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Review

The novel avian influenza A (H7N9) virus: origin, evolution, biological features and pandemic potential

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ABSTRACT

In the spring of 2013, a novel reassortant avian H7N9 influenza virus containing 6 internal genes from avian influenza H9N2 virus was first identified in the Yangtze Delta region of China. This virus has not been detected in humans or animals prior to that. Thus its emergence led to global public health concerns. In contrast to other previous human H7 infections that mainly manifested as mild illness or conjunctivitis, most H7N9 patients were critically ill and presented severe pneumonia and acute respiratory distress syndrome. Although the first epidemic wave declined quickly after the implementation of active control measurements including the closure of live poultry markets, the second epidemic wave, which began in October 2013 caused almost 300 human infections by the end of April 2014, compared with the first wave's total of 136. With the rapidly increasing human cases, and the expansion of affected regions, more efforts should be made to prepare for the potential pandemic.

KEYWORDS: influenza A (H7N9) avian viruses, infection source, pathogenicity, transmissibility

INTRODUCTION

Wild aquatic birds are the natural reservoir of influenza A viruses. All the 16 HA and 9 NA subtypes of influenza A viruses have been identified in wild birds, while subtypes H17-18 and N10-11 were found in bats [1-5]. Only H1, H2, H3 and N1, N2 subtypes have been stably introduced into the human population, which cause the seasonal influenza in humans every year. Since most of the human influenza pandemics were caused as a result of genetic reassortment events with the circulating human influenza virus acquiring one or more gene segments of avian origin, any human infection with avian influenza viruses raised global public concerns.

Sporadic human infections with avian influenza viruses including H5N1, H7N2, H7N3, H9N2, and H10N7, have been recorded [6-10]. In 2013, three novel avian influenza viruses were isolated from humans one after another: H7N9, H6N1 and H10N8 [11]. Among them, the avian influenza A (H7N9) viruses draw the global attention for both the human infection with high mortality and the special biological features such as “dual-receptor” binding profile. Although the first epidemic wave declined quickly after the implementation of active control measurements including the closure of live poultry markets in summer 2013, the second epidemic wave has caused almost 300 human infections so far. Meanwhile, during September to December, 2013, virological and serological surveillance in the animals around the country indicated that the novel H7N9 viruses may have already spread to Northern and Southern regions of China, including those where

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no human infection has been reported [12]. This intense situation raised many questions and global concerns, such as the virus origin and evolution, the human infection source, transmissibility and pathogenicity and so on. In this perspective, we review the current researches exploring the risk assessment of the novel avian H7N9 influenza viruses and highlight the remaining issues which urgently need to be addressed.

Etiology discovery

On March 25th, 2013, Chinese National Influenza Center (CNIC) received clinical throat swab samples from Shanghai Public Health Clinical Center, Shanghai Center for Disease Control and Prevention (CDC) and Anhui CDC. The throat swabs were collected from two patients from Shanghai city and one from Anhui Province. All the three patients were hospitalized with severe pneumonia. After excluding SARS-CoV, MERS, seasonal influenza viruses (H1, H3 or B), and H5N1 viruses, all the three patients were finally confirmed to be infected with a novel avian origin influenza A (H7N9) virus using real-time RT-PCR, viral isolation, and full genome sequencing. Three strains, named A/Shanghai/1/2013 (H7N9), A/Shanghai/2/2013 (H7N9) and A/Anhui/1/2013 (H7N9), were isolated [11].

Virus origin and evolution

Based on the full genome sequences of the first 3 virus isolates, and comparison with the available sequence data from GenBank, all eight genes of the virus isolates were determined to be of avian origin with six internal genes from avian influenza A (H9N2) virus. The HA gene might have originated from H7N3 virus in ducks and the NA gene likely derived from H7N9 virus isolated from wild bird. Moreover, the introduction of the foreign NA segment into the novel H7N9 virus could be attributed to the seasonal migration of wild birds along the East Asian flyway [13]. Further analysis demonstrated that the six internal genes of the H7N9 virus probably originated from two different groups of H9N2 avian influenza viruses, which were all isolated from chickens [13]. Hence we proposed that the novel H7N9 virus could have originated from three different subtypes including H7N3, H7N9, and H9N2 avian influenza viruses.

