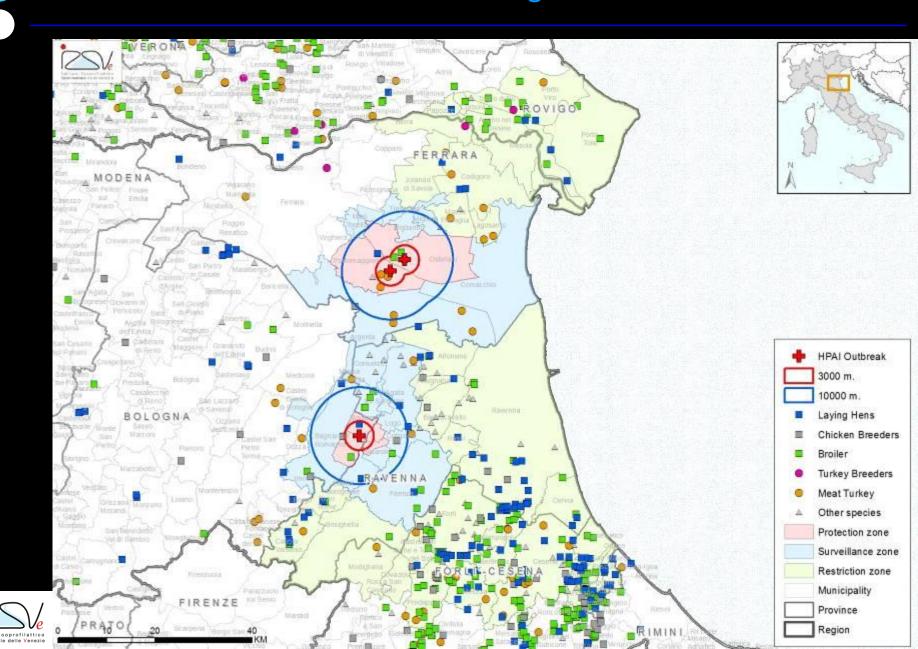
H7N7 HPAI outbreaks in Italy

Updated to 9 September 2013

Istituto Zooprofilattico Sperimentale delle Venezie,
National and OIE Reference Laboratory for avian influenza and Newcastle disease
FAO Reference Centre for animal influenza and Newcastle disease



ITALY – H7N7 AI OUTBREAKS 14-25 August 2013



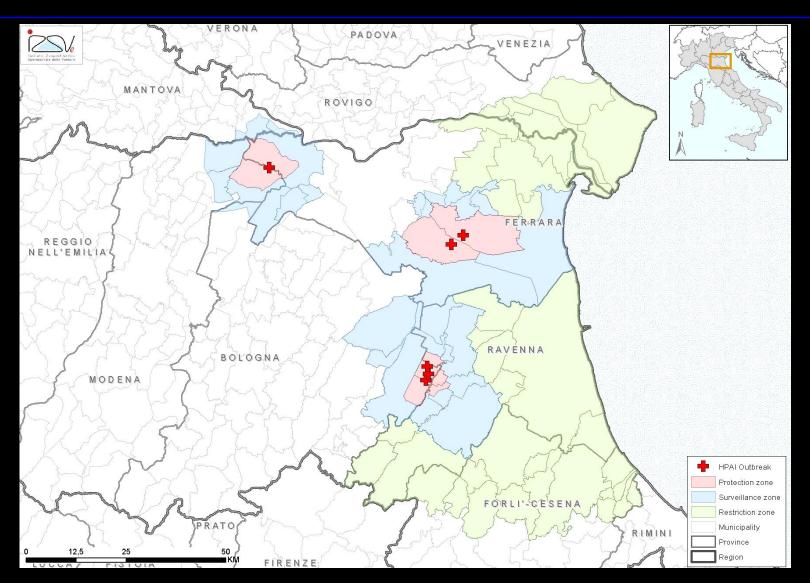


ITALY – H7N7 AI OUTBREAKS 14-25 August 2013

ADSN number	Region	Province	Holding type	N° of birds	Confirmation date	Depopulation date
2013/0001	Emilia - Romagna	Ferrara	Laying hen (Industrial farm)	128.000	14/08/2013	27/08/2013
2013/0002	Emilia - Romagna	Bologna	Laying hen (Industrial farm)	584.900	21/08/2013	08/09/2013
2013/0003	Emilia - Romagna	Ferrara	Meat turkey (Industrial farm)	19.850	23/08/2013	27/08/2013



ITALY - H7N7 AI OUTBREAKS 14 August - 9 September 2013





Fourth outbreak – Farm characteristics

- Layer holding located in Mordano municipality, Bologna province, belonging to the same poultry company
- A packaging centre that before 21 August received table eggs from 3 layer farms of the same vertically integrated group
- Located in the Protection Zone of the second outbreak
- Number of birds: 121.705 laying hens (5 sheds)
- Restriction measures enforced on 27/08/2013
- Protection and surveillance zones established on 28/08/2013
- Depopulation completed on 08/09/2013



