

How the H7N9 Influenza A Virus Adapted to Become A Human Pathogen?

Madison Hoes¹, Gerard Hoyne^{1,2,3}

¹School of Health Sciences, University of Notre Dame Australia, Fremantle, Western Australia, ²Institute of Health Research, University of Notre Dame Australia, Fremantle, Western Australia, ³School of Biomedical Sciences, The University of Western Australia, Nedlands, Western Australia

ABSTRACT

The influenza A (H7N9) virus is a zoonotic disease that arose in China in 2013. Most previously studied H7N9 viruses were found to have low pathogenicity; however, the genetic reassortment of several avian-origin influenza viruses gave rise to a novel H7N9 pathogen that could spread to humans to cause severe pneumonia, acute respiratory distress syndrome and, in some cases, death. Akin to other influenza viruses, the H7N9 virus, could induce strong inflammatory immune responses with the increased secretion of cytokines and chemokines, and pre-existing immunity to the influenza subtype in humans appeared to be limited. In this review, we will examine the changes that occurred in the H7N9 virus, namely the potential mutations in hemagglutinin, polymerase basic protein 2, and neuraminidase, that led to the virus' increased pathogenicity and virulence. The H7N9 virus could bind to both avian type and human type sialic acid receptors, but person-person transmission of the H7N9 virus was low. These findings raise the importance for continual vigilance in maintaining enhanced surveillance of H7N9 viruses to monitor their spread and limit the chance of pandemic infections.

Key words: Influenza virus, H7N9, HA, basic protein 2, immune response

INTRODUCTION

Influenza A viruses are important pathogens in humans with the capacity to cause pandemic infections. According to the World Health Organization, influenza epidemics cause 3–5 million severe cases and 300,000–500,000 deaths globally each year.^[1] The major global outbreaks of influenza A infections that occurred in the 20th century included the 1918 Spanish flu caused by the H1N1 virus that killed around 4 million people worldwide and the 1957 Asian flu (H2N2 virus) and 1968 Hong Kong (H2N3) flu which caused fewer deaths but still represented significant global pandemics. There is an on-going race between host immunity and viral evolution, and influenza viruses are subjected to continual antigenic change caused by “antigenic drift.”^[2] Control strategies for influenza infections rely on annual immunizations that must be updated to account for seasonal changes that occur in the virus due to

the pressure of host immune responses.^[1] Birds represent the major reservoir of influenza A viruses, but the flu virus can infect other hosts such as swine and humans. However, for the virus to move from birds to animals or to humans, it needs to adapt to its specific host.

The H7N9 influenza A virus caused the first cases of serious human infection in China in early 2013.^[3] Before this, H7 viruses circulated in poultry, but human cases were rare and usually resulted in mild symptoms such as conjunctivitis.^[3,4] In contrast, H7N9 is a virulent and pathogenic virus that has caused multiple epidemics in China with severe respiratory illness and a high mortality rate of $\geq 30\%$.^[5-7] The H7N9 virus that led to the outbreaks in China arose through the genetic reassortment of three different avian viruses (H7N3, H7N9, and H9N2); this result highlights the virus ability to adapt to humans and develop pandemic potential.^[8,9]

Address for correspondence: Gerard F. Hoyne, School of Health Sciences, University of Notre Dame Australia, 19 Mouat St. Fremantle 6959, Western Australia. Phone: 61-8-94330236. Fax: 61-8-94330210.
E-mail: gerard.hoyne@nd.edu.au

© 2018 The Author(s). This open access article is distributed under a Creative Commons Attribution (CC-BY) 4.0 license.

Several molecular changes in H7N9 are proposed to have taken place in the virus to enable it to transmit to humans and cause infection. This review will discuss the changes in a range of key viral proteins of the H7N9 virus that facilitated its adaptation to the human host.

