VIRUS EVOLUTION

New hemagglutinin dual-receptor-binding pattern of a human-infecting influenza A (H7N9) virus isolated after fifth epidemic wave

Lei Guo,[1,*] Nan Li,[1,*] Wenlong Li,[2] Jienan Zhou,[3] Ruotong Ning,[1] Min Hou,[2,†,‡] and Longding Liu[1,†,‡]

[1]Institute of Medical Biology, Chinese Academy of Medical Science, 935# Jiaoling Road, Kunming, Yunnan 650118, China, [2]Kunming City Center for Disease Control and Prevention, 4# Ziyun Road, Kunming, Yunnan 650228, China and [3]Yunan Center for Disease Control and Prevention, 158# Dongsi Street, Kunming, Yunnan 650022, China

[‡]Corresponding authors: E-mail: longdingl@gmail.com; houmin7250@163.com
[*]These authors contributed equally to this work.
[†]These authors were co-principal investigators.

Abstract

Since 2013, influenza H7N9 virus has caused five epidemic waves of human infection. The virus evolved from low pathogenic to highly pathogenic in wave 5, 2017, while the prevalence of host receptor-binding tropism in human-infecting viruses maintained dual-receptor-binding property with preference for avian receptor. A human-infecting H7N9 virus was isolated after the fifth epidemic wave and possessed an avian and human dual-receptor specificity, with a moderately higher affinity for human receptor binding. A V186I (H3 numbering) substitution in the receptor-binding site of the hemagglutinin (HA) molecule is responsible for the alteration of the dual-receptor-binding tropism. Viral strains which contain I186 amino acid of avian- and human-infecting H7N9 viruses were all isolated during or after wave 5, and their HA genes clustered in a same phylogenetic clade together with 2018–9 H7N9 isolates, highlights a new evolutionary path for human adaption of natural H7N9 viruses.

Key words: influenza; H7N9; hemagglutinin; dual-receptor binding; human infection..

1. Introduction

Since the human infection outbreak caused by influenza A virus (IAV) H7N9 in China in 2013, the virus has been constantly infecting humans in five epidemic waves and caused 1,567 human infections, including 615 deaths (WHO.int. 2018). The receptor-binding specificity of the IAV hemagglutinin (HA) protein in the viral envelope is a key factor that determines the host tropism from avians to humans. Generally, human IAV strains preferentially bind to the α2,6-linked sialic acid receptor, while avian IAV strains favor the α2,3-linked sialic acid receptor

(Shi et al. 2014). In the 2013 H7N9 infection outbreak, the prevalent viral strains isolated from humans emerged dual-receptor-binding capacity, with a lower affinity for human receptor (Shi et al. 2013; Zhou et al. 2013). Since then, H7N9 viruses with this dual-receptor-binding property have been dominantly circulating in human infection cases and some of the avian isolates. No pandemic H7N9 strain, which would preferentially recognize the human α2,6-linked sialic acid receptor, has been detected until now. However, H7N9 IAVs are still continuously evolving, especially in wave 5 (winter 2016 to spring 2017) while highly pathogenic (HP) H7N9 viruses emerged (Su et al. 2017;

Quan et al. 2018). Here, we characterized a human-infecting HP H7N9 IAV which isolated from post-wave 5, and focused on the receptor-binding property which is distinguished from current prevalent human-infecting H7N9 strains.

2. Materials and methods

2.1 Epidemiological investigation

Epidemiological investigation was carried out by staff from the Kunming City Center for Disease Control and Prevention. Information about epidemiological history, symptoms, medical records, and close contacts were obtained by inquiring the patient and collecting records from the local hospital.

2.2 Viral isolation, identification, and phylogenetic analysis

A nasopharyngeal swab specimen was collected from a 64-year-old chicken farmer in Xundian district of Kunming, Yunnan, China, December 2017, by the Kunming City Center for Disease Control and Prevention. The H7N9 virus from the sample was isolated via inoculation in Madin–Darby canine kidney (MDCK) cells cultured in a reduced serum culture medium (Opti-MEM, Thermo Fisher, USA) supplemented with TPCK-trypsin (Worthington Biochemical Corporation, USA) in a biosafety level 3 laboratory of Centers for Disease Control and Prevention, Kunming, Yunnan, China. The genomic sequences of 8 full-length segments of the virus were amplified by a two steps reverse transcription PCR with universal primers (Hoffmann et al. 2001) and obtained by Sanger sequencing, and designated as A/China/LN/2017(H7N9) (GenBank accession nos MN170546–MN170553). Phylogenetic trees of 2013–9 H7N9 viral genes in mainland China obtained from the GenBank and Global Initiative on Sharing Avian Influenza Data (GISAID) databases were built using neighbor-joining analysis with 1,000 bootstrap replicates (MEGA, version 7.0).