4th H7N7 HPAI outbreak - Chronology

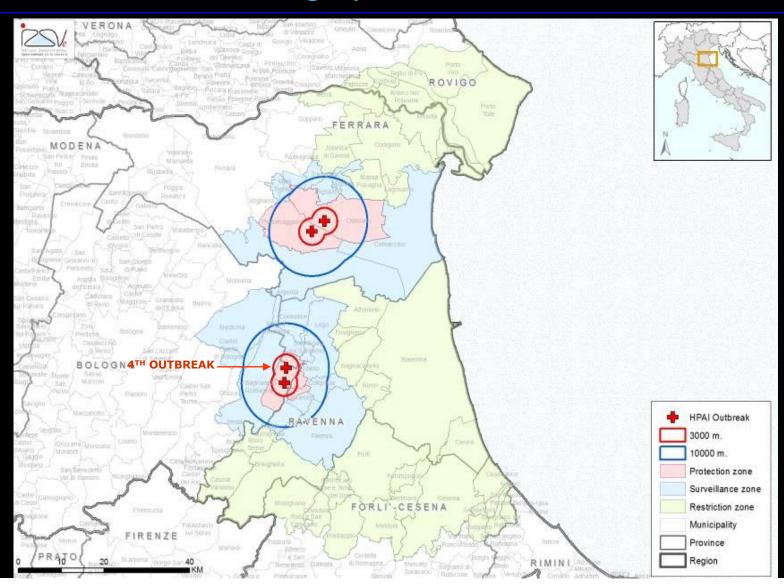
23 August – Negative AI tests carried out on 30 samples per shed

27 August - Increased mortality rate in shed number 5

28 August - H7N7 HPAI positive

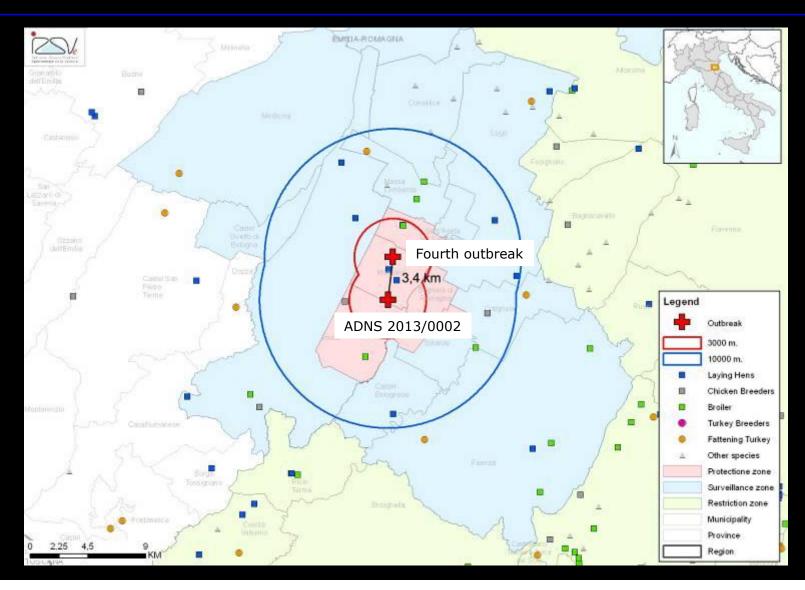


4th outbreak – Geographical location





4th outbreak – Proximity with the 2nd outbreak







4th outbreak - Results of epidemiological investigation

From 07/08/2013 to 21/08/2013 indirect contacts with the second infected farm (outbreak n. 2) of the same company:

- transport of manure



5th outbreak – Farm characteristics

- Pullet holding located in Mordano municipality, Bologna province, belonging to the same poultry company as the previous outbreaks in Mordano and Ostellato (outbreaks n. 1, 2, 4)
- Located in the Protection Zone between the 2nd and 4th outbreaks (about 1.5 Km from both farms)
- Number of birds: 98.200 pullets
- Depopulation completed on 8 September 2013



