Influenza A Cleavage Sites

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Background:

As specified by the OIE **Terrestrial Animal Health Code**, H5 and H7 avian influenza viruses classified as:

- high pathogenicity avian influenza viruses have an IVPI in six-week-old chickens greater than 1.2 or, as an alternative, cause at least 75% mortality in four-to eight-week-old chickens infected intravenously. H5 and H7 viruses which do not have an IVPI of greater than 1.2 or cause less than 75% mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the haemagglutinin molecule (HA0); if the amino acid motif is similar to that observed for other high pathogenicity avian influenza isolates, the isolate being tested should be considered as high pathogenicity avian influenza virus;

- low pathogenicity avian influenza viruses are all influenza A viruses of H5 and H7 subtypes that are not high pathogenicity avian influenza viruses

Avian influenza viruses attach to host cells using the viral hemagglutinin (HA) protein. Progeny influenza viruses contain an HA_0 protein, which has to be cleaved to form HA_1 and HA_2 to become functional. This cleavage occurs at the HA_0 cleavage site by cellular proteases of the host. The type of cellular proteases that can cleave HA_0 depends on the type of cleavage site in the HA_0 protein:

- **Mono–basic cleavage sites** contain one basic amino acid in the critical position (-1; e.g. **PEKQTR/GLF**) of the cleavage site and are cleaved by few cellular proteases. Therefore these viruses can grow only in limited areas of the poultry host: generally the intestinal and the respiratory systems.

- **Multi–basic cleavage sites** contain several basic amino acids in the critical position (-1 and immediately preceding; e.g. **PQRESRRKK/GLF**) of the cleavage site, some having insertions of 1 or more amino acids that lengthen the cleavage site. They are cleaved by several common cellular proteases. Therefore these viruses have the potential to grow systemically (throughout the body) of the host.

How to use this cleavage site information:

Use **Table 1 (H5 HPAI)** for an overview of the very consistent multi-basic cleavage sites of H5 HPAI viruses of the Goose/Guangdong lineage (Asia, Africa, Europe and North America). This currently includes subtypes H5N1, H5N2, H5N3, H5N5, H5N6 and H5N8 derived from the H5 Gs/GD lineage by reassortment.

Use **Tables 2 (other H5 HPAI)** and **3 (H7 HPAI)** for previously reported multi-basic cleavage sites from outbreaks throughout the world.

- The presence of a previously reported multi-basic cleavage site strongly indicates that the new virus is HPAI.

- The presence of any insertion in the cleavage site (even if not previously reported) should be discussed with your regional reference center.

Table 4 lists **unusual, 2-3 residue multi-basic cleavage sites**.

- Please **contact** your **FAO or OIE** reference laboratory (http://www.offlu.net/index.php?id=78) **for further advice**.

How to determine whether a cleavage site is HP using molecular methods:

1. Determine whether your new cleavage site exactly matches a previously reported HPAI virus cleavage site

    a. Table 1 for HPAI H5Nx of the H5 Gs/GD lineage
    b. Table 2 for other HPAI H5
    c. Table 3 for HPAI H7
    d. Table 4 for unusual, 2-3 residue multi-basic cleavage sites

If your cleavage site has previously been reported, you should report the new virus as HPAI and/or seek advice from your FAO or OIE reference laboratory.

(Remember all H5 and H7 viruses are reportable regardless of cleavage site)

2. If your cleavage site shows **more than one basic amino acid** compared to the LPAI cleavage site (first row of Tables 1, 2, 3), **contact** your **FAO or OIE** reference laboratory (http://www.offlu.net/index.php?id=78) **for further advice**.

3. If your cleavage site shows **any insertions of amino acids** compared to the LPAI cleavage site (first row of Tables 1, 2, 3), **contact** your **FAO or OIE** reference laboratory (http://www.offlu.net/index.php?id=78) **for further advice**.

Table 1: Multi-basic cleavage sites of recently circulating Goose/Guangdong-lineage H5Nx HPAI viruses.

