

Avian influenza A virus adaptation to the equine host and identification of host-specific markers

V. MUCHA, J. HOLLÝ, E. VAREČKOVÁ, F. KOSTOLANSKÝ*

Institute of Virology, Biomedical Research Center Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovak Republic

Received December 15, 2017; accepted July 6, 2018

Summary. – Avian influenza A viruses (IAVs) are able to overcome the interspecies barrier and adapt to the new non-avian host. The process of adaptation requires the adaptive changes of IAV genome resulting in amino acid substitutions. The aim of this work was the description of amino acid substitutions in avian influenza A viruses (IAVs) occurring during their adaptation to equine host. Today, viruses of the equine influenza H3N8 subtype, first isolated in 1963, represent a single genetic lineage of IAV causing a respiratory disease in horses. We compared the amino acid sequences of the conserved proteins PB2, PB1, PA, NP, M1, M2, NS1 and NEP of equine influenza H3N8 subtype IAV with sequences of avian viruses, both available in the NCBI's Influenza Virus Resource Database. The amino acid substitutions persisting in equine IAV isolates and occurring in avian IAV at <5% frequency were included into the list of equine host-specific markers. We documented amino acid substitutions in the examined IAV proteins of equine IAV isolates in the period 1963–2013 and observed their quasi-linear accumulation. The substitution rate for the virus ribonucleoprotein (RNP) complex (PB2, PB1, PA, and NP) was calculated as $R = 0.69 \times 10^{-3}$ per position per year. For other examined proteins (M1, M2, NS1, and NEP), calculated R-values ranged from 0.48×10^{-3} to 1.30×10^{-3} per position per year. We identified 127 equine host markers distributed among all internal virus proteins, 38 of which were present already in 1963 and other 89 accumulated during the period since 1963 until 2013. Ten equine host marker positions overlap with known human marker positions (Miotto *et al.*, 2010) and five of them are occupied with identical amino acids in IAV of both hosts.

Keywords: equine influenza virus; equine H3N8 virus lineage; adaptation to host; amino acid substitution; host-specific marker

Introduction

Waterfowl is considered to be a reservoir of influenza A viruses (IAVs), which are potentially able to replicate and spread to new hosts after overcoming the interspecies barrier (Webster *et al.*, 1992). The best-described examples are the influenza pandemic that spread in humans in the 20th century, the repeated transmissions of highly pathogenic avian H5N1 viruses (Subbarao and Shaw, 2000), and the

recently described H7N9 influenza zoonotic infections of humans (Gao *et al.*, 2013). As these zoonotic IAV infections are considered to be a major epidemic threat for humans, the study of the evolutionary process of IAVs and their adaptation to new mammalian hosts is of high importance. Relevant data on amino acid changes accumulated in viral proteins during the adaptation of avian IAVs to humans were collected using a large number of IAV nucleotide genome sequences and amino acid sequences of the corresponding IAV proteins. The analysis of IAV human host markers is of great interest because disclosing the possible adaptation changes occurring over time can help to defend humans against zoonoses with pandemic potential. Sixty-eight human host markers in the PB2, PB1, PA, NP, M1, M2, NS1, NS2 (NEP) proteins were described by Miotto (Miotto *et al.*,

*Corresponding author. E-mail: virufkos@savba.sk; phone: +421-2-59302454.

Abbreviations: IAV(s) = influenza A virus(es); RNP = ribonucleoprotein

2010). These markers distinguish human IAV strains from those circulating among birds.

IAVs cause respiratory diseases also in horses. The first equine isolate of IAV was obtained in 1956 in Czechoslovakia and was characterized as an H7N7 subtype (A/eq/Prague/56) (Sovinova *et al.*, 1958). Equine H7N7 represented a distinct line, which disappeared after the year 1977, when the last equine H7N7 virus was isolated. During the outbreak of respiratory disease of horses in Florida in 1963, IAV of the H3N8 subtype (A/equine/Miami/1/1963[H3N8]) was isolated for the first time (Waddell *et al.*, 1963). Since the late 1970s until present, the H3N8 subtype has been the dominant equine IAV and persisted in horses until now (Yoon *et al.*, 2014).

Aquatic birds are considered to be a reservoir not only of human, but also of equine IAV. During bird-to-horse IAV transmission, and subsequent adaptation to equine host, viruses undergo changes in the genome and consequently in the encoded proteins, resulting in the ability to replicate and spread in equine hosts.

Utilizing the NCBI's Influenza Virus Resource database, we analysed IAVs of the H3N8 subtype isolated from equine hosts from the period 1963 up to the present, in order to describe the natural course of avian-to-equine IAV adaptation. We determined conserved equine IAV marker amino acids, which were characterized with respect to the time of their appearance, uniqueness for equine host, incidence in individual proteins, the type of amino acid substitution and the possible influence on the function of a particular protein. A comparison of equine host markers with those described in human IAV was also performed.

Materials and Methods

Equine IAV H3N8 viruses. Amino acid sequences of the conserved proteins (i.e., PB2, PB1, PA, NP, M1, M2, NS1, and NEP) of all equine influenza A H3N8 isolates (between 1963, when the first isolate was obtained, and 2013) found in the NCBI's Influenza Virus Resource database (Bao *et al.*, 2008) were utilized to perform the alignments.

Alignment of influenza A H3N8 equine lineage proteins. Accession numbers and alignments of aa sequences of analysed proteins of virus isolates with identified equine host markers are given in Supplementary Tables.

The amino acid sequences of a working set of each protein of interest were sorted by the year of isolation. Then, sequence alignment of the individual proteins was executed online at <http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html>.

Substitution rate determination. For each protein, a plot was constructed that showed the accumulation of substitutions in the lineages of individual proteins from 1963 to 2013. The alignment plots were assessed. Outliers, evidently not belonging to the lineage (e.g., the particular sequence was too distant from the H3N8 lineage),

were removed. To calculate the intrinsic amino acid substitution rate of influenza A H3N8 equine lineage, a number of substitutions accumulated in a recent (A/equine/Xuzhou/01/2013(H3N8)) in relation to an early A/equine/Miami/1/1963(H3N8) member of the lineage were counted. This approach was employed due to a quasi-linearity of substitutions that accumulated over time. The substitution rate, i.e., the number of amino acid substitutions per position per year was determined using the formula $R = S/(L \times Y)$, where S is the number of substitutions between two aligned sequences, L is the length of the protein (number of amino acids), and Y is time between isolations of the two aligned sequences, measured in years.

For the purpose of calculation of the number of substitutions in NS1 protein, a deleted part of C-terminal amino acids of NS1 protein was taken as a single substitution.

Equine markers identification. To delineate amino acid positions characterizing equine lineages in each internal protein, we compared the sequences of equine lineages with sequences from all avian hosts isolated until 2013, found in the database. This limitation was chosen to reduce the number of isolates, as no more than 10,000 sequences are allowed as input to the Analyze Sequence Variation (SNP) tool at <http://www.fludb.org>. First, we delineated the accumulation of amino acid substitutions in all internal proteins studied from equine isolates during the period 1963–2013. The year of appearance of any amino acid change that persisted at a given position was determined.

For each amino acid position, the consensus amino acid of the influenza A H3N8 equine lineage together with accumulated substitutions and their frequencies among avian isolates were determined. This data was obtained from the NIAID IRD (Squires *et al.*, 2012) at <http://www.fludb.org>. Within the influenza A H3N8 equine lineage we have been looking for amino acid sequence positions occupied with residues considerably distinct from those prevailing in avian isolates that fulfilled the following criteria. We identified amino acid positions that were conserved throughout the whole monitored time-span (1963–2013) and, at the same time, amino acids at these positions differed from those in the avian “consensus” sequence. Several of these amino acid substitutions are fully conserved in equine IAVs, i.e., they have no (or only a few) alternatives since 1963, and they occur among avian sequences at <5% frequency. These we refer to as “early” equine-specific markers. Moreover, we identified additional long-lasting conserved substitutions that emerged in years after 1963 and occurred among the avian sequences at <5% frequency, which we refer to as “recent” equine-specific markers.

Results

The sequences of over 100 equine IAVs isolated since 1963 are available in the NCBI's Influenza Virus Resource database. All sequences in the database of equine H3N8 isolated between 1963 and 2013 belonged to the genotype

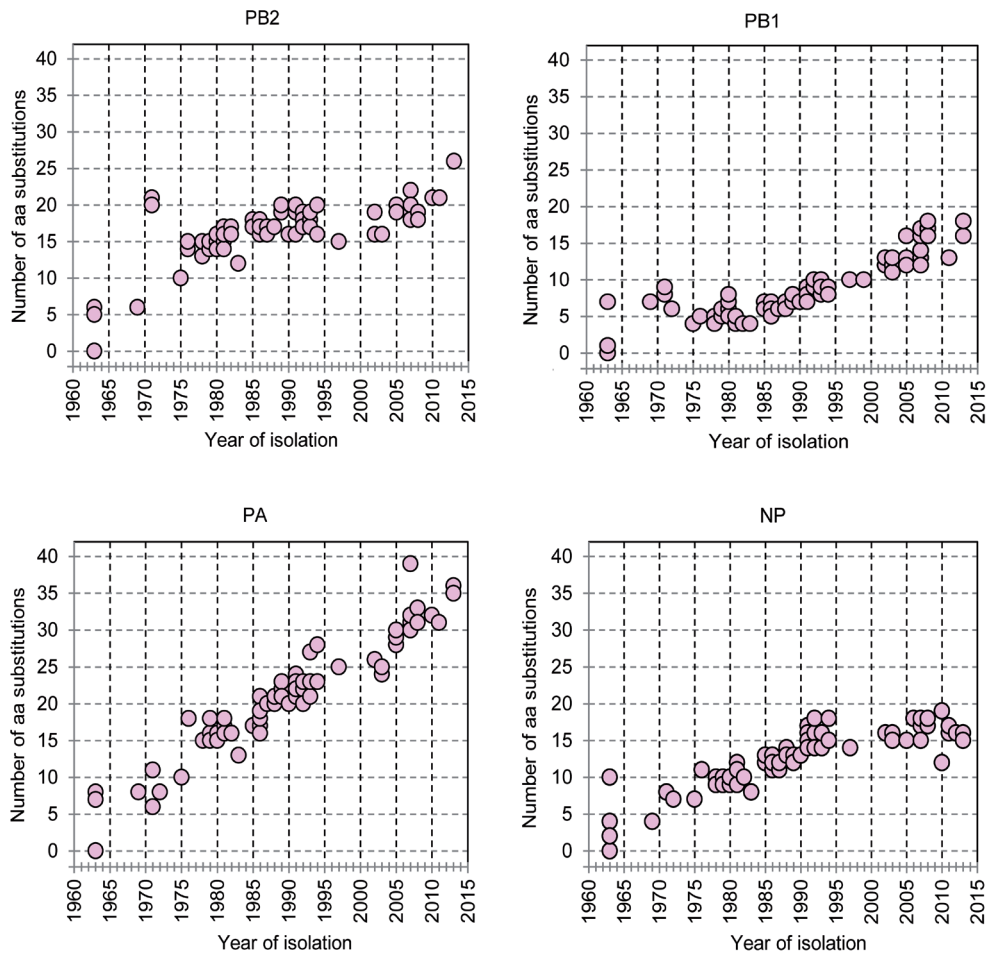


Fig. 1

Accumulation of amino acid substitutions in proteins of the RNP complex of the influenza A H3N8 equine lineage

Each point represents a particular sequence; some sequences are condensed to a single point due to identical coordinates. The number of amino acid substitutions in each isolate was counted with reference to the A/equine/Miami/1/1963 (H3N8) isolate.

comprising individual lineages of virus RNA segments (C/seg1, I/seg2, G/seg3, 3F/seg4, C/seg5, 8B/seg6, E/seg7, 1D/seg8) (Lu *et al.* 2007). Therefore, here we have used the term influenza A H3N8 equine lineage for sequences of this genotype.

Accumulation of substitutions in conserved proteins of the influenza A H3N8 equine lineage

We analysed amino acid sequences of conserved IAV proteins, i.e., proteins of the polymerase complex: PB2, PB1, PA, and NP; proteins encoded by segment 7 (M1 and M2); as well as by segment 8 (NS1, NEP) of H3N8 equine isolates available in the NCBI's Influenza Virus Resource database (Bao *et al.*, 2008). The amino acid sequences of particular proteins were sorted by the year of isolation. Their alignments showed gradual accumulation of substitutions in

proteins over a period of 50 years. Throughout the monitored time-span (1963–2013), the principal influenza A H3N8 equine lineage underwent mutations at several sequence locations, which subsequently became fixed. The plots in Fig. 1 and Fig. 2 demonstrate the number of substitutions within each isolate in comparison with an early virus isolate, A/equine/Miami/1/1963 (H3N8).

The number of substitutions occurring between the first sequenced isolate, A/equine/Miami/1/1963 (H3N8), and the latest isolate A/equine/Xuzhou/01/2013 (H3N8) was determined to calculate the amino acid substitution rate, i.e., the number of substitutions per position per year, for each protein. Among proteins in the polymerase complex, the highest substitution rate was found for PA ($R = 0.98 \times 10^{-3}$ amino acid substitutions per position per year), while the lowest rate was found in the PB1 protein with an amino acid substitution rate $R = 0.47 \times 10^{-3}$ (Table 1). Substitu-

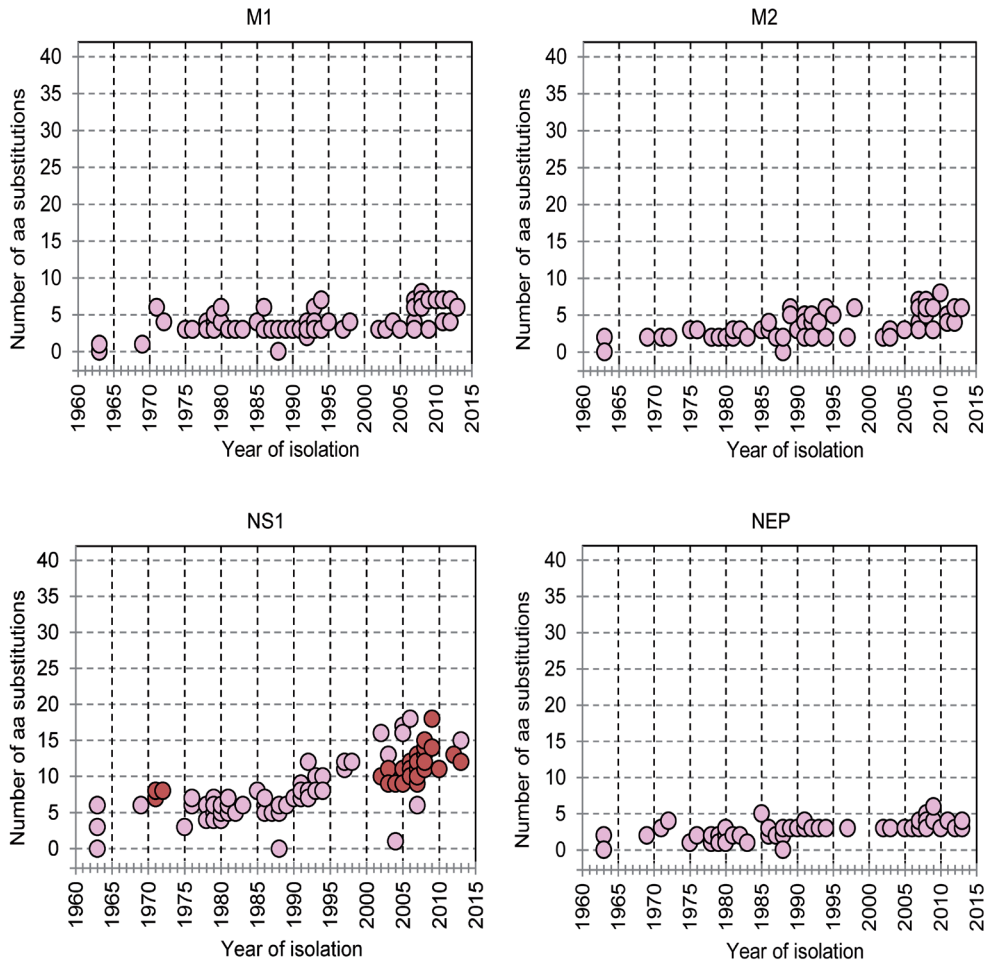


Fig. 2

Accumulation of amino acid substitutions in the M1, M2, NS1, and NEP proteins of the influenza A H3N8 equine lineage

Each point represents a particular sequence; some sequences are condensed to a single point due to identical coordinates. The number of amino acid substitutions in each isolate was counted with reference to the A/equine/Miami/1/1963 (H3N8) isolate. Dark-coloured circles (in NS1) stand for sequences in which C-terminal deletions were counted as a single substitution.

tion rates of NP (0.64×10^{-3}) and PB2 (0.69×10^{-3}) and the substitution rate for the entire polymerase complex (PB1, PB2, PA, and NP), $R = 0.69 \times 10^{-3}$ per position per year, were calculated. The following substitution rates were calculated for proteins encoded by segments 7 and 8: $R = 0.48 \times 10^{-3}$ for M1, $R = 1.24 \times 10^{-3}$ for M2, $R = 1.30 \times 10^{-3}$ for NS1, and $R = 0.66 \times 10^{-3}$ for NEP (Table 1). However, the linearity of amino acid substitution accumulation in proteins coded by segments 7 and 8 was not as apparent as it was in proteins of the RNP complex (compare Fig. 1 and Fig. 2).

Equine host markers

The amino acid sequences of PB2, PB1, PA, NP, M1, M2, NS1, and NEP of influenza A H3N8 equine lineage were

aligned with influenza A sequences isolated from avian hosts, which have been deposited in the NIAID Influenza Research database (IRD) at <http://www.fludb.org> (Squires *et al.*, 2012). Protein sequence variance analysis provided the “consensus” sequences of IAVs (all subtypes) isolated from avian hosts and a survey of alternative amino acids occurring at individual positions.

Amino acids defined as equine markers, which distinguish equine viruses from avian strains, are summarized in Table 2, 3, and 4. The frequency of an equine-specific amino acid at a given position among avian IAVs (<5%) reflects the equine host marker potency: the lower is the occurrence of an equine-specific amino acid among avian sequences, the higher is its marker potency. In proteins PB2, PB1, PA, NP, NS1, and NEP, we found both early and recent equine-

Table 1. Amino acid substitutions accumulated in proteins of the influenza A H3N8 equine lineage (period 1963–2013)

	Protein							
	PB2	PB1	PA	NP	M1	M2	NS1	NEP
L	759	757	716	498	252	97	230 ^a	121
S	26	16	36	16	6	6	15 ^b	4
S/Y	0.52	0.32	0.70	0.32	0.12	0.12	0.70	0.08
R	0.69 × 10⁻³	0.42 × 10⁻³	0.98 × 10⁻³	0.64 × 10⁻³	0.48 × 10⁻³	1.24 × 10⁻³	1.30 × 10⁻³	0.66 × 10⁻³

L = length of the protein (number of amino acids); S = number of substitutions between 2 aligned sequences, the early isolate A/equine/Miami/1/1963 (H3N8) and the late isolate A/equine/Xuzhou/01/2013 (H3N8); Y = time between the isolations of the 2 aligned sequences (50 years); R = substitution rate, i.e., the number of amino acid substitutions per position per year, calculated using the formula shown in the Methods section; ^aFull length of NS1 (without deletions in the C-terminus); ^bC-terminal deletions occurring in some isolates were counted up as a single substitution.

Table 2. Equine-specific markers identified in the equine influenza H3N8 virus proteins PB2 and PB1

Equine-specific markers in PB2					Equine-specific markers in PB1				
Pos.	Marker (%) ^a	Year of isolation	Marker potency	Avian	Pos.	Marker (%) ^a	Year of isolation	Marker potency	Avian
				Consensus					Consensus
Early equine markers					Early equine markers				
530	I (100)	1963	0.0005	T	157	S (100)	1963	0.0020	A
559	I (89)	1963-2013	0.0307	T	214	R (97)	1963	0.0355	K
	F (55)	1989-1994	0.0001		261	R (100)	1963	0.0000	S
586	R (100)	1963	0.0113	K	383	D (100)	1963	0.0216	E
701	N (100)	1963	0.0009	D	429	R (100)	1963	0.0062	K
Recent equine markers					Recent equine markers				
12	L (100)	2002	0.0080	S	61	I (100)	1975	0.0006	T
105	A (72)	2007	0.0334	T	114	I (100)	1997	0.0133	V
109	I (98)	1975	0.0463	V	119	M (76)	2007	0.0036	V
147	V (100)	1976	0.0285	I	164	M (100)	1975	0.0025	I
251	K (72)	2007	0.0150	R	198	R (100)	1988	0.0048	K
299	K (99)	1975	0.0191	R	221	T (100)	1997	0.0004	A
344	M (98)	1985	0.0173	V	317	I (100)	1997	0.0254	M
511	I (100)	1985	0.0496	V	329	R (76)	2007	0.0000	Q
575	I (99)	1976	0.0070	M	377	E (76)	2007	0.0017	D
588	T (99)	1975	0.0282	A	397	V (100)	1986	0.0022	I
613	A (100)	1975	0.0109	V	457	G (71)	2007-2008	0.0023	E
715	K (100)	1975	0.0005	N	587	T (100)	1975	0.0070	A
717	T (60)	1971	0.0213	A	618	D (76)	2007	0.0102	E
N = 98					N = 9609 (2011)				
					621 K (81) 1975-2013 0.0152				
					738 D (100) 1997 0.0017 E				
					N = 102				

^aPer cent of occurrence of a marker amino acid in a group of equine isolates since indicated year up to 2013 or as indicated. Marker potency is printed in bold if an equine marker amino acid occurs among avian influenza sequences at less than 1% frequency. Consensus sequence of avian IAV refers to the most frequent amino acid at a given position. N designates the number of analysed sequences, and (yyyy) means that all utilized avian strains were isolated until the indicated year.

specific markers. In contrast, no early markers were identified in the M1 and M2 protein sequences.

We did not include into the list of equine markers (Table 2, 3, and 4) those substitutions, which occurred during the monitored time-span, and reverted to the avian consensus sequence, nor substitutions that occurred after the year 2008, the stability of which is hard to predict.

The number of marker positions per unit length of the protein, i.e. the density of equine marker positions for individual proteins was determined as follows: NS1 (7.4%), NP (5.0%), PA (4.8%), NEP (4.1%), M2 (4.1%) PB1 (2.7%), PB2 (2.2%), M1 (1.6%).

Comparison of the influenza A H3N8 equine lineage to avian influenza sequences revealed 127 equine-specific

Table 3. Equine-specific markers identified in the equine influenza H3N8 virus proteins PA and NP

Equine-specific markers in PA					Avian	Equine-specific markers in NP					Avian
Pos.	Marker (%) ^a	Year of isolation	Marker potency	Consensus		Pos.	Marker (%) ^a	Year of isolation	Marker potency	Consensus	
Early equine markers						Early equine markers					
61	V (98)	1963	0.0342	I		41	V (100)	1963	0.0250	I	
63	I (99)	1963	0.0338	V		117	M (99)	1963	0.0003	R	
99	R (95)	1963	0.0104	G		146	T (100)	1963	0.0062	A	
217	H (100)	1963-1972	0.0000	Q		245	G (98)	1963	0.0021	S	
	Y (100)	1975-2013	0.0000			305	K (100)	1963	0.0065	R	
270	I (82)	1963-1994	0.0007	L		345	N (100)	1963	0.0003	S	
	M (100)	1997-2013	0.0003			351	K (100)	1963	0.0451	R	
272	IM (100)	1963-2013	0.0010	D		359	A (83)	1963-2013	0.0049	S	
	G (100)	1963	0.0164				T ^b (54)	2002-2012	0.0001		
336	Q (100)	1963	0.0002	L			AT (100)	1963-2013	0.0050		
689	S (100)	1963	0.0010	A		374	I (99)	1963	0.0216	M	
Recent equine markers						Recent equine markers					
55	N (100)	1975	0.0116	D		453	S (97)	1963	0.0041	P	
57	L (100)	1972	0.0003	R		496	F (99)	1963	0.0146	Y	
62	V (100)	1997	0.0282	I		Recent equine markers					
64	D (75)	2007	0.0012	E		16	D (98)	1988	0.0005	G	
86	I (75)	2007	0.0112	M		50	N (98)	1976	0.0192	S	
100	A (96)	1975	0.0187	V		100	K (98)	1985	0.0030	R	
114	K (78)	2008	0.0035	E		111	H (99)	1976-2012	0.0018	Y	
118	V (99)	1975	0.0229	I			Q (100)	2013-2013	0.0000		
158	R (60)	2007	0.0147	K		136	I (67)	2007	0.0010	L	
213	K (97)	2002	0.0128	R		257	T (67)	2007	0.0081	I	
216	N (99)	1976	0.0206	D		286	T (98)	1988	0.0001	A	
231	V (100)	1987	0.0005	A		293	K (100)	1975	0.0113	R	
237	K (75)	2007	0.0206	E		312	I (100)	1975	0.0032	V	
244	S (100)	1975	0.0002	G		319	K (100)	1975	0.0038	N	
261	S (100)	1975	0.0013	L		344	L (100)	1985	0.0103	S	
277	H (99)	1976	0.0006	S		384	K (95)	1997	0.0181	R	
343	E (97)	1997	0.0002	A		397	S (97)	1975	0.0262	N	
345	I (97)	1997	0.0053	L		498	S (99)	1975	0.0157	N	
353	R (94)	2002	0.0175	K		N = 110					N = 9580 (2011)
400	T (99)	1975	0.0092	PSQ							
437	Y (100)	1975	0.0180	H							
450	I (97)	1994	0.0054	V							
476	T (75)	2007	0.0008	A							
479	E (100)	1985	0.0022	D							
532	F (98)	1987	0.0000	L							
683	I (100)	1975	0.0123	L							
N = 100				N = 9580 (2011)							

^aPer cent of occurrence of a marker amino acid in a group of equine isolates since indicated year up to 2013 or as indicated. ^bAmino acid T (NP protein) has co-circulated with a since 2002. Marker potency is printed in bold if an equine marker amino acid occurs among avian influenza sequences at less than 1% frequency. Consensus sequence of avian IAV refers to the most frequent amino acid at a given position. N designates the number of analysed sequences, and (yyyy) means that all utilized avian strains were isolated until the indicated year.

marker amino acids in the analysed proteins. Of these, 38 early markers persisted since 1963 and additional 89 recent markers continued to emerge after 1963. Among all markers, 69 are of high potency (21 early and 48 recent), having <1% occurrence among avian influenza amino acid sequences (Table 2, 3, and 4).

