



**OFFLU Literature Review paper<sup>1</sup>**

## **Virus-specific factors associated with zoonotic and pandemic potential**

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## 1. Introduction

Influenza A viruses (IAVs) cause recurrent epidemics and global pandemics (11). The emergence of a novel H1N1 swine-origin virus (H1N1 S-OIV) in 2009, and the ongoing occurrence of human cases of infection with avian H5N1 IAVs are only recent examples of the zoonotic and pandemic potential of IAVs (19, 60, 139). Different mechanisms are believed to be able to transform an animal virus to a human pandemic strain (64) and these include a constellation of viral evolutionary events which are still to be thoroughly investigated (31, 84, 85). By enlarge, swine and avian influenza viruses cause the greatest concerns for public health. Understanding the molecular evolution of IAVs in the animal reservoir and understanding the mechanisms associated with interspecies transmission would improve our knowledge and prediction skills on relevant characteristics of zoonotic and pandemic influenza viruses (36, 98).

## 2. Biology of IAVs

IAVs are members of the *Orthomyxoviridae* family (17). On the basis of the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA), they currently cluster into sixteen HA (H1-H16) and nine NA (N1-N9) subtypes (109). IAVs consist of eight segmented, single-stranded, RNA-genomes of negative polarity, encoding 11 proteins: polymerase polypeptides PB1, PA, PB2 (polymerase complex), HA and NA, nucleocapsid protein (NP), matrix protein (M1), ionic channel protein (M2), non-structural protein 1 (NS1), nuclear export protein (NEP) and mitochondria-associated protein (PB1-F2) (91, 131). The HA glycoprotein is critical for binding to cellular host receptors and for the fusion of the viral and endosomal membranes (143). Replication and transcription of viral RNAs is carried out by the three polymerase subunits PB1, PB2 and PA, and by the NP (146). Newly synthesized viral ribonucleoproteins (RNP) complexes are exported from the nucleus to the cytoplasm by the NEP and M1, and are assembled into virions at the plasma membrane (126). The NA facilitates the virus release from infected cells by removing sialic acid (SA) from cellular and viral HA and NA proteins (90). The functions of NS1 and PB1-F2 proteins will be further discussed.

## 3. Molecular mechanism of host range restriction: receptor specificity and viral polymerase complex

### 3.1 Receptor distribution

The receptor binding site (RBS) of the HA glycoprotein recognizes the SA bond attached to galactose (Gal) in either alpha 2-3 or alpha 2-6 linkage (92, 137). IAVs recognize mainly two species of SAs, NeuAc (N-acetylneuraminic acid) and NeuGc (N-glycolylneuraminic acid), which are attached to galactose in SA-alpha 2-3Gal or SA-alpha 2-6Gal linkages. For instance, avian viruses preferably recognize SA-alpha 2-3Gal linkages, which are mainly found in the intestine and respiratory epithelia of birds (119, 143), whereas human influenza viruses recognize SA-alpha 2-6Gal linkages which mainly populate the human upper respiratory tract (URT) epithelia (74, 99, 108,137). However, towards the lower epithelial tract of humans, there is a relative increase of SA-alpha 2-3Gal expression (89, 94, 104), and this has been associated with severe pulmonary pathology observed in some cases of H5N1 infection (59, 102).

Pigs are known to exhibit a dual expression of both SA linkages in the respiratory tract (72, 119, 135), however recent studies indicate that receptor distribution is similar to the one found in humans, suggesting that the classical 'mixing vessel' hypothesis regarding the unique role played by pigs needs further discussion (89, 135). Concerning other species, the presence of SA-alpha 2-6Gal in the alveoli of dogs, cats, tigers, pigs and ferrets (130) and in the trachea of chickens and ducks has been reported (65); in the meantime, both types are present throughout the respiratory tract of pheasant and quails (149).

