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Review

Influenza virus proteins as factors involved in interspecies transmission

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Abstract

Influenza A viruses cause recurrent epidemics and global pandemics. One of the unique features of influenza virus is the ability to overcome interspecies barrier. Reassortment of viral genes and the accumulation of mutations contribute to the emergence of new influenza virus variants. The replication of influenza A virus in a specific host depends on many factors e.g. activity of viral proteins, host response system and environmental conditions. In this review the role of viral proteins as a condition for crossing the species barriers is discussed.

Key word: influenza virus, interspecies transmission, virus proteins

Introduction

Influenza viruses belong to the *Orthomyxoviridae* family. Their genome consists of 8 negative-sense, single-stranded RNA segments encoding well characterized proteins: PB2, PB1, PB1-F2, PA, HA (hemaglutynin), NP (nucleoprotein), NA (neuraminidase), M1(matrix protein), M2, NS1, NEP (NS2) as well as the newly discovered proteins: N40, PA-X, PA-N182, PA-N155 and M42 (Muramato et al. 2013). Influenza viruses are classified into three types: A, B and C on the basis of identity of the internal proteins NP and M1. Influenza A viruses cause severe and fatal acute respiratory disease, emerging in epidemics and sometimes world-wide pandemics. Influenza A viruses are classified into subtypes based on two proteins of the viral envelope: HA and NA (Baigent and McCauley 2003). There are 18 types of HA and 11 types of NA. New serotypes: H17N10 and H18N11 were isolated from bats in 2010 in Guatemala and Peru (Tong et al. 2012, Tong et al. 2013). The natural reservoir of influenza A virus are waterfowl (order *Anseriformes*) and shorebirds (order *Charadriformes*). Different animal species may be infected by different serotypes of influenza virus. Viruses of all HA and NA subtypes were found in avian species, but the serotype combination present in mammals is limited. The influenza A virus subtypes naturally occurring in pigs are H1N1, H1N2, H3N1 and H3N2. Subtypes circulating widely in humans are H1N1, H1N2 and H3N2. Horses can be infected with H3N8 and H7N7. Influenza virus has the ability to cross interspecies barriers. It is known that humans can be infected also by avianlike influenza H6N1, H5N1, H7N9, H7N7, H9N2 or H3N2, H1N1 of swine origin. Pigs can be infected also by avianlike H5N1, H4N6, H4N8, H9N2, H6N6, by humanlike H1N1 or H3N8 of equine origin. Sporadic infections of dogs with H3N8, H1N1, H3N2 and H5N1 were reported (Taubenberger and Kash 2010). Evolution of influenza viruses occurs through different mechan-

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isms, including point mutations (antigenic drift) and gene reassortment (antigenic shift). Three basic mechanisms of interspecies transmission of influenza virus are possible. The first is the direct transfer of an essentially unaltered virus from one species to another. The second mechanism is based on recombination as a consequence of the segmented nature of the genome. Simultaneous co-infection of a host with viruses from different species can result in reassortment of viral genes and the generation of a reassortant virus with the ability to infect other species. The third mechanism can be the result of point mutations. Multiple virus-host interactions are necessary for virus replication leading to adaptation to a new host (Belser et al. 2010, Romero-Tejeda and Capua 2013).

The role of hemagglutynin in influenza virus host specificity and virulence

The hemagglutinin is one of the key viral proteins determining the host range of influenza A viruses since it mediates attachment and entry of the virus into the target cells. The cleavage of HA0 monomer is a crucial step in the virus replication. The HA0 is cleaved into two subunits: HA1 and HA2 by cellular proteases, which recognize either a monobasic or multibasic cleavage site. The HA0 of HPAI (Highly Pathogenic Avian Influenza) H5 and H7 can be cleaved by ubiquitously expressed protein convertases of the subtilisin family like furin, facilitating systemic replication in chicken. The HA0 of LPAI (Low Pathogenic Avian Virus) and human influenza virus can be cleaved by trypsine like proteases. These enzymes are present only in the respiratory and intestinal tracts, thus replication of influenza virus is limited to these sites. Host specificity, tissue tropism and virulence are associated with the combination of HA receptor specificity and NA activity. HA is a trimeric protein localized on the influenza virus membrane. The globular head domain contains a receptor binding site (RBS) which lies near the membrane distal tip of each HA subunit that binds to sialic acid (SA) attached to galactose on target cells (Suzuki 2005). Receptor-binding specificity is also determined by the number and the position of N-linked oligosaccharides around the receptor-binding site. Variation of glycosylation sites were found to determine the biological properties of the HA by interfering with antibody binding and the binding with the receptor. Changes of the glycosylation around the receptor-binding site were also observed as a step in adaptation of the virus to a new host species or cell line. Residues 98, 131-134, 136-138, 153-155,183-190, 194-195, 218-220, 224 and 226-228 have been postulated to be key amino acids that constitute Receptor Binding Domain (RBD) (Skehel and Wiley 2000). However, the exact location of the RBS depends on the individual HA subtype. Amino acids at 134, 136 and 153 are preserved throughout the evolution of influenza viruses in different hosts. On the other hand, those at positions 190, 225, 226 and 228 are more variable and more prone to mutations. Ability of the virus to replicate in different host species is influenced by both SA forms in the host receptor and by amino acids at positions 226 and 228 in the RBS of the HA (Rogers et al. 1993). Gln226 in avian viruses correlates with SA α 2,3Gal receptor and Leu226 in human viruses correlates with SA α 2,6Gal. In H1 viruses of swine and humans, substitution of Glu190 to Asp and Gly225 to Glu are associated with acquisition of SA α 2,6Gal specificity during adaptation of avian viruses to these hosts. In human viruses all HA subtypes show association of Leu226 with Ser228 and in equine and avian viruses (with the exception of H13) Gln226 is associated with Gly228. Thus, these pairs of amino acids may be required for proper orientation of SAs of different species in the RBS (Matrosovich et al. 2000). Thus the ability of viruses to infect different host species depends on the type of SA binding site on the host cell. Avian and equine influenza virus preferentially binds the SA α 2,3Gal. Receptors for avian influenza viruses are found mostly in the avian intestinal epithelium. Due to the expression of SA α 2,3Gal receptors on the surface of epithelial cells of the trachea, horses show susceptibility to the virus circulating in birds, dogs and pigs. Horses are not susceptible to human influenza virus because the human tracheal epithelium has a primarily SA α 2,6Gal. Both α 2,6- and α 2,3- linked sialic acids can be found on cells within the human respiratory tract but in different locations. SA α 2,6-linked sialic acids are preferentially expressed in the human upper respiratory tract, while SA α 2,3-linked receptors are found on cells deeper in the lungs. Replication in the upper airways may be required for efficient transmission and initiation of a productive infection in humans (Zhang 2009). Pigs have been considered as intermediate hosts or mixing vessel for the reassortment of avian and human influenza viruses owing to their susceptibility to infection with both avian and human isolates. Initially, tracheal epithelial cells from pigs were reported to express substantial amounts of both types of receptors (Ito and Kawaoka 2000).