HEMAGGLUTININ (HA) MUTATIONS

A number of amino acid substitutions in the HA surface glycoprotein of H7N9 are responsible for driving the virus' adaptation to, and infectivity of, humans. Influenza viruses have two major glycoproteins, HA and neuraminidase (NA), that are incorporated into the viral envelope that surrounds the virion particle.^[10] These glycoproteins constitute the major antigenic proteins of influenza, and they play fundamental roles in influenza infection; HA glycoproteins are associated with adhesion, while NA proteins are involved in viral release.^[10] Although both are crucial to successful influenza pathogenicity, it appears that changes to the HA protein were more important than changes to NA in the adaptation of H7N9 virus to humans.

HA is a three-subunit, integral membrane glycoprotein where each subunit has a receptor-binding site that binds to, and is specific for, sialic acid (SA).^[10] These SA-binding sites are formed by several conserved and variable amino acids that comprise the central binding pocket and a number of secondary structures, including 130, 140, 150, and 220 loops and a 190 helix, that surround the pocket.^[10,11]

The general structure and binding of the HA ligand to the SA receptor are similar for all influenzas; however, the specific interaction varies according to the host species. SAs are glycan-attached monosaccharides that are found on the plasma membranes of many animal and human cells.^[12] Due to their widespread distribution, SAs constitute a diverse group with a large number of different types.^[12] The HA glycoproteins expressed by influenza viruses are specific for the Neu5Ac-galactose-linked SAs.^[11] Humans predominantly have Neu5Ac-galactose SA receptors in their respiratory tissues, whereas birds, the sources of most influenza viruses, have a significant number of these receptors in their intestines.^[10,13] The variation in the specific binding of HA to these Neu5Ac-galactose SAs is due to the different -galactose linkages that occur in the latter SAs. For example, mammals, including humans, primarily have HA-recognized SA receptors that are attached to galactose through 2,6-bonds.^[10] Avian species, on the other hand, have Neu5Ac SA receptors that are 2,3-linked.^[10] Birds also exclusively contain 2,3 linked SA (avian) receptors, whereas humans have an extensive number of 2,6 linked SA (mammalian) receptors in their upper and lower respiratory tracts and a small number of avian receptors in their lower respiratory tissues and lungs.^[12,13] Due to the nature of their -galactose linkages, 2,3- and 2,6-SAs are structurally different molecules. Antigens and

receptors must be specific for each other on a conformational level for successful binding to occur. As such, 2,3- and 2,6-SA receptors are specific for, and bind to, disparate types of HA glycoproteins found on different influenza viruses. In other words, influenza viruses can be "avian," with HA glycoproteins that specifically bind to avian 2,3 SA receptors, or mammalian 2,6 SA receptors.^[10]

This difference in host receptor specificity is important due to its role in adhesion. Adhesion is the most crucial step of the infection process because microbes have to securely attach to host cells to enter them.^[14] A virus cannot invade, replicate, or produce new virion particles (processes that enable survival and transmission) until that virus has adhered to a host cell.^[14] Since receptor binding facilitates adhesion for influenzas, the specificity of the HA protein for its receptor will determine not only the host range but also the specific cell type it can infect.^[12] This has implications for H7N9 adaptation to humans since influenza viruses with avian-specific HAs can only infect cells that express avian 2,3 SA receptors (e.g., intestinal epithelial cells in birds), while influenzas with mammalian-specific HAs can only infect cells that express the 2,6 SA receptor (e.g., human respiratory epithelial cells).^[12]

