

H7N7 HPAI outbreaks in Italy

14 - 25 August 2013

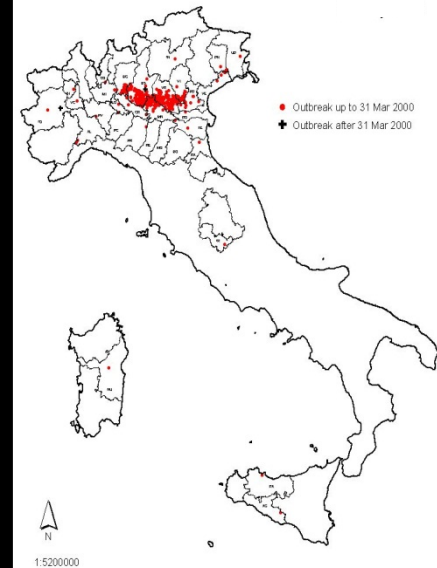
*Istituto Zooprofilattico Sperimentale delle Venezie,
National and OIE Reference Laboratory for avian influenza and Newcastle disease
FAO Reference Centre for animal influenza and Newcastle disease*

ITALY – AI OUTBREAKS FROM 1997 TO 2006

- 1997-1998 HPAI: H5N2 8 outbreaks, backyard flocks, prompt eradication
- 2000 LPAI-HPAI: H7N1, total costs € 500 million
- 2000-2001 LPAI: H7N1 78 outbreaks
- 2002-2003 LPAI: H7N3 388 outbreaks
- 2004 LPAI: H7N3 28 outbreaks (September-November)
- 2005 LPAI: H5N2 15 outbreaks (April-May)

