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REVIEW ARTICLE

# H1N1 Influenza Virus (Swine Flu): A Comprehensive Insight into Escalating Catch-22 Scenarios

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## Abstract

**Introduction:** Viruses have always been a major cause of various disastrous pandemics in mankind's history. H1N1 became a threat when its original strain was first discovered back in the swine flu pandemic of 2009. It became highly catastrophic on a large scale because none of the therapeutic interventions and methodologies that were already present at the time were effective against the virus.

**Methods:** A vast amount of literature and research is available regarding H1N1 influenza from different reputable sources online. The data was gathered following the contrasting and relative situations of 1918 as well as the 2009 pandemic in mind. The overall extracted material provides comprehensive insights into the ups and downs of H1N1 influenza from 1918 up to 2009.

**Results:** H1N1 virus comprises of a huge potential to cause a pandemic of Influenza type A. The illness caused by the virus has a varying degree of severity depending on the immune function of the individual being under attack. The virus exploits droplet-based transmission mode for its spread from one host to another. The major center of escalation of the subtypes of virus mostly originates from different avian and swine species. Most notably subtypes H9N2 and H5N1 of influenza A, which aren't easily transmissible among humans. Furthermore, the droplet-based transmission takes comparably less time to infect a population of thousands if not millions. This ultimately increases the overall death toll by several folds by initiating a constant wave of pro-inflammatory cytokine release among affected hosts.

**Conclusion:** Since its discovery in 2009, researchers have developed antiviral drugs and vaccines to fight the virus, most of which have proven to be very successful in treating the interconnected complications. The present-day strategies are only efficacious until the current strains of influenza A do not produce resistance against these drugs. All the therapeutic techniques and methodologies that have been developed to confront the virus up until now have been described in this ample review.

## Introduction

H1N1 is classified as the serotype of the species Influenza A virus, a descendant of the family Orthomyxoviridae. H1N1 caused the disastrous swine flu pandemic of 2009 that infected 60.8 million people along with 12,469 deaths and 274,304 hospitalizations just within a span of a little more than 1.5 years. It is the same type that caused the infamous influenza pandemic of 1918.[1] Other serotypes of Influenza A are H3N1, H2N3, H1N2, and H3N2. Influenza B and C do not have serotypes. H5N1, H7N5, and other serotypes cause Influenza in avian species. Other strains include H4N6 and H9N2 that infect swine species just like H1N1. he classification for subtypes of Influenza A is based on the presence of the glycoprotein Hemagglutinin and Neuraminidase. In the reproductive cycle of the virus, Hemagglutinin performs the clumping action on the red blood cells that helps the virus to bind and infect the cells effortlessly.[2] Locomotion and the budding action in the infected cell are executed with the help of Neuraminidase. Neuraminidase belongs to the category of glycoside hydroxylase enzymes. In general, the H1N1 influenza A does not always lead to the viral infection upon any type of contact to the swine population but if the contact leads to the infection, it is termed as zoonotic swine flu.[3] Experimentally, it has been observed that the virus generally shows its symptoms in swine within four days and can be easily transmitted among the individuals of different swine species, mostly through proper contact.[4–6]

There are different variants of the virus based on the genotype. For example, the recently discovered strain in China is G4 H1N1. It is a genotype 4 variant of the original strain that mainly affects the swine and has a striking resemblance with the Eurasian avian H1N1 strain. There is little evidence about this variant causing infection in humans. Severe cases of the infection of H1N1 mostly happen with pregnant women.[7,8] Fetus related hormonal changes in the body of pregnant women cause a drop in the function of the immune system that paves the way for the virus to cause infection. This is the reason it is mostly recommended for pregnant women to get vaccinated to avoid any H1N1-related complications.[9–12]

Humanity has already faced this kind of H1N1 influenza-related flu pandemic in the past. 1917-18 marks the era of the great disastrous influenza pandemic in the pages of history with an approximate death toll of over 50 million people worldwide. The advanced medical and therapeutic innovations that we've today weren't present back then. So, the medical copping facilities at that time were minute relatively. This was one of the main reasons why the severity of the 1918 pandemic was enormous as compared to the 2009 influenza pandemic. The mortality rate was far too high in 1918 but dropped exponentially as time passed away. However, the original strains of 1918 H1N1 are still circulating in the population today. The status of the immune system of the affected hosts was far better in 2009 than it was in 1918. Lower immune health with low cellular immunity as well as a low number of antiviral cross-reactive antibodies contributed to the severe pro-inflammatory cytokine storm along with the spread of H1N1 in the affected hosts of the 1918 pandemic. Today, we're also facing an exponential increase in the new strain of influenza (like H5N1 and H7N9) that have evolved since 1918 but the medical advancements are also on a whole different level.[13–15]

*2009 Swine Flu: A Brief Overview*

During the swine flu pandemic of 2009, cases of pneumonia were showing up consistently because of bacterial co-infection along with the virus. A study was performed during the pandemic to quantify the ratio of co-infection. Although several proportions were reported, approximately 25% of the total cases were appearing to have a co-infection. Dealing with this co-infection situation, was a challenge in 2009 due to the severely compromised immune systems of the patients.[16] Later on, experiments were performed by taking frequent samples from herds of swine to map out the lineage of the viruses that were evolving. It was concluded that swine are a "mixing vessel" of several serotypes that ultimately helps the virus to become unresponsive to a lot of newly developed human vaccines. A total of 17 sequenced whole genomes were used in this particular spatiotemporal distribution study.[17–18] Similarly, another study demonstrated in 2011 that the swine flu virus is antigenically evolved as compared to the viral strain of the 2009 pandemic. The experiments were performed using ferrets that were immune to the original strain but were least immune to this new strain. The concept is the same in the case of humans who were immune to an old strain, but are vulnerable to this mutated strain.[19–22] Real-time Polymerase Chain Reaction (PCR) techniques were approved by WHO (World Health Organization) in 2020 to detect the presence of the virus. Many new methods, like the use of biosensors (FET biosensors, PEDOT with galactose, Surface plasmon resonance, and AuNP immunosensor), have shown high sensitivity to the presence of DNA (Deoxyribonucleic Acid). The RT-LAMP (Reverse Transcription Loop-mediated Isothermal Amplification) technique that investigates antigen-antibody related interactions to confirm the presence of the virus was also utilized.[23] Biosensors are a recent development in this field and are very efficient as compared to other conventional detection methods. Biosensors usually exploit immune-chromatographic techniques. The most modern technology uses a bacterial quench-body that is an immuno-protein adhering to different antibodies labeled with specific fluorophores. The detec-

