Molecular signatures of mammalian adaptation of avian influenza viruses

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EXECUTIVE SUMMARY

Preamble

This report summarizes mutations reported to be associated with the mammalian adaptation of avian influenza viruses. This report builds on an excellent review published by Suttie e*t al*. (Inventory of molecular markers affecting biological characteristics of avian influenza A viruses. *Virus Genes* **55**: 739–768. 2019). Data up to 2019 was extracted directly from the Suttie *et al*. publication, and new data was captured using an analogous search strategy conducted on July 31, 2024. New data extracted for publications captured in our search are shown in **blue font** in the tables below. Although all subtypes of avian influenza virus were included in the search strategy, there is a bias in data for highly pathogenic avian influenza viruses, and those that more frequently cause spillover infections in humans.

Considerations for interpretation of data

Unlike clinical data, whose quality can be evaluated using standardized approaches such as GRADE, there is no analogous framework for evaluating the quality of experimental and ecological data used to identify molecular markers of viral adaptation. However, several overarching scientific principles can be considered when evaluating the relevance and reliability of this data. For example, the more of the following criteria that are met for a particular molecular signature, the greater the reliability of the data: repeatability (i.e., multiple independent reports), observation of mutation in natural isolates, and experimental validation of phenotype *in vitro* and *in vivo*.

Interpretation of molecular signatures of mammalian adaptation of avian influenza viruses must therefore be interpreted with caution. For example, a mutation that results in adaptation of a particular strain/subtype may not necessarily confer adaptation in the context of a different strain/subtype. Likewise, mutations that result in adaptation in a particular mammalian species (e.g., mice) may not necessarily confer adaptation to other mammalian species, including humans.

The hemagglutinin (HA) protein of influenza viruses can differ in length depending on subtype (and occasionally, by isolate within a given subtype). Unfortunately, a universal convention for HA numbering has not been established – though several studies have proposed frameworks. For subtypes that are commonly studied, specific reference strains are most often used to facilitate sequence alignment [H1 = A/Puerto Rico/8/1934 (H1N1), H3 = A/Aichi/2/1968 (H3N2), H5 =

A/Vietnam/1203/2004 (H5N1)], however, reference strains can differ for lesser-studied subtypes and primary literature must be examined to identify the reference strain.

In the report that follows, we summarize the molecular signatures reported to be associated with mammalian adaptation of avian influenza viruses. Each of the 8 genomic segments of influenza virus is summarized in a discrete section of the report. We have also added additional tables at the conclusion of the report (Tables 9-11) that summarize mutations and phenotypes associated with viral isolates from human and mammalian hosts from 2019-onwards.

MAIN REPORT

Hemagglutinin (HA) (Segment 4)

HA is the primary viral glycoprotein responsible for binding host cell sialic acid receptors and mediating entry. Many of the mutations in hemagglutinin associated with mammalian adaptation either shift receptor binding preference from α2,3 sialic acid (SA) (avian) to α2,6 sialic acid (mammalian), alter the pH required for fusion, or change the thermostability of HA. For example, T160A and Q226L (H3 numbering) have been well-characterized to shift receptor binding preference towards α2,6 sialic acid binding across a variety of influenza virus subtypes. The R-X-R/K-R motifs found at positions 326-329 (H3 numbering) or between 323-330 (H5 numbering) constitute the polybasic cleavage site of H5 and H7 highly pathogenic avian influenza viruses and confer the ability for HA to be cleaved by more promiscuous proteases that can also facilitate replication in tissues outside of the respiratory tract of humans. In the context of A(H5N1), the K387I mutation decreases the pH required for fusion, stabilizing HA and has been shown to enhance virulence in mice.

Table 1. Molecular markers/motifs in the hemagglutinin (HA; segment 4) gene of influenza virus involved in avian influenza virus (AIV) pathogenicity, receptor binding, infectivity, and transmission in (non-human) mammals†.

Mutation/motif [b]		Phenotype	Subtypes tested	References
H3 numbering	**H5 numbering**			
N2S (H4 numbering)		Increased infectivity in mammalian cells Decreased binding to α2,3	H4N2	[1]
Y17H		Decreased fusion pH of HA Reduced virulence in ferrets	H9N2	[2]
H18Q	H8Q	Decreased infectivity in mammalian cells Increased thermostability of HA	H5N1 (PR8 backbone)	[3]

N18D (H4 numbering)		Decreased thermostability and fusion pH of HA Decreased binding to α2,3	H4N2	[1]
	N27	Reducing binding to DC-SIGN Reduced infectivity in mammalian cells	H5N1	[4]
	N39	Reduced binding to DC-SIGN Reduced infectivity in mammalian cells	H5N1	[4]
A44S		Increased infectivity in mammalian cells	H4N5	[5]
K64E		Increased infectivity in mammalian cells Increased fusion pH of HA	H7N9	[6]
L67V		Increased virulence in mice (in combination with PB2 E627K)	H1N3	[7]
	E91G	Increased binding to α2,6*	H5N1	[8]
D101N	D94N	Increased binding to α2,6	H5N1	[9]
	D94S	Increased virus binding to α2,6*	H5N1	[10]
H110Y	H103Y	Decreased infectivity in mammalian cells Increased thermostability of HA	H5N1 (PR8 backbone)	[3]
A110V		Increased infectivity in mammalian cells No change in binding to α2,3 Decreased fusion pH of HA Increased virulence in mice and guinea pigs	H6N2	[11]
N110S		Mammalian adaption marker*	H5N1	[12]
S110N		Human receptor binding specificity*	H5N1	[13]
I111L (H4 numbering)		Increased infectivity in mammalian cells	H4N5	[5]
A125T (H7 numbering)		Increased infectivity in mammalian cells Decreased binding to α2,6 Immune escape from ferret sera	H7N9	[14,15]
S126N	S121N	Increased virus binding to α2,6	H5N1	[16]
	S123P	Increased binding to α2,6*	H5N1	[10]
D127N (H9 numbering)		Addition of glycosylation site Decreased virulence in mice	H9N2	[17]
N127D		Increased binding to α2,6 Increased virulence in mice	H9N2	[17]

(H9 numbering)				
S127P		Increased α2,6 binding*	H5N6	18
	S128P	Increased binding to α2,6*	H5N6	19
S128P		Associated with antigenic drift/immune escape*	H5N1	12
G133E (H7 numbering)		Increased infectivity in mammalian cells Immune escape from ferret sera	H7N9	14
A135V		Decreases susceptibility to neutralization*	H7N9	20
A135E		Increased binding to α2,3 NeuGc No change in binding to NeuAc	H15N8	21
		Increased binding to α2,3 NeuGc No change in binding to NeuAc	H7N3	21
A135T		Increased receptor binding specificity*	H7N7	22
V135T		Addition of glycosylation site Immune escape from chicken sera	H7N9	23
T136S		Increased receptor binding specificity*	H7N7	22
S137K		Stabilizes SA binding pocket of HA	H9N2	24
S137A	S133A	Increased pseudovirus binding to α2,6 Increased binding to α2,6*	H5N1	25 10,26,27
		Increased binding to α2,6*	H5N6	18,28
A138V	A134V	Increased infectivity in SIAT cells Mammalian adaptation marker*	H5N1	29,30 31
N138H		Stabilized HA binding pocket	H9N2	24
	Q/H/I138N	Increased replication in mammals*	H5N3	32
P139L		Human receptor binding specificity*	H5N1	13
G140R		Increased infectivity in mammalian cells Increased virulence in mice	H7N1	33
R140K		Decreases susceptibility to neutralization*	H7N9	20
N141K		Increased thermostability of HA Decreased infectivity in mammalian cells	H9N2	34

		Decreased α2,6 binding		
T141A		Associated with antigenic drift/immune escape*	H5N1	12
G143R	G139R	Increased virus binding to α2,6	H5N1	35
	E142G	Addition of glycosylation site*	H5N1	8
N144S		Addition of glycosylation site Decreased binding to α2,3 Decreased thermostability of HA	H5N1	36
N149D (H7 numbering)		Increased infectivity in mammalian cells Increased α2,6 binding	H7N9	14
T150A (H9 numbering)		Increased thermostability of HA Increased virulence in mice	H9N2	37
A151T (H7 numbering)		Increased infectivity in mammalian cells Decreased α2,6 binding	H7N9	15
155T		Increased binding to α2,6*	H3N8	38
		Increased binding to α2,6*	H9N2	39
S158N	S154N	Increased virus binding to α2–6	H5N1	16 27
N158D	N154D	Decreased virulence in mice	H9N2	40
		Decreased virulence in mice Increased thermostability of HA	H5N6	41
	D154N	Increased virulence in mice and polymerase activity*	H5N3	42
S159N	S155N/D	Increased virus binding to α2,6	H5N1	16
		Increased virulence in mice*	H5N3	42
		Increased virus binding to α2,6*	H5N1	10
	S158N	Increased binding to α2,6*	H5N6	28
T160[a]	T156[a]	Addition of glycosylation site Immune escape from chicken sera	H7N9	23
T160A	T156A	Increased binding to human-type receptors*	H3N8	43
		Increased virus binding to α2,6, Increased transmission in guinea pigs Decreased infectivity in mammalian cells	H5N1	16,44 26,27,45,46

		In concert with PB2 E627K, increased adaptation in mice*	H5N2	42
		Increased virulence in mice and polymerase activity*	H5N8	42
		Removal of glycosylation site at 158 Increased binding to α2,6*	H5N6	28,47
		Increased binding to α2,6*	H6N2	11
		Increased binding to α2,6*	H7N7	22
		Increased binding to human type receptors*	H6Nx	48
A160T	A156T	Addition of glycosylation site at 158 Increased virulence in mice	H5N6	47
R164Q (H9 numbering)		Increased infectivity in mammalian cells Increased thermostability of HA Immune evasion from chicken sera	H9N2	37
N166D (H9 numbering)		Immune evasion from chicken sera Decreased virulence in mice	H9N2	37
A169T (H7 numbering)		Addition of glycosylation site Immune escape from mouse sera	H7N9	49
	K172T	Increased binding to α2,6*	H5N1	8
	Y173H	Recognizes sialic acid* Increased binding to α2,6*	H5N1	8
A180V (H9 numbering)		Immune escape from sera	H9N2	50
G186V	G182V	Increased binding to α2,6	H7N9	51 52,53
N186K/D	N182K/D	Increased virus binding to α2,6	H5N1	16,30,35,44
V186N	V182N	Increased binding to α2,6 Decreased binding to α2,3*	H5N1	27
		Increased binding to α2,6 Decreased binding to α2,3	H13N6	54
N186[a]	N183[a]	Increased binding to α2,6*	H9N2	55

P186L	P182L	Decreased binding to α2,3	H6N1	56
N186K	N182K	Increased human receptor binding*	H5N1	45
D187G	D183G	Increased virus binding to α2,6	H5N1	57
	D183N	Increased virus binding to α2,6	H5N1	10
E190G	E186G	Increased virus binding to α2,6, maintained α2,3 binding, decreased virulence in mice	H5N1	57,58
E190A/K	E186A/K	Increased infectivity in mammalian cells	H5N1	45
E190V	E186V	Decreased binding to α2,3 and α2,6	H6N2	59
T190V	T186V	Enhances binding affinity to mammalian cells and replication in mammalian cells	H9N2	60
T192I	T188I	Increased pseudovirus binding to α2,6	H5N1	25
K193R/T	K189R/T	Increased virus binding to α2,6	H5N1	16,61
K193N	K189N	Decreased infectivity in mammalian cells	H5N1 (PR8 backbone)	3
N193[a]	N189[a]	Increased receptor binding*	H3N8	62
N193D	N189D	Increases infectivity in mammalian cells Increased infectivity in primary human cells Increases binding to α2,6 Increased virulence, viral dissemination outside of respiratory tract, tissue dissemination to brain, in mice Increased virulence, increased contact transmission, in ferrets	H5N1	63
	V190[a]	Increased replication in mice*	H9N2	55
A/V190E		Stabilizes HA binding pocket	H9N2	24
V190A		Stabilizes HA binding pocket	H9N2	24
H191[a]		Increased binding to α2,6* Increased infectivity in mammalian cells*	H9N2	64
T192I		Increased binding to α2,6*	H5N6	65

