Third Wave of Influenza A(H7N9) Virus from Poultry, Guangdong Province, China, 2014– 2015

Technical Appendix

Additional Information on Influenza A(H7N9) Viruses and their Internal Genes, Guangdong Province, China, 2014–2015

Technical Appendix Table 1. Influenza A(H7N9) viruses isolated and sequenced in this study, Guangdong Province, China*

ID	Virus	Site	Collection date	Host
EPI_ISL_176816	A/Chicken/Guangdong/DG478/2014(H7N9)	LPM, Houjie, Dongguan	2014 Nov 25	Chicken
EPI_ISL_176817	A/Chicken/Guangdong/DG479/2014(H7N9)	LPM, Guancheng, Dongguan	2014 Nov 30	Chicken
EPI_ISL_176818	A/Duck/Guangdong/DG527/2014(H7N9)	LPM, Houjie, Dongguan	2014 Dec 2	Duck
EPI_ISL_176819	A/Chicken/Guangdong/DG592/2014(H7N9)	LPM, Houjie, Dongguan	2014 Dec 8	Chicken
EPI_ISL_176820	A/Chicken/Guangdong/DG593/2014(H7N9)	LPM, Guancheng, Dongguan	2014 Dec 12	Chicken
EPI_ISL_176824	A/Chicken/Guangdong/HZ098/2015(H7N9)	Farm, Boluo, Huizhou	2015 Jan 1	Chicken
EPI_ISL_176828	A/Duck/Guangdong/DG103/2015(H7N9)	LPM, Humen, Dongguan	2015 Jan 7	Duck
EPI_ISL_176830	A/Environment/Guangdong/DG104/2015(H7N9)	LPM, Houjie, Dongguan	2015 Jan 16	Environment
EPI_ISL_176832	A/Chicken/Guangdong/DG120/2015(H7N9)	LPM, Guancheng, Dongguan	2015 Jan 15	Chicken
EPI_ISL_176833	A/Environment/Guangdong/DG127/2015(H7N9)	LPM, Humen, Dongguan	2015 Feb 20	Environment
EPI_ISL_176834	A/Chicken/Guangdong/GZ068/2015(H7N9)	LPM, Jianggao, Guangzhou	2015 Jan 1	Chicken
EPI_ISL_176835	A/Chicken/Guangdong/CZ145/2015(H7N9)	LPM, Chaozhou	2015 Feb 1	Chicken
EPI_ISL_176836	A/Chicken/Guangdong/SW153/2015(H7N9)	LPM, Shanwei	2015 Feb 5	Chicken
EPI_ISL_176837	A/Chicken/Guangdong/SW154/2015(H7N9)	LPM, Shanwei	2015 Feb 10	Chicken

*ID, identification; LPM, live poultry market.

Technical Appendix Table 2. Mutation analysis of critical and apparent amino acid residues of influenza A(H7N9) virus isolates, Guangdong Province, China*

	HA			NA	PE	32	M2
	186	226		289			
Virus	(H3 numbering)	(H3 numbering)	Cleavage sites	(N9 numbering)	627	701	31
A/Chicken/Guangdong/DG478/2014(H7N9)	А	L	PEIPKGRG	R	Е	D	Ν
A/Chicken/Guangdong/DG479/2014(H7N9)	А	L	PEIPKGRG	R	Е	D	Ν
A/Duck/Guangdong/DG527/2014(H7N9)	А	L	PEIPKGRG	R	Е	D	Ν
A/Chicken/Guangdong/DG592/2014(H7N9)	А	L	PEIPKGRG	R	Е	D	Ν
A/Chicken/Guangdong/DG593/2014(H7N9)	А	L	PEIPKGRG	R	Е	D	Ν
A/Chicken/Guangdong/HZ098/2015(H7N9)	А	L	PEIPKGRG	R	Е	D	Ν
A/Duck/Guangdong/DG103/2015(H7N9)	A	L	PEIPKGRG	R	Е	D	Ν
A/Environment/Guangdong/DG104/2015(H7N9)	А	L	PEIPKGRG	R	Е	D	Ν
A/Chicken/Guangdong/DG120/2015(H7N9)	А	L	PEIPKGRG	R	Е	D	Ν
A/Environment/Guangdong/DG127/2015(H7N9)	А	L	PEIPKGRG	R	Е	D	Ν
A/Chicken/Guangdong/GZ068/2015(H7N9)	V	L	PEIPKGRG	R	Е	D	Ν
A/Chicken/Guangdong/CZ145/2015(H7N9)	V	L	PEIPKGRG	R	Е	D	Ν
A/Chicken/Guangdong/SW153/2015(H7N9)	V	L	PEIPKGRG	R	Е	D	Ν
A/Chicken/Guangdong/SW154/2015(H7N9)	V	L	PEIPKGRG	R	Е	D	Ν

