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Emergence and Pandemic Potential of Avian Influenza A (H7N9) Virus

Wen Su and Hongxuan He

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Abstract

New genotypes of influenza A virus arise quickly and frequently around the world due to continued mutation and reassortment. Novel influenza A viruses pose a direct threat to immunologically naïve humans. A prime example is the recent emergence of avian-origin H7N9 viruses that have become enzootic in China. Three waves of the H7N9 breakout that began in March 2013 have resulted in 571 human cases and over 212 deaths as of 23 February 2015. Real-time influenza surveillance at the wild bird–human interface is essential to limit the outbreak in scale and geographic distribution and to understand the pandemic potential of the H7N9 avian influenza.

Influenza A viruses continue to evolve via several mechanisms, especially by point mutation and reassortment [1], which conduce to the emergence of new strains with epidemic or pandemic potential. In the last 100 years, influenza A viruses have transmitted to and spread among humans, resulting in at least four pandemics [2]. These outbreaks lead to huge economic losses. The recent outbreak of H7N9 viruses following the pandemic spread of 2009 pandemic influenza A(H1N1)pdm09 virus has caused more than 600 human infections, with nearly 30% mortality (http://www.who.int/influenza/human_animal_interface/influenza_h7n9/en/). Multiple distinct lineages of H7N9 viruses have established in chickens [3]. Of note, the increasing epidemiological evidences indicated that H7N9 viruses have limited transmissibility in a family cluster [4]. Therefore, a comprehensive understanding of the mechanisms that determine viral transmissibility, pathogenicity, and evolution is of importance for pandemic preparedness.

Keywords: Influenza, H7N9, evolution, re-assortment

1. Introduction

1.1. Influenza A viruses

Influenza A viruses belong to the family Orthomyxoviridae. The genome of influenza A viruses is comprised of eight segments of single-stranded, negative-sense RNA. The total genome size is about 13,588 bases. There are two viral surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), in the viral membrane. On the basis of the antigenic variation of the HA and NA, influenza A viruses were classified into different subtypes. Thus far, 18 HA subtypes (H1–H18) and 11 NA subtypes (N1–N11) have been detected in the wild [5]. In theory, influenza A viruses can yield up to 198 combinations. Currently, investigation confirmed that more than nine combinations (H3N2, H1N1, H5N1, H7N7, H7N9, H5N6, H10N8, H2N2, and H9N2) were able to infect humans (Figure 1).
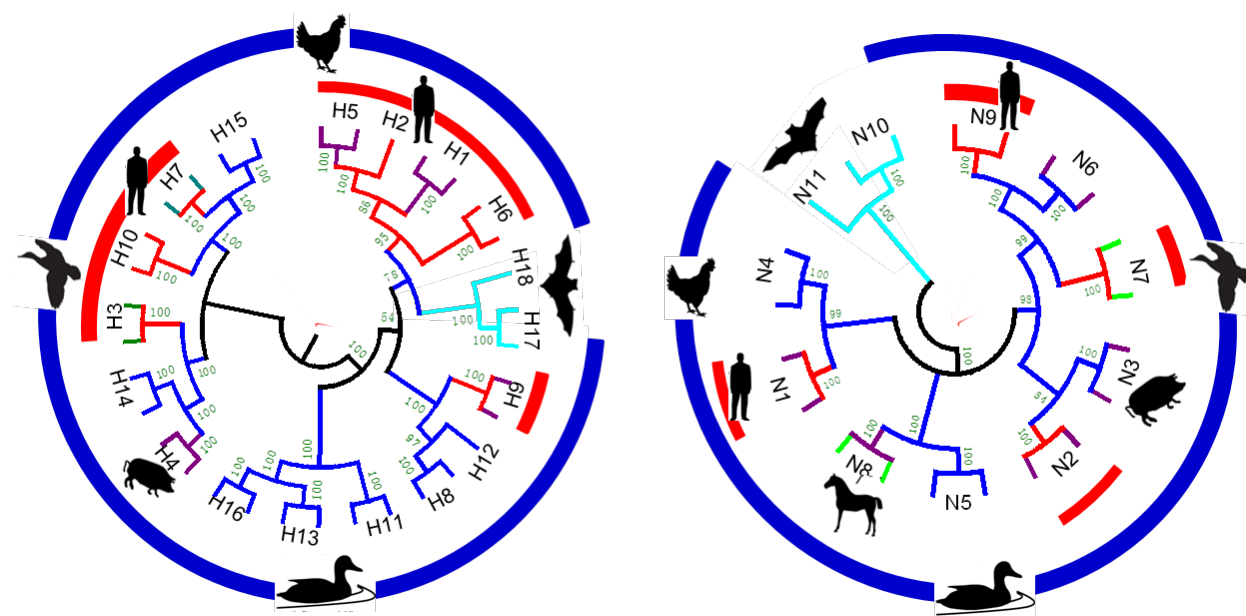