Relationship of equine and human host markers

We compared equine host marker positions described above with those located at ‘characteristic sites’ identified in human influenza viruses by other authors (Finkelstein *et al.*, 2007; Miotto *et al.*, 2010) (Table 5). While Miotto found 68 human characteristic sites in PB2, PB1, PA, NP, M1, M2,

Table 4. Equine-specific markers identified in the equine influenza H3N8 virus proteins M1, M2, NS1, and NEP

Equine-specific markers in M1				Avian
Pos.	Marker (%) ^a	Year of isolation	Marker potency	Consensus
Recent equine markers				
80	I (79)	2007	0.0015	V
85	S (99)	1975	0.0027	N
95	K (79)	2007	0.0243	R
	K (86)	1976-2013	0.0000	
208	R (99)	2003-2013	0.0003	Q
	KR (99)	1976-2013	0.0003	
No. = 146				N = 9938 (2010)

Equine-specific markers in NS1				Avian
Pos.	Marker (%) ^a	Year of isolation	Marker potency	Consensus
Early equine markers				
96	D (96)	1963	0.0007	E
139	E (99)	1963	0.0044	D
156	V (98)	1963	0.0032	I
	H (90)	1963-2013	0.0006	
207	Y (29)	2006-2007	0.0000	N
	HY (96)	1963-2013	0.0006	
209	N (98)	1963	0.0326	D
213	S (96)	1963	0.0345	P
214	F (100)	1963	0.0090	L
Recent equine markers				
45	R (70)	2008-2009	0.0005	G
48	I (87)	2007	0.0068	S
59	H (88)	1997	0.0217	R
67	Q (97)	1981	0.0087	R
71	K (90)	1997	0.0116	E
84	I (84)	2006	0.0120	V
86	T (88)	1997	0.0470	A
186	K (98)	1975	0.0059	E
194	I (82)	1990	0.0150	V
216	S (88)	1997	0.0123	P
N = 169				N = 9621 (2009)

NS1, and NEP, only 10 equine-specific marker positions (out of total 127) are identical with them in both hosts. However, of these 10 positions, five are occupied by identical amino acids in human and equine IAVs, namely PA-55, PA-100, NP-16, NP-136, and NP-305 (Table 6). In spite of this, great majority of equine marker amino acids described here are located at positions differing from those found by Miotto *et al.* (2010) for human influenza viruses.

Equine-specific markers in M2				Avian
Pos.	Marker (%) ^a	Year of isolation	Marker potency	Consensus
Recent equine markers				
48	S (72)	2007	0.0017	F
50	F (99)	1975	0.0057	C
85	S (78)	2007	0.0000	D
89	S (76)	2007	0.0217	G
N = 144				N = 9571 (2010)

Equine-specific markers in NEP				Avian
Pos.	Marker (%) ^a	Year of isolation	Marker potency	Consensus
Early equine markers				
35	L (100)	1963	0.0160	F
63	E (99)	1963	0.0102	G
Recent equine markers				
33	I (95)	1976	0.0071	T
34	R (96)	1988	0.0283	Q
89	N (99)	1975	0.0083	I
N = 130				N = 9010 (2009)

^aPer cent of occurrence of a marker amino acid in a group of equine isolates since indicated year up to 2013 or as indicated. Marker potency is printed in bold if an equine marker amino acid occurs among avian influenza sequences at less than 1% frequency. Consensus sequence of avian IAV refers to the most frequent amino acid at a given position. N designates the number of analysed sequences, and (yyyy) means that all utilized avian strains were isolated until the indicated year.

Table 5. Number of marker positions of equine and human influenza viruses

Protein	Number of equine markers		Number of human markers ^a
	Early, already since 1963	All, fixed until 2013	
PB2	4	17	17
PB1	6	21	1
PA	8	34	17
NP	11	25	12
M1	0	4	3
M2	0	4	9
NS1	7	17	6
NEP	2	5	3
All	38	127	68

^aHuman markers refer to sites characteristic of human-to-human transmission, as reported by Miotto *et al.* (2010).

Table 6. Marker positions common for equine and human viruses

Protein/ position	Equine marker	^a Human marker	Prevailing amino acid in avian host
PB2/588	T(1975)	I	A
PA/55 ^d	N(1975)	N	D
PA/57	L(1972) ^b	Q	R
PA/100 ^d	A(1975)	A	V
PA/400	T(1975) ^b	L	PSQ
NP/16 ^d	D(1988) ^b	D	G
NP/100	K(1985) ^b	VI	R
NP/136 ^d	IM ^c	MI	L
NP/305 ^d	K(1963) ^b	K	R
NS1/84	I(2006)	TA	VS

^aSites characteristic of human-to-human transmission, as reported by Mitto *et al.* (2010). ^bAmino acids occurring in the avian IAV sequences at a <1% frequency, i.e., markers of high potency. ^cAmino acid M at position NP/136 occurs in the avian IAV sequences at a 8.4% frequency. ^dMarker position where the same amino acid both in human and equine virus strains are present.

Discussion

Aquatic birds are considered to be a reservoir of IAVs, which can overcome interspecies barriers under suitable conditions and infect mammals, including humans. Interspecies transmission of avian viruses requires adaptation changes in the genome, characteristic for a particular new host, to enable optimal replication. The accumulation of adaptive changes in the genome of IAV establishes a new virus lineage associated with the new host. Currently, the best studied human influenza virus lineages are the H1N1 (1918), H2N2 (1957), H3N2 (1968), and H1N1 (2009) subtypes. Less studied are the non-avian IAV lineages of other animal hosts.

During the last 50 years, the sequences of over 100 equine IAV isolates have been determined and are available in public databases. In this study, we analysed sequences of the influenza A H3N8 equine lineage, the only equine lineage currently in circulation. We focused on the conserved proteins, i.e. proteins of the RNA polymerase complex (PB2, PB1, PA, and NP), as well as the M1, M2, NS1, and NEP proteins. The aim was to describe the evolutionary dynamics of these proteins and to identify equine host markers, i.e. their positions and the amino acids characteristic for the equine host, absent or occurring very rarely (<5%) among avian influenza sequences. Such “persistent host markers” were described for avian-to-human IAV adaptation (Finkelstein *et al.*, 2007; Miotto *et al.*, 2010).

Our analysis showed that some of equine host markers were already present in 1963, when influenza A H3N8 virus was isolated from horses for the first time. IAV of subtype H7N7 circulated in horses before 1963 and was first isolated

in 1956. This lineage was of a different genotype and was competitively eliminated in 1970's by the onset of the H3N8 lineage (Murcia *et al.* 2011). The presence of several equine host markers in equine H3N8 viruses identified in isolates from 1963 indicates that equine IAVs branched off earlier.

According to our findings, the substitution rate R for all proteins of the polymerase complex (PB2, PB1, PA, and NP) of equine influenza virus equals 0.69×10^{-3} substitutions per year per amino acid position. Our observations did not show any reassortment event in the equine H3N8 lineage, as continuity of accumulation of amino acid substitutions was not interrupted since 1963 in any of examined internal genes. We showed that the accumulation of amino acid substitutions over time was linear, as was also shown for the human H1N1 lineage (Carter and Sanford, 2012). Comparison of markers defined here for equine H3N8 isolates with those of human H(1, 2, 3)N(1, 2) isolates showed that the equine and human lineages are evolutionarily distinct. In the equine H3N8 lineage, similar to human viruses, short-lived side branches were present for a certain period of time and then disappeared, and only one main lineage continues. Our results based on the sequence alignment show that differences between human and equine lineages have continued to grow over time. Accumulation of new equine markers has taken place mainly at positions where human markers are not present, namely at 117 positions out of a total of 127 positions.

Some amino acid substitutions described here as equine host markers can have an impact on the RNA-polymerase activity of the virus, progressively reducing its restrictions in the new host.

In the H3N8 equine lineage, we found a few amino acid substitutions occurring in the region of the **PB1-PA** interface. An amino acid substitution in PB1 (T61I) emerged in the Influenza A H3N8 equine lineage in 1975. This position is part of the region involved in the PB1 interaction with the PA-linker (aa 196–257) (Pflug *et al.*, 2014). In particular, at PA position 217, histidine was present already in 1963 but was substituted with tyrosine (H217Y) in 1975, and both amino acids have remained exclusively characteristic of equine viruses to date. Position 217 is localized inside a cluster of equine-specific markers (213, 216, 217, 231, 237, and 244) in the nuclear localisation signal 2 region of PA (aa 186–247) (Nieto *et al.*, 1994; Hu and Liu, 2015).

The E627K substitution in the **PB2** protein is a well-known adaptive change of IAV in mammalian hosts. Ninety-seven per cent of avian isolates have a glutamic acid (E) residue at this position. This substitution overcomes mammalian antiviral RIG-I impairment of vRNPs (Weber *et al.*, 2015). Surprisingly, all equine isolates also exclusively show an E (identically with avian IAVs) at this position. In contrast to avian IAVs, equine isolates possess an additional substitution – D701N. This mutation plays an important role in viral adaptation to the nuclear import machinery

and enhances the nuclear import of newly synthesized PB2 (Gabriel *et al.*, 2008). The N701 equine marker in PB2 was present already in 1963. Residue N701 is involved in binding mouse host protein importin $\alpha 5$ (Naffakh *et al.*, 2008) and was also described in human H5 and H7 isolates (Gabriel and Fodor, 2014). However, it was not found in any human H1N1 or H3N2 lineages. Other equine-characteristic substitutions N715K and A717T had occurred already in 1975 and 1971, respectively, and these positions, similar to position 701, are localized in the binding region for the host protein importin alpha 5 (Naffakh *et al.*, 2008). The equine-characteristic amino acids at positions 701 and 715 mentioned above are present only in <0.1% of avian isolates; therefore, we consider them to be of high importance for IAV replication in equine host. PB2 protein plays also an important role in recognizing host mRNA caps, essential during IAV replication. Changes in the cap-binding domain of PB2 can influence host specificity. In equine IAV isolates, we found a V344M substitution, localized in the cap-binding domain of PB2 (residues 318–483) described by Guilligay *et al.* (2008) and by Pflug *et al.* (2014).

The region restricted by amino acid positions 107–134, the putative active endonuclease site of PA (Yuan *et al.* 2009), has involved equine host markers K114 and V118, which have been present in equine isolates since 2008 and 1975, respectively.

The NP N319K substitution described here as an equine host marker was shown to be associated with enhanced binding of the NP protein to importin alpha 1 in mammalian cells (Gabriel *et al.*, 2008). We identified an early equine marker V41 on NP. This position was described in the context of enhanced H7N9 IAV replication at lower temperature as potentially contributing to viral transmissibility (Zhu *et al.*, 2015).

NP of IAV plays a crucial role in the resistance to interferon-inducible Mx protein, an antiviral host factor. Similar to human MxA, equine Mx1 is phylogenetically distant from the avian Mx protein (Haller *et al.*, 2015). Therefore, equine IAVs are also under constant evolutionary pressure by Mx1 restriction. Amino acid substitutions in NP associated with MxA resistance among human IAVs can be only partially found in the equine NP. The human MxA-resistance NP amino acid cluster is thought to be determined mainly by V/I100, P283, and Y313 with additional amino acids associated with MxA resistance: N52, D53, H289, Y/V313, K305, M316, K350, K351, I353, K357 (Mänz *et al.*, 2013; Riegger *et al.* 2015; Götz *et al.*, 2016). Importantly, it is believed that MxA-resistance amino acid substitutions must be accompanied by compensatory stabilizing mutations to maintain viral fitness. Such compensatory mutations were determined to occur at amino acids D16, C385, and Y101 (Götz *et al.*, 2016).

We found only three equine-specific amino acids at positions associated with MxA resistance matching the equine NP sequence: K305, K351, and I353. We identified two of

them (K305 and K351) as early equine-adaptation markers. The third substitution, V353I, occurs at >5% frequency among avian isolates, therefore, we did not include it into the list of equine-specific substitutions. The R100K substitution is a recent marker that may also play a role in overcoming Mx1 restriction in further virus-adaptation processes. Moreover, we identified a recent equine-adaptation marker (G16D substitution), which is still the only documented compensatory stabilizing substitution that does not abrogate Mx1 resistance acquired by substitutions mentioned above (Götz *et al.*, 2016). It is possible that some early equine markers on NP could represent further stabilizing mutations, like G16D, contributing to viral fitness. There is also an indication that the Mx1 polymorphism may be associated with equine resistance to IAVs (Manuja *et al.*, 2014).

Until the 1970's, influenza M proteins were not involved in the adaptation process to a new equine host, in contrast to other virus proteins. All equine host markers in the **M1 and M2** proteins that can influence the functional properties of M1/M2 were introduced in the 1970s or later, which means that in these proteins are present only recent markers. The first adaptation changes in the M1 protein within the influenza a equine H3N8 lineage occurred in 1975 (N85S) and 1976 (Q208K). Recent equine markers identified in the M1 protein (80I, 85S, and 95K) are localized in the amino acid region 76–115, which is essential for NEP binding (Shimizu *et al.*, 2011). In the M2 protein the substitutions C50F and G89S may influence phosphorylation (Holsinger *et al.*, 1995; Schnell *et al.*, 2008), but do not influence M2 ion channel activity. Therefore, changes at these positions are probably not essential for avian IAV adaptation to equine host.

The **NS1** protein is composed of two main parts – the effector domain and the RNA-binding domain. These domains comprise numerous sites mediating the various activities of the NS1 protein (Marc, 2014). The equine marker positions in NS1 are almost all localized outside of the known active sites. The only exception is the substitution E96D, which is positioned in the $\alpha 4$ helix of the effector domain, the binding site for phosphatidylinositol 3-kinase. Amino acid E96 is highly conserved in birds, bats, and human isolates, but the equine H3N8 lineage possesses aspartic acid (D) in this position, which was already present in 1963 isolates. Another amino acid substitution (E186K occurring in 1975) is localized in NS1 contact area with a cellular 30-kDa subunit of cleavage and polyadenylation specificity factor CPSF30 (Das *et al.*, 2008). Both equine markers in NS1 – 96D and 186K – are of high potency. The mutation G45R in NS1 was described to induce increased virulence and an earlier robust pro-inflammatory response in mice (Kaewborisuth *et al.*, 2017). The same mutation appeared as a high-potency marker in equine strains in 2008–2009.

Shortened form of NS1 protein, due to the deletion of eleven amino acids at C-terminus, appeared in 2002 in the

majority of equine lineage isolates. Such shortened form of this protein occurs in avian isolates permanently with a 10% frequency.

In NEP, the equine marker I89N (emerged in 1975) forms part of the interaction site with M1 and functions in the nuclear export of progeny vRNPs (Shimizu *et al.*, 2011). The nuclear export signals, NES1 and NES2, in NEP are recognized by chromosome region maintenance protein 1, which mediates export of the viral RNP complex out of the nucleus. The T33I, Q34R, and F35L substitutions, determined as equine-adaptation changes, reside inside NES2 (31-IITQFESLKI-40) and can affect the export of RNP proteins and, consequently, the budding of progeny virions (Li *et al.*, 2015).

Conclusion

In conclusion, we identified 127 equine host markers that distinguish present-day equine H3N8 viruses from avian IAVs. Thirty-eight of them were already present in 1963 and another 89 accumulated during the period of 1963–2013. Among all 127 equine markers, 69 (21 early and 48 recent) are of high-potency, i.e., the particular amino acid at the given position occurs among avian strains at <1% frequency, or they do not occur at all.

We identified 10 overlapping human and equine marker positions in proteins PB2, PA, NP, and NS1, 5 of which represent identical amino acid residues in corresponding positions. This indicates that some common (mammalian?) features arose during the process of adaptation of avian IAV to human or equine host. However, as follows from the comparative analysis of human and equine markers, the great majority of them localize to distinct positions, in a strictly host-specific manner. Such a divergence of markers indicates the breadth of the evolutionary diversity of equine and human IAVs.

Acknowledgments. This work was supported by grants VEGA 2/0146/15 and VEGA 2/106/17 from the Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences and by grant APVV-0250-10 from the Slovak Research and Development Agency.

Supplementary information is available in the online version of the paper and in the EXCELL format from corresponding author.

References

- Bao Y, Bolotov P, Dernovoy D, Kiryutin B, Zaslavsky L, Tatusova T, Ostell J, Lipman D (2008): The influenza virus resource at the National Center for Biotechnology Information. *J. Virol.* 82, 596–601. <https://doi.org/10.1128/JVI.02005-07>
- Carter RW, Sanford JC (2012): A new look at an old virus: patterns of mutation accumulation in the human H1N1 influenza virus since 1918. *Theor. Biol. Med. Model.* 9, 42. <https://doi.org/10.1186/1742-4682-9-42>
- Das K, Ma LC, Xiao R, Radvansky B, Aramini J, Zhao L, Marklund J, Kuo RL, Twu KY *et al.* (2008): Structural basis for suppression of a host antiviral response by influenza A virus. *PNAS* 105, 13093–13098. <https://doi.org/10.1073/pnas.0805213105>
- Finkelstein DB, Mukatira S, Mehta PK, Obenauer JC, Su X, Webster RG, Naeye CW (2007): Persistent host markers in pandemic and H5N1 influenza viruses. *J. Virol.* 81, 10292–10299. <https://doi.org/10.1128/JVI.00921-07>
- Gabriel G, Fodor E (2014): Molecular determinants of pathogenicity in the polymerase complex. *Curr. Top. Microbiol. Immunol.* 385, 35–60. https://doi.org/10.1007/82_2014_386
- Gabriel G, Herwig A, Klenk H-D (2008): Interaction of polymerase subunit PB2 and NP with importin $\alpha 1$ is a determinant of host range of influenza A virus. *PLoS Pathog.* 4, e1–e11. <https://doi.org/10.1371/journal.ppat.0040011>
- Gao R, Cao B, Hu Y, Feng Z, Wang D *et al.* (2013): Human infection with a novel avian-origin influenza A (H7N9) virus. *N. Engl. J. Med.* 368, 1888–1897. <https://doi.org/10.1056/NEJMoa1304459>
- Götz V, Magar L, Dornfeld D, Giese S, Pohlmann A, Höper D, Kong BW, Jans DA, Beer M *et al.* (2016): Influenza A viruses escape from MxA restriction at the expense of efficient nuclear vRNP import. *Sci. Rep.* 6, 23138. <https://doi.org/10.1038/srep23138>
- Guilligay D, Tarendeau F, Resa-Infante P, Coloma R, Crepin T, Sehr P, Lewis J, Ruigrok RWH, Ortin J, Hart DJ, Cusack S (2008): The structural basis for cap binding by influenza virus polymerase subunit PB2. *Nat. Struct. Mol. Biol.* 15, 500–506. <https://doi.org/10.1038/nsmbl.1421>
- Haller O, Staeheli P, Schwemmler M, Kochs G (2015): Mx GTPases: dynamin-like antiviral machines of innate immunity. *Trends Microbiol.* 23, 154–163. <https://doi.org/10.1016/j.tim.2014.12.003>
- Holsinger LJ, Shaughnessy MA, Micko A, Pinto LH, Lamb RA (1995): Analysis of the posttranslational modifications of the Influenza virus M2 protein. *J. Virol.* 69, 1219–1225.
- Hu J, Liu X (2015): Crucial role of PA in virus life cycle and host adaptation of influenza A virus. *Med. Microbiol. Immunol.* 204, 137–149. <https://doi.org/10.1007/s00430-014-0349-y>
- Kaewborisuth C, Kaplan B, Zanin M, Finkelstein D, Webby RJ, Lekcharoensuk P (2017): G45R on nonstructural protein 1 of influenza A virus contributes to virulence by increasing the expression of proinflammatory cytokines in mice. *Arch. Virol.* 162, 45–55. <https://doi.org/10.1007/s00705-016-3072-8>
- Li J, Yu M, Zheng W, Liu W (2015): Nucleocytoplasmic Shuttling of Influenza A virus Proteins. *Viruses* 7, 2668–2682. <https://doi.org/10.3390/v7052668>
- Lu G, Rowley T, Garten R, Donis RO (2007): FluGenome: a web tool for genotyping influenza A virus. *Nucleic Acids Res.* 35, Web Server issue W275–W279. <https://doi.org/10.1093/nar/gkm365>

- Manuja BK, Manuja A, Dahiya R, Singh S, Sharma RC, Gahlot SK (2014): Diversity of interferon inducible Mx gene in horses and association of variations with susceptibility vis-à-vis resistance against equine influenza infection. *Infect. Genet. Evol.* 27, 142–148. <https://doi.org/10.1016/j.meegid.2014.07.018>
- Mänz B, Dornfeld D, Götz V, Zell R, Zimmermann P, Haller O, Kochs G, Schwemmler M (2013): Pandemic Influenza A Viruses Escape from Restriction by Human MxA through Adaptive Mutations in the Nucleoprotein. *PLoS Pathog.* 9, e1003279. <https://doi.org/10.1371/journal.ppat.1003279>
- Marc D (2014): Influenza virus non-structural protein NS1: interferon antagonism and beyond. *J. Gen. Virol.* 95, 2594–2611. <https://doi.org/10.1099/vir.0.069542-0>
- Miotto O, Heiny AT, Albrecht R, García-Sastre A, Tan TW, August JT, Bruscia V (2010): Complete-proteome mapping of human influenza A adaptive mutations: implications for human transmissibility of zoonotic strains. *PLoS ONE* 5, e9025. <https://doi.org/10.1371/journal.pone.0009025>
- Murcia PR, Wood JL, Holmes EC (2011): Genome-scale evolution and phylodynamics of equine H3N8 influenza A virus. *J. Virol.* 85, 5312–5322. <https://doi.org/10.1128/JVI.02619-10>
- Naffakh N, Tomoiu A, Rameix-Welti MA, van der Werf S (2008): Host restriction of avian influenza viruses at the level of the ribonucleoproteins. *Annu. Rev. Microbiol.* 62, 403–424. <https://doi.org/10.1146/annurev.micro.62.081307.162746>
- Nieto A, de la Luna S, Bfircena J, Portela A, Ortin J (1994): Complex structure of the nuclear translocation signal of influenza virus polymerase PA subunit. *J. Gen. Virol.* 75, 29–36. <https://doi.org/10.1099/0022-1317-75-1-29>
- Pflug A, Guilligay D, Reich S, Cusack S (2014): Structure of influenza A polymerase bound to the viral RNA promoter. *Nature* 516, 355–360. <https://doi.org/10.1038/nature14008>
- Riegger D, Hai R, Dornfeld D, Mänz B, Leyva-Grado V, Sánchez-Aparicio MT, Albrecht RA, Palese P, Haller O *et al.* (2015): The nucleoprotein of newly emerged H7N9 influenza A virus harbors a unique motif conferring resistance to antiviral human MxA. *J. Virol.* 89, 2241–2252. <https://doi.org/10.1128/JVI.02406-14>
- Schnell JR, Chou JJ (2008): Structure and mechanism of the M2 proton channel of influenza A virus. *Nature* 451, 591–595. <https://doi.org/10.1038/nature06531>
- Shimizu T, Takizawa N, Watanabe K, Nagata K, Kobayashi N (2011): Crucial role of the influenza virus NS2 (NEP) C-terminal domain in M1 binding and nuclear export of vRNP. *FEBS Lett.* 585, 41–46. <https://doi.org/10.1016/j.febslet.2010.11.017>
- Sovinova O, Tumova B, Pouska F, Nemeč J (1958): Isolation of a virus causing respiratory disease in horses. *Acta Virol.* 2, 52–61.
- Squires RB, Noronha J, Hunt V, García-Sastre A, Macken C, Baumgarth N, Suarez D, Pickett BE, Zhang Y *et al.* (2012): Influenza Research Database: an integrated bioinformatics resource for influenza research and surveillance. *Influenza Other Respir. Viruses* 6, 404–416. <https://doi.org/10.1111/j.1750-2659.2011.00331.x>
- Subbarao K, Shaw MN (2000): Molecular aspects of avian influenza (H5N1) viruses isolated from humans. *Rev. Med. Virol.* 10, 337–348. [https://doi.org/10.1002/1099-1654\(200009/10\)10:5<337::AID-RMV292>3.0.CO;2-V](https://doi.org/10.1002/1099-1654(200009/10)10:5<337::AID-RMV292>3.0.CO;2-V)
- Waddell GH, Teigland MB, Sigel MM (1963): a new influenza virus associated with equine respiratory disease. *J. Am. Vet. Med. Assoc.* 143, 587–590.
- Weber M, Sediri H, Felgenhauer U, Binzen I, Bänfer S, Jacob R, Brunotte L, García-Sastre A, Schmid-Burgk JL *et al.* (2015): Influenza virus adaptation PB2-627K modulates nucleocapsid inhibition by the pathogen sensor RIG-I. *Cell Host Microbe* 17, 309–319. <https://doi.org/10.1016/j.chom.2015.01.005>
- Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y (1992): Evolution and ecology of influenza A viruses. *Microbiol. Rev.* 56, 152, 179.
- Yoon SW, Webby RJ, Webster RG (2014): Evolution and Ecology of Influenza A Viruses. In Compans RW, Oldstone MBA (Eds): *Influenza Pathogenesis and Control – Volume I*, Springer International Publishing Switzerland, pp. 359–375. https://doi.org/10.1007/82_2014_396
- Yuan P, Bartlam M, Lou Z, Chen S, Zhou J, He X, Lv Z, Ge R, Li X *et al.* (2009): Crystal structure of an avian influenza polymerase PA(N) reveals an endonuclease active site. *Nature* 458, 909–913. <https://doi.org/10.1038/nature07720>
- Zhu W, Zou X, Zhou J, Tang J, Shu Y (2015): Residues 41V and/or 210D in the NP protein enhance polymerase activities and potential replication of novel influenza (H7N9) viruses at low temperature. *Virol. J.* 12, 71–75. <https://doi.org/10.1186/s12985-015-0304-6>

Supplementary information

Avian influenza A virus adaptation to the equine host and identification of host-specific markers

V. MUCHA, J. HOLLÝ, E. VAREČKOVÁ, F. KOSTOLANSKÝ

Institute of Virology, Biomedical Research Center Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovak Republic

Received December 15, 2017; accepted July 6, 2018

Supplementary Tables. Acc. Nos. alignments of aa sequences of analysed proteins of virus isolates with identified equine host markers (these supplementary tables are also available in Excell format from the corresponding author F. Kostolanský, E-mail: virufkos@savba.sk)

Legend:

- 1. Alternatives:** aa different from consensus , e.g., Q1 means that Q occurred in one isolate
- 2. Score:** Data taken from the Database describing measure of variability.
- 3. Alignment Details:** number of occurrences of various amino acids.
- 4. High-potency and Low-potency markers** are colored green and yellow, respectively.

