American BioDefense Institute

Congressional Briefing: Novel G4 H1N1 Influenza

An Emerging Biosecurity Threat

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Introduction

The high degree of national vulnerability to infectious respiratory disease observed during the COVID-19 pandemic brings into focus the need for rapid identification of similar emerging biothreats, to both enable early containment and development of countermeasures. The H1N1 influenza virus represents just such a threat – it is a known killer with a deadly history that possesses the ability to evolve serotypes that are both highly infectious and for which there is no established herd immunity. The possibility of a widespread H1N1 outbreak during the COVID-19 pandemic would compound the strain on regional medical systems, as there is no cross-protection between COVID-19 and H1N1 influenza immunity.

H1N1 is an influenza A subtype whose strains had been responsible for several known major flu epidemics, including the deadliest known flu pandemic in 1918-19. Different strains of the H1N1 virus are endemic to humans, pigs, and birds.

There have been three pandemics of influenza in the 20th century and one in the 21st century (Kilbourne, 2006). The H1N1 virus caused a significant and best-known pandemic in 1918-19 and the most recent one in 2009-10. In addition, there were three other notable epidemics in the 20th century that were not considered pandemics: (i) a pseudo pandemic in 1947 (that was not considered pandemic due to low death rates), (ii) 1977 epidemic that was pandemic in children, (iii) an abortive epidemic of swine flu in 1976 in Fort Dix, NJ. All of these were caused by strains of the H1N1 virus.

An antigenic variation of H1N1 caused the 1947 epidemic. One remarkable feature of this epidemic was the complete failure of the H1N1 vaccine with the 1943 H1N1 strain due to the antigenic drift of the 1947 virus. Millions of U.S. military personnel that were vaccinated were found to have no protection against the new strain of the H1N1 virus. Luckily, the epidemic caused relatively few deaths and is, therefore, considered to be a pseudo pandemic.

The 1976 outbreak of H1N1 was confined to Fort Dix, NJ, although it triggered a mass vaccination program that planned to vaccinate 43 million Americans. The vaccination was suspended after repeated reports of Guillain-Barré syndrome affecting vaccinated individuals in around a dozen states and seven times the higher reported incidence of swine flu in vaccinated individuals. There were indications that reporting bias was to blame for at least part of these cases, as there were no specific tests for Guillain-Barré syndrome, and the doctors were aware of the reported link between the vaccination and the syndrome. In any case, the vaccination program was not reinstated, and the virus did not spread. This outbreak became a cautionary tale about overreacting to the danger of an epidemic by initiating a pre-emptive vaccination program with a hastily developed and tested vaccine.

In 1977, there was an outbreak of the 1947 H1N1 virus in the Soviet Union, which spread worldwide. Because the older population was already exposed to the strain in 1947-1957, the epidemic primarily affected the population under 25. Since both haemagglutinin (HA) and neuraminidase (NA) antigens were very similar to the 1947 strain, this lack of antigenic drift led to speculations that this strain of H1N1 virus escaped from a laboratory somewhere in the USSR.

The latest 2009-10 pandemic caused around 500,000 cases and 18,500 deaths, although it has been speculated that these numbers are significantly higher: CDC estimated around 284,000 (Roos, 2012). For comparison, reported deaths from influenza worldwide are 200,000-500,000 annually. Unlike most influenza viruses, this strain of the H1N1 virus did not affect disproportionately people over 60 years old.

New H1N1 Swine Flu Strain

A recently published study on influenza virus surveillance of pigs from 2011 to 2018 in China identified a recently emerged genotype 4 (G4) reassortant Eurasian avian-like (EA) H1N1 virus, which contains 2009 pandemic (pdm/09) and triple-reassort ant-derived internal genes (Sun et al., 2020). This virus has been predominant in China's swine population since 2016. Similar to the pdm/09 virus, G4 viruses bind to human-type receptors, produce much higher progeny virus in human airway epithelial cells, and show efficient infectivity and aerosol transmission in ferrets. Moreover, low antigenic cross-reactivity of human influenza vaccine strains with G4 reassort ant EA H1N1 virus indicates that preexisting population immunity does not protect against G4 viruses.

