

Antigenic and Molecular Characterization of Avian Influenza A(H9N2) Viruses, Bangladesh

Technical Appendix

Technical Appendix Table 1. Genes of influenza A(H9N2) viruses from Bangladesh that were either not sequenced (X) or only partially sequenced (P)

Virus isolate	Influenza virus gene						
	NA	NP	NS	PA	PB1	PB2	HA
A/Environment/Bangladesh/907/2009							
A/Environment/Bangladesh/1041/2009					X		
A/Duck/Bangladesh/1231/2009							
A/Chicken/Bangladesh/2075/2009							
A/Pigeon/Bangladesh/4303/2009							
A/Environment/Bangladesh/5144/2009							
A/Chicken/Bangladesh/5209/2009							
A/Environment/Bangladesh/5745/2010							
A/Environment/Bangladesh/8202/2010							
A/Chicken/Bangladesh/8411/2010						P	
A/Chicken/Bangladesh/8413/2010						P	
A/Chicken/Bangladesh/8415/2010						P	
A/Environment/Bangladesh/8463/2010						P	
A/Environment/Bangladesh/8465/2010						P	
A/Chicken/Bangladesh/8725/2010					X		
A/Chicken/Bangladesh/8731/2010				X		P	
A/Chicken/Bangladesh/8996/2010						P	
A/Chicken/Bangladesh/9029/2010						P	
A/Environment/Bangladesh/9306/2010				X			
A/Chicken/Bangladesh/9334/2010			X				
A/Environment/Bangladesh/9350/2010						P	
A/Environment/Bangladesh/9457/2010						P	
A/Environment/Bangladesh/10234/2011							
A/Environment/Bangladesh/10306/2011							
A/Environment/Bangladesh/10307/2011						P	
A/Environment/Bangladesh/10313/2011							
A/Environment/Bangladesh/10316/2011			X				
A/Chicken/Bangladesh/10401/2011							
A/Chicken/Bangladesh/10411/2011							X
A/Chicken/Bangladesh/10450/2011						P	
A/Chicken/Bangladesh/10897/2011							
A/Chicken/Bangladesh/11154/2011							
A/Environment/Bangladesh/11173/2011							
A/Chicken/Bangladesh/11309/2011						X	
A/Chicken/Bangladesh/11315/2011							
A/Environment/Bangladesh/11597/2011							
A/Environment/Bangladesh/12068/2011		X					
A/Environment/Bangladesh/12077/2011							
A/Environment/Bangladesh/12093/2011		X			X		
A/Environment/Bangladesh/12103/2011		X		X			
A/Environment/Bangladesh/12116/2011	X			X			
A/Environment/Bangladesh/12119/2011				X			
A/Chicken/Bangladesh/13916/2011				X			
A/Chicken/Bangladesh/13962/2011				X			

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Technical Appendix Table 2. Amino acid substitutions* in hemagglutinin and neuraminidase gene products that determine host specificity of influenza A(H9N2) viruses from Bangladesh

