Adapting Global Influenza Management Strategies to Address Emerging Viruses.

Diana L. Noah and James W. Noah*

Southern Research Institute, 2000 9th Avenue South, Birmingham, AL 35205

*Corresponding Author Email: j.noah@southernresearch.org

Copyright © 2013 by the American Physiological Society.
**Key Summary Points:**

Influenza remains a critical factor in global health and economics, and recent pandemics and outbreaks of highly lethal avian virus strains (including the recent novel H7N9 virus strain) have increased public awareness and altered annual vaccine production strategies.

Influenza has caused four pandemics in the last century, and the most recent 2009 H1N1 pandemic virus infected up to 20% of the global population, depending on region, with an estimated 60 million infections in the U.S. alone.

The influenza pandemic of 2009 and continued emerging outbreaks emphasize the need for standardization of global surveillance methods. The WHO is addressing this by preparing a manual of global standards and working towards improving the tools for reporting both surveillance and vaccine distribution data.

Vaccination remains the principle means for controlling influenza, but antivirals are widely available for therapy and prophylaxis. Vaccine strategies are blunted by antigenic drift and shift, while increasing resistance reduces the long-term utility of currently approved antivirals.

New and adaptable strategies are needed to combat emerging influenza virus strains. These include the advancement of new antiviral inhibitors for unexploited viral or host immunomodulatory targets, novel adjuvants and alternate delivery systems, and the development of universal protein, DNA, or multivalent vaccines that are designed to increase immune responsiveness and enhance public health response times.
Abstract

Death by respiratory complications from influenza infections continues to be a major global health concern. Antiviral drugs are widely available for therapy and prophylaxis, but viral mutations have resulted in resistance that threatens to reduce the long-term utility of approved antivirals. Vaccination is the best method for controlling influenza, but vaccine strategies are blunted by virus antigenic drift and shift. Genetic shift in particular has led to four pandemics in the last century, which have prompted the development of efficient global surveillance and vaccination programs. Although the influenza pandemic of 2009 emphasized the need for the rapid standardization of global surveillance methods and the preparation and dissemination of global assay standards for improved reporting and diagnostic tools, outbreaks of novel influenza strains continue to occur, and current efforts must be enhanced by aggressive public education programs to promote increased vaccination rates in the global population. Recently, a novel H7N9 avian influenza virus with potential to become a pandemic strain recently emerged in China and was transmitted from animals to humans with a demonstrated >20% mortality rate. Sporadic outbreaks of highly lethal avian virus strains have already increased public awareness and altered annual vaccine production strategies to prevent the natural adaption of this virus to human-to-human transmission. Additional strategies for combating influenza include advancement of new antivirals for unexploited viral or host cellular targets, novel adjuvants and alternate vaccine delivery systems, and development of universal protein, DNA, or multivalent vaccines designed to increase immune responsiveness and enhance public health response times.
Introduction

Influenza type A and B virus infections in humans result in an estimated 150,000-200,000 hospitalizations and 30,000-50,000 deaths in the United States (U.S.) annually (79, 80) and many thousands more globally. In addition, annual epidemics have a large economic impact, costing more than $11 billion in direct medical costs, $16 billion in indirect loss of earnings and life, and $88 billion per year total (hospitalization costs and lost productivity) in the U.S. alone (49). In 2009, a highly contagious pandemic virus with low pathogenicity emerged in the unlikely region of Mexico and spread across the globe during months that are not typically part of the Northern Hemisphere influenza season. In the U.S., an estimated 44,000 deaths were directly attributable to this pandemic virus, which had a larger rate of morbidity, (particularly in the very young) (87), and is considered to have resulted in a substantial health burden in the U.S. during circulation (in terms of mortality). This pandemic justified the need for continued research in a myriad of areas that, while not idiosyncratic for influenza, contribute significantly to our understanding of the virus epidemiology, management, and most importantly, the protection of the population.

In April 2013, a new influenza H7N9 virus strain emerged in China (28) and has caused considerable concern because of the >20% mortality rate in humans and the uncertain modes of transmission. Unlike the avian H5N1 viruses which contain a neuraminidase subtype (N1) that is also present in the annual, seasonal human virus strains, this novel avian virus contains a hemagglutinin (H7) and neuraminidase (N9) that have not previously circulated in the human population. Because of this, the world’s population is likely immunogenically naïve, and this virus is considered a candidate for evolution into a pandemic strain. In the event of a highly-pathogenic pandemic, the Centers for Disease Control and Prevention (CDC) has predicted a 3-7 fold increase in hospitalization and mortality rates and at least a 20-fold increase in economic impact in the U.S. alone. The effect would be many times more devastating in regions of the
world where the health care system is not comparably advanced (45, 46). Finally, the
decimation of the domestic fowl (60, 85) and swine (62) populations by pathogenic influenza or
preventative culling would have a tremendous economic impact on worldwide markets. These
factors have prompted the NIAID to classify the influenza virus as a Category C priority
pathogen and the CDC and U.S. Department of Agriculture to classify highly pathogenic avian
influenza as a select agent (86). In light of this recent emergence of H7N9 influenza, here we
review the importance of influenza in the global environment and recent efforts for influenza
research in the realms of epidemiology and global surveillance, vaccine production, antiviral
use, and new research strategies.