2.3 Generation of recombinant viruses by reverse genetics

Recombinant viruses were generated by reverse genetics as described previously (DE Wit et al. 2004). Briefly, HA and NA segments were from the A/China/LN/2017(H7N9) virus, while the other six internal gene segments were from H1N1 PR8 strain as the backbone. HA gene was either substituted with A138/I186/P221/Q226, A138/G186/P221/Q226, A138/V186/P221/Q226, or A138/I186/P221/L226 amino acids. Mutated HA genes were verified by sequencing. The eight viral genes were cloned into a dual-promoter plasmid, pHW2000. MDCK and 293 T cells were co-cultured and transfected with the eight plasmids using TransIT-LT1 reagent (Mirus, USA) in Opti-MEM medium. The transfected cells were cultured in Opti-MEM medium containing 1 μg/ml TPCK-trypsin. The supernatant from the transfected cells was inoculated in MDCK cells to rescue the recombinant virus. The genomes of the rescued viruses were verified by Sanger sequencing. The viral stock was propagated in MDCK cells with Opti-MEM supplemented with TPCK-trypsin. Viral concentrations were determined by using a HA titer assay with 1 per cent (vol/vol) chicken red blood cells. All experiments with live viruses were performed in a biosafety level 3 containment laboratory as aforementioned.

2.4 Receptor-binding specificity by a solid-phase-binding assay

Two biotinylated glycans were used in this assay, Neu5Acα2-3Galβ1-4GlcNAcβ-PAA-biotin (3′ SLN) and Neu5Acα2-6Galβ1-4GlcNAcβ-PAA-biotin (6′ SLN) (GlycoTech Corporation, USA). Receptor-binding specificity was analyzed by a solid-phase direct-binding assay as described previously (Shi et al. 2013; Guo et al. 2018), with some modifications. Briefly, a 96-Well High-Binding Flat-Bottom Microplate (Corning, USA) was incubated with different concentrations (2-fold serial dilutions starting from the 256 HA titer) of viruses at 4 °C overnight; PBS was used as a negative control. After removal of the virus supernatant, the plates were washed four times with ice-cold PBS containing 0.1 per cent Tween 20 (PBST) and blocked with 0.2 ml of PBS containing 5 per cent bovine serum albumin (BSA) at room temperature for 2 h. After washing with ice-cold PBST, the plates were incubated with PBS containing either the 3′ SLN or 6′ SLN glycan (100 μl of 0.24 μM) at 4 °C overnight. After washing with PBST, the plate was incubated at room temperature for 1 h with horse-radish peroxidase-conjugated streptavidin (ab7403, Abcam, UK). The plate was then incubated with 200 μl of a tetramethylbenzidine (TMB; Sigma) solution for 15 min at room temperature. Finally, the reaction was stopped with 100 μl of 0.5 M H_2SO_4. Absorbance was determined at 450 nm using a plate reader.

3. Results and discussion

3.1 Case description and virus characterization

In 12 December 2017, after the fifth epidemic wave, we collected a nasopharyngeal swab sample from a 64-year-old chicken farmer in Xundian district of Kunming, Yunnan, China. The farmer developed flu-like symptoms and became severely ill before going to a local hospital. Later, he recovered after 2 weeks of treatment in the hospital. No close contacts of the farmer were found to be IAV positive. The virus isolated from the sample was designated as A/China/LN/2017(H7N9), and had a nucleotide identity of 96.56–99.87 per cent with the A/chicken/Yunnan/SD210/2017 virus via online blast analysis (Table 1). Phylogenetic analysis of 2013–9 H7N9 viruses isolated in mainland China obtained from the GenBank and GISAID databases was performed. The results showed that nine viral genes (except the polymerase, PA) of the two viruses were highly homologous and in the same clade (Figs 1 and 2 and Table 1). Considering that the two viruses were isolated from the same district of Kunming, Yunnan, in the same post-wave 5 period (November to December 2017), and the chicken farmer did not

Table 1. Sequence comparisons between the A/China/LN/2017 (H7N9) and A/chicken/Yunnan/SD210/2017(H7N9) viruses.