Figure 1. Phylogenetic trees of surface genes of all subtypes of influenza A viruses. The rooted phylogenetic tree was generated based on a full-length nucleotide sequence comparison among influenza A viruses. The representative sequence of each subtype was obtained from the Influenza Virus Resource of NCBI for multiple alignments, and the phylogenetic tree was generated by the ClustalW algorithm in Mega version 6.0 using UPGMA method. The bootstrap values are given at each node. Animal symbols denote the host of influenza A viruses. Subtypes of influenza A viruses have been identified in different hosts, as indicated: blue, birds; red, human; purple, pig; cyan, bat; green, horse.

The genome of influenza A viruses can code for up to 14 proteins [6], which are involved in the influenza virus life cycle [7]. HA mediates the binding to the receptors on host cells and the fusion of the virus and host cell membranes for vRNA release into the cytoplasm. NA is responsible for the efficient release of progeny viruses from infected cells. Replication and transcription and viral RNAs (vRNP) are carried out by the ribonucleoprotein complexes (RNPs) containing three polymerase proteins, basic polymerase 2 (PB2), basic polymerase 1 (PB1), and acidic polymerase (PA), and the nucleocapsid protein (NP). The PB1 also encodes other polypeptides in overlapping open reading frames: PB1-F2 and PB1-N40. PB1-F2 protein,

encoded by the +1 reading frame of PB1, functions to kill host immune cells, responding to influenza virus infection [8]. The role of PB1-N40 is associated with PB1 and PB1-F2 [9]. PA gene can code a previously unknown PA-X, which functions to repress cellular gene expression [10]. M1 is the viral matrix structural protein and the ion-channel protein M2 is incorporated in the viral membrane. M42 was identified, which functionally complements M2 [11]. NS gene encodes two proteins: xlinkand NEP. xlink is an interferon antagonist that blocks the activation of transcription factors and NEP is involved in the nuclear export of RNPs into the cytoplasm before virus assembly. The roles of HA and PB2 proteins in viral transmissibility are discussed in more detail later.

2. Highly pathogenic avian influenza viruses

On the basis of the virulence in chickens, influenza A viruses of the H5 and H7 subtypes can be divided into highly pathogenic avian influenza (HPAI) and low-pathogenic avian influenza (LPAI) viruses. In the field, a few H5 and H7 subtype avian influenza viruses are the HPAI phenotype. In general, HPAI viruses are considered to emerge in poultry infected by LPAI viruses. Since 1997, when it was first reported in Hong Kong [12, 13], the highly pathogenic avian influenza A(H5N1) viruses have spread through wild and domestic bird populations across Asia and into Europe, the Middle East, and Africa [14]. Currently, HPAI Asian-origin H5N1 viruses have become endemic in poultry in six countries (Bangladesh, China, Egypt, India, Indonesia, and Vietnam) [15].

H5N1 viruses continue to undergo dynamic evolution [14]. HPAI A(H5N1) viruses have resulted in outbreaks in waterfowl, the natural reservoir of influenza A viruses [16]. Ten years since highly pathogenic Asian-origin avian influenza A (H5N1) viruses began to infect migratory waterfowl in Qinghai Lake in western China in 2005 [16, 17], there is an unprece-dented panzootic in more than 63 countries across three continents [18]. They have led to severe economic damage to poultry industry, and have transmitted to humans with lethal conse-quences. As of 31 March 2015, 826 human infections with 440 deaths have been reported in 16 countries (http://www.who.int/influenza/human_animal_interface/EN_GIP_201503031 cumulativeNumberH5N1cases.pdf?ua=1). Compared with seasonal influenza epidemics and the 2009 H1N1 pandemic, the Asian-origin HPAI H5N1 virus is strikingly severe and fatal, with 52.3% mortality rate. Although several family clusters of H5N1 virus infection have been reported [19], sustained human-to-human infection has not been detected.