**Influenza overview**

Influenza viruses belong to the family of orthomyxoviruses, and contain a negative-sense,
segmented RNA genome (54). Influenza A viruses continue to emerge from the aquatic avian
reservoir and cause annual seasonal epidemics and infrequent pandemics (88). Influenza also
occurs in sporadic outbreaks of varying extent at any time of the year. The variation in the
antigenic properties of the influenza viruses is a major factor contributing to this epidemiological
pattern. The subsequent viral spread depends upon multiple factors, including transmissibility of
the virus and the susceptibility of the population. Although both influenza A and B types infect
humans, the restricted reservoir for influenza B does not make it a candidate for pandemics.
Among influenza A viruses that infect and circulate in humans, three major subtypes of
hemagglutinins (H1, H2, and H3) and two subtypes of neuraminidases (N1 and N2) have been
described. Avian influenza strains considered to have pandemic potential include H5, H7, and
H9 hemagglutinins and the N7 and N9 neuraminidase variants. Influenza A viruses, in
particular, have a remarkable ability to undergo periodic drifts in the antigenic characteristics of
their envelope glycoproteins HA and NA, and more extensive shifts of their genetic segments
Antigenic drifts are associated with more localized outbreaks of varying extent, whereas antigenic shifts are associated with epidemics and pandemics of influenza A.

The virus typically infects the upper respiratory tract and produces fever (> 37.8°C), headache, myalgia, malaise, sore throat, non-productive cough, sneezing and nasal drainage. Otitis media, nausea and vomiting are commonly observed in children. Fatalities directly due to infection are generally low, except in those with chronic lung, heart conditions or immunocompromisations, but pulmonary complications of influenza can include pneumonia (viral and bacterial), croup, asthma and bronchitis. Opportunistic bacterial pneumonia post-influenza infection is a leading cause of death worldwide.

On April 1, 2013, the World Health Organization (WHO) first reported 3 human infections with a new influenza A (H7N9) virus in China. Between 1996 and 2012, H7 influenza virus (H7N2, H7N3, and H7N7) infections were reported in humans in the Netherlands, Italy, Canada, U.S., Mexico, and the United Kingdom (but not in China). Because these virus strains widely circulate in wild fowl and domestic poultry, most of the human infections coincided with poultry outbreaks. Historically, these infections have caused mild upper respiratory symptoms, with the exception of one death in the Netherlands. Unlike these prior outbreaks, the current H7N9 has a much higher rate of human lethality in comparison. At the time of this writing, 130 human infection cases have been reported in China, with 31 fatalities. No cases involving this particular strain of H7N9 have been identified outside of China. Investigations by the WHO have uncovered no evidence of human-to-human transmission at this time, but because all cases do not involve individuals with direct exposure to poultry, human transmissibility is not ruled out at this time. The age of the patients ranges from 4-87, and the fatalities are characterized by early and acute respiratory distress syndrome, complications due to bacterial co-infections, and death due to refractory hypoxemia. Late stage antiviral therapy (on days 6-7) has not been shown to be beneficial.
The sequences of the viruses isolated from the first three fatal human infections have been made available to the public by China through the Global Initiative on Sharing Avian Influenza Data (GISAID) (29). This strain of H7N9 is a novel reassortant that has sequential characteristics that differ from previous H7 outbreak strains. Most of the isolates appear to be susceptible to the influenza antiviral drugs oseltamivir and zanamivir, with one proving resistant to oseltamivir by virtue of a neuraminidase mutation R294K (R232K) (56). All of the viruses also possessed the S31N mutation in the influenza matrix 2 (M2) protein that grants adamantane resistance. The sequence of the cleavage region of hemagglutinin (HA) only possess a single basic amino acid, which promotes low pathogenicity in birds (in contrast to the HA multibasic cleavage site of highly pathogenic H5N1 influenza (28)). Lastly, a majority of the viruses sequenced contained the Q226L (Q235L) mutation, and all possessed the G195V and D285 mutations in the hemagglutinin (HA) protein receptor binding site (33). These genetic changes have been associated with increased transmissibility of other avian influenza viruses to mammals based studies involving ferrets (63). In particular, the Q226L mutation has been shown in laboratory-generated virus strains to shift the preference from an avian host (with α2,3 sialyl glycans on the target cell surfaces) to a human host (with α2,6 sialyl glycan presentations) (35, 64, 71). Lastly, sequencing of the PB2 genes from H7N9 strains isolated in China have revealed the E677K mutation is all examined human isolates. Paradoxically, this mutation has not been found in H7N9 strains isolated from infected animals, suggesting that the mutation may be idiosyncratically acquired by the virus once human infection has occurred (36). In PB2, the 627 residue is an important determinant of host range restriction (76) and virulence and grants replicative efficiency in animal models (72). PB2 residue 627 has also been identified as the virulence determinant in the H5N1 and H9N2 viruses is associated with systemic infection and impaired T-cell receptor activation and diminished proinflammatory cytokines in the lungs of mice, most like caused by higher plasma glucocorticoid levels (81). However, while this consistent with the reported disease progression of patients infected with the current H7N9 virus
(where reported symptoms are mild and protracted at first), the progressive intensification of the
disease in a minority of patients suggests that the cytokine storm observed with H5N1 and
1918-like strains also contributes to H7N9 mortality (13). Together, the presence of these
mutations suggests that the virus may be more readily transmitted by aerosol to humans.