Release from cell surface – role of the NA

The NA molecule consists of a box-like catalytic head, centrally attached stalk with a hydrophobic

transmembrane fragment and a short cytoplasmic tail of six amino acids. The NA operates as a homotetramer, facilitating the mobility of virions by removing sialic acid residues from virions and infected cells during both virus entry and release from cells. NA can cleave sialic acid in α 2,3 and/or α 2,6 linkages (Blok and Air 1982). The mechanism of substrate specificity is not well understood but, for example, specificity of N8 NA is associated with the amino acid at position 275, close to the active site and with glycosylation at Asn144 (Munier et al. 2010). Many studies have documented that influenza virus particles with low NA enzymatic activity cannot be efficiently released from infected cells resulting in the accumulation of large aggregates of progeny virions on the cell surface. Since the formation of aggregates results from HA binding to sialic acid receptors on cellular and viral surfaces, a balance of HA and NA activities appears critical. In brief, the sufficient level of NA activity to ensure the release of progeny virus should compensate the HA binding activity (Wagner et al. 2002). The NA stalk which separates the head region with the enzymatic center from the cell surface and cytoplasmic domains varies in the length depending on the virus. Typically, shortened stalks result in less efficient virus release since the active site in the head region cannot efficiently access its substrate (Luo et al. 1993). However, naturally occurring avian viruses with shortened stalks are virulent in poultry. In avian species, the intestinal tract is the primary site of replication, whereas in humans, the replication is typically restricted to the respiratory tract. The NA activity of avian H1N1 viruses is more resistant to the low pH environment in the upper digestive tract than the human- or swine-derived counterpart. In line with this finding highly pathogenic H5N1 viruses can replicate in the human intestine causing gastrointestinal symptoms and are shed in large amounts in stool (Baum and Paulson 1991, Kobasa et al. 1999). A shortened NA stalk can decrease virulence in mice. Evolution of these viruses also correlates with decreased enzymatic activity of NA due to amino acid substitutions in and near the active site. Since affinity of human virus HAs for their respective receptors is lower than that of avian virus it is likely that reduction in specific activity of NA is required to maintain an optimal balance between HA and NA activity during evolution of these viruses in man (Baum and Paulson 1991).

A novel mechanism of influenza A virus attachment and membrane fusion during entry into host cells was postulated in a flat-faced fruit bat (H18N11). Structural analysis of HA and NA indicates that sialic acid is neither a receptor for virus attachment nor a substrate for virus release. Comparison of the A/bat/Peru/2010 N11 (NA like protein, NAL) with other NAs reveals surprising structural similarity despite low sequence identity (29.6%). Analyses of bat NAL showed substitutions of catalytic centre and no sialidase activity. These data may suggest that bat influenza virus envelope proteins mediate host cell entry and release via different mechanisms compared to other influenza A viruses. This fact may indicate that bats are potentially important reservoir for influenza viruses. (Tong et al. 2012, Tong et al. 2013).

Antiviral drugs can also influence the adaptation of influenza viruses to a new host. For example oseltamivir binds to the active site of NA present on the surface of an infected cell preventing it from removing sialic acid residues and causing virus aggregation. Mutation in neuraminidase H274Y induces resistance of the virus to the drug. This mutation makes that oseltamivir cannot bind to the enzyme and in consequence the virus can replicate smoothly. Drug induced variations in influenza virus may result in emergence of drug-resistant mutants, so in fact new virus variants may appear. This, in turn, may result in acquisition of the ability to cross the interspecies barrier (Ives et al. 2002).

M2-proton channel

The M2 protein is an integral envelope ion channel protein of the influenza A virus. This protein is a homotetramer consisting of four polypeptide chains of 97 residues with a 24-residue N-terminal extra-cellular domain, an internal hydrophobic domain that acts as a transmembrane domain (TM) and forms the pore of the proton selective ion channel, and a 54-residue cytoplasmic tail. The M2 protein has an important role in both the early and late replication cycle of the influenza A virus. The M2 proton channel regulates pH across the viral membrane during cell entry and across the trans-Golgi membrane of infected cells during viral maturation (Holsinger 1993). As virus enters the host cell by receptor mediated endocytosis the endosomal acidification takes place. This low pH activates the M2 channel. M2 now brings protons into the virion core. Acidification of virus interior leads to weakening of electrostatic interaction and to dissociation of M1 (matrix protein) and viral ribonucleoprotein (RNP) complexes. Subsequent membrane fusion releases into the cytoplasm the uncoated RNPs which are imported to the nucleus to start viral replication (Rossman et al. 2010). Mutagenesis studies have identified two residues important for channel function in the imidazole ring of His37 and in the indole ring of Trp41. These residues determine proton selectivity and unidirectional conduction of the channel. Cytoplasmic regions of M2 protein have

been suggested to play a role during viral assembly. At low pH the His37 H+ selectivity filter becomes protonated and the indole ring of Trp41 rotates, inducing protons moving. The M2 ion channel alters the pH within the Golgi apparatus to prevent premature conformational change of the HA protein (Wang et al. 1995). M2 ion channel in different species may be activated at different pH. This fact may suggest hipotetical role of M2 protein in mechanism of crossing of species barrier in a transmission by influenza virus.

