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REVIEW

Human H7N9 avian influenza virus infection: a review and pandemic risk assessment

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China is undergoing a recent outbreak of a novel H7N9 avian influenza virus (nH7N9) infection that has thus far involved 132 human patients, including 37 deaths. The nH7N9 virus is a reassortant virus originating from the H7N3, H7N9 and H9N2 avian influenza viruses. nH7N9 isolated from humans contains features related to adaptation to humans, including a Q226L mutation in the hemagglutinin cleavage site and E627K and D701N mutations in the PB2 protein. Live poultry markets provide an environment for the emergence, spread and maintenance of nH7N9 as well as for the selection of mutants that facilitate nH7N9 binding to and replication in the human upper respiratory tract. Innate immune suppression conferred by the internal genes of H9N2 may contribute to the virulence of nH7N9. The quail may serve as the intermediate host during the adaptation of avian influenza viruses from domestic waterfowl to gallinaceous poultry, such as chickens and related terrestrial-based species, due to the selection of viral mutants with a short neuraminidase stalk. Infections in chickens, common quails, red-legged partridges and turkeys may select for mutants with human receptor specificity. Infection in *Ratitae* species may lead to the selection of PB2-E627K and PB2-D701N mutants and the conversion of nH7N9 to a highly pathogenic avian influenza virus.
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INTRODUCTION

China is undergoing a recent outbreak of human nH7N9 infection that has involved 132 patients, including 37 deaths, as of 31st May 2013.[1] Nearly 84% of confirmed human cases of this outbreak are from Eastern China.[2] The median age of these patients was 61 years with a male to female ratio of approximately 2:1. More than 60% of the patients had one or more coexisting medical conditions.[3] More than 80% of these patients required treatment in an intensive care unit. Two-thirds of the patients had adult respiratory distress syndrome secondary to viral pneumonia, 24% had septic shock and 15% had acute renal failure.[4] An N31S mutation in the nH7N9 M2 protein confers resistance to amantadine and rimantadine.[5,6] Although most nH7N9 viruses are sensitive to oseltamivir, a mutation in the neuraminidase (NA) gene that conferred resistant to both oseltamivir and zanamivir was detected in two patients who received steroid therapy.[7] It has been reported that oseltamivir initiated at symptom onset to control disease progress was ineffective in a family cluster.[8] nH7N9 is a novel reassortant avian-origin influenza A virus. The hemagglutinin (HA) gene of this virus might have originated from H7N3 avian influenza viruses of duck origin. The NA gene of this virus might have been transferred from an H7N9 avian influenza virus of migratory birds. The six internal genes (non-structural (NS), nucleoprotein, polymerase acidic (PA), polymerase basic 1 (PB1), PB2 and matrix) most likely originated from H9N2 avian influenza viruses endemic in brambling birds or chickens in China. Phylogenetic analysis has indicated the possibility of an as yet unidentified intermediate host.[9–11] nH7N9 viruses isolated from humans contain features related to human adaptation, such as a Q226L mutation in the HA and E627K and D701N mutations in the PB2 protein.[12,13] These mutations enhanced nH7N9 binding to and replication in the human upper respiratory tract.[14–17] nH7N9 can be transmitted efficiently via direct contact among ferrets and can replicate in both the upper and lower respiratory tracts of these infected animals.[18] Live poultry markets were suspected as the source of the recent human nH7N9 outbreak.[19] The absence of multiple basic amino acids at the HA cleavage site of these low pathogenic nH7N9 viruses probably led to subclinical infections among poultry and favored the silent spread of nH7N9 in the live poultry market.[20–23] nH7N9 viruses encode a deletion at position 69–73 of the NA stalk region, a characteristic related to adaptation of an avian influenza virus from aquatic birds to terrestrial domestic poultry, particularly chickens. This deletion is also associated with increased virulence in mammals.[5] Hence, nH7N9 may have been circulating among poultry for some time before the current outbreak. Twenty percent of human nH7N9 infections were associated with farm exposure, especially in rural China.[24] The effectiveness of culling infected poultry and closing the live poultry markets in preventing human outbreaks of nH7N9 was demonstrated in Hangzhou, Shanghai and Zhejiang. The suspension of transport and trading of live birds carrying the virus may limit further geographical spread of nH7N9 to other uninvolved provinces.[25,26]

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THE EMERGENCE, SPREAD AND MAINTENANCE OF nH7N9 IN LIVE POULTRY MARKETS