**Epidemiology and surveillance strategies**

The goals of influenza epidemiological surveillance include a description of the start and
seasonality of influenza in the region, establishment of baseline trends to estimate annual
changes in disease severity, generation of data used to understand disease burden and impact
(relative to other diseases), identification of high-risk groups, and prioritization of resources for
treatment. Virological surveillance objectives include surveying types and subtypes of influenza
viruses and their relations to global and regional circulation patterns, characterizing the
antigenic and genetic features of circulating viruses, monitoring antiviral resistance rates,
investigating relationships between virus strain and severity, and providing candidate viruses for
vaccine selection and production (91).

The epidemiology of influenza is well studied. Influenza occurs in erratic, isolated cases,
local outbreaks, regional epidemics or global pandemics (1). Epidemics annually occur during
the late fall and winter seasons in the northern and southern hemispheres (32), and at any time
equatorially (23). There is evidence that more severe, global pandemics have regularly occurred
at generational intervals since the 16th century (32). Recent pandemics occurred in 1968-1969
(Hong Kong flu, and H3N2 strain), and is estimated to have caused 34,000 deaths in the U.S.
(42). Another took place in 1957-1958 (Asian flu) that was caused by influenza virus A subtype
H2N2, and is estimated to have caused 70,000 deaths in the U.S. (42). The 2009 H1N1
pandemic infected up to 20% of the global population, depending on region (75, 92). In the U.S.
alone, the pandemic virus infected approximately 60 million people and caused an estimated
270,000 hospitalizations and 12,270 deaths (10). Even though the mortality rate was less than
0.5%, both morbidity and mortality were higher in young adults and less common for adults over 60 years old (26, 31). Person-to-person transmission was similar to that of seasonal influenza (pandemic H1N1 transmission factor $R_0 = 1.4$ to $1.6$ v. seasonal $R_0 = 0.9$ to $2.1$) (14, 92). By comparison, the 1918 H1N1 influenza pandemic is estimated as having infected 500 million people globally, with 10-20% mortality (77) with an $R_0 = 2-3$ (48). Since the Hong Kong influenza (H3N2) pandemic, the number of influenza-associated hospitalizations has typically been greater during seasonal influenza epidemics caused by influenza A/H3N2 viruses than during seasons in which other influenza A virus subtypes have predominated (41).

Influenza infects primarily humans, swine, horses, domestic and wild avian species (predominantly ducks), geese, and shorebirds (88). Over the last century, humans have become the main reservoir of human influenza A viruses. Wild fowl remains the reservoir of avian influenza A viruses, but other animal reservoirs have proved to be new sources of new human subtypes, and influenza A viruses are also frequently isolated in swine (67). Swine have been shown to present both human and avian influenza virus receptors and therefore are considered bioreactors for reassorted human and avian viruses (95), producing strains which may be infectious to humans with idiosyncratic antigenic characteristics for which the human population is immunologically naïve (67). The 2009 pandemic H1N1 virus transmission from swine to humans again demonstrated that zoonotic infection may occur frequently in those involved directly or indirectly in swine farming and that the illness severity may vary drastically (55).