The G4 virus has different antigenicity from the current human influenza viruses. Like the pdm/09 virus, the G4 virus preferentially binds human-like SAα2,6Gal receptors and effectively transmits in the ferret model. The G4 virus also shows increased pathogenicity, based on the present ferret study and other reports in mice (Cao et al., 2019; Wang et al., 2019; Pulit-Penaloza et al., 2019). A limited serological investigation found that the general population, who had little opportunity to contact pigs, lacked antibodies against the G4 virus. Still, swine-exposed adult populations showed elevated seroprevalence (10.4%, 35/338), which further supports our hypothesis of G4 virus transmission from pigs to humans. It is of concern that human infection of the G4 virus will further human adaptation and increase the risk of a human pandemic. Its infectivity greatly enhances the opportunity for virus adaptation in humans and raises concerns for the possible generation of pandemic viruses.

A non-peer-reviewed preprint of research in the EA H1N1 virus showed that the replacement of a 271T marker in PB2 protein with a 271A marker significantly increases viral replication in both mammalian and avian cell lines (Feng et al., 2020). The mutation also increased viral infectivity, virulence, and pathogenicity in mice. It also increased the activity of viral polymerase in mammalian cells. It indicates that this mutation could significantly increase the likelihood that the EA H1N1 virus could infect humans and then transmit from human to human and trigger an epidemic.

Detection of H1N1 influenza A virus

Currently, WHO recommended methods for the detection of swine flu include real-time PCR in specific testing centers that take 3-4 hours (Dalal et al., 2020). More recently, several methods, such as Antigen-Antibody or RT-LAMP and DNA biosensors, have also been developed that are rapid and more sensitive.

Table 1. Overview of testing kits for H1N1 detection (Dalal et al., 2020)

Name of diagnostic method	Target	Sensitivity	Specificity	Reference
Immunochromatographic assay based rapid diagnostic kits	Nucleoprotein (NP)	2 × 105 viral copies/kit	100.00%	Miyoshi-Akiyama et al, 2010
SD Bioline Influenza Ag A/B/A(HINI) Pandemic	–	77.00%	100.00%	Choi et al, 2010
RapidSTRIPE test	Hemagglutinin (HA)	88.00%	94.00%	Patel et al., 2011
Immunochromatography (IC) rapid diagnostic test kits	HA, NP	49.4%, 79.5%	93%, 100%	Mizuike et al., 2011
Rapid fluorescent immunochromatographic strip test	NP	85.29%	100.00%	Yu et al., 2018

It takes 2-3 days to show its full symptomatic condition, and early-stage detection becomes necessary for early treatment. The old methods are time-consuming, mostly based on antigen-antibody titer, which makes them less sensitive and has a problem of false-negative results. Various kits have been developed for swine flu detection that is based on immunochromatographic techniques, as shown in Table 1, which also shows average results but not as informational as provided by real-time PCR.

A recent study reported the development of anti-HA Fab, a quench body (an immunosensor protein, which is fluorophore-labeled antibody or segment of an antibody. It shows a fluorescence response after quenching of dye from an antibody as a response of interaction between the quench body and the antigen) shows high fluorescence in the presence of HA antigen with the potential for sensor development [106]. Several biosensors have been reported, which are promising for specific results with high sensitivity, as reported in Table 2.

Table 2. Overview of biosensors for the detection of H1N1 (Dalal et al., 2020)

Sensor type	**Gene/protein**	**Sensitivity/L.O.D**	**Sample type**	**Detection time**	**References**
AuNP immunosensor	HA, NA antibody	50.5 pg/ml	H1N1 virus	–	Ahmed et al., 2017
Fluorescent immunosensor	HA/fusion antibody	Not reported	H1N1 Virus	–	Lee et al., 2012
Surface plasmon resonance	HA	4.5 pmol l-1	H1N1 Virus	–	Critchley et al., 2004
Immunosensor /SPR	HA /antibody	30 PFU/ml	labelled anti-HA	20 min	Su et al., 2012
Immunosensor	M1/polyclonal antibody	80–100 virions/µl	H1N1 virus	30 min	Nidzworski et al., 2014
PEDOT with galactose	HA binding	0.12, 0.013 HAU	H1N1 virus	–	Hai et al., 2017
BDD	M1 antibody	1 fg/ml	antibody-M1	5 min	Nidzworski et al., 2017
SiO2-IO	HA antibody	103–105 PFU	H1N1 virus	–	Lee et al., 2018
SWCNT immunoassay	anti- HIN1	180 TCID50ml	H1N1 Virus	–	Lee et al., 2011
Impedance aptasensor	DNA aptamer	0.9 pg/µl	H1N1 virus	–	Bai et al., 2018
DPM-coated gold electrode	His6-H1 HA	1 × 10^9 to 1 × 10^8 fold	HA antibody in sera	–	Mikula et al., 2018
QCM immunosensor	Anti-MA	1 × 10^3 pfu/ml	H1N1 virus	>100 min	Hewa et al., 2009
FET Biosensor	HA binding	6000 HA mol/20 µl	HA protein	–	Hideshima et al., 2013

Notes: HAU, hemagglutinin unit; TCID, tissue culture infective dose; PFU, plaque-forming unit; PEDOT, Poly (3,4-ethylene dioxythiophene); DPM, dipyrromethene; BDD, Boron doped diamond; HA, hemagglutinin; MA. Matrix.