Total direct costs: 170 million euros

HPAI (H7NI) Epidemic in Italy



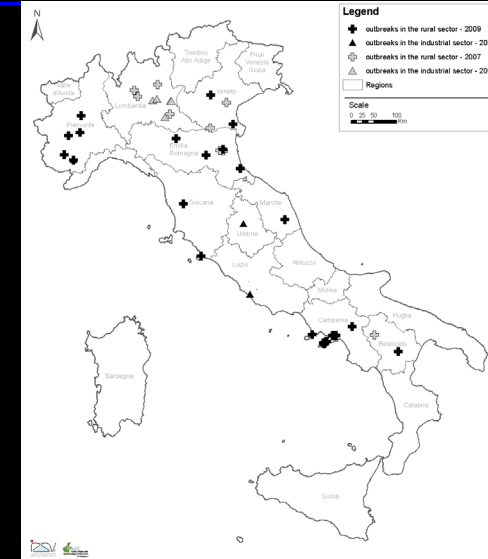


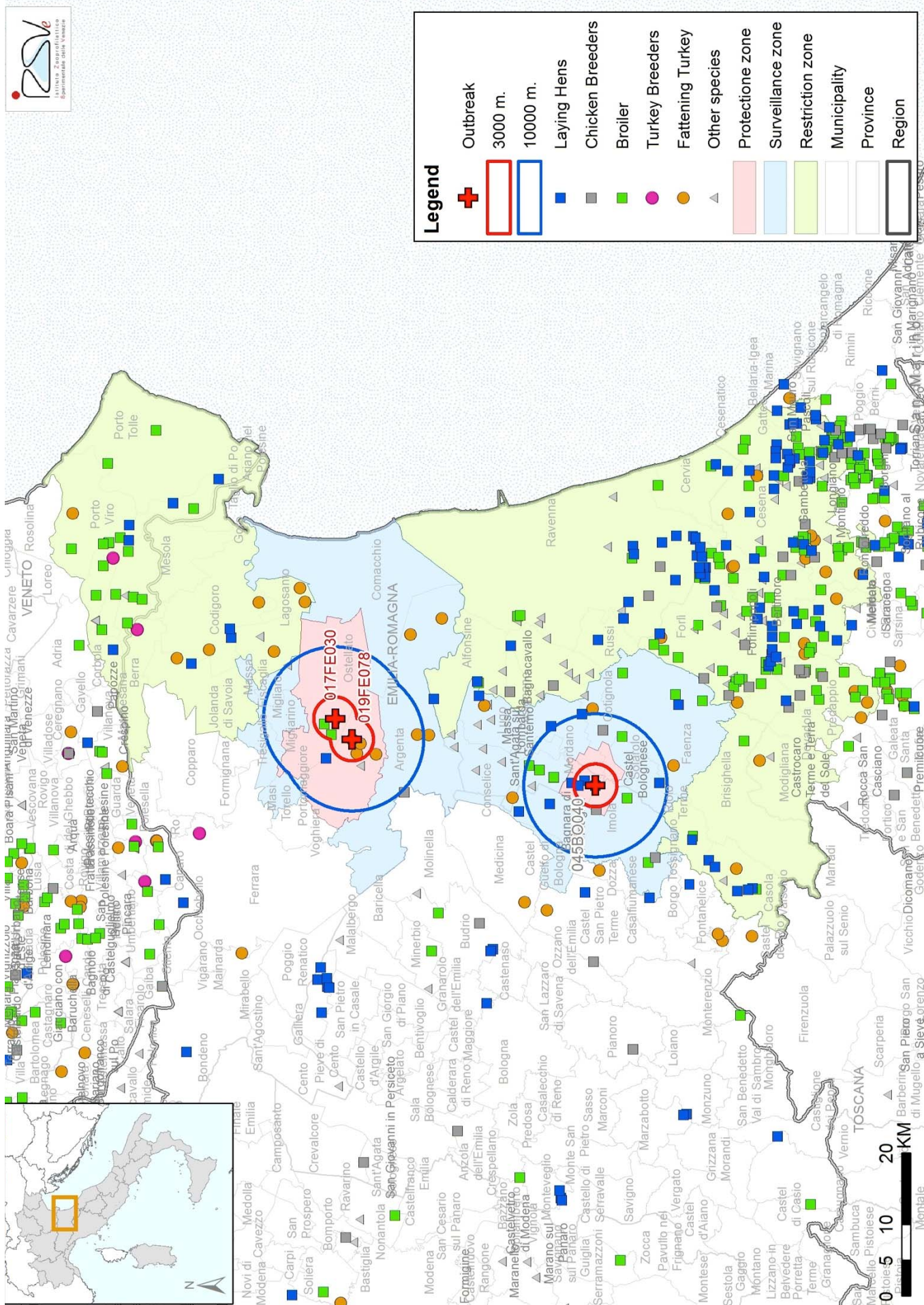
AI CHALLENGE IN NORTH-EASTERN ITALY

- Significant concentrations of highly susceptible species (turkeys) in a limited area (Densely Populated Poultry Area - DPPA)
- Wetlands and resting sites for migratory waterfowl in close proximity of this DPPA
- Multiple introductions of LPAI viruses from the wild reservoir resulting in some cases in major epidemics

ITALY – AI OUTBREAKS FROM 2007 TO 2012

- 2007-2008 LPAI: H7N3 19 outbreaks, in the rural sector and in a few meat turkey farms
- 2009-2011 LPAI: H7N3 51 outbreaks in the rural sector and in two meat turkey farms
- 2010-2012 LPAI: H5N2 27 outbreaks, in the rural sector and in 2012 also in 7 meat turkey farms in the DPPA





First H7N7 HPAI outbreak

Index case (Ostellato municipality – FE – Emilia Romagna Region)