Several studies indicated that HPAI Asian-origin H5N1 viruses that are capable of respiratory droplet transmission among mammals can be generated by mutation and reassortment. Herfst et al. introduced two well-known substitutions (Q226L, G228S in H3 HA numbering) in the receptor-binding site (RBS) of HA and a well-known glutamic acid (Glu) to lysine substitution at position 627 (E627K) of PB2 into a human-origin H5N1 isolate [20]. The H5N1 mutant acquired the ability to be transmitted by airborne transmissible among ferrets by further acquiring substitutions after serial passage [20]. Linster et al. stated that two sets of five mutations are identified (E627K in PB2; H99Y in PB1; H103Y, T156A, and either Q222L or

G224S in HA), either of which is sufficient to confer ferret transmissibility on a human H5N1 isolate. Using a combination method, a reassortant H5 HA/H1N1 virus—comprising H5 HA (from an H5N1 virus) with four mutations and the remaining seven gene segments from a 2009 pandemic H1N1 virus—was transmissible between ferrets by respiratory droplet [21]. In contrast, Zhang et al. created many transmissible H5N1 hybrid viruses derived from a lethal H5N1 virus and a 2009 pandemic influenza A(H1N1)pdm09 virus among guinea pigs [22]. However, sustained human-to-human transmission of Asian-origin H5N1 virus has not yet been confirmed in the field.

3. Swine-origin 2009 H1N1 influenza viruses

An outbreak of influenza-like respiratory illness started in a Mexican town in mid-February 2009 [23]. By the end of April, WHO raised repeatedly the influenza alert level from phase 3 to phase 4, and finally, to phase 6 (the pandemic phase) due to the rapid spread of this virus around the world. Most of reported cases outside Mexico and the United States were induced by travelers from both countries. As of May 30, 2010, more than 214 countries across seven continents had reported laboratory confirmed cases of pandemic influenza H1N1 2009, including over 18,138 deaths (http://www.who.int/csr/don/2010_06_04/en/). These viruses had a mortality rate comparable to that of seasonal influenza virus. Currently, influenza A(H1N1)pdm09 continues to circulate as a seasonal virus (http://www.who.int/csr/disease/swineflu/notes/briefing_20100810/en/).

Complete genomic coding sequences indicated influenza A(H1N1)pdm09 viruses emerged from three origins by means of genetic reassortment (i.e., avian/human/swine "triple" reassortants) [24]. These viruses possess avian-origin PB2 and PA genes, a human-origin PB1 gene, and swine-origin HA (H1), NP, NS, NA (N1), and M genes. The human-origin PB1 gene and the avian-origin PB2 and PA genes have been circulating in pigs [7]. However, the factors that contribute to the genesis of influenza A(H1N1)pdm09 viruses remain to be determined.

4. Outbreak of avian-origin H7N9 viruses in China

On 31 March 2013, the national health and family planning commission of China (NHFPCC) first reported human infections caused by a novel avian-origin avian influenza A(H7N9) virus [25]. On April 5, 2013, the Ministry of Agriculture of China first isolated this virus in poultry at an agricultural wholesale market in Shanghai [26]. Since the initial report, three waves of this flu have coursed through the country. Currently, H7N9 viruses have become enzootic in China.

Epidemiological investigations of human cases by the avian influenza A(H7N9) virus showed that most of patients had a history of recent exposure to poultry or a visit to live poultry market, suggesting a close relationship between live poultry market and the spread of flu [27]. The source of avian influenza A(H7N9) virus was immediately traced to live poultry market. After

the implementation of compulsory shutdown measures in avian influenza A(H7N9) virus–positive live poultry markets, the government quickly controlled the spread of the virus. However, the elimination of avian influenza A(H7N9) virus is a huge and long-term challenge due to its avirulent nature in poultry.

Phylogeny analysis indicated that the novel avian influenza A (H7N9) virus resulted from the reassortment of recent avian H7N3 viruses and $H_{X(2\ or\ 11)}N9$ influenza viruses with avian influenza A (H9N2) viruses originated from two different groups [28]. As a result, these viruses possess an HA gene of duck-origin H7N3 virus, an NA gene of migratory bird-origin $H_{X(2\ or\ 11)}N9$ virus, and the remaining six viral genes (PB2, PB1, PA, NP, M, and NS) of H9N2 viruses. However, important questions are when, where, and how the avian influenza A (H7N9) viruses are established in poultry and transmitted to humans in China.