Previous analyses of influenzas of the H7 subtype included the H7N3 and H7N7 viruses. In general, H7 alleles are associated with avian specificity through binding to avian 2,3-SAs.^[3] Before the emergence of the H7N9 virus in humans in 2013, the H7 influenzas had been restricted to cause disease in poultry and other birds but had rarely infected humans due to their inability to bind to the 2,6-SAs found in the human upper respiratory tract.^[3] The H7N9 virus, however, has undergone several molecular changes that have increased its HA affinity for mammalian receptors. The most significant of these changes is the Q226L substitution in the SA-binding site's 220 loop, where most H7N9 viruses have leucine (L) rather than glutamine (Q).^[15] This substitution is repeatedly observed within studies as the hallmark change that enabled H7N9 adaptation and binding to 2,6-SAs found in humans.^[11] Although this mutation is undoubtedly a key change, it does not confer a complete switch to mammalian specificity for H7N9.^[11] Watanabe *et al.* found that H7N9 isolates contain only the HA Q226L mutation bound to both 2,3 and 2,6 SA receptors in human bronchial cells, whereas isolates with 226L and a basic protein 2 (PB2) mutation (discussed later) adhered strongly and more selectively to 2,6 SA receptors.^[16] Three other studies noted that mutated H7N9 viruses dually bind to both avian and mammalian receptors, and that, compared to human adapted strains such as H1N1. The H7N9 viruses often exhibit weaker or less frequent binding to 2,6 SA receptors.^[5,11,13] These findings suggest that, although the Q226L mutation is a significant step toward human adaptation for H7N9 (i.e., it enables the virus to adhere to 2, 6 SA receptors and infect human cells),

the mutation does not completely switch the conformation of the virus H7 glycoproteins to mammalian 2,6-SAs, and residual avian specificity remains. Aside from Q226L, other HA mutations have also been flagged other HA mutations have been identified to facilitate binding to human 2,6 SA receptors. Huang *et al.* observed a HA mutation T160A that weakened the H7N9 virus' affinity for 2.3 linked SA receptors,^[5] while Ke *et al.* found a HA G186V mutation in a patient infected with the H7N9 virus.^[17] R156K, L244Q, and V195A were identified by Xiang *et al.* as important HA mutations for determining specificity and host shift for the H7N9 virus.^[6] Likewise, Tharakaraman *et al.* found that a 220 loop G228S mutation significantly increased the degree and strength of H7N9 HA binding to human respiratory tissues such as the trachea.^[11] In terms of the latter mutation, de Vries *et al.* concluded that a particular combination of mutations involving G228S with (1) V186G/K and K193T or (2) V186N and N224K caused H7N9 to lose specificity toward avian 2,3 linked SA receptors and gain affinity to human 2,6 SA receptors.^[15] However, the G228S mutation is yet to be seen naturally in H7N9 viruses.^[4,11]

POLYMERASE PB2 MUTATIONS

In addition to the H7 substitutions that have increased the affinity of H7N9 HAs for mammalian receptors, several substitutions in the virus polymerase PB2 are also closely associated with H7N9 human adaptation. PB2 is one of the three protein subunits that form the polymerase complex in influenza viruses.^[18] Viral replication relies on this complex which is packaged into virion particles alongside the virus RNA genomes and nucleoproteins.^[19] Each of the subunits in the complex has a role in catalyzing the steps of replication; PB2, for example, is responsible for binding to the capped RNA genomic segments.^[19] Given that the complex is fundamental to viral replication, a process that enables invading virions to grow to sufficient numbers to cause host damage, it follows that this complex and its subunits are implicated in the ability of influenzas, such as H7N9, to infect humans. Arai *et al.* suggested as such in their proposition that the polymerase complex and the mutational changes it experiences are essential to determine the spectrum of host cells that an influenza can invade.^[18] Although there are three subunits in the complex that can influence the enzyme's functioning, the source of the key adaptive mutations that increase H7N9's replication efficiency in humans is PB2; according to Yamayoshi *et al.*, there is a substitution in the PB2 region of every H7N9 virus that has been retrieved from humans.^[9]