Protein	Amino acid sequence identities (% discrepancy/total)
Hemagglutinin (HA)	99.46 (3/563)
Neuraminidase (NA)	96.56 (16/465)
MatrixM (M1 and M2)	99.71 (1/349, in M1)
Nonstructural protein NS (NS1 and NEP)	99.41 (2/338, in NS1)
Nucleoprotein (NP)	99.80 (1/498)
Polymerase (PA)	98.04 (14/716)
Polymerase basic 1 (PB1)	99.87 (1/757)
Polymerase basic 2 (PB2)	99.34% (0/759)

HA

NA

Figure 1. Phylogenetic analysis of the hemagglutinin (HA) and neuraminidase (NA) gene sequences of influenza virus A/China/LN/2017(H7N9) virus from Kunming, China and reference H7N9 viruses from 2013 to 2019 in mainland China. Phylogenetic trees were performed using neighbor-joining method with 1,000 bootstrap replicates (MEGA, version 7.0). The A/China/LN/2017(H7N9) virus was labeled with red circle. Viruses which harbor the I amino acid at position 186 (H3 numbering) were labeled with black triangle.
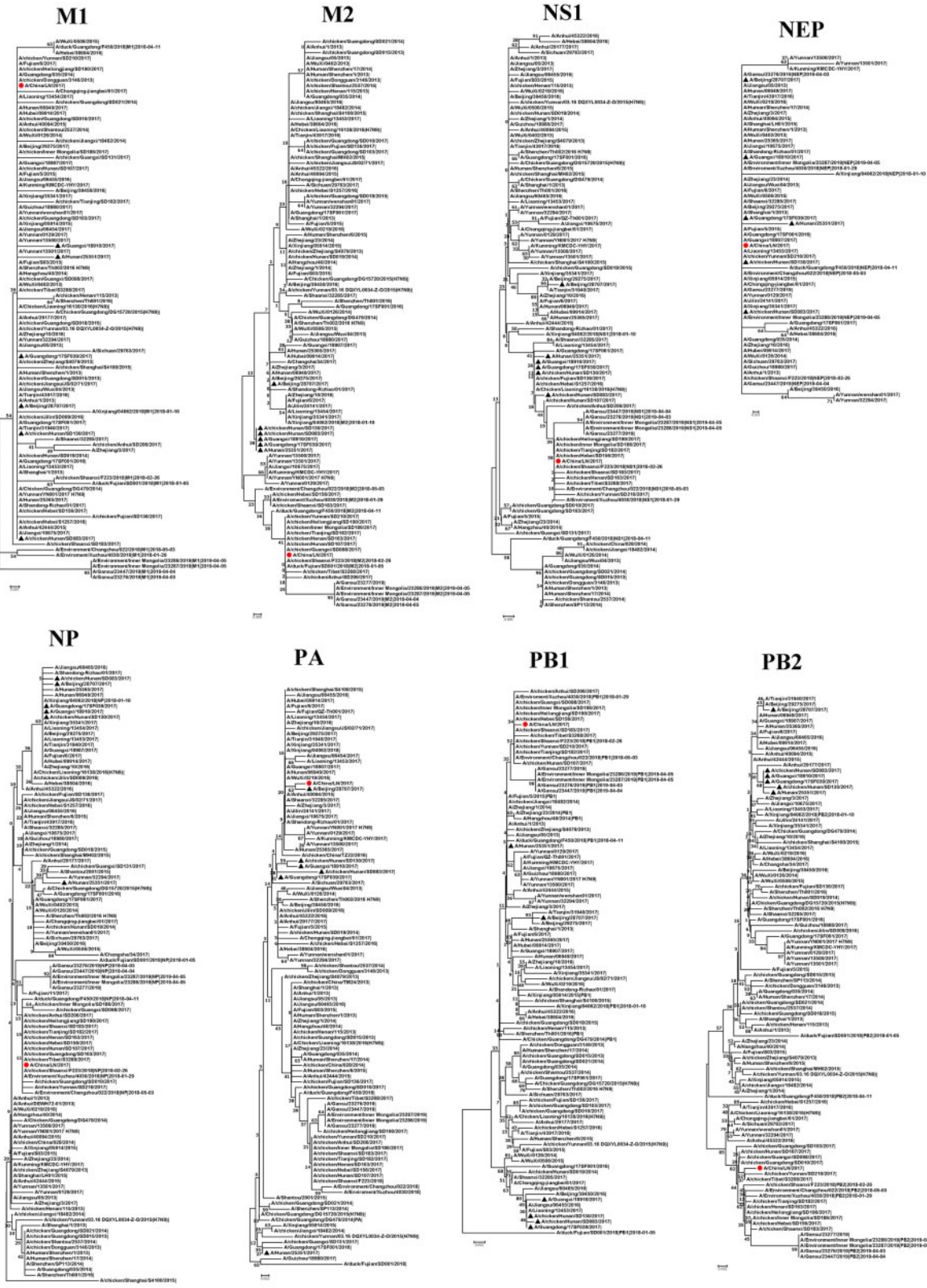
Figure 2. Phylogenetic analysis of the polymerase (PA, PB1, and PB2), nucleoprotein (NP), matrix protein (M1 and M2), and nonstructural protein (NS1 and NEP) gene sequences of influenza virus A/China/LN/2017(H7N9) virus from Kunming, China and reference H7N9 viruses from 2013 to 2019 in mainland China. Phylogenetic trees were performed using neighbor-joining method with 1,000 bootstrap replicates (MEGA, version 7.0). The A/China/LN/2017(H7N9) virus was labeled with red circle. Viruses which harbor the I amino acid at position 186 (H3 numbering) were labeled with black triangle.