CDC reaction

The CDC reported that the G4 swine flu viruses in China described in the PNAS report have a mix of genes from the influenza viruses found in humans, birds, and pigs (CDC, 2020a). Five genes of the G4 virus came from the 2009 H1N1 virus that caused the 2009 flu pandemic. Based on laboratory transmission studies in ferrets, the G4 viruses can spread via direct contact or respiratory droplets. The CDC tested a closely related G5 virus in its laboratories and observed transmission like that reported in this study. It's important to note that person-to-person transmission of the G4 viruses has not yet been reported, and they have not been detected in pigs or people in the United States.

The G4 viruses likely resulted from a process called "reassortment," which occurs when two or more influenza viruses infect a single host and exchange genetic material. This can sometimes lead to the emergence of new influenza viruses in people or animals. Pigs have been identified as a sort of "mixing vessel" for reassortment to occur because pigs are susceptible to infection with influenza viruses found in pigs, birds, and humans. The 2009 H1N1 pandemic arose from a reassortment event between pigs, birds, and human influenza A viruses.

The PNAS study showed that among 338 swine works whose blood was tested for antibodies, about 10% had evidence of past infection with G4 viruses (Sun et al., 2020). A higher rate was observed among 18-35-year-olds swine workers. Regular households also were sampled, and about 4% of 230 people from the general population had antibodies to G4 viruses. This data suggests that these viruses may have acquired an increased capability of infecting humans. The study authors caution that continued circulation of these viruses in pigs and exposure to humans may allow for additional reassortment events to occur and that these viruses and infections should be monitored closely.

Experts believe most people would lack immunity against the G4 viruses. Despite seasonal flu vaccines protecting against the 2009 H1N1 virus, the G4 viruses are different enough that seasonal flu vaccines would be unlikely to provide protection or prevent onward human-to-human transmission.

A prototype candidate vaccine virus (CVV) from the closely related EA avian-like H1N1 G5 swine flu virus was originally developed by the World Health Organization Collaborating Center at the China CDC. A comparison of the genome of this virus to that of G4 viruses reveals that these two viruses are similar. Studies are planned to assess cross-reactivity between this vaccine virus and the G4 viruses. If needed, the CDC will work to create a new CVV made specifically against the G4 viruses.

Influenza Risk Assessment Tool (IRAT)

The Influenza Risk Assessment Tool (IRAT) is an evaluation tool developed by the CDC and external influenza experts that assesses the potential risk posed by influenza A viruses that currently circulate in animals but not in humans (CDC, 2020b). The IRAT assesses potential pandemic risk based on two different scenarios: "emergence" and "public health impact."

"Emergence" refers to the risk of a novel (i.e., new in humans) influenza virus acquiring the ability to spread quickly and efficiently in people. "Public health impact" refers to the potential severity of human disease caused by the virus (e.g., deaths and hospitalizations) as well as the burden on society (e.g., missed workdays, a strain on hospital capacity and resources, and interruption of basic public services) if a novel influenza virus were to begin spreading efficiently and sustainably among people.

The IRAT uses ten scientific criteria to measure the potential pandemic risk associated with each of these scenarios. These ten criteria can be grouped into three overarching categories: "properties of the virus," "attributes of the population," and "ecology & epidemiology of the virus." Influenza subject matter experts evaluate novel influenza viruses based on each of these ten criteria. Each of the ten criteria is then weighted statistically based on its significance to each of the two scenarios. A composite score for each virus is then calculated based on the given scenario. These composite scores provide a means to rank and compare influenza viruses to each other in terms of their potential pandemic risk for each of the two scenarios (CDC, 2020c).

As we learn more about influenza A viruses, these ten criteria may change, other criteria may be added, or some current criteria may be dropped. The IRAT is designed to be flexible and responsive to current scientific advances.

The IRAT is intended to do the following:

- Prioritize and maximize investments in pandemic preparedness by helping to determine which novel (new) influenza viruses to develop vaccines against and capitalizing on surveillance efforts and in-country capacity building activities.