The PB2 of the H7N9 virus is avian derived which indicates that the polymerase complex of this virus is adapted to induce replication at the higher temperature of 37°C in avian intestinal and respiratory tracts.^[7,20] The environment

of the human upper respiratory tract is different with an ambient temperature of 33°C.^[7,20] This temperature difference represents a barrier to the adaptation of H7N9 and other avian influenzas to humans because RNA replication is temperature dependent.^[20] In other words, virus replication is controlled by the PB2 polymerase complex and its enzymatic function may not be optimal at 33°C compared to 37°C. As such, to adapt to humans, the H7N9 PB2 has to acquire the ability to initiate replication at the lower temperature in humans. Several mutations in PB2 appear to have facilitated this outcome. The most important and prevalent are a change from glutamic acid (E) to lysine (K) at position 627.^[9,21] This substitution has been repeatedly shown to increase replication efficiency in humans and confer human adaptability. Chan *et al.* demonstrated that H7N9 viruses containing the 627K mutation efficiently replicated in human bronchus and lung cultures (i.e., to a similar level to human-adapted H1N1), while viruses with 627E did not infect these tissues.^[22] Likewise, Zhang *et al.* found that 627K resulted in a 20-fold higher replication rate in human cells compared to 627E.^[23] This group also showed that polymerase efficiency is enhanced in cells infected with 627K viruses because the substitution promotes replication at a temperature of 33°C.^[23] Chen *et al.* similarly found very low polymerase activity of avian 627E in human cells which provides further evidence for the necessity of the E627K transformation to allow H7N9 virus to adapt to replication in humans.^[21] In addition to its effects in increasing polymerase replication in humans, E627K is also the most commonly occurring PB2 mutation; Gao *et al.* found three H7N9 isolates from infected patients with the mutation,^[4] while Chen *et al.* identified the E627K substitution in 102 of 147 analyzed PB2 segments.^[21] As Chen *et al.*'s data showed, however, human adaptation can still occur in the absence of the E627K mutation.^[21] Other significant mutations in PB2, namely Q591K and D701N, can also confer efficient replication in humans, although these mutations are less common and only occur when 627E is retained and appeared to play a compensatory role.^[9,21] Chen *et al.*, for example, restored 65.9% and 70.2% of polymerase activity in human cells that were infected with 591K and 701N viruses, respectively, that had been reverted back to avian 627E.^[21] These findings indicate that E627K is the most important molecular change in PB2 for increasing viral replication in, and adaptation to, human cells, but that Q591K and D701N allow H7N9 to infect humans, although with lesser efficiency, when the E627K mutation does not occur. It is also important to note that, while the latter three mutations occur independently of one another, other potentially significant human-adapting cosubstitutions, such as M535L, have also been identified when 627 is reverse mutated.^[21] Further research is needed to determine the capacity of such cosubstitutions for human adaptation.

Other mutations to facilitate human infection by H7N9 virus

The substitutions in the genes that code for HA and PB2 are the most important molecular changes that enable H7N9 to adapt to humans; however, they are not the only mutations that can occur.^[6] Across human H7N9 isolates, Xiang *et al.* identified 18 and 34 different genomic sites where positive selection and parallel evolution were occurring, respectively.^[6] Chen *et al.* located 104 sites with ≥ 7 mutations in the viruses they analyzed.^[21] Some of these changes potentially contribute to H7N9 adaptation and pathogenicity in humans; however, more research is required to ascertain their prevalence and specific functions.^[6] Nonetheless, some studies have still identified a number of mammalian-like changes in H7N9 virulence factors aside from HA and PB2. Chen *et al.* found a significant number of PA substitutions, including V100A, K356R, and S409N,^[21] while Gao *et al.* identified PB1-I368V substitutions that were associated with increased virus transmission in ferrets and M1-N30D and T125A mutations that were related to increased virulence in mice.^[4] Zaraket *et al.* also observed a high number of H7N9 viruses with mutations in PB1-D76N, NP-I365V, NA-E73K, and NA-I300V that exhibited efficient droplet transmission in ferrets.^[7]