*HA, hemagglutinin; NA, neuraminidase; PB2, polymerase basic 2; M2, matrix 2. HA 186: G \rightarrow V increases binding affinity for α (2–6)–linked sialic acid receptor (1,2). HA 226: Q \rightarrow L increases binding affinity for α (2–6)–linked sialic acid receptor (3–5). NA 289: R \rightarrow K reduces susceptibility to oseltamivir (6). PB2 627: E \rightarrow K enhances viral replication and virulence in mice model (7). PB2 701: D \rightarrow N causes mammalian adaptation (8). M2 31: S \rightarrow N causes resistance to adamantanes (9).

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Technical Appendix Figure 1. Phylogenetic relationships of polymerase basic 2 genes of influenza A(H7N9) viruses from poultry, Guangdong Province, China, 2014–2015. Trees were constructed by using the neighborjoining method in MEGA (<u>http://www.megasoftware.net/</u>). Red triangles indicate newly sequenced viruses isolated from poultry in Guangdong during the third wave. Scale bar indicates nucleotide substitutions per site.



Technical Appendix Figure 2. Phylogenetic relationships of polymerase basic 1 genes of influenza A(H7N9) viruses from poultry, Guangdong Province, China, 2014–2015. Trees were constructed by using the neighborjoining method in MEGA (<u>http://www.megasoftware.net/</u>). Red triangles indicate newly sequenced viruses isolated from poultry in Guangdong during the third wave. Scale bar indicates nucleotide substitutions per site.



Technical Appendix Figure 3. Phylogenetic relationships of polymerase acidic genes of influenza A(H7N9) viruses from poultry, Guangdong Province, China, 2014–2015. Trees were constructed by using the neighborjoining method in MEGA (<u>http://www.megasoftware.net/</u>). Red triangles indicate newly sequenced viruses isolated from poultry in Guangdong during the third wave. Scale bar indicates nucleotide substitutions per site.



Technical Appendix Figure 4. Phylogenetic relationships of nonstructural protein genes of influenza A(H7N9) viruses from poultry, Guangdong Province, China, 2014–2015. Trees were constructed by using the neighborjoining method in MEGA (<u>http://www.megasoftware.net/</u>). Red triangles indicate newly sequenced viruses isolated from poultry in Guangdong during the third wave. Scale bar indicates nucleotide substitutions per site.



Technical Appendix Figure 5. Phylogenetic relationships of nucleoprotein genes of influenza A(H7N9) viruses from poultry, Guangdong Province, China, 2014–2015. Trees were constructed by using the neighbor-joining method in MEGA (<u>http://www.megasoftware.net/</u>). Red triangles indicate newly sequenced viruses isolated from poultry in Guangdong during the third wave. Scale bar indicates nucleotide substitutions per site.



Technical Appendix Figure 6. Phylogenetic relationships of matrix genes of influenza A(H7N9) viruses from poultry, Guangdong Province, China, 2014–2015. Trees were constructed by using the neighbor-joining method in MEGA (<u>http://www.megasoftware.net/</u>). Red triangles indicate newly sequenced viruses isolated from poultry in Guangdong during the third wave. Scale bar indicates nucleotide substitutions per site.