Equine Pos	PB1 Consensus	H3N8 No of Alternatives	Permanent aa change	From To		Year of substitution	Marker	Avian PB1 all subtypes			Alignment Details	#
				From	To			Pos	Consensus	Score		
1	M	0						1	M	0	M=9597	9597
2	D	0						2	D	2	A=2, D=9586, E=4, G=4, S=1, V=1	9598
3	V	0						3	V	2	A=2, C=1, I=8, L=1, F=1, V=9585	9598
4	N	0						4	N	3	N=9582, D=1, Del=5, H=2, I=5, K=2, F=1, S=1, T=2, Y=1	9602
5	P	0						5	P	2	R=1, Del=5, Q=2, L=2, F=1, P=9594, S=1	9606
6	T	0						6	T	3	A=9, D=3, Del=5, I=1, P=1, S=1, T=9587	9607
7	L	0						7	L	2	Del=6, E=1, L=9593, F=7, S=1	9608
8	L	0						8	L	3	Del=6, I=7, L=9585, F=7, P=1, T=1, V=1	9608
9	F	0						9	F	1	L=1, F=9604, S=2, Y=1	9608
10	L	0						10	L	1	Del=2, L=9601, F=1, P=1, S=2, V=1	9608
11	K	0						11	K	3	R=13, N=2, Del=1, E=8, K=9584	9608
12	V	0						12	V	13	D=1, Del=1, E=2, G=1, I=96, L=3, M=28, S=2, V=9473, Xaa=1	9608
13	P	1	Q1					13	P	0	H=1, P=9607	9608
14	A	0						14	A	67	A=7948, G=1, S=1, T=4, V=1654	9608
15	Q	0						15	Q	1	A=1, R=1, Q=9603, H=2, P=1	9608
16	N	0						16	N	3	N=9580, D=4, S=24	9608
17	A	0						17	A	1	A=9603, G=3, V=2	9608
18	I	0						18	I	1	I=9597, L=1, V=10	9608
19	S	0						19	S	1	N=1, D=1, C=1, G=1, S=9604	9608
20	T	0						20	T	11	A=7, I=123, P=1, S=2, T=9475	9608
21	T	0						21	T	0	P=2, T=9606	9608
22	F	0						22	F	0	I=1, F=9606, Xaa=1	9608
23	P	0						23	P	0	P=9607, T=1	9608
24	Y	0						24	Y	0	S=1, Y=9607	9608
25	T	0						25	T	0	A=1, R=1, I=1, T=9605	9608
26	G	0						26	G	0	E=1, G=9607	9608
27	D	0						27	D	2	A=3, N=8, D=9592, E=3, G=2	9608
28	P	0						28	P	0	A=1, K=2, P=9605	9608
29	P	0						29	P	0	P=9607, S=1	9608
30	Y	0						30	Y	1	H=6, F=1, S=2, Y=9599	9608
31	S	0						31	S	0	N=2, C=1, S=9605	9608
32	H	0						32	H	0	R=1, H=9607	9608
33	G	0						33	G	0	G=9608	9608
34	T	0						34	T	0	A=1, E=1, P=1, T=9605	9608
35	G	1	E1					35	G	0	G=9608	9608
36	T	0						36	T	0	A=1, P=1, T=9606	9608
37	G	0						37	G	0	G=9608	9608
38	Y	0						38	Y	2	C=3, H=9, Y=9595, V=1	9608
39	T	0						39	T	1	A=10, I=1, P=1, T=9596	9608
40	M	0						40	M	4	I=25, L=7, M=9575, T=1, Xaa=1	9609
41	D	0						41	D	0	D=9607, E=2	9609
42	T	0						42	T	0	A=1, P=1, T=9607	9609
43	V	0						43	V	1	A=2, I=3, F=1, V=9603	9609
44	N	0						44	N	5	N=9567, D=2, I=2, S=21, T=17	9609
45	R	0						45	R	0	R=9607, G=1, I=1	9609
46	T	0						46	T	0	A=1, T=9608	9609
47	H	0						47	H	0	R=1, H=9607, L=1	9609
48	Q	0						48	Q	13	N=2, Q=9449, H=2, L=1, K=154, P=1	9609
49	Y	0						49	Y	0	C=3, Y=9606	9609
50	S	0						50	S	1	A=3, P=1, S=9605	9609
51	E	0						51	E	1	N=1, D=1, E=9601, G=3, K=2, Xaa=1	9609
52	K	0						52	K	18	R=166, N=32, Q=4, E=10, K=9391, M=6	9609
53	G	0						53	G	0	G=9609	9609
54	K	0						54	K	45	R=701, N=8, Q=1, E=65, I=1, K=8832, V=1	9609
55	W	0						55	W	0	R=2, Del=1, W=9606	9609
56	T	0						56	T	13	A=141, I=8, K=2, M=1, S=2, T=9455	9609
57	T	0						57	T	16	A=13, Q=2, I=26, K=122, M=20, T=9426	9609
58	N	0						58	N	1	N=9598, H=2, S=8, Xaa=1	9609
59	T	0						59	T	81	A=2, I=2, L=1, M=1, P=11, S=2271, T=7321	9609
60	E	0						60	E	2	D=4, E=9596, G=2, K=2, V=5	9609
61	I	8	T8	T	I	1975	0,0006	61	T	1	A=1, I=6, S=1, T=9601	9609
62	G	0						62	G	17	R=38, N=6, D=1, E=36, G=9416, K=110, Y=2	9609
63	A	0						63	A	0	A=9607, S=1, V=1	9609
64	P	2	L2					64	P	10	A=9, R=1, H=3, L=73, P=9506, S=15, T=2	9609
65	Q	0						65	Q	1	Q=9602, H=1, L=1, P=5	9609
66	L	0						66	L	1	L=9605, F=1, V=3	9609
67	N	0						67	N	1	N=9603, D=1, H=1, S=4	9609
68	P	0						68	P	0	Q=1, P=9608	9609
69	I	0						69	I	3	I=9577, T=1, V=30, Xaa=1	9609
70	D	0						70	D	1	N=3, D=9602, E=1, G=2, Xaa=1	9609
71	G	0						71	G	0	G=9608, Xaa=1	9609
72	P	0						72	P	0	L=1, P=9607, Xaa=1	9609
73	L	0						73	L	1	Q=1, I=3, L=9603, V=1, Xaa=1	9609
74	P	0						74	P	1	P=9599, S=9, Xaa=1	9609
75	E	0						75	E	22	D=198, E=9339, G=15, L=1, K=13, V=42, Xaa=1	9609
76	D	0						76	D	17	N=188, D=9391, E=5, G=22, S=1, Y=1, Xaa=1	9609
77	N	0						77	N	4	N=9571, D=17, H=18, I=1, T=1, Xaa=1	9609
78	E	0						78	E	8	D=82, Q=1, E=9522, G=2, K=1, Xaa=1	9609
79	P	0						79	P	0	P=9608, Xaa=1	9609
80	S	0						80	S	5	R=2, N=26, C=5, I=3, S=9561, T=11, Xaa=1	9609
81	G	0						81	G	0	R=1, G=9607, Xaa=1	9609
82	Y	0						82	Y	0	S=2, Y=9606, Xaa=1	9609
83	A	0						83	A	2	A=9591, G=2, S=13, T=1, V=1, Xaa=1	9609
84	Q	0						84	Q	1	R=2, Q=9605, H=1, Xaa=1	9609
85	T	0						85	T	1	A=4, M=2, T=9602, Xaa=1	9609
86	D	0						86	D	3	D=9584, E=23, G=1, Xaa=1	9609
87	C	0						87	C	0	C=9608, Xaa=1	9609
88	V	0						88	V	0	V=9608, Xaa=1	9609
89	L	0						89	L	1	I=6, L=9602, Xaa=1	9609
90	E	0						90	E	0	E=9607, K=1, Xaa=1	9609
91	A	0						91	A	1	A=9601, D=1, S=1, V=5, Xaa=1	9609
92	M	0						92	M	1	I=1, L=2, M=9603, V=2, Xaa=1	9609
93	A	0						93	A	0	A=9606, D=1, S=1, Xaa=1	9609
94	F	13	L12S1					94	F	8	C=3, L=33, F=9535, S=14, Y=21, Xaa=3	9609
95	L	0						95	L	1	L=9605, M=1, Xaa=3	9609
96	E	0						96	E	2	D=13, E=9589, G=4, Xaa=3	9609
97	E	0						97	E	12	A=3, R=1, N=18, D=12, E=9493, G=9, K=65, V=4, Xaa=4	9609
98	S	0						98	S	1	A=2, C=1, S=9601, T=2, Xaa=3	9609
99	H	0						99	H	1	N=1, Q=1, H=9603, Xaa=4	9609
100	P	0						100	P	2	P=9594, S=12, Xaa=3	9609
101	G	0						101	G	1	R=4, E=1, G=9601, Xaa=3	9609
102	I	0						102	I	6	I=9552, L=49, F=1, V=4, Xaa=3	9609
103	F	0						103	F	1	C=1, L=1, F=9599, Y=5, Xaa=3	9609
104	E	0						104	E	12	A=1, D=14, E=9469, G=119, K=1, V=1, Xaa=4	9609
105	N	0						105	N	10	R=2, N=9513, D=6, H=28, K=1, S=23, T=32, Xaa=4	9609
106	S	0						106	S	1	L=1, S=9604, Xaa=4	9609
107	C	0						107	C	1	R=1, C=9603, G=1, Xaa=4	9609
108	L	0						108	L	7	R=2, I=54, L=9540, F=5, P=1, V=3, Xaa=4	9609
109	E	0						109	E	1	A=1, D=1, E=9602, G=1, Xaa=4	9609
110	T	0						110	T	3	A=18, M=6, P=1, T=9580, Xaa=4	9609
111	M	1	V1					111	M	11	I=125, M=9475, T=1, V=4, Xaa=4	9609
112	E	0						112	E	1	E=9604, K=1, Xaa=4	9609
113	V	0						113	V	78	A=13, I=1939, M=2, F=16, T=9, V=7625, Xaa=5	9609
114	V	35	I33	V	I	1997	0,0133	114	V	12	A=6, I=128, L=4, F=1, V=9467, Xaa=3	9609
115	Q	0						115	Q	1	Q=9604, H=1, K=1, Xaa=3	9609
116	Q	0						116	Q	1	R=1, Q=9605, Xaa=3	9609

Marker (%)	Percent of occurrence of equine characteristic amino acid(s) at a given position in a group of equine virus isolates (in the column colored in magenta) since indicated year up to 2013 or during years of isolation.																																										
Avian "consensus" aa:	I	K	E	L	R	D	L	M	S	Q	S	R	T	R	E	I	L	T	K	T	T	V	D	H	M	A	I	I	K	K	Y	T	S	G	R	Q	E	K	N	P			
Equine Marker aa:	L																																										
Equine influenza A H3N8 consensus:	M	E	R	I	K	E	L	R	D	L	M	S	Q	S	R	T	R	E	I	L	T	K	T	T	V	D	H	M	A	I	I	K	K	Y	T	S	G	R	Q	E	K	N	P
Accession Country Year Strain	L																																										
ABY81502 USA 1963 Influenza2	L																																										
ACD85274 Brazil 1963 Influenza2	L																																										
ACD85428 Uruguay 1963 Influenza2	L																																										
ACD85395 Brazil 1969 Influenza2	L																																										
ACI25745 Japan 1971 Influenza2	L																																										
AM60157 Japan 1971 Influenza2	L																																										
ACA24926 USA 1975 Influenza2	L																																										
ABY81524 USA 1976 Influenza2	L																																										
ABY81513 USA 1976 Influenza2	L																																										
ACF41712 unknown 1978 Influenza2	L																																										
ABY81447 USA 1978 Influenza2	L																																										
ACD85406 France 1979 Influenza2	L																																										
AM60123 USA 1979 Influenza2	L																																										
AM60134 USA 1979 Influenza2	L																																										
ACD85373 Switzerland 1979 Influenza2	L																																										
ABY81469 USA 1980 Influenza2	L																																										
ACF22125 USA 1980 Influenza2	L																																										
ABY81458 USA 1980 Influenza2	L																																										
ACD85384 Romania 1980 Influenza2	L																																										
ABY81535 USA 1981 Influenza2	L																																										
ABY81568 USA 1981 Influenza2	L																																										
ABY81579 USA 1981 Influenza2	L																																										
ABY81546 USA 1981 Influenza2	L																																										
ABY81557 USA 1981 Influenza2	L																																										
ABY81491 USA 1981 Influenza2	L																																										
ABY81480 USA 1981 Influenza2	L																																										
ACA24545 USA 1981 Influenza2	L																																										
ACA96551 USA 1981 Influenza2	L																																										
ABY81590 USA 1981 Influenza2	L																																										
ABY81601 USA 1982 Influenza2	L																																										
ACA24556 USA 1982 Influenza2	L																																										
ABY81612 USA 1983 Influenza2	L																																										
ACD85285 Argentina 1985 Influenza2	L																																										
ACD85296 USA 1985 Influenza2	L																																										
ACF22147 South Afri 1986 Influenza2	L																																										
ACA24567 USA 1986 Influenza2	L																																										
AAA43133 USA 1986 Influenza2	L																																										
ACD85175 USA 1986 Influenza2	L																																										
ACD85186 USA 1986 Influenza2	L																																										
ACA24666 USA 1986 Influenza2	L																																										
ACA24578 USA 1987 Influenza2	L																																										
ACD56145 USA 1987 Influenza2	L																																										
ACZ45460 USA 1987 Influenza2	L																																										
ACA24589 USA 1988 Influenza2	L																																										
ACA24600 USA 1988 Influenza2	L																																										
ACA24611 USA 1988 Influenza2	L																																										
ACD85417 Germany 1989 Influenza2	L																																										
ACD97446 United Ki 1989 Influenza2	L																																										
ACI48775 United Ki 1989 Influenza2	L																																										
ACD97435 United Ki 1989 Influenza2	L																																										
ACA24622 USA 1990 Influenza2	L																																										
ACA24655 USA 1991 Influenza2	L																																										
ABM21948 USA 1991 Influenza2	L																																										
ACD85362 Italy 1991 Influenza2	L																																										
ACD85307 Italy 1991 Influenza2	L																																										
ACA24677 USA 1991 Influenza2	L																																										
ACD85351 Italy 1991 Influenza2	L																																										
ACA24633 USA 1991 Influenza2	L																																										
ACD85329 Austria 1992 Influenza2	L																																										
ACD85318 Italy 1992 Influenza2	L																																										
ACA24644 USA 1992 Influenza2	L																																										
ACI48795 United Ki 1993 Influenza2	L																																										
ACI48785 United Ki 1993 Influenza2	L																																										
ACD85340 Switzerland 1993 Influenza2	L																																										
ACA24688 USA 1994 Influenza2	L																																										
ACE81879 China 1994 Influenza2	L																																										
ACA96810 USA 1997 Influenza2	L																																										
ACA96821 USA 2002 Influenza2	L																																										
AAX23572 USA 2002 Influenza2	L																																										
ACZ45401 USA 2002 Influenza2	L																																										
AAZ23565 USA 2003 Influenza2	L																																										
ACI48806 United Ki 2003 Influenza2	L																																										
ABB17182 USA 2003 Influenza2	L																																										
ADM29294 USA 2005 Influenza2	L																																										
ADM29283 USA 2005 Influenza2	L																																										
ADM29261 USA 2005 Influenza2	L																																										
ADM29305 USA 2005 Influenza2	L																																										
ADM29272 USA 2005 Influenza2	L																																										
ADM29250 USA 2005 Influenza2	L																																										
ADM29624 USA 2005 Influenza2	L																																										
AGV74272 United Ki 2007 Influenza2	L																																										
ADM29635 USA 2007 Influenza2	L																																										
ACE81868 China 2007 Influenza2	L																																										
ACE81857 China 2007 Influenza2	L																																										
ACE81846 China 2007 Influenza2	L																																										
ADM29558 USA 2007 Influenza2	L																																										
ACE81824 China 2007 Influenza2	L																																										
ACE81835 China 2007 Influenza2	L																																										
ADC34564 China 2007 Influenza2	L																																										
AGV74280 United Ki 2007 Influenza2	L																																										
ACE81934 China 2008 Influenza2	L																																										
ACE81923 China 2008 Influenza2	L																																										
ACE81890 China 2008 Influenza2	L																																										
ACE81912 China 2008 Influenza2	L																																										
ACE81901 China 2008 Influenza2	L																																										
AGR45341 China 2010 Influenza2	L																																										
AFU83168 South Ko 2011 Influenza2	L																																										
AHA98365 China 2013 Influenza2	L																																										

Legend:

1. Outlier strains which obviously do not fit into the H3N8 equine lineage are shaded grey and are not included on plots.
2. Both early and recent marker positions are colored magenta (Row 5).
3. The virus strains utilized for amino acid substitution rate calculation of influenza A H3N8 equine lineage, A/equine/Miami/1/1963(H3N8) and A/equine/Xuzhou/01/2013(H3N8), are highlighted in magenta.

Legend:
 1. **Alternatives:** aa different from consensus , e.g., Q1 means that Q occurred in one isolate
 2. **Score:** Data taken from the Database describing measure of variability.
 3. **Alignment Details:** number of occurrences of various amino acids.
 4. High-potency and Low-potency **markers** are colored green and yellow, respectively.

Equine Pos	PB2 H3N8 Consensus	H3N8 No of Diffs	Alternatives	Permanent aa change			Marker potency	Avian PB2 all subtypes			Alignment Details	# Sequences	
				From	To	Year of substitution		Pos	Consensus	Score			
1	M	0						1	M	0	Del=1, M=9579	9580	
2	E	0						2	E	13	N=23, D=113, Del=1, E=9436, G=5, K=2	9580	
3	R	0						3	R	2	R=9567, Del=1, G=2, I=1, K=5, S=4	9580	
4	I	0						4	I	2	N=2, D=2, I=9563, L=1, K=1, M=2, T=2, V=6, Xaa=1	9580	
5	K	2	I2					5	K	3	R=15, N=1, Q=1, E=5, K=9558	9580	
6	E	0						6	E	3	A=1, D=6, Q=1, E=9554, G=12, K=4, V=1, Xaa=1	9580	
7	L	0						7	L	0	I=1, L=9577, F=2	9580	
8	R	2	K2					8	R	2	R=9561, I=3, K=15, S=1	9580	
9	D	0						9	D	13	N=75, D=9437, C=1, E=57, G=5, Y=5	9580	
10	L	0						10	L	1	I=3, L=9574, M=1, S=1, V=1	9580	
11	M	0						11	M	1	R=1, I=2, L=2, M=9574, V=1	9580	
12	S	31	L31	S	L		2002	0,0080	12	S	9	A=2, L=77, P=1, S=9489, T=10, W=1	9580
13	Q	0						13	Q	3	R=8, Q=9552, H=6, L=5, K=9	9580	
14	S	0						14	S	2	A=1, F=7, P=7, S=9564, T=1	9580	
15	R	0						15	R	1	R=9569, C=7, G=2, H=2	9580	
16	T	1	I1					16	T	1	A=2, I=2, T=9576	9580	
17	R	0						17	R	3	R=9555, C=4, H=17, S=4	9580	
18	E	0						18	E	2	E=9560, G=20	9580	
19	I	0						19	I	1	I=9569, M=1, T=1, V=9	9580	
20	L	0						20	L	0	L=9580	9580	
21	T	0						21	T	3	A=12, I=6, K=1, M=1, P=1, T=9559	9580	
22	K	0						22	K	19	R=247, N=3, Q=9, K=9320, Xaa=1	9580	
23	T	0						23	T	0	N=1, I=1, S=1, T=9577	9580	
24	T	0						24	T	1	A=1, N=2, P=2, S=4, T=9571	9580	
25	V	0						25	V	0	A=1, G=1, V=9578	9580	
26	D	0						26	D	0	A=1, D=9577, G=2	9580	
27	H	0						27	H	0	N=1, H=9579	9580	
28	M	0						28	M	2	I=9, M=9561, T=5, V=5	9580	
29	A	2	T2					29	A	2	A=9567, P=3, S=3, T=4, V=3	9580	
30	I	0						30	I	5	I=9536, I=10, M=3, V=31	9580	
31	I	1	X1					31	I	1	G=1, I=9574, L=3, F=1, V=1	9580	
32	K	0						32	K	1	R=7, Q=1, E=2, K=9569, Xaa=1	9580	
33	K	0						33	K	1	R=8, G=1, K=9571	9580	
34	Y	0						34	Y	1	C=3, H=1, F=2, S=5, Y=9569	9580	
35	T	0						35	T	1	P=3, S=1, T=9576	9580	
36	S	0						36	S	1	P=1, S=9574, T=5	9580	
37	G	0						37	G	0	E=1, G=9579	9580	
38	R	0						38	R	1	R=9575, G=1, I=1, K=1, S=1, T=1	9580	
39	Q	0						39	Q	1	R=1, Q=9575, E=1, L=1, K=1, Xaa=1	9580	
40	E	0						40	E	0	E=9579, G=1	9580	
41	K	0						41	K	1	R=4, N=2, K=9573, T=1	9580	
42	N	0						42	N	0	N=9579, S=1	9580	
43	P	0						43	P	0	L=1, P=9578, S=1	9580	
44	A	2	S2					44	A	103	A=4968, Del=4580, S=29, T=3	9580	
45	L	0						45	L	100	Del=4580, L=5000	9580	
46	R	0						46	R	0	R=9577, I=1, K=1, S=1	9580	
47	M	0						47	M	1	N=1, I=1, M=9573, V=5	9580	
48	K	0						48	K	1	R=8, E=1, K=9571	9580	
49	W	0						49	W	0	W=9580	9580	
50	M	0						50	M	5	R=1, Del=1, I=37, M=9533, T=3, V=5	9580	
51	M	0						51	M	0	L=1, M=9578, V=1	9580	
52	A	0						52	A	0	A=9578, S=1, T=1	9580	
53	M	0						53	M	1	I=2, M=9573, T=2, V=2, Xaa=1	9580	
54	K	1	N1					54	K	3	R=32, I=1, K=9547	9580	
55	Y	0						55	Y	2	H=15, F=2, Y=9563	9580	
56	P	0						56	P	1	Del=1, Q=1, P=9570, S=8	9580	
57	I	0						57	I	2	Del=1, I=9565, M=5, F=1, T=2, V=6	9580	
58	T	0						58	T	3	A=22, I=1, M=1, P=2, S=1, T=9552, Xaa=1	9580	
59	A	0						59	A	0	A=9579, Del=1	9580	
60	D	0						60	D	7	A=1, N=25, D=9518, E=14, G=21	9580	
61	K	1	R1					61	K	6	R=60, N=1, D=1, I=1, K=9516, M=1	9580	
62	R	0						62	R	13	R=9429, Q=1, G=68, I=1, K=81	9580	
63	I	0						63	I	5	I=9525, L=1, M=1, T=4, V=49	9580	
64	M	3	I3					64	M	59	I=1023, L=13, M=8450, T=61, V=33	9580	
65	E	8	G7K1	G	E		1975	0,0031	65	E	24	N=3, D=290, E=9250, G=30, K=5, T=1, V=1	9580
66	M	1	V1					66	M	16	A=5, I=29, L=5, M=9402, T=16, V=122, Xaa=1	9580	
67	I	1	N1					67	I	13	I=9422, L=1, M=3, T=1, V=153	9580	
68	P	1	S1					68	P	0	P=9580	9580	
69	E	0						69	E	1	A=3, D=2, E=9572, G=1, K=2	9580	
70	R	0						70	R	2	R=9558, G=1, K=21	9580	
71	N	0						71	N	0	N=9577, I=2, K=1	9580	
72	E	0						72	E	1	D=1, E=9576, V=2, Xaa=1	9580	
73	Q	5	H5					73	Q	4	R=11, Q=9546, E=1, H=12, K=10	9580	
74	G	0						74	G	2	D=1, C=1, E=5, G=9568, S=5	9580	
75	Q	0						75	Q	0	Q=9579, H=1	9580	
76	T	1	S1					76	T	22	A=40, I=124, K=54, M=21, S=5, T=9335, V=1	9580	
77	L	1	P1					77	L	0	L=9579, F=1	9580	
78	W	0						78	W	0	W=9580	9580	
79	S	1	N1					79	S	8	R=2, N=15, G=7, I=6, S=9503, T=47	9580	
80	K	0						80	K	18	R=247, N=2, Q=2, I=1, K=9327, M=1	9580	
81	T	5	M3I2					81	T	12	A=61, I=46, L=1, K=3, M=7, S=6, T=9456	9580	
82	N	0						82	N	16	R=1, N=9395, H=8, K=15, S=138, T=23	9580	
83	D	0						83	D	1	D=9576, Del=1, V=2, Xaa=1	9580	
84	A	0						84	A	0	A=9578, V=1, Xaa=1	9580	
85	G	0						85	G	0	G=9579, Xaa=1	9580	
86	S	0						86	S	1	L=1, P=1, S=9576, T=1, Xaa=1	9580	
87	D	0						87	D	6	N=35, D=9529, E=4, G=10, I=1, Xaa=1	9580	
88	R	0						88	R	2	R=9566, G=3, K=10, Xaa=1	9580	
89	V	0						89	V	7	E=2, I=10, L=26, M=24, V=9517, Xaa=1	9580	
90	M	0						90	M	11	I=98, L=1, K=1, M=9460, T=3, V=15, Xaa=2	9580	
91	V	0						91	V	1	A=1, I=2, L=1, V=9575, Xaa=1	9580	
92	S	0						92	S	0	A=1, P=1, S=9577, Xaa=1	9580	
93	P	0						93	P	5	L=5, P=9532, S=27, T=14, Xaa=2	9580	
94	L	0						94	L	1	R=1, L=9574, M=1, P=1, Xaa=3	9580	
95	A	0						95	A	2	A=9568, T=7, V=1, Xaa=4	9580	
96	V	0						96	V	3	A=1, I=19, V=9556, Xaa=4	9580	
97	T	0						97	T	1	A=2, I=1, T=9573, Xaa=4	9580	
98	W	0						98	W	1	W=9576, Xaa=4	9580	
99	W	0						99	W	1	R=3, W=9573, Xaa=4	9580	
100	N	0						100	N	1	N=9573, D=1, I=2, Xaa=4	9580	
101	R	0						101	R	1	R=9572, K=4, Xaa=4	9580	
102	N	0						102	N	9	N=9492, D=1, K=16, S=65, T=2, Xaa=4	9580	
103	G	0						103	G	1	A=1, G=9575, Xaa=4	9580	
104	P	0						104	P	2	Q=4, L=4, P=9564, S=2, T=1, Xaa=5	9580	
105	T	13	A13	T	A		2007	0,0334	105	T	31	A=320, R=1, I=40, K=13, M=15, P=6, S=2, T=9172, V=5, Xaa=6	9580
106	T	2	A2					106	T	25	A=291, N=16, I=2, K=4, S=24, T=9238, Xaa=5	9580	
107	S	0						107	S	22	R=2, N=211, D=19, G=29, I=2, S=9310, T=2, Xaa=5	9580	
108	T	0						108	T	28	A=431, I=1, T=9135, V=8, Xaa=5	9580	
109	I	9	V9	V	I		1975	0,0463	109	I	31	A=12, G=2, I=444, L=3, F=10, V=9102, Xaa=7	9580
110	H	0						110	H	2	R=1, N=1, Q=1, H=9566, Y=4, Xaa=7	9580	
111	Y	0						111	Y	1	C=1, H=2, F=1, Y=9569, Xaa=7	9580	
112	P	0						112	P	4	Q=1, L=2, P=9550, S=17, T=3, Xaa=7	9580	
113	K	0						113	K	8	R=12, N=1, Q=49, E=1, L=2, K=9507, T=1, Xaa=7	9580	
114	V	0						114	V	9	I=101, V=9472, Xaa=7	9580	
115	Y	0						115	Y	1	N=1, H=1, F=1, S=1, Y=9569, Xaa=7	9580	
116	K	0						116	K	13	R=143, N=1, Q=1, E=2, K=9425, T=1, Xaa=7	9580	
117	T	0						117	T	12	A=38, N=16, I=3, P=30, S=19, T=9467, Xaa=7	9580	
118	Y	0						118	Y	1	C=2, H=1, F=1, Y=9569, Xaa=7	9580	
119	F	0						119	F	1	F=9573, Xaa=7	9580	
120	E	0						120	E	6	D=44, E=9519, G=8, K=2, Xaa=7	9580	
121	K	0						121	K	3	R=15, K=9558, Xaa=7	9580	
122	V	0						122	V	15	A=39, G=1, I=70, M=31, F=3, V=9429, Xaa=7	9580	
123	E	0						123	E	22	A=11, D=72, Q=3, E=9340, G=66, K=70, V=12, Xaa=6	9580	
124	R	0											

