Highly Pathogenic Avian Influenza Subtype H7N7 Italy, August 2013

Laboratory and molecular findings

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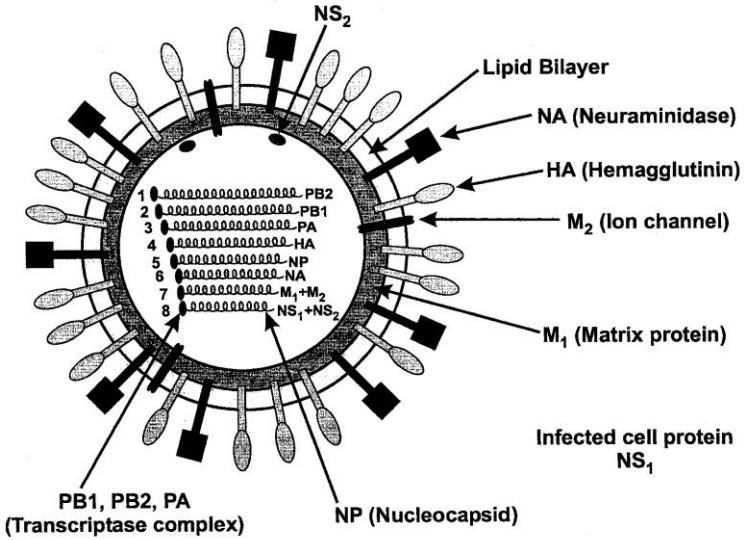
FAO Reference Centre for animal influenza and Newcastle disease

Avian Influenza - introduction

- Family *Orthomyxoviridae*
- Enveloped, negative stranded RNA viruses
- Segmented genome
- Genera *Influenzavirus A*
- Classified in 17 and 9 subtypes on the basis of the haemagglutinin (HA) and neuraminidase (NA) antigens, respectively



Avian Influenza - introduction

- In birds it causes two distinct diseases on the basis of the clinical signs determined in susceptible birds :
  - Highly Pathogenic AI (**HPAI**)
  - Low Pathogenicity AI (**LPAI**)

- LPAI is caused by viruses belonging to all H subtypes, and is a mild disease of poultry

- HPAI is caused only by some viruses of the H5 and H7 subtypes, and is an acute and severe disease of poultry

HPAI outbreak in Italy – August 2013
Laboratory findings

Virus Haemagglutinin (HA) & Neuraminidase (NA) typing

Based on molecular (RT-PCR) and serological (HA/HI/NI) methods
HA = **H7** NA = **N7**

Pathotyping

Molecular methods (Sanger sequencing)

PKRKRR*G
PKRRERR*G

Phenotypic test

IVPI = 3

HPAI outbreak in Italy – August 2013
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Serology

Haemagglutination inhibition (HI) test according to standard methods (EU AI Diagnostic Manual).

Antigens used in the HI test
(provided by the EU-RL AHVLA, UK)

A/African starling/England/983/79 (H7N1) *
A/turkey/England/647/77 (H7N7)

** Preliminary tests indicate that this antigen provides results equivalent to the homologous antigen (H7N7)*

HPAI outbreak in Italy – 13th of August and 3rd of September 2013

Phylogeny – HA gene

All the viruses identified so far cluster together and with:

•LPAI H7 viruses collected from wild birds in Belgium, Poland, the Czech Republic and Italy from 2009 to 2013

•LPAI H7 viruses sporadically isolated from poultry in Northern Europe (The Netherlands and Germany) between 2010 and 2012 (similarity ranged from 97,5% to 98,4% for the HA gene and from 97,4% and 99,1% for the NA gene).

•Genetically similar to the H7 genome sequence revealed in a wild bird (Anas crecca) sampled in Southern Italy and submitted to IZSVe in March 2013 (A/Anas crecca/Italy/13VIR1292-304/2013 – marked with a red asterisk in the HA phylogenetic tree). Isolation of this virus was unsuccessful at the time of submission and only a short partial sequence of the HA gene was obtained.