Mutations in the influenza virus polymerase complex

The complex is comprised of three proteins PB2, PB1 and PA and interacts with the influenza virus NP and host cellular elements to transcribe and replicate the virus genome (Labadie et al. 2007). PB1 is the catalytic component of the complex. PB2 binds the cap structure of host pre-mRNA molecules as part of the mechanism known as "cap snatching". PA has been shown to possess the endonuclease activity required for this mechanism. Mutations in these proteins can affect transcription and replication (Boivin and Hart 2011). PB2 seems to be a critical component of the viral polymerase in a host restriction. The PB2 domains include the N-terminal peptide (residues 1-35) that interacts with the C-terminal end of PB1, the cap-binding domain (residues 320-483), the 627 domain (residues 538-677) and C-terminal domain (residues 678-759) bearing a bipartite nuclear localization sequence (NLS) (Foeglein et al. 2011).

Influenza virus replication efficiency in mammalian cells is significantly affected by the amino acid at position 627 in PB2 and the role of this aa position is extensively studied. The mutation E627K dramatically improves the function of an avian polymerase complex and enhances viral growth in mammalian cells. Almost all human viruses have 627K, whereas avian viruses have 627E in PB2 (Subbarao et al. 1993). However, the molecular mechanism by which any of these host specific sites determine host range is unknown. One hypothesis is that residue 627 mediates interactions with host factors involved in RNA transcription and replication that differs between mammalian and avian species (Crescenzo-Chainge et al. 2002). A second hypothesis suggests that position 627 of PB2 determines the temperature sensitivity of the vRNA replication (Massin et al. 2001). Human influenza viruses replicate in the upper respiratory tract at an approximate temperature of 33°C, whereas avian influenza viruses prefer the intestinal tract, at a temperature of nearly 41°C. Viruses with PB2 627K can efficiently replicate in the mammalian upper respiratory tract whereas those that possess PB2 627E cannot. A PB2 E627K mutation enhances avian virus replication in mammalian cells at 33°C but not at 37°C or 41°C (Massin et al. 2001). Structural analysis of PB2 627-domain indicates that mutation in PB2 (E627K) modifies electrostatic surface potential of this domain. The change in surface potential is hypothesized to influence the interaction with cellular elements such as α-importins (Kuzuhara et al. 2009). The relationships between NP and PB2 in host adaptation are very close and mutations in PB2 correlate with origin of NP. Whereas NP of avian origin induces the exchange of E627K in PB2 (H5N1) during infection of mammalian cells, NP of a human origin does not. The unstable binding between PB2 and NP of avian influenza virus observed in mammalian cells can be compensated by mutation E627K of PB2 (Bogs et al. 2011). Recent study also has shown that the D701N mutation enhances the binding of PB2 to importin α1 and therefore increases the level of PB2 in the nucleus of mammalian cells. Similar effect causes mutation N319K in NP. As a result, both mutations interact each other in mammalian cells. A direct contact observed between D701 and the flexible NLS-containing C-terminus of PB2 suggests a role in modulating the PB2-importin interaction and nuclear import efficiency. Subsequently it was shown that the substitution D701N significantly affects the interaction of PB2 with importin α1 in mammalian but not in avian cells. This fact suggests that the adaptation of the viral polymerase to the nuclear import machinery plays an important role in the interspecies transmission of influenza virus (Resa-Infante and Gabriel 2013). Mutation of PB2-D701N causes the change in the affinity of the avian viruses from importin alpha 3 to importin alpha 7, which increases viral replication and pathogenicity in mice (Gabriel et al. 2009). Additionally, the D701N PB2 virus has correlated with reduced secretion of the antiviral IFN-λ and showed enhanced replication in primary human alveolar epithelial cell relative to the wild H1N1 virus. These findings suggest that the obtaining of the D701N substitution in PB2 by H1N1 viruses may result in more severe disease or increased transmission rate in humans (Zhou et al. 2013). It was confirmed that the amino acid in position 271 in PB2 enhances mammalian adaptation of recombinant viruses both in vitro and in vivo. Threonine to alanine mutation at residue 271 of PB2 plays a key role in virus growth in mammalian hosts (Bussey et al. 2010). Other mutations including A588I, Q591K also play a similar function (Yamada et al. 2010). Another important cellular factor e.g. DDX17,

a member of the DEAD-box RNA helicase family regulates influenza A virus polymerase activity. Avian DDX17 appears to be essential for avian virus polymerase activity but human DDX17 inhibits avian polymerase activity. Optimal replication of avian influenza viruses in mammalian cells may depend on DDX17 and its interaction with PB2 (Bortz et al. 2011).

Recent study focused on influenza Nuclear Export Protein (NEP) as a new adaptive factor involved in interspecies transmission. The NEP was described as a mediator of vRNA nuclear export. The mechanism by which NEP increases vRNA synthesis and regulates polymerase activity remains unclear (Manz et al. 2012). It was found that mutations in NEP are also involved in host adaptation and regulation polymerase activity. The adaptive mutations M16I, Y41C (in N terminal domain) and E75G (in C terminal domain) in the NEP can increase polymerase activity of avian viruses in mammalian cells (Robb et al. 2009). Probably conformational changes between NEP domains induced by these mutations may be essential for the enhancement of replication of avian virus (Manz et al. 2013).

NS1 role in suppression of the host immune response

The NS1 protein consists of two functional domains. The N-terminal RNA binding domain encompasses amino acids 1-73. It binds nonspecifically to RNA and is also required for interaction with specific cellular proteins. The C-terminal effector domain (ED) includes amino acids 86-230/237 and also interacts with a variety of cellular proteins (Qian et al. 1995). This protein plays a role in suppression of the host immune response by counteracting the cellular interferon (IFN). NS1 binds to double-stranded RNA, thereby suppressing the activation of double-stranded RNA-activated protein kinase – the stimulator of type I IFN production. Type I IFN is a key element in the innate immune response to infection with influenza virus (Lin et al. 2007). Additionally, NS1 has been found to be associated with inhibition pro-inflammatory cytokine production in humans. The NS1 protein is currently postulated to be responsible for the enhanced virulence of highly pathogenic avian H5N1 viruses (Forbes et al. 2012). The single amino acid change D92E in NS1 increased the pathogenicity of the H5N1 influenza virus. The presence of glutamic acid in position 92 of NS1 allows the viral replication in the presence of IFN and is linked to pathogenicity in pigs (Seo et al. 2002). The change at the position 42 of NS1 (P42S) in H5N1 causes an increase in virulence in the mouse model and reduced levels of IFN-α/β production in vitro (Jiao et al. 2008).Two mutations: L103F and I106M in the NS1 of H5N1 viruses increase NS1 binding to the cellular pre-mRNA, and increase yield of viral replication probably by suppressing expression of IFN-α/β mRNAs (Twu et al. 2007). It is clear that NS1 also modulates other important aspects of the virus replication cycle including viral RNA replication, viral protein synthesis, and general host-cell physiology (Lin et al. 2007).