- Identify key gaps in information and knowledge, which can be the basis to prompt additional studies. (For example, if the information is not available for one of the ten criteria used by the IRAT additional studies could be done or resources allocated to provide the needed information).

- Document in a transparent manner the data and scientific process used to inform management decisions associated with pandemic preparedness.

- Provide a flexible means to easily and regularly update the risk assessment of novel influenza viruses as new information becomes available.

- Be a useful communications tool for policymakers and the influenza community.

- Provide a means to weigh the ten evaluation criteria differently depending on whether the intent of the risk assessment is to measure the ability of an influenza virus to "emerge" as a pandemic capable virus (i.e., become capable of efficient human-to-human spread) or "impact" the human population if it did emerge.

The IRAT cannot predict the next pandemic and is not intended to do so. Furthermore, the IRAT is not intended to eliminate the need for subject matter expertise. In fact, subject matter experts are needed to carefully analyze the ten criteria of the IRAT to make determinations of pandemic risk and to rank the importance of the criteria according to the specific risk question or situation. Lastly, the IRAT is not intended to make exact risk estimates. For example, many risk assessments generate a quantitative measure that describes the likelihood of exposure or disease risk. The IRAT focuses on the perceived pandemic potential of novel influenza viruses as estimated by subject matter experts using the IRAT evaluation criteria and available data. Estimates of the risk level expressed as IRAT scores should be incorporated into the planning and development of rapid countermeasures to emerging infectious disease threats.

Public Perceptions of Swine Flu Pandemic Response

A study by researchers at the University of Southampton looked at the public perception of government advice and management of the H1N1 influenza pandemic of 2009 (Teasdale et al., 2011). The study revealed several things:

- There was widespread skepticism about the feasibility and appropriateness of self-diagnosis of pandemic flu, even with detailed guidelines and recommendations.

- There was a widespread belief that flu vaccination was not 100% effective and can be unnecessary as people build up natural immunity.

- There were doubts about the recommendation to stay at home if symptomatic.

- People were anxious about missing work if confined to their homes.

- People perceived H1N1 flu as a mild illness and, therefore, tended to follow general guidelines for mild illnesses rather than specific recommendations from health officials.

- There was general skepticism towards media and government messages about the pandemic.

- The publicity surrounding the pandemic was seen as exaggerated and causing unnecessary panic.

This study was conducted during the pandemic. Therefore, it can be considered that it reflects the actual pandemic attitudes much more accurately than the studies conducted in a non-pandemic context. The conclusion of the study was that:

"Government advice is a specialized form of health communication with members of the public. People actively engage in skeptical evaluation of government advice, particularly in terms of its feasibility, credibility, and costs, which can influence whether they adopt the recommended actions."

The results of this study show the importance of communication during a pandemic. Since 2009, there has been a significant increase in the number of people who get their information primarily from the social networks and the internet overall. It means that they could easily be exposed to false and potentially life-threatening information without the counterbalance of reliable information from trusted sources in a pandemic.

This increases the responsibility of the government and other medical professionals to provide accurate and relevant information on time to counter rumors and misinformation. During the 2009 H1N1 flu pandemic, the symptoms of the illness progressively got milder on average as the epidemic spread around the world, creating the public perception that swine flu is relatively mild and harmless. This preconception would be damaging to the efforts to fight a new pandemic if the illness caused by the new H1N1 virus proves to be more serious with a significantly higher death rate.

Conclusions

The H1N1 influenza virus is well known and has been the cause of several pandemics or large epidemics in the past. The G4 strain of the EA H1N1 virus poses a danger as it has been shown that the current flu vaccines do not protect against it. However, the latest 2009-2010 pandemic response has been tainted by accusations of conflict of interest on the side of WHO; with some of their experts being accused of having financial ties with the companies producing vaccines and antiviral drugs (Godlee, 2010). This created significant public resistance to pre-emptive flu vaccination that persists today in many countries around the world. It is impossible to predict when the G4 strain of the EA H1N1 virus will begin human-to-human transmission or community infiltration. Therefore, the best precaution against a potential epidemic would be a rapid response intervention or countermeasure that could be quickly adapted to the G4 strain of the EA H1N1 virus and deployed at the first sign of an outbreak in order to help suppress the spread of influenza.