Limitations in adaptation

Thus far, the H7N9 virus has undergone a number of changes that have increased its capacity to adhere to, enter, and replicate within human cells. These changes have enabled avian H7N9 virus to adapt to and cause infection in humans. Notwithstanding the observed changes in HA, PB2, PB1, M1, NP, and NA proteins of the H7N9 virus, the newly emerging pathogen is still limited in the scope of infection that it can cause because the virus is not fully adapted to humans and continues to lack some key changes. The most important of these missing adaptations is an ability to transmit efficiently between human hosts, despite its capacity to transmit directly from bird reservoirs to humans.^[8,17] The virus is most limited in terms of airborne transmission, which is the most common and effective transmission method in humans.^[24]

As demonstrated by a number of ferret models and human cases, the H7N9 virus may be capable of very limited human-to-human transmission by direct contact and aerosol droplets. Importantly, studies using ferrets seem to indicate higher levels of H7N9 viral transmission compared to the more sporadic transmission that is observed in humans. For example, Richard *et al.* demonstrated airborne transmission in three of four of their ferrets.^[24] In contrast, a study conducted by Qi *et al.* found that H7N9 likely transferred to the daughter of an infected man who had direct contact with his body fluids.^[25] However, this same study also reported no signs of infection in the man's 43 other close contacts.^[25] Likewise, Ke *et al.* monitored and found no evidence of disease in the 70 close

contacts of the case-patient they studied,^[17] and Gao *et al.* reported that none of the contacts of the three patients that they assessed tested positive for H7N9.^[4] As such, in humans, H7N9 virus transmission appears to be rare, and if it can be transmitted, the infected human host becomes a "dead end" host.^[25]

These results suggest that H7N9 has not experienced the necessary molecular changes that are required to enable the virus to efficiently transmit between humans. Given that the virus has also failed to completely adapt to human hosts, the capacity of the H7N9 virus to cause a widespread pandemic is limited. The overriding view of scientists is that the poor transmissibility of the H7N9 virus is not due to a single genetic change caused by a mutation but rather due to changes in a number of viral characteristics. For example, Richard *et al.* and colleagues hypothesized that the partial binding of H7N9 virus to 2,3 SA receptors, the tendency of its virions to fuse with cell membranes at a higher pH, and the relative instability of its HA glycoproteins in more acidic environments (like the human respiratory tract) may account for H7N9 poor transmission between humans.^[24]

CONSEQUENCES OF COMPLETE ADAPTATION

In its current forms, the inability of H7N9 virus to cause widespread infection in humans is limited by its poor transmission capacity. Despite this restriction, H7N9 is consistently reported as a virulent, severe, and rapidly-acting influenza virus in human patients.^[11,13] As such, it is likely that any further mutations in the virus, particularly those that improve its transmission efficiency, have the potential to increase the virulence of the virus and its risk of causing a pandemic. This is due to a number of inherent host and viral characteristics that, despite H7N9 incomplete adaptation to humans, still enable it to cause infection and severe disease in humans.

It appears that humans have negligible adaptive immunity against H7N9 viruses. Zhou *et al.* analyzed serum antibody levels before and following seasonal influenza vaccination in 90 subjects and found no pre-existing or cross-reactive antibodies in all cases.^[13] Similarly, a larger study of 500 individuals conducted by Watanabe *et al.* measured no serum antibodies against a H7N9 isolate.^[16] With minimal ability to elicit fast, strong, and highly effective secondary immune responses against the virus and no additional protection from vaccines, the current human population is very susceptible to the full spectrum of H7N9 disease.^[13] Given the naive nature of the subtype and the mostly sporadic occurrence of related H7 influenza viruses in humans before the first H7N9 outbreak, this lack of immunity is not surprising but indicates

that H7N9 has the potential to cause more severe infection if it becomes more adapted to the human host.^[4]

Second, H7N9 infections can provoke excessively strong or maladapted cytokine and chemokine responses (e.g., “cytokine storms”) that have been observed with H1N1 influenza infections. Several studies indicate that the levels of certain cytokines, including IP10, MIP-1, MCP1, IL6, and IL8, are increased after H7N9 infection.^[5,13] These levels are often comparable to those seen in human infections with highly lethal H5N1 virus.^[5,13] Furthermore, the latter studies suggest a correlation between higher cytokine concentrations and more severe disease presentations and outcomes, where these pro-inflammatory molecules likely act to exaggerate the body’s natural immune responses to worsen the symptoms of infection.^[5,13] Like H5N1, then, this association with cytokines might make H7N9 more dangerous and virulent to humans.