The mixing of multiple species of poultry along with mammals in live poultry markets provides an ideal environment that favors reassortment among avian influenza viruses of different subtypes and interspecies viral transmission, including transmission from avian species to humans. Duck and geese are natural reservoirs of avian influenza viruses.[27,28] Quail may serve as the intermediate host for the adaptation of novel reassortant avian influenza viruses from domestic waterfowl to gallinaceous poultry and humans.[29,30] Chickens, common quail, red-legged partridges, and turkeys have both human-like sialic acid alpha-2,6-galactose-linked receptors and avian-like sialic acid alpha-2,3-galactose-linked receptors in their trachea and intestine.[31–34] The ability of some low pathogenic H9 and H7 avian influenza viruses to bind to both avian and human receptors has conferred a survival advantage to these subtypes over other avian influenza viruses in these poultry species,[35] which may explain why endemic H9N2[36], H7N2[37] and H7N7[38] viruses in poultry have been shown to acquire human receptor specificity and have caused outbreaks in humans. The asymptomatic infection of poultry with these pathogenic influenza viruses that have acquired human receptor specificity in live poultry markets facilitates the transmission of these pathogenic reassortant influenza viruses to humans.[39] There is a link between human highly pathogenic H5N1 avian influenza virus (HPH5N1) infection and live poultry markets in China.[40] Influenza infection of ostrich and emu can lead to the selection of PB2-E627K and PB2-D701N mutants,[41] and ratite-origin isolates of low pathogenicity can easily be converted to highly pathogenic avian influenza viruses in chickens.[42–44] The mixing of new birds with unsold birds in live poultry markets sustains the circulation of these reassortant viruses. Hence, the live poultry market is important in the emergence, spread and maintenance of these pathogenic reassortant influenza viruses.[45]

THE CONTRIBUTION OF THE INTERNAL GENES FROM THE H9N2 AVIAN INFLUENZA VIRUS TO THE VIRULENCE OF nH7N9

The interferon-mediated antiviral response and the species specificity of the NS segment are important in determining tissue tropism and virulence of influenza viruses.[46–48] Interferon-beta (IFN-β) plays a critical role in the defense against an influenza virus that cannot be compensated by IFN-α.[49] The NS of HPH5N1 is able to inhibit constitutive IFN-β release[50] and inducible IFN-β production at the pre-transcriptional[51] and post-transcriptional levels[52] and to induce cytokine dysregulation.[53] The intense innate immune suppression enhances tropism of HPH5N1 for human tissues and allows HPH5N1 to invade and replicate in human tissues without the need for the avian sialic acid alpha-2,6-galactose receptor.[54–57] Introduction of the HPH5N1 NS into the low pathogenic H7N1 avian influenza virus enabled H7N1 to replicate efficiently in different human cell lines without prior adaptation due to potent suppression of IFN-β production.[58] This observation shows that the NS of HPH5N1 is able to increase the virulence and enhance the adaptation of avian influenza viruses to human hosts. An optimal gene constellation is important for the virulence of NS in HPH5N1 infection.[59,60] The viral polymerase protein PA and the nucleocapsid protein are important for the stabilization of the CPSF30–NS1 complex.[61] The three influenza virus polymerases (PA, PB1 and PB2) and the PB1-F2 protein enhance NS-mediated interferon antagonism.[62,63] The interaction between matrix and NS leads to down-regulation of the inflammasome and produces a delayed apoptosis of respiratory epithelial cells.[64,65] This delayed apoptosis and potent suppression of the innate immune response allows HPH5N1 to proliferate rapidly during the stealth phase of influenza infection[66] and results in a high viral load,[67] cytokine dysregulation[68] and high mortality.[69] The NS of HPH5N1 comes from an ancestor H7N7 avian influenza virus, which has become incorporated into the H9N2 of the quail population in China. The introduction of NS and the other five internal genes (nucleoprotein, PA, PB1, PB2 and matrix) from the H9N2 of the quail population into HPH5N1 was responsible for the outbreak of the HPH5N1 infection in 1997 in chickens and humans in Hong Kong.[70] This NS-associated gene constellation that led to the outbreak of HPH5N1 in 1997 is also present in some of the H9N2 avian influenza viruses endemic in China.[71,72] The outbreak of human nH7N9 infection after reassortment of the six internal genes from H9N2 into nH7N9 has been corroborated by the outbreak of the human HPH5N1 infection in 1997 following the reassortment of these six internal genes from H9N2 that are capable of suppressing the innate immune response into HPH5N1 in Hong Kong. The potent IFN-suppressing capability of this NS-associated gene constellation may have contributed to the virulence of nH7N9 in humans. Because low pathogenic H7 avian influenza virus can cause asymptomatic infections in poultry and has an increased ability to acquire human receptor specificity by selective pressure in poultry species that contain both human and avian binding sites in their trachea and intestine, the reassortment of this NS-associated gene constellation of H9N2 into nH7N9 may be more disastrous than the outbreak of HPH5N1 in 1997 in terms of pandemic risk.