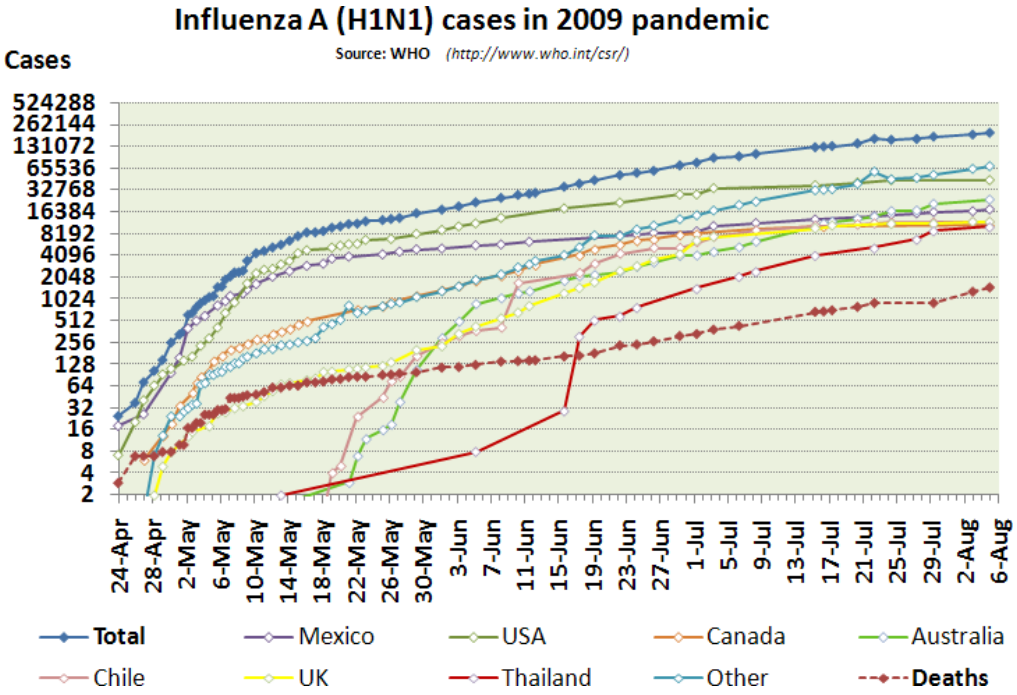


**Figure 1.** A semi-logarithmic chart of laboratory-confirmed A (H1N1) influenza cases by date according to WHO (World Health Organization) reports.[34]

tion is based on the interaction between antigen and the quench-body. Examples of quench-body include anti-HA Fab with ATTO520 (attachment of a Cys-tag at locus N-terminus of heavy chain in the expression vector of Fab), anti-BGP Fab, anti-Flag Fab, and anti-His Fab.[24–27]

All of these detection methods were developed to cope with the mutated strains of Influenza A that arose as a result of the phenomenon of divergence. Here, divergence specifies the outward spread of the mutated strains from the center of origin. Recent studies have observed the presence of the virus in Australians with divergent origins from the main human population. The diversity in the genotype of different whole-genome sequenced samples showed that all strains are somewhat different from each other. The samples that were taken between 2012 and 2016 revealed that limited diversity is the main hurdle for risk assessment as well as for the control procedures. The diversity problem poses new challenges in the further development of new treatment methods.[28] Although the 2009 pandemic had disastrous effects on the population as the cases rise with time (**Figure 1**), it led to an improvement of strategies to manage future infections. In Mexico, the health shocks of the pandemic directed to better health control measures that helped health authorities to regulate the causes of diarrhea in young adults. Furthermore, aftershocks of the pandemic motivated some people to quit smoking and improve their hygiene habits.[29–32] One phylodynamic study also displayed that after the pandemic, there was a reduction in the diversity of the virus globally due to its transitioning towards immune-driven selection instead of host-adaptation mechanisms.[33]

There are some sequence-based studies with strong evidence that 3 out of 5 pandemics of flu have a possible avian origin. These studies utilized polymerase derived gene segments that showed that diverse lineages in the swine population of North America are due to the re-introduction of the original American avian strains. There was a 70% average match of antigenic sites with the original strain of the 2009 pandemic, suggesting that the original strain had an avian origin. This circulation and mutation of strains in wild avian species can play a significant role in future pandemics.[35–37] Another study displayed that the original strain of the 2009 pandemic was still circulating in Russia. Randomized trials of healthy and influenza-infected people showed that a small number of neuraminidase antibodies were present even in the healthy individuals, which supports the hypothesis of circulation of strains in the general population. It was also observed in swine that antibody responses were extremely different in different individuals of the species.[38–41] Climate also had a very crucial role in driving seasonal influenza, as described. The analysis of different Influenza serotypes affecting several regions of Australia revealed that the seasonal drives