Finally, H7N9 demonstrates resistance to a number of antiviral drugs that are commonly used to treat influenza A and is potentially developing resistance to others. H7N9 isolates consistently have S31N substitutions in their matrix (M2) proteins that confer resistance to adamantane-derived drugs such as amantadine.^[4,17] In addition to this established resistance, some isolates are beginning to display mutations in their surface NA glycoproteins, such as H274K and R292K, that result in diminished susceptibility and budding resistance to antivirals such as oseltamivir, peramivir, and zanamivir.^[3-5,17] Since antiviral treatment is essential to dampening down the symptoms and duration of influenza, the poor responses of H7N9 to many antivirals only increase its ability to cause more serious and lethal infections.^[3]

CONCLUSION

H7N9 is an avian influenza that has developed the ability to adapt to, and cause infection in, humans as a consequence of several molecular changes. These changes are primarily key substitutions in the viral proteins HA and PB2 which have functions in viral adherence to host SA receptors and replication efficiency in host cells, respectively. Currently, the pandemic potential of H7N9 is restricted by its lack of adaptation to human-human and airborne transmission. However, if further changes occur in the virus to increase this capacity, H7N9 is likely to cause severe and widespread infection as a result of poor immunity, strong cytokine responses, and resistance to several antivirals. As such, close monitoring of H7N9 adaptation and outbreak occurrence is essential to ensure a rapid response to any H7N9 pandemics. Further research should focus on identifying and understanding the function of existing and new molecular changes in H7N9. Such research will enable H7N9, as well as any future influenzas with similar mutations, to be better managed. The latter is, especially, important given that

studies investigating the nature, adaptation, containment, and prevention of influenza viruses are potentially limited by the existence of mutations that are still unknown to the scientific community.

REFERENCES

1. Paules CI, Sullivan SG, Subbarao K, Fauci AS. Chasing seasonal influenza—the need for a universal influenza vaccine. *N Engl J Med* 2018;378:7-9.
2. Viboud C, Epstein SL. First flu is forever. *Science* 2016;354:706-7.
3. Jernigan DB, Cox NJ. H7N9: Preparing for the unexpected in influenza. *Annu Rev Med* 2015;66:361-71.
4. Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, *et al.* Human infection with a novel avian-origin influenza A (H7N9) virus. *N Engl J Med* 2013;368:1888-97.
5. Huang DT, Lu CY, Chi YH, Li WL, Chang LY, Lai MJ, *et al.* Adaptation of influenza A (H7N9) virus in primary human airway epithelial cells. *Sci Rep* 2017;7:11300.
6. Xiang D, Shen X, Pu Z, Irwin DM, Liao M, Shen Y, *et al.* Convergent evolution of human-isolated H7N9 avian influenza A viruses. *J Infect Dis* 2018;217:1699-707.
7. Zaraket H, Baranovich T, Kaplan BS, Carter R, Song MS, Paulson JC, *et al.* Mammalian adaptation of influenza A(H7N9) virus is limited by a narrow genetic bottleneck. *Nat Commun* 2015;6:6553.
8. Lam TT, Wang J, Shen Y, Zhou B, Duan L, Cheung CL, *et al.* The genesis and source of the H7N9 influenza viruses causing human infections in China. *Nature* 2013;502:241-4.
9. Yamayoshi S, Fukuyama S, Yamada S, Zhao D, Murakami S, Uraki R, *et al.* Amino acids substitutions in the PB2 protein of H7N9 influenza A viruses are important for virulence in mammalian hosts. *Sci Rep* 2015;5:8039.
10. Gamblin SJ, Skehel JJ. Influenza hemagglutinin and neuraminidase membrane glycoproteins. *J Biol Chem* 2010;285:28403-9.
11. Tharakaraman K, Jayaraman A, Raman R, Viswanathan K, Stebbins NW, Johnson D, *et al.* Glycan receptor binding of the influenza A virus H7N9 hemagglutinin. *Cell* 2013;153:1486-93.
12. Varki A. Sialic acids in human health and disease. *Trends Mol Med* 2008;14:351-60.
13. Zhou J, Wang D, Gao R, Zhao B, Song J, Qi X, *et al.* Biological features of novel avian influenza A (H7N9) virus. *Nature* 2013;499:500-3.
14. Jiang L, Changsom D, Lerdsamran H, Wiriyarat W, Masamae W, Noisumdaeng P, *et al.* Immunobiological properties of influenza A (H7N9) hemagglutinin and neuraminidase proteins. *Arch Virol* 2016;161:2693-704.
15. de Vries RP, Peng W, Grant OC, Thompson AJ, Zhu X, Bouwman KM, *et al.* Three mutations switch H7N9 influenza to human-type receptor specificity. *PLoS Pathog* 2017;13:e1006390.
16. Watanabe T, Kiso M, Fukuyama S, Nakajima N, Imai M, Yamada S, *et al.* Characterization of H7N9 influenza A viruses isolated from humans. *Nature* 2013;501:551-5.
17. Ke C, Mok CK, Zhu W, Zhou H, He J, Guan W, *et al.* Human infection with highly pathogenic avian influenza A(H7N9) virus, China. *Emerg Infect Dis* 2017;23:1332-40.

