate, re-assort and give rise novel variants to evade host immunity and vaccination strategies. The emergence of antiviral mutants has further worsened the worldwide control measures. Although management of influenza has been a challenging task owing to its large reservoir and ability to mutate rapidly, the disease can be controlled in the animal source to decrease the risk to human population. With advancements in modern diagnostic methods, vaccination and antiviral strategies, the annual epidemics and occasional pandemics can be managed efficiently.

## 9. Future perspectives

The public health threats from influenza viruses have always been a global concern. They are not only responsible for annual epidemics throughout the world, but also affect quality of life and have negative impacts on the economy due to frequent school and work place absenteeism. The frequencies of influenza infections have further increased due to co-mingling in shared human-animal environment. The virus is known to acquire antigenic shift and drifts and thus pose challenges in control measures and management. Advancements in vaccination strategies, discovery of novel drugs and antiviral therapeutics along with development of a universal influenza vaccine are promising approaches toward the management of future epidemics and pandemics.

## Disclosure of potential conflicts of interest

All authors declared that they have no conflict of interest (financial or non-financial).

## Abbreviations

CDC	centers for disease control and prevention
ECDC	European Centre for Disease Prevention and Control
FDA	Food and Drug Administration
GISRS	global influenza surveillance and response system
HA	hemagglutinin
H5N1	highly pathogenic avian influenza
IAV	influenza A virus
IIV	inactivated influenza vaccine
LAIV	live-attenuated influenza vaccine
LPAI	low pathogenic avian influenza
mAb	monoclonal antibody
NA	neuraminidase
NAI	neuraminidase inhibitors
PMO	phosphorodiamidate morpholino oligomer
QIV	quadrivalent influenza vaccine
RNA	ribo nucleic acid
SIV	swine influenza virus
WHO	World Health Organization



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