	HA									NA									
	HA RBS					HA1/HA2 Cleavage site	Glycosylation site		Stalk Deletion			Hemabsorbing site							
Residues at HA RBS (H9N2 numbering)	166	191	197	198	232		234	235	236	399	206	218	38-39	46-50	62-64	372	402	403	431-433
H3 Residues at HA RBS (H3 numbering)	158	183	189	190	224	226	227	228	391										
A/Qa/HK/G1/97	S	H	T	E	N	L	Q	G	K	RSSR	NDTT	NRTF	Yes	No	No	S	I	R	PQE
A/Ck/HK/G9/97	N	N	T	A	N	L	Q	G	K	RSSR	No	NRTF	No	No	No	S	N	S	PKE
A/Dk/HK/Y280/97	N	N	T	T	N	L	Q	G	K	RSSR	No	NRTF	No	No	No	S	N	W	PQE
A/Ck/Bei/1/94	N	N	T	V	N	Q	Q	G	K	RSSR	No	NRTF	No	No	No	S	N	W	PQE
A/Dk/HK/Y439/97	S	H	T	E	N	Q	Q	G	K	ASYS	No	NRTF	No	No	No	S	N	W	PQE
A/HK/1073/99	S	H	T	E	N	L	Q	G	K	RSSR	NDTT	NRTF	Yes	No	No	S	N	W	PQE
A/Env/BD/907/2009	N	H	T	T	N	L	I	G	K	KSSR	No	No	No		A	N	W	PQE	
A/Env/BD/1041/2009	S	H	T	E	N	Q	Q	G	K	ASDR	No	NRTF			S	N	W	PQE	
A/Dk/BD/1231/2009	N	H	T	A	N	L	I	G	K	KSSR	No	No			A	N	W	PQE	
A/Ck/BD/2075/2009	N	H	T	A	N	L	I	G	K	KSSR	No	No			A	N	W	PQE	
A/Pg/BD/4303/2009	N	H	T	A	N	L	I	G	K	KASR	No	No			A	N	W	PQE	
A/Env/BD/5144/2009	N	H	T	T	N	L	I	G	K	KSSR	No	No			A	N	W	PQE	
A/Ck/BD/5209/2009	N	H	T	A	N	L	I	G	K	KSSR	No	No			A	N	W	PQE	
A/Env/BD/5745/2010	N	H	T	A	N	L	I	G	K	KSSR	No	No			A	N	W	PQE	
A/Env/BD/8202/2010	N	H	T	A	N	L	I	G	K	KSSR	No	No			A	N	W	PQE	
A/Ck/BD/8411/2010	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	
A/Ck/BD/8413/2010	N	H	T	A	N	L	I	G	K	KSSR	No	No			A	H	W	PQE	
A/Ck/BD/8415/2010	N	H	T	A	N	L	I	G	K	KSSR	No	No			A	H	W	PQE	
A/Env/BD/8463/2010	N	H	T	A	N	L	I	G	K	KSSR	No	No			A	N	W	PQE	
A/Env/BD/8465/2010	N	H	T	A	N	L	I	G	K	KSSR	No	No			A	N	W	PQE	
A/Ck/BD/8725/2010	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	
A/Ck/BD/8731/2010	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	
A/Ck/BD/8996/2010	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	
A/Ck/BD/9029/2010	N	H	T	A	N	L	I	G	K	KSSR	No	No			A	N	W	PQE	
A/Env/BD/9306/2010	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	
A/Ck/BD/9334/2010	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	
A/Env/BD/9350/2010	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	
A/Env/BD/9457/2010	N	H	T	A	N	L	I	G	K	KSSR	No	No			A	H	W	PQE	
A/Env/BD/10234/2011	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	N	W	PQE	
A/Env/BD/10306/2011	D	H	T	A	N	Q	T	G	K	KSKR	No	No			A	H	W	PQE	
A/Env/BD/10307/2011	N	H	T	T	N	L	T	G	K	KSKR	No	No			A	H	W	PQE	
A/Env/BD/10313/2011	N	H	T	A	N	L	T	G	K	KSKR	No	No			A	H	W	PQE	
A/Env/BD/10316/2011	N	H	T	A	N	L	T	G	K	KSKR	No	No			A	H	W	PQE	
A/Ck/BD/10401/2011	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	N	W	PQE	
A/Ck/BD/10411/2011	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	
A/Ck/BD/10450/2011	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	
A/Ck/BD/10897/2011	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	
A/Ck/BD/11154/2011	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	
A/Env/BD/11173/2011	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	
A/Ck/BD/11309/2011	N	H	T	A	N	L	I	G	K	KSKR	No	No			A	H	W	PQE	