589	R	0						589	R	3 R=9558, K=5, Xaa=17	9580	
590	S	26	G26	G	S			590	G	54 A=2, R=1, N=8, C=40, G=8560, S=950, Xaa=19	9580	
591	Q	1	R1					591	Q	13 R=18, Q=9459, H=27, L=45, K=11, P=3, Xaa=17	9580	
592	Y	0						592	Y	2 D=1, Y=9561, Xaa=18	9580	
593	S	0						593	S	2 N=1, S=9562, Xaa=17	9580	
594	G	0						594	G	2 G=9563, Xaa=17	9580	
595	F	0						595	F	2 L=1, F=9562, Xaa=17	9580	
596	V	0						596	V	3 A=1, I=7, V=9556, Xaa=16	9580	
597	R	0						597	R	2 R=9562, G=1, K=1, Xaa=16	9580	
598	T	1	I1					598	T	21 A=39, I=3, M=22, T=9328, V=171, Xaa=17	9580	
599	L	0						599	L	2 L=9563, Xaa=17	9580	
600	F	0						600	F	3 L=1, F=9559, S=3, Y=2, Xaa=15	9580	
601	Q	0						601	Q	2 Q=9565, H=1, P=1, Xaa=13	9580	
602	Q	0						602	Q	1 Q=9567, Xaa=13	9580	
603	M	0						603	M	2 I=1, M=9561, T=1, V=4, Xaa=13	9580	
604	R	0						604	R	2 R=9567, S=1, Xaa=12	9580	
605	D	0						605	D	1 D=9567, Xaa=13	9580	
606	V	0						606	V	7 I=67, V=9501, Xaa=12	9580	
607	L	0						607	L	6 R=1, I=25, L=9524, M=5, F=4, W=2, V=7, Xaa=12	9580	
608	G	0						608	G	1 G=9568, Xaa=12	9580	
609	T	0						609	T	2 K=1, T=9567, Xaa=12	9580	
610	F	0						610	F	3 L=8, F=9559, S=1, Xaa=12	9580	
611	D	0						611	D	10 A=1, N=15, D=9484, E=63, G=5, Y=1, Xaa=11	9580	
612	T	0						612	T	3 A=3, N=3, I=6, F=2, S=3, T=9553, Xaa=10	9580	
613	A	7	V5I2	V	A		1975	0,0109	613	V	16 A=104, I=72, V=9394, Xaa=10	9580
614	Q	0						614	Q	1 Q=9568, H=1, P=1, Xaa=10	9580	
615	I	1	M1					615	I	7 I=9507, L=2, M=4, T=3, V=54, Xaa=10	9580	
616	I	0						616	I	4 I=9547, M=7, T=3, V=13, Xaa=10	9580	
617	K	0						617	K	1 R=2, K=9568, Xaa=10	9580	
618	L	0						618	L	4 R=1, Del=1, L=9546, M=22, Xaa=10	9580	
619	L	0						619	L	2 I=5, L=9564, V=1, Xaa=10	9580	
620	P	0						620	P	1 P=9569, T=1, Xaa=10	9580	
621	F	0						621	F	2 C=1, L=2, F=9566, V=1, Xaa=10	9580	
622	A	0						622	A	3 A=9554, S=2, T=14, Xaa=10	9580	
623	A	0						623	A	4 A=9541, D=1, S=27, T=1, Xaa=10	9580	
624	A	0						624	A	2 A=9562, D=1, S=6, T=1, Xaa=10	9580	
625	P	0						625	P	1 P=9569, Xaa=11	9580	
626	P	0						626	P	1 P=9568, S=1, Xaa=11	9580	
627	E	0						627	E	29 A=1, E=9174, G=3, K=315, V=75, Xaa=12	9580	
628	Q	0						628	Q	3 R=2, Q=9553, H=6, L=3, K=2, P=4, Xaa=10	9580	
629	S	0						629	S	5 R=2, N=35, G=2, S=9531, T=1, Xaa=9	9580	
630	R	0						630	R	6 R=9521, K=50, Xaa=9	9580	
631	M	0						631	M	2 I=2, L=6, K=1, M=9562, Xaa=9	9580	
632	Q	0						632	Q	2 R=3, Q=9567, H=1, Xaa=9	9580	
633	F	0						633	F	1 L=1, F=9569, Y=2, Xaa=8	9580	
634	S	0						634	S	4 F=15, P=1, S=9550, T=2, Y=2, Xaa=10	9580	
635	S	0						635	S	1 F=2, P=2, S=9569, Xaa=7	9580	
636	L	0						636	L	2 I=1, L=9567, M=6, F=1, Xaa=5	9580	
637	T	0						637	T	3 A=3, N=2, I=10, S=2, T=9558, V=1, Xaa=4	9580	
638	V	0						638	V	1 G=1, I=4, L=1, V=9570, Xaa=4	9580	
639	N	1	X1					639	N	5 N=9537, I=2, K=1, S=28, T=8, Xaa=4	9580	
640	V	0						640	V	3 A=1, I=24, L=2, V=9549, Xaa=4	9580	
641	R	0						641	R	2 R=9564, G=8, K=4, Xaa=4	9580	
642	G	0						642	G	1 A=1, G=9575, Xaa=4	9580	
643	S	0						643	S	7 A=1, L=2, S=9509, T=64, Xaa=4	9580	
644	G	0						644	G	1 R=2, E=1, G=9573, Xaa=4	9580	
645	M	1	L1					645	M	3 I=1, L=19, M=9554, V=3, Xaa=3	9580	
646	R	0						646	R	1 R=9573, K=4, Xaa=3	9580	
647	I	0						647	I	6 I=9524, L=4, M=7, T=1, V=41, Xaa=3	9580	
648	L	0						648	L	19 A=1, I=12, L=9353, M=7, F=7, P=27, V=170, Xaa=3	9580	
649	V	0						649	V	38 I=669, L=4, M=4, V=8899, Xaa=4	9580	
650	R	0						650	R	1 R=9574, K=1, S=1, Xaa=4	9580	
651	G	0						651	G	1 A=1, R=1, C=2, G=9573, Xaa=3	9580	
652	N	0						652	N	3 N=9558, H=4, S=15, Xaa=3	9580	
653	S	0						653	S	1 C=1, F=3, S=9574, Xaa=2	9580	
654	P	0						654	P	2 H=1, P=9564, S=13, Xaa=2	9580	
655	V	0						655	V	10 A=83, G=3, I=1, L=16, M=2, V=9473, Xaa=2	9580	
656	F	0						656	F	1 F=9576, S=2, Xaa=2	9580	
657	N	0						657	N	3 N=9561, D=1, I=1, K=4, M=1, S=6, T=2, Y=2, Xaa=2	9580	
658	Y	0						658	Y	4 D=1, C=3, H=17, F=7, Y=9550, Xaa=2	9580	
659	N	0						659	N	6 N=9521, D=1, H=3, I=1, M=1, S=50, T=1, Xaa=2	9580	
660	K	10	R9X1					660	K	12 R=143, N=1, Q=1, K=9431, M=1, Xaa=3	9580	
661	A	0						661	A	22 A=9314, E=1, G=8, S=42, T=171, V=42, Xaa=2	9580	
662	T	2	N2					662	T	8 A=3, N=5, G=31, I=10, S=19, T=9510, Xaa=2	9580	
663	K	1	N1					663	K	6 R=37, N=5, Q=10, E=2, G=1, K=9523, Xaa=2	9580	
664	R	0						664	R	1 R=9575, Del=1, K=1, T=1, W=1, Xaa=1	9580	
665	L	0						665	L	1 D=2, I=7, L=9569, F=1, V=1	9580	
666	T	0						666	T	9 A=48, I=34, M=1, S=1, T=9493, V=3	9580	
667	V	0						667	V	25 A=2, R=1, I=390, V=9187	9580	
668	L	0						668	L	2 I=9, L=9567, F=3, Xaa=1	9580	
669	G	0						669	G	0 R=1, E=1, G=9577, Xaa=1	9580	
670	K	0						670	K	1 R=3, K=9574, T=2, Xaa=1	9580	
671	D	0						671	D	2 N=13, D=9562, E=2, G=2, Xaa=1	9580	
672	A	0						672	A	1 A=9576, C=1, T=2, Xaa=1	9580	
673	G	0						673	G	1 R=2, G=9574, S=3, Xaa=1	9580	
674	A	1	T1					674	A	22 A=9330, E=22, G=5, P=1, S=123, T=81, V=15, Xaa=3	9580	
675	L	0						675	L	0 L=9577, K=1, M=2	9580	
676	T	3	I3				1971	676	T	65 A=357, R=7, N=19, E=2, I=248, K=6, M=201, S=19, T=8678, V=43	9580	
677	E	0						677	E	15 D=38, E=9416, G=102, K=23, Xaa=1	9580	
678	D	0						678	D	3 N=8, D=9552, G=14, Y=3, V=2, Xaa=1	9580	
679	P	0						679	P	5 R=1, Q=14, L=14, P=9538, S=11, T=2	9580	
680	D	0						680	D	19 N=73, D=9365, E=113, G=22, Y=3, V=3, Xaa=1	9580	
681	E	0						681	E	2 D=10, E=9564, G=5, K=1	9580	
682	G	0						682	G	2 A=1, R=1, D=2, C=1, G=9565, S=10	9580	
683	T	0						683	T	19 A=131, R=1, I=27, K=20, M=5, S=24, T=9370, V=2	9580	
684	A	2	T2				1971	684	A	15 A=9413, D=2, E=2, G=7, S=13, T=128, V=14, Xaa=1	9580	
685	G	0						685	G	0 R=2, G=9578	9580	
686	V	2	I2				1971	686	V	5 G=1, I=48, L=1, M=1, V=9529	9580	
687	E	0						687	E	0 D=1, E=9579	9580	
688	S	0						688	S	5 A=1, F=46, S=9529, Xaa=4	9580	
689	A	0						689	A	0 A=9577, G=1, P=2	9580	
690	V	0						690	V	0 D=1, I=2, V=9577	9580	
691	L	0						691	L	0 L=9578, F=1, W=1	9580	
692	R	0						692	R	1 R=9576, G=2, K=2	9580	
693	G	0						693	G	0 G=9580	9580	
694	F	0						694	F	0 L=3, F=9577	9580	
695	L	0						695	L	0 I=1, L=9579	9580	
696	I	0						696	I	2 I=9565, T=1, V=14	9580	
697	L	0						697	L	1 I=3, L=9575, P=2	9580	
698	G	0						698	G	2 A=1, G=9566, S=13	9580	
699	K	0						699	K	6 R=39, N=12, Q=5, K=9523, M=1	9580	
700	E	0						700	E	2 D=1, E=9561, G=17, V=1	9580	
701	N	1	D1			Early marker - 1963	0,0009	701	D	2 N=9, D=9564, E=6, V=1	9580	
702	K	0						702	K	14 R=159, N=9, Q=3, G=1, I=1, K=9405, T=2	9580	
703	R	2	K2					703	R	1 R=9573, G=3, K=4	9580	
704	Y	0						704	Y	4 C=1, H=2, F=32, Y=9545	9580	
705	G	0						705	G	1 R=1, Del=2, G=9576, S=1	9580	
706	P	0						706	P	1 Del=1, P=9576, S=3	9580	
707	A	0						707	A	4 A=9540, P=2, S=36, T=2	9580	
708	L	0						708	L	1 L=9567, F=13	9580	
709	S	0						709	S	1 R=1, N=2, G=2, I=1, S=9571, T=2, Xaa=1	9580	
710	I	0						710	I	1 I=9573, T=3, V=4	9580	
711	N	0						711	N	13 N=9427, D=38, K=2, S=112, T=1	9580	
712	E	0						712	E	0 E=9578, G=1, Xaa=1	9580	
713	L	0						713	L	1 R=1, Del=1, I=1, L=9570, M=7	9580	
714	S	0						714	S	12 N=3, G=128, I=1, S=9439, T=9	9580	
715	K	7	N7	N	K		1975	0,0005	715	N	12 N=9444, D=2, H=2, I=1, K=5, S=120, T=5, Y=1	9580
716	L	0						716	L	2 H=1, I=6, L=9565, F=7, P=1	9580	
717	T	42	A42	A	T		1971	0,0213	717	A	19 A=9334, S=5, T=204, V=36, Xaa=1	

739	R	0		739	R	1 R=9576, P=4	9580
740	D	0		740	D	2 N=18, D=9559, E=1, G=1, Xaa=1	9580
741	S	0		741	S	3 A=2, F=21, S=9556, Xaa=1	9580
742	S	0		742	S	0 G=1, S=9577, T=2	9580
743	I	0		743	I	1 I=9568, L=12	9580
744	L	0		744	L	0 L=9579, F=1	9580
745	T	0		745	T	1 I=9, P=1, T=9570	9580
746	D	0		746	D	0 D=9580	9580
747	S	0		747	S	1 R=1, N=1, C=1, G=1, I=1, S=9575	9580
748	Q	0		748	Q	1 R=1, Q=9576, H=1, L=1, P=1	9580
749	T	0		749	T	0 P=1, T=9579	9580
750	A	0		750	A	0 A=9577, G=1, P=2	9580
751	T	1	S1	751	T	0 N=1, P=2, T=9577	9580
752	K	0		752	K	1 R=3, Q=1, I=1, K=9574, T=1	9580
753	R	0		753	R	1 R=9573, N=1, K=5, S=1	9580
754	I	0		754	I	2 H=1, I=9561, L=3, F=3, T=2, V=10	9580
755	R	0		755	R	1 R=9571, Q=3, G=2, P=2, W=2	9580
756	M	0		756	M	5 R=4, D=1, I=4, L=7, M=9537, S=1, T=6, V=20	9580
757	A	0		757	A	2 A=9561, D=2, G=1, P=3, S=1, V=12	9580
758	I	0		758	I	5 N=2, I=9528, M=1, F=2, T=44, V=2, Xaa=1	9580
759	N	2	S2	759	N	9 N=9495, D=1, H=1, I=18, K=11, F=1, S=2, T=2, Y=48, V=1	9580

Legend:
 1. **Alternatives:** aa different from consensus , e.g., Q1 means that Q occurred in one isolate
 2. **Score:** Data taken from the Database describing measure of variability.
 3. **Alignment Details:** number of occurrences of various amino acids.
 4. High-potency and Low-potency **markers** are colored green and yellow, respectively.

Equine Pos	PA H3N8 Consensus	No of Diff's	Alternatives	Permanent aa change			Marker potency	Avian PA all subtypes			# Sequences
				From	To	Year of substitution		Pos	Consensus	Score	
1	M	0						1	M	0 M=9726	9726
2	E	0						2	E	4 A=1,Q=1,E=9708,G=22,K=3,V=4	9739
3	D	2	N1G1					3	D	14 A=1,R=2,N=121,D=9582,E=25,G=5,H=1,Y=2	9739
4	F	0						4	F	2 L=18,F=9720,V=1	9739
5	V	0						5	V	5 A=10,D=1,G=1,L=13,M=14,V=9700	9739
6	R	0						6	R	0 A=1,R=9738	9739
7	Q	0						7	Q	2 A=1,R=3,Q=9728,H=1,K=2,P=2,S=1,T=1	9739
8	C	0						8	C	3 C=9717,G=1,S=10,W=2,Y=9	9739
9	F	0						9	F	1 L=3,F=9733,Y=1,V=1,Xaa=1	9739
10	N	1	Y1					10	N	4 N=9701,D=2,H=1,I=2,S=32,T=1	9739
11	P	0						11	P	1 L=1,P=9735,S=3	9739
12	M	0						12	M	2 I=14,M=9723,V=2	9739
13	I	2	M1V1					13	I	15 R=1,I=9565,M=1,T=80,V=90,Xaa=2	9739
14	V	0						14	V	7 A=44,I=27,F=1,V=9666,Xaa=1	9739
15	E	1	V1					15	E	2 Q=1,E=9717,G=20,K=1	9739
16	L	0						16	L	0 H=1,L=9737,Xaa=1	9739
17	A	0						17	A	1 A=9728,G=1,S=5,T=3,V=1,Xaa=1	9739
18	E	2	G1K1					18	E	2 D=3,E=9726,G=8,K=2	9739
19	K	0						19	K	6 R=40,N=13,E=2,K=9683,S=1	9739
20	A	3	T3					20	A	12 A=9597,E=2,S=3,T=134,V=3	9739
21	M	0						21	M	1 I=3,M=9733,T=1,V=2	9739
22	K	1	R1					22	K	4 R=36,I=1,K=9702	9739
23	E	0						23	E	0 E=9736,G=2,V=1	9739
24	Y	0						24	Y	4 D=2,H=34,Y=9702,Xaa=1	9739
25	G	0						25	G	0 G=9738,W=1	9739
26	E	0						26	E	1 D=1,Q=1,E=9733,G=3,K=1	9739
27	D	1	N1					27	D	26 A=8,N=286,D=9389,E=4,G=3,K=1,S=24,T=8,Y=10,V=6	9739
28	P	0						28	P	3 Q=1,H=1,L=10,P=9712,S=11,T=4	9739
29	K	0						29	K	6 R=44,N=1,Q=1,E=6,G=1,I=1,K=9684,F=1	9739
30	I	0						30	I	13 A=1,I=9600,L=34,F=1,T=24,Y=1,V=78	9739
31	E	0						31	E	1 D=1,E=9731,G=3,K=4	9739
32	T	1	M1					32	T	5 A=5,N=14,I=3,K=1,M=11,S=5,T=9700	9739
33	N	0						33	N	1 N=9734,D=1,K=2,S=1,T=1	9739
34	K	0						34	K	0 R=1,K=9737,T=1	9739
35	F	0						35	F	3 L=28,F=9709,P=1,S=1	9739
36	A	0						36	A	5 A=9697,S=9,T=28,V=4,Xaa=1	9739
37	A	0						37	A	9 A=9635,S=103,T=1	9739
38	I	0						38	I	4 I=9696,L=1,M=4,T=1,V=37	9739
39	C	0						39	C	0 R=1,C=9736,S=1,Y=1	9739
40	T	0						40	T	2 A=7,I=8,T=9724	9739
41	H	0						41	H	0 R=1,Q=1,H=9737	9739
42	L	0						42	L	2 I=2,L=9723,M=10,F=2,S=2	9739
43	E	0						43	E	1 D=2,Q=8,E=9728,G=1	9739
44	V	0						44	V	11 A=9,G=1,I=120,F=1,V=9607,Xaa=1	9739
45	C	0						45	C	0 C=9737,S=1,W=1	9739
46	F	0						46	F	0 L=1,F=9738	9739
47	M	0						47	M	0 M=9739	9739
48	Y	0						48	Y	0 F=1,Y=9738	9739
49	S	0						49	S	1 A=3,L=4,S=9730,T=2	9739
50	D	0						50	D	0 D=9738,H=1	9739
51	F	0						51	F	2 L=14,F=9724,Xaa=1	9739
52	H	0						52	H	0 H=9738,L=1	9739
53	F	0						53	F	0 L=2,F=9736,S=1	9739
54	I	0						54	I	2 I=9718,L=1,M=3,T=2,V=15	9739
55	N	8	D8	D	N	1975	0,0116	55	D	9 N=113,D=9626	9739
56	E	0						56	E	2 D=8,E=9722,G=3,V=6	9739
57	L	6	R6	R	L	1972	0,0003	57	R	14 R=9570,Q=148,G=1,L=3,K=5,W=12	9739
58	G	1	S1					58	G	46 N=14,D=5,E=5,G=8860,S=855	9739
59	E	1	G1					59	E	11 A=2,N=5,D=34,E=9635,G=14,K=47,V=1,Xaa=1	9739
60	S	0						60	S	2 A=1,L=1,P=9,S=9727,Xaa=1	9739
61	V	4	I2M1X1	I	V	1997	0,0342	61	I	53 R=1,I=8978,L=10,K=14,M=96,T=304,W=1,V=333,Xaa=2	9739
62	I	33	V33	I	V	1997	0,0282	62	I	20 I=9449,L=3,M=2,T=9,V=275,Xaa=1	9739
63	I	3	V3	I	V	2007	0,0338	63	V	33 A=56,I=329,L=27,M=45,V=9281,Xaa=1	9739
64	E	15	D15	E	D	2007	0,0012	64	E	2 A=4,D=12,Q=1,E=9721,G=1	9739
65	S	1	F1					65	S	16 A=14,L=5,F=72,P=37,S=9575,T=13,Y=7,V=14,Xaa=2	9739
66	G	0						66	G	14 R=2,N=1,D=27,C=1,Del=1,E=9,G=9584,S=111,V=1,Xaa=2	9739
67	D	0						67	D	2 A=4,N=10,D=9722,G=1,Xaa=2	9739
68	P	0						68	P	7 Q=21,H=1,L=15,P=9679,S=20,T=1,Xaa=2	9739
69	N	0						69	N	1 N=9730,H=4,S=2,T=1,Y=1,Xaa=1	9739
70	A	0						70	A	18 A=9503,M=1,S=3,T=27,V=204,Xaa=1	9739
71	L	1	X1					71	L	2 I=1,L=9722,F=1,P=13,Xaa=2	9739
72	L	0						72	L	1 L=9735,M=2,V=1,Xaa=1	9739
73	K	0						73	K	3 R=11,Del=1,Q=1,E=1,I=2,K=9719,T=3,Xaa=1	9739
74	H	0						74	H	0 H=9738,Xaa=1	9739
75	R	0						75	R	0 R=9737,Q=1,Xaa=1	9739
76	F	0						76	F	0 C=1,L=1,F=9736,Xaa=1	9739
77	E	0						77	E	1 D=3,E=9733,G=2,Xaa=1	9739
78	I	0						78	I	3 I=9706,L=30,T=1,V=1,Xaa=1	9739
79	I	0						79	I	2 I=9719,L=2,M=5,F=1,V=11,Xaa=1	9739
80	E	0						80	E	0 E=9738,Xaa=1	9739
81	G	0						81	G	0 R=2,G=9736,Xaa=1	9739
82	R	0						82	R	1 R=9735,I=1,K=2,Xaa=1	9739
83	D	0						83	D	1 N=3,D=9730,E=1,G=4,Xaa=1	9739
84	R	0						84	R	0 R=9737,Xaa=2	9739
85	T	2	A2					85	T	20 A=118,N=75,I=26,M=1,P=6,S=3,T=9509,Xaa=1	9739
86	M	15	I15	M	I	2007	0,0112	86	M	21 I=109,L=68,K=2,M=9499,S=1,T=1,V=58,Xaa=1	9739
87	A	0						87	A	1 A=9732,D=1,G=1,V=4,Xaa=1	9739
88	W	0						88	W	1 R=3,C=1,W=9735	9739
89	T	0						89	T	1 A=7,S=1,T=9730,Xaa=1	9739
90	V	2	I1X1					90	V	9 A=31,G=4,I=16,L=5,M=30,V=9653	9739
91	V	0						91	V	5 G=1,I=41,L=1,M=4,V=9692	9739
92	N	0						92	N	1 N=9734,S=2,Y=2,Xaa=1	9739
93	S	0						93	S	1 R=1,C=1,G=1,I=3,S=9731,T=2	9739
94	I	0						94	I	7 I=9671,L=16,M=1,V=50,Xaa=1	9739
95	C	0						95	C	3 C=9709,F=1,S=27,Y=2	9739
96	N	0						96	N	9 R=5,N=9644,H=5,K=59,P=1,S=24,T=1	9739
97	T	1	I1					97	T	5 A=9,N=4,I=16,S=11,T=9699	9739
98	T	0						98	T	4 A=34,I=1,M=1,P=1,T=9702	9739
99	R	7	K4G3			Early marker - 1963	0,0104	99	G	14 R=101,E=55,G=9575,K=2,V=6	9739
100	A	13	V8T3I2	V	A	1975	0,0187	100	V	24 A=182,D=2,I=129,F=1,T=3,V=9422	9739
101	E	1	D1					101	E	82 A=1,N=26,D=1675,E=7875,G=137,K=20,Y=2,V=3	9739
102	K	0						102	K	0 R=1,K=9737,Xaa=1	9739
103	P	0						103	P	1 L=1,P=9735,S=3	9739
104	K	0						104	K	13 R=144,N=9,E=1,I=1,K=9578,T=6	9739
105	F	0						105	F	15 C=1,L=51,F=9578,S=32,Y=76,Xaa=1	9739
106	L	0						106	L	1 H=1,I=8,L=9729,V=1	9739
107	P	0						107	P	0 P=9738,S=1	9739
108	D	0						108	D	1 N=3,D=9734,E=1,G=1	9739
109	L	0						109	L	1 L=9735,M=1,S=1,W=1,V=1	9739
110	Y	0						110	Y	0 F=2,Y=9737	9739
111	D	0						111	D	1 D=9735,E=1,H=2,Xaa=1	9739
112	Y	0						112	Y	0 C=1,Y=9738	9739
113	K	0						113	K	2 R=18,N=1,K=9719,T=1	9739
114	E	12	K12	E	K	2008	0,0035	114	E	4 E=9696,G=9,K=34	9739
115	N	0						115	N	22 N=9507,D=74,G=14,H=3,K=60,S=63,T=8,Y=9,Xaa=1	9739
116	R	0						116	R	1 R=9729,Q=7,H=1,W=2	9739
117	F	0						117	F	0 F=9739	9739
118	V	9	I9	I	V	1975	0,0229	118	I	29 I=9369,L=101,M=2,S=1,T=43,V=223	9739
119	E	0						119	E	0 D=1,E=9738	9739
120	I	0						120	I	6 I=9674,M=1,V=64	9739
121	G	0						121	G	0 R=2,G=9737	9739
122	V	0						122	V	1 I=8,L=2,V=9728,Xaa=1	9739
123	T	0						123	T	0 A=1,T=9737,Xaa=1	9739
124	R	0						124	R	0 R=9737,K=1,Xaa=1	9739
125	R	0						125	R	0 R=9736,K=2,Xaa=1	9739
126	E	0						126	E	2 D=3,E=9722,G=13,Xaa=1	9739
127	V	0						127	V	8 A=9,C=1,I=85,V=9644	9739
128	H	2	N1Y1					128	H	2 R=4,N=5,Q=2,H=9720,P=4,Y=4	9739
129	I	0						129	I	57 A=2,I=8737,L=13,M=72,T=831,V=83,Xaa=1	9739
130	Y	0						130	Y	0 Y=9739	9739
131	Y	0						131	Y	0 C=1,Y=9738	9739
132	L	0						132	L	1 Q=1,I=2,L=9730,M=5,S=1	9739
133	E	0						133	E		

Legend:
 1. **Alternatives:** aa different from consensus , e.g., Q1 means that Q occurred in one isolate
 2. **Score:** Data taken from the Database describing measure of variability.
 3. **Alignment Details:** number of occurrences of various amino acids.
 4. High-potency and Low-potency markers are colored green and yellow, respectively.