5th H7N7 HPAI outbreak - Chronology

22-23-24-26-28-30 August – Negative Al tests on carcasses

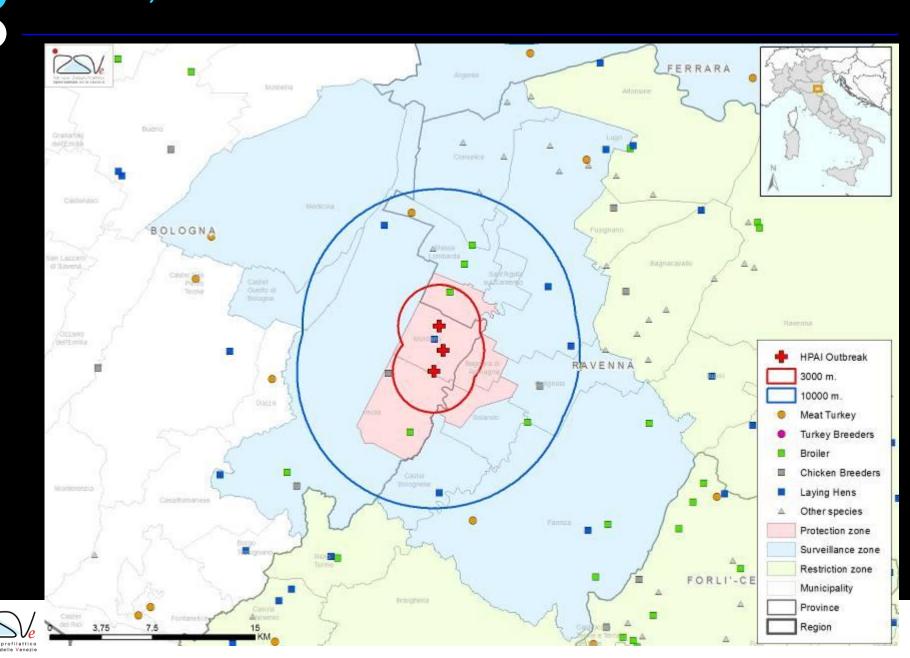
30 August - Pre-emptive killing planned

02 September - Increased mortality

04 September - H7N7 HPAI



Second, Fourth and Fifth outbreaks - Restriction zones

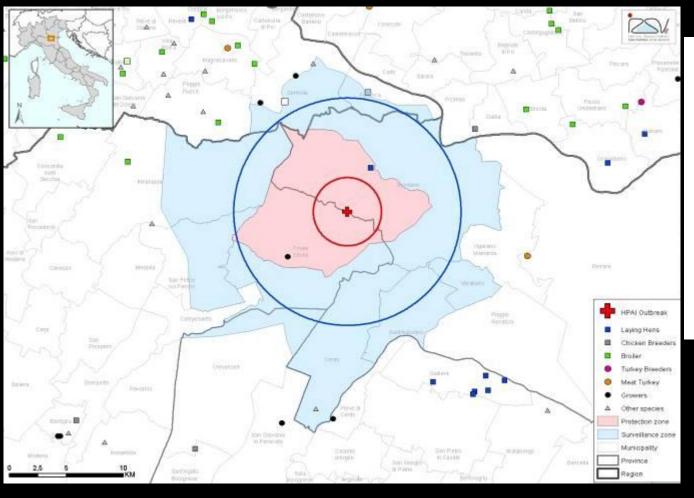


6th outbreak – Farm characteristics and chronology

- Rural free-range farm in Bondeno municipality, Ferrara province (Emilia Romagna Region)
- Number of birds : 6 layers
- 4 layers died starting from the last week of August
- 02/09/13: one carcass sent to IZSLER Ferrara
- 05/09/13: H7N7 HPAI positive
- Depopulation measures completed on 5 September 2013



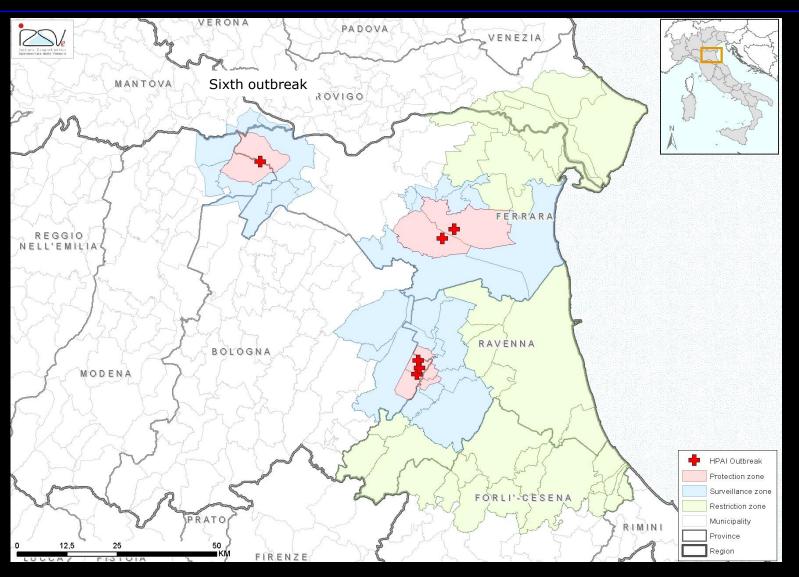
6th outbreak – Restriction zones



3 km radius	S
Type of holding	N. of poultry farms
Layer	1 (9.000 birds)
Dealer	1
10 km	
Type of	N. of poultry
holding	farms
Other	2



6th outbreak – Geographical location





Control measures – Grower and dealer holdings

- Due to the detection of H7N7 HPAI virus in a rural poultry farm (outbreak n. 6):
- Compulsory checks on dealers and growers in the all Emilia Romagna region have been established: every 15 days all farms should be virologically and serologically tested (20 swabs and 20 blood samples)
- Official checks on dealer and grower farms have been applied also in <u>DPPAs</u> in other Regions