	M1		M2		M2 drug resistance			NS		NP				PA		PB1		PB1-F2					PB2				
	15	16	20	28	55	26	31	Deletion of amino acid ⁸⁰ TIASV ⁸⁴	NS1 (227)	PL motif	33	109	214	313	372	57	409	13	336	375	Size (aa)	66	68	73	79	82	81
A/Env/BD/8465/2010	I	V	S	F	F	L	N	K	KPEV	V	I	K	F	D	R	S	P	V	N	90	N	T	K	L	L	T	K
A/Ck/BD/8725/2010	I	V	S	F	F	L	N	K	KSEV	V	I	K	F	D	R	S	na	na	na	na	na	na	na	na	na	T	K
A/Ck/BD/8731/2010	I	V	S	F	F	L	N	K	KSEV	V	I	K	F	D	na	na	P	V	N	90	N	T	K	L	L	T	K
A/Ck/BD/8996/2010	I	V	S	F	F	L	N	K	KSEV	V	V	K	F	D	R	N	P	V	S	90	N	T	K	L	L	I	K
A/Ck/BD/9029/2010	I	V	S	F	F	L	N	K	KPEV	V	I	K	F	D	R	S	P	V	T	90	N	T	K	L	L	I	K
A/Env/BD/9306/2010	I	V	S	F	F	L	N	K	KSEV	V	I	K	F	D	na	na	P	V	N	90	N	T	K	L	L	T	K
A/Ck/BD/9334/2010	I	V	S	F	F	L	N	na	na	I	I	K	F	D	R	S	P	V	N	90	N	T	K	L	L	T	K
A/Env/BD/9350/2010	I	V	S	F	F	L	N	K	KSEV	V	I	K	F	D	R	S	P	V	N	90	N	T	K	L	L	T	K
A/Env/BD/9457/2010	I	V	S	F	F	L	N	K	KSEI	V	V	K	F	D	R	S	P	V	N	90	N	T	K	L	L	I	K
A/Env/BD/10234/2011	I	V	S	F	F	L	N	K	KSEV	V	V	K	F	D	R	S	P	V	S	90	N	T	K	L	L	I	K
A/Env/BD/10306/2011	I	V	S	F	F	L	N	K	KSEV	V	V	K	F	D	R	S	P	V	N	90	N	I	K	L	L	I	K
A/Env/BD/10307/2011	I	V	S	F	F	L	N	K	KSEV	V	I	K	F	D	R	S	P	V	N	90	N	T	K	L	L	*	K
A/Env/BD/10313/2011	I	V	S	F	F	L	N	K	KSEV	V	I	K	F	D	R	S	P	V	N	90	N	T	K	L	L	I	K
A/Env/BD/10316/2011	I	V	S	F	F	L	N	na	na	V	I	K	F	D	R	S	P	V	N	90	N	T	K	L	L	I	K
A/Ck/BD/10401/2011	I	V	S	F	F	L	N	K	KPEV	V	I	K	F	D	R	N	P	V	T	90	N	T	K	L	L	I	K
A/Ck/BD/10411/2011	I	V	S	F	F	L	N	K	KSEV	V	I	K	F	D	R	S	P	V	N	90	N	T	K	L	L	T	K
A/Ck/BD/10450/2011	I	V	S	F	F	L	N	K	KSEV	V	V	K	F	D	R	S	P	V	N	90	N	T	K	L	L	T	K
A/Ck/BD/10897/2011	I	V	S	F	F	L	N	K	KSEV	V	I	K	F	D	R	S	P	V	S	90	N	T	K	L	L	T	K
A/Ck/BD/11154/2011	I	V	S	F	F	L	N	K	KSEV	V	V	K	F	D	R	S	P	V	N	90	N	T	K	L	L	T	K
A/Env/BD/11173/2011	I	V	S	F	F	L	N	K	KSEV	V	V	K	F	D	R	S	P	V	N	90	N	T	K	L	L	I	K
A/Ck/BD/11309/2011	I	V	S	F	F	L	N	K	KSEV	V	V	K	F	D	R	S	P	V	N	90	N	T	K	L	L	na	na
A/Ck/BD/11315/2011	I	V	S	F	F	L	N	K	KSEV	V	V	K	F	D	R	S	P	V	N	90	N	T	K	L	L	I	K
A/Env/BD/11597/2011	I	V	R	F	F	L	N	K	KSEV	V	I	K	F	D	R	S	P	V	N	90	N	T	K	L	L	I	K
A/Env/BD/12068/2011	I	V	S	F	F	L	N	K	KSEV	na	na	na	na	na	R	S	P	V	T	90	N	T	K	L	L	I	K
A/Env/BD/12077/2011	I	V	S	L	F	L	S	K	KSEV	V	I	K	F	D	Q	S	P	V	N	90	N	T	K	L	L	T	K
A/Env/BD/12093/2011	I	V	S	F	F	L	N	K	KSEV	na	na	na	na	na	R	S	na	na	na	na	na	na	na	na	na	I	K
A/Env/BD/12103/2011	I	V	S	F	F	L	N	K	KSEV	na	na	na	na	na	na	na	P	V	N	90	N	T	K	L	L	I	K
A/Env/BD/12116/2011	I	V	S	F	F	L	N	K	KSEV	V	V	K	F	D	na	na	P	V	N	90	N	T	K	L	L	I	K
A/Env/BD/12119/2011	I	V	S	F	F	L	N	K	KSEV	V	V	K	F	D	na	na	P	V	N	90	N	T	K	L	L	I	K
A/Ck/BD/13916/2011	I	V	S	F	F	L	N	K	KSEV	V	I	K	F	D	na	na	P	I	N	90	N	T	K	L	L	T	K
A/Ck/BD/13962/2011	I	V	S	F	F	L	N	K	KSEV	V	V	K	F	D	na	na	P	V	N	90	S	T	R	L	L	I	K

*Key amino acid substitutions are formatted in **boldface**; na, sequence not available for analyses.

Abbreviations: D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; I, isoleucine; K, lysine; L, leucine; N, asparagine; M, methionine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine.



Technical Appendix Figure. Phylogenetic relations of key internal genes of influenza A(H9N2) viruses from Bangladesh. Full-length sequencing, starting from the first codon, was used. The phylogenetic trees were generated by PhyML within the maximum-likelihood framework. Numbers above the branches indicate bootstrap value, and only values greater than 60 are shown. Influenza A(H9N2) viruses from Bangladesh are shown in red.