Equine Pos	NP H3N8 Consensus	No of Diffs	Alternatives	Permanent aa change			Marker potency	Avian NP all subtypes			Alignment Details	# Sequences
				From	To	Year of substitution		Pos	Consensus	Score		
1	M	0						1	M	0 M=9239	9239	
2	A	0						2	A	0 A=9236, T=1, V=2	9239	
3	S	0						3	S	12 A=5, L=90, F=11, P=7, S=9119, T=5, Y=2	9239	
4	Q	0						4	Q	1 R=2, Q=9232, E=1, H=2, P=2	9239	
5	G	0						5	G	1 A=1, G=9232, S=6	9239	
6	T	0						6	T	0 A=3, T=9236	9239	
7	K	0						7	K	0 K=9239	9239	
8	R	0						8	R	0 R=9239	9239	
9	S	0						9	S	4 G=1, F=2, P=26, S=9202, T=8	9239	
10	Y	0						10	Y	11 H=132, L=1, Y=9106	9239	
11	E	0						11	E	0 D=1, E=9237, G=1	9239	
12	Q	0						12	Q	2 Q=9222, H=1, L=1, K=13, M=1, Xaa=1	9239	
13	M	0						13	M	0 M=9239	9239	
14	E	0						14	E	0 D=3, E=9236	9239	
15	T	0						15	T	2 A=1, N=1, I=11, P=1, T=9225	9239	
16	D	49	G49	G	D	1988	0,0005	16	G	32 N=1, D=5, C=1, Del=110, G=8802, S=320	9239	
17	G	0						17	G	0 D=1, G=9238	9239	
18	E	0						18	E	3 D=17, E=9218, G=2, K=1, V=1	9239	
19	R	0						19	R	0 R=9236, N=1, H=2	9239	
20	Q	0						20	Q	2 Q=9227, H=1, P=10, Xaa=1	9239	
21	N	0						21	N	5 N=9191, D=30, K=5, S=13	9239	
22	A	0						22	A	7 A=9181, P=1, S=27, T=18, V=11, Xaa=1	9239	
23	T	0						23	T	9 N=51, I=1, P=2, S=35, T=9150	9239	
24	E	0						24	E	1 D=4, E=9234, V=1	9239	
25	I	0						25	I	1 I=9233, S=1, T=1, V=4	9239	
26	R	0						26	R	0 R=9239	9239	
27	A	0						27	A	4 A=9204, S=17, T=17, Xaa=1	9239	
28	S	0						28	S	0 S=9238, T=1	9239	
29	V	0						29	V	1 A=1, G=3, I=5, V=9230	9239	
30	G	0						30	G	0 R=2, E=1, G=9236	9239	
31	R	0						31	R	3 R=9209, G=5, K=25	9239	
32	M	0						32	M	0 M=9239	9239	
33	V	0						33	V	28 A=2, I=442, V=8795	9239	
34	G	0						34	G	64 A=1, R=1, N=60, D=4, C=1, G=7911, S=1258, V=3	9239	
35	G	0						35	G	1 A=3, R=4, G=9232	9239	
36	I	0						36	I	2 N=1, I=9222, L=1, F=2, T=1, V=11, Xaa=1	9239	
37	G	0						37	G	0 R=2, G=9236, Xaa=1	9239	
38	R	0						38	R	8 R=9147, K=91, Xaa=1	9239	
39	F	0						39	F	1 L=2, F=9235, Y=1, Xaa=1	9239	
40	Y	0						40	Y	0 Y=9238, Xaa=1	9239	
41	V	1	I1			Early marker - 1963	0,0250	41	I	18 A=3, I=8998, M=1, T=5, V=231, Xaa=1	9239	
42	Q	0						42	Q	0 R=2, Q=9236, Xaa=1	9239	
43	M	0						43	M	2 I=6, M=9221, T=6, V=5, Xaa=1	9239	
44	C	0						44	C	0 C=9236, G=1, S=1, Xaa=1	9239	
45	T	0						45	T	0 T=9238, Xaa=1	9239	
46	E	0						46	E	1 D=1, E=9235, K=1, V=1, Xaa=1	9239	
47	L	0						47	L	4 H=1, I=19, L=9205, F=13, Xaa=1	9239	
48	K	0						48	K	4 R=24, N=3, Q=2, G=1, I=1, K=9207, Xaa=1	9239	
49	L	0						49	L	2 I=6, L=9226, F=5, P=1, Xaa=1	9239	
50	N	7	S6G1	S	N	1976	0,0192	50	S	22 N=177, D=16, G=56, I=1, S=8983, T=5, Xaa=1	9239	
51	D	0						51	D	8 N=18, D=9161, E=58, Y=1, Xaa=1	9239	
52	H	0					0,0652	52	Y	62 N=217, C=4, Q=122, H=602, F=1, S=1, Y=8291, Xaa=1	9239	
53	E	0						53	E	1 D=5, Q=1, E=9231, Xaa=2	9239	
54	G	0						54	G	2 C=8, G=9224, S=5, W=1, Xaa=1	9239	
55	R	0						55	R	1 R=9231, G=1, M=6, Xaa=1	9239	
56	L	0						56	L	1 L=9230, M=1, V=7, Xaa=1	9239	
57	I	1	T1					57	I	12 I=9109, L=46, T=1, Y=1, V=81, Xaa=1	9239	
58	Q	0						58	Q	0 Q=9237, H=1, Xaa=1	9239	
59	N	0						59	N	0 N=9236, K=1, T=1, Xaa=1	9239	
60	S	0						60	S	0 G=1, S=9236, T=1, Xaa=1	9239	
61	I	0						61	I	9 R=1, I=9142, L=4, M=79, V=11, Xaa=2	9239	
62	T	0						62	T	1 A=1, P=2, T=9235, Xaa=1	9239	
63	I	0						63	I	9 I=9156, L=23, M=14, T=1, V=44, Xaa=1	9239	
64	E	1	G1					64	E	0 E=9237, K=1, Xaa=1	9239	
65	R	0						65	R	1 R=9231, K=6, S=1, Xaa=1	9239	
66	M	0						66	M	1 I=2, M=9235, V=1, Xaa=1	9239	
67	V	0						67	V	9 A=8, I=55, L=26, V=9148, Xaa=2	9239	
68	L	0						68	L	0 L=9236, F=2, Xaa=1	9239	
69	S	0						69	S	0 F=1, S=9236, Xaa=2	9239	
70	A	0						70	A	1 A=9231, S=7, Xaa=1	9239	
71	F	0						71	F	0 F=9237, S=1, Xaa=1	9239	
72	D	0						72	D	0 D=9236, E=1, G=1, Xaa=1	9239	
73	E	0						73	E	0 D=1, E=9236, G=1, Xaa=1	9239	
74	R	0						74	R	0 R=9236, G=1, K=1, Xaa=1	9239	
75	R	0						75	R	0 R=9238, Xaa=1	9239	
76	N	0						76	N	0 N=9238, Xaa=1	9239	
77	K	0						77	K	78 R=2073, N=1, Q=2, I=1, K=7161, Xaa=1	9239	
78	Y	0						78	Y	0 Y=9238, Xaa=1	9239	
79	L	0						79	L	0 L=9236, M=1, P=1, Xaa=1	9239	
80	E	0						80	E	0 D=1, E=9237, Xaa=1	9239	
81	E	0						81	E	2 D=11, E=9221, G=5, K=1, Xaa=1	9239	
82	H	0						82	H	7 R=1, N=65, H=9168, P=1, S=1, Y=2, Xaa=1	9239	
83	P	0						83	P	0 L=1, P=9237, Xaa=1	9239	
84	S	0						84	S	13 R=7, N=97, H=1, I=1, S=9104, T=28, Xaa=1	9239	
85	A	0						85	A	11 A=9127, D=1, S=13, T=81, V=15, Xaa=2	9239	
86	G	0						86	G	2 R=7, G=9223, K=8, Xaa=1	9239	
87	K	0						87	K	9 R=104, K=9134, Xaa=1	9239	
88	D	0						88	D	0 N=1, D=9236, G=1, Xaa=1	9239	
89	P	0						89	P	1 H=6, P=9232, Xaa=1	9239	
90	K	0						90	K	2 R=15, N=1, K=9221, T=1, Xaa=1	9239	
91	K	0						91	K	0 N=1, K=9237, Xaa=1	9239	
92	T	0						92	T	0 T=9238, Xaa=1	9239	
93	G	0						93	G	0 G=9237, V=1, Xaa=1	9239	
94	G	0						94	G	0 G=9238, Xaa=1	9239	
95	P	0						95	P	1 P=9230, S=8, Xaa=1	9239	
96	I	0						96	I	5 I=9194, L=4, M=1, F=1, T=4, V=34, Xaa=1	9239	
97	Y	0						97	Y	0 H=1, S=1, Y=9236, Xaa=1	9239	
98	R	0						98	R	20 R=8952, G=1, K=285, Xaa=1	9239	
99	R	2	K2					99	R	1 R=9233, K=4, M=1, Xaa=1	9239	
100	K	23	R23	R	K	1985	0,0030	100	R	6 R=9182, I=6, K=28, V=22, Xaa=1	9239	
101	D	0						101	D	17 A=1, N=11, D=9018, E=208, Xaa=1	9239	
102	G	0						102	G	1 A=3, R=3, G=9230, V=2, Xaa=1	9239	
103	K	0						103	K	5 R=49, N=2, E=1, K=9186, Xaa=1	9239	
104	W	0						104	W	1 L=5, W=9233, Xaa=1	9239	
105	M	10	I8V2					105	M	124 A=17, Q=6, I=330, L=12, K=4, M=5528, T=70, V=3269, Xaa=3	9239	
106	R	0						106	R	1 R=9234, K=4, Xaa=1	9239	
107	E	0						107	E	1 A=1, E=9233, G=4, Xaa=1	9239	
108	L	0						108	L	1 I=1, L=9233, M=4, Xaa=1	9239	
109	I	0						109	I	23 I=8962, L=1, M=3, S=3, T=97, V=172, Xaa=1	9239	
110	L	0						110	L	0 I=2, L=9236, Xaa=1	9239	
111	H	14	Y11Q3	Y	H	1976	H:0.0018 Q:0.0000	111	Y	9 N=1, C=12, H=17, F=50, Y=9158, Xaa=1	9239	
112	D	0						112	D	4 N=16, D=9208, E=10, G=2, H=1, Y=1, Xaa=1	9239	
113	K	0						113	K	0 Q=1, K=9237, Xaa=1	9239	
114	E	0						114	E	10 A=1, D=116, E=9120, G=1, Xaa=1	9239	
115	E	0						115	E	0 E=9236, G=1, K=1, Xaa=1	9239	
116	I	0						116	I	7 I=9172, L=44, T=1, V=21, Xaa=1	9239	
117	M	2	I1R1			Early marker - 1963	0,0003	117	R	3 R=9218, G=1, K=14, M=3, S=1, T=1, Xaa=1	9239	
118	R	0						118	R	2 R=9224, K=14, Xaa=1	9239	
119	I	3	V3					119	I	12 N=32, D=1, I=9117, L=8, M=3, T=2, V=75, Xaa=1	9239	
120	W	0						120	W	0 W=9237, Xaa=2	9239	
121	R	0						121	R	3 R=9219, C=2, G=1, H=13, P=1, S=2, Xaa=1	9239	
122	Q	1	R1					122	Q	2 R=7, Q=9228, L=2, P=1, Xaa=1	9239	
123	A	0						123	A	1 A=9235, R=1, S=1, V=1, Xaa=1	9239	
124	N	0						124	N	2 N=9221, D=1, H=13, S=2, T=1, Xaa=1	9239	
125	N	0						125	N	4 N=9208, D=1, H=1, S=26, T=1, Y=1, Xaa=1	9239	
126	G	0						126	G	1 R=1, E=2, G=9234, V=1, Xaa=1	9239	
127	E	0						127	E	15 N=1, D=191, E=9042, G=3, K=1, Xaa=1	9239	
128	D	0						128	D	5 N=8, D=9193, E=33, G=3, Y=1, Xaa=1	9239	
129	A	0						129	A	9 A=9140, D=1, G=2, S=87, T=7, V=1, Xaa=1	9239	
130	T	0						130	T	17 A=15, N=64, I=1, L=1, P=107, S=1, T=9049, Xaa=1	9	

137	M	0				137	M	0	R=1,M=9238	9239
138	I	0				138	I	0	1 I=9233,L=1,V=5	9239
139	W	0				139	W	0	0 W=9238,Xaa=1	9239
140	H	0				140	H	0	0 R=1,H=9238	9239
141	S	0				141	S	0	0 P=1,S=9236,T=1,Xaa=1	9239
142	N	0				142	N	0	0 A=1,N=9237,S=1	9239
143	L	0				143	L	2	2 I=1,L=9227,M=4,V=7	9239
144	N	0				144	N	1	1 N=9234,D=1,H=2,K=1,Y=1	9239
145	D	0				145	D	1	1 N=1,D=9235,E=1,G=2	9239
146	T	1	A1	Early marker - 1963	0,0062	146	A	9	9 A=9148,S=30,T=57,V=4	9239
147	T	0				147	T	0	0 A=1,I=2,T=9236	9239
148	Y	0				148	Y	0	0 S=1,Y=9238	9239
149	Q	0				149	Q	1	1 Q=9233,H=5,Xaa=1	9239
150	R	0				150	R	0	0 R=9237,S=1,T=1	9239
151	T	0				151	T	0	0 P=1,T=9237,Xaa=1	9239
152	R	0				152	R	0	0 R=9236,G=3	9239
153	A	0				153	A	0	0 A=9237,G=1,P=1	9239
154	L	0				154	L	1	1 H=2,I=2,L=9233,F=1,V=1	9239
155	V	0				155	V	0	0 A=1,I=1,V=9237	9239
156	R	0				156	R	0	0 R=9239	9239
157	T	34	A34			157	T	15	15 A=16,P=1,S=173,T=9049	9239
158	G	0				158	G	0	0 G=9238,Xaa=1	9239
159	M	0				159	M	0	0 L=1,M=9237,V=1	9239
160	D	0				160	D	0	0 D=9237,G=2	9239
161	P	0				161	P	0	0 A=1,P=9237,T=1	9239
162	R	0				162	R	1	1 R=9233,I=1,K=2,M=1,T=1,Xaa=1	9239
163	M	0				163	M	1	1 M=9231,T=6,Xaa=2	9239
164	C	0				164	C	1	1 R=1,C=9235,G=1,Xaa=2	9239
165	S	0				165	S	1	1 C=1,F=1,P=1,S=9234,Xaa=2	9239
166	L	0				166	L	1	1 I=1,L=9235,M=1,Xaa=2	9239
167	M	1	T1			167	M	1	1 K=1,M=9234,T=1,Xaa=3	9239
168	Q	0				168	Q	1	1 Q=9233,H=1,L=1,K=1,P=1,Xaa=2	9239
169	G	0				169	G	1	1 A=1,R=1,G=9235,Xaa=2	9239
170	S	0				170	S	0	0 P=1,S=9236,Xaa=2	9239
171	T	0				171	T	1	1 A=1,N=1,S=1,T=9234,Xaa=2	9239
172	L	0				172	L	1	1 I=1,L=9232,F=2,P=2,Xaa=2	9239
173	P	0				173	P	0	0 P=9236,Xaa=3	9239
174	R	0				174	R	1	1 R=9234,K=1,T=1,Xaa=3	9239
175	R	0				175	R	0	0 R=9236,K=1,Xaa=2	9239
176	S	0				176	S	1	1 P=1,S=9235,Xaa=3	9239
177	G	0				177	G	0	0 R=1,G=9236,Xaa=2	9239
178	A	0				178	A	1	1 A=9233,D=1,F=1,S=1,V=1,Xaa=2	9239
179	A	0				179	A	0	0 A=9237,Xaa=2	9239
180	G	0				180	G	1	1 R=1,G=9235,V=1,Xaa=2	9239
181	A	0				181	A	1	1 A=9231,S=5,Xaa=3	9239
182	A	1	S1			182	A	2	2 A=9224,G=1,S=8,T=3,Xaa=3	9239
183	V	2	I2			183	V	21	21 A=1,I=218,L=1,M=42,V=8974,Xaa=3	9239
184	K	0				184	K	1	1 R=1,K=9234,Xaa=4	9239
185	G	0				185	G	1	1 G=9235,V=1,Xaa=3	9239
186	V	0				186	V	24	24 I=361,V=8875,Xaa=3	9239
187	G	0				187	G	1	1 R=3,G=9233,Xaa=3	9239
188	T	0				188	T	1	1 A=1,Q=1,P=1,T=9233,Xaa=3	9239
189	M	3	I3			189	M	7	7 I=58,L=3,M=9172,V=3,Xaa=3	9239
190	V	10	I10			190	V	8	8 A=68,G=2,I=5,T=2,V=9159,Xaa=3	9239
191	M	0				191	M	1	1 L=5,M=9229,V=2,Xaa=3	9239
192	E	0				192	E	1	1 E=9235,Xaa=4	9239
193	L	0				193	L	2	2 I=14,L=9220,M=1,V=1,Xaa=3	9239
194	I	0				194	I	20	20 N=1,I=8975,F=1,T=7,V=252,Xaa=3	9239
195	R	1	Q1			195	R	1	1 R=9235,Q=1,Xaa=3	9239
196	M	0				196	M	1	1 I=1,M=9233,T=2,Xaa=3	9239
197	I	0				197	I	6	6 I=9178,T=3,V=55,Xaa=3	9239
198	K	0				198	K	3	3 R=12,N=2,K=9219,S=1,T=2,Xaa=3	9239
199	R	0				199	R	1	1 R=9233,Q=2,G=1,Xaa=3	9239
200	G	0				200	G	1	1 R=3,E=1,G=9232,Xaa=3	9239
201	I	3	V3			201	I	26	26 A=1,N=1,I=8856,M=1,T=4,V=373,Xaa=3	9239
202	N	0				202	N	1	1 N=9231,D=1,H=1,S=2,T=1,Xaa=3	9239
203	D	0				203	D	1	1 A=1,N=2,D=9232,I=1,Xaa=3	9239
204	R	0				204	R	1	1 R=9231,Q=1,P=3,W=1,Xaa=3	9239
205	N	0				205	N	1	1 N=9234,K=1,S=1,Xaa=3	9239
206	F	0				206	F	1	1 F=9233,Xaa=6	9239
207	W	0				207	W	1	1 W=9234,Xaa=5	9239
208	R	0				208	R	1	1 R=9233,G=1,Xaa=5	9239
209	G	0				209	G	1	1 G=9234,Xaa=5	9239
210	E	0				210	E	13	13 D=157,E=9075,G=1,P=1,Xaa=5	9239
211	N	0				211	N	4	4 N=9208,D=2,S=14,T=10,Xaa=5	9239
212	G	0				212	G	1	1 E=2,G=9232,Xaa=5	9239
213	R	0				213	R	1	1 R=9233,P=1,Xaa=5	9239
214	R	5	K5			214	R	8	8 R=9148,L=1,K=85,Xaa=5	9239
215	T	0				215	T	2	2 A=4,N=1,P=2,T=9227,Xaa=5	9239
216	R	0				216	R	3	3 R=9214,G=1,K=16,M=2,W=1,Xaa=5	9239
217	I	0				217	I	27	27 A=2,N=3,I=8909,L=1,M=5,F=1,T=222,V=91,Xaa=5	9239
218	A	0				218	A	1	1 A=9230,G=1,P=1,T=2,Xaa=5	9239
219	Y	0				219	Y	1	1 C=1,Y=9233,Xaa=5	9239
220	E	0				220	E	2	2 D=8,E=9224,K=1,Xaa=6	9239
221	R	1	K1			221	R	1	1 R=9233,G=1,Xaa=5	9239
222	M	0				222	M	1	1 I=2,M=9232,Xaa=5	9239
223	C	0				223	C	1	1 C=9232,S=1,Y=1,Xaa=5	9239
224	N	0				224	N	1	1 N=9232,K=1,T=1,Xaa=5	9239
225	I	0				225	I	1	1 I=9229,V=5,Xaa=5	9239
226	L	0				226	L	1	1 L=9233,P=1,Xaa=5	9239
227	K	0				227	K	1	1 K=9234,Xaa=5	9239
228	G	0				228	G	1	1 R=2,G=9232,Xaa=5	9239
229	K	0				229	K	1	1 K=9232,T=1,Xaa=6	9239
230	F	0				230	F	3	3 I=2,L=14,F=9216,S=2,Xaa=5	9239
231	Q	0				231	Q	1	1 R=2,Q=9229,P=3,Xaa=5	9239
232	T	0				232	T	3	3 A=2,I=12,T=9219,Xaa=6	9239
233	A	0				233	A	1	1 A=9234,Xaa=5	9239
234	A	0				234	A	5	5 A=9195,I=1,S=31,T=3,V=4,Xaa=5	9239
235	Q	0				235	Q	1	1 Q=9234,Xaa=5	9239
236	R	0				236	R	3	3 R=9212,G=1,K=21,Xaa=5	9239
237	A	0				237	A	4	4 A=9208,G=15,S=1,T=7,V=3,Xaa=5	9239
238	M	0				238	M	1	1 I=3,L=1,M=9229,T=1,Xaa=5	9239
239	M	0				239	M	13	13 R=1,G=1,I=2,M=9083,T=4,V=142,Xaa=6	9239
240	D	0				240	D	1	1 A=1,N=1,D=9231,I=1,Xaa=5	9239
241	Q	0				241	Q	1	1 Q=9232,H=1,K=1,Xaa=5	9239
242	V	0				242	V	1	1 A=4,I=1,V=9229,Xaa=5	9239
243	R	0				243	R	1	1 R=9231,Q=2,K=1,Xaa=5	9239
244	E	0				244	E	1	1 E=9233,K=1,Xaa=5	9239
245	G	3	S3	Early marker - 1963	0,0021	245	S	3	3 N=1,G=19,S=9214,Xaa=5	9239
246	R	0				246	R	2	2 R=9227,G=1,K=4,T=1,Xaa=6	9239
247	N	0				247	N	9	9 N=9149,D=1,G=1,H=1,S=79,T=3,Xaa=5	9239
248	P	0				248	P	1	1 P=9233,T=1,Xaa=5	9239
249	G	0				249	G	1	1 R=2,E=2,G=9229,V=1,Xaa=5	9239
250	N	0				250	N	1	1 N=9233,S=1,Xaa=5	9239
251	A	0				251	A	3	3 A=9208,V=26,Xaa=5	9239
252	E	0				252	E	1	1 E=9234,Xaa=5	9239
253	I	0				253	I	5	5 I=9194,M=2,T=4,V=34,Xaa=5	9239
254	E	0				254	E	1	1 E=9233,G=1,Xaa=5	9239
255	D	0				255	D	1	1 N=1,D=9229,E=1,G=2,H=1,Xaa=5	9239
256	L	0				256	L	1	1 I=2,L=9229,F=1,V=2,Xaa=5	9239
257	I	17	T16S1	I T	2007 0,0081	257	I	9	9 A=1,I=9151,L=3,S=1,T=75,V=3,Xaa=5	9239
258	F	0				258	F	1	1 F=9233,V=1,Xaa=5	9239
259	L	0				259	L	2	2 R=2,Q=1,I=2,L=9224,P=1,S=4,Xaa=5	9239
260	A	4	T4			260	A	2	2 A=9226,T=7,Xaa=6	9239
261	R	0				261	R	1	1 R=9234,Xaa=5	9239
262	S	0				262	S	1	1 C=1,S=9232,Xaa=6	9239
263	A	0				263	A	1	1 A=9232,P=1,Xaa=6	9239
264	L	0				264	L	1	1 I=1,L=9231,P=2,Xaa=5	9239
265	I	1	V1			265	I	3	3 I=9216,L=1,V=15,Xaa=7	9239
266	L	0				266	L	2	2 Q=1,L=9227,M=1,P=2,V=2,Xaa=6	9239
267	R	0				267	R	1	1 R=9232,K=1,Xaa=6	9239
268	G	0				268	G	1	1 R=2,E=2,G=9229,Xaa=6	9239
269	S	0				269	S	10	10 A=98,P=2,S=9131,T=2,Xaa=6	9239
270	V	0				270	V	11	11 A=2,I=78,L=2,M=21,V=9130,Xaa=6	9239
271	A	0				271	A	1	1 A=9229,G=1,P=2,S=1,Xaa=6	9239
272	H	0				272	H	1	1 H=9232,L=1,Xaa=6	9239
273	K	0				273	K	1	1 K=9233,Xaa=6	9239
274	S	0				274	S	1	1 S=9233,Xaa=6	9239
275	C	0				275	C	2	2 C=9225,S=5,W=1,Y=1,Xaa=7	9239
276	L	0				276	L	2	2 L=9226,M=4,P=1,W=1,Xaa=7	9239
277	P	0				277	P	2	2 A=1,H=2,P=9228,S=1,Xaa=7	9239
278	A	0								