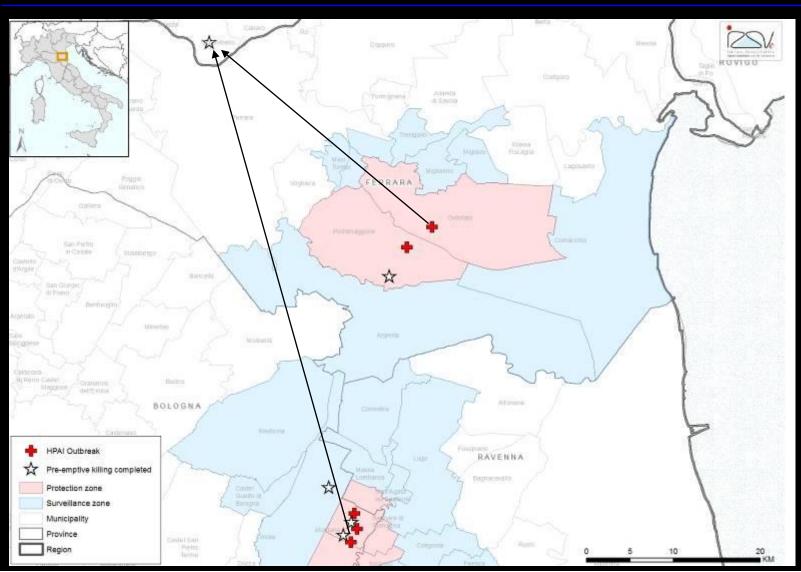


ITALY – H7N7 HPAI OUTBREAKS 26 August–9 September 2013

ADSN number	Region	Province	Holding type	N° of birds	Confirmation date	Depopulation date
2013/004	Emilia - Romagna	Bologna	Layers (Industrial farm)	121,705	28/08/2013	08/09/2013
2013/0005	Emilia - Romagna	Bologna	Pullets (Industrial farm)	98,200	04/09/2013	08/09/2013
2013/0006	Emilia - Romagna	Ferrara	Backyard flock	3	05/09/2013	05/09/2013



Pre-emptive killing applied on farms at risk





Pre-emptive killing – Farms stamped out

Farm	Province/R egion	Holding type	N° of birds	Reason	Date of Depopulation
033RO011	Occhiobello (RO) Veneto	Layers	220.063	Indirect contacts	29/08/2013
045BO037	Mordano (BO)/Emilia Romagna	Layers	1.043	Neighbourhood	09/09/2013
032BO255	Imola(BO)/Emil ia Romagna	Layers	83.000	Indirect contacts	08/09/2013
045BO001	Mordano (BO)/Emilia Romagna	Broilers	1.660	Neighbourhood	09/09/2013
019FE041	Portomaggiore (FE)/Emilia Romagna	Meat turkeys	65.000	Neighbourhood	01/09/2013



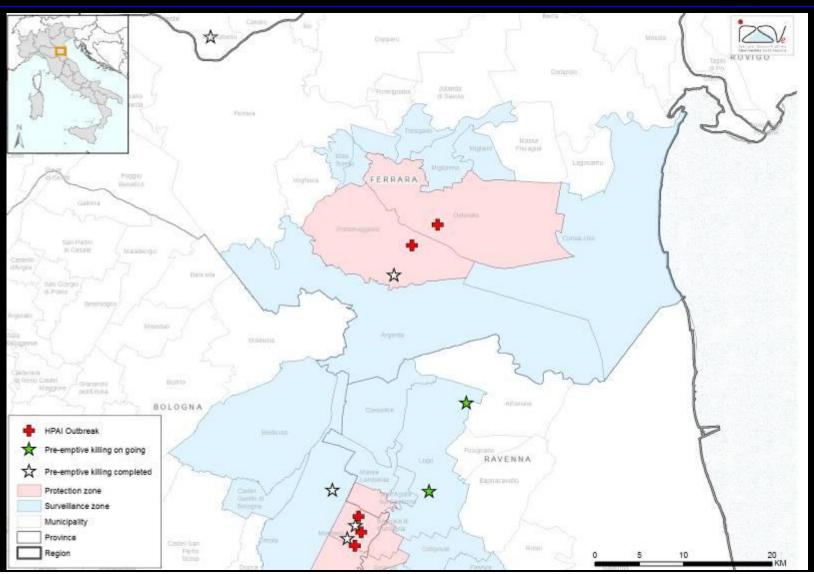


Further depopulation procedures

Pre-emptive killing of all birds in one layer and one pullet farm (contact farms) of the same vertically integrated company located in the municipality of Lugo, Ravenna province is on going



Further depopulation measures





• Fu

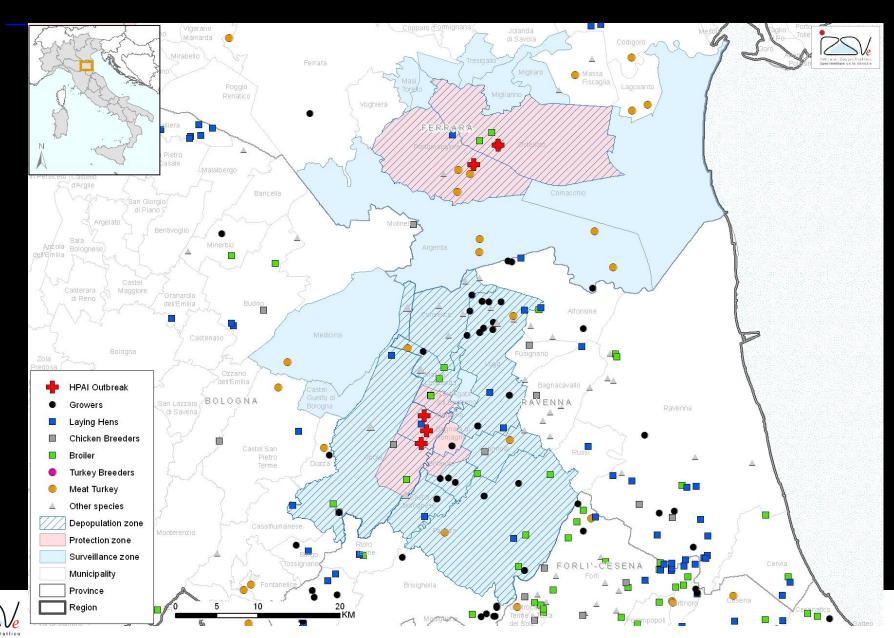
Further depopulation procedures

Depopulation of the following poultry farms have been planned:

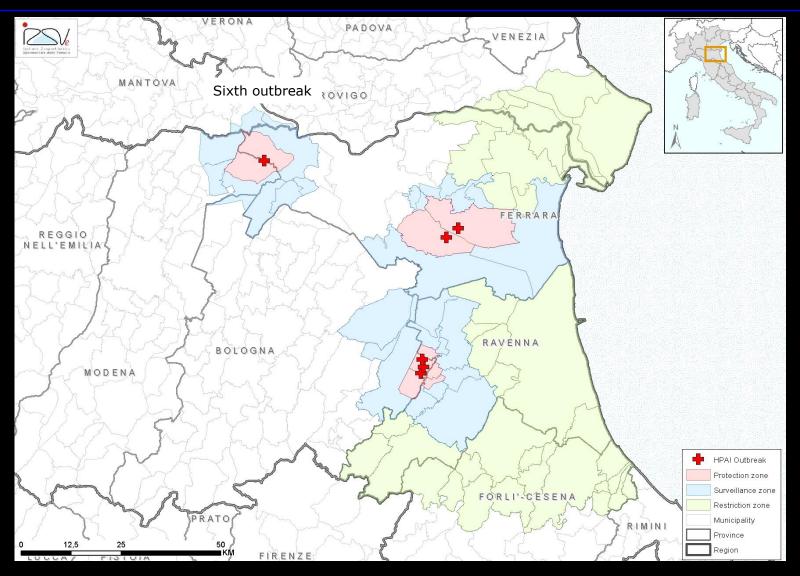
- All poultry farms, with the exception of one breeder farm (Gallus gallus), located in the:
 - ✓ protection zone of outbreaks n. 1 and 3,
 - ✓ protection zone of outbreaks n. 2, 4 and 5
- Poultry farms, with the exception of breeder farms, located in an area which includes all or part of the following municipalities: Castel Bolognese, Solarolo, Faenza, Cotignola, Lugo, Conselice, Imola and Massa Lombarda



Further depopulation measures



Restriction zones





Protection and surveillance zones

	Holding type	N° of farms	Farms controlled		Virological monitoring		Serological mo	onitoring	Carcasses		
	Holding type	N Of fairing	n	%	n° of inspections	n° of tests	n° of inspections	n° of test	n° of inspection	n° of carcasses	
Surveillance Zone	Layer	8	8	100,00%	13	960	13	960	6	38	
	Pullet	1	1	100,00%	4	240	4	240	4	16	
	Meat turkey	2	2	100,00%	5	980	5	980	0		
	Chicken breeder	3	3	100,00%	3	130	3	140	0		
	other species	26	26	100,00%	29	983	28	983	0		
Total		40	40	100,00%	54	3293	53	3303	10	54	
	Layer	5	5	100,00%	5	630	5	600	3	15	
	Pullet	1	1	100,00%					6	55	
Protection Zone	Meat turkey	2	2	100,00%	3	440	3	440	1	7	
_	Chicken breeder	1	1	100,00%	0		1	10	0		
	other species	5	5	100,00%	7	128	7	128	0	0	
Total		14	14	100,0%	15	1198	16	1178	10	77	





Further restricted zone - Art. 16 (4) 2005/94/EC

Based on art. 32 of Directive 2005/24/EC, some of the measures provided for in art. 7 (2) have been enforced on poultry farms located in the further restricted zone established adjacent to the surveillance and protection zones

The competent authority may grant derogations from the measures provided for movement of live poultry and poultry products

Layer, breeder and meat turkey holdings situated must be officially checked with the collection of 30 swabs and 30 blood samples per shed. The testing shall be repeated **after 21 days**