Furthermore, the virulence of the NS of an influenza virus in host cells is species specific. The species specificity of NS was first discovered during testing of the individual gene segments of the 1918 H1N1 Spanish influenza pandemic virus in mice. The NS that is virulent to human cells is less virulent than the corresponding wild-type control virus in mice.[47,73] The 2009 novel H1N1 influenza virus (2009PV), which induced a potent cytokine dysregulation of the human host and produced an enhanced mortality among young adult humans,[74] caused only modest disease in ferrets[75] and asymptomatic infection in pathogen-free miniature swine.[76] The asymptomatic infection of 2009PV in pathogen-free miniature swine explains the absence of a detectable outbreak of 2009PV infection in the swine population before the virus surfaced in humans. HPH5N1 is highly pathogenic to humans but has heterogeneous virulence in mice and ferrets.[77] Despite the outbreak of an HPH5N1 infection that had a mortality rate of 80% in Indonesia, HPH5N1 was detected in asymptomatic infections in an Indonesian swine population.[78] Therefore, tests assessing the pathogenicity of nH7N9 in humans should be conducted in human cell lines.

THE CONTRIBUTION OF nH7N9 MUTANTS SELECTED IN LIVE POULTRY MARKETS TO HUMAN INFECTION

Live poultry markets provide an ideal environment for viral reassortment and interspecies transmission of avian influenza viruses. Ducks and quail can act as mixing vessels to facilitate reassortment of influenza viruses of different subtypes, which may result in the generation of novel reassortant avian influenza viruses and the selection of mutants that may expand their host range to mammals.[79,80] nH7N9 has a five-amino-acid deletion in the NA stalk that may be associated with increased virulence.[5,6] Influenza viruses with a long NA stalk have a selective advantage in aquatic birds. Sustained circulation of subtype

H5 and H7 influenza viruses in terrestrial poultry often selects for viruses with a shorter neuraminidase stalk. Those HPH5N1 viruses with a short NA stalk have increased virulence in mice and humans. Among the 162 HPH5N1 viruses isolated from humans, only two viruses contain a long NA stalk.[81] Serial passage of a duck-origin avian influenza virus in quail led to the acquisition of mutations in HA commonly found in human seasonal influenza viruses and stepwise stalk deletions in NA. These quail-adapted duck-origin influenza viruses were able to replicate in human bronchial epithelial cells.[82] Serial passage of H9N2 viruses into quails and chickens can lead to the production of mutants with short NA stalks that can infect mice without prior adaptation and result in the selection of PB2-E627K mutants.[83] These quail- and chicken-adapted mutants with short NA stalks can efficiently replicate in the respiratory tract of chickens and be transmitted via respiratory contact. The susceptibility of quails to multiple subtypes of influenza viruses facilitates reassortment among these viruses. The role of quail as an intermediate host in the adaptation of avian influenza viruses from domestic waterfowl, such as ducks and geese, to gallinaceous poultry, such as chickens and related terrestrial-based species, and the ability of quail to shed these novel reassortant viruses via respiratory aerosols led to their removal from live poultry markets in Hong Kong in 2002.[84,85]