HPAI outbreak in Italy
Whole genome analysis

Viruses showing the highest similarity with the virus A/chicken/Italy/13VIR4527-11/2013 causing the first outbreak and selected as representative:
(A/Anas crecca/Italy/13VIR1292-304/2013 virus is not included)

Gene	Virus	Subtypte	Nt identity
HA	A/ck/Netherlands/12014794/2012	H7N7	98.4%
NA	A/ck/Netherlands/12014794/2012	H7N7	99.1%
PB2	A/mallard/France/090360/2009	H9N2	97.9%
PB1	A/shoveler/Egypt/101574-NAMRU3/07	H10N4	98.2%
PA	A/wild duck/Mongolia/1-241/2008	H7N9	97.2%
NP	A/mallard/Czech Repubblic/14333-1K/2011	H3N8	99.1%
M	A/mallard/Germany/R193/09	H1N1	99.3%
NS	A/mallard/Sweden/2009	H6N2	99.1%

Number of nucleotide differences between the seven Italian HPAI H7N7 collected between 13th of August and 3rd of September 2013

Num. of substitutions

Sample number	Farm code	Location	Collection date	N. of nt differences compared to the index case							
				PB2	PB1	PA	HA	NP	NA	M	NS
13VIR4527-11*	017FE030	Ostellato (FE)	13 August								
13VIR4541-34	017FE030	Ostellato (FE)	13 Aug	1	1	1	2	-	1	-	
13VIR4603	045BO040	Mordano (BO)	19 August	1	1	-	-	-	2	2	-
13VIR4678-1	019FE078	Portomaggiore (FE)	21 August	2	1	-	-	1	2	1	-
13VIR4774	045BO042	Mordano (BO)	27 August	2	3	-	1	-	1	-	-
13VIR5091-1	NA	Bondeno (FE)	2 September	3	2	-	-	1	2	2	-
13VIR5051-3	045BO044	Mordano (BO)	3 September	1	1	1	-	-	1	-	-

Nucleotide substitution: position and type

Segment	1 (PB2)				2 (PB1)				3 (PA)		4 (HA)			5 (NP)	6 (NA)		7 (M)		
Position	232	279	333	1044	183	1215	1882	2133	9	1251	471	1018	1410	72	541	1347	573	818	884
A/chicken/Italy/13VIR4527-11/2013*	C	T	T	C	C	T	C	C	C	G	A	G	G	G	T	A	G	A	G
A/chicken/Italy/13VIR4541-34/2013*	T	.	.	.	.	.	.	T	t	.	G	A	.	.	A	.	.	R	.
A/chicken/Italy/13VIR4603/2013	T	.	.	.	.	.	.	T	.	.	.	.	.	.	A	G	A	G	.
A/turkey/Italy/13VIR4678-1/2013	T	.	.	T	.	.	.	T	.	.	.	.	.	A	A	G	.	G	.
A/chicken/Italy/13VIR4774/2013	T	.	C	.	.	G	T	T	.	.	.	.	A	.	A	.	.	.	.
A/chicken/Italy/13VIR5091-1/2013	T	C	.	T	T	.	.	T	na	.	.	.	.	A	A	G	.	G	A
A/chicken/Italy/13VIR5051-3/2013	T	.	.	na	.	.	.	T	.	A	.	.	.	.	A	.	.	.	.

* Index case

. Sequence identical to the index case (13VIR4527-11)

- Nucleotide differences in 19 positions were identified in the eight gene segments
- The sequences of **ALL** the viruses analysed to date are **closely related between each other** with nucleotide similarity ranging from **99.8% to 100%** for all the gene segments
- The virus from backyard (13VIR5091-1; identified in red in the table above) showed the highest similarity with the sample 13VIR4678-1 (nucleotide similarity ranged between 99.9% and 100%)

Number of amino acid differences between the seven Italian HPAI H7N7 collected between 13th of August and 3rd of September 2013