PB1-F2 induction apoptosis

The PB1 F2 protein of infuenza A viruses was discovered in a search for CD8+ T-cell epitopes and it is encoded in the +1open reading frame of the PB1 gene. PB1-F2 preferentially localizes to the mitochondria of infected cells inducing intrinsic apoptosis and cell death. It can induce inflammation by recruitment of inflammatory cells, binds to PB1, increases the activity of the virus polymerase, elicits an antibody response, and forms protein ion channels with no noticeable selectivity in membranes (McCauley et al. 2010). PB1-F2 may be one of virulence factors but the mechanism by which it might enhance disease severity is not understood. Full-length PB1-F2 proteins are found in almost all avian influenza viruses but often become truncated during adaptation to mammalian hosts. Shorter PB1-F2 (with deleted mitochondrial targeting sequence located at C terminus) with less efficient mitochondrial localization and PB1 binding is connected with the lower virus virulence (Pasricha et al. 2013). The amino acid change N66S in PB1-F2 was found to be associated with the high virulence of H5N1 avian viruses. This mutation increases the secretion of proinflammatory cytokines, such as TNF and enhances virus titers in lungs. (Yamada et al. 2004, Conenello et al. 2011). Position 66 is part of the α-helical region of the PB1-F2, in the mitochondrial targeting sequence (MTS). The location of aa 66 in the C-terminal MTS of the protein could affect PB1-F2 interactions with ANT3 and VDAC1 potentially increasing the induction of apoptosis by PB1-F2 (Smith and McCullers 2013).

Transmission data are documented in Table 1. The table shows the most important cases of crossing interspecies barriers by influenza virus. Evidence in the table is directly focused on molecular mechanism of the interspecies transmission. The information includes mutation within internal proteins and surface proteins, names of the strains, localization, date of transmission and species names.

Table 1. Mutations and signatures associated with interspecies transmission of influenza A virus.

Species infected	Transmission	Subtype/Places/ Year of isolation	Effect transmission and function	References
1	2	3	4	5
Human	From avian	H5N1 Hong Kong (1997)	HA closely related to *A/Goose/Guangdong/1/96;* mutation E627K in PB2 enhances polymerase activity in mammals	(Subbarao et al. 1998)
		HN2 Hong Kong (2003)	HA of A/*Hong Kong/2108/03* has Q226L and G228S (preferential binding to α 2,6 receptors); E627K in PB2	(Butt et al. 2005)
		H5N1 Vietnam (2004)	HA Q192R preferential binding to α 2,6 receptors; PB2 E627K	(Tran et al. 2004)
		H7N7 Netherlands (2007)	HA A143T increases viral attachment to human macrophages PB2: D710N, D714R, E627K and PB1 L13P enhances polymerase activity in mammals. Four aa substitutions in NA, increased activity replication in mammals	(Koompans et al 2004)
		H9N2 Hong Kong (2009)	HA Q226L preferential binding to α 2,6 receptors; PB2 E627K	(Sorell et al. 2009)
		H7N9 China (2013)	HA Q226L was found in the *A/Anhui/1/2013* and *A/Shanghai/2/2013;* a deletion of five aa in NA stalk region was found in three viruses	(Gao et al. 2013)
		H6N1 Taiwan (2013)	HA G228S preferential binding to α 2,6 receptors	(Lei and Shi 2011)
	From swine	H3N2 Canada (2006	*A/Canada/1158/2006* deletion of 4 aa in HA1(156--159), substitutions in HA1(G7, K142, S162), HA2 (T77, Q139, M149, E150, N160) and NA (P45, K74, N150, M349, L354) increases virulence	(Robinson et al. 2007)
		H1N1 United States (2009)	HA S206T, NA V91I and N233D may affect the infectivity in humans	(Jasin et al. 2009)
Swine	From avian	H4N6 Canada (1999)	HA of *A/Swine/Ontario/1999*: Q226L and G228S preferential binding to α 2,6 receptors	(Karasin et al. 2000)
		H9N2 China (2003)	*A/Swine /Shandong/1/2003;* HA: H58R, V205A	(Xu et al. 2004)
		H5N1 Indonesia (2005)	PB2 (E627K)	(Nidom et al. 2010)
		H9N2 China (2008)	HA of *A/Swine/Hubei/2008* was closely related to *A/Duck/Hong Kong/768/97* (H10N3); NA was closely related to *A/Chicken/Hubei/119/1983* (H10N5); PB2 D701N	(Cong et al. 2007)
		H6N6 China (2010)	*A/Swine/Guangdong/K6/2010* originated from domestic aquatic birds. PB2 close to H5N1(HPAIV); HA: A222V and G228S preferential binding to α 2,6 receptors	(Zhang et al. 2011)
		H4N8 China (2011)	*A/Swine/Guandong/2011;* HA was closely related to A/*Avian/Japan/8K10185/2008* (H4N6); HA Q226L, G228S preferential binding to α 2,6 receptors. NA was closely related to *A/chicken/Guangxi/2117/2010* (H3N8)	(Su et al. 2012)
	From human	H1N1 Japan (1992)	HA and NA *A/Swine/Obihiro5/92* were closely related to those of current human H1N1 isolated between 1990 and 1992	(Katsuda et al. 1995)
		H1N1 China (2006)	*A/Swine/Guangdong/96/06* derived from human H1N1 isolated in 2000	(Yu et al. 2007)