leave town during this period, it is possible that the farmer acquired H7N9 virus infection from local poultry.

3.2 Genomic comparative analysis

Comparative genetic analysis of the HA molecule of A/China/LN/2017 and A/chicken/Yunnan/SD210/2017 viruses revealed that the A/China/LN/2017 strain carried the same HP avian IAV cleavage motif (PEVPKRKRTAR/GL) in the HA cleavage site as did the A/chicken/Yunnan/SD210/2017 strain, which belongs to the G2 genotype (Shi et al. 2018) (Table 2). Residue mutations in the receptor-binding site (RBS) of HA molecules play vital roles in the change of receptor-binding preference of IAV (De Graaf and Fouchier 2014). Four amino acid substitutions within RBS of HA, S138A/G186V/T221P/Q226L (H3 numbering), have been demonstrated to be responsible for the human receptor-binding capacity by H7N9 virus. Mutations in these four amino acids conferred a human receptor-binding capacity to the A/Anhui/1/2013 strain from the wave 1 outbreak, which developed an avian/human dual-receptor-binding property, in contrast to the unmutated A/Shanghai/1/2013 strain with a typical avian receptor-binding specificity (Shi et al. 2013; Xiong et al. 2013; Xu et al. 2013) (Table 2). Sequence analysis revealed that the A/China/LN/2017 strain contained the A138/I186/P221/Q226 amino acids at the four positions in RBS (Table 2). Notably, an uncommon amino acid, isoleucine (Ile, I), occurred at the 186 position, instead of glycine (Gly, G), or valine (Val, V). Seven strains of H7N9 that harbor the I amino acid at position 186 (I186) were found in the GenBank and GISAID databases since 2013 (Table 2), five of which are human infection isolates, and the other two are chicken infection isolates. All of the seven viral strains were isolated in mainland China until 2017, which means that occurrence of the I186 substitution started at wave 5. Among them, A/chicken/Hunan/SD083/2017, A/chicken/Hunan/SD130/2017, and A/Hunan/25351/2017 were all isolated in Hunan province, central China, while the others were isolated from north China (Beijing) to south China (Guangdong, Guangxi, Yunnan). Phylogenetic analysis of the HA genes showed that the Hunan, Guangdong and Guangxi isolates grouped into a sub-clade, which together with A/China/LN/2017 strain belonged to a distinct cluster of H7N9 virus isolated during 2018–9 (Yu et al. 2019) (Fig. 1). Phylogenetic analysis of the other nine viral genes showed that the A/China/LN/2017 strain was divergent from the other I186 strains. In contrast, the A/China/LN/2017 strain still clustered together with the 2018–9 H7N9 isolates in the neuraminidase (NA), nucleoprotein (NP), polymerase (PB1 and PB2), matrix protein (M1 and M2), and non-structural protein (NS1 and NEP) genes (Figs. 1 and 2). These results indicate that HA genes of the I186 H7N9 strains are highly homologous and evolutionary close to the recent 2018–9 H7N9 isolates, and the A/China/LN/2017 strain shares a most close evolutionary pattern to the 2018–9 virus strains.