Num. of aa substitutions

Sample number	Farm code	Location	Collection date	N. aa differences compared to the index case							
				PB2	PB1	PA	HA	NP	NA	M	NS
13VIR4527-11*	017FE030	Ostellato (FE)	13 August								
13VIR4541-34*	017FE030	Ostellato (FE)	13 Aug	1	-	-	1+ins	-	1	-	
13VIR4603	045BO040	Mordano (BO)	19 August	1	-	-	-	-	1	1§	-
13VIR4678-1	019FE078	Portomaggiore (FE)	21 August	1	-	-	-	-	1	1§	-
13VIR4774	045BO042	Mordano (BO)	27 August	1	-	-	-	-	1	-	-
13VIR5091-1	NA	Bondeno (FE)	2 September	1	1^	-	-	-	1	2§	-
13VIR5051-3	045BO044	Mordano (BO)	3 September	1	-	-	-	-	1	-	-

*index case
§ M2
^ PB1-F2

AA substitution: position and type

Protein	PB2	PB1-F2	HA	NA	M2	
Position	78	30	340	181	44	66
A/chicken/Italy/13VIR4527-11/2013*	R	P	E	S	N	E
A/chicken/Italy/13VIR4541-34/2013*	W	.	K	T	.	.
A/chicken/Italy/13VIR4603/2013	W	.	.	T	D	.
A/turkey/Italy/13VIR4678-1/2013	W	.	.	T	D	.
A/chicken/Italy/13VIR4774/2013	W	.	.	T	.	.
A/chicken/Italy/13VIR5091-1/2013	W	L	.	T	D	K
A/chicken/Italy/13VIR5051-3/2013	W	.	.	T	.	.

- Amino acid differences in 6 positions were identified in the eight gene segments.
- The virus from backyard (13VIR5091-1; identified in red in the table above) showed two unique amino acid substitutions in the PB1-F2 and M2 proteins compared to previously detected viruses. None of these mutations have been previously described as molecular markers for H7 adaptation to poultry

Molecular features

- Multiple basic amino acid motif at the HA cleavage site: PKRRERR*G or PKRKRR*G
- Known molecular markers for H7 adaptation to poultry (e.g. additional glycosylation sites in the HA or stalk deletion in the NA) were not detected in the six samples.
- No mutations associated with resistance towards adamantanes and neuraminidase inhibitors
- No mutations associated with an increased virulence or modified host range (i.e. PB2 627K, PB2 701N, NS1 92E)
- No amino acid signatures of human influenza viruses were identified (Chen and Shih, EID 2009; Miotto et al., PlosOne 2010)
- No amino acid signatures of the novel influenza H7N9 virus that causes an outbreak in humans in China were observed (Liu et al., Microbes and Infection 2013)