The novel avian influenza A(H7N9) virus has several remarkable features. First, this virus has resulted in lethal infections in human and mammalian species, but this virus was low pathogenic for poultry including chickens [29]. Second, this virus possessed a truncated NA protein with a deletion of five amino acids in the stalk, which was associated with virus virulence [30, 31]. Third, some H7N9 human isolates have a naturally occurring Q226L substitution in the receptor-binding site HA, a pivotal site for the switch of binding receptor [30, 31]. Fourth, PB2 proteins from some human H7N9 isolates have mutations Lys at positions 627 and Asn at 701 [30, 31], whereas the PB2 proteins from bird H7N9 isolates retain Glu at position 627 and Asp at 701. They are important for viral replication at the upper airway of mammalian hosts [32, 33]. These findings suggested that the mutation is positively selected upon replication in the human host.

5. Role of HA in cross-species transmission

HA has a major role in the interspecies transmission of influenza A viruses. The HA of human influenza virus (e.g. influenza A(H1N1)pdm09) preferentially recognizes sialic acid linked to galactose by 6-linkages (Siaa2,6Gal) (human-type receptor), whereas the HA of avian-origin influenza A virus preferentially binds to sialic acid linked to galactose by 2,3-linkages (Siaa2,3Gal) (avian-type receptor). The receptor-binding properties of HA are determined by the amino acid residues in the receptor-binding pocket, which creates microenvironment responsible for viral binding to receptor of host cells. Viruses with leucine (Leu) at position 226 and serine (Ser) at position 228 preferentially bind to human-type receptors, whereas those with glutamine (Gln) and glycine (Gly) at these positions bind to avian-type receptors [34, 35].

Glycan microarray analysis by using a solid-phase binding assay with receptor analogs, revealed that highly pathogenic avian-origin H5N1 virus, in general, specifically binds the avian receptor [34]. When two amino acid substitutions (Q226L and G228S) were introduced in the receptor-binding site (RBS) of H5 HA, highly pathogenic avian-origin H5N1 virus acquired an increased ability to bind to human-type receptors [34], which was due to a cis/trans conformational change in the glycosidic linkage [36]. Several reports have showed that a change of receptor specificity from avian-type receptor to human-type receptor is important for conferring airborne transmission on avian-origin H5N1 virus among mammals [20, 37].

Influenza A(H1N1)pdm09 virus naturally binds the human receptor [38]. This virus is transmissible virus among humans. In the direct glycan receptor-binding assay, the HA of influenza A(H1N1)pdm09 virus exhibited a dose-dependent binding to human-type receptors. Zhang et al. revealed that glutamine at position 226 of HA is a key factor on the transmissibility of influenza A(H1N1)pdm09 virus between mammals [39].

Receptor-binding properties of avian influenza A(H7N9) viruses are diverse. Some H7N9 viruses preferentially bind the avian receptor analog, whereas some have dual receptor-binding property [29, 40-42]. Avian influenza A(H7N9) virus can retain dual receptor properties, although a naturally occurring Q226L of the HA, a pivotal amino acid that mediates receptor-binding specificity was mutated [41], suggesting other factors of avian influenza A(H7N9) virus are probably involved in the receptor-binding specificity. Two substitutions (Q226L and G228S) in the host receptor-binding protein HA contributed to a switch in receptor specificity of an avian H7N9 isolate from dual receptor property to human receptor-binding property (unpublished data). H7N9 influenza virus that binds both avian and human receptor analogs can be transmissible by respiratory droplet among ferrets [29, 40]. However, the transmissibility of this virus is limited and unsustained [4, 40].

6. Role of PB2 in viral transmissibility

The amino acid at position 627 of the PB2 protein is important for airborne transmission of influenza A viruses among mammals. Influenza A viruses with lysine (Lys) at residue 627 of PB2 protein, but not those possessing glutamic acid (Glu) at this position, increased virus replication in mammalian cells at relatively low temperatures [33]. Lys at residue 627 of PB2 is one of five substitutions that proved to be sufficient to confer transmissibility on H5N1 virus [37]. The amino acid at residue 627 of PB2 could increase polymerase activity [37]. In fact, lysine to glutamic acid substitution at position 627 (E627K) of the PB2 could reduce the transmissibility of human influenza viruses [43].