3.3 Dual-receptor-binding property of a/China/LN/2017 virus

To further determine the contribution of V186I substitution on HA receptor-binding property, a solid-phase-binding assay was conducted as described previously (Shi et al. 2013; Guo et al. 2018). The results showed that the virus possessed an avian and human dual-receptor specificity but had a higher affinity for human receptor binding. The usage of different detection anti-sera or antibodies against a virus in the binding assay can, to some extent, affect the binding specificity outcome, as we and others described in previous studies (Zhu et al. 2017; Guo et al. 2018). Thus, to compare the dual-receptor specificity of the A/China/LN/2017 virus to that of the prevalent dual-receptor-binding H7N9 virus in a more rigorous way, we modified the solid-phase-binding assay by detecting glycans as described in the methods. The results demonstrated that the A/China/LN/2017 virus could bind to both avian and human receptors, with a moderately higher affinity for the human receptor (Fig. 3A). Compared with the typical prevalent dual-receptor-binding H7N9 virus, A/Kunming/KMCDC-YHY/2017 (Guo et al. 2018), the receptor-binding capacity of the A/China/LN/2017 virus was significantly lower for 3′ SLN (avian receptor) (peak value: 0.5–1), while that 6′ SLN (human receptor) was slightly enhanced (peak value: 0.6–0.7) (Fig. 3A). The results indicated that the avian receptor-binding capacity was reduced while the human receptor-binding capacity of the A/China/LN/2017 virus was comparable to that of typical prevalent dual-receptor-binding H7N9 viruses. In terms of the four key amino acids in the HA RBS, the A/Kunming/KMCDC-YHY/2017 strain possessed A138/V186/P221/L226, while A/China/LN/2017 harbored A138/I186/P221/Q226 (Table 2). The substitutions included V186I and L226Q. A recent published work has revealed that the G186V substitution alone is sufficient for avian H7N9 viruses to acquire a human receptor-binding capacity, while the Q226L substitution only favors binding to both avian and human receptors when paired with A138/V186/P221 amino acids (Xu et al. 2019). It is likely that the V186I substitution contributed to the acquisition of the human receptor-binding capacity by the A/China/LN/2017

Table 2. Genetic comparison of HA molecules from different H7N9 isolates.

Viral strains	Collection date	Host	Cleavage peptide	Pathogenic	138aa	186aa	221aa	226aa[a]
A/Shanghai/1/2013	March, 2013	Human	PEIPKGR/GL	LP	S	G	T	Q
A/Anhui/1/2013	February, 2013	Human	PEIPKGR/GL	LP	A	V	P	L
A/Kunming/KMCDC-YHY/2017	March, 2017	Human	PEIPKGR/GL	LP	A	V	P	L
A/chicken/Yunnan/SD210/2017	November, 2017	Chicken	PEVPKRKRTAR/GL	HP	A	V	P	Q
A/China/LN/2017	December, 2017	Human	PEVPKRKRTAR/GL	HP	A	I	P	Q
A/chicken/Hunan/SD083/2017	February, 2017	Chicken	PEVPKRKRTAR/GL	HP	A	I	P	Q
A/chicken/Hunan/SD130/2017	March, 2017	Chicken	PEVPKRKRTAR/GL	HP	A	I	P	Q
A/Guangdong/17SF039/2017	February, 2017	Human	PEVPKRKRTAR/GL	HP	A	I	P	Q
A/Guangxi/18910/2017	March, 2017	Human	PEVPKRKRTAR/GL	HP	A	I	P	Q
A/Hunan/25351/2017	April, 2017	Human	PEVPKRKRTAR/GL	HP	A	I	P	H
A/Beijing/28707/2017	June, 2017	Human	PEIPKGR/GL	LP	A	I	P	L