Q196R/H	Q192R/H	Increased virus binding to α2,6	H5N1	35,57,66
	K193T	Increased binding to human-type receptors*	H13N6	67
N197K	N193K	Increased virus binding to α2,6	H5N1	35
	N198S	Increases α2,6 binding*	H5N1	8
V214I/A	V210I/A	Increased virus binding to α2,6	H5N1	66 10,12
V214I	V210I	Increased polymerase activity* Increased virulence in mice	H5N8	42
	K212E/R/G	Enhances replication in mammals*	H5N3	32
M214V	M225V	Decreased virulence in mice	H5N6	68
	K218Q	Increased virus binding to α2,3 and α2,6	H5N1	10
I220T (H9 numbering)		Increased infectivity in mammalian and avian cells Decreased thermostability of HA Immune escape from chicken and mice sera Increased virulence in mice	H9N2	37
R220G/I		Decreased binding to α2,3, and α2,6 Increased infectivity in mammalian cells Decreased NA activity	H7N9	69
K222Q	K218Q	Decreased infectivity in mammalian cells	H5N1 (PR8 backbone)	3
I223V		Increased thermostability of HA	H5N1	36
N224K		Increased affinity to human type receptors*	H5N1	70
G225W	G236W	Decreased infectivity in mammalian cells Increased fusion pH of HA Decreased thermostability of HA Decreased virulence in mice	H5N6	68
G225D	G221D	Increased virus binding to α2,6	H6N1	71
G225[a]		Increased virulence in mammals*	H5N6	72
Q226L	Q222L	Increased virus binding to α2,6	H4N6	73
		Increased virus binding to α2,6	H6N2	59
		Increased virus binding to α2,6, decreased binding to α2,3	H5N1	74 45,70

		Increased virus binding to α2,3, decreased binding to α2,6	H7N9	75,76
		Increased virus binding to α2,6 Enhanced replication in mammalian cells and ferrets Enhanced contact transmission in ferrets	H9N2	77,78 39,79
		Loss of binding to α2,3	H10N8	80–82
		Increased binding to human-type receptor*	H5N6	65
		Increased binding to α2,6 Increased infectivity in mammalian cells	H4N5	5
		Increased affinity for α2,6 receptors*	H7N9	52,83
		Increased binding to α2,6 Decreased binding to α2,3 Increased infectivity in mammalian cells Increased pH stability of HA Increased virulence and dissemination outside respiratory tract, increased inflammatory response, in mice	H9N2	84–86
		Increased binding to α2,6	H10N3	87
Q226K	Q222K	Decreased binding to α2,3 Decreased binding to α2,6 Decreased infectivity in mammalian cells Decreased thermostability of HA	H5N1	45
Q226M	Q222M	Increased binding to a,2,6 Decreased infectivity in mammalian and avian cells Decreased thermostability of HA	H9N2	84
Q226R	Q222M	Increased infectivity in mammalian cells	H4N4	5

		Decreased infectivity in mammalian cells Decreased thermostability of HA	H5N1	45
226Q	222Q	Increased binding to α2,6* Increased binding to α2,3*	H9N2	55
L226Q	L222Q	Increased infectivity in mammalian cells Increased virulence in mice Increased stability of HA binding pocket	H9N2	24,88
	L217Q (H7 numbering)	Decreased α2,6 receptor binding Increased thermostability of HA Decreased fusion pH of HA Immune escape*	H7N9	14,15,89
T226A	T222A	Increased binding to α2,6*	H5N1	8
L226I	L222I	Decreased binding to α2,3	H7N9	75
W226	W222	Increased receptor binding specificity*	H3N8	62
S227N	S223N	Increased virus binding to α2,6 and increased infectivity in mammalian cells	H5N1	30,57,74,90,91 45,92
S227R	S223R	Increased binding to α2,6*	H5N6	65
		Increased virus binding to α2,3 and α2,6	H5N1	10
S227G	S223G	Increased infectivity in mammalian cells Decreased binding to α2,6	H5N1	45
S227[a]	S223[a]	Receptor binding pocket*	H3N8	62
H227Q	H223Q	Increased stability of HA binding pocket	H9N2	24
G228A/S	G224A/S	Increased binding to α2,6, dual receptor specificity	H4N6	73
G228S	G224S	Increased viral replication in mammalian cells and virulence in mice	H1N2	93
		Increased virus binding to α2,6	H5N1	16,56,74,94
		Decreased virus binding to α2,3	H6N2	59
		Decreased binding to α2,3 and α2,6 receptors	H7N9	95

		Decreased binding to α2,3, no binding to α2,6	H10N8	80,82
		Human host adaptation*	H3N8	62
		Increased binding to α2,6*	H10N3	96
G228	G224	Increased binding to α2,6 Increased binding to α2,3* Mammalian adaption marker*	H9N2	55
G228S/D	G224S/D	Increased binding to α2,6	H5N1	45
			H13N6	67
	L226Q	Increased binding to RBCs*	H7N9	97
R229I		Increased infectivity in mammalian cells Increased NA activity Increased binding to α2,3 Increased binding to α2,6 Increased virulence in mice Immune escape from ferret sera	H7N9	98
L234		Mammalian adaption marker*	H9N2	64
P239S	P235S	Increased virus binding to α2,6	H5N1	66
	Q238L	Increased binding to α2,6* Decreased binding to α2,3* Mammalian adaption marker*	H5N1	8
	Q238R	Increased binding to α2,6* Decreased binding to α2,3* Mammalian adaption marker*	H5N1	8
R246K		Increased infectivity in mammalian cells Increased pH stability of HA Increased virulence, viral dissemination outside of	H9N2	85

		respiratory tract and to brain, increased inflammatory response, in mice		
E255K	E251K	Increased virus binding to α2,6	H5N1	57
E267K		Human receptor binding specificity*	H5N1	13
T318I	T315I	Decreased fusion pH of HA Increased thermostability of HA*	H5N1	3,70
K319A (H9 numbering)		Increased fusion pH of HA	H9N4	99
K319G (H9 numbering)		Increased fusion pH of HA	H9N2	99
K319N (H9 numbering)		Increased fusion pH of HA	H9N5	99
K319S (H9 numbering)		Increased fusion pH of HA	H9N3	99
326 to 329	323 to 330 (R-X-R, K-R)	Polybasic cleavage motif sequence required for high pathogenicity avian influenza viruses	H5Nx	100–107
			H7Nx	108–110
K387I	K388I	Decreased pH of fusion, increased HA stability, increased replication efficiency and virulence in mice	H5N1	111–113
K393E	K394E	Increased pH of fusion, decreased HA stability, decreased virulence in mice	H7N9	6
M404V		Human receptor binding specificity*	H5N1	13
L513S		Human receptor binding specificity*	H5N1	13
A452T (H7 numbering)		Increased infectivity in mammalian cells Increased virulence in mice	H7N9	114
V537A		Decreased virulence in mice	H5N6	68

T197, G520 (H9 numbering)		Increased aerosol transmission in ferrets*	H9N2	79
I223V, N144S		Increased thermostability of HA Increased infectivity in cells	H5N1	36
S128T, A135E		Increased binding to N-Glycolylneuraminic acid (NeuGc) Decreased binding to N-Acetylneuraminic acid (NeuAc)	H7N3	21
I30V, A135E		Increased binding to NeuAc Decreased binding to NeuGc	H7N3	21
T189A, A135E, K193R		Increased binding to NeuGc Decreased binding to NeuAc	H7N3	21
K193R, A135E		Binding to NeuAc and NeuGc	H7N3	21
A135E, D189A, K193R		Increased binding to NeuGc Decreased binding to NeuAc	H15N8	21
Q226L, A138, V186, P221		Increased binding to α2,6	H7N9	53
T40I, T71I, T157I, V223I		Increased binding to α2,6	H7N9	115
T71I, V223I		Increased binding to α2,6	H7N9	115
E83K, S128P	E75K, S123P	Increased virus binding to α2,6	H5N1	35
E83K, S128P, R496K	E75K, S123P, R497K	Increased virus binding to α2,6	H5N1	35
E83K, S128P, N197K, R496K	E75K, S123P, N193K, R497K	Increased virus binding to α2,6	H5N1	35
E83K, N197K	E75K, N193K	Increased virus binding to α2,6	H5N1	35
E83K, N197K, R496K	E75K, N193K, R497K	Increased virus binding to α2,6	H5N1	35
E83K, R496K	E75K, R497K	Increased virus binding to α2,6	H5N1	35
H110Y, T160A, Q226L, G228S (with PB2: E627K; PB1: H99Y)	H103Y, T156A, Q222L, G224S (with PB2: E627K; PB1: H99Y)	Airborne transmissible in ferrets	H5N1	116,117

S114R, T115I	S107R, T108I	Increased virulence in chickens and mice, increased pH of fusion	H5N1	118 27
S119R, I155T, D145G/N, Q156R, H183N, Q226L, Q227M A190T		Preference for human receptor binding*	H9N2	119
S128P, N197K	S123P, N193K	Increased virus binding to α2,6	H5N1	35
S128P, N197K, R496K	S123P, N193K, R497K	Increased virus binding to α2,6	H5N1	35
S128P, R496K	S123P, R497K	Increased virus binding to α2,6	H5N1	35
Δ[c], A138V	L129V, A134V	Increased virus binding to α2,6	H5N1	120
Δ, I155T	L129del, I151T	Increased virus binding to α2,6	H5N1	66,120
S137A, T192I	S133A, T188I	Increased pseudovirus binding to α2,6	H5N1	25
G143R, N186K	G139R, N182K	Decreased binding to α2,3, increased virus binding to α2,6	H5N1	35,74
N158D, N224K, Q226L, T318I	N154D, N220K, Q222L, T315I	Transmissible among ferrets	H5N1	121
N158S, Q226L	N154S, Q222L	Increased virus binding to α2,6	H5N1	122
N158S, Q226L, N248D	N154S, Q222L, N244D	Increased virus binding to α2,6	H5N1	56,122
S159N, T160A	S155N, T156A	Increased virus binding to α2,6	H5N1	16,56
S159N, T160A, S227N	S155N, T156A, S223N	Increased virus binding to α2,6, reduced lethality and systemic spread in mice	H5N1	123
T160A, K193T, N224K, Q226L	T156A, K189T, N220K, Q222L	Increased virus binding to α2,6	H5N1	61
T160A, Q226L	T156A, Q222L	Increased virus binding to α2,6	H5N1	16,56

T160A, Q226L, G228S	T156A, Q222L, G224S	Increased virus binding to α2,6	H5N1	16,56,124
T160A, S227N	T156A, S223N	Increased virus binding to α2,6	H5N1	16,56
T160A, G186V, G186A, Q226L		Increased binding to α2,6*	H7N9	125
V186N, N228K	V182N, N224K	Increased virus binding to α2,6	H7N9	95
V186K/G, K193T, G228S	V182 K/G, K189T, G224S	Increased virus binding to α2,6	H7N9	95
V186N, N224K, G228S	V182N, N220K, G224S	Increased virus binding to α2,6	H7N9	95
N186K, Q196R, Q226L, S227N, G228S	N182K, Q192R, Q222L, S223N, G224S	Increased virus binding to α2,6	H5N1	74
N186K, Q226L, S227N, G228S	N182K, Q222L, S223N, G224S	Increased virus binding to α2,6	H5N1	74
N186K, Q226L, G228S	N182K, Q222L, G224S	Increased virus binding to α2,6	H5N1	74
E187G, E190D, K193S, Q226L, G228S	E183G, E186D, K189S, Q222L, G224S	Increased virus binding to α2,6	H5N1	126
E187G, Q226L, G228S	E183G, Q222L, G224S	Increased virus binding to α2,6	H5N1	57
D187G, S227N	D183G, S223N	Increased virus binding to α2,6	H5N1	57
T189A, G192R	T185A, G188R	Enhanced replication in ferrets, transmitted via aerosols among ferrets	H9N2 (with human H3N2 backbone)	127