286	T	49	A49	A	T	1988	0,0001	286	A	6 A=9181, L=2, P=3, S=1, T=1, V=44, Xaa=7	9239
287	S	1	T1					287	S	11 N=16, G=96, I=1, S=9118, T=1, Xaa=7	9239
288	G	0						288	G	1 A=2, R=1, G=9230, Xaa=6	9239
289	Y	1	H1					289	Y	6 H=38, F=11, Y=9184, Xaa=6	9239
290	D	0						290	D	10 A=1, N=67, D=9142, E=14, G=1, H=1, S=3, V=1, Xaa=8	9239
291	F	0						291	F	1 L=3, F=9230, Xaa=6	9239
292	E	0						292	E	4 Q=3, E=9210, G=15, K=1, V=3, Xaa=7	9239
293	K	9	R9	R	K	1975	0,0113	293	R	10 R=9124, G=2, I=3, K=104, Xaa=6	9239
294	E	0						294	E	2 A=2, D=3, E=9225, G=1, K=2, Xaa=6	9239
295	G	0						295	G	1 R=4, G=9229, Xaa=6	9239
296	Y	0						296	Y	4 H=26, S=1, Y=9207, Xaa=5	9239
297	S	0						297	S	1 L=1, P=1, S=9232, Xaa=5	9239
298	L	0						298	L	1 I=9231, P=1, S=1, V=1, Xaa=5	9239
299	V	1	I1					299	V	1 I=2, V=9232, Xaa=5	9239
300	G	0						300	G	1 R=1, G=9233, Xaa=5	9239
301	I	7	V6T1					301	I	15 R=6, N=1, I=9079, L=18, K=1, M=12, P=4, V=113, Xaa=5	9239
302	D	0						302	D	2 A=2, N=1, D=9223, E=2, G=3, Xaa=8	9239
303	P	0						303	P	2 L=1, P=9226, S=7, Xaa=5	9239
304	F	0						304	F	2 L=1, F=9227, S=3, Y=3, Xaa=5	9239
305	K	1	R1		Early marker - 1963		0,0065	305	R	8 R=9165, H=7, K=60, S=2, Xaa=5	9239
306	L	0						306	L	2 L=9226, M=5, P=1, V=2, Xaa=5	9239
307	L	0						307	L	1 L=9233, F=1, Xaa=5	9239
308	Q	0						308	Q	4 Q=9207, K=27, Xaa=5	9239
309	N	0						309	N	2 A=1, N=9224, D=1, K=4, S=2, T=2, Xaa=5	9239
310	S	0						310	S	4 N=1, G=2, S=9205, T=23, V=3, Xaa=5	9239
311	Q	0						311	Q	1 R=1, Q=9233, Xaa=5	9239
312	I	7	V7	V	I	1975	0,0032	312	V	4 A=1, I=30, V=9203, Xaa=5	9239
313	F	1	L1					313	F	9 I=8, L=53, F=9151, S=14, Y=2, V=6, Xaa=5	9239
314	S	0						314	S	1 C=2, S=9232, Xaa=5	9239
315	L	0						315	L	4 I=31, L=9202, P=1, Xaa=5	9239
316	I	0						316	I	5 I=9194, L=1, M=7, T=1, V=31, Xaa=5	9239
317	R	0						317	R	1 R=9231, S=3, Xaa=5	9239
318	P	0						318	P	18 Q=1, L=4, K=1, P=9017, S=207, T=4, Xaa=5	9239
319	K	9	N9	N	K	1975	0,0038	319	N	6 N=9182, D=1, I=1, K=35, S=12, T=3, Xaa=5	9239
320	E	0						320	E	1 D=1, E=9231, G=1, K=1, Xaa=5	9239
321	N	0						321	N	3 N=9212, I=1, K=4, S=15, T=1, Y=1, Xaa=5	9239
322	P	0						322	P	3 A=2, Q=1, P=9211, S=19, T=1, Xaa=5	9239
323	A	0						323	A	8 A=9163, P=1, S=33, T=11, V=25, Xaa=6	9239
324	H	0						324	H	1 Q=1, H=9232, Xaa=6	9239
325	K	0						325	K	1 R=1, Q=1, K=9231, Xaa=6	9239
326	S	0						326	S	2 G=9, I=1, S=9224, Xaa=5	9239
327	Q	0						327	Q	1 R=1, Q=9232, P=1, Xaa=5	9239
328	L	0						328	L	1 L=9230, M=1, F=2, V=1, Xaa=5	9239
329	V	0						329	V	11 A=1, E=1, I=110, M=2, V=9120, Xaa=5	9239
330	W	0						330	W	1 G=1, W=9233, Xaa=5	9239
331	M	0						331	M	5 I=21, L=7, M=9200, T=1, V=5, Xaa=5	9239
332	A	0						332	A	1 A=9232, T=2, Xaa=5	9239
333	C	0						333	C	1 C=9233, S=1, Xaa=5	9239
334	H	0						334	H	1 R=1, N=2, H=9229, P=1, Y=1, Xaa=5	9239
335	S	0						335	S	3 A=6, F=2, P=2, S=9220, T=1, Xaa=8	9239
336	A	0						336	A	2 A=9224, P=2, S=3, T=3, V=2, Xaa=5	9239
337	A	0						337	A	2 A=9226, S=1, T=3, V=5, Xaa=4	9239
338	F	0						338	F	1 F=9231, S=1, Y=2, Xaa=5	9239
339	E	0						339	E	1 E=9234, Xaa=5	9239
340	D	0						340	D	1 N=2, D=9231, G=1, H=1, Y=1, Xaa=3	9239
341	L	2	M2					341	L	1 L=9230, M=6, Xaa=3	9239
342	R	1	K1					342	R	1 R=9232, G=2, K=1, S=1, Xaa=3	9239
343	V	0						343	V	3 A=1, I=15, L=1, M=6, V=9212, Xaa=4	9239
344	L	30	S30	S	L	1985	0,0103	344	S	9 A=1, L=95, F=1, S=9136, T=3, Xaa=3	9239
345	N	1	S1		Early marker - 1963		0,0003	345	S	2 N=3, G=8, S=9224, T=1, Xaa=3	9239
346	F	0						346	F	2 L=13, F=9221, Xaa=5	9239
347	I	0						347	I	3 I=9214, L=2, V=20, Xaa=3	9239
348	R	0						348	R	1 R=9233, G=1, K=2, Xaa=3	9239
349	G	0						349	G	1 R=1, G=9235, Xaa=3	9239
350	T	0						350	T	37 A=492, I=23, K=30, M=2, P=1, S=7, T=8681, Xaa=3	9239
351	K	1	R1		Early marker - 1963		0,0451	351	R	27 R=8816, G=1, I=2, K=417, Xaa=3	9239
352	V	1						352	V	27 A=7, I=93, L=39, M=175, V=8921, Xaa=4	9239
353	I	9	V9	V	I	1975	0,0641	353	V	51 A=52, I=592, L=124, F=6, S=1, V=8461, Xaa=3	9239
354	P	0			vmuc: Not a marker (>0.05)			354	P	1 P=9235, S=1, Xaa=3	9239
355	R	0						355	R	0 R=9236, Xaa=3	9239
356	G	0						356	G	1 R=3, E=1, G=9232, Xaa=3	9239
357	Q	3	R2K1					357	Q	6 R=4, Q=9178, K=53, T=1, Xaa=3	9239
358	L	0						358	L	2 Q=1, L=9223, M=10, T=1, Y=1, Xaa=3	9239
359	A	20	T19S1	A	AT	Early marker - 1963 AT from 2002	A:0.0049 T:0.0001	359	S	6 A=45, C=1, P=1, S=9186, T=1, Y=1, V=1, Xaa=3	9239
360	T	0						360	T	1 A=1, P=2, T=9232, Xaa=4	9239
361	R	0						361	R	1 R=9232, G=1, I=1, K=2, Xaa=3	9239
362	G	0						362	G	1 R=2, G=9234, Xaa=3	9239
363	V	0						363	V	14 A=5, I=165, L=1, F=2, V=9064, Xaa=2	9239
364	Q	0						364	Q	1 Q=9233, E=1, L=2, K=1, Xaa=2	9239
365	I	0						365	I	14 N=1, I=9057, M=1, T=1, V=177, Xaa=2	9239
366	A	0						366	A	1 A=9232, S=6, Xaa=1	9239
367	S	0						367	S	1 L=1, F=1, P=1, S=9234, W=1, Xaa=1	9239
368	N	0						368	N	3 N=9210, K=3, F=1, S=24, Xaa=1	9239
369	E	0						369	E	1 E=9235, G=1, K=2, Xaa=1	9239
370	N	0						370	N	3 N=9212, H=2, S=24, Xaa=1	9239
371	M	0						371	M	40 A=1, I=91, L=5, M=8720, T=140, V=281, Xaa=1	9239
372	E	0						372	E	20 D=277, Del=1, E=8957, G=4	9239
373	T	0						373	T	74 A=1444, Del=1, I=44, L=3, M=29, P=3, S=11, T=7691, V=13	9239
374	I	2	M2		Early marker - 1963		0,0216	374	M	18 Del=1, T=200, L=3, M=9009, V=26	9239
375	D	0						375	D	24 N=80, D=8985, Del=1, Q=1, E=99, G=23, S=42, V=7, Xaa=1	9239
376	S	1						376	S	1 A=2, Del=1, H=1, F=1, S=9233, Y=1	9239
377	S	4	C2N2					377	S	67 R=4, N=1485, D=1, C=1, Del=1, G=4, H=1, I=3, M=1, P=1, S=7725, T=12	9239
378	T	0						378	T	1 A=1, Del=1, P=2, S=1, T=9234	9239
379	L	1	V1					379	L	1 Del=1, I=1, L=9235, P=1, Xaa=1	9239
380	E	0						380	E	1 D=1, Del=1, E=9235, G=1, K=1	9239
381	L	0						381	L	1 Del=1, L=9232, M=5, P=1	9239
382	R	0						382	R	1 R=9232, Del=1, G=1, L=1, K=4	9239
383	S	0						383	S	1 N=1, G=1, I=1, S=9235, Xaa=1	9239
384	R	44	K44	R	K	1997	0,0181	384	R	14 R=9065, Q=4, I=1, K=167, Xaa=2	9239
385	Y	0						385	Y	1 H=4, F=1, Y=9232, Xaa=2	9239
386	W	0						386	W	1 R=1, C=1, G=1, W=9235, Xaa=1	9239
387	A	0						387	A	0 A=9237, S=1, V=1	9239
388	I	0						388	I	0 I=9238, K=1	9239
389	R	0						389	R	6 R=9173, K=65, W=1	9239
390	T	0						390	T	1 A=1, N=1, I=1, M=1, P=3, S=1, T=9231	9239
391	R	1	K1					391	R	3 R=9212, Q=1, G=1, I=2, K=22, P=1	9239
392	S	0						392	S	0 R=1, G=1, S=9237	9239
393	G	0						393	G	0 G=9238, Xaa=1	9239
394	G	0						394	G	0 R=1, G=9237, K=1	9239
395	N	0						395	N	10 N=9142, D=3, Del=1, H=1, K=1, S=16, T=75	9239
396	T	0						396	T	6 A=10, N=40, Del=1, P=1, S=1, T=9186	9239
397	S	12	N12	N	S	1975	0,0262	397	N	19 N=8988, D=1, Del=1, H=1, I=3, K=1, S=242, T=1, Xaa=1	9239
398	Q	1	K1					398	Q	5 R=9, N=1, Q=9193, H=25, L=1, K=8, Xaa=2	9239
399	Q	0						399	Q	8 R=1, N=10, Q=9161, H=67	9239
400	R	3	K3					400	R	17 R=9019, I=1, K=216, S=1, W=1, Xaa=1	9239
401	A	0						401	A	2 A=9221, E=1, G=3, S=6, T=5, V=3	9239
402	S	0						402	S	3 A=1, F=6, P=1, S=9220, T=2, Y=3, Xaa=6	9239
403	A	0						403	A	0 A=9236, T=3	9239
404	G	0						404	G	0 G=9239	9239
405	Q	0						405	Q	0 Q=9238, H=1	9239
406	I	0						406	I	14 I=9059, L=1, T=3, V=176	9239
407	S	0						407	S	0 R=1, S=9237, Xaa=1	9239
408	V	0						408	V	30 I=482, L=2, T=1, V=8753, Xaa=1	9239
409	Q	0						409	Q	0 Q=9238, Xaa=1	9239
410	P	0						410	P	0 R=1, P=9237, Xaa=1	9239
411	T	0						411	T	2 A=8, P=1, S=2, T=9227, Xaa=1	923

433	T	0					433	T	31	A=186,N=112,I=11,M=5,P=30,S=23,T=8871,Xaa=1	9239
434	E	0					434	E	1	D=6,E=9232,Xaa=1	9239
435	G	2	R2				435	G	1	A=1,C=1,G=9234,S=2,Xaa=1	9239
436	R	0					436	R	0	R=9237,D=1,Xaa=1	9239
437	T	2	A2				437	T	2	A=1,I=13,F=1,T=9223,Xaa=1	9239
438	S	0					438	S	1	R=1,F=5,S=9229,T=1,Y=1,Xaa=2	9239
439	D	0					439	D	1	D=9235,E=1,H=1,Xaa=2	9239
440	M	0					440	M	1	E=1,L=6,M=9230,V=1,Xaa=1	9239
441	R	0					441	R	1	R=9234,G=2,K=1,T=1,Xaa=1	9239
442	T	3	M3				442	T	5	A=9,E=1,S=30,T=9198,Xaa=1	9239
443	E	0					443	E	1	E=9235,G=1,I=1,V=1,Xaa=1	9239
444	I	0					444	I	5	I=9189,F=1,T=3,V=45,Xaa=1	9239
445	I	0					445	I	1	I=9230,V=8,Xaa=1	9239
446	R	0					446	R	28	R=8811,G=3,I=1,K=419,M=2,S=1,Xaa=2	9239
447	M	0					447	M	7	I=9,L=5,M=9179,T=41,V=4,Xaa=1	9239
448	M	0					448	M	1	R=1,I=1,L=1,M=9235,Xaa=1	9239
449	E	1	G1				449	E	0	D=1,E=9238	9239
450	N	20	S20				450	N	101	R=1,N=5571,D=3,G=13,H=1,I=14,K=1,S=3633,T=2	9239
451	A	0					451	A	8	A=9164,C=1,S=40,T=22,V=12	9239
452	K	53	R53				452	R	42	R=8468,Q=3,G=4,K=761,S=2,T=1	9239
453	S	4	P4				453	P	7	A=4,Q=1,L=6,P=9179,S=38,T=9,Xaa=2	9239
454	E	1	G1				454	E	8	D=81,E=9150,G=7,K=1	9239
455	D	0					455	D	3	N=7,D=9215,E=15,G=1,V=1	9239
456	V	1	A1				456	V	9	A=3,L=57,M=26,V=9153	9239
457	S	0					457	S	0	F=1,S=9238	9239
458	F	0					458	F	0	F=9236,S=2,Xaa=1	9239
459	Q	0					459	Q	2	R=3,Q=9226,H=8,K=1,Xaa=1	9239
460	G	0					460	G	1	R=5,E=1,G=9233	9239
461	R	0					461	R	0	R=9237,P=1,W=1	9239
462	G	0					462	G	0	A=1,G=9238	9239
463	V	0					463	V	0	A=3,V=9236	9239
464	F	0					464	F	0	R=1,F=9237,S=1	9239
465	E	0					465	E	1	A=1,E=9235,G=2,K=1	9239
466	L	1	F1				466	L	5	I=27,L=9191,F=20,V=1	9239
467	S	0					467	S	1	A=2,P=1,S=9231,T=3,W=1,Y=1	9239
468	D	0					468	D	1	A=1,N=1,D=9234,E=2	9239
469	E	0					469	E	1	D=2,E=9234,K=1,S=1,Xaa=1	9239
470	K	0					470	K	1	R=2,N=1,E=1,K=9233,T=2	9239
471	A	0					471	A	0	A=9237,R=1,I=1	9239
472	T	0					472	T	4	A=33,R=1,K=1,M=1,S=1,T=9202	9239
473	N	0					473	N	4	N=9202,H=2,K=1,S=33,T=1	9239
474	P	0					474	P	1	P=9229,S=9,T=1	9239
475	I	0					475	I	2	I=9223,T=2,V=14	9239
476	V	0					476	V	1	A=3,L=1,V=9235	9239
477	P	0					477	P	0	P=9239	9239
478	S	0					478	S	2	F=9,P=3,S=9227	9239
479	F	1	L1				479	F	2	L=14,F=9224,V=1	9239
480	D	0					480	D	3	N=10,D=9214,E=14,G=1	9239
481	M	0					481	M	6	H=1,I=15,L=3,K=2,M=9182,T=1,V=35	9239
482	S	5	N4G1				482	S	71	N=1713,D=1,C=1,Del=2,E=1,G=2,I=1,S=7511,T=7	9239
483	N	0					483	N	27	N=8829,D=4,K=404,S=1,Y=1	9239
484	E	0					484	E	3	D=27,E=9209,G=2,V=1	9239
485	G	0					485	G	1	R=4,E=1,G=9233,S=1	9239
486	S	0					486	S	1	I=1,F=1,P=1,S=9232,T=2,Y=1,Xaa=1	9239
487	Y	0					487	Y	0	N=1,H=2,Y=9236	9239
488	F	1	Y1				488	F	1	F=9232,S=7	9239
489	F	1	Y1				489	F	1	L=1,F=9234,S=4	9239
490	G	0					490	G	0	E=2,G=9236,V=1	9239
491	D	0					491	D	1	A=3,N=2,D=9230,E=3,G=1	9239
492	N	0					492	N	5	N=9203,D=4,H=7,I=2,K=3,S=16,T=3,Y=1	9239
493	A	1	T1				493	A	0	A=9239	9239
494	E	0					494	E	4	D=21,E=9201,G=2,K=14,Xaa=1	9239
495	E	0					495	E	3	D=20,E=9213,G=1,K=5	9239
496	F	2	Y2				496	Y	12	H=1,I=3,F=135,S=1,Y=9098	9238
497	D	0					497	D	17	A=1,N=1,D=9026,E=202,G=2,H=1,T=2,Y=3	9238
498	S	8	N8				498	N	14	N=9073,D=1,H=5,I=5,K=5,S=145,Y=1,Xaa=3	9238

vmuc:
Not a marker
(>0.05)

vmuc:
Not a marker
(>0.05)

N S

R K

Early marker - 1963

2007 0,3932

1990 0,0824

0,0041

Early marker - 1963

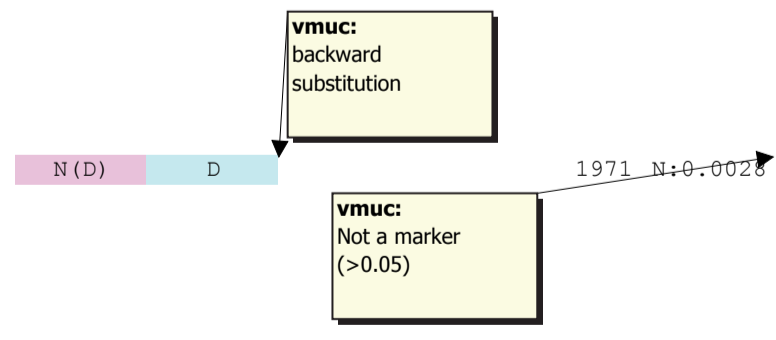
0,0146

1975 0,0157

- Legend:**
- 1. Alternatives:** aa different from consensus , e.g., Q1 means that Q occurred in one isolate
 - 2. Score:** Data taken from the Database describing measure of variability.
 - 3. Alignment Details:** number of occurrences of various amino acids.
 - 4. High-potency and Low-potency markers** are colored green and yellow, respectively.