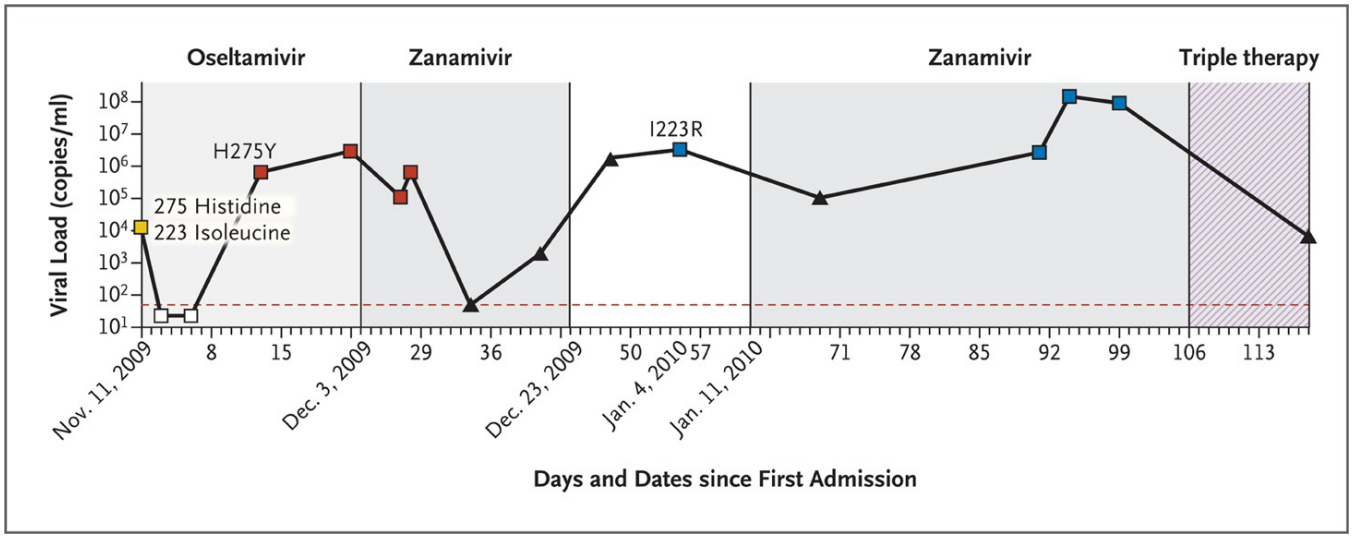


**Figure 2.** Viral load, antiviral therapy, and resistance detection during hospitalization of a patient infected with pandemic influenza A (H1N1) virus. [Colored squares indicate the absence of resistance mutations (Yellow, Red and Blue), White squares indicate virus-negative samples. Triangles indicate samples from which no neuraminidase sequence could be obtained. The dashed red line indicates the lower limit of detection of influenza A semi-quantitative RT-PCR (Real-Time Polymerase Chain Reaction) assay. The duration of oseltamivir monotherapy and zanamivir monotherapy is indicated by light-gray and dark-gray shading, respectively, and the duration of triple therapy.] [58]

of the virus are most active in subtropical areas. Although the pattern of all inter-seasonal transmissions is far more complex to comprehend using simple techniques, it is believed that climate is also a major factor in the drive of seasonal infections.[42–44]

Epidemiological techniques on a molecular level were used in Europe to quantify the different strains of the virus that were in circulation after the pandemic of 2009. All the strains within four years after the pandemic were analyzed. It was revealed that most of the strains diverged from the original strain and made the vaccination process more challenging. The reason behind this is that some of the lineages of the strains were unresponsive to vaccines and antivirals that were developed for the virus.[45–47]

*Advancement of Modern Antivirals*

One of the main potent actions of an antiviral drug is the inhibition of strain-specific binding receptors. The blocking of these receptor sites disrupts the infection cycle and ultimately stops the spread of the virus within its host. One of these compounds that have strain-specific activity against the virus is iminosugars such as NN-DNJ (N-Nonyldeoxynojirimycin) and NB-DNJ (N-Butyldeoxynojirimycin). Both of these have shown a high strain-specific potency when their activity was observed by experimenting on a large number of Influenza A viruses. NN-DNJ is an $\alpha$-glucosidase inhibitor that acts on influenza viruses by disrupting the main glycan processing between Neuraminidase and Hemagglutinin. Hemagglutinin is an essential factor for the proper inhibition of the cycle.[48] Common antiviral treatment options include oseltamivir and zanamivir. Both of these antiviral drugs are neuraminidase inhibitors and are highly effective against H1N1 related infections.[49–50]

Recent studies have shown that most of the modern strains have developed a significant amount of resistance to both of these drugs. Other drugs like rimantadine and amantadine (adamantanes) have also been developed but these drugs come with their side-effects like embryonic toxicity and teratogenic abnormalities in the developing fetus during the gestation period.[51–53] Adamantanes are only used in those cases where Neuraminidase inhibitors based drugs fail to show any viable potency against the infection. Sometimes, the combination of adamantanes and Neuraminidase inhibitors is more efficient due to their synergistic effects as displayed by various mice-based models.[54–56] Early cases of oseltamivir resistance were reported back in 2017 (**Figure 2**) in Myanmar when H1N1 infected patients in a local com-munity outbreak were analyzed.[57]

*Vaccine Mediated Therapies*

The most recent development in the field of vaccines is the use of the AS03 (Adjuvant System 03) type adjuvant system that exploits $\alpha$-tocopherol oil and water-based emulsion of a very precise ratio. A study based on randomized and controlled trials in adults has shown that by utilizing the AS03 type adjuvant systems of vaccine administration, the negative effects of conven-

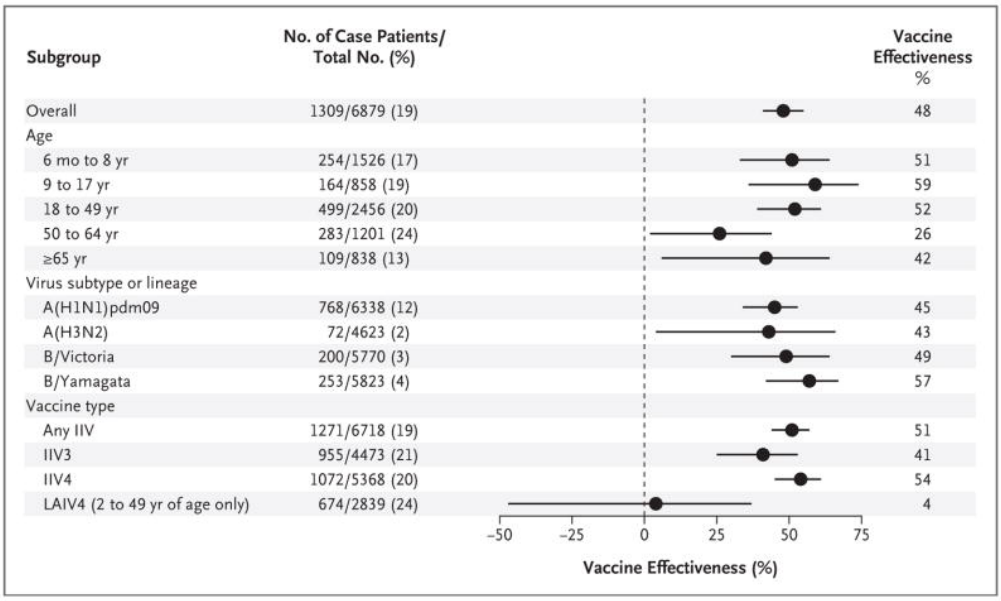


**Figure 3.** Adjusted estimates of influenza vaccine effectiveness [2016–17], overall and stratified according to age, virus subtype or lineage, and vaccine type.[75]

tional vaccination procedures can be significantly reduced with a relatively higher persistence rate. This produces both humoral and cell-mediated immune responses following the induction of CD8 T-cells (Cluster of Differentiation 8 Thymus cells).[59-60]