E190G, Q226E, G228S	E186G, Q222E, G224S	Increased virus binding to α2,6	H5N1	57
T187P, M227L, PB2-E627K		Increased binding to α2,6 Decreased binding to α2,3 Increased infectivity in mammalian cells Increased virulence in mice Increased contact transmission in guinea pigs	H9N2	128
K193T, G228S	K189T, G224S	Dual α2,3 and α2,6 binding	H7N9	95
K193R, Q226L, G228S	K189R, Q222L, G224S	Increased virus binding to α2,6	H5N1	124,126
Q196R, Q226L, S227N, G228S	Q192R, Q222L, S223N, G224S	Increased virus binding to α2,6	H5N1	74
Q196R, Q226L, G228S	Q192R, Q222L, G224S	Increased virus binding to α2,6	H5N1	57,74
Q196R, S227N	Q192R, S223N	Increased virus binding to α2,6	H5N1	57,74
T192I, K193R		Increased binding to α2,6*	H5N1	129
N197K, R496K	N193K, R497K	Increased virus binding to α2,6	H5N1	35
K222Q, S227R	K218Q, S223R	Increased virus binding to α2,3 and α2,6	H5N1	130 27
N224K, G228S	N220K, G224S	Increased virus binding to α2,6	H7N9	95
Q226L, S227N, G228S	Q222L, S223N, G224S	Increased virus binding to α2,6	H5N1	74
Q226L, G228S	Q222L, G224S	Increased virus binding to α2,6	H4N6	73
		Increased virus binding to α2,6; decreased antiviral response in host; reduced tissue tropism in guinea pigs	H5N1	16,44,56,57,74,76,94, 122,124,126,131,132
		Increased virus binding to α2,6	H7N7 (human isolate)	133

		Loss of binding to α2,3, no gain of binding to α2,6	H10N8 (human isolate)	80,81,134
		Increased binding to α2,6	H6N6	135
K222Q, S227R		Increased binding to α2,3*	H5N1	136
L331I/G453R		Increased virulence in mice	H4N6	137

[a] These residues have been ascribed with a particular phenotype when present at the indicated position without reference to the wildtype (WT) residue at that position

[b] To facilitate rapid synthesis, mutations found in reports from 2019-onwards are reported using the HA numbering as reported in the accompanying citation. For subtypes that are commonly studied, specific reference strains are most often used to facilitate sequence alignment [H1 = A/Puerto Rico/8/1934 (H1N1), H3 = A/Aichi/2/1968 (H3N2), H5 = A/Vietnam/1203/2004 (H5N1)], however, reference strains can differ for lesser-studied subtypes and primary literature must be examined to identify the reference strain.

*indicates that the phenotype for the mutation has been previously described, but not experimentally verified by the source.

†Mutations in **black font** are directly extracted from Suttie *et al.* Mutations in **blue font** are newly extracted using an analogous search strategy.

Neuraminidase (NA) (Segment 6)

Neuraminidase (NA) is the other major viral glycoprotein. It is typically less abundant on the viral surface than hemagglutinin but plays an essential role in egress by cleaving sialic residues on the host cell surface to prevent aggregation of newly formed viral particles. NA also plays a role in entry by cleaving sialic acid residues on mucins, facilitating viral access to respiratory epithelial cells. Deletions in NA are often observed during adaptation of avian influenza viruses as they jump into poultry or mammalian hosts. NA is also the target of the largest class of antivirals used to treat influenza (NA inhibitors), and many mutations have been described that contribute to resistance against this class of drugs (E.g. H274Y, R292K).

Table 2. Molecular markers/motifs in the neuraminidase (NA; segment 6) gene of avian influenza A viruses associated with enhanced virulence and antiviral resistance†.

Mutation/ motif (N2 numbering)	Phenotype	Subtypes tested	References
49–68 deletion	Enhanced virulence in mice	H5N1 (with H1N1 backbone)	138,139
	Enhanced virulence in mice	H7N9	140
54–72 deletion	Enhanced virulence in mice but not chickens	H5N1	141
	Enhanced virulence in mice	H7N9	140
	Enhanced virulence in mice and chickens	H1N1	142
54–73 deletion	Enhanced virulence in mice	H7N9	140
54–75 deletion	Enhanced virulence in chickens but not ducks	H7N1	143
54–81 deletion	Enhanced replication in chicken, but not duck, cell line. Enhanced replication in respiratory tract of chickens.	H2N2	144
57–65 deletion	Enhanced virulence in mice	H7N9	145
60-68 deletion	Increased infectivity in mammalian cells* Increased virulence in mice*	H6N6	135
69-73 deletion	Increased virulence in mammals*	H7N9	125

A48D	Increased binding to α2,6 Reduced viral titers in the brain in mice	H5N1	146
V116A	Reduced susceptibility to oseltamivir and zanamivir	H5N1	147,148
I117T	Reduced susceptibility to oseltamivir and zanamivir	H5N1	149
		H13N6	67
E119A	Reduced susceptibility to zanamivir	H4N2	150
	Reduced susceptibility to oseltamivir and zanamivir	H5N1	151,152
	Reduced susceptibility to oseltamivir, zanamivir, peramivir, and laninamivir	H7N9	153
E119D	Reduced susceptibility to zanamivir	H4N2	150
	Reduced susceptibility to oseltamivir, zanamivir, and peramivir	H5N1	151
	Reduced susceptibility to oseltamivir, zanamivir, peramivir, and laninamivir	H7N9	153
	Reduced susceptibility to zanamivir	H9N2	154
E119G	Reduced susceptibility to zanamivir	H4N2	150
	Reduced susceptibility to zanamivir and peramivir	H5N1	151
	Reduced susceptibility to zanamivir, peramivir, and laninamivir	H7N9	153
Q136L	Reduced susceptibility to oseltamivir and zanamivir	H5N1	155
	Reduced susceptibility to zanamivir, peramivir, and laninamivir	H7N9	153
R143K	Increased replication in mammals*	H5N3	32
R152K	Reduced susceptibility to laninamivir	H7N9	153
H155Y	Reduced susceptibility to neuraminidase inhibitors	H5N1	12
D198G	Reduced susceptibility to oseltamivir and zanamivir	H5N1	156
V202I	Increased infectivity in mammalian cells	H5N6	41
L204M	Decreased NA activity Decreased infectivity in primary human cells Increased virulence and viral dissemination to the brain, in mice	H5N1	146
I222M	Reduced susceptibility to oseltamivir	H5N1	156

I222K	Reduced susceptibility to oseltamivir, zanamivir, peramivir, and laninamivir	H7N9	153
I222R	Reduced susceptibility to oseltamivir and laninamivir	H7N9	153
I222T	Reduced susceptibility to oseltamivir and peramivir	H7N9	157
T223I	Increased replication in mammals*	H5N3	32
S246N	Reduced susceptibility to oseltamivir	H5N1	148 12
T247P	Reduced susceptibility to oseltamivir and zanamivir	H7N9	153
H274Y	Reduced susceptibility to oseltamivir and peramivir	H5N1	151–153,156,158,159
	Reduced susceptibility to oseltamivir	H7N9	153
	Reduced susceptibility to oseltamivir and peramivir Decreased NA activity		157
275H	Reduced susceptibility to NA inhibitors*	H5N1	160
E276D	Reduced susceptibility to oseltamivir, zanamivir, peramivir, and laninamivir	H7N9	153
E277Q	Reduced susceptibility to oseltamivir	H5N1	161
R289K	Decreased susceptibility to NA inhibitors*	H7N9	89
R292K	Reduced susceptibility to zanamivir	H4N2	150
	Reduced susceptibility to oseltamivir	H6N2	162
	Reduced susceptibility to oseltamivir, zanamivir, peramivir, and laninamivir	H7N9	163,164 165,166
	Reduced susceptibility to oseltamivir	H9N2	154
N294S	Reduced susceptibility to oseltamivir, zanamivir, and peramivir	H5N1	151,152,158,167
	Reduced susceptibility to zanamivir	H7N9	153
S319F	Increased virulence, viral dissemination to brain, in mice Increased binding to α2,6 No change in binding to α2,3	H5N1	146
S327A	Mammalian adaptation marker*	H9N2	64
N366S	Associated with antigenic drift/immune escape*	H5N1	12
369I	Disruption to second sialic acid binding site*	H5N1	27

R371K	Reduced susceptibility to oseltamivir, zanamivir, peramivir, and laninamivir	H7N9	153
A395E	Associated with antigenic drift/immune escape*	H5N1	12
A401T	Increased virus binding to α2–3	H7N9	168
R403W	Mammalian adaptation marker*	H9N2	64
S430G	Increased virulence, viral dissemination to brain, in mice Increased binding to α2,6 No change in binding to α2,3	H5N1	146
K432T	Reduced susceptibility to zanamivir	H5N1	169
V202I, T160A (in HA)	Increased infectivity in mammalian cells Increased virulence in mice	H5N6	41
V202I, A160T (in HA)	Decreased virulence in mice	H5N6	41
A46D, L204M, S319F, S430G	Increased infectivity in primary human cells Increased virulence, viral dissemination to brain, in mice Increased binding to α2,6 No change in binding to α2,3 Decreased NA activity	H5N1	146
I117V, I314V	Reduced susceptibility to oseltamivir	H5N1	147
E119V, E222V	Reduced susceptibility to oseltamivir	H7N9	170
E119A/D/G, H274Y	Reduced susceptibility to oseltamivir, zanamivir, and peramivir	H5N1	151
I222L, S246N	Reduced susceptibility to oseltamivir	H5N1	169
I222M/V, H274Y	Reduced susceptibility to oseltamivir and peramivir	H5N1	156
K150N, I222L, S246N	Reduced susceptibility to oseltamivir	H5N1	148
72T, 370L, 392I, 427I	Confers ability to replicate in murine cells*	N9N2	86

*indicates that the phenotype for the mutation has been previously described, but not experimentally verified by the source.

†Mutations in **black font** are directly extracted from Suttie *et al.* Mutations in **blue font** are newly extracted using an analogous search strategy.

Polymerase Basic Protein 2 (PB2) (Segment 1)

The polymerase basic protein 2 (PB2) is one of three virally-encoded proteins that form the viral polymerase complex. PB2 recognizes the 5' cap of host mRNAs, which are then cleaved by the viral polymerase complex and added to viral mRNAs to enhance their stability. PB2 interacts with several host proteins and thus adaptive mutations in PB2 are often found when influenza virus jumps into new hosts. Q591K, E627K and D701N are examples of prominent markers of mammalian adaptation in PB2 that have been found across multiple influenza virus subtypes. These mutations tend to enhance the activity of the viral polymerase, leading to more efficient replication in mammals and often increasing virulence.

Table 3. Molecular markers/motifs in the polymerase basic protein 2 (PB2; segment 1) gene of avian influenza A virus associated with polymerase activity, virulence, and transmissibility†.