cont. table 1

1	2	3	4	5
	From equine	H3N8 China (2004, 2006)	One substitution caused the loss of a potential glycosylation site; two substitutions located at the cleavage site and adjacent to the receptor-binding pocket	(Tu et al. 2009)
Avian	From swine	H3N2 United States (2003)	HA and NA *A/Turkey/NC/16108/03 and A/Turkey/MN/764/03* were closely related to *A/Swine/North Carolina/29974/02*	(Choi et al. 2004)
Dog	From avian	H5N1 Thailand (2004)	*A/Canine/Thailand/Ku-08/04*; HAQ226L, G228S preferential binding to α 2,6; NA had 20 aa deletion at positions 49-68 and contained histidine at position 274 indicating the absence of an oseltamivir- resistant residue; PB2 E627K	(Songserm et al. 2006)
		H3N2 Korea (2007)	*A/Canine/Korea 01/2007*, *A/Canine/Korea 02/2007*, *A/Canine/Korea 03/2007*; HA and NA *A/canine/ /Korea/01/2007 (H3N2)* were closely related to avian influenza virus (H3N2) from South Korea	(Song et al. 2008)
		H5N2 China (2009)	*A/Canine/Shandong/J101/2009;* HA was closely related to *A/Swine Fujian/F1/2001* (H5N1); NA gene was closely related to *A/turkey/Wisconsin/66 (H9N2)*	(Zhan et al. 2012)
	From equine	H3N8 Floryda (2004)	HA substitutions (N54K, N83S, W222L and N483T) enhance receptor binding to a new host	(Crawdford et al. 2005)
		H3N8 Australia (2007)	The HA and NA were closely related to *A/Equine/ /Kanazawa/1/2007* and *A/Equine/Ibaraki/1/2007*	(Kirkland et al. 2010)
Equine	From avian	H3N8 China (1989)	*A/Equine/Jilin/1/89* HA was closely related to avian influenza viruses	(Guo et al.1992)
Other species	From avian to seal	H3N8 New England (2011)	*A/Harbor seal/Massachusetts/1/2011* HA L226 Q and S228 G, preferential binding α 2,3 receptors; PB2 D701N	(Anthony et al. 2012)
	From avian to blow fly	H5N1 Japan (2004)	*A/Blow fly/Kyoto/93/2004* show high homology to chicken *A/Chicken/Kyoto/3/2004* and crows *A/Crows/ /Kyoto/53/2004* derived during this outbreak period in Kyoto in 2004	(Sawabe et al. 2009)
	From avian to raccoon dog	H5N1 China (2005)	*Raccon dog/Shandong/sd1/2005*, *Raccon dog/Shandong/ /sd2/2005;* HA: Q222L, G224S, S223N, N182K, Q192R, L129V preferential binding of H5N1 to α 2,6 receptor; PB2 E627K and D701N	(Qi et al. 2009)
	From avian to tiger	H5N1 Thailand (2003)	*A/Tiger/Thailand/Cu-T3/2004*, *A/Tiger/Thailand/Cu-T7/ 2004* ; PB2 E627K; contained a 20 aa deletion in the NA stalk (49-68)	(Keawcharoen et al. 2004)
	From avian to domestic cat	H5N1 Thailand (2004)	NA had 20 aa deletion (49-68) and contained His at position 274, indicating absence of antiviral drug resistant residues	(Songerm et al. 2006)
	From dog to domestic cat	H3N2 Korea (2010)	The complete genome *A/Feline/Korea/01/2010* identical to H3N2 canine influenza virus of avian origin *A/Canine /Korea/01/2007 (H3N2)*	(Seong-Jun Park et al. 2013)
	From human to anteaters	H1N1 South Africa (2007)	HA and NA closely related to human influenza virus *A /Tennessee/UR06-0119/2007* (H1N1) and other isolated in 2006 and 2007 in the United States	(Nofs et al. 2009)
	From human to cheetach	H1N1 California (2009)	HA and NA show a 100% homology to the other virus isolated from human during pandemic H1N1 in 2009	(Crossley et al. 2012)

References

Anthony SJ, St Leger JA, Pugliares K, Ip HS, Chan JM, Carpenter ZW, Navarrete-Macias I, Sanchez-Leon M, Saliki JT, Pedersen J, Karesh W, Daszak P, Rabadan R, Rowles T, Lipkin WI (**2012**) Emergence of fatal avian influenza in New England harbor seals. MBio 3: e00166-12.

Baigent SJ, McCauley JW (**2003**) Influenza type A in humans, mammals and birds: determinants of virus virulence, host-range and interspecies transmission. Bioessays 25: 657-671.

Belser JA, Maines TR, Tumpey TM, Katz JM (**2010**) Influenza A virus transmission: contributing factors and clinical implications. Expert Rev Mol Med 12: e39.

Blok J, Air GM (**1982**) Variation in the membrane-insertion and "stalk" sequences in eight subtypes of influenza type A virus neuraminidase. Biochemistry 21: 4001-4007.

Bogs J, Kalthoff D, Veits J, Pavlova S, Schwemmle M, Manz B, Mettenleiter TC, Stech J (**2011**) Reversion of PB2-627E to -627K during replication of an H5N1 clade 2.2 virus in mammalian hosts depends on the origin of the nucleoprotein. J Virol 85: 10691-10698

Bortz E, Westera L, Maamary J, Steel J, Albrecht RA, Manicassamy B, Chase G, Martinez-Sobrido L, Schwemmle M, Garcia-Sastre A (**2011**) Host- and strain-specific regulation of influenza virus polymerase activity by interacting cellular proteins. Mbio e00151-11

Bussey KA, Bousse TL, Desmet EA, Kim B, Takimoto T (**2010**) PB2 residue 271 plays a key role in enhanced polymerase activity of influenza A viruses in mammalian host cells. J Virol 84: 4395-4406.

Butt KM, Smith GJ, Chen H, Zhang LJ, Leung YH, Xu KM, Lim W, Webster RG, Yuen KY, Peiris JS, Guan Y (**2005**) Human infection with an avian H9N2 influenza A virus in Hong Kong in 2003. J Clin Microbiol 43: 5760-5767.

Chan PK (**2002**) Outbreak of avian influenza A (H5N1) virus infection in Hong Kong in 1997 Clin Infect Dis 2: 58-64.

Choi YK, Lee JH, Erickson G, Goyal SM, Joo HS, Webster RG, Webby RJ (**2004**) H3N2 influenza virus transmission from swine to turkeys, United States. Emerg Infect Dis 10: 2156-2160.

Conenello GM, Tisoncik JR, Rosenzweig E, Varga ZT, Palese P, Katze MG (**2011**) A single N66S mutation in the PB1-F2 protein of influenza A virus increases virulence by inhibiting the early interferon response in vivo. J Virol 85: 652-662.