10 August - Increased mortality rate particularly in shed 2 (AI suspected)

13 August – Official samples and carcasses sent IZSLER in Forlì

13 August - PCR AIV Type A positive at IZSLER

14 August – IZSve-NRL H7 PCR positive – Pathotype confirmed molecularly (HPAI), NA typing (N7) by RT-PCR

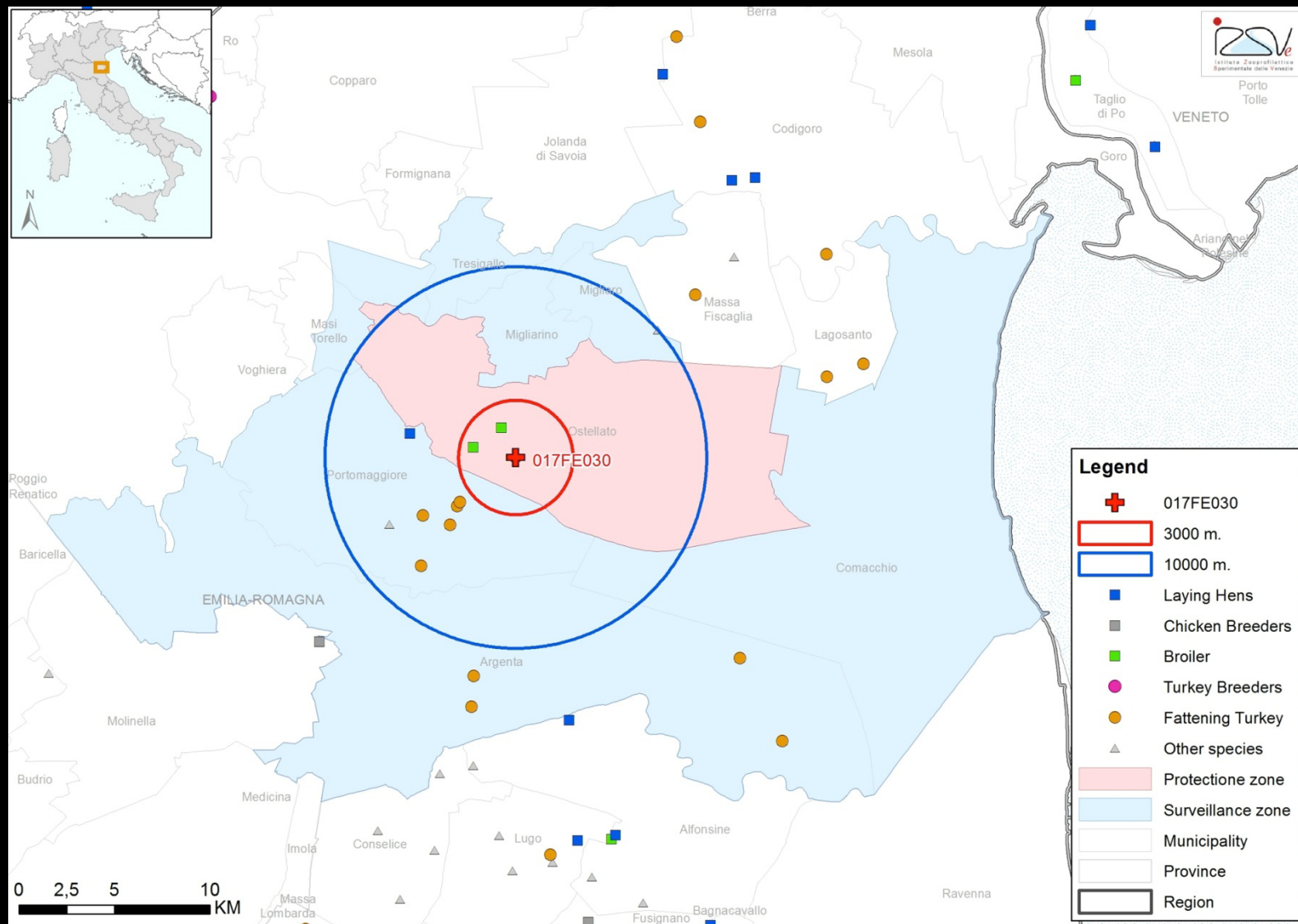
17 August – IVPI concluded (score=3)



First outbreak

- Layer holding located in Ostellato municipality, Ferrara province (Emilia Romagna Region)
- Number of birds : 128.000 laying hens
- Restriction measures enforced on 13/08/2013
- Protection and surveillance zones established on 14/08/2013
- Depopulation measures completed on 24/08/2013

First outbreak - Restriction zones





First outbreak - Restriction zones

Poultry population size in 3 and 10 km radius

3 km radius

Type of holding	N. of poultry farms	Number of birds
Meat turkey	0	0
Layer	0	0
Broiler	2	378400

10 km

Type of holding	N. of poultry farms	Number of birds
Meat turkey	4	243500
Layer	1	17500
Broiler	0	0



First outbreak - Results of epidemiological investigation

Identification of risk factors for disease introduction

- The farm has 5 sheds – Two sheds (1 and 7) with external paddocks and no nets
- The Ostellato municipality is a wetland area and a resting site for migratory birds in the Po delta with a high waterfowl density