References

Ahmed, S.R., Kim, J., Suzuki, T., Neethirajan, S., Lee, J., and Park, E.Y. (2017). *In situ self-assembly of gold nanoparticles on hydrophilic and hydrophobic substrates for influenza virus-sensing platform*. Sci. Rep. 7, 44495, https://doi.org/10.1038/srep44495

Bai, C., Lu, Z., Jiang, H., Yang, Z., Liu, X., Ding, H. et al. (2018). *Aptamer selection and application in multivalent binding-based electrical impedance detection of inactivated H1N1 virus*. Biosens. Bioelectron. 110, 162–167, https://doi.org/10.1016/j.bios.2018.03.047

Cao, Z., Zeng, W., Hao, X., Huang, J., Cai, M., Zhou, P. and Zhang, G. (2019). *Continuous evolution of influenza A viruses of swine from 2013 to 2015 in Guangdong, China*. PloS one, 14(7), p.e0217607.

CDC, (2020a). CDC Takes Action to Prepare Against "G4" Swine Flu Viruses in China with Pandemic Potential, Available at: https://www.cdc.gov/flu/spotlights/2019-2020/cdc-prepare-swine-flu.html Accessed on July 06, 2020

CDC, (2020b). Influenza Risk Assessment Tool (IRAT), Available at: https://www.cdc.gov/flu/pandemic-resources/national-strategy/risk-assessment.htm, Accessed on July 06 2020

CDC, (2020c). Summary of Influenza Risk Assessment Tool (IRAT) Results, Available at: https://www.cdc.gov/flu/pandemic-resources/monitoring/irat-virus-summaries.htm, Accessed on July 06 2020

Choi, Y.J., Kim, H.J., Park, J.S., Oh, M.H., Nam, H.S., Kim, Y.B. (2010). *Evaluation of new rapid antigen test for detection of pandemic influenza A/H1N1 2009 virus*. J. Clin. Microbiol. 48, 2260–2262, https://doi.org/10.1128/JCM.02392-09

Critchley, P. and Dimmock, N.J. (2004). *Binding of an influenza A virus to a neomembrane measured by surface plasmon resonance*. Bioorg. Med. Chem. 12, 2773–2780, https://doi.org/10.1016/j.bmc.2004.02.042

Dalal, A., Mohan, H., Prasad, M. and Pundir, C.S. (2020). *Detection methods for influenza A H1N1 virus with special reference to biosensors: a review*. Bioscience reports, 40(2), p.BSR20193852.

Feng, Z., Zhu, W., Zhou, L., Chen, Y., Li, X., Gao, R., Liu, J., Wang, D. and Shu, Y. (2020). *The substitution of T271A in PB2 protein could enhance the infectivity and pathogenicity of Eurasian avian-like H1N1 swine influenza viruses in mice.*

Godlee, F. (2010*). Conflicts of interest and pandemic flu*. BMJ 340, c2947

Hai, W., Goda, T., Takeuchi, H., Yamaoka, S., Horiguchi, Y., Matsumoto, A. (2017). *Specific recognition of human influenza virus with PEDOT bearing sialic acid-terminated trisaccharides*. ACS Appl. Mater. Interfaces 9, 14162–14170, https://doi.org/10.1021/acsami.7b02523

Hewa, T.M., Tannock, G.A., Mainwaring, D.E., Harrison, S. and Fecondo, J.V. (2009). *The detection of influenza A and B viruses in clinical specimens using a quartz crystal microbalance*. J. Virol. Methods 162, 14–21, https://doi.org/10.1016/j.jviromet.2009.07.001

Hideshima, S., Hinou, H., Ebihara, D., Sato, R., Kuroiwa, S., Nakanishi, T. et al. (2013). *Attomolar detection of influenza A virus hemagglutinin human H1 and avian H5 using glycan-blotted field-effect transistor biosensor*. Anal. Chem. 85, 5641–5644, https://doi.org/10.1021/ac401085c

Kilbourne, E.D. (2006). Influenza pandemics of the 20th century. Emerging infectious diseases, 12(1), p.9.