Sub-type	Clade[1]	no. of sequences 2009-2017	Cleavage site consensus[2]	critical basic aa[3]	Size of insert
H5	LP	415	PQRETR/GLF	1	0
H5N1	Gs/Gd-lineage	83[4]	**PQRERRRKKR/GLF**	6	4
H5N1	Clade 1	121	**PQREERRKKR/GLF** **PQREGRRKKR/GLF**	5	4
H5N1	Clade 2.1	140	**PQRESRRKK/GLF**	4	3
H5N1	Clade 2.2	684	**PQGEKRRKKR/GLF** **PQGERRRKKR/GLF** **PQGEGRRKKR/GLF**	5-6	4
H5N1	Clade 2.3.1	20[5]	**PQRERRRKR/GLF**	5	3
H5N1	Clade 2.3.2	1024	**PQRERRRKR/GLF**	5	3
H5N1	Clade 2.3.3	30[5]	**PQRERRRKR/GLF**	5	3
H5N1, H5N2, H5N3, H5N5, H5N6, H5N8	Clade 2.3.4	640	**PLRERRRKR/GLF** **PLREKRRKR/GLF**	5	3
H5N1	Clade 2-like	8[5]	**PQRERRRKKR/GLF**	6	4
H5N1	Clade 3	18[5]	**PQRERRRKKR/GLF**	6	4
H5N1	Clade 4	6[5]	**PQRERRRKKR/GLF**	6	4
H5N1	Clade 5	9[5]	**PQREIRRKKR/GLF**	5	4
H5N1	Clade 6	8[5]	**PQRERRRKKR/GLF**	6	4
H5N1	Clade 7	35	**PQIEGRRRKR/GLF**	5	4
H5N1	Clade 9	24[5]	**PQRERRRKKR/GLF**	6	4

[1] LP – low pathogenic. Gs/Gd = A/goose/Guangdong/1/1996-lineage highly pathogenic avian influenza H5N1; clades include all higher order subclades (i.e clade 7 includes clade 7, 7-like, 7.1, 7.2) unless otherwise specified.

[2] Consensus sequence generated from all complete segment of H5 HA sequences in IRD clade-search tool 2009-2017, recovered September 2017, using MUSCLE alignment and Geneious® 8.1.7; variants with a population frequency greater than 10% were included; red color indicates critical basic residues; / indicates cleavage position (38).

[3] Basic residue at the -1 position and basic amino acids immediately preceding this position.

[4] No new sequences available for 2009-2017 as these subclades are extinct, therefore the consensus sequences were generated on data from 1996-2008.

[5] no sequences available for 2009-2017 therefore the consensus sequences were generated on data from 2000-2008.

Table 2: Multi-basic cleavage sites of sporadic H5 HPAI avian influenza A viruses

Sub-type	Pheno-type[1]	location	year	Type virus	cleavage site[2]	Critical basic aa[3]	Size of insert	Accession number[4]	Ref.
H5	LP				PQRETR/GLF	1	0		38
H5N2	HP	South Africa	2011	A/ostrich/SA/AI2114/11 A/ostrich/SA/AI2887/11	**PQRRKKR/GLF** **PQRRRKR/GLF**	4 4	1 1	JX069081.1 JX069097.1	18
H5N2	HP	South Africa	2006	A/ostrich/SA/AI1091/06	**PQREKRRKKR/GLF**	6	4	EF591749.1	1,16
H5N1	HP	Germany	2006	A/gull/Germany/R882/06	**PQGERRRKKR/GLF**	6	4	AM408215.1	29
H5N2	HP	Italy	1997	A/chicken/Italy/1485/97	**PQRRRKKR/GLF**	5	2	GU052473.1	6,30
H5N2	HP	Mexico	1994	A/chicken/Puebla/8623-607/94	**PQRRKRKTR/GLF** **PQRKRKRKTR/GLF**	5 6	3 4	AB558473.1	4,5,6
H5N2	HP	Mexico	1994	A/chicken/Puebla/8624-602/94	**PQRKRKTR/GLF**	4	2		5
H5N1	HP	England	1991	A/turkey/England/50-92/91	**PQRKRRKTR/GLF**	5	3	EU636692.1	10
H5N8	HP	Ireland	1983	A/turkey/Ireland/1378/83	**PQRKRKKR/GLF**	5	2	CY015089.1	6,31
H5N9	HP	Canada	1966	A/turkey/Ontario/7732/66	**PQRRRKKR/GLF**	5	2	CY107859.1	6,32
H5N3	HP	South Africa	1961	A/tern/South Africa/61	**PQRETRRQKR/GLF**	4	4	CY107861.1	6,10

[1] LP = low pathogenic, HP = highly pathogenic as determined by IVPI.
[2] Cleavage site between HA1 and HA2; red color indicates critical basic residues; residue insertions are underlined.
[3] Number of basic residue at immediately preceding and including the -1 position; excludes the -4 position found in the LPAI cleavage site consensus motif.
[4] GenBank record number

Table 3: Multi-basic cleavage sites of sporadic H7 HPAI avian influenza A viruses

