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Reassortant Avian Influenza A(H9N2) Viruses in Chickens in Retail Poultry Shops, Pakistan, 2009–2010

Technical Appendix

Technical Appendix Table 1. Comparison of critical amino acid substitutions in hemagglutinin and neuraminidase proteins in H9N2 isolates from samples collected from chickens in live poultry retail shops, Pakistan, 2009–2010*

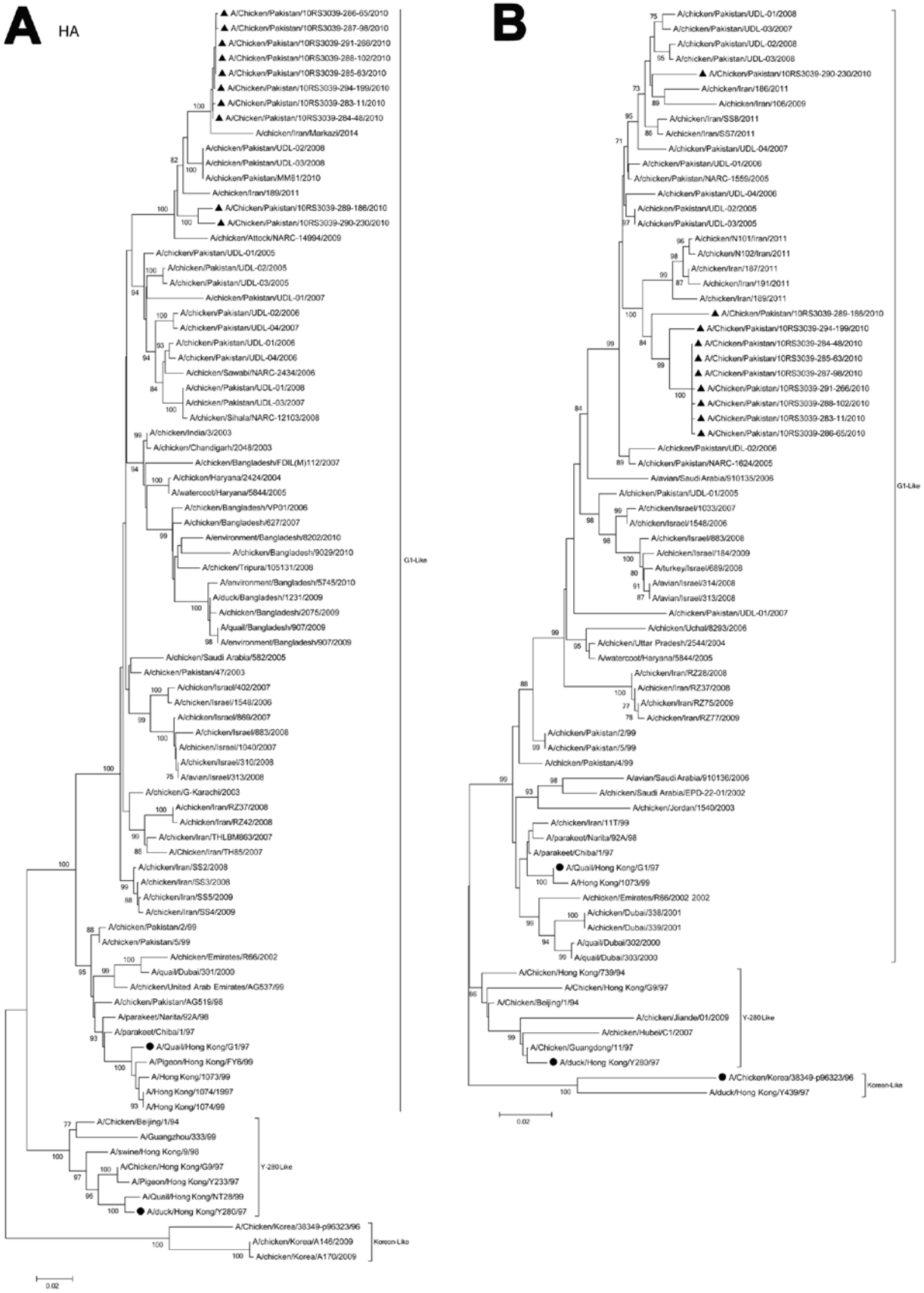
	HA						NA						
	HA RBS					HA1/HA2 cleavage site	Glycosylation site	Stalk deletion				Semiabsorbing site	
Residues at HA RBS (H9N2 numbering)	191	198	234	235	236	335-338	218	38-39	46-50	62-64	372	402	403
H3 Residues at HA RBS (H3 numbering)	183	190	226	227	228	326-329	210	–	–	–	–	–	–
A/Qa/HK/G1/97	H	E	L	Q	G	RSSR	Yes	Yes	No	No	S	I	R
A/duck/HK/Y280/97[2]	N	T	L	Q	G	RSSR	Yes	No	No	No	S	I	R
A/ck/Korea/38349-p96323/96[3]	H	E	Q	Q	G	ASYR	Yes	No	No	No	S	N	W
A/Hong Kong/1073/99[4]	H	E	L	Q	G	RSSR	Yes	Yes	No	No	S	N	W
A/ck/Pak/UDL-01/07[5]	H	A	L	I	G	RSSR	No	No	No	No	S	N	W
A/ck/Pak/10RS3039-283-11/2010	H	V	L	I	G	KSSR	No	No	No	No	A	N	R
A/ck/ Pak/10RS3039-284-48/2010	H	A	L	I	G	KSSR	No	No	No	No	A	N	R
A/ck/Pak/10RS3039-285-63/2010	H	A	L	I	G	KSSR	No	No	No	No	A	N	R
A/ck/ Pak/10RS3039-286-65/2010	H	A	L	I	G	KSSR	No	No	No	No	A	N	R
A/ck/ Pak/10RS3039-287-98/2010	H	A	L	I	G	KSSR	No	No	No	No	A	N	R