Mutation /motif	Phenotype	Subtypes tested	References
D9N	Increased virulence in mice	H5N1	171,172 79
V25A	Increased virulence in mice	H5N1 backbone with H1N1 NS	173
K61R	Decreased PB2 protein expression in mammalian cells	H9N2	174
I63T	Decreased pathogenicity in mice	H5N1	141
T63I	Increased virulence in mice*	H7N3	175
L89V	Increased polymerase activity* Increased virulence in mice*	H5N2 (and H5N3, H5N8)	42
	Increased virulence in mice*	H5N8	176
	Increased polymerase activity* Increased virulence in mice*	H7N3	175
	Increased polymerase activity* Increased virulence in mice*	H11N9	177
	Increased virulence in mice*	H9N2	39
	Increased polymerase activity in mammalian cells* Increased virulence in mice*	H13N6	67

S155N	Increased infectivity in mammalian cells Increased virulence in mice	H9N2	178
E158G	Increased polymerase activity in mammalian cell line, increased virulence in mice	H5N2	179
		H5N9	179
E158K	Increased polymerase activity and replication in mammalian cell line, increase virulence in mice	H4N6	137
I76T	Increased polymerase activity, increased infectivity, in mammalian cells	H13N8	180
S181A	Decreased stability of PB2 Increased polymerase activity in mammalian cells Decreased virulence, increased inflammatory immune response, in mice	H5N1	181
S181E	Decreased stability of PB2 Decreased polymerase activity, decreased infectivity, in mammalian cells Decreased virulence, increased inflammatory immune response in mice	H5N1	181
K187Q	Decreased polymerase activity in mammalian cells Increased virulence, increased inflammatory immune response, in mice	H5N1	181
K187R	Decreased polymerase activity in mammalian cells	H5N1	181
E192K	Increased polymerase activity in mammalian and avian cell line, increased virulence in mice	H5N1	182
A199S	Increased virulence in mice	H5N1	172
Q236H	Increase polymerase activity in mammalian cells*	H5N1	27
M238I	Decreased polymerase activity, decreased infectivity in mammalian cells Decreased virulence in mice	H5N1	183
M283L	Increased polymerase activity, increased infectivity in mammalian cells Increased virulence in mice	H5N1	183

K251R	Increased virulence in mice*	H7N3	175
D253N	Increased polymerase activity in mammalian cell line	H9N2	184
D256G	Increased polymerase activity in mammalian cell line	H5N1 backbone with pH1N1 PB2	185
T271A	Increase polymerase activity in avian and mammalian cell line*	H3N2 (avian)	186
		H5N1	187 26,27,188
		H7N9	189
I292V	Increased polymerase activity in mammalian cell line, increased virulence in mice	H9N2	190
	Increased polymerase activity, increased infectivity, decreased antiviral immune response, in mammalian cells Increased virulence, increased mortality, in mice	H9N2	190
	Increased polymerase activity in mammalian cell line	H10N8	191
	Increased pathogenicity and replication in mammalian host*	H13N6	67
V292I	Decreased polymerase activity in mammalian cells Decreased aerosol transmission in guinea pigs	H7N9	192
	Mammalian adaptation marker*	H5N1	12
G309D	Increased polymerase activity* Increased virulence in mice*	H5N2	42
	Increased polymerase activity* Increased virulence in mice*	H7N3	175
	Increased virulence in mice*	H9N2	39
	Increased polymerase activity* Increased virulence in mice*	H11N9	177
	Increased polymerase activity in mammalian cells* Increased virulence in mice*	H13N6	67
T339K	Increased polymerase activity* Increased virulence in mice*	H5N2 (and H5N3, H5N8)	42
	Increases pathogenicity in mammals*	H5N6	19

	Increased polymerase activity* Increased virulence in mice*	H11N9	177
E358V	Decreased virulence in mice	H7N3	193
K389R	Increased polymerase activity and replication in mammalian cell line	H7N9	194
	Increased polymerase activity*	H5N1	26,27
L339T	Decreased polymerase activity and decreased virulence in mice	H5N1	195
Q368R	Increased polymerase activity* Increased virulence in mice*	H7N3	175
M473T	Decreased polymerase activity, decreased infectivity, in mammalian cells Decreased virulence, decreased lethality, in mice	H7N9	196
H447Q	Increased polymerase activity* Increased virulence in mice*	H7N3	175
I471T	Change in electrostatic potential*	H7N3	175
R477G	Increased polymerase activity* Increased virulence in mice*	H5N2 (and H3N2, H5N8)	42
	Increased polymerase activity* Increased virulence in mice*	H7N3	175
	Increased virulence in mice*	H9N2	39
K482R	Increased polymerase activity in mammalian cell line	H7N9	197,198
I495V	Increased polymerase activity* Increased virulence in mice*	H5N8 (and H5N2, H5N3)	42
	Increased polymerase activity* Increased virulence in mice*	H7N3	175

	Increased virulence in mice	H9N2	39
I504V	Increased polymerase activity in mice*	H13N6	67
K526R	Increased polymerase activity in mammalian cell line	H5N1	199 27,200
	Increased polymerase activity in mammalian cell line	H7N9	199
	Increased infectivity in mammalian cells*	H7N9	89
M535I	Restores viral polymerase activity in mammalian cells in the absence of PB2-E627K*	H5N1	201
M535L	Increased polymerase activity in mammalian cell line	H7N9	202
	Restoration in polymerase activity	H7N9	89
I559T	Increased polymerase activity, increased infectivity, in mammalian cells	H13N8	180
K562R	Increased polymerase activity Increased infectivity in mammalian cells Increased virulence in mice	H9N2	174
A588T	Can increase the polymerase activity in human cells	H5N1	27
A588V	Increased polymerase activity and replication in mammalian and avian cell lines, increased virulence in mice	H7N9	191
		H9N2	191 39,174
		H10N8	191 203
	Increased virulence in mice*	H7N9	125
Q591K	Increased polymerase activity in mammalian and avian cell line, increased replication in mammalian cell line, increased virulence in mice	H5N1	182,204

	Increased infectivity in mammalian cells* Increased virulence in mice*	H5N1	10
	Increased virulence, increased mortality, in mice	H5N8	205
	Increased polymerase activity in mammalian and avian cell lines	H7N9	189,202 196
	Increased polymerase activity and replication in mammalian cell line, increased virulence in mice	H9N2	206
V598T/I	Increased polymerase activity and replication in mammalian cells, increased virulence in mice	H7N9	194
		H5N1	27
E627K	Increased polymerase activity and replication in mammalian cell line, increase virulence in mice	H4N6	137
	Enhanced polymerase activity, increased virulence in mice, contributes to airborne pathogenicity of IAVs in ferrets and contact transmission in guinea pigs. Decreases polymerase activity and replication in avian cell lines. Decreases virulence in chickens.	H5N1	116,158,172,185,207–216 13,26,27,200,217
	Increased polymerase activity in mammalian cell lines, increase virulence in mice	H7N7	218,219

	Increased polymerase activity and replication in mammalian cell lines, increased virulence in mice	H7N9	218,220 221
	Enhanced virus replication*	H7N9	125
	Increased polymerase activity in mammalian cell line, increased virulence in mice	H9N2	218,222 174
E627V	Increased polymerase activity and replication in mammalian cell lines, increased virulence in mice	H5N1	182 26
	Increased polymerase activity and increased infectivity in mammalian cell lines Increased virulence in mice	H9N2	174
K627E	Decreased polymerase activity, decreased infectivity, in mammalian cells Decreased aerosol transmission in guinea pigs	H7N9	192
	Decreased infectivity in mammalian cells Decreased virulence, decreased mortality, in mice	H7N9	196
K627E	Increased virulence in chickens	H5N1	223
A676T	Increased polymerase activity* Increased virulence in mice*	H5N8 (and H5N2, H5N3)	42
		H7N3	175
	Increased polymerase activity in mammalian cells* Increased virulence in mice*	H13N6	67
D701N	Increased viral replication in mammalian cells and virulence in mice	H1N2	93

	Increased polymerase activity*	H3N8	43
	Increased polymerase activity, enhanced replication efficiency, increased virulence and contact transmission in guinea pigs, increased virulence in mice	H5N1	44,182,224–226 26
	Increased polymerase activity* Increased virulence in mice*	H5N6	65
	Increased infectivity in mammalian cells Increased virulence, viral dissemination outside of respiratory tract, in mice	H5N8	205,227
	Increased polymerase activity in mammalian cell line	H7N9	189,202 196
	Enhanced adaptability in mammals*	H7N9	125
	Increased polymerase activity in mammalian cell line	H9N2	218
	Enhance replication activity of avian influenza RNA polymerase in humans*	H10N3	87
D701V	Increased polymerase activity and replication in mammalian cell lines, increased virulence in mice	H5N1	182
K702R	Increases polymerase activity* Increased virulence in mice*	H5N1	228

	Decreased PB2 protein expression in mammalian cells	H9N2	174
S714R	Increased polymerase activity and replication in mammalian cell line	H7N7	218,229,230
	Increased polymerase activity in mammalian cell line	H9N2	218
S715N	Decreased virulence in mice	H5N1	231
A717T	Decreased PB2 protein expression in mammalian cells	H9N2	174
Q73E, K526R, L183S, P112L, N100S	Increased polymerase activity	H5N1	232
G74R, F323V, Q507R	Increased polymerase activity	H5N1	232
L89V, G309D	Increased polymerase activity in mammalian cells* Increased virulence in mice*	H5N1	27
L89V, A676T, K627E, I495V, R477G, T339K, G309D	Increased polymerase activity*	H11N9	233
E158K, E627K,	Increased polymerase activity, increased infectivity in mammalian cells Increased virulence in mice	H4N6	137
M28I, A274T, K526R, I553V, L607V	Decreased polymerase activity in mammalian cell line	H5N1	234
I283M, K526R	Increased infectivity in mammalian cells Increased inflammatory immune response in mice	H5N8	235

I292V, R389K, A588V, T598M, L648V, T676M	Increased polymerase activity Increased virulence in mice	H10N8	203
L89V, G309D	Increased polymerase activity in mammalian cell line and increased virulence in mice	H5N1	236
L89V, G309D, T339K, R477G, I495V, K627E, A676T	Increased polymerase activity in mammalian cell line and increased virulence in mice	H5N1	236 79
M147L, E627K	Increased polymerase activity in mammalian cell line and virulence in mice	H9N2	237
I147T, K339T, A588T	Increased polymerase activity in mammalian cell line and virulence in mice	H5N1	238
I283M, K526R	Increased infectivity in mammalian cells Increased virulence, increased mortality, in mice	H5N8	239
V292I, E627K	Increased polymerase activity, increased infectivity, in mammalian cells	H7N9	240
S489P, NP-V408I	Increased polymerase activity in mammalian cells Increased virulence in ferrets	H5N1	217
K526R, E627K	Increased polymerase activity and viral replication in mammalian cell lines, increased virulence in mice	H5N1	199
		H7N9	199
K526R, R288Q, R288Q/K526R	Increased polymerase activity, increased infectivity, in mammalian cells Increased virulence in mice	H5N1	241

E627K, D701N	Increased polymerase activity in mammalian cell line	H7N9	242
E627K, S714R	Increased polymerase activity in mammalian cell lines	H7N7	218
		H7N9	218
		H9N2	218
E627K, D701N, S714R	Increased polymerase activity in mammalian cells, increased virulence in mice	H9N2	218
E627K, PB1-V719M	Increased polymerase activity, increased infectivity, in mammalian cells Increased virulence, increased inflammatory immune response, increased mortality in mice	H7N9	243
E627K, PA-N444D	Increased polymerase activity, increased infectivity, in mammalian cells Increased virulence, increased inflammatory immune response, increased mortality in mice	H7N9	243
E627K, PB1-V719M, PA-N444D	Increased polymerase activity, increased infectivity, in mammalian cells Increased virulence, increased inflammatory immune response, increased mortality in mice	H7N9	243
E627K, PB1-E739G	Increased infectivity in mammalian cells Increased virulence in mice	H6N1	244
E627K, HA-A110V	Increased polymerase activity, increased infectivity, in mammalian cells Increased virulence in mice	H5N6	11
D701N, S714R	Increased polymerase activity in mammalian cell line, increased virulence in mice	H5N1	245
	Increased polymerase activity in mammalian cell lines	H7N7	218
	Increased polymerase activity in mammalian cell lines	H7N9	218
	Increased polymerase activity in mammalian cell lines	H9N2	218
E627K (with HA: H110Y, T160A,	Enable airborne transmissibility between ferrets and contact transmission between guinea pigs	H5N1	116,117

Q226L, G228S; PB1: H99Y)			

* indicates that the phenotype for the mutation has been previously described, but not experimentally verified by the source.
†Mutations in **black font** are directly extracted from Suttie *et al.* Mutations in **blue font** are newly extracted using an analogous search strategy.