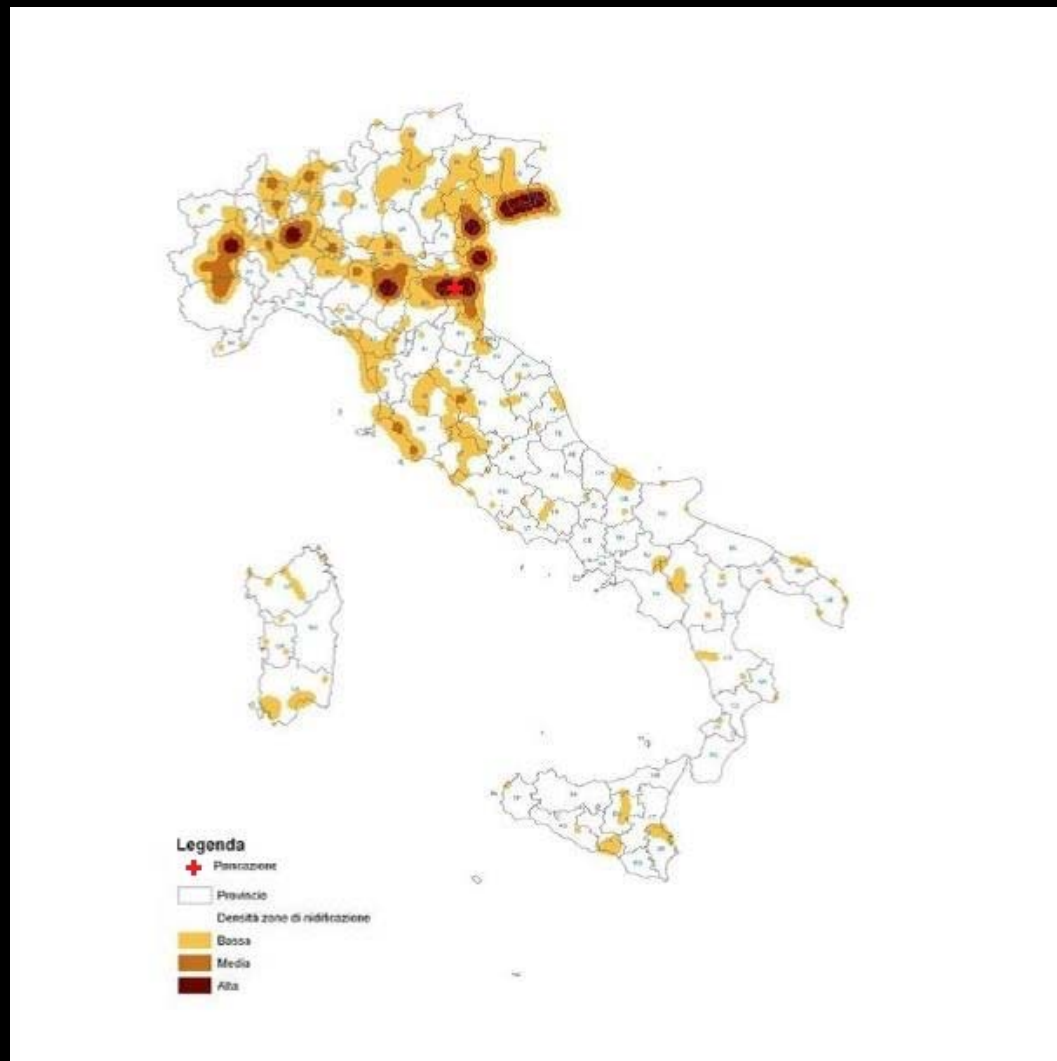
First outbreak - Results of epidemiological investigation