Global surveillance relies on the willing participation of countries to share their influenza surveillance data, and is continually coordinated by the WHO, which supports and maintains a global influenza surveillance and monitoring program for collecting and analyzing virological and epidemiological data from countries, areas, and territories. This allows the organization to provide information about the global spread of influenza so that national policy makers may make informed decisions regarding recommendations for vaccination and treatment.
Surveillance also provides descriptions of critical features of influenza epidemiology (including risk groups, transmission characteristics, and impact), changing global trends in influenza transmission, and the progress of vaccine production. Lastly, surveillance has included the establishment of the WHO Influenza Surveillance and Response System, which includes a collaborative network of national and WHO laboratories for surveillance, epidemiology, ecology, and regulatory control of influenza research and reference standards. Figure 1 shows a global map with surveillance laboratories in participating countries. The influenza pandemic of 2009 emphasized the need for standardization of data collection and reporting systems, for which the WHO organized a global consultation to review influenza surveillance standards and the current data-sharing and reporting tools, with the goal of preparing a manual of global standards and improving the reporting tools. The WHO also provides recommendations on the selection and location of sentinel sites which largely depend on regional demographics and geography.

**Vaccine perspective**

Vaccination remains the principle strategy for controlling influenza, and is implemented through annual vaccination programs using either inactivated, or live, attenuated vaccines (3). Vaccine composition can vary as a result of global surveillance data (53), and new vaccines (such as the H5N1 or 2009 H1N1 pandemic vaccine) might be developed based on the perceived threats to global health. For example, during the 2009 H1N1 pandemic, an adjuvanted inactivated vaccine was developed by Glaxo Smith-Kline which was approved for use by the European Commission, with priority given to at risk populations, pregnant women, health care workers, and those in close contact with immunocompromised individuals (58). In the U.S., the FDA approved four vaccines against the pandemic 2009 H1N1: inactivated vaccines manufactured by Sanofi Pasteur, Novartis Vaccines and Diagnostics Limited, CSL Limited; and a live attenuated intranasal vaccine manufactured by Medimmune LLC (73).
Current seasonal vaccines contain strains that antigenically match the annually recommended strains, and are tri- or quadrivalent, with one influenza A H3N2 virus, one influenza A (H1N1) virus, and one or two influenza B viruses (one each from Yamagata and Victoria lineages). Vaccines in the U.S. for 2012–13 contained A/California/7/2009 H1N1-like, A/Victoria/361/2011 (H3N2)-like, and B/Wisconsin/1/2010-like (Yamagata lineage) antigens. The influenza A (H3N2) and B antigens differ from the respective 2010–11 and 2011–12 seasonal vaccine antigens (83, 84) in that the influenza A (H1N1) vaccine virus strain was derived from an influenza A (2009 H1N1) virus and was included in the 2009 H1N1 monovalent pandemic vaccine, as well as the 2010–11 and 2011–12 seasonal vaccines.

Constant surveillance contributes heavily to the selection process for the strains recommended for the vaccine composition. Every year in January, the FDA's Vaccines and Related Biological Products Advisory Committee (VRBPAC) reviews global surveillance data, and recommends one or more of the strains to be included in the vaccine for the subsequent influenza season. By mid-February, the World Health Organization (WHO) completes its review and makes recommendations for the Northern Hemisphere vaccine. The WHO repeats this process in September for Southern Hemisphere vaccine recommendations. In March, VRBPAC meets to finalize the recommendations for the U.S. influenza vaccine. The effectiveness of the resultant multivalent vaccine depends upon how well the chosen strains match the actual circulating strains in the new season. Although there is no guarantee that the strains picked for the vaccine will be the circulating strains during the subsequent flu season, there is an expected 90% match between vaccine strains and circulating strains each season (83).

Vaccination rates are also crucial to outbreak management, and even with a moderate effectiveness of about 60%, vaccination can reduce influenza-related illness and death, antibiotic use, time lost from work, and hospitalizations. However, influenza vaccination rates in the US are below 50%, which negatively impacts population protection (70). The CDC's 2013 vaccine efficacy estimates (9) indicate that 56% of patients were protected from influenza-
related medical intervention by vaccination in, all age groups (95% confidence interval: 47% to 63%). Influenza virus specific vaccine efficacy (against influenza A H3N2 virus – which was the primary virus in circulation this season) – was estimated to be 47% (95% CI: 35% to 58%), while effectiveness against influenza B was 67% (95% CI: 51% to 78%) for all ages. There were not enough influenza A H1N1 cases detected early in the season to provide an estimate of vaccine efficacy. These results indicate that vaccination with the 2012-2013 flu season vaccine reduced the risk of flu-associated medical visits from H3N2 viruses by one half and from flu B viruses by two-thirds for most of the population. Overall, vaccine efficacy estimates suggest that the 2012-2013 flu vaccine has moderate effectiveness for most people against the flu viruses spreading in the U.S., similar to previously published reports. However, the vaccine efficacy among people 65 and older against H3N2 viruses was exceptionally lower than expected, at 9% (95% CI: -84% to 55%), primarily against H3N2 viruses. For the other age groups, the vaccine effectiveness estimates are within the range expected during typical influenza seasons when circulating virus strains match vaccine strains, as is the case this season (57).