With more sequence data of H7 and H9 viruses being further available, Lam *et al.* further proposed that HA gene of the novel H7N9 viruses may have derived from the H7 viruses which was probably transferred from domestic duck to chicken population in China, while NA gene was more likely related to H11N9 and H2N9 viruses which had been found in migratory birds in Hong Kong in 2010-2011 [14]. Although all internal genes of these human H7N9 viruses originated from H9N2 viruses, they clustered into different groups, generating distinct genotypes [15-17]. The data indicated that current H7N9 viruses might have originated through multiple reassortments with different lineages of H9N2 viruses. One of the viruses, A/Anhui/1/2013, represented the dominantly circulated H7N9 genotype in both humans and avians. Another isolate, A/Shanghai/5/2013, was recognized as the closest ancestor H7N9 virus [16]. Further studies showed that the internal genes of the novel H7N9 viruses were generated by at least two-step sequential reassortments involving distinct H9N2 donor viruses in different hosts. The first reassortant event likely occurred in wild birds, and the second in domestic birds in east China in early 2012 [18]. An unrecognized H7N7 lineage virus has also been detected in Southern China recently. This lineage virus was similar to the novel H7N9 viruses except for the NA gene, and can also infect ferret efficiently [14]. These findings indicated that reassortant events among different influenza virus subtypes and different hosts are far more complicated than thought before.

Human infection sources

Once the human infections with novel avian H7N9 influenza virus were confirmed, the first question that arose was how they got infected. After testing approximately ten thousand avian and environment samples collected from Middle and Eastern China, 52 H7N9 viruses were isolated and they shared high homology with H7N9 human isolates [19, 20]. Of these, 50 isolates were from samples collected from live poultry market, indicating that live poultry market might be the main source of the novel avian H7N9 influenza viruses. Additional studies showed that human infection with the novel H7N9 virus had an epidemiologic association with exposure to chickens

from live-animal markets [21-23], which echoed well with previous epidemiological investigations, which showed that 77% of the patients with H7N9 infection had a history of direct exposure to live animals, including chickens [24]. A serological study also shows that incidence of sero-positivity for antibodies against novel H7N9 virus is significantly higher in poultry workers than in general population in Zhejiang province, which had the highest number of H7N9 virus infection cases in China [25]. Efficient replication and transmission of H7N9 virus in chickens also indicated the birds as the spreaders of H7N9 viruses in live poultry markets [19]. Complete suspension of the live poultry markets may have contributed to the reducing number of human cases in these regions [26, 27].

Epidemiological characteristics and transmissibility of H7N9

Based on several epidemiological reports [24, 28], males are the dominant patients in all the human H7N9 virus infection cases, with the majority of them being urban residents. Unlike most H5N1 virus infections detected in younger people, most of the patients with H7N9 virus infection were older adults [29-31]. By monitoring close contacts of 82 confirmed H7N9 patients, real-time PCR tests showed that no samples were positive for the novel H7N9 viruses, supporting its non-sustainable transmissibility as well [24]. However, at least five family-clustered cases have been identified in Shanghai municipality, Jiangsu, Shandong and Zhejiang Provinces [32, 33]. Genome sequence and phylogenetic analyses of the viruses isolated from the Jiangsu index patient and his daughter showed that both viruses were almost genetically identical, indicating the limited human-to-human transmission of H7N9 viruses [33].

The *ex vivo* infection using trachea and lung explants demonstrated that more efficiently replication is observed in lung than that in trachea tissues, which might be one of the limitations for the efficient transmissibility of the virus [34]. By using the ferret model, the H7N9 viruses were efficiently transmitted via direct contact, but less efficiently by droplets [35-38]. Furthermore, pigs could be productively infected by A/Shanghai/2/2013 (H7N9) but were unable to transmit the virus to ferrets or other uninfected pigs [35].

Molecular mechanism of human infection

Avian influenza viruses such as H5N1 bind to avian-type (α2, 3-linked sialic acid) receptors while seasonal influenza virus exhibited high affinity to human-type (α2, 6-linked sialic acid) receptors. Human upper respiratory tissues and trachea contain mainly α2, 6 receptors, whereas lung tissues contain a mixture of α2,3- and α2,6-linked sialic acid. Previous studies have shown that the presence of Q226L mutation in the HA protein has been associated with reduced binding to avian-like receptors [39], and potentially an increasing ability to bind to mammalian-like receptors located in the human upper airway. HA Q226L has also been shown to be associated with increased transmissibility of HPAI H5N1 viruses in ferrets. The HA Q226L mutation was detected in most of the novel H7N9 viruses indicating that the H7N9 viruses could bind to both avian-type and human-type receptors [34, 38, 40], and enable the novel H7N9 virus to infect both human upper and lower airway respiratory tracts. Further studies also demonstrated that the HA G186V mutation also helped the H7N9 virus to increase the human-type receptor binding affinity [40-41]. This special feature was confirmed by biological receptor binding characterization and crystal structure analysis [40-42]. These findings indicated that H7N9 would infect humans easier than H5N1 viruses. Besides, the H7N9 viruses isolated from human cases also have E627K substitution in PB2 protein, which has also been associated with mammalian adaptation and respiratory-droplet transmission of HPAI H5N1 virus in ferrets [43]. However, most of the H7N9 viruses isolated from birds or the environmental samples collected from poultry markets contained 627E in PB2, which indicated the E627K mutation occurred during human infection [19].