First outbreak - Results of epidemiological investigation



Italy - Density of wild waterfowl





Free range areas marked in red



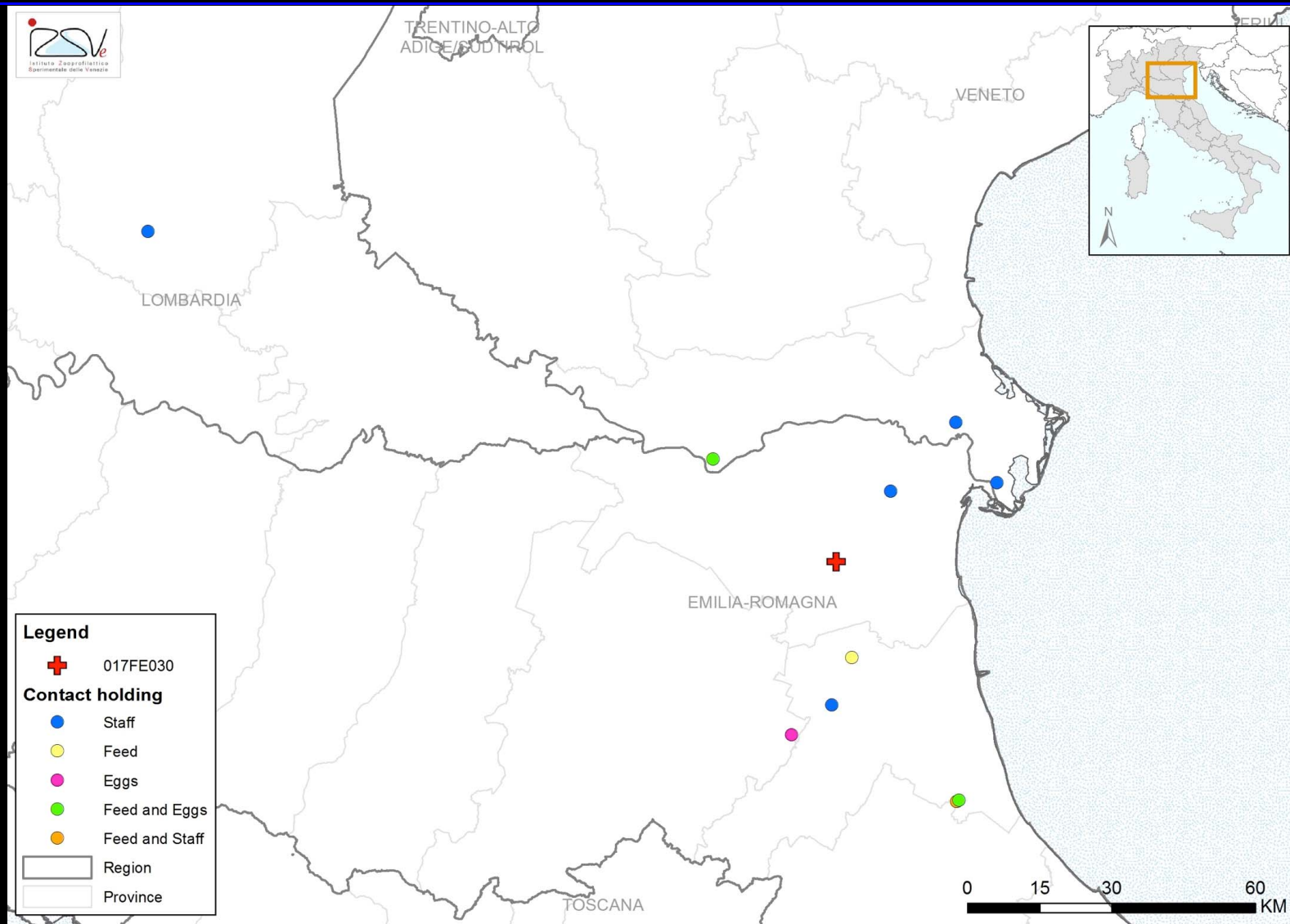
First outbreak - Results of epidemiological investigation

Identification of risk factors for disease introduction

- Epidemiological data suggest that a LPAI virus entered the farm via wild birds where it mutated to a HPAI strain

First outbreak - Results of epidemiological investigation

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 must be officially checked every week 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 must be transmitted to the competent authority