The vaccination rates in other areas of the globe vary significantly depending on the region. For example, in 2009, European Union vaccine coverage rates ranged from 1.1% in Estonia to 82.6% in the Netherlands amongst the elderly, clinical risk groups, and health care workers (47). In China, surveys in Beijing indicated that an average annual vaccination rate of 18.5% was observed for the years 2007-2010 (93), but there is no information available for vaccination rates in rural China.

Because the data available for vaccination rates is incomplete, reported vaccination coverage by government regulatory agencies to the World Health Organization is one area that still requires improvement (47). Clearly, raising vaccination rates is another strategy for mitigating seasonal influenza outbreaks and remains a high priority as part of pandemic preparedness plans. Room for additional improvement in community awareness also exists, and the recent H7N9 virus outbreak justifies aggressive education programs that encourage...
increased vaccination participation. Lastly, significant flexibility and capacity must be built into the vaccine production strategies so that vaccines for off-season outbreaks can be rapidly and safely produced. Currently, influenza vaccine manufacturers around the world are developing H7N9 vaccine seed strains to serve as templates for bulk immunization production, should it be required (4).

**Antiviral perspective**

Although commercial vaccines are available for influenza A subtypes H1N1 and H3N2 (23), chemoprophylactic drugs are widely recommended and used in the control or prevention of influenza. In most cases, antiviral prophylaxis must be initiated within 3 days of the detected illness to be effective in slowing transmission (42). Several antiviral chemotherapeutic agents are approved for use that are used either alone or in combination for the treatment and prophylaxis of influenza infection, and include the neuraminidase and M2 proton channel inhibitors. The only FDA approved small molecule therapeutics for the treatment of influenza infections are oseltamivir, zanamivir, and the adamantanes. The influenza-specific neuraminidase inhibitors (oseltamivir, zanamivir, and peramivir) prevent release of the virus from infected cells by competing for the enzyme active site with the natural substrate, sialic acid, found on the surface proteins of normal host cells (30). By blocking the activity of the viral neuraminidase enzyme, neuraminidase inhibitors prevent new viral particles from being released by infected cells. The ability of the virus to evade the antiviral effect of oseltamivir (Tamiflu) by introducing mutations into the viral neuraminidase is the primary concern with its continued use. In 2008, 86% of circulating H1N1 human virus strains were resistant to oseltamivir (6). Zanamivir (Relenza) inhibits by the same mechanism (24, 30), and similar to oseltamivir, exhibits no toxic, teratogenic and/or embryocidal effects in animals. However, it is not greatly bioavailable if taken orally, and is therefore administered by inhalation, limiting its widespread use. Of the circulating H1N1 human virus strains that were resistant to oseltamivir
in 2008, 100% were still susceptible to zanamivir, most likely because oseltamivir is in much
greater, widespread use as an anti-influenza drug than zanamivir. Lastly, peramivir is approved
in Japan (as Rapiacta) and also available in South Korea as Peramiflu, and is used to treat
patients with influenza A and B viruses, including H1N1 and avian influenza. The drug is in
extended Phase III studies in the U.S. and a new drug application by the parent company,
BioCryst, is expected. Because peramivir is not as bioavailable as either oseltamivir or
zanamivir, it is administered by injection (22) and it is only prescribed if oseltamivir resistance
develops and the patient is unable to take zanamivir by the inhaled route.

M2 proton channel inhibitors (amantadine and rimantadine) prevent the virus from
uncoating inside the endosome, and are approved in the US for treatment of influenza (61).
However, the reassortment between viruses and the overuse of these drugs resulted in
resistance in 98% of H3N2 virus strains isolated in 2006, (8). In addition, the CDC found that
100% of 2009 pandemic flu samples tested possessed resistance mutations to the
adamantanes, prompting an alert to doctors to prescribe the neuraminidase inhibitors
oseltamivir and zanamivir instead of amantadine and rimantadine for treatment of pandemic
circulating flu (7). Amantadine is commercially sold as Symmetrel, and is marketed for use both
as an antiviral and an anti-Parkinsonian drug. Amantadine and rimantadine are not currently
recommended for first-line use because of the high levels of resistance to these drugs among
circulating influenza A viruses, but are still considered valuable because of the reemergence of
adamantane-susceptible strains (22).