Cong YL, Pu J, Liu QF, Wang S, Zhang GZ, Zhang XL, Fan WX, Brown EG, Liu JH (**2007**) Antigenic and genetic characterization of H9N2 swine influenza viruses in China. J Gen Virol 88: 2035-2041.

Crawford PC, Dubovi EJ, Castleman WL, Stephenson I, Gibbs EP, Chen L, Smith C, Hill RC, Ferro P, Pompey J, Bright RA, Medina MJ, Johnson CM, Olsen CW, Cox NJ, Klimov AI, Katz JM, Donis RO (**2005**) Transmission of equine influenza virus to dogs. Science 310: 482-485.

Crescenzo-Chaigne B, van der Werf S, Naffakh N (**2002**) Differential effect of nucleotide substitutions in the 3' arm of the influenza A virus vRNA promoter on transcription/replication by avian and human polymerase complexes is related to the nature of PB2 amino acid 627. Virology 303: 240-252.

Crossley B, Hietala S, Hunt T, Benjamin G, Martinez M, Darnell D, Rubrum A, Webby R (**2012**) Pandemic (H1N1) 2009 in captive cheetah. Emerg Infect Dis 18: 315-317.

Foeglein Á, Loucaides EM, Mura M, Wise HM, Barclay WS, Digard P (**2011**) Influence of PB2 host-range determinants on the intranuclear mobility of the influenza A virus polymerase. J Gen Virol 92: 1650-1661.

Forbes NE, Ping J, Dankar SK, Jia JJ, Selman M, Keleta L, Zhou Y, Brown EG (**2012**) Multifunctional adaptive NS1 mutations are selected upon human influenza virus evolution in the mouse. PLoS One 7: e31839.

Gabriel G, Herwig A, Klenk HD (**2008**) Interaction of polymerase subunit PB2 and NP with importin alpha1 is a determinant of host range of influenza A virus. PLoS Pathog 4: e11

Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, Chen J, Jie Z, Qiu H, Xu K, Xu X, Lu H, Zhu W, Gao Z, Xiang N, Shen Y, He Z, Gu Y, Zhang Z, Yang Y, Zhao X, Zhou L, Li X, Zou S, Zhang Y, Li X, Yang L, Guo J, Dong J, Li Q, Dong L, Zhu Y, Bai T, Wang S, Hao P, Yang W, Zhang Y, Han J, Yu H, Li D, Gao GF, Wu G, Wang Y, Yuan Z, Shu Y (**2013**) Human infection with a novel avian-origin influenza A (H7N9) virus. N Engl J Med 368: 1888-1897.

Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A, Sessions WM, Xu X, Skepner E, Deyde V, Okomo-Adhiambo M, Gubareva L, Barnes J, Smith CB, Emery SL, Hillman MJ, Rivailler P, Smagala J, de Graaf M, Burke DF, Fouchier RA, Pappas C, Alpuche-Aranda CM, López-Gatell H, Olivera H, López I, Myers CA, Faix D, Blair PJ, Yu C, Keene KM, Dotson PD Jr, Boxrud D, Sambol AR, Abid SH, St George K, Bannerman T, Moore AL, Stringer DJ, Blevins P, Demmler-Harrison GJ, Ginsberg M, Kriner P, Waterman S, Smole S, Guevara HF, Belongia EA, Clark PA, Beatrice ST, Donis R, Katz J, Finelli L, Bridges CB, Shaw M, Jernigan DB, Uyeki TM, Smith DJ, Klimov AI, Cox NJ (**2009**) Antigenic and genetic characteristics of swine-origin 2009 A (H1N1) influenza viruses circulating in humans. Science 325: 197-201.

Guo Y, Wang M, Kawaoka Y, Gorman O, Ito T, Saito T, Webster RG (**1992**) Characterization of a new avian-like influenza A virus from horses in China. Virology 188: 245-255.

Holsinger LJ, Nichani D, Pinto LH, Lamb RA (**1994**) Influenza A virus M2 ion channel protein: a structure-function analysis. J Virol 68:1551-1563.

Ives JA, Carr JA, Mendel DB, Tai CY, Lambkin R, Kelly L, Oxford JS, Hayden FG, Roberts NA (**2002**) The H274Y mutation in the influenza A/H1N1 neuraminidase active site following oseltamivir phosphate treatment leave virus severely compromised both in vitro and in vivo. Antiviral Res 55: 307-317.

Jain S, Kamimoto L, Bramley AM, Schmitz AM, Benoit SR, Louie J, Sugerman DE, Druckenmiller JK, Ritger KA, Chugh R, Jasuja S, Deutscher M, Chen S, Walker JD, Duchin JS, Lett S, Soliva S, Wells EV, Swerdlow D, Uyeki TM, Fiore AE, Olsen SJ, Fry AM, Bridges CB, Finelli L (**2009**) Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. N Engl J Med 361: 1935-1944.

Jiao P, Tian G, Li Y, Deng G, Jiang Y, Liu C, Liu W, Bu Z,

Kawaoka Y, Chen H (**2008**) A single-amino-acid substitution in the NS1 protein changes the pathogenicity of H5N1 avian influenza viruses in mice. J Virol 82: 1146-1154.

Karasin AI, Brown IH, Carman S, Olsen CW (**2000**) Isolation and characterization of H4N6 avian influenza viruses from pigs with pneumonia in Canada. J Virol 74: 9322-9327.

Katsuda K, Sato S, Shirahata T, Lindstrom S, Nerome R, Ishida M, Nerome K, Goto H (**1995**) Antigenic and genetic characteristics of H1N1 human influenza virus isolated from pigs in Japan. J Gen Virol 76: 1247-1249.

Keawcharoen J, Oraveerakul K, Kuiken T, Fouchier RA, Amonsin A, Payungporn S, Noppornpanth S, Wattanodorn S, Theambooniers A, Tantilertcharoen R, Pattanarangsan R, Arya N, Ratanakorn P, Osterhaus DM, Poovorawan Y (**2004**) Avian influenza H5N1 in tigers and leopards. Emerg Infect Dis 10: 2189-2191.

Kirkland PD, Finlaison DS, Crispe E, Hurt AC (**2010**) Influenza virus transmission from horses to dogs, Australia. Emerg Infect Dis16: 699-702.