A/ck/ Pak/10RS3039-288-102/2010	H	A	L	I	G	KSSR	No	No	No	No	A	N	R
A/ck/ Pak/10RS3039-289-186/2010	H	A	L	I	G	KSSR	No	No	No	No	A	N	R
A/ck/ Pak/10RS3039-199-199/2010	H	A	L	I	G	KSSR	No	No	No	No	A	N	R
A/ck/ Pak/10RS3039-290-230/2010	H	A	L	I	G	KSSR	No	No	No	No	A	N	R
A/ck/ Pak/10RS3039-291-266/2010	H	A	L	I	G	KSSR	No	No	No	No	A	N	R

*Residual differences and similarities are compared with reference strains of lineages: G1-lineage (1), Y280-like (2), and Korea-like (3), vaccine strain (4), and previous H9N2 isolate originating in Pakistan (5).HA, hemagglutinin,; NA, neuraminidase; RBS, receptor binding site; –, not applicable; A, alanine; D, aspartic acid; E, glutamic acid; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; N, asparagine; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine.

Technical Appendix Table 2. Comparison with validated host-specific amino acids residue and pathogenicity determinants in the M1, NP, PB2, PB1, and NS1 protein in H9N2 viruses isolated from 280 composite samples in 280 live poultry retail shops of Lahore, Pakistan*

	M1	M2				M2 drug resistance		NS			NP							PA		PA-X		PB1		PB1-F2					PB2	
x	15	16	20	28	55	26	31	Deletion of amino acid 80-84	NS1 (227)	PL motif	33	109	214	313	372	57	409	108	134	13	336	Size (aa)	73	79	82	87	256	355