Furthermore, antigen selection and cross-protection process can be improved by using a nomenclature system that is easy to comprehend and is based on the phylogeny of Hemagglutinin based lineages. A total of 7,070 H1 sequences were used for the development of the nomenclature annotation tool that yields 99% accurate results most of the time.[61–62] Modern technological development has made it possible to study the interactions of vaccines by using computational simulation analysis and constructing various Mathematical models. These computationally optimized vaccines have shown their activity over a broad range of Influenza A viruses isolated from human and swine subjects.[63–67] Recently developed COBRA (Computationally Optimized Broadly Reactive Antigen) vaccines with HA (Influenza Hemagglutinin) antigen are optimized in such a way that most zoonotic infections were prevented in farmworkers as shown in a study by Morris and colleagues.[68–70] miRNA (micro Ribonucleic Acid) alteration technologies are a kind of new approach to deal with the virus with a unique strategy. It has been observed that especially developed miRNA such as miR-let-7b-MRE enhances the biosafety for developing more operative and stable vaccines by decreasing the replication and virulence of the virus.[71–74]

Neutralization can be achieved by using monoclonal antibodies that have performed well in experimental assays to study antigen-antibody interactions (**Figure 3**). Such an example is murine-based high-affinity MA2077 neutralizing monoclonal antibody that inhibits the 2009 strain of Influenza A in a specifically developed experimental assay. The specificity of this monoclonal antibody lies with the 'Sa' site in an in vitro medium.[76] Chicken eggs are generally used as a basic medium to reproduce viruses and then to produce vaccines along with, but a new type of approach utilizes attenuated metabolically active E. coli to produce an enormous amount of antibodies before primary immunization in a short span of about three weeks. This method can be used as an efficient way to study vaccines without applying any rigorous purification procedures for deriving antigen. On the other hand, this method is much faster than other lengthy conventional methods because it only employs E. Coli based harvested Hemagglutinin. This Hemagglutinin produces a detectable amount of antibodies when observed in MDCK (Madin-Darby Canine Kidney) cells. Furthermore, this immunization technique doesn't require any exogenous adjuvant as a booster dose.[77–80]

*Modern Research and Progress*

There is a reinforcement problem that exists in the cases of co-infection such as Influenza A Virus in a combination with the hRSV (Human Respiratory Syncytial Virus). It has been observed that during normal seasonal peaks of both viruses, the ferrets with co-infection show an increase in the levels of pro-inflammatory cy-

tokines. This happened when the inoculum dose was increased more than normal with the same ratio of hRSV. This phenomenon is mostly termed as viral interference that increases the challenges while dealing with a subject of co-infection. Immune mediators with other chemokines were also released upon increasing the dosage that poses the possibility of antigen-independent mechanisms that drive the interference process.[81] Later, it was found that autophagy is a process that defines the interaction of dendritic cells with several strains of influenza viruses as observed in mice models.[82] Although the seasonal viral spread can be explained with a reverse zoonosis strategy that promotes the development of new lineages, viral interference is not perfectly understood yet.[83–84]

A recent study in hosts other than birds and swine showed that host jumping of several strains of Influenza A is a major threat in control of any future pandemics. Canines and Equines usually have a very stable lineage of Influenza A viruses. The samples that were taken from different southern China (2013–15), upon sequencing have shown that the virus is slowly mutating when the interaction of these species with major reservoirs of Influenza A virus occurs. It means that host jumping can contribute to the development of new lineages in minor hosts as well.[85–87] Similarly, experimental studies also support the transmission of the virus from different avian species into mammals. The virus adapts to the host body by exploiting specific HA-receptors.[88]

Modern methods of characterization mostly depend upon the genetics of the agent that is being examined. One of the profound examples of using genetic methods was the characterization of a strain of Influenza A present in native pigs of Ontario, CA. The 99% triple re-assortment based identity match of subtypes like H3N2 with the original strain of 2009 confirmed their origin from pigs. Furthermore, the Ontario based strain posed significant resistance to adamantanes but was immensely responsive to Neuraminidase inhibitors based drugs.[89–92] Other methods still work effectively as shown in an experiment (mice and ferret models) of combined factor vaccines (both Hemagglutinin as well as Neuraminidase) that were utilized to produce a significant amount of immune responses in research subjects.[93–94] Song and colleagues conducted a pathogenic study on the corresponding lineages of Chinese swine and humans (NS, PA, PB2, NP, M, NA, and PB1) by using several techniques and methods to differentiate strains in the laboratory. The study concluded that the viral strain in the Chinese swine sample was homologous with the strain that was previously circulating in North American regions. On the other hand, there were several variations in the pathogenicity when compared to other mammal-infecting strains. Mice models were used for histopathological examinations that showed that the viruses (AV1522, AV1523) progress over time due to the re-assortment in Chinese swine. It ultimately affects the respiratory as well as the nervous system of the infected individuals.[95–98] Furthermore, the continual resistance of the virus against modern antivirals drugs and vaccines makes it more challenging for researchers to develop new treatment strategies. The new anti-influenza agents that are still evolving and are in rigorous development include RNA (Ribonucleic Acid) inhibitors, RdRp (RNA-dependent RNA polymerase) inhibitors, V-ATPase (Vacuolar-type Adenosine Tri-Phosphatase) inhibitors as well as some species of modified antioxidants.[99–103]

## Conclusion

Although the present-day scenario is far more different from the circumstances during the pandemic of 2009, we should always be ready to deal with the unforeseen circumstances. The complications related to the infection caused by the different strains of the virus are now under control due to the rapidly evolved technology that enabled us to develop more effective treatments and advanced therapeutic methodologies. These developments helped people on a global scale in recent decades.[104] If we discover a new strain of the virus with a pandemic causing potential, the management strategies would be less complex because of our far more developed global management system. Overall, we are optimistic that we shall find a cure for this infection as we always have.