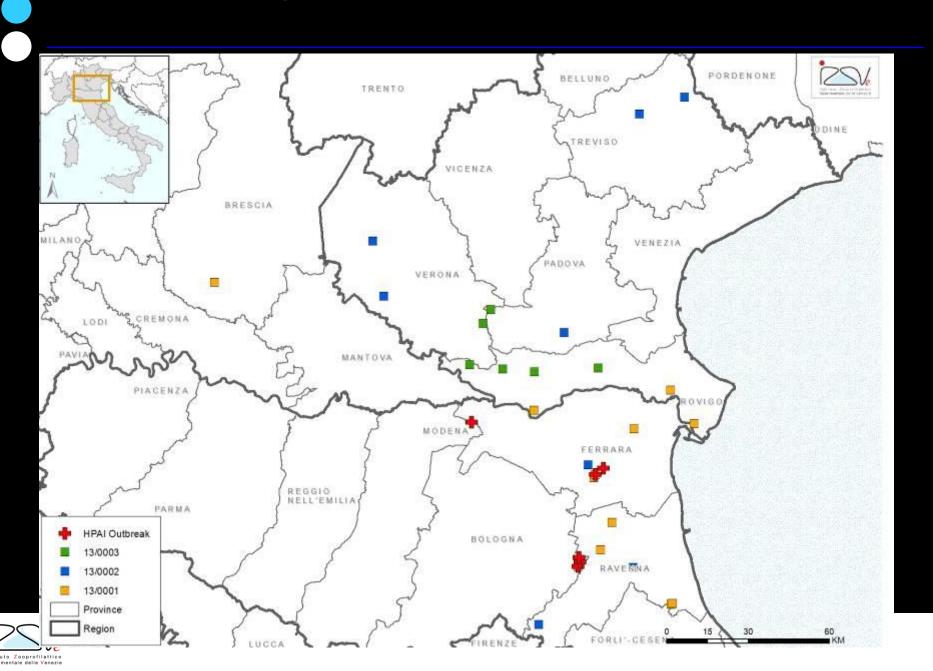


Monitoring measures – Further restricted zone

	Holding type	N° of farms	Farms controlled		Virological monitoring		Serological mo	onitoring	Carcasses		
	noiding type	N OI Iaiiiis	n	%	n° of inspections	n° of tests	n° of inspections	n° of test	n° of inspection	n° of carcasses	
	Layer	43	43	100,00%	72	4529	87	5415	24	323	
	Pullet	20	20	100,00%	45	2505	43	2165	5	40	
	Meat Turkey	18	18	100,00%	26	2242	27	2360	0		
Restriction Zone	Turkey	1	1	100,00%	1	120	1	120	0		
Nestriction Zone	Breeder	1	1	100,00%	1	120	1	120	U		
	Chicken	18	18	100,00%	22	2070	33	2262	0		
	breeder	10	10	100,00%	22	2070	33	2202	U		
	Other Species	53	53	100,00%	91	1795	85	1645	0		
Totale		153	153	100,00%	257	13261	276	13967	29	363	



Contact holdings



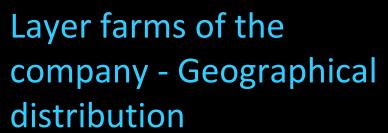
Control measures – Contact holdings

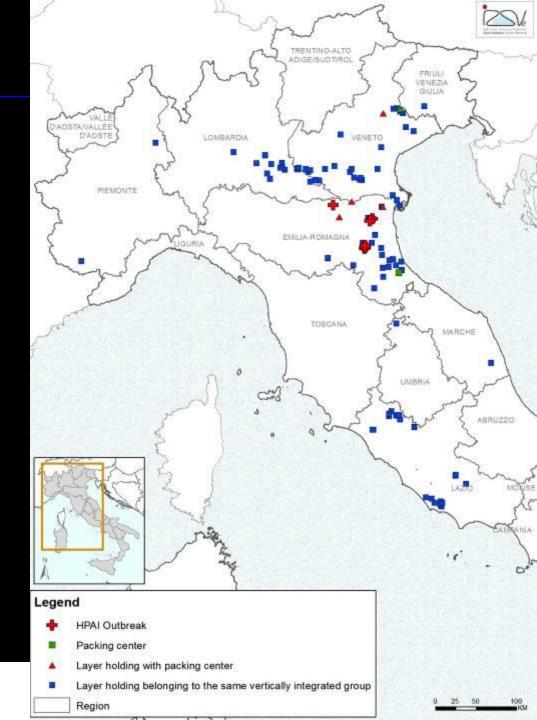
Some of the measures provided for in Article 7 (2) of Directive 2005/94/EC have been applied on contact holdings

All contact holdings have been officially checked with the collection of 30 swabs and 30 blood samples per shed and 15 dead hens per farm

On 9 September, <u>all the 21 contact farms</u> have been tested with <u>negative results</u>