Predicted Avian	V	E	S	I	L	NA	NA	NA	E	NA	V	I	R	F	E	R	T	D	K	L	V		K	R	L	E	D	K
Predicted Mammalian	I	G/D	N	I/V	F	NA	NA	NA	R/K	NA	I	V	K	Y	D	Q	S			P	I		R	Q	S	G	G	Q
Predicted amantadine resistant	NA	NA	NA	NA	NA	F	N/G	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Predicted amantadine sensitive	NA	NA	NA	NA	NA	L	S	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A/Qa/HK/G1/97[1]	U	U	U	U	U	U	U	No	E	EPEV	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
A/duck/HK/Y280/97[2]	U	U	U	U	U	U	U		E	EPEV	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U
A/Hong Kong/1073/99[3]	I								E	EPEV		I	R	F	E	R	S			P	V							
A/ck/ Pak/10RS3039-283-11/2010	I	**G**	K	I	**F**	L	S	No	**K**	**K**SEI	V	I	R	F	**D**	R	S	D	K	**P**	V	90	K	R	L	E	D	R
A/ck/ Pak/10RS3039-284-48/2010	I	**G**	K	I	**F**	L	S		**K**	**K**SEI	V	I	R	F	**D**	R	S	D	K	**P**	V	90	K	R	L	E	D	R
A/ck/ Pak/10RS3039-285-63/2010	I	**G**	K	I	**F**	L	S		**K**	**K**SEI	V	I	R	F	**D**	R	S	D	K	**P**	V	90	K	R	L	E	D	R
A/ck/ Pak/10RS3039-286-65/2010	I	**G**	K	I	**F**	L	S		**K**	**K**SEI	V	I	R	F	**D**	R	S	D	K	**P**	V	90	K	R	L	E	D	R
A/ck/ Pak/10RS3039-287-98/2010	I	**G**	K	I	**F**	L	S		**K**	**K**SEI	V	I	R	F	**D**	R	S	D	K	**P**	V	90	K	R	L	E	D	R
A/ck/ Pak/10RS3039-288-102/2010	I	**G**	K	I	**F**	L	S		**K**	**K**SEI	V	I	R	F	**D**	R	S	D	K	**P**	V	90	K	R	L	E	D	R