HA gene

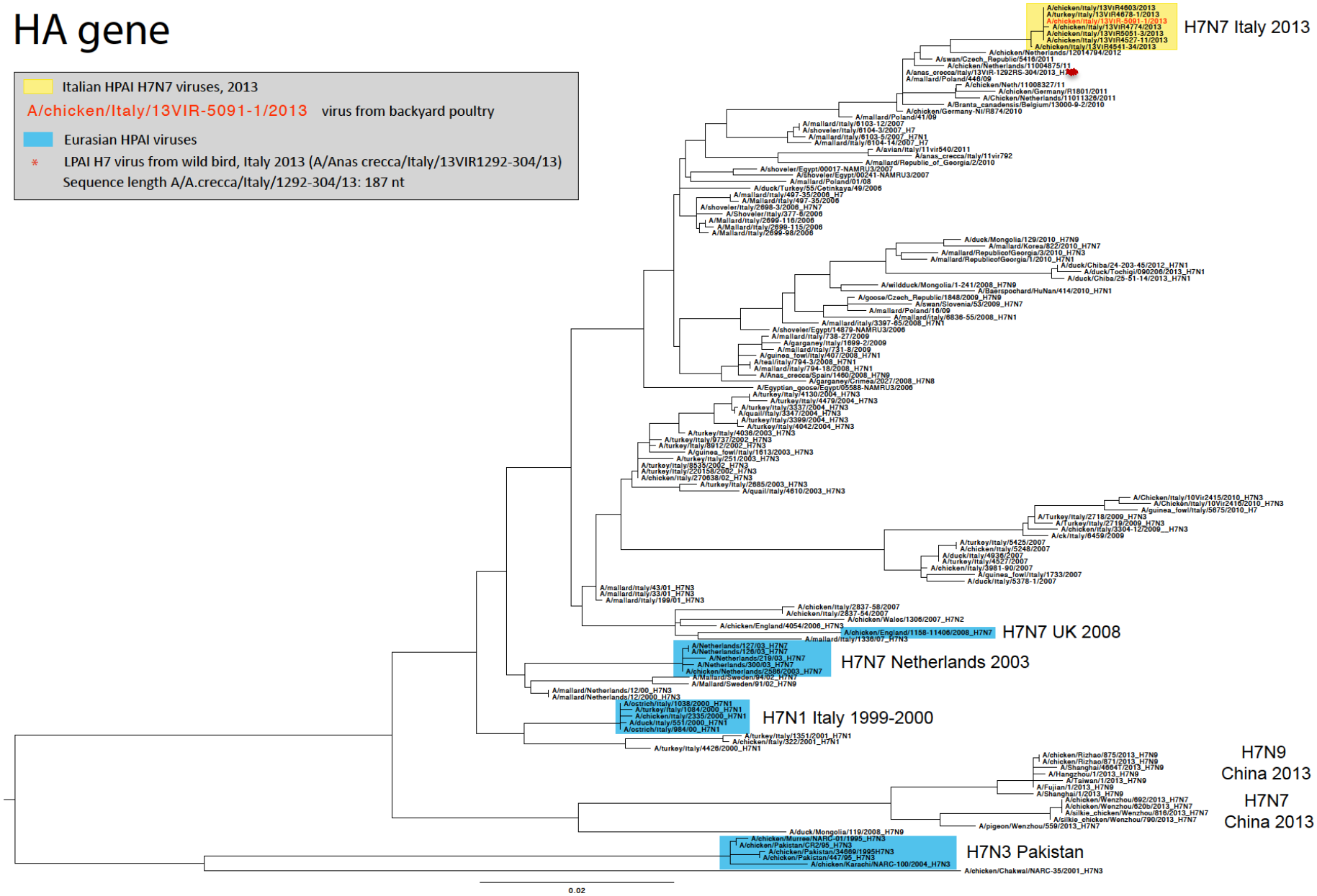
Italian HPAI H7N7 viruses, 2013

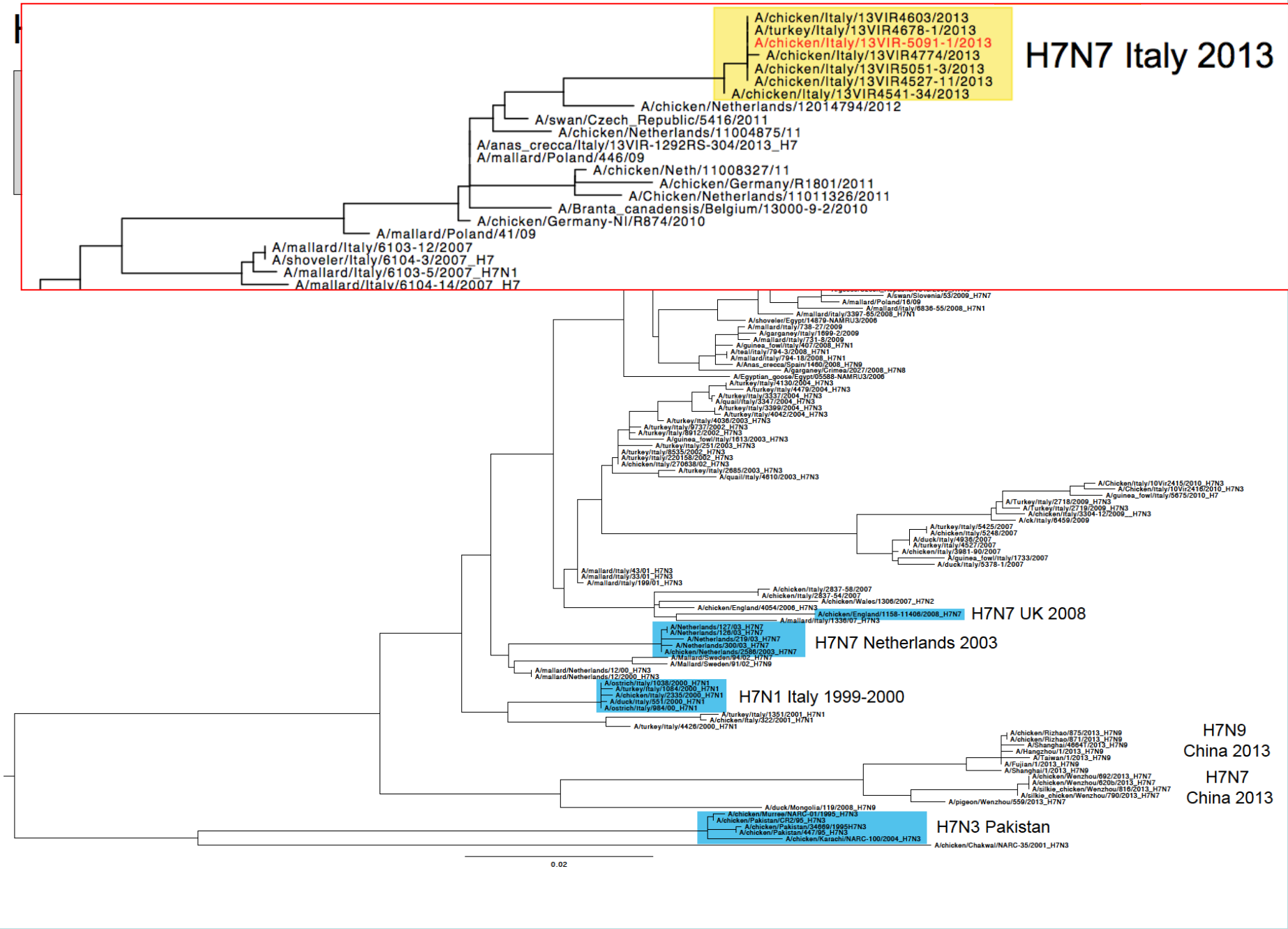
A/chicken/Italy/13VIR-5091-1/2013 virus from backyard poultry

Eurasian HPAI viruses

* LPAI H7 virus from wild bird, Italy 2013 (A/Anas crecca/Italy/13VIR1292-304/13)