**New research opportunities**

**Universal vaccines:** Universal influenza vaccines have been in development for more
than a decade (52), but have been slow in materializing. Although the annually-produced
season influenza vaccines are widely available and effective, influenza still poses a
considerable threat to global health because the antigenic drift inherent in influenza viruses
reduces population immunity. In addition, multiple strains of influenza must be incorporated into an annual vaccine in order to provide protection against most circulating strains. Occasionally, the vaccine composition does not match circulating strains well, reducing overall protection, and revaccination is required each year due to antigenic drift. The effectiveness of vaccines is generally reduced for older adults, who fall into the high-risk category and are one of the main target groups for vaccination. Novel approaches to universal vaccine generation has focused on highly-conserved regions of influenza A subtype viral antigens in hopes of providing broad immunity not only to seasonal viruses, but also to potential pandemic virus strains that may spread rapidly and cause high mortality. Experimental vaccines have been specifically developed against the matrix 2 protein (M2) and the conserved stem region of the hemagglutinin that have provided broad protection in animal models, while multivalent compositions and adjuvants also continue to improve.

Several of these universal vaccines have now entered into clinical development, and are listed in Table 1. An M2 vaccine candidate (M2e) recently completed phase 1 clinical trials (21). It uses the cytoplasmic domain of the M2 protein (M2e), an ion channel protein on the surface of influenza A viruses, as the antigenic epitope. M2e is the most highly conserved surface protein on the virus and is expected to undergo the least annual genetic drift, so it is anticipated that this strategy would eliminate the need for annual re-definition of the influenza vaccine (68). In a different approach, researchers at NIAID are working to develop a DNA-based universal influenza vaccine. In recent experiments, a two-step immunization approach of priming (with a DNA vaccine that encodes the conserved stem region of HA) followed by boosting (with an inactivated seasonal vaccine) induced broad immunity against multiple influenza virus strains in mice, ferrets, and monkeys (89). A third strategy has directly exploited protein epitopes containing conserved regions of the viral hemagglutinin head and stem to induce the production of broadly-neutralizing antibodies which have demonstrated cross-reactivity among different
influenza strains (18). This was done to identify monoclonal antibodies that may contribute to a flu vaccine for all influenza A and B viruses (39).

Alternate vaccine production strategies: In an effort to decrease the response time to emerging influenza strains, vaccine manufacturers have developed novel methods for vaccine and antigen production. Although some of these platform strategies have been utilized for other vaccines, their utilization for influenza vaccine production offers advantages for rapid production in the event of a pandemic or urgent outbreak. Protein Sciences Corporation has created the trivalent HA epitope vaccine (Flublok) which is the only licensed and approved flu vaccine that does not use eggs, antibiotics, or live influenza virus in any part of the manufacturing process. Their system uses an insect virus (baculovirus) expression system to produce influenza antigenic proteins (82). Medicago Inc. is in Phase II clinical trials with an influenza virus-like particle vaccine expressed in and purified from tobacco (37) and using a process that can reduce vaccine production times by 33%. Novavax, Inc. is also in Phase II trials with their platform that uses virus-like particles (VLP) expressed through the insect virus system (5). VaxInnate's Toll-like Receptor technology utilizes a bacterial expression system to produce influenza vaccines that contain viral protein epitopes (M2e or HA) fused to the bacterial protein, flagellin, which is a potent stimulator of TLR-5. These vaccines are in Phase 1/2 clinical trials (78). Lastly, VaxArt is working to generate a broad immune response to influenza using a non-replicating adenovirus type 5 (Ad5) vector backbone which expresses hemagglutinin from avian influenza and a TLR3 ligand as an adjuvant that can be delivered together orally, and is currently in Phase I trials (59, 66).

Novel antiviral developments: Several experimental influenza antiviral drugs are currently in phase 2 or 3 clinical trials and are anticipated as being available for additional therapeutic or prophylaxis options within several years. These are favipiravir, laninamivir, and DAS181.
Favipiravir (developed by Toyama Chemical Co., Ltd.) is a substituted pyrazine compound that is effective against seasonal A and B, H5N1, and 2009 H1N1 influenza viruses, and several other RNA viruses. It differs from approved anti-influenza drugs in that it targets influenza viral polymerase. The anti-influenza virus activity of favipiravir in vitro is attenuated by addition of purines and purine nucleosides, including adenosine, guanosine, inosine, and hypoxanthine, whereas pyrimidines do not affect its activity. However, favipiravir does not have an influence on cellular DNA or RNA synthesis, suggesting that favipiravir functions as a specific inhibitor of influenza virus RNA polymerase (27). Promising pre-clinical studies for oral administration are underway in Japan and in the U.S. simultaneously.