Polymerase Basic Protein 1 (PB1) (Segment 2)

Polymerase basic protein 1 (PB1) is another component of the viral polymerase complex that encodes the polymerase activity required for viral mRNA synthesis and genome replication. In some strains of influenza virus, the PB1 segment also encodes an accessory protein, PB1-F2, which has pleotropic activities, including regulation of apoptosis and modulation of the antiviral response. Most well-characterized mutations in PB1 enhance the activity of the viral polymerase in mammalian cells, which tends to increase viral replication and virulence (e.g. D3V, D622G). There are only limited reports of signatures associated mammalian adaptation in PB1-F2 to-date.

Table 4. Molecular markers/motifs in the polymerase basic protein 1 (PB1; segment 2) gene of influenza A virus associated with polymerase activity, replication, virulence, and transmissibility†.

Protein	Mutation /motif	Phenotype	Subtypes tested	References
PB1	D3V	Increased polymerase activity and viral replication in avian and mammalian cell lines	H5N1	246 26
		Increased polymerase activity* Increased virulence in mice*	H5N2	42
		Increased polymerase activity* Increased virulence in mice*	H7N3	175
		Increased polymerase activity and replication in mammalian and avian cell lines*	H9N2	79
		Increased polymerase activity and replication in mammalian and avian cells*	H13N6	67
	L13P	Increased polymerase activity* Increased virulence in mice*	H7N3	175
		Enhanced replication in mammals*	H9N2	119
	N66S	Enhanced virulence in mammals*	H6Nx	48
	N105S	Increase polymerase activity and replication in mammalian cell line, Increased virulence in mice	H5N1	182

	K207R	Decreased polymerase activity in mammalian cell line	H5N1	247
	R207K	Increased polymerase activity* Increased virulence in mice*	H7N3	175
	269S	Increased viral polymerase activity and mammalian virulence*	H3N2	248
	K328N	Increased polymerase activity* Increased virulence in mice*	H7N3	175
	I368V	Increased transmission in ferrets*	H7N9	52
	S375N	Increased polymerase activity* Increased virulence in mice*	H7N3	175
		Increased virulence*	H5N1	12
	N375S	Increased virulence*	H5N1	12
	H436Y	Increased polymerase activity* Increased virulence in mice* Increased virulence in ferrets*	H7N3	175
		Increased polymerase activity* Increased virulence in mice* Increased virulence in ferrets*	H11N9	177
		Increased polymerase activity in mammalian cells* Increased virulence in ducks, ferrets and mice*	H13N6	67
	Y436H	Decreased polymerase activity in mammalian cell line; decreased virulence in ducks, ferrets and mice	H5N1	247

	A469T	Increased aerosol transmission in guinea pigs*	H7N3	175
	L473V	Increased polymerase activity	H7N3	175
	V473L	Decreased polymerase activity and replication efficiency in mammalian cells	H1N1 with PB2, PB1, PA NP from H5N1	249
	S524G	Increased virulence in mice Increased airborne transmission in ferrets	H3N8	38
	K577E	Increased polymerase activity and virulence in mice	H9N2	250
	V598P	Decreased polymerase activity and replication efficiency in mammalian cells	H1N1 with PB2, PB1, PA NP from H5N1	249
	D622G	Increased polymerase activity and virulence in mice	H5N1	251 12,26,27
		Increased polymerase activity* Increased virulence in mice*	H5N2	42
		Increased polymerase activity in mammalian cell lines*	H9N2	79
		Increased polymerase activity, increased virulence in mice*	H13N6	67
	V652A	Increased virulence in mice*	H7N3	175
	M677T	Increased virulence in mice*	H7N3	175

		Increased viral polymerase activity and mammalian virulence*	H3N2	248
	T677M	Increased polymerase activity in mammalian cell line, decreased replication efficiency, decreased virulence in mice	H5N1	141
	S678N	Increased replication in avian and mammalian cell lines	H7N7	230
	P708S	Increased virulence in mice	H5N8	205
	V3A, N328K, N375S	Decreased replication efficiency and virulence in ferrets	H5N1	252
	I368V, S409N	Increases transmission in mammals*	H9N2	119
	V473L, P598L	Decreased polymerase activity and replication in mammalian cells	H1N1 with PB2, PB1, PA NP from H5N1	249
	P596S, L598P	Increased polymerase activity	H5N1	232
	I674T, I724V, S720P, F696L, I674S, I674L	Increased polymerase activity	H5N1	232
	H99Y (with HA: H110Y, T160A, G226L, G228S; PB2: E627K)	Airborne transmissible in ferrets	H5N1	116,117

	N66S	Enhanced replication, virulence and antiviral response in mice	H5N1	253,254
PB1-F2	N66S	Increased virulence, increased antiviral immune response, in mice*	H5N1	27
		Increased virulence in mice*	H5N2 *and H5N8)	42
		Increased virulence in mice*	H7N3	175
	T51M, V56A, E87G	Decrease polymerase activity, replication and virulence in ducks	H5N1	255

* indicates that the phenotype for the mutation has been previously described, but not experimentally verified by the source.
†Mutations in **black font** are directly extracted from Suttie *et al.* Mutations in **blue font** are newly extracted using an analogous search strategy.

Polymerase (PA) (Segment 3)

The polymerase protein (PA) is the third and final member of the viral polymerase complex. It is responsible for cleaving 5' caps from host mRNAs. Adaptive mutations in PA most often enhance activity of the polymerase complex, leading to more efficient viral replication in mammalian hosts (e.g., T97I, N383D). PA is also the target of the newest antiviral targeting influenza virus (baloxavir marboxil), and mutations in PA can contribute to antiviral resistance (e.g., A36V). Some strains of influenza also encode PA-X, an accessory protein which contributes to host shut-off by degrading host mRNAs, and attenuating virulence. Therefore, mutations that result in loss of PA-X expression/function have often been associated with increased virulence.

Table 5. Molecular markers/motifs in the polymerase (PA; segment 3) gene of avian influenza A viruses associated with polymerase activity, replication, virulence, and host inflammatory response†.

Protein	Mutation /motif	Phenotype	Subtypes tested	References
PA	E26K	Decreased infectivity in mammalian and avian cells Increased polymerase activity, Decreased host shutoff	H3N8	256
	K26E	Associated with mammalian adaptation*	H3N8	62
		Increased infectivity in mammalian cells Increased host shutoff		256
	P28L	Mammal adaptation marker*	H5N1	12
	32T	Increased infectivity and polymerase activity in mammalian cells Decreased antiviral response in mammalian cells	H5N8	257
	T32M	Decreased infectivity in mammalian cells Increased antiviral immune responses in mammalian cells	H5N8	257
	A36V	Drug resistance*	H5N1	12
	S37A	Increased virulence in mammals*	H5N3	32
		Increased polymerase activity in mammalian cells*	H5N1	26,27
		Increased infectivity and polymerase activity in mammalian cells*	H7N3	175

		Increased polymerase activity in mammalian cell line	H7N9	197
		Increased polymerase activity in mammalian cells*	H13N6	67
	A37S	Decreased polymerase activity in mammalian cell line	H7N7	258
	38D	Increased polymerase activity in mammalian and avian cells*	H9N2	64
	S49Y	Increased infectivity in mammalian cells Increased virulence and inflammatory response in mice	H9N2	178
	V63I	Increase polymerase activity and enhanced replication in mammalian cell line, increased virulence in mice	H7N7	258,259
	A70V	Increased polymerase activity*	H9N2	119
	T85A	Increased polymerase activity in mammalian cells*	H5N1	8
	T97I	Increased virulence in mice*	H3N8	43
		Increased polymerase activity and replication in mammalian cell line, increased virulence in mice	H5N1	182
		Increased polymerase activity in mammalian cell line and enhanced replication in mice	H5N2	260
		Increased polymerase activity in mammalian cells	H6N1	261
		Increased infectivity and polymerase activity in mammalian cells Increased virulence in mice enhanced viral polymerase activity in mammalian cells	H9N2	88
	I118T	Decreased host shutoff	H9N2	256
	K142N/E	Increased virulence in mice	H5N1	172 27

	K158R	Increased polymerase activity in mammalian cell line	H5N1	246
	V160D	Associated with mammalian adaptation*	H3N8	62
	K185R	Increased polymerase activity in mammalian cells*	H5N1	27
		Enhanced pathogenicity and transmission*	H6N2	11
	P190S	Decreased virulence in mice	H7N3	193
	E199D	Decreased sensitivity to PA inhibitors*	H9N2	79
	H266R	Increased polymerase activity* Increased virulence in birds and mammals*	H7N3	175
	F227S	Mammalian host adaption marker*	H7N3	175
	C278Q	Mammalian host adaptation marker*	H7N3	175
	L336M	Increased infectivity and polymerase activity in mammalian cells	H5N1	232
	D347G	Increased infectivity and polymerase activity in mammalian cells Increased virulence and inflammatory response in mice	H9N2	178
	G347D	Increased viral polymerase activity and mammalian virulence*	H3N2	248
	K356R	Mammalian host adaptation marker* Increased virulence and polymerase activity in mammals*	H3N8	62,262
		Increase polymerase activity and enhanced replication in mammalian cell line, Increased virulence in mice Increases replication in mammals*	H9N2	263 119
	R367K	Increased infectivity in mammalian cells and primary human cells Increased virulence in mice	H5N1	264
	N383D	Increased viral polymerase activity and mammalian virulence*	H3N2	248

		Increased polymerase activity in mammalian and avian cell lines Increased virulence in mammals*	H5N1	265,266 26,129
		Increased virulence and transmission in mammals*	H6N2	11
		Increased virulence in mice*	H7N3	175
		Increased polymerase activity in mammalian and avian cell lines*	H13N6	67
	Q400P	Decreased virulence in mice	H7N3	193
	N409S	Increased polymerase activity and replication in mammalian cell line	H7N9	197
	N409S S409N	Increased polymerase activity in mammalian cells*	H5N1	26,27
		Increased polymerase activity and replication in mammalian cell line*	H13N6	67
		Increased virulence*	H10N3	87
		Increased virulence and polymerase activity in mammals*	H3N8	262
	S409N S421I	Potential for enhanced infectivity in humans Potential increased pathogenicity*	H10N3	87
		Increased virulence in mice	H5N1	172

	R443K	Increased virulence in mice	H5N1	173
	K497R	Increased polymerase activity in mammalian cell line	H7N9 (human isolate)	198
	T515A	Decreased polymerase activity in mammalian cell line, decreased virulence in ducks	H5N1	247
	A515T	Increased polymerase activity, increased virulence in mammals*	H11N9	177
	S/A515T	Increased infectivity in mammals*	H5N3	32
	S/A515T I545V	Increased polymerase activity, increased virulence in mammals and birds*	H7N3	175
		Increased infectivity in mammalian cells Increased virulence in mice	H9N2	88
	I550L	Increased infectivity and polymerase activity in mammalian cells Decreased antiviral response in mammalian cells Increased virulence in mice	H5N8	67,257
	L550I	Decreased infectivity and polymerase activity in mammalian cells Increased antiviral response in mammalian cells Decreased virulence in mice	H5N8	257
	Q556R	Increased virulence in mice*	H5N8	205
	K615N	Increased infectivity in mammalian cells Increased virulence in mice	H9N2	88