Sub-type	Pheno-type[1]	location	year	Type virus	cleavage site[2]	Critical basic aa[3]	Size of insert	Accession number[4]	Ref.
H7	LP				**PEIPKGR/GLF, PELPKGK/GLF, PEPPKGR/GLF, PENPKTR/GLF PESPKTR/GLF**	1	0		38
H7N9	HP	China	2017	A/chicken/Guangdong/GD4/2017	**PEVPKGKRTAR/GLF**	3	4	KY855518	43-46
				A/chicken/Guangdong/GD15/2016	**PEVPKRKRTAR/GLF**	4	4	EPI960361	
				A/Guangdong/Th005/2017	**PEVPKGKRIAR/GLF**[5]	3	4	EPI926825	
H7N9	HP	USA	2017	A/chicken/Tennessee/17-007147-2/2017	**PENPKTDRKSRHRRIR/GLF**	6	9	KY818811	42
H7N7	HP	Italy	2016	A/chicken/Italy/16VIR-1873/2016	**PELPKGRKRR/GLF**	4	3	EPI220955	IZSVenezie
H7N8	HP	USA	2016	A/turkey/Indiana/16-001403-1/2016	**PENPKKRKTR/GLF**	4	3	KU558906.1	41
H7N7	HP	UK	2015	A/chicken/England/26352/2015	**PEIPRHRKGR/GLF**	4	3	EPI623939	APHA
H7N7	HP	Germany	2015	A/chicken/Germany/AR1386/2015	**PEIPKRKRR/GLF**	5	3	TBA	FLI
H7N7	HP	Italy	2013	A/chicken/Italy/13VIR4527_11/13	**PETPKRRERR/GLF**	4	3	KF569186.1	39
H7N3	HP	Mexico	2012	A/chicken/Jalisco/CPA1/12	**PENPKDRKSRHRRTR/GLF**	6	8	JX397993.1	23
				A/chicken/Puebla/CPA-04451/16	**PENPKDRKNRHRRTR/GLF**	6	8	KX351916.1	
				A/chicken/Jalisco/CPA-01859/16	**PENPKGKKSRHRKTR/GLF**	6	8	KX351892.1	
H7N7	HP	Spain	2009	A/chicken/Spain/6279-2/2009	**PELPKGTKPRPRR/GLF**	4	6	GU121458.1	24
H7N7	HP	UK	2008	A/chicken/England/1158-11406/08	**PEIPKRKKR/GLF**	4	2	FJ476173.1	25,26
H7N3	HP	Canada	2007	A/chicken/Saskatchewan/HR-00011/07	**PENPKTTKPRPRR/GLF**	4	6	EU500860.1	17
H7N7	HP	North Korea	2005	A/chicken/North Korea/1/2005	**PEIPKGRHRRPKR/GLF**	5	6		13
H7N3	HP	Canada	2004	A/chicken/Canada/rv504/04	**PENPKQAYRKRMTR/GLF**	4	7	CY015006.1	13
					PENPKQAYQKRMTR/GLF	3	7		
					PENPKQAYKKRMTR/GLF	4	7		
					PENPKQAYHKRMTR/GLF	3	7		
					PENPKQAHQKRMTR/GLF	3	7		
					PENPRQAYRKRMTR/GLF	4	7		
					PENPKQACQKRMTR/GLF	3	7		
H7N7	HP	Netherlands	2003	A/chicken/Netherlands/219/03	**PEIPKRRRR/GLF**	4	2	AY338459.1	9,27
H7N3	HP	Chile	2002	A/chicken/Chile/4322/02	**PEKPKTCSPLSRCRETR/GLF**	3	10	AY303631.1	7,28
					PEKPKTCSPLSRCRKTR/GLF	4	10		
H7N1	HP	Italy	1999	A/chicken/Italy/444/99	**PEIPKGSRVRR/GLF**	3	4	AJ704810.1	12
					PEIPKGSRMRR/GLF	3	4		
					PEIPKRSRVRR/GLF	4	4		
H7N4	HP	Australia	1997	A/chicken/New South Wales/2/97	**PEIPRRRKR/GLF**	4	2	CY022693-	19,20
					PEIPRKRKR/GLF	4	2	CY022700	
H7N3	HP	Pakistan	1995	A/chicken/Pakistan/447/95	**PETPKRKRKR/GLF**	5	3	AF202226	2
				A/chicken/Pakistan/CR2/95	**PETPKRRKR/GLF**	4	2	AF202230	
				A/chicken/Pakistan/16/99/95	**PETPKRRNR/GLF**	3	2	AF202233	
H7N3	HP	Australia	1994	A/chicken/Queensland/94	**PEIPRKRKR/GLF**	4	2	CY022685	11,20
H7N3	HP	Australia	1992	A/chicken/Victoria/224/92	**PEIPKKKKR/GLF**	4	2	CY025077-	20
					PEIPKRKKR/GLF	4	2	CY025084	
					PEIPKKRKKR/GLF	5	3		
					PEIPKKKKKKR/GLF	6	4		
H7N7	HP	Australia	1985	A/chicken/Victoria/85	**PEIPKKREKR/GLF**	4	3	CY025069	10,20
H7N7	HP	Germany	1979	A/chicken/Leipzig/79	**PEIPKKKKR/GLF**	4	2	U20459.1	21
				A/goose/Leipzig/137-8/79	**PEIPKRKKR/GLF**	4	2	L43913.1	
				A/goose/Leipzig/192-7/79	**PEIPKKRKKR/GLF**	5	3	L43915.1	
				A/goose/Leipzig/187-7/79	**PEIPKKKKKR/GLF**	6	4	L43914.1	
H7N7	HP	Australia	1976	A/chicken/Victoria/76	**PEIPKKREKR/GLF**	4	3	CY024786	10,20
H7N3	HP	England	1963	A/turkey/England/63	**PETPKRRRR/GLF**	4	2	AF202238	1,10,14