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## References


**1.** Lim BH, Mahmood TA. Influenza A H1N1 2009 (swine flu) and pregnancy. J Obstet Gynecol India **2011**; 61:386–393. Available at: https://pubmed.ncbi.nlm.nih.gov/22851818/. Accessed 8 March 2021.

**2.** Humans May Give Swine Flu To Pigs In New Twist To Pandemic. 2009. Available at: https://www.sciencedaily.com/releases/2009/07/090709201847.htm. Accessed 14 March 2021.

**3.** Weekly U.S. Influenza Surveillance Report — CDC. 2021. Available at: https://www.cdc.gov/flu/weekly/index.htm. Accessed 8 March 2021.

**4.** New Study Expands Understanding of Influenza-associated Complications. 2020. Available at: https://www.cdc.gov/flu/spotlights/2019-2020/study-flu-complications.html. Accessed 8 March 2021.

**5.** Recently publicized swine flu not new, under surveillance since 2011: WHO expert - Xinhua — English.news.cn. 2020. Available at: http://www.xinhuanet.com/english/2020-07/02/c_139181551.htm. Accessed 8 March 2021.

**6.** Sun H, Xiao Y, Liu J, Wang D, Li F, Wang C. Prevalent Eurasian avian-like H1N1 swine influenza virus with 2009 pandemic viral genes facilitating human infection. Proc Natl Acad Sci U S A **2020**; 117:17204–17210.

**7.** Cohen J. Swine flu strain with human pandemic potential increasingly found in pigs in China. 2020. Available at: https://www.sciencemag.org/news/2020/06/swine-flu-strain-human-pandemic-potential-increasingly-found-pigs-china. Accessed 8 March 2021.

**8.** Li S, Liu SS, Zhu AQ, et al. [The mortality burden of influenza in China: a systematic review]. Zhonghua Yu Fang Yi Xue Za Zhi **2019**; 53:1049–1055.

**9.** de Jesus EG. 4 reasons not to worry about that 'new' swine flu in the news. 2020. Available at: https://www.sciencenews.org/article/swine-flu-influenza-pandemic-reasons-not-to-worry. Accessed 8 March 2021.

**10.** Threat Assessment Brief: Eurasian avian-like A(H1N1) swine influenza viruses. 2020. Available at: https://www.ecdc.europa.eu/en/publications-data/threat-assessment-brief-eurasian-avian-ah1n1-swine-influenza-viruses. Accessed 8 March 2021.

**11.** CDC Takes Action to Prepare Against "G4" Swine Flu Viruses in China with Pandemic Potential. 2020. Available at: https://www.cdc.gov/flu/spotlights/2019-2020/cdc-prepare-swine-flu.html. Accessed 14 March 2021.

**12.** Ives M. Scientists Say New Strain of Swine Flu Virus Is Spreading to Humans in China. The New York Times. 2020; Available at: https://www.nytimes.com/2020/06/30/world/asia/h1n1-swine-flu-virus-china-pig.html. Accessed 14 March 2021.

**13.** Short KR, Kedzierska K, van de Sandt CE. Back to the Future: Lessons Learned From the 1918 Influenza Pandemic. Front Cell Infect Microbiol **2018**; 8:343.

**14.** Mills CE, Robins JM, Lipsitch M. Transmissibility of 1918 pandemic influenza. Nature **2004**; 432:904–906.

**15.** Carter RW, Sanford JC. A new look at an old virus: patterns of mutation accumulation in the human H1N1 influenza virus since 1918. Theor Biol Med Model **2012**; 9:42.

**16.** MacIntyre CR, Chughtai AA, Barnes M, Ridda I, Seale H, Toms R. The role of pneumonia and secondary bacterial infection in fatal and serious outcomes of pandemic influenza a(H1N1)pdm09. BMC Infect Dis **2018**; 18:637.

**17.** Chastagner A, Herve S, Bonin E, Queguiner S, Hirchaud E, Henritzi D. Spatiotemporal Distribution and Evolution of the A/H1N1 2009 Pandemic Influenza Virus in Pigs in France from 2009 to 2017: Identification of a Potential Swine-Specific Lineage. J Virol **2018**; 92:e00988-18.

**18.** Chauhan RP, Gordon ML. A systematic review analyzing the prevalence and circulation of influenza viruses in swine population worldwide. Pathog (Basel, Switzerland) **2020**; 9:E355.

**19.** Bahl J, Nelson MI, Chan KH, Chen R, Vijaykrishna D, Halpin RA. Temporally structured metapopulation dynamics and persistence of influenza A H3N2 virus in humans. Proc Natl Acad Sci U S A **2011**; 108:19359–19364.

**20.** Bedford T, Suchard MA, Lemey P, Dudas G, Gregory V, Hay AJ. Integrating influenza antigenic dynamics with molecular evolution. Elife **2014**; 3:e01914.

**21.** Liu M, Zhao X, Hua S, Du X, Peng Y, Li X. Antigenic Patterns and Evolution of the Human Influenza A (H1N1) Virus. Sci Rep **2015**; 5:14171.

**22.** Luksza M, Lassig M. A predictive fitness model for influenza. Nature **2014**; 507:57–61.

**23.** R R. Dalal A, Mohan H, Prasad M, Pundir CS. Detection methods for influenza A H1N1 virus with special reference to biosensors: a review. Biosci Rep **2020**; 40.

**24.** Veguilla V, Hancock K, Schiffer J, Gargiullo P, Lu X, Aranio D. Sensitivity and specificity of serologic assays for detection of human infection with 2009 pandemic H1N1 virus in U.S. populations. J Clin Microbiol **2011**; 49:2210–2215.