Equine Pos	M1 Consensus	H3N8 No of Diffs	Alternatives	Permanent aa change			Marker potency	Avian M1 all subtypes			Alignment Details	# Sequences
				From	To	Year of substitution		Pos	Consensus	Score		
1	M	0						1	M	0 M=9864, S=1	9865	
2	S	0						2	S	1 R=2, N=1, D=1, Del=1, E=2, I=2, S=9928, T=1	9938	
3	L	0						3	L	3 C=1, H=1, I=1, L=9912, F=15, P=3, Xaa=5	9938	
4	L	0						4	L	1 R=1, L=9931, F=1, P=1, S=3, Xaa=1	9938	
5	T	0						5	T	3 I=6, P=19, S=2, T=9908, Y=2, Xaa=1	9938	
6	E	0						6	E	2 R=11, D=1, E=9921, G=3, K=2	9938	
7	V	0						7	V	1 I=3, L=1, V=9934	9938	
8	E	0						8	E	2 A=14, E=9919, G=3, K=1, F=1	9938	
9	T	0						9	T	1 A=1, K=2, T=9934, V=1	9938	
10	Y	0						10	Y	1 L=1, F=2, S=3, Y=9932	9938	
11	V	0						11	V	1 A=1, G=1, I=1, S=1, V=9934	9938	
12	L	0						12	L	2 L=9926, F=8, P=2, V=1, Xaa=1	9938	
13	S	0						13	S	1 A=1, F=3, P=2, S=9931, Xaa=1	9938	
14	I	3	V3					14	I	6 I=9880, L=1, F=1, T=17, V=39	9938	
15	V	41	I37L3M1	V	I	2007	0,3305	15	V	93 I=3285, L=2, F=1, T=11, V=6638, Xaa=1	9938	
16	P	0						16	P	2 R=4, L=2, P=9922, S=10	9938	
17	S	1	A1					17	S	1 A=1, P=1, S=9934, T=2	9938	
18	G	0						18	G	1 A=1, R=2, C=2, G=9932, S=1	9938	
19	P	0						19	P	0 H=1, L=2, P=9935	9938	
20	L	0						20	L	1 H=2, I=3, L=9927, F=2, P=4	9938	
21	K	0						21	K	1 A=1, R=1, Q=2, K=9934	9938	
22	A	0						22	A	1 A=9930, D=1, S=4, T=2, V=1	9938	
23	E	0						23	E	1 R=1, D=2, Q=4, E=9930, K=1	9938	
24	I	0						24	I	0 I=9936, T=1, V=1	9938	
25	A	0						25	A	0 A=9938	9938	
26	Q	1	R1					26	Q	2 R=4, Q=9926, G=1, H=2, L=5	9938	
27	R	0						27	R	62 R=8448, G=1, K=1485, S=1, T=2, Xaa=1	9938	
28	L	0						28	L	3 H=3, I=18, L=9913, F=2, P=2	9938	
29	E	0						29	E	0 E=9937, G=1	9938	
30	D	6	N6					30	D	2 N=5, D=9923, E=2, G=2, S=4, Y=2	9938	
31	V	0						31	V	1 C=1, I=7, M=1, F=1, V=9927, Xaa=1	9938	
32	F	0						32	F	1 A=1, L=4, F=9932, S=1	9938	
33	A	0						33	A	4 A=9896, S=35, V=6, Xaa=1	9938	
34	G	0						34	G	0 R=1, G=9937	9938	
35	K	2	N1R1					35	K	1 R=10, N=1, E=1, K=9926	9938	
36	N	0						36	N	3 N=9910, D=4, K=3, S=16, T=5	9938	
37	T	2	A2					37	T	17 A=125, I=8, P=3, S=65, T=9732, V=5	9938	
38	D	0						38	D	1 A=1, N=2, D=9933, E=1, H=1	9938	
39	L	0						39	L	1 I=1, L=9931, F=5, P=1	9938	
40	E	0						40	E	2 D=10, Q=1, E=9921, G=6	9938	
41	A	0						41	A	6 A=9877, G=3, S=1, V=56, Xaa=1	9938	
42	L	0						42	L	2 I=14, L=9918, F=3, P=1, T=1, Xaa=1	9938	
43	M	0						43	M	1 Q=1, M=9934, T=3	9938	
44	E	0						44	E	1 D=2, E=9934, G=2	9938	
45	W	0						45	W	1 R=3, C=1, G=1, W=9933	9938	
46	L	0						46	L	17 Q=1, I=243, L=9692, T=1, Xaa=1	9938	
47	K	0						47	K	1 R=3, E=1, K=9933, M=1	9938	
48	T	0						48	T	1 A=3, P=5, T=9930	9938	
49	R	0						49	R	0 R=9936, G=2	9938	
50	P	1	S1					50	P	0 A=1, L=1, P=9936	9938	
51	I	0						51	I	1 N=1, I=9931, K=4, M=1, T=1	9938	
52	L	0						52	L	1 R=3, Q=1, L=9932, M=1, P=1	9938	
53	S	0						53	S	1 A=1, Q=1, P=1, S=9934, T=1	9938	
54	P	1	S1					54	P	7 H=2, L=3, P=9858, S=74, Xaa=1	9938	
55	L	0						55	L	1 L=9932, P=4, Xaa=2	9938	
56	T	0						56	T	2 I=7, S=7, T=9923, Xaa=1	9938	
57	K	0						57	K	1 R=5, N=2, I=1, K=9930	9938	
58	G	0						58	G	0 E=2, G=9936	9938	
59	I	0						59	I	47 I=9077, M=765, T=1, V=94, Xaa=1	9938	
60	L	0						60	L	0 L=9936, S=1, W=1	9938	
61	G	0						61	G	0 R=2, E=1, G=9935	9938	
62	F	0						62	F	2 L=18, F=9918, V=2	9938	
63	V	0						63	V	3 E=1, I=30, V=9907	9938	
64	F	0						64	F	1 I=1, L=1, F=9934, Y=2	9938	
65	T	0						65	T	0 T=9938	9938	
66	L	0						66	L	0 L=9938	9938	
67	T	0						67	T	0 A=1, S=1, T=9936	9938	
68	V	0						68	V	0 G=1, M=1, V=9936	9938	
69	P	0						69	P	1 A=1, H=1, L=3, P=9927, S=1, T=5	9938	
70	S	0						70	S	2 N=24, S=9914	9938	
71	E	0						71	E	1 Q=1, E=9934, G=3	9938	
72	R	1	Q1					72	R	2 R=9924, Q=13, P=1	9938	
73	G	0						73	G	0 R=2, G=9936	9938	
74	L	0						74	L	1 R=2, Q=1, L=9932, M=1, P=1, V=1	9938	
75	Q	0						75	Q	0 Q=9938	9938	
76	R	0						76	R	1 R=9931, C=2, G=1, S=4	9938	
77	R	0						77	R	2 R=9923, I=4, K=8, T=1, Xaa=2	9938	
78	R	0						78	R	1 R=9932, Q=3, G=1, H=1, L=1	9938	
79	F	0						79	F	0 L=1, F=9937	9938	
80	V	30	I30	V	I	2007	0,0015	80	V	2 I=15, V=9923	9938	
81	Q	0						81	Q	0 Q=9936, H=1, L=1	9938	
82	N	0						82	N	4 N=9903, D=1, H=3, I=1, K=2, S=19, T=8, Y=1	9938	
83	A	0						83	A	2 A=9921, P=3, S=13, V=1	9938	
84	L	0						84	L	0 H=1, L=9936, S=1	9938	
85	S	13	N13	N	S	1975	0,0027	85	N	5 N=9889, D=15, S=27, T=7	9938	
86	G	0						86	G	1 E=1, G=9933, V=4	9938	
87	N	0						87	N	1 N=9931, H=2, K=1, S=4	9938	
88	G	0						88	G	0 A=1, E=2, G=9935	9938	
89	D	0						89	D	1 A=1, N=1, D=9933, H=2, L=1	9938	
90	P	0						90	P	1 A=5, P=9929, S=3, T=1	9938	
91	N	0						91	N	11 N=9821, D=1, H=3, I=2, S=92, T=15, Y=4	9938	
92	N	0						92	N	2 N=9927, D=3, H=1, I=1, K=1, T=2, Y=2, Xaa=1	9938	
93	M	0						93	M	2 I=17, M=9920, Xaa=1	9938	
94	D	0						94	D	5 A=26, N=4, D=9894, E=1, G=10, H=2, Xaa=1	9938	
95	R	34	K34	R	K	2007	0,0243	95	R	17 R=9691, G=3, I=1, K=241, T=1, Xaa=1	9938	
96	A	0						96	A	0 A=9937, T=1	9938	
97	V	1	I1					97	V	1 A=1, I=12, V=9925	9938	
98	K	0						98	K	2 R=5, N=6, Q=1, E=2, K=9921, M=1, T=1, Xaa=1	9938	
99	L	0						99	L	1 I=3, L=9931, M=4	9938	
100	Y	0						100	Y	0 C=1, F=2, Y=9935	9938	
101	R	6	K6					101	R	85 R=7223, G=1, K=2713, T=1	9938	
102	K	0						102	K	0 E=1, K=9937	9938	
103	L	0						103	L	2 I=2, L=9922, M=12, V=2	9938	
104	K	0						104	K	1 R=2, Q=1, E=1, I=1, K=9933	9938	
105	R	0						105	R	0 R=9937, S=1	9938	
106	E	1	D1					106	E	0 E=9936, G=1, Xaa=1	9938	
107	I	1	V1					107	I	47 I=8997, M=921, T=1, V=18, Xaa=1	9938	
108	T	0						108	T	1 H=1, I=2, P=4, T=9931	9938	
109	F	0						109	F	2 L=3, F=9920, Y=14, V=1	9938	
110	H	0						110	H	2 N=3, Q=1, H=9919, P=1, Y=14	9938	
111	G	0						111	G	3 R=23, E=6, G=9907, V=2	9938	
112	A	0						112	A	3 A=9908, D=1, G=3, K=1, S=22, T=2, V=1	9938	
113	K	0						113	K	2 R=13, N=2, I=1, K=9922	9938	
114	E	0						114	E	1 D=6, E=9929, G=3	9938	
115	V	0						115	V	4 A=1, E=1, G=2, I=22, L=3, M=6, F=1, V=9902	9938	
116	A	0						116	A	14 A=9745, G=1, M=1, P=3, S=186, T=1, Xaa=1	9938	
117	L	0						117	L	5 H=2, I=24, L=9892, F=9, P=3, V=6, Xaa=2	9938	
118	S	1	G1					118	S	8 R=3, N=1, G=89, I=1, M=1, S=9841, Xaa=2	9938	
119	Y	0						119	Y	0 C=2, F=1, Y=9935	9938	
120	S	0						120	S	1 P=4, S=9929, T=5	9938	
121	T	1	A1					121	T	15 A=214, P=3, T=9721	9938	
122	G	0						122	G	0 G=9938	9938	
123	A	0						123	A	1 A=9932, S=6	9938	
124	L	0						124	L	0 R=1, L=9937	9938	
125	A	0						125	A	5 A=9888, P=1, S=2, T=46, V=1	9938	
126	S	0						126	S	3 G=34, S=9903, T=1	9938	
127	C	0						127	C	0 C=9937, W=1	9938	
128	M	0						128	M	1 R=1, I=1, L=4, M=9931, Xaa=1	9938	
129	G	0						129	G	0 C=1, G=9936, S=1	9938	
130	L	0						130	L	1 I=5, L=9930, P=1, V=2	9938	
131	I	0						131	I	2 I=9923, L=13, T=1, V=1	9938	
132	Y	0						132	Y	0 Y=9938	9938	
133	N	0						133	N	2 N=992		

139	T	0							139	T	11 A=46,N=73,I=1,P=5,S=1,T=9812	9938
140	T	0							140	T	19 A=272,S=9,T=9657	9938
141	E	0							141	E	0 E=9935,G=2,Xaa=1	9938
142	V	0							142	V	41 A=40,G=441,S=146,V=9309,Xaa=2	9938
143	A	1	S1						143	A	1 A=9934,T=2,V=1,Xaa=1	9938
144	F	0							144	F	49 L=1054,F=8883,Xaa=1	9938
145	G	0							145	G	0 D=1,G=9936,Xaa=1	9938
146	L	0							146	L	0 L=9937,Xaa=1	9938
147	V	0							147	V	2 A=1,I=2,L=8,M=2,V=9924,Xaa=1	9938
148	C	0							148	C	0 C=9937,Xaa=1	9938
149	A	0							149	A	1 A=9933,S=2,T=1,V=1,Xaa=1	9938
150	T	0							150	T	2 A=2,I=8,M=2,S=1,T=9924,Xaa=1	9938
151	C	0							151	C	0 C=9935,S=1,Xaa=2	9938
152	E	0							152	E	0 E=9936,G=1,Xaa=1	9938
153	Q	0							153	Q	1 R=1,Q=9933,H=2,P=1,Xaa=1	9938
154	I	1	X1						154	I	0 N=1,I=9935,T=1,Xaa=1	9938
155	A	0							155	A	1 A=9933,G=1,S=1,V=2,Xaa=1	9938
156	D	0							156	D	1 N=1,D=9931,E=4,H=1,Xaa=1	9938
157	S	0							157	S	31 A=547,G=2,L=1,S=9386,T=1,Xaa=1	9938
158	Q	0							158	Q	23 R=1,Q=9581,H=354,Xaa=2	9938
159	H	0							159	H	0 Q=1,H=9936,Xaa=1	9938
160	R	1	T1						160	R	1 R=9934,Q=2,K=1,Xaa=1	9938
161	S	1	A1						161	S	4 A=31,F=2,P=3,S=9901,Xaa=1	9938
162	H	3	Y3						162	H	1 Q=5,H=9932,Xaa=1	9938
163	R	0							163	R	1 R=9929,G=8,Xaa=1	9938
164	Q	0							164	Q	1 R=1,Del=1,Q=9930,L=3,K=2,Xaa=1	9938
165	M	1	I1						165	M	2 I=8,L=2,M=9926,V=1,Xaa=1	9938
166	V	1	A1						166	V	88 A=2590,I=37,M=8,T=10,V=7292,Xaa=1	9938
167	T	1	I1						167	T	32 A=493,N=5,I=12,S=8,T=9417,V=1,Xaa=2	9938
168	T	5	I5						168	T	65 A=18,I=1568,K=1,P=1,T=8349,Xaa=1	9938
169	T	0							169	T	2 N=1,I=13,S=2,T=9921,Xaa=1	9938
170	N	0							170	N	1 N=9929,D=1,S=5,T=2,Xaa=1	9938
171	P	0							171	P	0 P=9936,T=1,Xaa=1	9938
172	L	0							172	L	1 I=2,L=9932,M=3,Xaa=1	9938
173	I	0							173	I	1 I=9934,T=1,V=2,Xaa=1	9938
174	R	0							174	R	5 R=9888,G=1,K=47,S=1,Xaa=1	9938
175	H	0							175	H	0 R=1,H=9936,Xaa=1	9938
176	E	0							176	E	0 D=1,E=9935,G=1,Xaa=1	9938
177	N	0							177	N	1 N=9929,D=2,K=1,S=4,T=1,Xaa=1	9938
178	R	0							178	R	0 R=9936,K=1,Xaa=1	9938
179	M	0							179	M	0 I=1,M=9935,V=1,Xaa=1	9938
180	V	0							180	V	0 G=1,V=9936,Xaa=1	9938
181	L	1	I1						181	L	6 I=20,L=9886,M=26,P=1,W=1,V=3,Xaa=1	9938
182	A	1	V1						182	A	1 A=9933,T=4,Xaa=1	9938
183	S	0							183	S	1 C=1,G=1,I=1,S=9934,Xaa=1	9938
184	T	0							184	T	1 A=1,H=1,I=4,P=2,T=9929,Xaa=1	9938
185	T	0							185	T	1 A=1,P=2,T=9934,Xaa=1	9938
186	A	0							186	A	1 A=9932,G=1,P=2,T=1,V=1,Xaa=1	9938
187	K	0							187	K	1 R=4,K=9933,Xaa=1	9938
188	A	6	T5X1						188	A	0 A=9937,Xaa=1	9938
189	M	0							189	M	1 I=1,M=9933,V=3,Xaa=1	9938
190	E	0							190	E	0 E=9936,G=1,Xaa=1	9938
191	Q	0							191	Q	2 R=3,Q=9923,H=4,L=2,K=1,Xaa=5	9938
192	M	4	V2X2						192	M	21 I=45,M=9641,V=250,Xaa=2	9938
193	A	0							193	A	1 A=9933,D=1,E=1,P=1,V=1,Xaa=1	9938
194	G	0							194	G	0 D=1,G=9936,Xaa=1	9938
195	S	0							195	S	2 A=10,L=1,P=1,S=9920,T=4,W=1,Xaa=1	9938
196	S	1	N1						196	S	2 N=18,I=2,K=1,S=9916,Xaa=1	9938
197	E	0							197	E	1 D=1,Q=2,E=9932,G=1,K=1,Xaa=1	9938
198	Q	0							198	Q	2 R=2,Q=9919,H=15,P=1,Xaa=1	9938
199	A	0							199	A	0 A=9936,Xaa=2	9938
200	A	0							200	A	5 A=9890,E=1,M=13,P=1,S=12,T=3,V=17,Xaa=1	9938
201	E	1	N1						201	E	2 D=6,E=9925,K=6,Xaa=1	9938
202	A	0							202	A	1 A=9931,R=3,P=1,T=1,V=1,Xaa=1	9938
203	M	0							203	M	2 I=8,M=9924,V=5,Xaa=1	9938
204	E	0							204	E	3 D=22,E=9913,K=2,Xaa=1	9938
205	V	4	I4						205	V	29 A=6,I=452,T=7,V=9471,Xaa=2	9938
206	A	0							206	A	1 A=9932,G=1,P=1,V=3,Xaa=1	9938
207	S	1	N1						207	S	77 N=1698,C=7,G=127,I=1,K=1,S=8102,T=1,Xaa=1	9938
208	K	32	Q14,R18	Q	K KR	1976 2003	0.0000 0.0003		208	Q	1 R=3,Q=9931,H=1,P=1,S=1,Xaa=1	9938
209	A	0							209	A	6 A=9875,R=1,G=2,T=60	9938
210	R	0							210	R	1 R=9931,K=7	9938
211	Q	0							211	Q	4 R=3,Q=9903,E=1,H=19,L=1,K=10,Xaa=1	9938
212	M	0							212	M	0 I=2,M=9935,V=1	9938
213	V	0							213	V	1 A=1,E=2,G=5,I=2,V=9928	9938
214	Q	0							214	Q	4 R=2,Q=9893,H=42,Xaa=1	9938
215	A	0							215	A	1 A=9933,S=1,T=4	9938
216	M	0							216	M	0 M=9937,V=1	9938
217	R	0							217	R	0 R=9936,I=1,K=1	9938
218	T	1	I1						218	T	20 A=289,N=1,I=1,P=1,S=4,T=9642	9938
219	I	1	V1						219	I	25 I=9540,M=2,T=1,V=395	9938
220	G	0							220	G	0 R=1,G=9936,V=1	9938
221	T	0							221	T	0 T=9938	9938
222	H	3	N3						222	H	26 R=4,N=17,Q=373,H=9540,L=2,S=1,Xaa=1	9938
223	P	0							223	P	2 H=22,P=9916	9938
224	S	1	N1						224	S	70 R=6,N=1806,G=2,K=3,S=8118,T=1,Y=1,Xaa=1	9938
225	S	0							225	S	25 C=2,I=1,F=1,S=9529,T=405	9938
226	S	0							226	S	1 R=5,I=1,S=9931,T=1	9938
227	A	2	T2						227	A	27 A=9495,D=1,Q=1,P=1,S=2,T=437,V=1	9938
228	G	1	R1						228	G	2 R=15,D=1,G=9919,I=1,S=2	9938
229	L	0							229	L	2 Q=1,L=9923,M=8,V=2,Xaa=4	9938
230	K	0							230	K	68 R=1811,K=8127	9938
231	D	7	N7						231	D	6 N=28,D=9877,E=31,H=1,V=1	9938
232	D	1	N1						232	D	67 N=1742,D=8196	9938
233	L	0							233	L	1 I=1,L=9933,F=3,V=1	9938
234	L	0							234	L	44 I=901,L=9037	9938
235	E	3	V2D1						235	E	3 D=7,E=9911,G=12,K=8	9938
236	N	0							236	N	2 N=9924,D=1,H=1,I=1,K=4,S=1,T=5,Y=1	9938
237	L	0							237	L	1 L=9933,S=2,W=2,V=1	9938
238	Q	0							238	Q	0 Q=9936,L=1,Xaa=1	9938
239	A	1	V1						239	A	4 A=9905,G=4,P=1,T=24,V=4	9938
240	Y	0							240	Y	2 H=2,F=11,Y=9925	9938
241	Q	0							241	Q	1 Q=9930,H=5,P=1,Xaa=2	9938
242	K	0							242	K	7 R=13,N=45,K=9869,S=1,T=10	9938
243	R	0							243	R	0 R=9935,P=2,W=1	9938
244	M	0							244	M	1 R=1,L=1,K=2,M=9934	9938
245	G	0							245	G	0 E=1,G=9937	9938
246	V	0							246	V	12 A=1,G=6,L=124,M=16,V=9791	9938
247	Q	0							247	Q	0 Q=9937,H=1	9938
248	M	5	L5						248	M	29 I=53,L=362,M=9510,V=12,Xaa=1	9938
249	Q	1	R1						249	Q	4 R=5,Q=9904,H=28,L=1	9938
250	R	0							250	R	0 R=9938	9938
251	F	0							251	F	3 L=25,F=9913	9938
252	K	0							252	K	2 R=13,Q=1,E=2,I=1,K=9920,M=1	9938



Legend:

- Alternatives:** aa different from consensus , e.g., Q1 means that Q occurred in one isolate
- Score:** Data taken from the Database describing measure of variability.
- Alignment Details:** number of occurrences of various amino acids.
- High-potency and Low-potency **markers** are colored green and yellow, respectively.

Equine M2 H3N8				Permanent aa change			Marker potency	Avian_M2 all subtypes			# Sequences	
Pos	Consensus	No of Diffs	Alternatives	From	To	Year of substitution		Pos	Consensus	Score		Alignment Details
1	M	0						1	M	0	M=9555	9555
2	S	0						2	S	0	I=1, S=9569, T=1	9571
3	L	0						3	L	2	C=1, H=1, L=9552, F=12, P=1, V=1, Xaa=3	9571
4	L	0						4	L	1	R=1, L=9566, P=1, S=2, Xaa=1	9571
5	T	0						5	T	2	N=1, P=7, S=2, T=9559, Y=2	9571
6	E	0						6	E	1	Del=1, E=9567, G=2, K=1	9571
7	V	0						7	V	1	Del=1, I=3, L=1, V=9566	9571
8	E	0						8	E	3	A=19, Del=1, E=9547, G=3, K=1	9571
9	T	0						9	T	1	A=1, R=1, Del=1, P=1, T=9567	9571
10	P	1	S1					10	P	53	Del=38, H=319, L=477, P=8737, S=1	9572
11	T	1	I1					11	T	38	Del=38, I=587, S=11, T=8935, Xaa=1	9572
12	R	1	K1					12	R	20	R=9314, Del=38, K=217, P=1, T=1, Xaa=1	9572
13	N	1	S1					13	N	54	A=2, N=8770, D=8, Del=37, H=6, I=1, K=136, S=526, T=85, Xaa=1	9572
14	G	4	E4					14	G	68	A=6, R=1, D=1, Del=37, E=1536, G=7989, V=2	9572
15	W	0						15	W	0	R=2, G=1, W=9569	9572
16	E	0						16	E	25	D=8, E=9206, G=337, K=1, V=20	9572
17	C	1	Y1					17	C	4	R=1, C=9532, G=1, F=1, Y=36, Xaa=1	9572
18	K	6	N5R1					18	K	117	R=3732, N=333, I=1, K=5496, S=9, Xaa=1	9572
19	C	0						19	C	5	C=9523, F=1, Y=47, Xaa=1	9572
20	S	3	N2D1					20	S	23	R=17, N=224, C=1, G=3, I=27, K=17, S=9282, Xaa=1	9572
21	G	68	D20V1					21	D	18	D=9330, G=231, V=11	9572
22	S	0						22	S	4	L=36, S=9536	9572
23	S	0						23	S	6	N=50, C=2, G=8, S=9510, Xaa=1	9571
24	D	0						24	D	5	N=47, D=9516, E=8	9571
25	P	1	L1					25	P	4	H=2, L=10, P=9536, S=12, T=9, Xaa=2	9571
26	L	0						26	L	22	R=1, I=278, L=9270, F=20, V=2	9571
27	V	1	I1					27	V	51	A=82, E=1, G=6, I=773, L=4, M=4, F=3, T=10, V=8686, Xaa=2	9571
28	I	2	A2					28	I	94	A=16, D=9, I=7378, L=34, M=1, F=181, S=3, T=17, Y=2, V=1930	9571
29	A	1	I1					29	A	11	A=9464, D=1, G=1, S=16, T=48, V=41	9571
30	A	0						30	A	3	A=9548, E=1, S=8, T=12, Xaa=2	9571
31	S	0						31	S	37	R=2, N=646, D=2, G=5, I=1, K=1, S=8913, Xaa=1	9571
32	I	0						32	I	20	I=9283, F=4, T=6, V=277, Xaa=1	9571
33	I	6	V5T1					33	I	5	I=9518, L=4, T=1, V=47, Xaa=1	9571
34	G	0						34	G	0	G=9571	9571
35	I	0						35	I	5	I=9523, M=1, S=6, T=1, V=38, Xaa=2	9571
36	L	0						36	L	1	L=9566, M=1, W=1, V=3	9571
37	H	0						37	H	0	D=1, Q=1, H=9568, P=1	9571
38	L	0						38	L	2	L=9555, K=1, F=15	9571
39	I	0						39	I	9	R=1, I=9488, K=2, M=11, T=28, V=41	9571
40	L	0						40	L	1	L=9564, M=2, F=3, S=1, W=1	9571
41	W	0						41	W	1	R=1, C=2, G=1, L=1, W=9566	9571
42	I	0						42	I	5	I=9526, M=9, S=1, T=24, V=10, Xaa=1	9571
43	L	1	F1					43	L	16	A=1, I=15, L=9381, F=164, P=1, S=3, T=6	9571
44	D	0						44	D	4	N=35, D=9531, E=1, Y=3, V=1	9571
45	R	0						45	R	3	R=9541, C=3, H=25, L=1, S=1	9571
46	L	0						46	L	2	I=8, L=9558, F=4, P=1	9571
47	F	1	S1					47	F	3	L=32, F=9538, Y=1	9571
48	F	26	S26		F	S	2007	48	F	3	L=8, F=9545, S=16, Y=1, V=1	9571
49	K	0						49	K	1	R=4, Q=2, K=9565	9571
50	F	13	C13		C	F	1975	50	C	19	R=1, C=9343, F=55, S=1, W=3, Y=168	9571
51	I	1	A1					51	I	17	A=1, I=9348, L=4, T=11, V=207	9571
52	Y	0						52	Y	14	N=1, C=86, H=52, F=10, S=4, Y=9418	9571
53	R	2	H2					53	R	1	R=9563, C=6, G=1, L=1	9571
54	R	2	H1L1					54	R	5	R=9531, C=17, H=13, I=1, L=3, S=5, Xaa=1	9571
55	L	2	F2					55	L	66	I=116, L=8186, F=1265, Xaa=4	9571
56	K	1	R1					56	K	7	R=72, E=5, I=1, K=9493	9571
57	Y	1	H1					57	Y	3	N=1, C=2, H=20, Y=9547, Xaa=1	9571
58	G	0						58	G	15	A=6, R=4, D=32, E=100, G=9416, S=7, V=6	9571
59	L	14	M14					59	L	1	L=9566, F=2, P=2, V=1	9571
60	K	1	Q1					60	K	2	R=3, Q=11, E=1, K=9556	9571
61	R	27	G26K1					61	R	29	R=9214, N=1, E=1, G=116, I=18, K=187, S=34	9571
62	G	0						62	G	2	R=7, E=1, G=9559, S=2, W=1, V=1	9571
63	P	0						63	P	1	L=1, P=9567, S=1, T=1, Xaa=1	9571
64	S	0						64	S	25	A=286, F=32, P=4, S=9234, T=5, Y=10	9571
65	T	0						65	T	9	A=10, R=7, K=28, M=33, S=1, T=9491, V=1	9571
66	E	1	G1					66	E	37	A=486, E=9003, G=24, K=32, T=24, V=1, Xaa=1	9571
67	G	1	V1					67	G	1	R=8, G=9563	9571
68	V	2	I1					68	V	20	A=22, I=65, L=28, M=99, T=2, V=9355	9571
69	P	1	R1					69	P	1	L=4, P=9567	9571
70	E	1	D1					70	E	16	R=1, D=1, Q=1, E=9374, G=3, K=182, T=8, V=1	9571
71	S	0						71	S	1	A=1, C=1, F=3, S=9564, Y=2	9571
72	M	0						72	M	0	I=2, M=9568, T=1	9571
73	R	0						73	R	1	R=9561, K=6, M=2, T=2	9571
74	E	1	D1					74	E	2	A=6, D=3, E=9555, G=2, K=4, Xaa=1	9571
75	E	0						75	E	2	D=2, Q=1, E=9559, G=2, K=7	9571
76	Y	2	F1					76	Y	1	N=1, C=2, F=3, S=1, Y=9564	9571
77	R	1	Q1					77	R	11	R=9433, Q=132, L=1, P=2, W=3	9571
78	Q	0						78	Q	4	R=1, Q=9537, E=2, H=23, L=4, K=1, F=1, P=2	9571
79	E	0						79	E	3	E=9544, K=26, S=1	9571
80	Q	0						80	Q	7	R=22, Q=9503, H=1, L=2, K=42, P=1	9571
81	Q	0						81	Q	3	R=10, Q=9545, H=8, L=4, K=2, P=1, Xaa=1	9571
82	N	9	S9		S	N	1971	82	S	56	N=1166, D=5, G=12, I=1, K=1, S=8381, T=4, Xaa=1	9571
83	A	12	T11D1					83	A	4	A=9536, G=1, T=29, V=5	9571
84	V	1	G1					84	V	4	A=9, G=14, L=1, M=4, W=1, V=9542	9571
85	D	30	S29N1		D	S	2007	85	D	6	N=18, D=9515, E=2, G=36	9571
86	V	0						86	V	2	A=5, I=5, F=2, S=1, V=9558	9571
87	D	2	E2					87	D	2	N=2, D=9558, E=4, H=1, T=2, Y=2, V=1, Xaa=1	9571
88	D	1	N1					88	D	26	A=6, R=1, N=196, D=9261, E=4, G=53, H=6, I=1, M=1, T=1, Y=15, V=25, Xa	9571
89	G	31	S31		G	S	2007	89	G	23	R=1, D=28, C=18, G=9288, S=208, V=26, Xaa=2	9571
90	H	0						90	H	2	R=3, N=1, Q=7, H=9555, P=3, T=1, Y=1	9571
91	F	0						91	F	2	A=1, L=14, F=9556	9571
92	V	0						92	V	6	A=60, C=5, L=1, F=1, V=9504	9571
93	N	1	Y1					93	N	6	N=9519, D=35, H=8, S=6, Y=3	9571
94	I	0						94	I	3	R=2, G=1, I=9549, L=1, K=1, M=4, T=3, V=10	9571
95	E	3	G2V1					95	E	8	A=5, D=1, Q=11, E=9498, G=5, K=21, S=1, V=26, Xaa=3	9571
96	L	0						96	L	6	Q=1, L=9515, K=3, M=32, F=2, P=17, Xaa=1	9571
97	E	1	K1					97	E	17	R=1, D=6, E=9358, G=38, K=167, V=1	9571

vmuc:
not a marker

Legend:

- 1. Alternatives:** aa different from consensus , e.g., Q1 means that Q occurred in one isolate
- 2. Score:** Data taken from the Database describing measure of variability.
- 3. Alignment Details:** number of occurrences of various amino acids.
- 4. High-potency and Low-potency markers** are colored green and yellow, respectively.