The substitution Glu to Lys in the PB2 proteins at position 627 was positively selected upon replication in human host. In general, the PB2 proteins from human-origin isolates have Lys at position 627, whereas those of viruses isolated from birds retain Glu at this position. The strong selection has been reported previously for influenza A(H7N7), A(H5N1), and novel A(H7N9) infections. For example, the PB2 proteins from most of human H7N9 isolates at positions 627 are Lys. In contrast, the PB2 proteins from H7N9 viruses isolated from birds retain Glu [44].

7. Other proteins in viral transmissibility

The viral ribonucleoprotein (RNP) complex—consisting of the polymerase proteins PB2, PB1, PA, and NP—is one of the most important mechanisms for replication, pathogenticity, and transmissibility of influenza A virus [45]. The levels of RNP activity at 33° and 37°C have an

important correlation with virus replication in the upper and lower respiratory tracts of mammalians [32, 33]. Previous studies indicated that high replication efficiency of influenza A virus in the upper respiratory of mammalian host tract contributed to viral transmission between mammalians [32, 33].

Avian-origin H5N1 virus could acquire an essential substitution (His to Tyr) at position 99 of PB1 after serial passage, which was associated with airborne transmission among ferrets [37]. The substitution in PB1 contributed to polymerase transcription and virus replication [20, 37]. Zhang et al. suggested that PA protein and NS protein played a critical role in the airborne transmission between guinea pigs [46]. Therefore, airborne transmission of avian influenza A(H5N1) virus remains a polygenic trait. Transmission of avian influenza A(H7N9) virus also is a polygenic trait [29].

8. Prevention and control

For the treatment of influenza A virus infections, two classes of antiviral drugs—M2 ion channel inhibitors and neuraminidase inhibitors—are at present approved for the control of viral infection. However, the emergence of drug-resistant strains is a challenge. Amantadine and rimantadine are licensed for use against influenza A viruses. However, many strains from multiple subtypes of influenza A virus are now resistant to two available antiviral drugs [7]. Based on a serine to asparagine substitution at position 31 (S31N) of the M2 protein, novel H7N9 viruses are predicted to be resistant to adamantane. Therefore, this drug is not recommended for use [25].

Two neuraminidase inhibitors, Tamiflu (oseltamivir phosphate) and Relenza (zanamivir), are influenza antiviral drugs used by health authorities against recently circulating influenza A viruses, including H7N9 influenza viruses. However, NA inhibitors-resistant influenza viruses are arising in clinical settings. Recently, the rate of oseltamivir-resistant H1N1 viruses, including some H5N1 viruses, is increasing around the world [47-49]. Equally alarming, oseltamivir-resistant H7N9 virus has been reported in a clinical specimen collected two days after commencement of oseltamivir therapy [30]. The H7N9 isolate encoding the NA R292K substitution is highly resistant to oseltamivir and peramivir and partially resistant to zanamivir [50].

In addition to antiviral drugs, inactivated and live attenuated vaccines are available. Traditional influenza vaccines made to protect against seasonal influenza viruses (H1N1 and H3N2 subtypes) are available. In general, vaccines need to be revised and/or developed due to significant antigenic diversity of influenza A viruses. For example, a comparison of novel avian influenza A(H7N9) virus with Eurasian or North American lineages of H7 subtype viruses revealed antigenic differences (http://www.who.int/influenza/vaccines/virus/201302_h5h7h9_vaccinevirusupdate.pdf), suggesting new vaccines need to be developed on the basis of the HA and NA genes of H7N9 candidate vaccine stains. Of note, several new strategies are now in various stages of development. In one example, a "universal" vaccine focuses on regions of viral proteins that are highly conserved across virus subtypes, and

provides broad protection against existing and newly emergent unmatched strains [51]. Antihuman C5a antibody treatment markedly reduced acute lung injury (ALI) and systemic inflammation induced by H7N9 virus infection [52].

9. The future

Currently, new strains of influenza A virus are arising quickly and frequently around the world due to continued evolution. In the past two years alone, at least five new subtypes of influenza A virus: A(H7N9), A(H10N8), A(H5N8), A(H6N1), and A(H5N6), have been reported in China. Little knowledge is known about key factors that determine reassortment, virulence, and transmissibility of influenza virus, although much has been learned [1]. Wild birds have served as an ecologic factor favoring emergence and maintenance of avian influenza viruses in natural ecosystems. Therefore, careful influenza surveillance, especially at the wild bird–human interface, remains essential for pandemic preparedness. From a scientific perspective, the opportunity to watch virus evolution in real time may help bridge this gap of knowledge.

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