### 3.2 Viral characteristics of Receptor Binding Site

The amino acid residues in the RBS of HA affect the virus host range (146). Glutamine (Q) at position 226 and glycine (G) at position 228 of H2 and H3 HAs confer binding to SA- $\alpha$ 2-3Gal, while leucine (L) and serine (S) at these positions determine binding to SA- $\alpha$ 2-6Gal. For H1 strains, glutamic acid (E) and glycine (G) at positions 190 and 225 confer binding to SA- $\alpha$ 2-3Gal, whereas aspartic acid (D) at the same positions confers binding to SA- $\alpha$ 2-6Gal (4, 11, 143, 147).

Influenza virus-receptor interactions are more complex than the simple  $\alpha$ 2-3 versus  $\alpha$ 2-6 dichotomy on the host range restriction, suggesting that glycan species (linked to SA) and their topology could also play an important role (8, 94, 119). The human respiratory tract expresses only NeuAc, whereas NeuGc is present in other species (72). For instance, avian, human and swine IAVs exhibit preference for NeuAc rather than NeuGc (119). Interestingly, NeuAc is abundant in swine trachea and this feature could set this species as a possible adaptation and/or intermediate virus reassortment host in the creation of novel viruses for humans (8, 119). On the other hand, SA glycans are classified as having 'umbrella-like' and 'cone-like' structural topology (137) and this may also influence virus-receptor affinity. Recently, it has been demonstrated that human-adapted HAs bind with high affinity to umbrella-like topology SAs, whereas avian and swine HAs preferentially recognize cone-like topology. These findings indicate that glycan composition and topological changes may also be important determinants in species-specific switch events (143).

### 3.3 Viral polymerase complex

Another determinant of host restriction is the IAV polymerase complex (78, 103). The amino acid residue 627 in the PB2 subunit regulates polymerase activity in a species-specific fashion (56, 68). The PB2 derived from human viruses mainly possesses lysine (K) at position 627 (PB2-K627), whereas glutamic acid (PB2-E627) predominates in avian viruses (24, 121), with the exception of most of the H5N1 "Qinghai" descendants (145). PB2-K627 correlates with enhanced polymerase activity, virus replication, transmission and pathogenicity in mammals (14, 34, 56, 100), as well as with virus replication at 33°C temperature (human URT temperature) (78, 107, 121).

## 4. Molecular basis of pathogenicity: role of HA cleavage, NS1 and PB1-F2 proteins

The HA protein is synthesized as a precursor protein that is cleaved into two subunits (HA<sub>1</sub> and HA<sub>2</sub>) by host cell proteases (4). This proteolytic cleavage is a prerequisite for fusion of the viral and endosomal membranes to release viral RNP to the cytoplasm (90). Low pathogenic avian influenza viruses (LPAI) possess a cleavage site with a monobasic motif recognized by trypsin-like proteases, which confine viral replication to the respiratory and gastrointestinal tracts (12). In contrast, highly pathogenic avian influenza (HPAI) viruses possess a polybasic HA cleavage site cleavable by the ubiquitous furin, supporting the systemic replication (37, 120). This polybasic HA cleavage of HPAI viruses has originated from LPAI precursors by acquisition of a multibasic cleavage site (MBCS) under both experimental and natural conditions (64, 85, 86).

The NS1 protein is an interferon antagonist (49, 150). The majority of IAVs NS1 proteins have a class 1 PDZ binding motif at the C-terminus, and its truncation results in attenuation of the virulence in mice, pigs and horses (49, 129), as well as in limited replication *in vitro* (54). Additionally, NS1 has been associated to an exacerbated pro-inflammatory cytokine production in humans (85, 99). On the other hand, PB1-F2 is a small protein encoded by the +1 alternate open reading frame (ORF) in the PB1 polymerase gene of some IAVs. This protein is thought to be playing a role as virulence factor by compromising mitochondrial function and eventually leading to apoptosis (77, 136).