[a]H3 numbering.
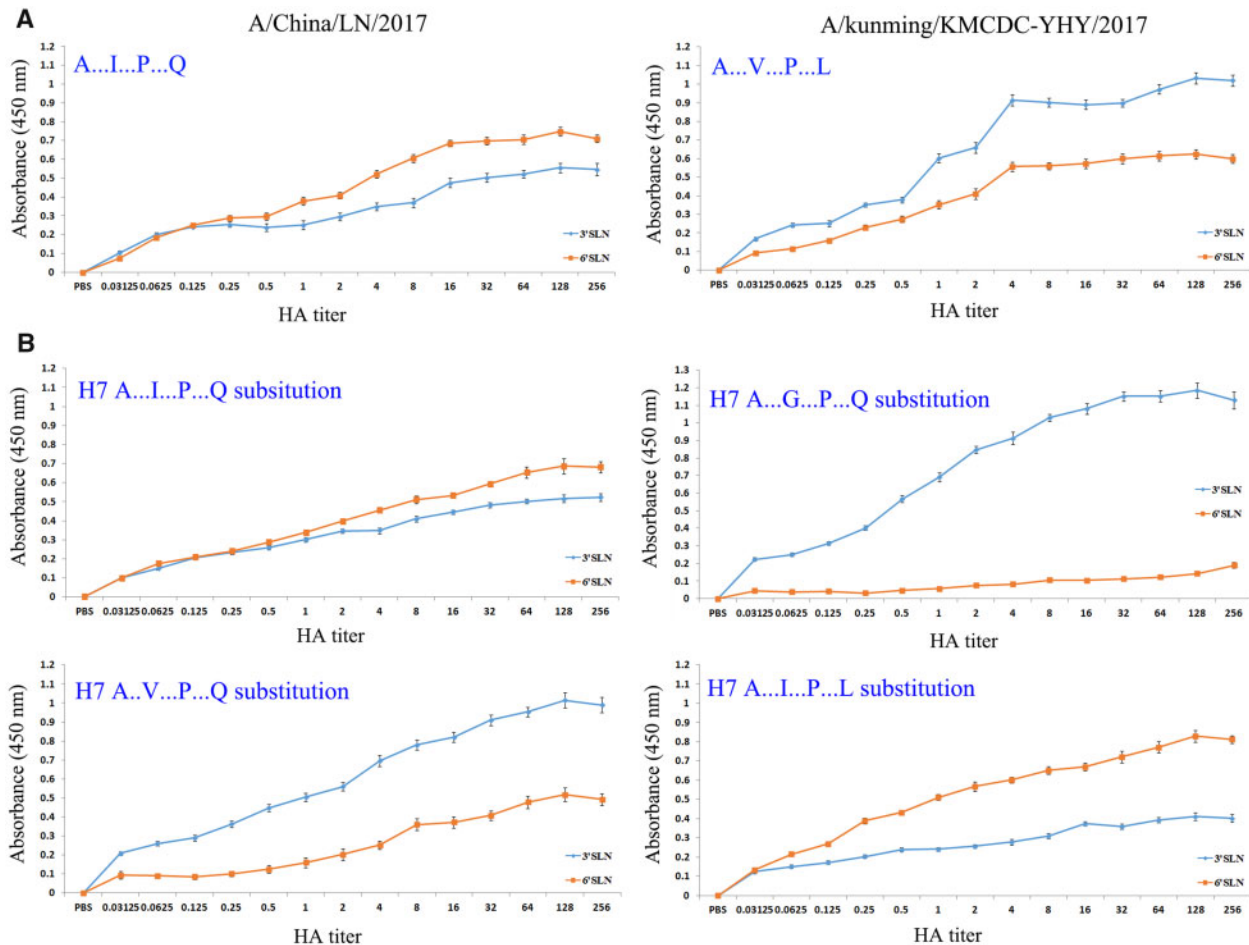HP, highly pathogenic; LP, low pathogenic.