**25.** Hideshima S, Hinou H, Ebihara D, Sato R, Kuroiwa S, Nakanishi T. Attomolar detection of influenza A virus hemagglutinin human H1 and avian H5 using glycan-blotted field effect transistor biosensor. Anal Chem **2013**; 85:5641–5644.

**26.** Jeong HJ, Dong J, Ueda H. Single-Step Detection of the Influenza Virus Hemagglutinin Using Bacterially-Produced Quenchbodies. Sensors (Basel) **2018**; 19:E52.

**27.** Poon LL, Chan KH, Smith GJ, Leung CS, Guan Y, Yuen KY. Molecular detection of a novel human influenza (H1N1) of pandemic potential by conventional and real-time quantitative RT-PCR assays. Clin Chem **2009**; 55:1555–1558.

**28.** Wong FY, Donato C, Deng YM, Teng D, Komadina N, Baas C. Divergent Human-Origin Influenza Viruses Detected in Australian Swine Populations. J Virol **2018**; 92:e00316-18.

**29.** Philipson T. Economic epidemiology and infectious diseases. In: Anthony J. Culyer, Joseph P. Newhouse, eds. Handbook of Health Economics. Amsterdam: Elsevier, 2000: 1761–1799.

**30.** Aguero JM, Beleche T. Health shocks and their long-lasting impact on health behaviors: evidence from the 2009 H1N1 pandemic in Mexico. J Health Econ **2017**; 54:40–55.

**31.** Harris JE, Balsa AI, Triunfo P. Tobacco control campaign in Uruguay: impact on smoking cessation during pregnancy and birth weight. J Health Econ **2015**; 42:186–196.

**32.** Ippolito PM, Mathios AD. Information and advertising: the case of fat consumption in the United States. Am Econ Rev **1995**; 85:91–95.

**33.** Su YC, Bahl J, Joseph U, Butt KM, Peck HA, Koay ES. Phylodynamics of H1N1/2009 influenza reveals the transition from host adaptation to immune-driven selection. Nat Commun **2015**; 6:7952.

**34.** WHO | Situation updates - Pandemic (H1N1) 2009. 2009. Available at: http://www.who.int/csr/disease/swineflu/updates/en/. Accessed 8 March 2021.

**35.** Kocer ZA, Krauss S, Stallknecht DE, Rehg JE, Webster RG. The potential of avian H1N1 influenza A viruses to replicate and cause disease in mammalian models. PLoS One **2012**; 7:e41609.

**36.** Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science (80- ) **2009**; 325:197–201.

**37.** Kocer ZA, Carter R, Wu G, Zhang J, Webster RG. The Genomic Contributions of Avian H1N1 Influenza A Viruses to the Evolution of Mammalian Strains. PLoS One **2015**; 10:e0133795.

**38.** Desheva Y, Sychev I, Smolonogina T, Rekstin A, Ilyushina N, Lugovtsev V. Anti-neuraminidase antibodies against pandemic A/H1N1 influenza viruses in healthy and influenza-infected individuals. PLoS One **2018**; 13:e0196771.

**39.** Gagnon A, Acosta JE, Madrenas J, Miller MS. Is antigenic sin always 'original?' Re-examining the evidence regarding circulation of a human H1 influenza virus immediately prior to the 1918 Spanish flu. PLoS Pathog **2015**; 11:e1004615.

**40.** Kitikoon P, Nelson MI, Killian ML, Anderson TK, Koster L, Culhane MR. Genotype patterns of contemporary reassorted H3N2 virus in US swine. J Gen Virol **2013**; 94:1236–1241.

**41.** Couch RB, Atmar RL, Franco LM, Quarles JM, Wells J, Arden N. Antibody correlates and predictors of immunity to naturally occurring influenza in humans and the importance of antibody to the neuraminidase. J Infect Dis **2013**; 207:974–981.

**42.** Hufnagel L, Brockmann D, Geisel T. Forecast and control of epidemics in a globalized world. Proc Natl Acad Sci U S A **2004**; 101:15124–15129.

**43.** Nelson MI, Gramer MR, Vincent AL, Holmes EC. Global transmission of influenza viruses from humans to swine. J Gen Virol **2012**; 93:2195–2203.

**44.** Patterson Ross Z, Komadina N, Deng YM, Spirason N, Kelly HA, Sullivan SG. Inter-Seasonal Influenza is Characterized by Extended Virus Transmission and Persistence. PLoS Pathog **2015**; 11:e1004991.

**45.** Starick E, Lange E, Fereidouni S, Bunzenthal C, Hoveler R, Kuczka A. Reassorted pandemic (H1N1) 2009 influenza A virus discovered from pigs in Germany. J Gen Virol **2011**; 92:1184–1188.

**46.** Vijaykrishna D, Poon LL, Zhu HC, Ma SK, Li OT, Cheung CL. Reassortment of pandemic H1N1/2009 influenza A virus in swine. Science (80- ) **2010**; 328:1529.

**47.** Watson SJ, Langat P, Reid SM, et al. Molecular Epidemiology and Evolution of Influenza Viruses Circulating within European Swine between 2009 and 2013. J Virol **2015**; 89:9920–9931.

**48.** Hussain S, Miller JL, Harvey DJ, Gu Y, Rosenthal PB, Zitzmann N. Strain-specific antiviral activity of iminosugars against human influenza A viruses. J Antimicrob Chemother **2015**; 70:136–152.

**49.** Ries J. New Swine Flu Discovered in China: Why You Don't Need To Worry Too Much Yet. 2020. Available at: https://www.healthline.com/health-news/new-swine-flu-discovered-in-pigs-in-china. Accessed 8 March 2021.

**50.** Machemer T. New Swine Flu Strain With Pandemic Potential Isn't Cause for Alarm. 2020. Available at: https://www.smithsonianmag.com/smart-news/new-flu-virus-pandemic-potential-isnt-cause-alarm-180975252/. Accessed 8 March 2021.

**51.** Saito S, Minakami H, Nakai A, Unno N, Kubo T, Yoshimura Y. Outcomes of infants exposed to oseltamivir or zanamivir in utero during pandemic (H1N1) 2009. Am J Obstet Gynecol **2013**; 209:130.e1–9.