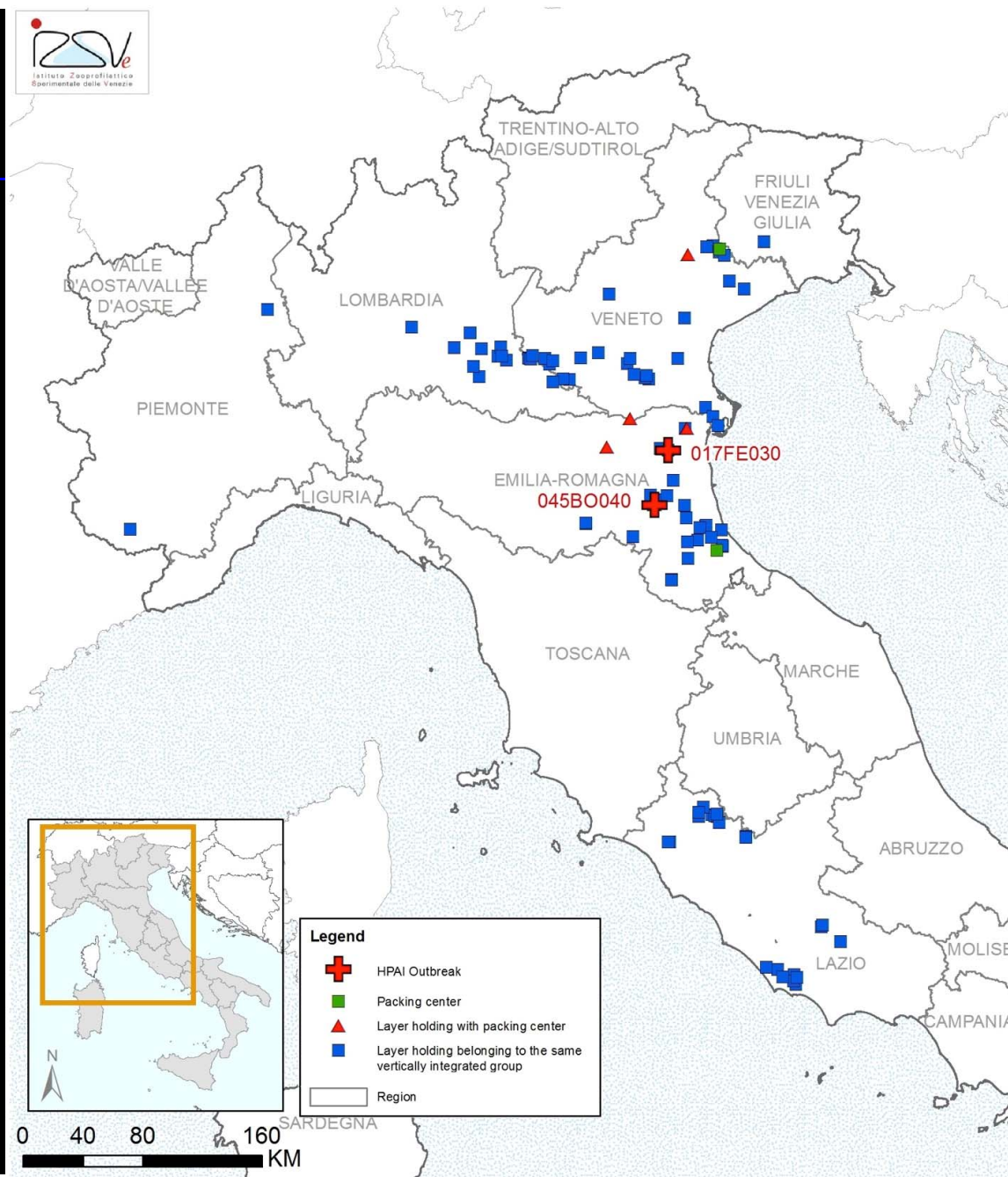


First outbreak - Results of epidemiological investigation

Identification of poultry holdings at risk of infection

- The infected farm belongs to a vertically integrated group specialized in the production of table eggs
- The group in Italy involves a total of 107 layer farms distributed in several Italian regions with a total of 10 million layers, 6 large packaging centres (4 located within a layer operation) collecting eggs from more than one layer farm

107 layer farms of the company - Geographical distribution





Control measures – All farms of the company

All the poultry (layer) holdings of the company must be officially checked every week 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 must be transmitted to the competent authority