18. Arai Y, Kawashita N, Hotta K, Hoang PV, Nguyen HL, Nguyen TC, *et al.* Multiple polymerase gene mutations for human adaptation occurring in Asian H5N1 influenza virus clinical isolates. *Sci Rep* 2018;8:13066.
19. Stevaert A, Naesens L. The influenza virus polymerase complex: An update on its structure, functions, and significance for antiviral drug design. *Med Res Rev* 2016;36:1127-73.
20. Aggarwal S, Dewhurst S, Takimoto T, Kim B. Biochemical impact of the host adaptation-associated PB2 E627K mutation on the temperature-dependent RNA synthesis kinetics of influenza A virus polymerase complex. *J Biol Chem* 2011;286:34504-13.
21. Chen GW, Kuo SM, Yang SL, Gong YN, Hsiao MR, Liu YC, *et al.* Genomic signatures for avian H7N9 viruses adapting to humans. *PLoS One* 2016;11:e0148432.
22. Chan LL, Bui CT, Mok CK, Ng MM, Nicholls JM, Peiris JS, *et al.* Evaluation of the human adaptation of influenza A/H7N9 virus in PB2 protein using human and swine respiratory tract explant cultures. *Sci Rep* 2016;6:35401.
23. Zhang H, Li X, Guo J, Li L, Chang C, Li Y, *et al.* The PB2 E627K mutation contributes to the high polymerase activity and enhanced replication of H7N9 influenza virus. *J Gen Virol* 2014;95:779-86.
24. Richard M, Schrauwen EJ, de Graaf M, Bestebroer TM, Spronken MI, van Boheemen S, *et al.* Limited airborne transmission of H7N9 influenza A virus between ferrets. *Nature* 2013;501:560-3.
25. Qi X, Qian YH, Bao CJ, Guo XL, Cui LB, Tang FY, *et al.* Probable person to person transmission of novel avian influenza A (H7N9) virus in Eastern China, 2013: Epidemiological investigation. *BMJ* 2013;347:f4752.

How to cite this article: Hoes M, Hoyne G. How the H7N9 Influenza A Virus Adapted to become A Human Pathogen? *Clin Res Immunol* 2018; 1(2):1-6.