Figure 3. Glycan receptor-binding specificity of A/Kunming/KMCDC-YHY/2017(H7N9) and A/China/LN/2017(H7N9) viruses and rescued H7N9 mutants. Binding of the viruses to glycans (3′ SLN, 6′ SLN) in a solid-phase-binding assay (see Section 2). (A) Binding of A/Kunming/KMCDC-YHY/2017(H7N9) and A/China/LN/2017(H7N9) viruses. (B) Binding of rescued H7N9 mutants harbored A138/I186/P221/Q226, A138/G186/P221/Q226, A138/V186/P221/Q226, or A138/I186/P221/L226 amino acids in the HA gene. HA and NA segments of the rescued viruses were based on the A/China/LN/2017(H7N9) virus. HA genes were substituted with the indicated amino acids at 138, 186, 221, 226 positions (H3 numbering). The error bars represent the standard deviation from three repeats.

virus and impaired its avian receptor-binding capacity. To further determine the contribution of V186I substitution in changing of the receptor-affinity, we rescued four recombinant H7N9 viruses with either A138/I186/P221/Q226, A138/G186/P221/Q226, A138/V186/P221/Q226, or A138/I186/P221/L226 amino acids combination in the HA gene of A/China/LN/2017 virus by reverse genetics technology, the results demonstrated that the receptor-binding pattern of the A/China/LN/2017 virus is correlated with the Ile186 substitution in the RBS of HA molecule (Fig. 3B). Of note, the affinity for the human receptor was even greater than that of the avian receptor of the A138/I186/P221/L226 recombinant virus compare to the A138/I186/P221/Q226 recombinant virus (Fig. 3B). It seems that Ile186 can further improve the affinity to human-type receptor in the presence of Leu226. Together, the findings suggest that the A138/I186/P221/Q226 amino acid substitution in the RBS of HA molecule contributes to a new dual-receptor-binding pattern which is distinguished to the prevalent dual-receptor-binding pattern of H7N9 viruses.

In conclusion, in the case of the A/China/LN/2017 strain described here, a new pattern of the dual-receptor-binding specificity was uncovered, in which affinity for the human receptor was moderately higher than that for the avian receptor, and the Ile186 mutation in RBS of the HA molecule is responsible for the

change in the receptor-affinity. Substitution from Gly to Ile at the 186 position requires a minimum of two-nucleotide change. It is more likely that Gly186 evolves to Ile186 via Val186. G186V requires a minimum of one-nucleotide change, and V186I requires a minimum of one-nucleotide change. Figure 1 also supports the notion that Ile186 evolves from Val186 rather than Gly186, because the closest neighbor of A/China/LN/2017 in the HA phylogenetic tree is A/chicken/Yunnan/SD210/2017, which carries a Val186. Therefore, evolution to Ile186 should be the next step after G186V, but is not independent of the G186V evolutionary pathway. V186I substitution is a forward evolutionary step for current circulating dual-receptor-binding H7N9 viruses. Considering that the human receptor-binding affinity of this virus is not substantially enhanced compared with that of the prevalent dual-receptor strain (Fig. 3), the virus did not evolve to an increased human tropism. However, when the V186I mutation combined with Q226L mutation in the A138/I186/P221/L226 recombinant virus resulted in even greater human-type receptor specificity, which slightly enhancing of human receptor-binding capacity is accompanied by decreasing of avian receptor-binding affinity and contributes to the relatively higher affinity for human receptor. It's worth noting that the A138/I186/P221/L226 substitution in natural H7N9 virus has

been isolated from one case in China during wave 5 (Table 2). H7N9 viruses which contain the I186 substitution in RBS of the HA molecule have already occurred in seven natural infections from wave 5, among which, six strains were isolated in wave 5, and the A/China/LN/2017 strain was isolated from post-wave 5 (potential wave 6) (Table 2). Notably, due to the considerable damage to the poultry industry in China during epidemic wave 5, the Chinese Ministry of Agricultural and Rural Affairs conducted a large-scale vaccination for chicken poultry at different provinces (including Yunnan province) in China with an H5/H7 bivalent inactivated vaccine in September 2017 (Zeng et al. 2018). After vaccination, the H7N9 virus isolation rate in poultry dropped by 93.3 per cent (Zeng et al. 2018), which also contributed to remarkable reduction of human cases after wave 5 (only four H7N9 human cases were reported between October 2017 and March 2019). Infections of 6 Ile186 mutation strains were occurred before the vaccination, while the A/China/LN/2017 strain emerged after the vaccination in one of the four human infection cases. Phylogenetic analysis showed that the A/China/LN/2017 strain belonged to the group of post-vaccination H7N9 isolates (Figs 1 and 2), which suggests that the Ile186 mutation H7N9 virus may still circulate in mainland China at a low level. In all, the occurrence of V186I substitution and the new dual-receptor-binding pattern suggests that H7N9 viruses are still undergoing human adaption evolution after fifth epidemic wave. Considering the re-emergence of HP H7N9 virus in humans in late March 2019 (Yu et al. 2019), there is a potential risk of H7N9 virus which still circulate in mainland China evolving into a human-specific virus in the future, and more attention need to be paid to surveillance and structural analysis of the 186 substitution of H7 HA molecule.