**52.** Key Facts About Seasonal Flu Vaccine. 2020. Available at: https://www.cdc.gov/flu/prevent/keyfacts.htm. Accessed 8 March 2021.

**53.** What You Should Know about Flu Antiviral Drugs. 2021. Available at: https://www.cdc.gov/flu/treatment/whatyoushould.htm. Accessed 8 March 2021.

**54.** Sidwell RW, Smee DF, Huffman JH, Barnard DL, Bailey KW, Morrey JD. In vivo influenza virus-inhibitory effects of the cyclopentane neuraminidase inhibitor RJW-270201. Antimicrob Agents Chemother **2001**; 45:749–757.

**55.** Smee DF, Wong MH, Bailey KW, Sidwell RW. Activities of oseltamivir and ribavirin used alone and in combination against infections in mice with recent isolates of influenza A (H1N1) and B viruses. Antivir Chem Chemother **2006**; 17:185–192.

**56.** Smee DF, Julander JG, Tarbet EB, Gross M, Nguyen J. Treatment of oseltamivir-resistant influenza A (H1N1) virus infections in mice with antiviral agents. Antiviral Res **2012**; 96:13–20.

**57.** Kyaw Win SM, Saito R, Win NC, Lasham DJ, Kyaw Y, Lin N. Epidemic of influenza A(H1N1)pdm09 analyzed by full genome sequences and the first case of oseltamivir-resistant strain in Myanmar 2017. PLoS One **2020**; 15:e0229601.

**58.** van der Vries E, Stelma FF, Boucher CAB. Emergence of a Multidrug-Resistant Pandemic Influenza A (H1N1) Virus. N Engl J Med 2010; 363:1381–1382.

**59.** van der Most RG, Clement F, Willekens J, Dewe W, Walravens K, Vaughn DW. Long-Term Persistence of Cell-Mediated and Humoral Responses to A(H1N1)pdm09 Influenza Virus Vaccines and the Role of the AS03 Adjuvant System in Adults during Two Randomized Controlled Trials. Clin Vaccine Immunol; CVI **2017**; 24:e00553-16.

**60.** Tungatt K, Dolton G, Morgan SB, Attaf M, Fuller A, Whalley T. Induction of influenza-specific local CD8 T-cells in the respiratory tract after aerosol delivery of vaccine antigen or virus in the Babraham inbred pig. PLoS Pathog **2018**; 14:e1007017.

**61.** Vemula S V, Sayedahmed EE, Sambhara S, Mittal SK. Vaccine approaches conferring cross-protection against influenza viruses. Expert Rev Vaccines **2017**; 16:1141–1154.

**62.** Anderson TK, Macken CA, Lewis NS, Scheuermann RH, Van Reeth K, Brown IH. A Phylogeny-Based Global Nomenclature System and Automated Annotation Tool for H1 Hemagglutinin Genes from Swine Influenza A Viruses. MSphere **2016**; 1:e00275-16.

**63.** Kirchenbaum GA, Carter DM, Ross TM. Sequential Infection in Ferrets with Antigenically Distinct Seasonal H1N1 Influenza Viruses Boosts Hemagglutinin Stalk-Specific Antibodies. J Virol **2015**; 90:1116–1128.

**64.** Lantos PM, Hoffman K, Hohle M, Anderson B, Gray GC. Are People Living Near Modern Swine Production Facilities at Increased Risk of Influenza Virus Infection? Clin Infect Dis **2016**; 63:1558–1563.

**65.** Li Y, Myers JL, Bostick DL, Sullivan CB, Madara J, Linderman SL. Immune history shapes specificity of pandemic H1N1 influenza antibody responses. J Exp Med **2013**; 210:1493–1500.

**66.** Sandbulte MR, Spickler AR, Zaabel PK, Roth JA. Optimal Use of Vaccines for Control of Influenza A Virus in Swine. Vaccines **2015**; 3:22–73.

**67.** Carter DM, Darby CA, Lefoley BC, Crevar CJ, Alefantis T, Oomen R. Design and Characterization of a Computationally Optimized Broadly Reactive Hemagglutinin Vaccine for H1N1 Influenza Viruses. J Virol **2016**; 90:4720–4734.

**68.** Yoshida T, Mei H, Dorner T, Hiepe F, Radbruch A, Fillatreau S. Memory B and memory plasma cells. Immunol Rev **2010**; 237:117–139.

**69.** Paccha B, Jones RM, Gibbs S, Kane MJ, Torremorell M, Neira-Ramirez V. Modeling risk of occupational zoonotic influenza infection in swine workers. J Occup Environ Hyg **2016**; 13:577–587.

**70.** Skarlupka AL, Owino SO, Suzuki-Williams LP, Crevar CJ, Carter DM, Ross TM. Computationally optimized broadly reactive vaccine based upon swine H1N1 influenza hemagglutinin sequences protects against both swine and human isolated viruses. Hum Vaccin Immunother **2019**; 15:2013–2029.

**71.** Morris SJ, Nightingale K, Smith H, Sweet C. Influenza A virus-induced apoptosis is a multifactorial process: exploiting reverse genetics to elucidate the role of influenza A virus proteins in virus-induced apoptosis. Virology **2005**; 335:198–211.

**72.** Shen X, Sun W, Shi Y, Xing Z, Su X. Altered viral replication and cell responses by inserting microRNA recognition element into PB1 in pandemic influenza A virus (H1N1) 2009. Mediators Inflamm **2015**; 2015:976575.

**73.** Wu X, Wu X, Sun Q, Zhang C, Yang S, Li L. Progress of small molecular inhibitors in the development of anti-influenza virus agents. Theranostics **2017**; 7:826–845.

**74.** Boivin S, Cusack S, Ruigrok RW, Hart DJ. Influenza A virus polymerase: structural insights into replication and host adaptation mechanisms. J Biol Chem **2010**; 285:28411–28417.

**75.** Jackson ML, Chung JR, Jackson LA, Phillips CH, Benoit J, Monto AS. Influenza Vaccine Effectiveness in the United States during the 2015-2016 Season. N Engl J Med **2017**; 377:534–543.