	K615N	Increased polymerase activity in mammalian cell line and increased virulence in mice	H7N7	229,230
	K615R	Mammalian adaption marker*	H5N1	8
	S631G	Increased virulence in mice*	H5N1	8
	L653P	Mammalian adaptation marker*	H7N3	175
	V100A, S409N	Human host marker*	H7N9	89
	32T, 550L	Increased infectivity and polymerase activity in mammalian cells Decreased antiviral response in mammalian cells Increased virulence in mice	H5N8	257
	T32M, L550I	Decreased infectivity and antiviral responses in mammalian cells Increased polymerase activity in mammalian cells	H5N8	257
	S37A, N383D	Increased polymerase activity in mammalian and avian cell line*	H9N2	79
	A343S, D347E	Increased polymerase activity in mammalian cell line, increase virulence in mice	H5N1	268

	P103H, S659L	Decreased polymerase activity replication in mammalian cell line, decreased virulence in mice	H7N7	269
	S224P, N383D	Increased polymerase activity and enhanced viral replication in duck and mouse cell lines, increased virulence in mice and ducks	H5N1	265,266
	S409N, F672L	Increased transmission in mammals*	H9N2	119
	K142R, I147V, I171V, M182L	Increased polymerase activity in mammalian cell line	H7N9	270
	V44I, V127A, C241Y, A343T, I573V	Increased replication in mammalian cell line virulence in mice	H5N1	271
	S149P, H266R, I357K, S515T	Increased polymerase activity in mammalian cell line	H5N1	234
	K356R (with PB2 E627K)	Increase polymerase activity, enhanced replication capacity in mammalian cell line, increased virulence in mice	H9N2	263
	Truncations resulting in loss of PA-X expression	Increased viral replication in mammalian and avian cell lines; increased inflammatory response in mice; increased virulence in mice, chickens, and ducks	H5N1	265,272–275
PA-X	Truncations resulting in loss of PA-X expression T118I	Decreased virulence in mice, inhibited host inflammatory response	H9N2	275
		Increased host shutoff	H5N1	276

	V127I	Increased host shutoff	H5N1	[276]
	R195K	Increased infectivity in mammalian and primary human cells Increased inflammatory responses in mammalian cells Decreased host shutoff in mammalian cells Increased virulence in mice Increased virulence, and contact and aerosol transmission, in ferret	H7N9	[277]
	K206R	Increased virulence in mice and ferrets	H9N2	[277]
	P210L	Increased virulence in mice and ferrets	H9N2	[277]

* indicates that the phenotype for the mutation has been previously described, but not experimentally verified by the source.
†Mutations in **black font** are directly extracted from Suttie *et al.* Mutations in **blue font** are newly extracted using an analogous search strategy.

Nucleoprotein (NP) (Segment 5)

The nucleoprotein (NP) of influenza virus interacts with viral RNA as part of the viral ribonucleoprotein complex. NP plays regulatory roles in viral RNA synthesis, facilitates nuclear import of viral RNA, and is essential for virus assembly. Many mutations in NP have phenotypes that enhance the viral polymerase activity and virulence (e.g., N319K, K470R).

Table 6. Molecular markers/motifs in the nucleoprotein (NP; segment 5) gene of influenza A virus associated with polymerase activity, virulence, and transmissibility†.

Mutation /motif	Phenotype	Subtypes tested	References
I41V[a]	Increased polymerase activity in mammalian cell line	H7N9	242
V41I	Increased viral transmissibility*	H7N3	175
K91R	Decreased polymerase activity in mammalian cell line	H5N1	278
M105V	Increased virulence in chickens	H5N1	279,280
I109T	Increased polymerase activity and viral replication in chickens (but not ducks), increased virulence in chickens	H5N1	279,280
A184K	Increased replication in avian cells and virulence in chickens, enhanced interferon (IFN)response	H5N1	281
K198R	Decreased polymerase activity in mammalian cell line	H5N1	278
E210D	Increased polymerase activity in mammalian cell line	H7N9	242
	Increased polymerase activity in mammalian cell line*	H9N2	79
D210E	Increased to viral transmissibility*	H7N3	175
K227R	Increased polymerase activity in mammalian cell line	H5N1	278

F253I	Decreased virulence in mice*	H7N9	175
N319K	Increased polymerase activity and replication in mammalian cell line	H7N7	229,282
	Increased polymerase activity in mammalian cells*	H5N1	26
I353V	Increased virulence in mice*	H7N3	175
M374V	Increased infectivity in mammalian cells	H5N8	283
E434K	Increased polymerase activity in mammalian cell line	H9N2	80
T437M	Decreased polymerase activity Decreased infectivity in mammalian cells	H7N9	284
K470R	Increased polymerase activity and replication in mammalian cell line, increased virulence in mice	H5N1	278
T437M, A286V	Decreased virulence in mice	H7N9	284
Q357L (with PB2: E627K)	Increased virulence in mice	H5N1	172
E434K (with HA: Q227P, D375E)	Enhanced contact transmission in guinea pigs	H9N2	80
E434K (with HA: Q227P, PB2: D195N)	Enhanced contact transmission in guinea pigs	H9N2	80
R99K, S345N (with HA: H110Y, T160A, Q226L, G228S; PB2: E627K; PB1: H99Y, I368V	Airborne transmissible in ferrets	H5N1	116

* indicates that the phenotype for the mutation has been previously described, but not experimentally verified by the source.
†Mutations in **black font** are directly extracted from Suttie *et al.* Mutations in **blue font** are newly extracted using an analogous search strategy.

Nonstructural protein (NS) (Segment 8)

The non-structural protein (NS) segment encodes NS1, the main antiviral antagonist protein encoded by influenza virus, and NS2/NEP, which supports nuclear export of viral ribonuclear proteins, regulates the balance of viral RNA synthesis/export, and interacts with M1 to support viral assembly. Mutations in NS1 often enhance the ability of NS1 to antagonize antiviral proteins in new hosts, leading to reduced antiviral responses and enhanced replication/virulence (e.g., P42S).

Table 7. Molecular markers/motifs in the non-structural protein (NS; segment 8) gene of influenza A virus associated with replication, virulence, pathogenicity, and antiviral response†.

Protein	Mutation /motif	Phenotype	Subtypes tested	References
NS1	P42S	Increased virulence in mice*	H3N8	43
		Increased virulence and decreased antiviral response in mice	H5N1	285 12,27
		Increased virulence and decreased antiviral response in mice*	H9N2	39,64,79
		Increased virulence in mice*	H6Nx	48
		increased virulence in mice*	H13N6	67
	D74N	Enhanced replication in mammalian cells and pathogenicity in mice	H7N1 backbone	286

			with H5N1 NS	
	80–84 deletion	Increased virulence in chickens	H1N1 (avian)	287
		Increased infectivity in mammalian cells Increased virulence in mice	H5N1	288
	80–84 deletion Y84F	Increased virulence in chickens and mice	H1N1 backbone with H5N1 HA, NA and NS	289
		Increased virulence in swine	H1N1 backbone with H5N1 NS	289
		Decreases replication in mammalian cells and enhances interferon response	H1N1 with H5N1 NS	290
	D92E	Increased virulence in swine and mice	H1N1 backbone with H5N1 NS	291,292
	D92E I106M	Increased virulence in chickens and mice	H1N1 backbone with H5N1 HA, NA and NS	289
		Increased viral replication in mammalian cells virulence in mice	H1N1 with all internal genes from H7N9	293
	I101M	Increased virulence in mice*	H6Nx	48
	L103F	Increased replication in mammals*	H5N6	18

	Y103F	Increased virulence in mice*	H5N1	10
	I106M	Increased virulence*	H5N1	12,27
		Increased virulence in mice*	H5N2 (and H5N3, H5N8)	42
		Improved replication in mammals*	H5N6	18
		Increased viral replication in mammalian cells and virulence in mice*	H9N2	79
	C138F	Increased replication in mammalian cells, decreased interferon response	H5N1	294 27
		Increased virulence in mice*	H5N2	42
	V149A	Increased virulence and decreased interferon response in chickens	H5N1	295 27
		Increased virulence and viral replication in mammalian cells*	H9N2	39,64
	I205N	Decreased antiviral immune response in ferrets*	H5N1	10
	V226I	Increased virulence*	H5N1	12
	T375G	Decreased infectivity in mammalian cells Decreased virulence in mice	H5N2	296
	P42S, E227/S228/E229/V230, N101D	Increased virulence in mice*	H11N9	233
	P42S, L103F, M106I, V149A, E227K	Increased virulence in mammals*	H9N2	119

	L103F, I106M	Increased virulence in mice	H5N1	297,298 27
		Increased virulence in mice*	H11N9	233
	N205S (with NS2: T47A)	Decreased antiviral response in ferrets	H5N1	299
	G210R (with: NS2 M51I)	Decreased antiviral response in ferrets	H5N1	299
	P3S, R41K, D74N	Enhanced replication in mammalian cells and pathogenicity in mice	H7N1 backbone with H5N1 NS	286
	R38A, K41A	Decreased replication in mammalian and avian cell line	H7N1	300
	42S, 103L, 184G	Increased virulence in mice, decreased interferon response in mice	H9N2	86
	K55E, K66E, C138F	Enhanced replication in mammalian cells, decreased interferon response	H5N1	294 27
	222–230 deletion	Increased replication in mammalian and avian cell lines	H5N1	301
	225–230 deletion	Increased viral replication in avian cell line	H7N1	111,302
	225–230 deletion ^{227}ESEV^{230} (PDZ domain)	No impact on viral replication in avian cell lines	H7N1	303
		Increased virulence in mice	H1N1 pdm09 virus with 'avian' PDZ motif	304
	^{227}ESEV^{230} (PDZ domain) ^{227}RSKV^{230}(PDZ domain)	Decreased viral replication in mammalian and avian cell lines	H5N1	301
		Increased viral replication and virulence in mice, decreased viral replication in human and duck cell lines	H7N1	305

		Increased viral replication in human and duck cell lines but no effect in murine cells	H7N1	305
	230–237 elongation	Increased replication and inflammatory cytokine production in chickens	H9N2	306
	M16I	Increased polymerase activity in mammalian cell line	H5N1	307
NS2/NEP	M16I, Y41C, E75G	Increased polymerase activity in mammalian cell line	H5N1	307
	T47A (with NS1: N205S)	Decreased antiviral response in ferrets	H5N1	299
	M51I (with NS1: G2010R)	Decreased antiviral response in ferrets	H5N1	299

* indicates that the phenotype for the mutation has been previously described, but not experimentally verified by the source.
†Mutations in **black font** are directly extracted from Suttie *et al.* Mutations in **blue font** are newly extracted using an analogous search strategy.

Matrix (M) (Segment 7)

The matrix segment encodes the M1 and M2 proteins. M1 is an essential structural protein that creates a proteinaceous "shell" beneath the viral membrane. It plays critical roles in virion assembly and regulates stability of the virus particle. Mutations in M1 (e.g., N30D) are often associated with changes in virion stability, assembly, and immune evasion. M2 is embedded in the viral membrane and forms a proton channel that helps to regulate viral uncoating in the endosome. Most mutations in M2 are associated with resistance to amantadine/rimantadine, antivirals that inhibit M2 ion channel activity, but are no longer widely used to treat seasonal influenza virus due to widespread resistance.