Data availablility

Data available at GenBank database accession nos MN170546–MM170553.

References

De Graaf, M., and Fouchier, R. (2014) 'Role of Receptor Binding Specificity in Influenza a Virus Transmission and Pathogenesis', *The EMBO Journal*, 33: 823–41.

De Wit, E. et al. (2004) 'Efficient Generation and Growth of Influenza Virus a/PR/8/34 from Eight cDNA Fragments', *Virus Research*, 103: 155–61.

Guo, L. et al. (2018) 'A Family Cluster of Two Fatal Cases Infected with Influenza A (H7N9) Virus in Kunming China, 2017', *Infection, Genetics and Evolution*, 66: 152–8.

Hoffmann, E. et al. (2001) 'Universal Primer Set for the Full-length Amplification of All Influenza a Viruses', *Archives of Virology*, 146: 2275–89.

Quan, C. et al. (2018) 'New Threats from H7N9 Influenza Virus: Spread and Evolution of High- and Low-pathogenicity Variants with High Genomic Diversity in Wave Five', *Journal of Virology*, 92: pii:e00301–18.

Shi, J. et al. (2018) 'Rapid Evolution of H7N9 Highly Pathogenic Viruses That Emerged in China in 2017', *Cell Host & Microbe*, 24: 558–68.

Shi, Y. et al. (2013) 'Structures and Receptor Binding of Hemagglutinins from Human-infecting H7N9 Influenza Viruses', *Science*, 342: 243–7.

—— et al. (2014) 'Enabling the "Host Jump": Structural Determinants of Receptor-binding Specificity in Influenza a Viruses', *Nature Reviews Microbiology*, 12: 822–31.

Su, S. et al. (2017) 'Epidemiology, Evolution, and Pathogenesis of H7N9 Influenza Viruses in Five Epidemic Waves Since 2013 in China', *Trends in Microbiology*, 25: 713–28.

WHO.int. (2018) *Human Infection with Avian Influenza A (H7N9) Virus—China: Update 5 September 2018* <http://www.who.int/csr/don/05-september-2018-ah7n9-china/en/> accessed 24 March 2020.

Xiong, X. et al. (2013) 'Receptor Binding by an H7N9 Influenza Virus from Humans', *Nature*, 499: 496–9.

Xu, R. et al. (2013) 'Preferential Recognition of Avian-like Receptors in Human Influenza a H7N9 Viruses', *Science*, 6163: 1230–5.

Xu, Y. et al. (2019) 'Avian-to-human Receptor-binding Adaptation of Avian H7N9 Influenza Virus Hemagglutinin', *Cell Reports*, 29: 2217–8.

Yu, D. et al. (2019) 'The Re-emergence of Highly Pathogenic Avian Influenza H7N9 Viruses in Humans in Mainland China, 2019', *Eurosurveillance*, 24: pii:1900273.

Zeng, X. et al. (2018) 'Vaccination of Poultry Successfully Eliminated Human Infection with H7N9 Virus in China', *Science China Life Sciences*, 61: 1465–73.

Zhou, J. et al. (2013) 'Biological Features of Novel Avian Influenza A (H7N9) Virus', *Nature*, 499: 500–3.

Zhu, W. et al. (2017) 'Biological Characterisation of the Emerged Highly Pathogenic Avian Influenza (HPAI) A (H7N9) Viruses in Humans, in Mainland China, 2016 to 2017', *Eurosurveillance*, 22: pii:30533.