Laninamivir is novel neuraminidase inhibitor that was developed by Biota Holdings and Diichi-Sankyo Japan as a therapeutic administered by inhalation. It has demonstrated an extremely long half-life, and has the potential as a single-dose therapeutic. It is active against seasonal A and B, H5N1, and 2009 H1N1 influenza viruses, and is currently in phase 3 clinical trials in the U.S., but is approved for use in Japan (11).

DAS181 is host-directed antiviral. It is a recombinant sialidase fusion protein composed of the active domain of *Actinomyces viscosus* sialidase and the human amphiregulin heparin binding sequence. It inhibits viral attachment by cleaving sialic acid molecules from target cell surfaces, thereby preventing the recognition and binding of these by viral hemagglutinin. It is potent against H3N2, H1N1 and B influenza viruses, and has been administered by inhalation in phase 2 clinical trials (51).

While approved antivirals are effective against seasonal human influenza, two complications when using antiviral drugs alone against highly pathogenic avian influenza infections are the limited success (16) and narrow time window available for effective treatment of patients (2). To address this, underexplored but growing research efforts have been made toward the mitigation of severe influenza or avian influenza symptoms through modulation of the host immune system, usually through the administration of anti-inflammatory agents. With the
emergence of the H5N1 avian influenza strains in 1997 and the experimental resurrection of the 1918 H1N1 pandemic influenza-like strains in 2005, the physiological elevation of pro-inflammatory cytokines caused by virus infections (i.e., the cytokine storm) has been recognized as a critical factor in viral pathogenesis in humans and animal models (15). The importance of the upregulation of individual cytokines during avian influenza infections is not well defined, but taken as a whole, cytokine dysregulation may account for the high mortality rates associated with the avian virus strains (12). Adapting therapeutic strategies to include immunomodulatory agents is valid, given the high prevalence of avian characteristics in recently emerged virus strains. Several classes of FDA approved drugs – (COX-2 inhibitors (94), CCR2 inhibitors (40), macrolides (65), statins (20), TNF-antagonists (74), and peroxisome proliferator-activated receptor agonists alone or in combination with AMP-activated protein kinase agonists (50)) have been shown to prevent avian influenza-induced acute lung injury in animal models.

While these therapies have great potential when managing outbreaks of avian influenza, their value in treating seasonal human influenza might be limited because the cytokine storm is not observed during seasonal H1N1 and H3N2 virus infections, and the 2009 pandemic H1N1 influenza virus has not been shown to cause this either (17). However, recent progress has been made in extending the survival of a pandemic influenza lethal mouse model by blocking Toll-like receptor 4 (TLR4)-dependent inflammation. This report indicates that the therapeutic administration of Eritoran (a lipid-polysaccharide-like antagonist of TLR4) blocks in lung pathology, clinical symptoms, cytokine and oxidized phospholipid expression, and influenza-induced lethality in mice, and results in decreased viral titers (69).

Continued transmission studies: So far, the recently isolated H7N9 virus has not been definitively shown to have the capability of transmitting from human to human, although this is being investigated. Although not readily transmissible in humans, the number of highly pathogenic avian virus human cases makes this virus a strong biodefense concern. Also, because of the frequent incidents of zoonosis and the selective pressure that induc
drift and genetic reassortment, it is possible this virus could naturally gain greater transmissibility within a single season, becoming a much greater threat to public health. The occurrence of high-mortality rate (>10%) avian influenza virus infections in humans is expected to continue and increase, making the understanding of the genetic factors in virus adaptation for human-to-human transmission of extreme importance. Therefore, influenza virus transmission studies are essential for outbreak, epidemic and pandemic preparedness, and recently researchers who have approval from their governments and institutions to conduct this research safely under appropriate biosecurity conditions have resumed human transmission studies for the highly pathogenic H5N1 avian influenza virus (25). Currently, these studies have measured the potential for spontaneous viral adaptation to a human-to-human transmitting virus (34), identified key molecular determinants that account for such an adaption, and estimated the rates of transmission should this occur (63). It is expected that these studies will be extended to additional pandemic influenza virus candidates, including the recent H7N9 strain.