**76.** Shembekar N, Mallajosyula V V, Mishra A, Yeolekar L, Dhere R, Kapre S. Isolation of a high affinity neutralizing monoclonal antibody against 2009 pandemic H1N1 virus that binds at the 'Sa' antigenic site. PLoS One **2013**; 8:e55516.

**77.** Khurana S, Verma S, Verma N, Crevar CJ, Carter DM, Manischewitz J. Properly folded bacterially expressed H1N1 hemagglutinin globular head and ectodomain vaccines protect ferrets against H1N1 pandemic influenza virus. PLoS One **2010**; 5:e11548.

**78.** Lankowski AJ, Hohmann EL. Killed but metabolically active Salmonella typhimurium: application of a new technology to an old vector. J Infect Dis **2007**; 195:1203–1211.

**79.** Liu PF, Wang Y, Liu YT, Huang CM. Vaccination with Killed but Metabolically Active E. coli Over-expressing Hemagglutinin Elicits Neutralizing Antibodies to H1N1 Swine Origin Influenza A Virus. J Nat Sci **2017**; 3:e317.

**80.** Mesonero A, Suarez DL, van Santen E, Tang DC, Toro H. Avian influenza in ovo vaccination with replication defective recombinant adenovirus in chickens: vaccine potency, antibody persistence, and maternal antibody transfer. Avian Dis **2011**; 55:285–292.

**81.** Chan KF, Carolan LA, Korenkov D, Druce J, McCaw J, Reading PC. Investigating Viral Interference Between Influenza A Virus and Human Respiratory Syncytial Virus in a Ferret Model of Infection. J Infect Dis **2018**; 218:406–417.

**82.** Zang F, Chen Y, Lin Z, Cai Z, Yu L, Xu F. Autophagy is involved in regulating the immune response of dendritic cells to influenza A (H1N1) pdm09 infection. Immunology **2016**; 148:56–69.

**83.** Rajao DS, Vincent AL, Perez DR. Adaptation of Human Influenza Viruses to Swine. Front Vet Sci **2019**; 5:347.

**84.** Nelson MI, Vincent AL. Reverse zoonosis of influenza to swine: new perspectives on the human-animal interface. Trends Microbiol **2015**; 23:142–153.

**85.** Russier M, Yang G, Marinova-Petkova A, Vogel P, Kaplan BS, Webby RJ. H1N1 influenza viruses varying widely in hemagglutinin stability transmit efficiently from swine to swine and to ferrets. PLoS Pathog **2017**; 13:e1006276.

**86.** Wang G, Dos Anjos Borges LG, Stadlbauer D, Ramos I, Bermudez Gonzalez MC, He J. Characterization of swine-origin H1N1 canine influenza viruses. Emerg Microbes Infect **2019**; 8:1017–1026.

**87.** Chen Y, Trovao NS, Wang G, Zhao W, He P, Zhou H. Emergence and Evolution of Novel Reassortant Influenza A Viruses in Canines in Southern China. MBio **2018**; 9:e00909-18.

**88.** Neumann G, Kawaoka Y. Transmission of influenza A viruses. Virology **2015**; 479–480:234–246.

**89.** Grgic H, Costa M, Friendship RM, Carman S, Nagy E, Poljak Z. Genetic Characterization of H1N1 and H1N2 Influenza A Viruses Circulating in Ontario Pigs in 2012. PLoS One **2015**; 10:e0127840.

**90.** Grgic H, Costa M, Friendship RM, Carman S, Nagy E, Wideman G. Molecular characterization of H3N2 influenza A viruses isolated from Ontario swine in 2011 and 2012. Virol J **2014**; 11:194.

**91.** Howard WA, Essen SC, Strugnell BW, Russell C, Barass L, Reid SM. Reassortant Pandemic (H1N1) 2009 virus in pigs, United Kingdom. Emerg Infect Dis **2011**; 17:1049–1052.

**92.** Conenello GM, Zamarin D, Perrone LA, Tumpey T, Palese P. A single mutation in the PB1-F2 of H5N1 (HK/97) and 1918 influenza A viruses contributes to increased virulence. PLoS Pathog **2007**; 3:1414–1421.

**93.** Stauft CB, Yang C, Coleman JR, Boltz D, Chin C, Kushnir A. Live-attenuated H1N1 influenza vaccine candidate displays potent efficacy in mice and ferrets. PLoS One **2019**; 14:e0223784.

**94.** Marcelin G, Bland HM, Negovetich NJ, Sandbulte MR, Ellebedy AH, Webb AD. Inactivated seasonal influenza vaccines increase serum antibodies to the neuraminidase of pandemic influenza A(H1N1) 2009 virus in an age-dependent manner. J Infect Dis **2010**; 202:1634–1638.

**95.** Shope RE. Swine influenza: III. Filtration experiments and etiology. J Exp Med **1931**; 54:373–385.

**96.** Song Y, Zhang Y, Zhang B, Chen L, Zhang M, Wang J. Identification, Genetic Analysis, and Pathogenicity of Classical Swine H1N1 and Human-Swine Reassortant H1N1 Influenza Viruses from Pigs in China. Viruses **2020**; 12:E55.

**97.** Tumpey TM, Basler CF, Aguilar P V, Zeng H, Solorzano A, Swayne DE. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. Science (80- ) **2005**; 310:77–80.

**98.** FAOSTAT. 2021. Available at: http://www.fao.org/faostat/en/#home. Accessed 14 March 2021.

**99.** Webster RG, Govorkova EA. Continuing challenges in influenza. Ann N Y Acad Sci **2014**; 1323:115–139.

**100.** Moscona A. Neuraminidase inhibitors for influenza. N Engl J Med **2005**; 353:1363–1373.

**101.** Davidson S. Treating Influenza Infection, From Now and Into the Future. Front Immunol **2018**; 9:1946.

**102.** Hayden FG, Shindo N. Influenza virus polymerase inhibitors in clinical development. Curr Opin Infect Dis **2019**; 32:176–186.

**103.** Hu CJ, Chen YT, Fang ZS, Chang WS, Chen HW. Antiviral efficacy of nanoparticulate vacuolar ATPase inhibitors against influenza virus infection. Int J Nanomedicine **2018**; 13:8579–8593.

**104.** Ferguson NM, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic. Nature **2006**; 442:448–452.