Kobasa D, Kodihalli S, Luo M, Castrucci MR, Donatelli I, Suzuki Y, Suzuki T, Kawaoka Y (**1999**) Amino acid residues contributing to the substrate specificity of the influenza A virus neuraminidase. J Virol 73: 6743-6751.

Kuzuhara T, Kise D, Yoshida H, Horita T, Murazaki Y, Nishimura A, Echigo N, Utsunomiya H, Tsuge H (**2009**) Structural basis of the influenza A virus RNA polymerase PB2 RNA-binding domain containing the pathogenicity-determinant lysine 627 residue. J Biol Chem 284: 6855-6860.

Krumbholz A, Philipps A, Oehring H, Schwarzer K, Eitner A, Wutzler P, Zell R (**2011**) Current knowledge on PB1-F2 of influenza A viruses. Med Microbiol Immunol 200: 69-75.

Labadie K, Dos Santos Afonso E, Rameix-Welti MA, van der Werf S, Naffakh N (**2007**) Host-range determinants on the PB2 protein of influenza A viruses control the interaction between the viral polymerase and nucleoprotein in human cells. Virology 362: 271-282.

Lei F, Shi W (**2011**) Prospective of Genomics in Revealing Transmission, Reassortment and Evolution of Wildlife-Borne Avian Influenza A (H5N1) Viruses. Curr Genomics 12: 466-474.

Lin D, Lan J, Zhang Z (**2007**) Structure and function of the NS1 protein of influenza A virus. Acta Biochim Biophys Sin (Shanghai) 39: 155-162.

Luo G, Chung J, Palese P (**1993**) Alterations of the stalk of the influenza virus neuraminidase: deletions and insertions. Vrus Res 29: 141-153.

Manz B, Brunotte L, Reuther P, Schwemmle M (**2012**) Adaptive mutations in NEP compensate for defective H5N1 RNA replication in cultured human cells. Nat Commun 3: 802.

Mänz B, Schwemmle M, Brunotte L (**2013**) Adaptation of avian influenza A virus polymerase in mammals to overcome the host species barrier. J Virol 87: 7200-7209.

Massin P, van der Werf S, Naffakh N (**2001**) Residue 627 of PB2 is a determinant of cold sensitivity in RNA replication of avian influenza viruses. J Virol 75: 5398-5404.

Matrosovich M, Tuzikov A, Bovin N, Gambaryan A, Klimov A, Castrucci MR, Donatelli I, Kawaoka Y (**2000**) Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. J Virol 74: 8502-8512.

McAuley JL, Zhang K, McCullers JA (**2010**) The effects of influenza A virus PB1-F2 protein on polymerase activity are strain specific and do not impact pathogenesis. J Virol 84: 558-564.

Munier S, Larcher T, Cormier-Aline F, Soubieux D, Su B, Guigand L, Labrosse B, Cherel Y, Quéré P, Marc D, Naffakh N (**2010**) A genetically engineered waterfowl influenza virus with a deletion in the stalk of the neuraminidase has increased virulence for chickens. J Virol 84: 940-945.

Muramoto Y, Noda T, Kawakami E, Akkina R, Kawaoka Y (**2013**) Identification of novel influenza A virus proteins translated from PA mRNA. J Virol 87: 2455-2462.

Nidom CA, Takano R, Yamada S, Sakai-Tagawa Y, Daulay S, Aswadi D, Suzuki T, Suzuki Y, Shinya K, Iwatsuki-Horimoto K, Muramoto Y, Kawaoka Y (**2010**) Influenza A (H5N1) viruses from pigs, Indonesia. Emerg Infect Dis 16: 1515-1523.

Nofs S, Abd-Eldaim M, Thomas KV, Toplon D, Rouse D, Kennedy M (**2009**) Influenza virus A (H1N1) in giant anteaters (Myrmecophaga tridactyla). Emerg Infect Dis 15: 1081-1083.

Qi X, Li X, Rider P, Fan W, Gu H, Xu L, Yang Y, Lu S, Wang H, Liu F (**2009**) Molecular characterization of highly pathogenic H5N1 avian influenza A viruses isolated from raccoon dogs in China. PloS One 4: e4682.

Qian XY, Chien CY, Lu Y, Montelione GT, Krug RM (**1995**) An amino-terminal polypeptide fragment of the influenza virus NS1 protein possesses specific RNA-binding activity and largely helical backbone structure. RNA 1: 948-956.

Resa-Infante P, Gabriel G (**2013**) The nuclear import machinery is a determinant of influenza virus host adaptation. Bioeasays 35: 23-27.

Robb NC, Smith M, Vreede FT, Fodor E (**2009**) NS2/NEP protein regulates transcription and replication of the influenza virus RNA genome. J Gen Virol 90: 1398-1407.

Robinson JL, Lee BE, Patel J, Bastien N, Grimsrud K, Seal RF, King R, Marshall F, Li Y (**2007**) Swine influenza (H3N2) infection in a child and possible community transmission, Canada. Emerg Infect Dis 13: 1865-1870.

Rogers GN, Paulson JC, Daniels RS, Skehel JJ, Wilson IA, Wiley DC (**1983**) Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity. Nature 304: 76-78.

Romero-Tejeda A, Capua I (**2013**) Virus-specific factors associated with zoonotic and pandemic potential. Influenza Other Respir Viruses 2: 4-14.

Rossman JS, Jing X, Leser GP, Balannik V, Pinto LH, Lamb RA (**2010**) Influenza virus m2 ion channel protein is necessary for filamentous virion formation. J Virol 84: 5078-5088.

Sawabe K, Tanabayashi K, Hotta A, Hoshino K, Isawa H, Sasaki T, Yamada A, Kurahashi H, Shudo C, Kobayashi M (**2009**) Survival of avian H5N1 influenza A viruses in Calliphora nigribarbis (Diptera: Calliphoridae). J Med Entomol 46: 852-855.

Seo SH, Hoffmann E, Webster RG (**2002**) Lethal H5N1 influenza viruses escape host anti-viral cytokine responses. Nat Med 8: 950-954.

Shimbo K, Brassard DL, Lamb RA, Pinto LH (**1996**) Ion selectivity and activation of the M2 ion channel of influenza virus. Biophys J 70: 1335-1346.