## 5. Evolutionary pathways and molecular mechanisms of IAVs involved in human adaptation

### 5.1 Mutations

Mutations result from the lack of a proof-reading activity of the IAV RNA polymerase (58). The rate of mutations during replication is of about 1 nucleotide change for every copied genome (4). The 'antigenic drift' is associated with single point mutations in the antigen-encoding genes and is prompted by immunological pressure, whereas 'antigenic shift' occurs through the reassortment with the generation of a novel combination of viral gene segments (36). For transmission to humans, animal IAVs need to acquire the ability to recognize SA-alpha 2-6Glu as a prerequisite to igniting a pandemic (5, 123, 147). Key mutations of HA at positions 138, 190, 194, 225, 226 and 228 (H3 numbering) affect receptor binding preference of several subtypes including H2, H3, H4 and H9 (7, 133), whereas the HAs from H1 human adapted viruses bear changes at positions 190 and 225 (*Table 1*) (124, 146).

Transmission of H5N1 HPAI virus from poultry to humans was first reported in Hong Kong in 1997 (48, 134). As of June 22<sup>nd</sup> 2011, 562 cases of H5N1 virus infections in humans and 329 deaths have been reported in 15 different countries (WHO, 2011). Even if human-to-human transmission has been limited, H5N1 is believed to be a significant health threat due to "spill-over" infections in humans associated with widespread infection in poultry populations (16, 111). The single mutation HA-Q192H in some H5N1 strains isolated from humans increased viral binding to SA-alpha 2-6Glu, correlating as well with an increased virulence in mice (139). However, mutations enhancing the binding to SA-alpha 2-6Glu are not in themselves sufficient for host switching and transmission, meaning that other virus factors may be involved (52, 71, 74, 133). In this regard, the adaptation of the IAVs polymerase to host factors is an important mechanism underlying interspecies transmission (64). Besides to the PB2-E627K mutation present in some H5N1 strains (35, 124), mutations as PB2-T271A (14), PB2-Q591K (145) and PB2-D701N (47, 82, 100, 121) have been associated with elevated avian polymerase activity in human cells, replication and transmissibility in guinea pigs and with an increased transport of PB2 into the nucleus of mammalian cells.

Prior to 2003, infection with H7 viruses was not considered a serious health threat, although some H7 outbreaks in poultry were sporadically associated with conjunctivitis in humans (10,116). However, the H7N7 HPAI outbreak in the Netherlands in 2003, during which 86 people involved in the culling operation and 3 in-contact persons were infected, prompted a re-evaluation of the human health risks attributed to this virus (9, 27). The majority of H7 infections in humans have resulted in self-limiting conjunctivitis with occasional mild respiratory illness (1, 18, 58), and this is linked to the presence of SA-alpha 2-3Glu linkages in corneal and conjunctival epithelial cells of the human eye (1, 10).

During the 2003 H7N7 Dutch outbreak, different mutations in the polymerase complex, HA, NA and NS1 were found in viruses isolated from a fatal case (FC) when compared to strains isolated from conjunctivitis cases (CC). Among these mutations, PB2-E627K was the main determinant of virus pathogenicity, whereas the HA-A143T mutation correlated with viral attachment to human alveolar macrophages (27). Additionally, viruses from FC presented the PB2-D701N, PB2-S714R and PA-K615N mutations, which conferred an increased polymerase activity in mammal cells at relatively low temperatures (27, 38, 64).

H9N2 LPAI viruses have become enzootic in domestic poultry populations of many Eurasian countries, causing human infections characterized by influenza-like symptoms (15, 138). Since H9N2 viruses have been isolated from pigs and humans (148), they are believed to be potential pandemic candidates (20, 114,122). Molecular characterization of H9N2 viruses circulating in the Middle East and Asia have revealed that more than 70% of the viruses contained the HA-L226 signature, which modifies receptor preference to SA-alpha 2-6Glu linkages (42, 115, 138). Along the same lines, Sorrell et al. (2009) demonstrated that the combination of four key amino acid residues at the RBS of the HA (H183, A189, E190 and L226) in a chimeric virus carrying the surface proteins of avian H9N2 in a human H3N2 backbone, are essential for transmission in ferrets. Additionally, the PB2-E627K mutation in mouse-adapted H9N2 viruses was correlated with increased virulence in mammals (141).