[1] LP = low pathogenic, HP = highly pathogenic as determined by IVPI.
[2] Cleavage site between HA1 and HA2; red color indicates critical basic residues; residue insertions are underlined.
[3] Number of basic residue at immediately preceding and including the -1 position; excludes the -4 position found in the LPAI cleavage site consensus motif.
[4] GenBank or GISAID record number
[5] This cleavage site is presumed HP, but awaits confirmation by IVPI testing in chickens

Table 4. Unusual, 2-3 basic residue multi-basic cleavage sites with variable LP and HP phenotypes

Sub-type	Pheno-type[1]	location	year	Type virus	cleavage site[2]	Critical basic aa[3]	Size of insert	Accession number[4]	Ref.
H5	LP				PQRETR/GLF	1	0		39
H5N1 **H5N2** **H5N9**	HP	France	2015	A/chicken/France/150169a/15 A/duck/France/150233/15 A/duck/France/150236/15	**HQRRKR/GLF**	3	0	KU310447.1 KX014878.1 KX014886.1	
H5N2	HP	Taiwan	2012	A/chicken/Taiwan/A1997/12	**PQRRKR/GLF**	3	0	KF193394.1	40
H5N2	HP	Taiwan	2008	A/chicken/Taiwan/K703-1/08	**PQRKKR/GLF**[5]	3	0	AB507264.1	34,35
H5N2	LP	USA	2004	A/chicken/Texas/298313/04	**PQRKKR/GLF**[6]	3	0	AY849793.1	6
	LP	Taiwan	2003	A/chicken/Taiwan/1209/03	**PQREKR/GLF**	2	0	AY573917.1	34,35
H5N2	LP/HP	USA	1983	A/chicken/PA/1370/83	**PQKKKR/GLF**[7]	3	0	CY107848.1	6,10,37
H5N1	HP	Scotland	1959	A/chicken/Scotland/59	**PQRKKR/GLF**[8]	3	0	GU052518.1	6
H7	LP				PEIPKGR/GLF	1	0		38
H7N1	LP	UAE	2004	A/houbara bustard/UAE/2004	**PELPKRR/GLF**	2	0		APHA-UK
H7N3	HP	Pakistan	1995	A/chicken/Pakistan/16/99/95	**PETPKRRNR/GLF**	3	2	AF202233	2
H7N7	HP	England	1979	A/turkey/England/199/79	**PEIPKKREK/GLF**	3	2		1,9,14
H7Nx	LP	Australia	1976	A/duck/Victoria/76	**PEIPKKR/GLF**	2	0	U20463.1	33

[1] LP = low pathogenic, HP = highly pathogenic as determined by IVPI. Where both are indicated, age of chickens affected IVPI score or presence/absence of a glycosylation site.
[2] Cleavage site between HA1 and HA2; red color indicates critical basic residues; residue insertions are underlined.
[3] Number of basic residue at immediately preceding and including the -1 position; excludes the -4 position found in the LPAI cleavage site consensus motif.
[4] GenBank record number
[5] Based on OIE mandated 6 week-old chickens, the IVPI = 1.86 and virus was declared HP. However, in 8 week-old chickens the IVPI = 0.89.
[6] LP in 4 week-old chickens (0/8). Parent virus had putative glycosylation site at position 11-13 (NST). Was declare HP based on HA cleavage site sequence similarity to A/chicken/Scotland/1959
[7] Required loss of putative glycosylation at position 11-13 (NSK) for highly pathogenic phenotype
[8] Lack of putative glycosylation site at position 11-13 (KST)

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