Table 8. Molecular markers/motifs in the matrix (M; segment 7) gene of influenza A virus associated with virulence and antiviral resistance

Protein	Mutation/motif	Phenotype	Subtypes tested	References
M1	V15I/T	Increased virulence in mice*	H7N3	175
	D30N	Increased infectivity in mammalian cells Affects virus morphology	H5N1	308
	N30D	increased viral polymerase activity and mammalian virulence*	H3N2	248
		Enhanced virulence in mice*	H3N8	43
		Increased virulence in mice	H5N1	309 12
		Increased virulence in mice*	H5N2 (and H5N3, H5N8)	42
		Increased virulence in mice*	H5N6	18
		Increased virulence in mice*	H7N3	175
		Increased virulence in mice*	H9N2	39
		Increased virulence in mice*	H11N9	177
		Increased virulence in mice*	H13N6	67
		Increased virulence in mice*	H6Nx	48
	S31N/G	Increased resistance to amantadine and rimantadine*	H5N1	27,87
	T37A	Increased infectivity in mammalian cells Increased virulence in mice	H5N6 H9N2	310
	I43M	Increased virulence in mice, chickens and ducks	H5N1	311
		Increased virulence in mice*	H5N2	42

	S82N	Mammalian adaption marker*	H5N1	12
	R95K	Increased infectivity in mammalian cells Increased virulence, increased mortality in mice	H9N2	312
	K102R	Decreased infectivity in mammalian cells Decreased virulence in mice	H5N6	313
	D156E	Decreased infectivity in mammalian cells Decreased aerosol transmission in guinea pigs Spheroidal virus production	H7N9	192
	A166V	Increased virulence in mice*	H7N3	175
	A215T	Decreased infectivity in mammalian cells	H5N1	308
	T215A	Increased virulence in mice	H5N1	309
		Increased viral polymerase activity and mammalian virulence*	H3N2	248
		Increased virulence in mice*	H5N1	12
			H3N8	43
			H5N2	42
			H5N6	18
			H7N3	175
			H9N2	39
			H11N9	177
			H13N6	67
			H6Nx	48
	K242N	Increased infectivity in mammalian cells Increased virulence in mice	H9N2	312
	N30D, T215A	Increased virulence in mice*	H11N9	233
	N30D, I43M, T215A	increased virulence in mice*	H9N2	79
	R95K, K242N	Increased infectivity in mammalian cells Increased virulence in mice	H9N2	312
	C148/C151/ H159/H162	Increased virulence in mice*	H11N9	233
M2	L26F	Increased resistance to amantadine and rimantadine	H5N1	314–316
	I/V27A/T/S	Increased resistance to amantadine and rimantadine	H5N1	314–318
			H5N2	
	A30V/T/S		H5N1	316–318

		Increased resistance to amantadine and rimantadine	H5N2	
			H7N2	
			H9N2	86
	S31N/G	Increased resistance to amantadine and rimantadine	H5N1	315–321 39,52,79,160
			H5N2	
			H6N2	
			H7N9	
			H9N2	
			H10N3	87
	G34E	Increased resistance to amantadine and rimantadine	H5N1	314
	S50C	Increased virulence in mice*	H11N9	233

* indicates that the phenotype for the mutation has been previously described, but not experimentally verified by the source.
†Mutations in **black font** are directly extracted from Suttie *et al.* Mutations in **blue font** are newly extracted using an analogous search strategy.

Experimentally validated mammalian adaptive phenotypes associated with constellations of mutations identified in avian influenza virus isolates

In certain cases, influenza isolates of various subtypes have been experimentally tested for adaptation to mammals however, the contribution of specific mutations to the observed phenotypes is not described. Instead, the strains themselves are characterized and the mutations (relative to parental avian isolates) are reported in aggregate. These are captured in the table below. Note that this type of data was not captured in the review by Suttie *et al.*, and so the data below is derived from publications spanning our search from 2019-present.

Table 9. Experimentally verified strains of influenza A virus isolated from avian species containing molecular markers/motifs associated with mammalian adaptation published 2019 – July 2024‡¶.

Subtype	Segment	Mutation/motif	Phenotype	References
H5N6	HA	**S137A**, **S158N**, **T160A**, I192M, **226Q**, **228G**	Increased infectivity in mammalian cells Increased virulence, viral dissemination to brain, in mice Decreased contact transmission in ducks	28
	NA	58-68 deletion, I26T, P48L, N59E, M50T, **T63I**, I64K, M65V, E70G, D100G, Q216H, K251I, R275G, T294I, R299M, K311T, D375N		

	PB1-F2	**N66S**		
H9N2	NA	I19T, M51V, N325S, G425R	Increased virulence in mice	322
	PB1	I322M		
	PA	N614S		
	M2	L47F		
H9N2	HA	T220I	Increased infectivity in mammalian cells Increased virulence in mice Increased binding to α2,3 Decreased binding to α2,6 Loss of glycosylation site Decreased thermostability of HA	323
	NA	R344I		
H9N2	HA	D166N, T198V, N201D, L234Q	Increased infectivity in mammalian cells Increased virulence in mice Increased binding to α2,3 Decreased binding to α2,6 Immune escape from chicken serum	
	NA	D125G, K296N		
H9N2	HA	D166N, A168T, T198V, L234Q	Increased infectivity in mammalian cells Increased virulence in mice Increased binding to α2,3 Decreased binding to α2,6 Immune escape from chicken serum	
	NA	R253K		
H7N9	HA	R57K, E129G, A189E, **Q226L**, R312K	Increased infectivity in mammalian cells Increased virulence, viral dissemination to brain, in mice Increased binding to α2,6 Decreased binding to α2,3	83
	NA	V102L, V233I, V236I, P264S, N322T, P386T		
	PB1	N213T		
	PB2	G74S, K191E, M402I, V511I, **M535L**, N559T, M570I, **E627K**, I647V, **D701N**		

	PA	R57Q, I118V, F268L		
	NS1	M27K, A86S		
H5N3	HA	T86V, **I138N**, 150A, 202E, 206L, **K212E**, 237G, 238Q, N376G	Infectivity in mammalian cells Modest virulence in mice	32
	PB1	A3V, **L13P**, **K328N**		
	PB1-F2	**N66S**		
	PB2	**Q368R, H447Q**		
	PA	**S37A**, I357T, **A515T**		
	NS1	M31P, D87P, L98I, I101E, A127R, S228Q		
H7N3	NA	I26V	Infectivity and polymerase activity in mammalian cells Modest virulence in mice	177
	PB1	**D3V**, **L13P**, **R207K**, **K328N**, **S375N**, **H436Y**, **A469T**, **L473V**, **V652A**, **M677T**		
	PB1-F2	**N66S**, T68I		
	PB2	**T63I**, **L89V**, **K251R**, **G309D**, **Q368R**, **H447Q, I471T, R477G**, I459V, **A676T**		
	PA	**S37A**, H226R, **F227S**, C278Q, **N383D**, **A515T**, **L653P**		
	NEP	P42S, A127N, V149A		
	NP	**V41I**, I109V, **D210E**, **F253I**, **I353V**		
	M1	**N30D**, A116V		
H3N8	HA	**T160A**	Increased virulence in mice Viral dissemination outside of respiratory tract, viral dissemination in brain, in mice Virulence and contact transmission in guinea pigs	43
	PB1	T413I, V609I		
	PB2	T235I, D408E, N448S, **D701N**		
	PA	**T97I**, P534S		
	NP	A27V		
	NS1	**P42S**		
	M1	**N30D**, T125A		
H3N8	PB2	R17C, T35A	Virulence in mice, Viral dissemination outside of respiratory tract, in mice	
	PA	**T97I**		
	M1	**N30D, T215A**		
H6N2	HA	**A110V, T160A**	Decreased fusion pH of HA	11

			Increased replication in mammalian cells Increased virulence in mice	
	PB2	G158E, **G309D**, T431M, **V598T, E627K**	Increased replication in mammalian cells Increased virulence in mice	
	PA	**K185R**, N338D		
	M1	**N30D, A166V**, T125A		
H11N9	PB2	**L89V, G309D, T339K**	Infectivity in mammalian cells Modest virulence in mice	177
	PB1	**H436Y**		
	PA	**A515T**		
	M1	**N30D, T215A**		
H5N2	HA	**S155N, T156A**	Infectivity in mammalian cells Virulence in mice	42
	PB2	**L89V, G309D, T339K, R477G, I495V, A676T**		
	PB1	**D3V, D622G**		
	PB1-F2	**N66S**		
	M1	**N30D, I43M, T215A**		
	NS1	**P42S, I106M, C138F**		
H5N3	HA	**D154N, S155N, T156A**	Infectivity in mammalian cells Virulence in mice	42
	PB2	**L89V, G309D, T339K, R477G, I495V, A676T**		
	PB1	**D3V, D622G**		
	M1	**N30D, I43M, T215A**		
H5N3	HA	**D154N, S155N, T156A**	Infectivity in mammalian cells Virulence in mice	42
	PB2	**L89V, G309D, T339K, R477G, I495V, A676T**		
	PB1	**D3V, D622G**		
	M1	**N30D, I43M, T215A**		
	NS1	**I106M, C138F**		
H5N8	HA	**D94N, S155N, T156A, V210I**	Infectivity in mammalian cells No virulence in mice	42
	PB2	**L89V, G309D, T339K, R477G, I495V, A676T**		
	PB1	**D3V, D622G**		
	PB1-F2	**N66S**		
	M1	**N30D, I43M, T215A**		
	NS1	**P42S, I106M, C138F**		
H5N6	HA	**S127P, S137A**	Virulent in mice Viral dissemination outside of respiratory tract in mice	18
	PB2	**L89V**		
	NA	**R289K**		
	M1	**N30D, T215A**		
	NS1	**P42S, L103F, I106M**		

H5N6	HA	R44G, D59N, S77R, Q120L, R125S, **S128P**, A146P, K174R, S188N, **V202I**, R288S, I274M, S325N, R372K, V400I, E484G, V532A, V537M	Increased infectivity in mammalian cells Increased binding to α2,6 Increased virulence in mice Viral dissemination outside of respiratory tract and viral dissemination in brain of mice Increased virulence and contact transmission in guinea pigs	23
	NA	N80S, K83R, H228Q, A267T, R287G, V304I, I306T		
	PB2	R62K, P112T, S137N, R144K, **T339K**, V384L, I457V, M473I, V495A, T524M, N559S, A613V, M636L, R699K, R755G		
	M	T34A, T183A, Y235C, S333N		
	PA	K216T, G359E, V546A		
	PB1	V22A, E62K		
H7N9	HA	A19S, E81K, I88V, T126K, V125T, S144P, **A160T,** K310R	Binding to α2,6 and α2,3 Virulent in mice Viral dissemination outside of respiratory tract and viral dissemination in brain of mice	23
H7N7	HA	V186G, **T160A**, **A135T, T136S**	Virulent in mice	22
	NA	E119P, R152T		
	M1	**N30D**, **T215A**		
	NS	**P42S**, D97E, L89Y		
	PB1	13P, 198K		
	PB2	**R389K**, N83D		
	PA	**V100A**		
H9N2	HA	183N, 190V, 225G, 228G	Virulent in mice Viral dissemination outside of respiratory tract and viral dissemination in brain of mice	55
	PB2	**E627K**, **N701D**		
	M2	**S31N**		
H1N3	PB2	**E627K**	Increased virulence and increased mortality in mice	7
	HA	**L67V**		
H7N9	PB1	H115Q	Increased infectivity in mammalian cells	324
	PB2	**E627K**		

			Increased virulence in mice Increased airborne transmission in guinea pigs	
H11N9	PB2	**K627E, N701D, L89V, G309D, T339K, R477G, I495V, K627E, A676T**	No change in virulence in mice Viral dissemination outside of respiratory tract and viral dissemination in brain of mice	233
	PB1	**Y436H**		
	PA	Q57R, V62I, L65S, A100V		
	NP	**A184K**		
	M1	**N30D**, **T215A**, C148, C151, H159, H162		
	M2	S50C		
	NS1	80–84 deletion, **P42S**, **N101D, L103F, I106M**, E227, S228, E229, V230		
H10N3	PB2	**E627V**	Binding to α2,6 and α2,3 Increased virulence in mice Increased contact transmission and increased aerosol transmission in guinea pigs	96
	HA	**G228S**		
	M1	**N30D, T215A**		
	M2	**S31N**		
H6Nx	HA	**T160A**	Infectivity in mammalian cells Virulent in mice	48
	PB1	**N66S**		
	M1	**N30D** **T215A**		
	NS1	**I101M** **P42S**		

‡Bolded mutations are present in tables 1-8.