To date, swine influenza viruses (SIAV) H1N1, H3N2 and H1N2 subtypes are circulating in swine all over the world (63, 79). Classical swine H1N1 viruses (cSIAV) presumably emerged from the 1918 pandemic, circulating and reassorting with other viruses to give rise to the 'triple reassortant' H3N2 SIAV. Independently, an 'avian-like' H1N1 SIAV emerged in Europe (21). Phylogenetic analysis of different SIAVs showed that cSIAVs analyzed possess the HA-E190D mutation (H3 numbering), which is required to switch the host-specificity. In addition, cSIAVs possess the 'avian signature' HA-225G, whereas in the European lineage this signature is variable (G225E or G225K). Interestingly, the European 'avian-like' H1N1 lineage possesses the PB2-D701N that may play a role in mammalian adaptation (32).

## 5.2 Reassortments

Since the genome of IAVs consists of eight separate RNA segments, co-infection of one host cell with two different strains can result in progeny viruses containing gene segments of both parental viruses (23, 41). Theoretically, there are 256 possible combinations of the eight genes segments between two viruses (126). Swine are considered as the main candidates for generating reassortant viruses between human and avian IAVs (17, 66). Available reports have demonstrated the isolation of whole avian and human IAVs in pigs (21, 95), meanwhile complete genomic analyses have confirmed the reassortment of swine, avian and/or human viruses in pigs worldwide (72, 88, 110), as recently reported in China (26). Importantly, swine are also capable of transmitting reassortant viruses to humans, as demonstrated during the last 2009 pandemic (13, 44).

The sporadic detection of H9N2 IAVs in domestic pigs and humans (28, 99), as well as their co-circulation with other IAVs, have provided the conditions to lead H9N2 viruses to evolve and generate multiple novel genotypes through reassortant events (29, 115). Fusaro et al., (2011) reported significant inter- and intra- subtype reassortments associated to specific amino acid substitutions that are believed to result in increased transmissibility in mammals. To date, an inter-subtype reassortment has been detected between H9N2, H5N1 HPAI and H7N3 viruses in China (148) and in Pakistan (2, 29, 53). *In vivo* studies have demonstrated that a reassortant virus containing the surface glycoprotein genes from H9N2 and the six internal genes of a human H3N2 virus (138), as well as a reassortant virus carrying the HA of H9N2 in the background of a H1N1 S-OIV (62), were both able to replicate and be transmitted from ferret to ferret.

Amongst reassortment dynamics of internal IAV gene segments, an avian-origin PB1 segment is present both in the H2N2/57 and in the H3N2/68 pandemic strains. This suggests that the reassortment of polymerase subunit genes between mammalian and avian IAVs might play a role for interspecies transmission (31). To test this hypothesis, Li et al. (2009) studied the compatibility between avian H5N1 and human H1N1 polymerases, observing that recombinant viruses carrying the PB2-H1N1 and PB1-H5N1 had a stronger polymerase activity in cell culture. Furthermore, a study demonstrated that *in vivo* co-infection with avian H5N1 and human H3N2 viruses of ferrets generated reassortant viruses containing genes from both progenitor viruses (55).