Clinical features and pathogenicity mechanism

Sporadic human infection with H7 subtype of influenza A virus has been reported and it usually develops into mild illness except for one fatal case reported before [7]. However, most human infections with the novel H7N9 virus developed into severe illness, including pneumonia and acute respiratory distress syndrome (ARDS) [11, 21, 28, 44, 45]. Fever and cough were the most common symptoms. Diarrhea or vomiting was observed in

a small number of patients. The white-cell count was normal or slightly decreased in most patients. Elevated levels of aspartate aminotransferase, creatine kinase, and lactate dehydrogenase were observed in nearly all the patients [46]. Based on a clinical report including 111 cases of H7N9 virus infection, a majority of patients (98/111) had lymphocytopenia, and 44 had thrombocytopenia. Chest radiography showed that pneumonia was developed in most patients [46]. Unlike seasonal influenza virus and previously reported H7 influenza virus infection in humans, 76.6% of the patients infected with H7N9 virus were admitted to the ICU [28]. Moderate-to-severe ARDS was the most common complication, followed by shock, acute kidney injury, and rhabdomyolysis. Children infected with the novel H7N9 virus developed milder diseases [47, 48].

As seen with the H5N1 viruses [21, 49], efficient replication of the H7N9 virus in type II pneumocytes may cause lung function deterioration, which might contribute to the severe disease in H7N9 human infections. Similar to HPAI H5N1 infection, cytokine storm was also observed in severe H7N9 human cases [21, 34], which may be one of the important contributors to clinical severity. Similarly, the ability of A/Shanghai/2/2013 to elicit proinflammatory cytokines in the lung and serum of mice was observed to be similar to HPAI H5N1 [50]. Age might contribute to higher risk of death in patients who were 65 years of age or older, because the enhanced expression of chemokine receptor was related to aging or chronic diseases [51-53]. Furthermore, host factors cannot be exclusive in the pathogenicity of the H7N9 infections. Weak immunity is known as a risk factor for severe seasonal/pandemic influenza virus infection, and may also contribute to the preference of H7N9 virus to the elders [54]. Moreover, host factors such as IFITM3 and CTL immunity have been reported to be associated with the H7N9 infection sensitivity and severe symptoms [55, 56]. Genetic polymorphisms such as CD55and SFTPB might also contribute to the severe infections [57, 58]. The high levels of angiotensin II in plasma are strongly correlated with mortality among H7N9 patients, making it a biomarker for lethality in H7N9 human infections [59].

Vaccine

H7N9 infections are associated with severe illness in humans. Because of its sudden emergence in humans, no specific vaccine is available. The serological surveys in general population and seasonal influenza vaccination groups showed that there was no pre-existing immunity to the novel H7N9 viruses existing in general population, and the current seasonal influenza vaccine could not provide cross protection to H7N9 virus [25, 34, 60, 61]. Due to the pandemic potential of H7N9 virus, specific vaccine is urgently needed. Based on both genetic and antigenic analysis, WHO has recommended A/Anhui/1/2013 (H7N9) as the candidate vaccine strain [62]. However, the poor immunogenicity of H7N9 in preliminary clinical trials raised the challenge for successful H7N9 vaccine development [63].

Antiviral drugs treatment and susceptibility

WHO recommended early antiviral therapy for patients suspected or confirmed with novel H7N9 virus infection. A reduction of viral load following antiviral treatment was found to correlate with improved outcome [64]. All of the H7N9 viruses contained S31N mutation in M2 protein, which proposed the amantadine drug resistance. R292K substitution in neuraminidase was also observed in H7N9 virus infected patients with corticosteroid treatment and correlated with adverse clinical prognosis, raising the importance of ongoing surveillance of antiviral resistance in A/H7N9 viruses [64].

CONCLUSION

Though sustained human-to-human transmission is not observed so far, the special “dual-receptor” binding feature, as well as its efficient replication and transmission in ferrets, raised global concern on the pandemic potential of novel H7N9 virus. The fact that the H7N9 viruses could not cause any clinical symptoms in poultry makes it very difficult to monitor the virus circulation in poultry [19]. Frequent contacts between humans and poultry in the live poultry markets would increase the chance of interspecies transmission of avian influenza A (H7N9) viruses to humans or other animals. Hence poultry breeding and consumption mode in China should be altered. Also the closure

of live poultry market and centralized slaughter of poultry is of absolute importance in the control of H7N9 virus spreading, and even to avoid pandemic occurrence.

As there is no pre-existing immunity in human population and seasonal influenza vaccines confer no cross protection, it is important to develop H7N9 vaccines. The antiviral therapy is of vital importance and should be implemented as soon as possible for patients suspected or confirmed with H7N9 virus infection. High alert to the emergence of antiviral resistant strains should also be kept.

Finally, it is essential to closely monitor the viruses in both humans and other animals, including wild birds. Meanwhile more detailed and systematic data should be analyzed in order to reveal the accurate picture of virus genetic diversity and evolution, which are critical for pandemic preparedness and response.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

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