Sequence length A/A.crecca/Italy/1292-304/13: 187 nt




A/chicken/Italy/13VIR4603/2013
A/turkey/Italy/13VIR4678-1/2013
A/chicken/Italy/13VIR-5091-1/2013
A/chicken/Italy/13VIR4774/2013
A/chicken/Italy/13VIR5051-3/2013
A/chicken/Italy/13VIR4527-11/2013
A/chicken/Italy/13VIR4541-34/2013
H7N7 Italy 2013
A/chicken/Netherlands/12014794/2012
A/swan/Czech_Republic/5416/2011
A/chicken/Netherlands/11004875/11
A/anas_crecca/Italy/13VIR-1292RS-304/2013_H7
A/mallard/Poland/446/09
A/chicken/Neth/11008327/11
A/chicken/Germany/R1801/2011
A/Chicken/Netherlands/11011326/2011
A/Branta_canadensis/Belgium/13000-9-2/2010
A/chicken/Germany-NI/R874/2010
A/mallard/Poland/41/09
A/mallard/Italy/6103-12/2007
A/shoveler/Italy/6104-3/2007_H7
A/mallard/Italy/6103-5/2007_H7N1
A/mallard/Italy/6104-14/2007_H7
A/swan/Slovenia/53/2009_H7N7
A/mallard/Poland/16/09
A/mallard/italy/6836-55/2008_H7N1
A/mallard/italy/3397-65/2008_H7N1
A/shoveler/Egypt/14879-NAMRU3/2006
A/mallard/Italy/738-27/2009
A/garganey/Italy/1699-2/2009
A/mallard/Italy/731-8/2009
A/guinea_fowl/Italy/407/2008_H7N1
A/teal/Italy/794-3/2008_H7N1
A/mallard/italy/794-18/2008_H7N1
A/Anas_crecca/Spain/1460/2008_H7N9
A/garganey/Crimea/2027/2008_H7N8
A/Egyptian_goose/Egypt/05588-NAMRU3/2006
A/turkey/Italy/4130/2004_H7N3
A/turkey/Italy/4479/2004_H7N3
A/turkey/Italy/3337/2004_H7N3
A/quail/Italy/3347/2004_H7N3
A/turkey/Italy/3399/2004_H7N3
A/turkey/Italy/4042/2004_H7N3
A/turkey/Italy/4036/2003_H7N3
A/turkey/Italy/9737/2002_H7N3
A/turkey/Italy/8912/2002_H7N3
A/guinea_fowl/Italy/1613/2003_H7N3
A/turkey/Italy/251/2003_H7N3
A/turkey/Italy/8535/2002_H7N3
A/turkey/Italy/220158/2002_H7N3
A/chicken/Italy/270638/02_H7N3
A/turkey/Italy/2685/2003_H7N3
A/quail/Italy/4610/2003_H7N3
A/Chicken/Italy/10Vir2415/2010_H7N3
A/Chicken/Italy/10Vir2416/2010_H7N3
A/guinea_fowl/Italy/5675/2010_H7
A/Turkey/Italy/2718/2009_H7N3
A/Turkey/Italy/2719/2009_H7N3
A/chicken/Italy/3304-12/2009__H7N3
A/ck/Italy/6459/2009
A/turkey/Italy/5425/2007
A/chicken/Italy/5248/2007
A/duck/Italy/4936/2007
A/turkey/Italy/4527/2007
A/chicken/Italy/3981-90/2007
A/guinea_fowl/Italy/1733/2007
A/duck/Italy/5378-1/2007
A/mallard/Italy/43/01_H7N3
A/mallard/Italy/33/01_H7N3
A/mallard/Italy/199/01_H7N3
A/chicken/Italy/2837-58/2007
A/chicken/Italy/2837-54/2007
A/chicken/Wales/1306/2007_H7N2
A/chicken/England/4054/2006_H7N3
A/chicken/England/1158-11408/2008_H7N7
H7N7 UK 2008
A/mallard/Italy/1336/07_H7N3
A/Netherlands/127/03_H7N7
A/Netherlands/126/03_H7N7
A/Netherlands/219/03_H7N7
A/Netherlands/300/03_H7N7
A/chicken/Netherlands/2586/2003_H7N7
A/Mallard/Sweden/94/02_H7N7
A/Mallard/Sweden/91/02_H7N9
H7N7 Netherlands 2003
A/mallard/Netherlands/12/00_H7N3
A/mallard/Netherlands/12/2000_H7N3
A/ostrich/Italy/1038/2000_H7N1
A/turkey/Italy/1084/2000_H7N1
A/chicken/Italy/2335/2000_H7N1
A/duck/Italy/551/2000_H7N1
A/ostrich/Italy/984/00_H7N1
H7N1 Italy 1999-2000
A/turkey/Italy/1351/2001_H7N1
A/chicken/Italy/322/2001_H7N1
A/turkey/Italy/4426/2000_H7N1
A/chicken/Rizhao/875/2013_H7N9
A/chicken/Rizhao/871/2013_H7N9
A/Shanghai/4664T/2013_H7N9
A/Hangzhou/1/2013_H7N9
A/Taiwan/1/2013_H7N9
A/Fujian/1/2013_H7N9
A/Shanghai/1/2013_H7N9
H7N9
China 2013
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A/chicken/Wenzhou/820b/2013_H7N7
A/silkie_chicken/Wenzhou/816/2013_H7N7
A/silkie_chicken/Wenzhou/790/2013_H7N7
A/pigeon/Wenzhou/559/2013_H7N7
H7N7
China 2013
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A/chicken/Murree/NARC-01/1995_H7N3
A/chicken/Pakistan/CR2/95_H7N3
A/chicken/Pakistan/34569/1995H7N3
A/chicken/Pakistan/447/95_H7N3
A/chicken/Karachi/NARC-100/2004_H7N3
H7N3 Pakistan
A/chicken/Chakwal/NARC-35/2001_H7N3
0.02

NA gene

Italian HPAI H7N7 viruses, 2013

A/chicken/Italy/13VIR-5091-1/2013 virus from backyard poultry