## 6. Genetic markers

The surveillance of genetic markers (changes in the viral genome) of adaptation could help the prediction of the risk of an epidemic emergence (98). Previous studies have reported 52 (22), 32 (33) and 17 (80) 'species-associated signatures' between avian and human IAVs. Unfortunately, these methods did not take into account the phylogenetic relationship of the isolates and treated each sequence as an independent observation, resulting in an over-estimation of statistical significance (123). Other studies reported 18 mortality markers (3), 172 markers under selective pressure during avian-to-human switch (124) and 68 conserved mutations in 8 internal proteins (81). In addition, 42 markers have been reported in mouse-adapted H9N2 viruses (141) and 10 in mouse-adapted H1N1 S-OIVs (51). Although the identification of genetic markers is not a trivial task and mechanisms of viral adaptation in mammals is thought to be polygenic, a great number of the mutations identified to date involve the IAV polymerase complex genes (38, 39, 80, 82, 145).

At present, the study of GC content in each gene segment has been referred to as possible indicator of the evolutionary process, showing that avian-origin IAVs have a higher GC content than human-adapted viruses (45, 57). Similar changes in nucleotide composition with a diminished in GC content were also evident in swine-adapted IAVs (32). The biological basis for these observations is still unclear (126).

## 7. Pandemic overview

To date, only viruses of the H1, H2 and H3 subtypes are known to have caused pandemics (10,125). It has been estimated that there have been at least 13 pandemics in the last 500 years, including 4 scientifically well-documented ones in the 20<sup>th</sup> century (64, 127). Although the origin of the “Spanish” Influenza pandemic (1917-18) has not been fully resolved, is thought that an avian-like H1N1 virus was involved (46, 128). Alternatively, it may have evolved in swine prior to its emergence (113). Since then, there have been two major influenza pandemics (1957 and 1968) caused by H2N2 and H3N2 subtypes, respectively. Both strains originated by reassortment between the existing “seasonal” strain and an animal virus. The human viruses seem to have acquired three avian segments (HA, NA and PB1), as in the case of the pandemic of 1957 (69,113), and two avian segments (HA, PB1) in the case of the pandemic of 1968 (30). The other segments are believed to have been circulating in humans and pigs since the 1918 pandemic. Until 2009, H3N2 and H1N1 (re-introduced in 1977) were still circulating in the human population (64).

In early April 2009, the H1N1 S-OIV emerged in Mexico and in the United States and spread rapidly around the world, causing the World Health Organization (WHO) to raise its pandemic alert from level 5 to level 6 (43, 73, 132). The H1N1 S-OIV derived its NA and M gene segments from the European ‘avian-like’ H1N1 lineage and its remaining six gene segments from the North American swine H1N2 ‘triple’ reassortant lineage (23). The HA, NP and NS genes segments derived from cSIAV H1N1, while the polymerase gene segments PB2 and PA derived from avian source and PB1 from a human seasonal H3N2 (112, 144). It was established that the virus had already been circulating in swine for more than 10 years (25, 30) and that the transmission from pigs to humans had occurred several months before the outbreak (40). The H1N1 S-OIV has evolved rapidly due to positive selection (67, 83, 105, 142). The H1N1 S-OIV has the D190/D225 signature, supporting the efficient transmissibility among humans, although some recent strains possess the HA-D225G/E mutation, which allow the viruses to have dual hosts (pigs and humans) (23). In addition, HA-D222G mutation has been involved in severe infection outcomes in humans (21, 61). On the other hand, HA-E391K mutation has been associated with the fitness of the virus (75, 76) and it has been reported that H1N1 S-OIV lacks both PB2-E627K and PB2-D701N mutations (24, 50).

On June 8<sup>th</sup> 2011, the first case of co-infection with seasonal H3N2 and H1N1 S-OIV, followed by *in vivo* reassortment in humans was reported in Canada. The phylogenetic analysis demonstrated that the reassortant virus consisted of HA and NA of H3N2 and the remaining genes of H1N1 S-OIV (proMED, 2011). Human mixed infections of H1N1 S-OIVs and seasonal H3N2 viruses were reported in China (70) while an infection with a triple-reassortant SIAV H1N1 distinct from H1N1 S-OIV containing the HA and NA genes of seasonal H1N1 virus was detected in Canada (6, 106). On the other hand, H1N1 S-OIV is able to re-infect swine (87, 140) and to reassort with other viruses circulating in swine herds, as reported to have occurred in Canada (93), Hong Kong (23) and China (151). A study demonstrated that reassortant viruses containing the HA gene from a seasonal H1N1 on a H1N1 S-OIV background showed enhanced growth in cell culture (97). However, a limited compatibility among polymerase subunits from different IAVs must be considered as a restricting factor for reassortment (96).