Control measures – All farms of the company

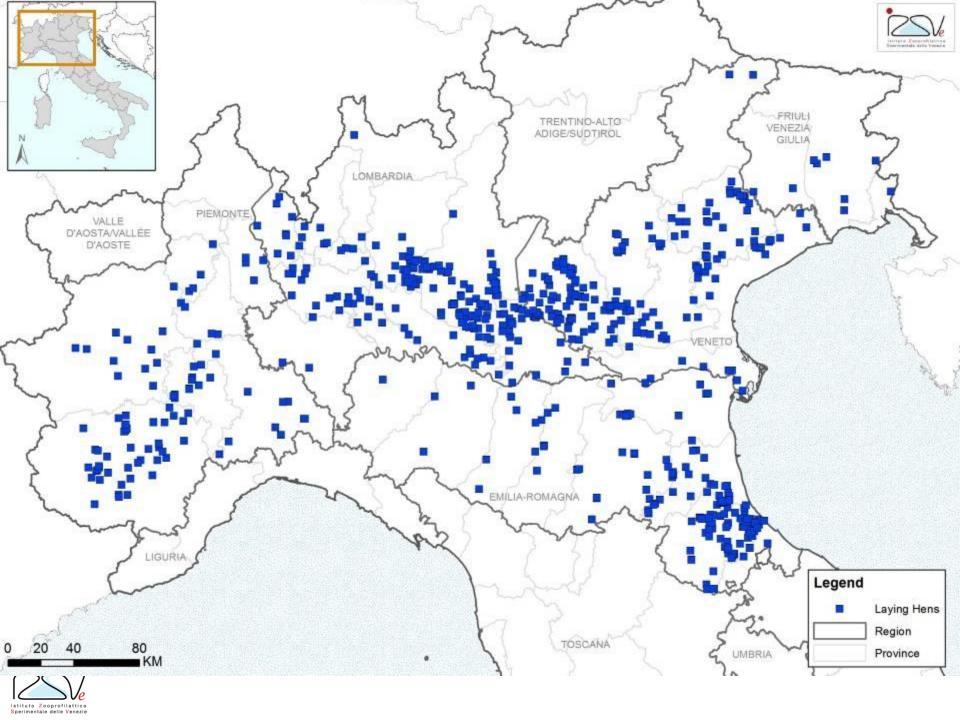
All the poultry (layer) holdings of the company have been officially checked <u>every week</u> with the collection of 30 swabs and 30 blood samples per shed and 15 dead hens per farm

Data on mortality rates, feed consumption and egg production have been transmitted to the competent authority

On 9 September, **86 layer farms** out of a total of 97* holdings have been tested with negative results

*Ownership of 11 farms located in Lazio Region is under scrutiny





Control measures – All layer farms in DPPA

All layer holdings situated in areas with a high density of poultry farms (DPPA) have been officially checked with the collection of 30 swabs and 30 blood samples per shed

In case of increased mortality at least 15 dead hens have been examined

Laboratory testing have been repeated **after 21 days**

On 9 September, 365 layer farms out of a total of 420 (86%) holdings have been tested with negative results



Characteristics of the H7N7 HPAI virus

Based on molecular (RT-PCR) and serological (HA/HI) methods

HA = H7 NA = N7
Pathotyping

Molecular methods (Sanger sequencing)

PKRKRR*G PKRRERR*G

Phenotypic test

IVPI = 3



Index case – Genomic characteristics of H7N7 virus strains

Number of nucleotide and amino acid differences between the two AI virus strains isolated in outbreak n. 1

	HA	NA	PB2	PB1	PA	NP	Σ	NS
Nucleotide differences	1+3 nt insertion	0	2	1	1	0	0	0
Amino acid differences	1+1 aa insertion*	0	2	0	0	0	0	0

^{*}both aa substitutions are in the HA cleavage site (PKRKRR*G; PKRRERR*G)

Viruses with distinct genetic characteristics were found in the same farm



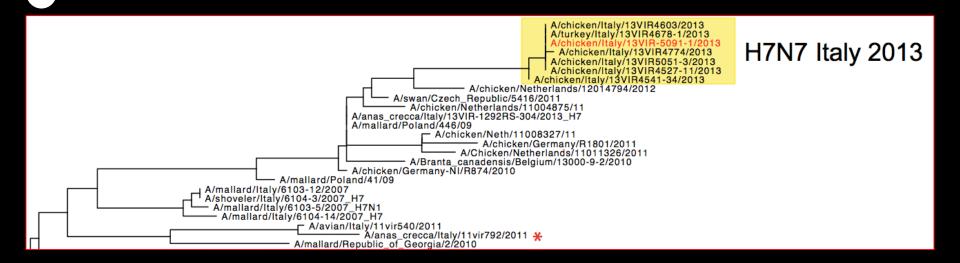
A/chicken/Italy/13VIR4603/2013 A/turkey/Italy/13VIR4678-1/2013 Archicken/taly/13ViF4676-7030 Archicken/taly/13ViF4676-7030 Archicken/taly/13ViF4774/2013 Archicken/taly/13ViF4774/2013 Archicken/taly/13ViF4774/2013 Archicken/taly/13ViF474/2013 Archicken/taly/13ViF4652-1172013 Archicken/taly/13ViF4652-1172013 Archicken/taly/13ViF4652-1172013 Archicken/taly/13ViF4762015 Archicken/taly/13ViF4762013 Arch HA gene H7N7 Italy 2013 A/mallard/flay/8103-1/2007 H7 Italian HPAI H7N7 viruses, 2013 A/chicken/Italy/13VIR-5091-1/2013 virus from backyard poultry **Eurasian HPAI viruses** Analladrika Amalladrika Amalla LPAI H7 virus from wild bird, Italy 2013 (A/Anas crecca/Italy/13VIR1292-304/13) A/shoveler/Egypt/00017-NAMRU3/2007 A/shoveler/Egypt/00017-NAMRU3/2007 A/shoveler/Egypt/00241-NAMRU3/2007 A/shoveler/Egypt/00241-NAMRU3/2007 A/mallard/flay/497-33/2006 A/mallard/flay/497-33/2006 A/shoveler/flay/371-9/2006 A/shoveler/flay/371-9/2006 A/Mallard/flay/2694-115/2006 A/Mallard/flay/2694-115/2006 A/Mallard/flay/2694-115/2006 A/Mallard/flay/2694-115/2006 Sequence length A/A.crecca/Italy/1292-304/13: 187 nt — Alduck/Mongolia/129/2010_H7N9 — Almailard/RepublicofGeorgia/12010_H7N7 — Almailard/RepublicofGeorgia/1/2010_H7N3 Almailard/RepublicofGeorgia/1/2010_H7N1 — Alduck/Chiba/24-203.45/2012_H7N1 — Alduck/Chiba/24-203.45/2012_H7N1 — Alduck/Chiba/25-51-14/2013_H7N1 A/Chikko/http://10/ir/2415/2010_H7N3 -/Chikko/http://10/ir/2415/2010_H7N3 -/Chikko/http://10/ir/2415/2010_H7N3 -/Chikko/http://10/ir/2415/2010_H7N3 -/Chikko/http://2115/2009_H7N3 -/Chikko/http://2115/2009_H7N3 -/Chikko/http://2115/2010_H7N3 -/Chikko/http:///Chikko/http://2115/20 A/turkey/italy/5425/2007 A/chicken/italy/5248/2007 A/duck/italy/4936/2007 A/duck/italy/4936/2007 A/chicken/italy/4936/2007 A/chicken/italy/3981-90/2007 A/duck/italy/5378-1/2007 A/chicken/Italy/2837-58/2007 A/chicken/Italy/2837-54/2007 A/chicken/Wales/1306/2007_H7N2 A/Netherlands/127/03_H7N7 A/Netherlands/127/03_H7N7 A/Netherlands/219/03_H7N7 A/Netherlands/300/03_H7N7 A/Netherlands/300/03_H7N7 A/Mallard/Sweden/94/02_H7N9 A/Mallard/Sweden/94/02_H7N9 H7N7 Netherlands 2003 A/mailard/Netherlands/12/00_H7N3 A/mailard/Netherlands/12/2000_H7N3 H7N1 Italy 1999-2000 A/turkey/Italy/1351/2001_H7N1 A/chicken/Italy/322/2001_H7N1 A/turkey/Italy/4428/2000_H7N1 H7N9 China 2013 H7N7 China 2013