¶Where the direction of change has not been indicated, the authors are reporting on descriptive phenotypes of an isolate without a comparison to WT virus.

Phenotypes associated with human isolates of avian influenza virus

Several studies have reported mutations found in avian influenza virus isolated from infected humans. In these studies, the authors have often not independently validated the phenotypes associated with particular mutations, but rather make note of those mutations present in the isolates for which phenotypes have previously been described. These mutations are captured in the table below. Note that this type of data was not captured in the review by Suttie *et al.*, and so the data below is derived from publications spanning our search from 2019-present.

Table 10. Molecular markers/motifs found in avian influenza A virus strains isolated from humans published 2019- July 2024‡.

Subtype	Segment	Mutation/motif	Phenotype (if known)	Country Isolation year/range	References
H7N4	HA	H242Q, N283D, K321E, I479M	N/A	China 2018	[325]
	NA	A137V, K296R			
	PB2	I385L, **E627K**, K683T			
	PB1-F2	N47S			
	PA	F105L, L441M			
	M1	M248I			
	M2	C19Y			
H7N9	HA	**L217Q**	Immune escape from serum*	China 2017	[89]
	NA	**R289K**	Reduced susceptibility to NA inhibitors*		
	PB2	**K526R** **M535L** **D701N**	Increased infectivity in mammalian cells* Increased polymerase activity* Increased virulence in mammals*		
	PA	**V100A** **S409N**	Host signature amino acids (avian to human)*		
	NP	V33I	Host signature amino acids (avian to human)*		
H5N1	PA	**T85A**	Increased polymerase activity in mammalian cells*	Cambodia 2010-2014	[8]
H5N1	PB2	**N348Y**	M7GTP cap binding site*		
	PA	**K615R**	Polymerase human adaptation marker*		

	HA	**E91G, K172T, T226A**	Increased binding to α2,6*		
	NS1	P159L	Nuclear export signal mask*		
H5N1	PB2	N392H	M7GTP cap binding site*		
	HA	A150V, **Y173H, N198S, Q238L**	Increased α2,6 binding* Mammalian transmission marker (Q238L)*		
H5N1	PB2	**E627K,** N701D	Increased mammalian replication*		
H5N1	PB2	V667I, S714G	Human association marker (V667I)* Increased pathogenicity (S714G)*		
	PB1	K353R, T566S	Increased pathogenicity*		
	HA	A150V, Q238R	Increased α2,6 binding* Mammalian transmission marker (Q238R)*		
H5N1	HA	**E142G, Q238L**	Glycosylation site (E142G)* Mammalian transmission marker (Q238L)*		
H5N1	PA	**S631G**	Increased virulence in mice*		
	NEP	E47G	Increased virulence in ferrets*		
H5N1	PB1	R211G	Interference with host machinery replication*		
	M2	C50Y	Increased virulence*		
H3N8	HA	N193, W222, S227, **G228S**	Increased α2,6 binding Associated with mammalian adaptation*	China 2022	[62]
	PB2	**I292V**, K318R, **E627K**	Associated with mammalian adaptation*		
	PB1	**L13P, L473V**			
	PA	**K26E, V160D,** K365R			
	NP	K398Q			

	NS1	**P42S**, E227K			
	M1	**V15I, N30D, T215A**			
H3N8	PA	K365R, S409	Increased virulence and polymerase activity in mammals*	China 2023	[262]
	M2	**S31N**	Decreased susceptibility to amantadine*		
H7N9	HA	G138V, **Q226L**	Increased binding to α2,6*	China 2017	[52]
	PB1	**I368V**	Increased transmission in ferrets*		
	PA	K365R, **S409N**	Species specific signatures*		
	NS1	**N205S**	Decreased host antiviral response*		
	M	**S31N**	Decreased susceptibility to amantadine*		
H5N6	HA	**T192I, Q226L, S2227N/R, G228S**	Altered receptor specificity*	China 2014	[65]
	NA	**E119V/A/D**	Reduced susceptibility to neuraminidase inhibitors*		
	M2	**V27A, A30V/T/S, S31N/G**	Reduced susceptibility to amantadine*		
	PA	I38M/T/S/L	Reduced susceptibility to endonuclease inhibitors*		
	PB2	**Q591K, E627K, D701N**	Increased virulence in mammalian models*		
	NS1	**D92E, L103F, I106M**	Altered virulence in mice*		
	M1	**N30D**, P41A, T139A, **T215A**	Altered virulence in mice*		
H10N3	HA	**Q226L**	Increased binding to α2,6*	China 2024	[87]
	PB2	**D701N**	Mammalian adaptation marker*		
	PB1	**I368V**	Increased transmission in ferrets*		
	PA	**S409N**	Host signature marker*		

	M2	**S31N**	Reduced susceptibility to M2 inhibitors		

* indicates that the phenotype for the mutation has been previously described, but not experimentally verified by the source.
‡Bolded mutations are present in tables 1-8.

Phenotypes associated with mammalian (non-human) isolates of avian influenza virus

As for Table 11, a number of studies have reported mutations found in avian influenza virus isolates that have infected other non-human mammals. The authors have often not independently validated the phenotypes associated with particular mutations, but rather make note of those mutations present in the isolates for which phenotypes have previously been described. These mutations are captured in the table below. Note that this type of data was not captured in the review by Suttie *et al.*, and so the data below is derived from publications spanning our search from 2019-present.

Table 11. Molecular markers/motifs found in avian influenza A virus strains isolated from mammals (excluding humans) published 2019- July 2024‡.

Subtype	Segment	Mutation/motif	Mammal	Phenotype (if known)	References
H5N1	PB2	**Q591K, D701N**	Sea lion	Increased virulence in mice* (Q591K) Mammalian adaptation* (D701N)	10
	PB1	L378M, S515A		Mammalian adaptation*	
	PA	R57Q, T85V, M86I			
	NP	Y289F			
	HA	**S123P, S133A**, S155D, D138N, V210A, **K218Q, S223R**			
	NA	S339P			
	M1	N87T			
	NS1	D26E, E60A, **Y103F**			
H5N1	PB2	**D701N**	Sea lion	Mammalian adaptation*	
	PB1	L378M			
	PA	R57Q, T85V			
	HA	H355R, **S123P**, **S133A**, S155D, D138N, V210A, **K218Q, S223R**			
	NA	A81T, S339P			
	M1	N87T			
	NS1	D26E, E60A, **Y103F, I205N**			
H5N1	PB1	S515A	Sea lion	Mammalian adaptation*	
	PA	R57Q, T85V			

	HA	H335R, **S123P, S133A**, S155D, D138N, V210A, **K218Q, S223R**			
	NP	Y289F			
	NA	A81T, S339P			
	M1	N87T			
	NS1	D26K, E60F, **Y103F**			
H5N1	PB2	T215M, N175T	Sea lion	Mammalian adaptation*	
	PB1	L378M			
	PA	**T85A**, M86I			
	HA	A496S, **S123P**, **S133A**, S155D, D138N, V210A, **K218Q, S223R**			
	NP	M222L, A428T, R452K			
	NA	V62I, A81T, S339P			
	M1	N87T			
	NS1	D26E, E60A, **Y103F**			
H5N1	PB1	L378M	Dolphin	Mammalian adaptation*	
	PA	**T85A**			
	HA	**S123P**, **S133A**, S155D, D138N, V120A, **K218Q, S223R**			
	NA	A81T, S339P			
	M1	N87T			
	NS1	D26E, E60A, **Y103F**			
H5N1	PB2	**T271A**	Fox	Meningoencephalitis in the brain Large numbers of infected cells in the cerebrum	[201]
H5N1	PB2	**M535I**	Fox		
H5N1	PB1-F2	T7I			
H5N1	PB2	**T271A**	Mink	N/A	[326]
	PB1	M317V, K388R			
	PA	M86T			
	HA	I390M			
	NA	F74S, V163L			

	NS2	D43G			
	PB1-F2	P30L			
H5N1	PB2	K526R, **E627K**	Domestic cat	Infectivity in mammalian cells	[327]
H5N1	PB2	**E627K**	Red fox	Dark red oedematous lungs Severe acute multifocal necrotizing pneumonia	[267]
	PA	**V387I**			
H5N1	PB2	**D701N**	Red fox		
	PA	K664R			
H5N1	PB2	I63V, G79S, F154L, K157E, V338T, I398V, T524I, **E627K**, **D701N**	Lynx	Multifocal acute hemorrhages in heart and lungs Sever necrotizing pneumonia	
	PA	L532I			
	HA	I335K			
	NA	**R220G**			
	M1	S272Y			
	M2	L43Y			
	NS1	N4K			
	NS2	N4K			
H5N1	HA	**K222Q**, 226L, **S227R**, 228S	Ezo red fox	Meningoencephalitis Immune infiltration in the brain Virus detected in brain, lungs, and trachea	[136]
	PB2	627E, 701D			
H5N1	HA	**K222Q**, 226L, **S227R**, 228S	Tanuki	Conjunctivitis and secondary bacterial infection	
	PB2	**627E**, 701D			
H5N1	HA	**S137A, T160A**, A185E, D195T, V198T, E268G, V210A	Red fox, skunk	Combinations of seizures, ataxia, lethargy, tremor, rolling on the ground, and other neurologic signs	[26]
	PB2	**L89V, G309D, T339K K389R**, R77G, I494V, **A676T**			
	PB1	**D3V, D622G**			
	PA	**S37A, N383D, N409S**			
	NP	**N319K**			

H5N1	HA	**S137A, T160A**, A185E, D195T, V198T, E268G, V210A	Red fox	Seizures	
	PB2	**L89V, T217A, G309D, T339K K389R**, R77G, I494V, **A676T**			
	PB1	**D3V, D622G**			
	PA	**S37A, N383D, N409S**			
	NP	**N319K**			
H5N1	HA	**S137A, T160A,** A185E, D195T, V198T, E268G, V210A	Red fox	Seizures, lethargy, tremor, and ataxia	
	PB2	**L89V, G309D, T339K K389R, E627K**, R77G, I494V, **A676T**			
	PB1	**D3V, D622G**			
	PA	**S37A, N383D, N409S**			
	NP	**N319K**			
H5N1	HA	**S137A, T160A**, A185E, D195T, V198T, E268G, V210A	Red fox	Neurological signs, seizures	
	PB2	**L89V, G309D, T339K K389R,** E627V, R77G, I494V, **A676T**			
	PB1	**D3V, D622G**			
	NP	**N319K**			
H5N1	HA	**S110N**, **P139L, L513S**	Red fox	Replicated in nasal cavity of swine, tissue pathology in lung in swine, direct contact transmission in swine	[13]
	PB2	**E627K**			
H5N1	HA	**S110N**, M40V	Racoon		

	PB2	**E627K**		Replicated in nasal cavity of swine Tissue pathology in lung in swine Direct contact transmission in swine	
H5N1	HA	**N110S**, T128P, T141S, V21A	Red fox	Neurological symptoms, seizures, depression, noisy crying, diarrhea, lethargy, salivating, and choking Virus present in brain, lungs, kidneys, and heart	[12]
	NA	**H155Y**, S248N, **N366S**, A39E			
	PB2	**V292I**			
	PB1	**N375S, D622G**			
	PA	**P28L, A36V**			
	NS	**P42S, I106M, V226I**			
	M	**N30D, S82N, T215A**			

* indicates that the phenotype for the mutation has been previously described, but not experimentally verified by the source.
‡Bolded mutations are present in tables 1-8.

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