Conclusion

Influenza is a chronic affliction for the entire world’s population, and modern society has enabled the virus to spread and adapt more rapidly than ever before. In light of the emergence of novel influenza viruses which cross from animals to humans, vigilance is paramount for rapid identification of more dangerous, pandemic-candidate strains that may be able to spread from human to human. Surveillance and vaccine production and distribution efforts must be heightened to prepare for and combat the statistical certainty of future influenza outbreaks, epidemics, and pandemics. Of equal importance, researchers must continue to learn more about the factors involved in zoonosis and human-to-human transmission of influenza. Efficient influenza outbreak management depends on rigorous surveillance and rapid identification of such events when they occur, streamlining of production effective vaccines, early antiviral application. Increased monitoring of the types and rates of novel influenza virus infections in
domestic animal populations is warranted, and the increased proactive application of vaccines in these populations may be a preventative approach to reactive culling of infected herds and flocks. Also, the administration of influenza vaccines (or antiviral prophylaxis) to humans that work closely with susceptible animal populations may be a viable strategy to reduce the incidence of zoonosis. Key innovations that result in new antivirals and new, broadly effective vaccines will contribute to increased public health, but aggressive education programs may be the most important factor in immediately leveraging current vaccines and antivirals, as these efforts will increase public awareness, hygiene practices, and most importantly, vaccination rates in the areas of the globe in which novel, pandemic-candidate virus strains are expected to originate. Lastly, critical transmission events between domesticated animal populations have occurred both in 2009 (for pandemic H1N1 influenza) and most recently in 2013. More funding and effort should be directed toward developing a better understanding of the factors involved in transmission of novel virus strains from animal-to-human and/or human-to-human.
References:


70. Singleton JA. Influenza Vaccination Distribution and Coverage, United States, 2010-11 and 2011-12 Seasons Meeting of the ACIP. 2011;October 26, 2011.


83. United States Food and Drug Administration. Influenza Virus Vaccine, Trivalent, Types A and B: Seasonal Influenza Vaccine. Influenza Virus Vaccine, Trivalent, Types A and B.


86. United States Department of Health and Human Services.


Table 1. Influenza vaccines and antivirals in development.

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Antigenic components</th>
<th>Stage of development in U.S.</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2e universal vaccine</td>
<td>Sanofi Pasteur</td>
<td>M2e viral protein epitope</td>
<td>Phase I/II clinical trials</td>
<td>(68)</td>
</tr>
<tr>
<td>DNA primed universal vaccine</td>
<td>NIH/NIAID</td>
<td>DNA encoding the stem region of HA and boosted with inactivated vaccine</td>
<td>Pre-clinical /Phase I clinical trials</td>
<td>(89)</td>
</tr>
<tr>
<td>Monoclonal antibody-based universal vaccine</td>
<td>N/A</td>
<td>HA-stem viral protein epitope</td>
<td>Pre-clinical</td>
<td>(18)</td>
</tr>
<tr>
<td>Flublok seasonal vaccine</td>
<td>Protein Sciences Corp.</td>
<td>HA viral protein epitopes</td>
<td>Approved for ages 18-49 years</td>
<td>(82)</td>
</tr>
<tr>
<td>VLP pandemic vaccine</td>
<td>Medicago Novavax</td>
<td>Recombinant VLPs displaying functional viral proteins</td>
<td>Phase II clinical trials</td>
<td>(5, 37)</td>
</tr>
<tr>
<td>Bacterial recombinant vaccines for seasonal and pandemic influenza</td>
<td>VaxInnate</td>
<td>Recombinant viral protein epitopes fused to bacterial flagelin</td>
<td>Phase I clinical trials</td>
<td>(78)</td>
</tr>
<tr>
<td>Oral avian influenza vaccine</td>
<td>VaxArt</td>
<td>Ad5 vector expressing hemagglutinin from avian influenza and a TLR3 ligand as an adjuvant</td>
<td>Phase I clinical trials</td>
<td>(59, 66)</td>
</tr>
<tr>
<td>Favipiravir</td>
<td>Toyama Chemical Co.</td>
<td>Viral polymerase inhibitor</td>
<td>Phase III clinical trials</td>
<td>(27)</td>
</tr>
<tr>
<td>Laninamivir</td>
<td>Biota Pharmaceuticals and Daiichi Sankyo</td>
<td>Neuraminidase inhibitor</td>
<td>Approved for use in Japan/Phase III clinical trials (U.S.)</td>
<td>(11)</td>
</tr>
<tr>
<td>DAS181</td>
<td>NexBio, Inc.</td>
<td>Recombinant sialidase</td>
<td>Phase II clinical trials</td>
<td>(51)</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1. Global Map of World Health Organization (WHO) Influenza Surveillance and Response Laboratories. As of 2012, the WHO Surveillance and Response Network consisted of four major centers and 122 collaborating institutions in 94 countries, which are recognized as WHO national centers for reference and research on influenza (90).