Lee, D., Chander, Y., Goyal, S.M. and Cui, T. (2011). *Carbon nanotube electric immunoassay for the detection of swine influenza virus H1N1*. Biosens. Bioelectron. 26, 3482–3487, https://doi.org/10.1016/j.bios.2011.01.029

Lee, K.G., Lee, T.J., Jeong, S.W., Choi, H.W., Heo, N.S., Park, J.Y. et al. (2012). *Development of a plastic-based microfluidic immunosensor chip for detection of H1N1 influenza*. Sensors 12, 10810–10819, https://doi.org/10.3390/s120810810

Lee, W., Kang, T., Kim, S.H. and Jeong, J. (2018). *An Antibody-Immobilized Silica Inverse Opal Nanostructure for Label-Free Optical Biosensors*. Sensors 18, 307, https://doi.org/10.3390/s18010307

Mikula, E., Silva, C.E., Kopera, E., Zdanowski, K., Radecki, J. and Radecka, H. (2018). *Highly sensitive electrochemical biosensor based on redox-active monolayer for detection of anti-hemagglutinin antibodies against swine-origin influenza virus H1N1 in sera of vaccinated mice*. BMC Vet. Res. 14, 328, https://doi.org/10.1186/s12917-018-1668-9

Miyoshi-Akiyama, T., Narahara, K., Mori, S., Kitajima, H., Kase, T., Morikawa, S. et al. (2010). Development of an immunochromatographic assay *specifically detecting pandemic H1N1 2009 influenza virus*. J. Clin. Microbiol. 48, 703–708, https://doi.org/10.1128/JCM.02262-09

Mizuike, R., Sasaki, T., Baba, K., Iwamoto, H., Shibai, Y., Kosaka, M. (2011). *Development of two types of rapid diagnostic test kits to detect the hemagglutinin or nucleoprotein of the swine-origin pandemic influenza A virus H1N1*. Clin. Vaccine Immunol. 18, 494–499, https://doi.org/10.1128/CVI.00269-10

Nidzworski, D., Pranszke, P., Grudniewska, M., Kro´ l, E. and Gromadzka, B. (2014). *Universal biosensor for detection of influenza virus*. Biosens. Bioelectron. 59, 239–242, https://doi.org/10.1016/j.bios.2014.03.050

Nidzworski, D., Siuzdak, K., Niedzialkowski, P., Bogdanowicz, R., Sobaszek, M., Ryl, J. (2017). *A rapid-response ultrasensitive biosensor for influenza virus detection using antibody modified boron-doped diamond*. Sci. Rep. 7, 15707, https://doi.org/10.1038/s41598-017-15806-7

Patel, P., Graser, E., Robst, S., Hillert, R., Meye, A., Hillebrand, T. et al. (2011). *rapidSTRIPE H1N1 test for detection of the pandemic swine origin influenza A (H1N1) virus*. J. Clin. Microbiol. 49, 1591–1593, https://doi.org/10.1128/JCM.02563-10

Pulit-Penaloza, J.A., Belser, J.A., Tumpey, T.M. and Maines, T.R. (2019). *Mammalian pathogenicity and transmissibility of a reassortant Eurasian avian-like A (H1N1v) influenza virus associated with human infection in China 2015*. Virology, 537, pp.31-35.

Roos, R. (2012). *CDC estimate of global H1N1 pandemic deaths: 284,000*. Available at: https://www.cidrap.umn.edu/news-perspective/2012/06/cdc-estimate-global-h1n1-pandemic-deaths-284000, Accessed on July 08 2020

Su, L.C., Chang, C.M., Tseng, Y.L., Chang, Y.F., Li, Y.C., Chang, Y.S. (2012). *Rapid and highly sensitive method for influenza A (H1N1) virus detection*. Anal. Chem. 84, 3914–3920, https://doi.org/10.1021/ac3002947

Sun, H., Xiao, Y., Liu, J., Wang, D., Li, F., Wang, C., Li, C., Zhu, J., Song, J., Sun, H. and Jiang, Z. (2020). *Prevalent Eurasian avian-like H1N1 swine influenza virus with 2009 pandemic viral genes facilitating human infection*. Proceedings of the National Academy of Sciences.

Teasdale, E. and Yardley, L. (2011). *Understanding responses to government health recommendations: public perceptions of government advice for managing the H1N1 (swine flu) influenza pandemic.* Patient education and counseling, 85(3), pp.413-418.

Wang, G., dos Anjos Borges, L.G., Stadlbauer, D., Ramos, I., Bermúdez González, M.C., He, J., Ding, Y., Wei, Z., Ouyang, K., Huang, W. and Simon, V. (2019). *Characterization of swine-origin H1N1 canine influenza viruses*. Emerging microbes & infections, 8(1), pp.1017-1026.

Yu, S.T., Bui, C.T., Nguyen, A.V., Trinh, T.T. and Yeo, S.J. (2018). *Clinical evaluation of rapid fluorescent diagnostic immunochromatographic test for influenza A virus (H1N1)*. Sci. Rep. 8, 13468, https://doi.org/10.1038/s41598-018-31786-8