A/duck/Mongolia/119/2008_H7N9
A/chicken/Murree/NARC-01/1995_H7N3
A/chicken/Pakistan/CR/295_H7N3
A/chicken/Pakistan/34699/1995H7N3
A/chicken/Pakistan/44795_H7N3
A/chicken/Pakistan/44795_H7N3

H7N3 Pakistan



H7N7 HPAI viruses – Phylogenetic analyses



- The sequences of <u>ALL</u> the viruses 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) showed the highest similarity with the strain isolated in the infected meat turkey farm (outbreak n. 3) 13VIR4678-1 (nucleotide similarity ranged between 99.9% and 100%)



H7N7 HPAI viruses – 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 <u>not</u> detected in the six virus strains
- 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)
- <u>No amino acid signatures</u> of the novel influenza <u>H7N9</u> virus that causes an outbreak in humans in China were observed (Liu et al., Microbes and Infection 2013)



Preventive measures for depopulation crews

 All workers taking part to depopulation measures on infected premises applied strict prevention procedures and wore protective clothing (overalls, rubber gloves, boots, turboventilated masks with FP3 filter, etc.)







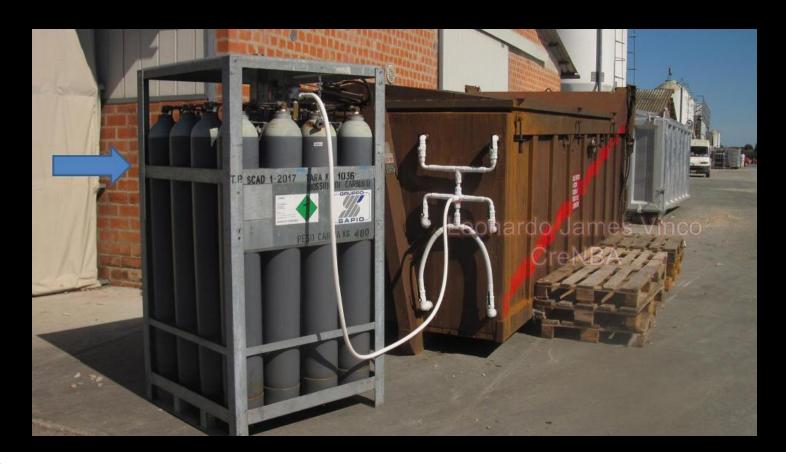
Human health implications

- They were regularly checked for symptoms of AI infection Three cases of H7N7 human infection were identified in three workers who took part to stamping out procedures on outbreaks n. 1 and 2:
- 28 August 2013 first human case of conjunctivitis
- 31 August 2013 second case of conjunctivitis and ILI
- 04 September third case of conjunctivitis
- No human to human transmission



Stamping out procedures – Use of containers

During the first two weeks live birds in the infected premises were killed in containers using CO₂ at a concentration of 70%







Stamping out procedures

After the first couple of weeks

- Birds were killed inside the poultry sheds using CO₂ at a concentration of at least 30%
- Dead birds were loaded in the containers and during loading continuous checks on the efficacy of the killing procedure were applied



Stamping out procedures