## 8. Summary

- The host-range restriction of IAVs is a multigenic trait, which includes genes that encode viral surface glycoproteins, proteins involved in the viral replication and in those that counteract the host immune response.

- SA receptors influence the host range. Some species other than swine could play an important role as 'mixing vessels' due to the dual presence of alpha 2-3 and alpha 2-6 receptors in their respiratory tract.
- New IAVs strains emerge through the accumulation of mutations, natural reassortment and adaptation to their new host. Mutations in the RBS of the HA protein of avian IAVs may change the binding preference towards the human host. However, the evolution and adaptation of IAVs is complex and polygenic, involving several viral genes and other unknown host factors.
- H5N1 HPAI viruses are still to be considered as a significant threat for public health. Some H9 avian AIVs have the ability to bind to alpha 2-6 receptors, and the strong evidence of reassortment with other IAVs emphasize their potential to emerge as possible pandemic strains.
- H1N1 S-OIV is evolving rapidly and reassorting with other IAVs that are currently circulating.
- Several genetic markers in IAVs genes have been reported, mainly associated with host restriction, tropism, virulence, modulation of host immunity, as well as with replication and transmission. However, the full understanding of the correlation between molecular markers and biological properties is yet to come.

### 9. Knowledge gaps and future work

- To investigate the role of animal species other than swine as possible 'mixing vessels', by studying their pattern of distribution of SA receptors. Due to non-specific reactions, it is necessary to optimize the use of lectins as probes for histochemistry tests.
- Further investigations are needed to clarify the factors governing reassortment in IAVs.
- To conduct researches on human and host genes involved in modulation of IAVs infection.
- Strengthening the use of molecular methods to study the IAVs evolution: (i) large-scale genomic sequencing to improve the surveillance of mutations and gene constellations showing pandemic potential in all IAVs subtypes; (ii) bioinformatic analyses to study the spatio-temporal evolution dynamics of IAVs, to identify mutations under positive selection and protein structural prediction; (iii) 'deep sequencing' in order to monitor within host viral population diversity.
- To update and share sequences of all IAVs in public databases. The addition of epidemiological and ecological data is also strongly recommended.
- There is a strong need to study the occurrence of infection of other IAVs (such as H1, H2, H3 and H9 subtypes) in humans and animals.
- To implement a systematic cross-sectional and longitudinal sampling in both host and reservoir in order to mapping the genetic changes occurred during viral adaptation.
- To strengthen the collaboration between public and veterinary health sectors to support the systemic and extensive surveillance of animal IAVs (including healthy animals) in order to provide early evidence of emerging viruses.

**Table 1.** Principal amino acid mutations and signatures associated with interspecies transmission of Influenza A viruses

Gene	Mutation	Effect	Strain	Reference
HA			H1N1/1918	
	E190D	-Viral strains with residues D190/D225 are human-specific, D190/G225 swine-specific and E190/G225 avian-specific.	H1N1 cSIAVs	23,32,
	G225D	-Mutations in these residues cause a switch in receptor binding preference from alpha 2-3 to alpha 2-6 SA	European 'avian-like' swine H1N1	124, 126
	D225G/E	-Enhances receptor binding to dual hosts (pigs and humans)	H1N1 S-OIV	23
	D222G	-Enhances binding to SA-alpha 2-3Glu receptors	H1N1 S-OIV	21,24,39,