A/ck/ Pak/10RS3039-289-186/2010	I	**G**	K	I	**F**	L	S		**K**	**K**SEI	V	I	R	F	**D**	R	S	D	K	**P**	V	90	K	R	L	E	D	R
A/ck/ Pak/10RS3039-199-199/2010	I	**G**	K	I	**F**	L	S		**K**	**K**SEI	V	I	R	F	**D**	R	S	D	K	**P**	V	90	K	R	L	E	D	R
A/ck/ Pak/10RS3039-290-230/2010	I	**G**	K	I	**F**	L	S		**K**	**K**SEI	V	I	R	F	**D**	R	S	D	K	**P**	V	90	K	R	L	E	D	R
A/ck/ Pak/10RS3039-291-266/2010	I	**G**	K	I	**F**	L	S		**K**	**K**SEI	V	I	R	F	**D**	R	S	D	K	**P**	V	90	K	R	L	E	D	R

*Residues differences and similarities are compared with reference strains of lineages (G1-lineage[1], Y280-like[2]), and vaccine strain[3]. Amino acids in boldface indicate the host-associated amino acid signature changes identical to those in influenza viruses isolated from humans. A, alanine; D, aspartic acid; E, glutamic acid; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; N, asparagine; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; NA, Not applicable; ND, No data; U, unknown.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A/chicken/Pakistan/UDL-03/2007
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A/chicken/India/3/2003
A/chicken/Chandigarh/2048/2003
A/chicken/Bangladesh/FDIL(M)112/2007
A/chicken/Haryana/2424/2004
A/watercoot/Haryana/5844/2005
A/chicken/Bangladesh/VP01/2006