Equine Pos	NS1 Consensus	H3N8 No of Alternatives Diffs	Permanent aa change			Marker potency	Avian NS1 all subtypes			Alignment Details	# Sequences
			From	To	Year of substitution		Pos	Consensus	Score		
1	M	0					1	M	0	M=9620,Xaa=1	9621
2	D	0					2	D	5	N=27,D=9577,E=6,G=8,K=1,Y=1,Xaa=1	9621
3	S	5 P3F2					3	S	13	G=1,L=3,F=18,P=127,S=9468,T=3,Y=1	9621
4	N	0					4	N	3	N=9600,D=1,H=2,I=5,K=4,S=6,T=3	9621
5	T	1					5	T	1	A=1,H=1,P=2,S=1,T=9616	9621
6	V	1					6	V	93	A=10,R=2,C=1,G=3,I=2240,L=2,M=105,P=1,T=41,V=7216	9621
7	S	1					7	S	91	A=4,L=131,F=1,P=1,S=7144,T=2339,V=1	9621
8	S	0					8	S	1	D=2,G=3,K=1,S=9614,T=1	9621
9	F	0					9	F	1	L=4,F=9614,Y=3	9621
10	Q	0					10	Q	1	Q=9614,H=2,L=2,K=2,S=1	9621
11	V	0					11	V	1	A=2,G=1,I=1,L=2,T=2,V=9612,Xaa=1	9621
12	D	0					12	D	1	D=9613,E=4,G=1,H=1,S=1,Xaa=1	9621
13	C	0					13	C	1	R=1,C=9612,G=1,H=1,S=5,Xaa=1	9621
14	F	1					14	F	81	F=7275,S=1,Y=2343,Xaa=2	9621
15	L	0					15	L	3	I=21,L=9594,F=5,Xaa=1	9621
16	W	0					16	W	1	L=4,W=9616,Xaa=1	9621
17	H	1					17	H	3	R=1,N=7,Q=9,H=9594,Y=9,Xaa=1	9621
18	V	2					18	V	82	A=1,I=2397,L=4,M=4,T=1,V=7213,Xaa=1	9621
19	R	0					19	R	0	R=9619,C=1,G=1	9621
20	K	0					20	K	4	R=24,N=5,E=5,I=1,K=9584,F=1,T=1	9621
21	R	1					21	R	84	R=7269,Q=21,H=1,I=13,L=2316,P=1	9621
22	F	1					22	F	89	I=97,L=2347,F=7169,P=1,S=1,V=6	9621
23	A	1					23	A	82	A=7280,N=2,G=2,S=2324,V=13	9621
24	D	2					24	D	81	A=1,D=7291,E=2,I=1,K=3,M=2320,T=1,V=2	9621
25	Q	2					25	Q	85	R=2353,N=25,D=1,Q=7228,H=1,L=1,K=9,P=1,S=1,W=1	9621
26	E	2					26	E	90	N=1,D=2383,E=7141,G=50,K=45,Xaa=1	9621
27	L	1					27	L	93	R=27,Q=3,I=8,L=6733,M=2841,F=1,P=2,S=1,T=2,V=3	9621
28	G	3					28	G	81	N=1,C=2331,G=7278,S=9,V=2	9621
29	D	0					29	D	0	D=9621	9621
30	A	0					30	A	1	A=9615,T=2,V=4	9621
31	P	0					31	P	0	P=9620,T=1	9621
32	F	0					32	F	1	L=2,F=9615,S=3,V=1	9621
33	L	1					33	L	84	R=1,N=13,D=2312,H=7,I=5,L=7276,M=1,F=1,P=4,S=1	9621
34	D	0					34	D	1	D=9617,V=4	9621
35	R	0					35	R	0	R=9618,M=2,P=1	9621
36	L	0					36	L	6	R=2,I=52,L=9563,F=3,P=1	9621
37	R	0					37	R	1	R=9612,C=3,K=4,S=2	9621
38	R	0					38	R	0	A=1,R=9618,G=1,K=1	9621
39	D	0					39	D	0	D=9620,H=1	9621
40	Q	0					40	Q	5	R=17,Q=9578,H=6,L=1,K=19	9621
41	K	0					41	K	11	R=137,K=9484	9621
42	S	1					42	S	80	A=2327,C=1,P=3,S=7290	9621
43	L	0					43	L	1	I=4,L=9615,P=2	9621
44	R	84 K43E1					44	R	86	R=7004,G=11,I=2,K=2602,M=1,T=1	9621
45	G	9 R9	G	R	2008-9	0,0005	45	G	1	R=5,G=9616	9621
46	R	0					46	R	0	R=9619,K=2	9621
47	G	4					47	G	34	A=1,C=25,G=9062,S=533	9621
48	S	47 I44N2G1	S	I	2007	0,0068	48	S	68	R=4,N=1433,C=2,G=3,H=1,I=65,S=8113	9621
49	T	1					49	T	1	A=3,D=1,T=9617	9621
50	L	0					50	L	1	I=11,L=9609,P=1	9621
51	G	3					51	G	2	D=2,E=3,G=9601,S=15	9621
52	L	2					52	L	13	Q=1,I=68,L=9477,M=73,F=1,V=1	9621
53	D	11					53	D	12	N=95,D=9491,E=15,G=16,H=1,S=1,Y=2	9621
54	I	1					54	I	83	I=7284,L=2299,M=3,P=27,T=4,V=4	9621
55	E	1					55	E	102	A=1,R=2921,N=1,D=15,Q=10,E=6557,G=38,K=75,S=2,Xaa=1	9621
56	T	5					56	T	97	A=177,R=5,N=1,I=24,K=5,M=15,P=3,T=7102,V=2289	9621
57	A	0					57	A	2	A=9599,S=20,T=2	9621
58	T	2					58	T	2	A=6,N=1,I=6,K=1,S=1,T=9606	9621
59	R	70 H68L1M1	R	H	1997	0,0217	59	R	135	R=6541,C=424,G=1,H=209,I=52,L=35,M=2243,S=35,T=28,Y=51,V=2	9621
60	A	2					60	A	109	A=6469,D=16,E=2928,G=13,I=3,K=5,M=9,S=57,T=49,V=72	9621
61	G	0					61	G	0	R=1,E=1,G=9619	9621
62	K	1					62	K	10	R=120,N=1,Q=1,K=9498,T=1	9621
63	Q	1					63	Q	143	R=170,N=2,Q=6247,E=1,H=610,I=9,L=305,K=2275,W=2	9621
64	I	1					64	I	26	I=9308,L=89,M=32,T=11,V=181	9621
65	V	0					65	V	19	A=3,G=2,I=181,L=9,M=37,F=1,V=9388	9621
66	E	3					66	E	7	A=1,N=1,D=4,Q=1,E=9559,G=23,K=32	9621
67	Q	27 R62G3N1W1	R	Q	1980	0,0087	67	R	106	R=7126,N=34,D=2157,Q=84,E=122,G=14,K=30,M=14,P=3,S=2,W=33,Y=	9621
68	I	0					68	I	5	A=1,I=9570,L=2,T=10,V=37,Xaa=1	9621
69	L	2					69	L	5	R=1,Q=17,I=6,L=9574,M=22,Xaa=1	9621
70	E	4					70	E	98	R=4,N=1,D=122,Q=3,E=6915,G=45,K=2528,V=3	9621
71	E	69 K67S1D1	E	K	1997	0,0116	71	E	123	A=3,R=84,N=138,D=40,Q=4,E=6836,G=211,I=2,K=112,S=2188,T=1,V=2	9621
72	E	1					72	E	3	D=1,E=9596,G=4,K=20	9621
73	S	1					73	S	91	A=4,N=23,K=1,M=12,F=4,P=80,S=7228,T=2262,Y=7	9621
74	D	7					74	D	18	N=150,D=9414,E=24,G=12,S=19,Y=1,V=1	9621
75	E	0					75	E	32	D=42,E=9161,G=28,K=381,W=3,V=5,Xaa=1	9621
76	A	6					76	A	101	A=7101,N=2263,D=4,E=61,H=6,I=47,K=1,P=1,S=25,T=111,V=1	9621
77	L	6					77	L	12	Del=2,G=1,I=12,L=9489,F=113,P=4	9621
78	K	0					78	K	20	R=233,N=2,Del=5,Q=8,E=10,K=9360,T=3	9621
79	M	2					79	M	90	R=1,N=2,Del=2,I=2361,L=1,K=4,M=7156,T=60,V=33,Xaa=1	9621
80	T	1					80	T	146	A=2365,N=97,D=1,Del=1333,G=3,I=9,L=1,S=57,T=5755	9621
81	I	1					81	I	76	Del=1332,I=8046,M=3,T=16,V=224	9621
82	A	0					82	A	68	A=8181,D=3,Del=1332,P=6,T=44,V=55	9621
83	S	0					83	S	60	Del=1332,P=14,S=8274,Y=1	9621
84	V	45 I44S1	V	I	2006	0,0120	84	V	162	A=25,R=2,N=66,C=2,Del=1332,G=10,I=115,L=23,M=63,S=2220,T=69,V=	9621
85	P	0					85	P	12	R=1,Q=4,H=14,L=21,F=1,P=9500,S=54,T=26	9621
86	T	91 A75V16	A	T	1997	0,0470	86	A	61	A=8729,N=4,D=41,G=9,I=4,F=2,S=217,T=452,V=161,Xaa=2	9621
87	S	1					87	S	99	A=35,Q=2,H=14,L=27,F=1,P=3042,S=6488,T=12	9621
88	R	0					88	R	8	R=9545,C=22,Q=7,H=41,L=2,S=3,Xaa=1	9621
89	Y	0					89	Y	1	H=3,S=1,Y=9617	9621
90	L	1					90	L	95	R=2,Q=8,I=2364,L=7093,M=56,F=1,P=1,V=96	9621
91	T	2					91	T	17	A=51,N=2,C=1,I=22,M=4,P=3,S=102,T=9436	9621
92	D	0					92	D	8	N=1,D=9541,E=67,G=10,K=1,V=1	9621
93	M	0					93	M	0	I=2,M=9618,T=1	9621
94	T	1					94	T	84	A=2,N=43,G=1,S=2324,T=7251	9621
95	L	3					95	L	98	A=1,R=1,I=2348,L=7064,M=40,F=7,P=141,T=4,V=15	9621
96	D	8 E6G2			Early marker - 1963	0,0007	96	E	2	D=7,E=9607,G=1,K=5,S=1	9621
97	E	0					97	E	1	D=2,E=9614,K=4,S=1	9621
98	M	4					98	M	81	I=2310,L=2,K=1,M=7301,T=1,V=6	9621
99	S	0					99	S	2	A=1,N=6,C=1,G=1,P=1,S=9605,T=6	9621
100	R	0					100	R	7	R=9551,G=4,I=1,K=64,M=1	9621
101	D	2					101	D	90	N=20,D=6990,E=2560,G=49,K=2	9621
102	W	0					102	W	1	C=3,G=1,L=1,W=9616	9621
103	F	1					103	F	116	C=1,H=4,I=2,L=673,F=6603,S=17,Y=2320,V=1	9621
104	M	1					104	M	2	I=6,L=10,M=9602,T=2,Xaa=1	9621
105	L	0					105	L	2	I=8,L=9608,F=3,P=1,Xaa=1	9621
106	M	0					106	M	39	R=3,I=683,M=8925,T=8,V=2	9621
107	P	0					107	P	1	H=2,L=1,P=9617,S=1	9621
108	K	2					108	K	81	R=2344,Q=2,G=1,K=7271,M=1,Xaa=2	9621
109	Q	0					109	Q	3	R=4,Q=9601,E=1,H=7,L=3,K=2,W=3	9621
110	K	0					110	K	2	R=11,N=1,Q=1,E=3,K=9604,T=1	9621
111	V	3					111	V	105	A=10,R=1,G=1,I=2031,L=13,M=263,F=137,T=4,V=7160,Xaa=1	9621
112	T	13 A11I2	A	T	1975	0,3437	112	A	103	A=6201,E=4,I=57,K=2,S=10,T=3307,V=39,Xaa=1	9621
113	G	0					113	G	3	R=5,D=6,C=3,E=1,G=9598,S=6,Xaa=2	9621
114	S	1					114	S	109	A=1,N=1,G=2209,F=1,P=578,S=6828,T=3	9621
115	L	0					115	L	1	I=2,L=9615,F=4	9621

vmuc:
not a marker
(>0.05)

- Legend:**
- Alternatives:** aa different from consensus, e.g., Q1 means that Q occurred in one isolate
 - Score:** Data taken from the Database describing measure of variability.
 - Alignment Details:** number of occurrences of various amino acids.
 - High-potency and Low-potency markers are colored green and yellow, respectively.

Equine Pos	PB1 Consensus	H3N8 No of Diffs	Alternatives	Permanent aa change			Marker potency	Avian PB1 all subtypes			# Sequences
				From	To	Year of substitution		Pos	Consensus	Score	
1	M	1	del1					1	M	0 M=9002, Xaa=1	9003
2	D	0						2	D	5 N=24, D=8965, E=6, G=8, K=1, Y=2, Xaa=1	9007
3	S	5	P3F2					3	S	13 G=1, L=3, F=16, Pro=123, S=8860, T=3, Y=1	9007
4	N	0						4	N	3 N=8985, D=1, H=2, I=5, K=4, F=1, S=6, T=3	9007
5	T	0						5	T	0 H=1, Pro=1, S=1, T=9004	9007
6	V	1	T1					6	V	93 A=8, R=2, Cys=1, G=3, I=2087, L=2, M=106, T=38, V=6760	9007
7	S	1	T1					7	S	91 A=4, L=127, F=1, Pro=1, S=6689, T=2184, V=1	9007
8	S	0						8	S	1 D=2, G=3, K=1, S=8999, T=2	9007
9	F	0						9	F	1 Del=1, L=4, F=8999, Y=3	9007
10	Q	0						10	Q	1 Del=4, Q=8997, H=2, L=2, K=1, S=1	9007
11	D	0						11	D	8 A=4, N=4, D=8929, Del=1, G=70	9008
12	I	0						12	I	3 I=8988, M=4, F=1, T=13, V=2	9008
13	L	0						13	L	5 L=8957, M=46, V=5	9008
14	M	2	K1Q1					14	M	163 A=13, R=14, Q=2073, E=91, G=1, I=77, L=21, K=91, M=5541, T=272, V=81	9008
15	R	0						15	R	0 R=9005, G=1, K=2	9008
16	M	0						16	M	1 I=2, M=9004, S=1, V=1	9008
17	S	0						17	S	0 S=9008	9008
18	K	0						18	K	2 R=7, N=3, K=8997, T=1	9008
19	M	0						19	M	5 N=1, I=4, L=25, M=8963, V=15	9008
20	Q	0						20	Q	19 A=1, R=246, Q=8758, E=1, H=1, Pro=1	9008
21	L	0						21	L	1 I=1, L=9000, M=2, S=2, W=3	9008
22	G	5	K2R2E1					22	G	138 A=1137, R=49, E=2345, G=5459, K=2, T=11, V=5	9008
23	S	0						23	S	14 A=3, F=11, Pro=28, S=8873, T=6, Y=86, V=1	9008
24	S	0						24	S	2 H=1, L=14, F=1, Pro=1, S=8991	9008
25	S	1	L1					25	S	4 L=23, Pro=10, S=8975	9008
26	E	4	G3V1					26	E	97 A=24, E=6639, G=190, K=1, M=4, V=2149, Xaa=1	9008
27	D	0						27	D	9 N=8, D=8912, G=87, V=1	9008
28	L	0						28	L	1 L=9002, S=2, W=4	9008
29	N	0						29	N	12 N=8871, D=2, K=1, S=131, T=1, Y=1, Xaa=1	9008
30	G	0						30	G	0 G=9007, V=1	9008
31	M	0						31	M	5 I=36, K=1, M=8961, V=10	9008
32	I	1	V1					32	I	4 I=8967, M=1, T=1, V=39	9008
33	I	17	T17	T	I	1976	0,0071	33	T	6 I=64, T=8944	9008
34	R	48	Q48	Q	R	1988	0,0283	34	Q	19 R=255, Q=8747, H=2, L=3, Pro=1	9008
35	L	2	F2				0,0160	35	F	12 L=144, F=8860, S=3, V=1	9008
36	E	0						36	E	32 D=5, Q=1, E=8600, G=249, K=152, Xaa=1	9008
37	S	1	R1					37	S	83 A=11, R=2159, Cys=8, G=1, H=4, F=3, S=6820, T=2	9008
38	L	0						38	L	0 L=9008	9008
39	K	0						39	K	18 R=229, E=2, I=1, K=8775, Xaa=1	9008
40	L	2	I2					40	L	94 R=1, I=2632, L=6311, K=1, M=2, F=34, T=18, V=9	9008
41	Y	0						41	Y	0 L=1, F=1, Y=9005, Xaa=1	9008
42	R	0						42	R	3 R=8985, I=3, K=20	9008
43	D	0						43	D	3 =1, A=1, N=14, D=8984, E=2, G=3, V=3	9008
44	S	0						44	S	23 A=1, L=179, M=1, S=8726, T=101	9008
45	L	0						45	L	2 I=3, L=8994, F=11	9008
46	G	0						46	G	1 E=5, G=9003	9008
47	E	0						47	E	6 A=2, R=4, D=5, Del=2, E=8953, G=5, K=37	9008
48	A	2	S1T1					48	A	108 A=6453, R=1, N=1, D=7, E=1, I=5, K=3, F=2, Pro=5, S=2182, T=285, V=59	9008
49	V	0						49	V	62 A=502, I=31, L=353, M=29, S=9, T=2, V=8081, Xaa=1	9008
50	M	0						50	M	10 R=1, I=33, M=8902, V=72	9008
51	R	0						51	R	0 R=9006, W=1, Xaa=1	9008
52	M	3	I3					52	M	45 I=276, L=54, M=8415, T=17, V=244, Xaa=2	9008
53	G	0						53	G	2 R=19, G=8989	9008
54	D	0						54	D	1 N=1, D=8998, E=6, G=2, V=1	9008
55	L	0						55	L	58 I=6, L=7787, F=1212, Pro=1, Y=2	9008
56	H	0						56	H	11 Q=1, H=8881, L=4, Y=122	9008
57	S	1	Y1					57	S	17 A=1, Q=4, H=1, L=31, F=99, Pro=2, S=8838, T=6, Y=26	9008
58	L	0						58	L	14 I=3, L=8845, F=157, Pro=1, S=1, V=1	9008
59	Q	0						59	Q	2 R=6, Q=8997, Pro=4, Xaa=1	9008
60	S	2	N2					60	S	132 A=3, R=11, N=1444, D=2, G=3, H=1, I=1109, S=6231, T=198, V=5, Xaa=1	9008
61	R	0						61	R	8 R=8918, K=90	9008
62	N	0						62	N	9 N=8910, K=1, T=97	9008
63	E	2	A1K1				0,0102	63	G	100 A=2170, R=30, E=92, G=6623, K=50, S=1, W=23, V=19	9008
64	K	1	T1					64	K	97 A=34, R=14, N=73, D=10, Q=7, E=10, I=9, K=6724, S=22, T=2104, Xaa=1	9008
65	W	0						65	W	0 Cys=3, W=9005	9008
66	R	0						66	R	0 R=9007, K=1	9008
67	E	3	K2D1					67	E	39 R=23, N=16, D=496, Q=1, E=8440, G=14, K=15, Y=3	9008
68	Q	3	K2E1					68	Q	83 R=5, Q=6824, E=2151, G=16, H=1, L=1, K=10	9008
69	L	0						69	L	0 L=9006, M=1, V=1	9008
70	S	1	G1					70	S	29 R=1, N=1, D=1, G=440, S=8563, Xaa=1	9007
71	Q	3	L2R1					71	Q	8 R=24, Q=8937, H=42, L=3, K=1	9007
72	K	0						72	K	2 R=8, N=5, Q=1, K=8993	9007
73	F	0						73	F	0 L=1, F=9005, S=1	9007
74	E	0						74	E	2 D=7, E=8989, G=10, V=1	9007
75	E	0						75	E	0 E=9004, K=3	9007
76	I	4	V3L1					76	I	8 I=8938, L=4, K=1, M=41, T=2, V=21	9007
77	R	0						77	R	21 R=8712, K=294, T=1	9007
78	W	0						78	W	1 G=2, L=3, W=9002	9007
79	L	0						79	L	5 L=8956, M=48, S=1, V=2	9007
80	I	0						80	I	4 I=8967, M=1, V=39	9007
81	E	2	A1D1					81	E	87 A=2163, D=4, E=6768, G=66, S=3, T=2, V=1	9007
82	E	0						82	E	2 A=1, D=2, E=8993, G=10, K=1	9007
83	V	1	C1					83	V	111 A=2, R=2, Cys=2168, I=371, L=7, M=71, F=1, T=3, V=6382	9007
84	R	0						84	R	0 R=9006, Q=1	9007
85	H	1	N1					85	H	95 A=7, R=27, N=2127, D=2, Q=10, H=6723, L=18, S=69, T=3, Y=21	9007
86	R	1	I1					86	R	96 R=6671, G=2, I=2136, K=157, M=7, S=1, T=3, V=30	9007
87	L	0						87	L	1 L=9003, F=1, W=3	9007
88	K	2	R1T1					88	K	98 A=36, R=173, N=13, Q=1, I=6, K=6659, M=3, S=1, T=2115	9007
89	N	11	I9K1A1	I	N	1975	0,0083	89	I	149 A=16, R=37, N=75, Q=14, I=5715, L=1, K=2115, M=15, F=17, S=22, T=123	9007
90	T	0						90	T	1 A=4, I=2, Pro=2, S=2, T=8997	9007
91	E	1	D1					91	E	1 D=1, Q=2, E=8999, G=5	9007
92	N	3	S3					92	N	9 N=8915, D=5, K=1, S=85, T=1	9007
93	S	0						93	S	2 R=3, G=15, S=8989	9007
94	F	0						94	F	4 L=1, F=8973, S=1, Y=32	9007
95	E	0						95	E	1 D=4, Q=3, E=8997, G=2, K=1	9007
96	Q	0						96	Q	1 R=3, Q=9001, H=3	9007
97	I	0						97	I	0 N=2, I=9004, T=1	9007
98	T	0						98	T	0 R=1, Pro=2, T=9004	9007
99	F	0						99	F	1 L=4, F=9003	9007
100	M	1	L1					100	M	88 I=45, L=2202, K=1, M=6733, T=1, V=24, Xaa=1	9007
101	Q	1	R1					101	Q	2 R=2, Q=8996, L=4, Pro=5	9007
102	A	0						102	A	1 A=9001, S=1, T=5	9007
103	L	0						103	L	0 G=1, L=9005, K=1	9007
104	Q	0						104	Q	2 Q=8992, H=10, K=4, Pro=1	9007
105	L	0						105	L	2 I=13, L=8993, V=1	9007
106	L	0						106	L	1 L=9001, F=1, Pro=1, W=1, Y=1, V=1, Xaa=1	9007
107	L	0						107	L	1 Q=1, L=9003, F=1, Pro=2	9007
108	E	0						108	E	1 D=1, Q=1, E=9000, G=5	9007
109	V	0						109	V	2 A=5, E=2, G=2, I=2, K=1, M=2, V=8993	9007
110	E	0						110	E	1 D=1, E=9000, G=4, K=2	9007
111	Q	1	S1					111	Q	86 R=1, N=59, Q=6823, G=4, H=1, L=5, K=3, Pro=3, S=2107, Xaa=1	9007
112	E	0						112	E	2 N=1, D=12, E=8993, G=1	9007
113	I	0						113	I	7 I=8934, M=72, Xaa=1	9007
114	R	0						114	R	2 R=8992, E=1, G=1, K=7, S=2, T=1, W=1, Xaa=2	9007
115	T	0						115	T	41 A=700, N=1, D=2, L=4, Pro=2, S=1, T=8297	9007
116	F	0						116	F	1 N=1, I=1, L=2, F=8999, S=2, Y=1, V=1	9007
117	S	0						117	S	1 A=1, S=9003, T=3	9007
118	F	0						118	F	1 L=4, F=8998, Y=2, V=3	9007
119	Q	0						119	Q	2 R=3, Q=8995, H=4, L=1, Pro=1, S=2, Xaa=1	9007
120	L	0						120	L	2 R=2, H=2, I=1, L=8990, F=7, Pro=1, S=1, Xaa=3	9007
121	I	0						121	I	1 I=8998, L=2, M=2, F=2, S=1, T=1, Y=1	9007