Skehel JJ, Wiley DC (**2000**) Receptor binding and mem-

brane fusion in virus entry: the influenza hemagglutinin. Annu Rev Biochem 69: 531-569.

Smith AM, McCullers JA (**2013**) Molecular signatures of virulence in the PB1-F2 proteins of H5N1 influenza viruses. Virus Res 178: 146-150.

Song D, Kang B, Lee C, Jung K, Ha G, Kang D, Park S, Park B, Oh J (**2008**) Transmission of avian influenza virus (H3N2) to dogs. Emerg Infect Dis 14: 741-746.

Songserm T, Amonsin A, Jam-on R, Sae-Heng N, Meemak N, Pariyothorn N, Payungporn S, Theamboonlers A, Poovorawan Y (**2006**) Avian influenza H5N1 in naturally infected domestic cat. Emerg Infect Dis 12: 681-683.

Sorrell EM, Wan H, Araya Y, Song H, Perez DR (**2009**) Minimal molecular constraints for respiratory droplet transmission of an avian-human H9N2 influenza A virus. Proc Natl Acad Sci U S A 106: 7565-7570.

Su S, Qi WB, Chen JD, Cao N, Zhu WJ, Yuan LG, Wang H, Zhang GH (**2012**) Complete genome sequence of an avian-like H4N8 swine influenza virus discovered in southern China. J Virol 86: 9542.

Subbarao EK, London W, Murphy BR (**1993**) A single amino acid in the PB2 gene of influenza A virus is a determinant of host range. J Virol 67: 1761-1764.

Suzuki Y (**2005**) Sialobiology of influenza: molecular mechanism of host range variation of influenza viruses. Biol Pharm Bull 28: 399-408.

Steel J, Lowen AC, Mubareka S, Palese P (**2009**) Transmission of influenza virus in a mammalian host is increased by PB2 amino acids 627K or 627E/701N. PLoS Pathog 5: e1000252

Taubenberger JK, Kash JC (**2010**) Influenza virus evolution, host adaptation, and pandemic formation Cell Host Microbe 7: 440-451.

Tong S, Li Y, Rivailler P, Conrardy C, Castillo DA, Chen LM, Recuenco S, Ellison JA, Davis CT, York IA, Turmelle AS, Moran D, Rogers S, Shi M, Tao Y, Weil MR, Tang K, Rowe LA, Sammons S, Xu X, Frace M, Lindblade KA, Cox NJ, Anderson LJ, Rupprecht CE, Donis RO (**2012**) A distinct lineage of influenza A virus from bats. Proc Natl Acad Sci U S A 109: 4269-4274.

Tong S, Zhu X, Li Y, Shi M, Zhang J, Bourgeois M, Yang H, Chen X, Recuenco S, Gomez J, Chen LM, Johnson A, Tao Y, Dreyfus C, Yu W, McBride R, Carney PJ, Gilbert AT, Chang J, Guo Z, Davis CT, Paulson JC, Stevens J, Rupprecht CE, Holmes EC, Wilson IA, Donis RO (**2013**) New world bats harbor diverse influenza A viruses. PLoS Pathog 9: e1003657.

Tran TH, Nguyen TL, Nguyen TD, Luong TS, Pham PM, Nguyen vV, Pham TS, Vo CD, Le TQ, Ngo TT, Dao BK, Le PP, Nguyen TT, Hoang TL, Cao VT, Le TG, Nguyen DT, Le HN, Nguyen KT, Le HS, Le VT, Christiane D, Tran TT, Menno de J, Schultsz C, Cheng P, Lim W, Horby P, Farrar J (**2004**) Avian influenza A (H5N1) in 10 patients in Vietnam. N Engl J Med 350: 1179-1188.

Tscherne DM, Garcta-Sastre A (**2011**) Virulence determinants of pandemic influenza viruses. J Clin Invest 121: 6-13.

Tu J, Zhou H, Jiang T, Li C, Zhang A, Guo X, Zou W, Chen H, Jin M (**2009**) Isolation and molecular characterization of equine H3N8 influenza viruses from pigs in China. Arch Virol 154: 887-890.

Twu KY, Kuo RL, Marklund J, Krug RM (**2007**) The H5N1 influenza virus NS genes selected after 1998 enhance virus replication in mammalian cells. J Virol 81: 8112-8121.

Wang C, Lamb RA, Pinto LH (**1995**) Activation of the M2 ion channel of influenza virus: a role for the transmembrane domain histidine residue. Biophys J 69: 1363-1371.

Wagner R, Matrosovich M, Klenk HD (**2002**) Functional balance between haemagglutinin and neuraminidase in influenza virus infections. Rev Med Virol 12: 159-166.

Xu C, Fan W, Wei R, Zhao H (**2004**) Isolation and identification of swine influenza recombinant A/Swine/Shandong/1/2003(H9N2) virus. Microbes Infect 6: 919-925.

Yamada H, Chounan R, Higashi Y, Kurihara N, Kido H (**2004**) Mitochondrial targeting sequence of the influenza A virus PB1-F2 protein and its function in mitochondria. FEBS Lett 578: 331-336.

Yu H, Zhang GH, Hua RH, Zhang Q, Liu TQ, Liao M, Tong GZ (**2007**) Isolation and genetic analysis of human origin H1N1 and H3N2 influenza viruses from pigs in China. Biochem Biophys Res Commun 356: 91-96.

Zhan GJ, Ling ZS, Zhu YL, Jiang SJ, Xie ZJ (**2012**) Genetic characterization of a novel influenza A virus H5N2 isolated from a dog in China. Vet Microbiol 155: 409-416.

Zhang H (**2009**) Tissue and host tropism of influenza viruses: importance of quantitative analysis. Sci China C Life Sci 52: 1101-1110.

Zhang G, Kong W, Qi W, Long LP, Cao Z, Huang L, Qi H, Cao N, Wang W, Zhao F, Ning Z, Liao M, Wan XF (**2011**) Identification of an H6N6 swine influenza virus in southern China. Infect Genet Evol 11: 1174-1177.

Zhou B, Pearce MB, Li Y, Wang J, Mason RJ, Tumpey TM, Wentworth DE (**2013**) Asparagine substitution at PB2 residue 701 enhances the replication, pathogenicity, and transmission of the 2009 pandemic H1N1 influenza A virus. PLoS One 8: e67616.