A/chicken/Bangladesh/627/2007
A/environment/Bangladesh/8202/2010
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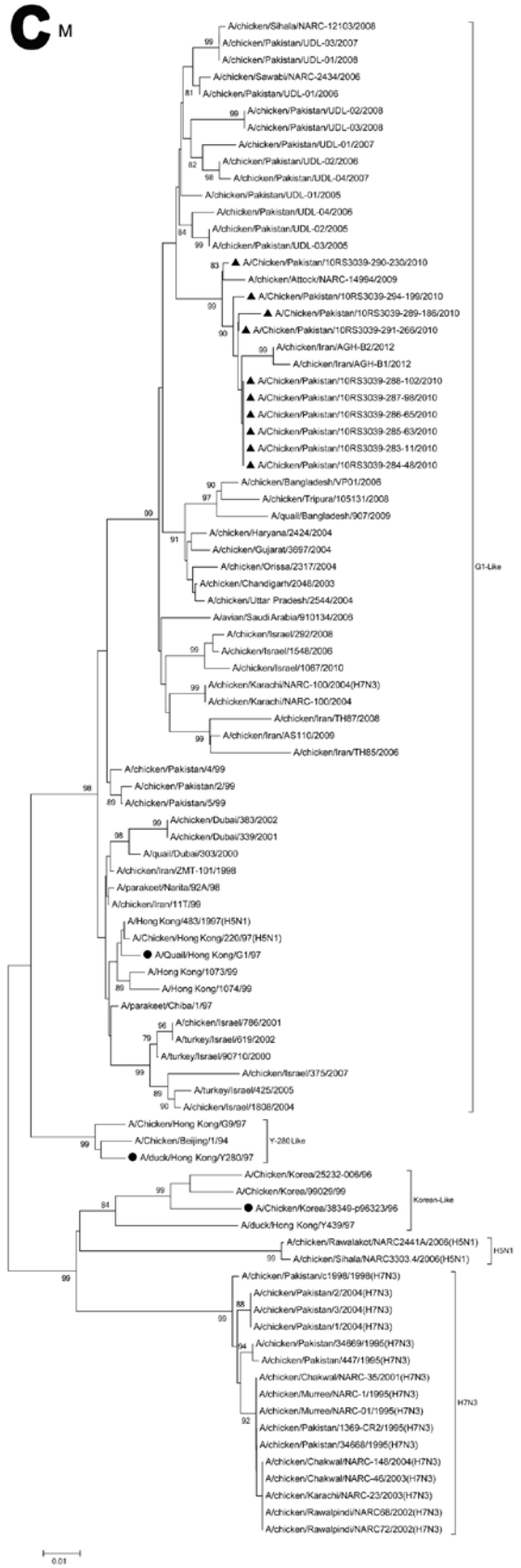
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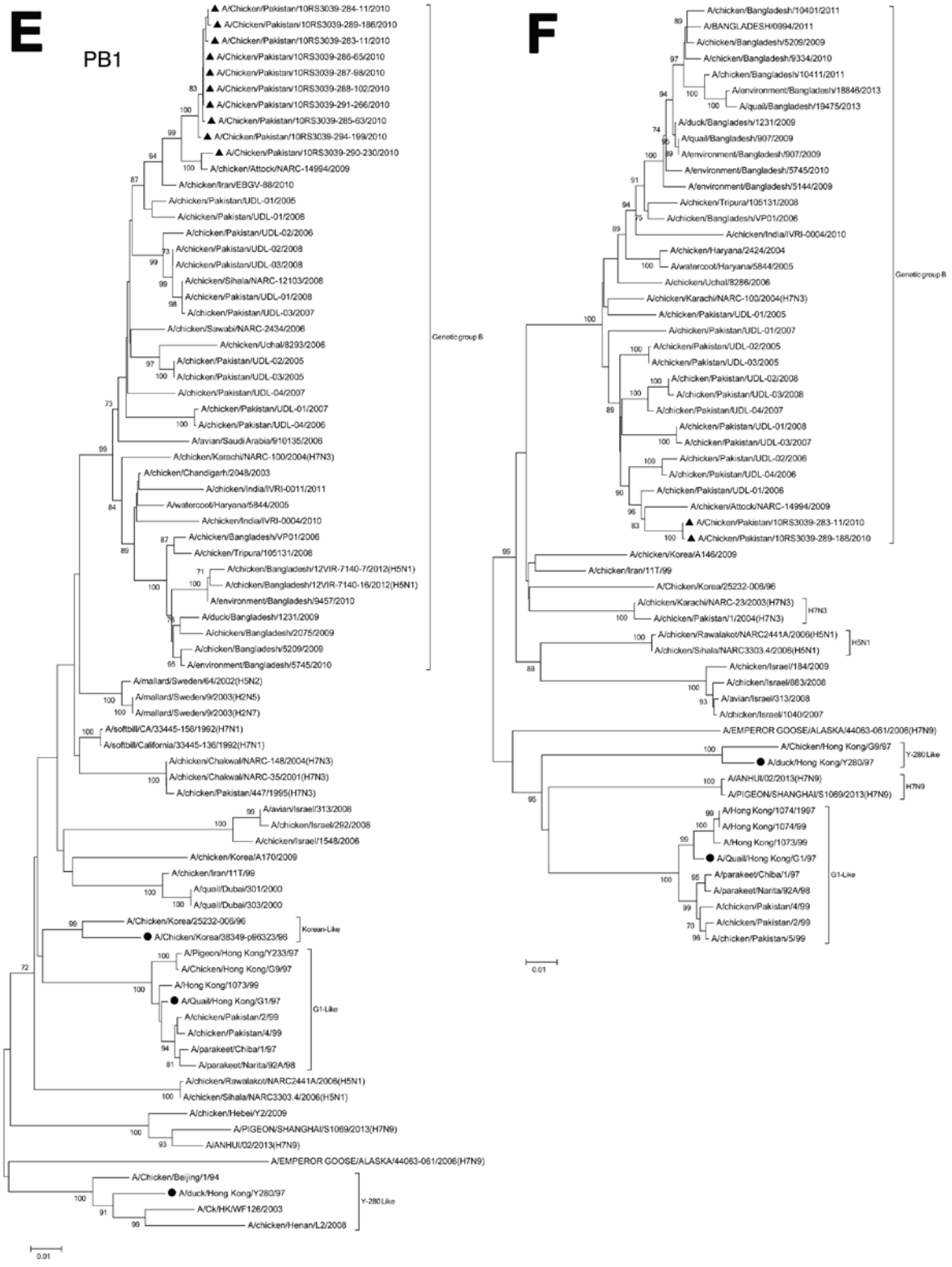
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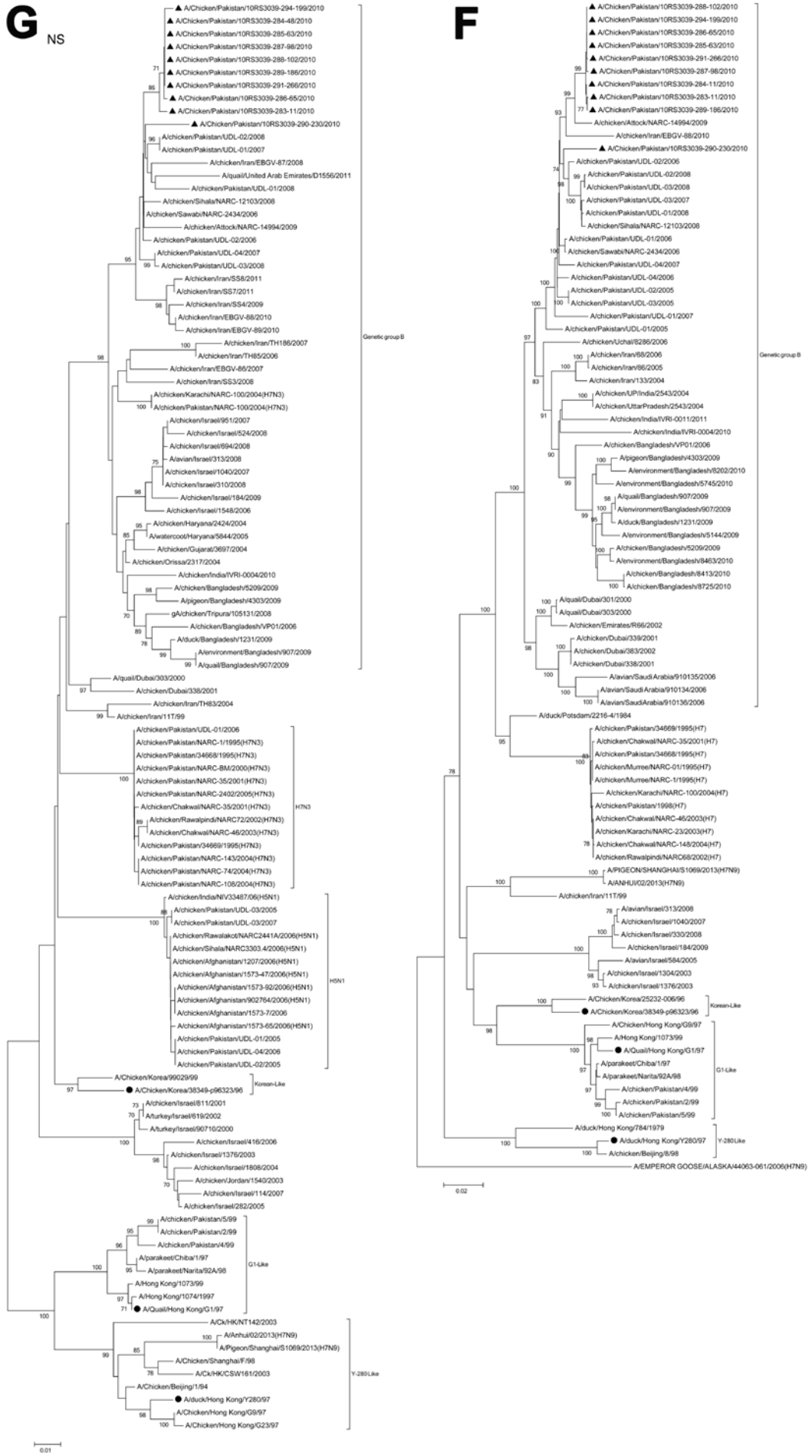
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Technical Appendix Figure. Phylogenetic distribution of 10 sequenced viruses (GenBank accession nos. KF975457–KF975503 and KP223678–KP223693) from 280 live poultry retail shops in selected towns of Lahore, Pakistan. The phylogenetic trees were generated by using the neighbor-joining method with maximum composite likelihood using the Tamura-Nei model (*5*) with MEGA (version 5.2.2). Number below branches indicates bootstrap value percentages from 1,000 replicates; bootstrap values of >70% were considered to be significant. Triangle shows viruses collected in current study; circles show the reference lineage viruses. Scale bars indicate nucleotide substitutions per site. Panels shown on subsequent pages: Hemagglutinin (HA); neuraminidase (NA); matrix (M); nucleoprotein (NP); polybasic 1 (PB1) polymerase (PA); nonstructural (NS); polybasic 2 (PB2).

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