nH7N9 isolated from humans contains features related to human adaptation, such as a Q226L mutation in the HA cleavage site and E627K and D701N mutations in the PB2 protein, that facilitate nH7N9 binding to and replication in the human upper respiratory tract. The Q226L mutation in HA facilitated the adhesion of nH7N9 to the human upper respiratory tract,[86,87] a property that enhanced the ability of this avian influenza virus to transmit via aerosols.[14–16] The PB2-E627K mutation increased the viral polymerase activity, replication efficiency and pathogenicity of this avian influenza virus in the mammalian host.[88,89] The PB2-E627K mutation also allowed efficient replication of the avian influenza virus at both 33 ℃ and 37 ℃. The ability to replicate at 33 ℃ facilitates avian influenza virus proliferation in the upper respiratory tract of humans, a property that may allow the virus to be readily spread by sneezing and coughing. Efficient replication at 37 ℃ allows this avian influenza virus to replicate in human lungs and to induce acute pulmonary complications.[90–92] The only reported fatal case of a low pathogenic H7N7 avian influenza virus was with a virus containing a PB2-E627K mutation, which is absent in those patients presenting with mild conjunctivitis.[93,94] The PB2-D701N mutation allows the avian influenza virus to cross the host species barrier and to infect mammalian cells.[95,96] The asymptomatic spread of nH7N9 among poultry and the rapid selection of nH7N9 mutants capable of human invasion indicates that the pandemic threat of nH7N9 may surpass that of HPH5N1. The live poultry market plays a key role in the recent outbreak of poultry and human infections with nH7N9 by providing an environment for amplification, maintenance and interspecies transmission of the nH7N9 virus. Closing of the live poultry markets and culling of poultry in infected live poultry markets are important events in the prevention of further dissemination of nH7N9 infection. A ban on keeping live poultry overnight in live poultry markets may reduce the circulation of reassortant influenza viruses.[97] Massive poultry vaccination and central poultry slaughtering should be considered if nH7N9 becomes an endemic disease among poultry.[24] However, such a vaccination program may lead to the development of multilineage antigenic drift in these low pathogenic avian influenza viruses.[98] The control of a human nH7N9 outbreak may prevent further adaptation of nH7N9 in humans because PB2-E267K and PB2-D701N mutants can be selected during replication in humans.[99] Proper disposal of infected poultry and animal carcasses is important in preventing the spread of nH7N9 via predatory or scavenger birds.[100–102]

Because influenza infection of ostrich and emu can lead to the selection of PB2-E267K and PB2-D701N mutants and because ratite-origin influenza isolates of low pathogenicity can easily be converted to a highly pathogenic avian influenza virus in chickens, meticulous care must be taken to prevent the spread of nH7N9 to ostrich and other members of the *Ratitae* family. China has undergone a boom in ostrich and emu farming due to the market demand for their high-quality feathers, leather, healthy low-cholesterol red meat, eggs, eggshells and oil. China is the largest ostrich producer in Asia.[103] In Inner Mongolia, producers are experimenting with the rearing of emus to replace sheep and goats in grasslands to alleviate the problem of overgrazing and desertification in China.[104–106] Outbreaks of avian influenza virus infections have been reported in ostrich and emu farms in China.[107,108] Direct contact between ostrich flocks and migratory birds via the free-range production systems in ostrich farms may play an important role in the mutual dissemination of mutant avian influenza viruses between ostriches and migrating birds.[109] Biosecurity measures should be implemented to minimize possible contact between ostriches or emus and migrating birds on these farms.[110] Regular surveillance programs on ostrich and emu farms are important for the detection and early control of the asymptomatic spread of nH7N9 among these species.[111] Wild birds carrying low-pathogenic nH7N9 may migrate towards Qinghai Lake.[112] The acquisition of the PB2-E627K mutation has been reported in a HPH5N1 virus at Qinghai Lake in 2005.[113,114] Descendants of the *Ratitae* family around Qinghai Lake have been implicated in the emergence of such mutant viruses as fossils of *Struthio asiaticus*[115] and ornithomimosaurs (ostrich-mimic dinosaurs)[116] have been detected in the Gansu Province of China. Hence, nH7N9 may undergo further reassortment or mutation at Qinghai Lake before spreading to the southern part of China and to the rest of the world during the coming winter season via migrating birds.[117,118]

CONCLUSION

Current epidemiological data suggest that an immediate human nH7N9 pandemic is unlikely,[119] although it is impossible to predict whether nH7N9 will become endemic among poultry in China. The world should be vigilant against nH7N9. Continued monitoring and surveillance to obtain additional epidemiological data of nH7N9 is important for the early detection of a potential human nH7N9 pandemic. Seroprevalence studies may delineate the magnitude of asymptomatic or mild nH7N9 cases in the community.[120] Unless long-term measures are taken, such as the enhancement of live poultry market hygiene and poultry farm biosecurity to eliminate the emergence, spread and maintenance of reassortant avian influenza viruses and the selection of mutants capable of human invasion, live poultry markets and poultry farms may serve as incubators for the emergence of highly lethal reassortant avian influenza viruses with pandemic potential.

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