	-Associated with severe infection outcome in humans		61, 105	
Q192R G139R N182K	-Enhance binding to human-type receptors <i>in vitro</i>	H5N1	4	
Q192H	-Increases binding to SA-alpha 2-6Glu and virulence in mice	H5N1	139	
Q226L G228S	-In H2 and H3 viruses 226Q and 228G correlate with binding to avian receptors; 226L and 228S correlate with the shift from avian to human receptor binding	Human isolates	4, 11, 146	
226L	-Signature which exhibits preferential binding to human-like SA-alpha 2-6Glu receptors. A key element for the successful infection of humans	H9N2	42	
A143T	-Increases viral attachment to human alveolar macrophages	H7N7	27	
E391K	-Associated with the fitness of the virus	H1N1 S-OIV	75, 76	
T160A	-Required to sustain the avian virus transmission in guinea pig model	H5N1	146	
K193R	-Decreases binding to SA-alpha 2-3Glu or increases binding to SA-alpha 2-6Glu	H5N1	11	
<b>PB2</b>	E627K	-Avian strains have 627E and human strains 627K signature. -Associated with increased transmission. Important determinant of host range -Increases polymerase activity in mammalian cells at relatively low temperatures	H1N1/1918 H5N1	38, 39, 78, 85, 101
		-Determinant of host range. Increases transcription at a low temperature	H7N7 isolated from human FC	27
		-Increases virulence in mammals	Mouse-adapted H9N2	115, 138, 141
	D701N	-Enhances the binding of PB2 to importin alpha 1, increasing the level of PB2 in the nucleus in mammalian cells. Important role in the interspecies transmission of IAVs -Increases polymerase activity in mammal cells at relatively low temperatures	Some HPAI H5N1 strains	14, 47, 68, 82, 100, 121
		-Involved in mammalian adaptation	European 'avian like' swine H1N1	32
		-Increases transmissibility of Influenza A viruses in guinea pig model	H5N1	145
		-Enhances the polymerase activity in mammalian cells	Avian- and mouse-adapted H7N7	14, 47, 64
	S714R	-Enhances the polymerase activity in mammalian cells	H7N7	64
	K318R	-Correlates with high pathogenicity in mice in the presence of additional mutations	H5N1	4
	T271A	-Enhances activity only at higher temperatures (37 and 39°C) -Contributes to avian polymerase adaptation to mammalian	H5N1	14

	hosts			
	Q591R/K	-Enhances viral replication in human cells and involved in mammalian adaptation -Compensates the lack of PB2-E627K mutation in the S-OIV	HPIA H5N1 H1N1 S-OIV	145
	G590S	-Associated with mammalian pathogenicity and enhanced replicative ability in mammals	H1N1 S-OIV	36, 78
	A684S	-Associated with host shift from avian to swine and the subsequent transfer to humans	Avian IAVs	124
	E158G	-Probably associated in the adaptation of avian PB2 genes to mammals (mouse model)	Avian H5N9 LPAI H5N2	152
<b>PB1</b>	L13P	-Enhances the activity of viral polymerase	H7N7	4
	G375S	-Associated with adaptation to a new species (swine to human)	H1N1	146
	K577E/M K578Q	-Increase virulence and polymerase activity in mouse model	H3N2 human isolates	101
<b>PA</b>	K615N	-Enhances activity of viral RNA polymerase and stimulates viral replication and pathogenicity in mouse model	H7N7	38,64
	K356R	-Associated with host shift from avian to swine and the subsequent transfer to humans	Avian IAVs	124
	T85I G186S L336M	-Multiple residues that contribute to the enhancement of avian polymerase activity in mammalian cells which is essential for mammalian host adaptation	H1N1 S-OIV	14
<b>NP</b>	N319K	-Increases binding to mammalian importin alpha 1 proteins and polymerase activity. Related to host range specificity.	H7N7	47
	V100I	-Increases transmissibility among humans	H1N1 S-OIV	23

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