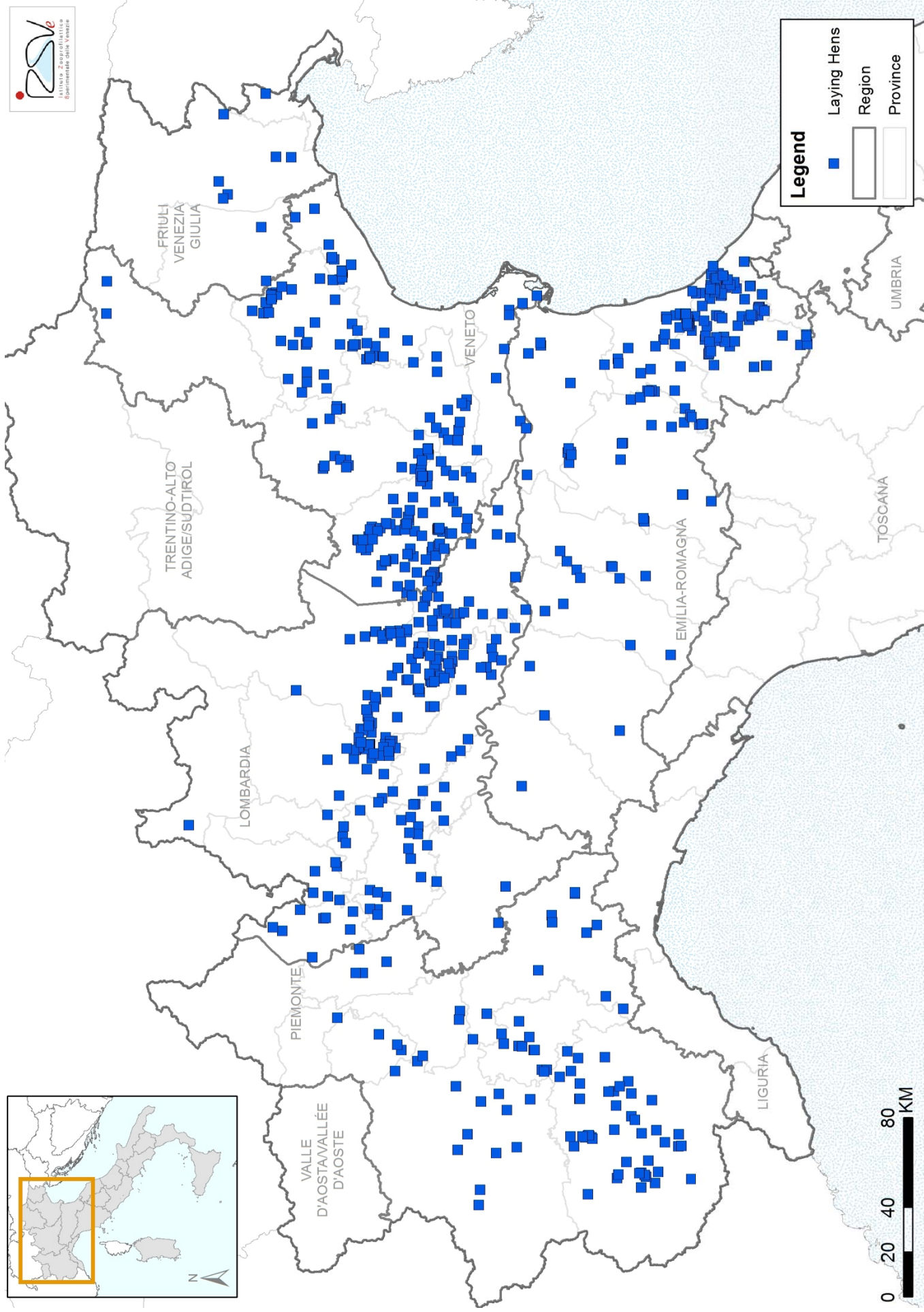
On 23 August, 75 layer farms out of a total of 107 have been tested with negative results

● Control measures – All layer farms in DPPA

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

In case of increased mortality at least 15 dead hens shall be examined

Laboratory testing shall be repeated after 21 days





Control measures – All the Italian territory

Pre-moving inspection and testing - layers and pullets shall be officially inspected and virologically and serologically tested (30 swabs and 30 blood samples) before loading

Table eggs shall not be delivered directly to a packaging centre or a thermal-treatment plant located within a poultry farm, with the exception of table eggs produced in the same farm where the packaging centre or the thermal-treatment plant is situated



Second HPAI H7N7 outbreak

In the frame of monitoring activities applied on contact holdings:

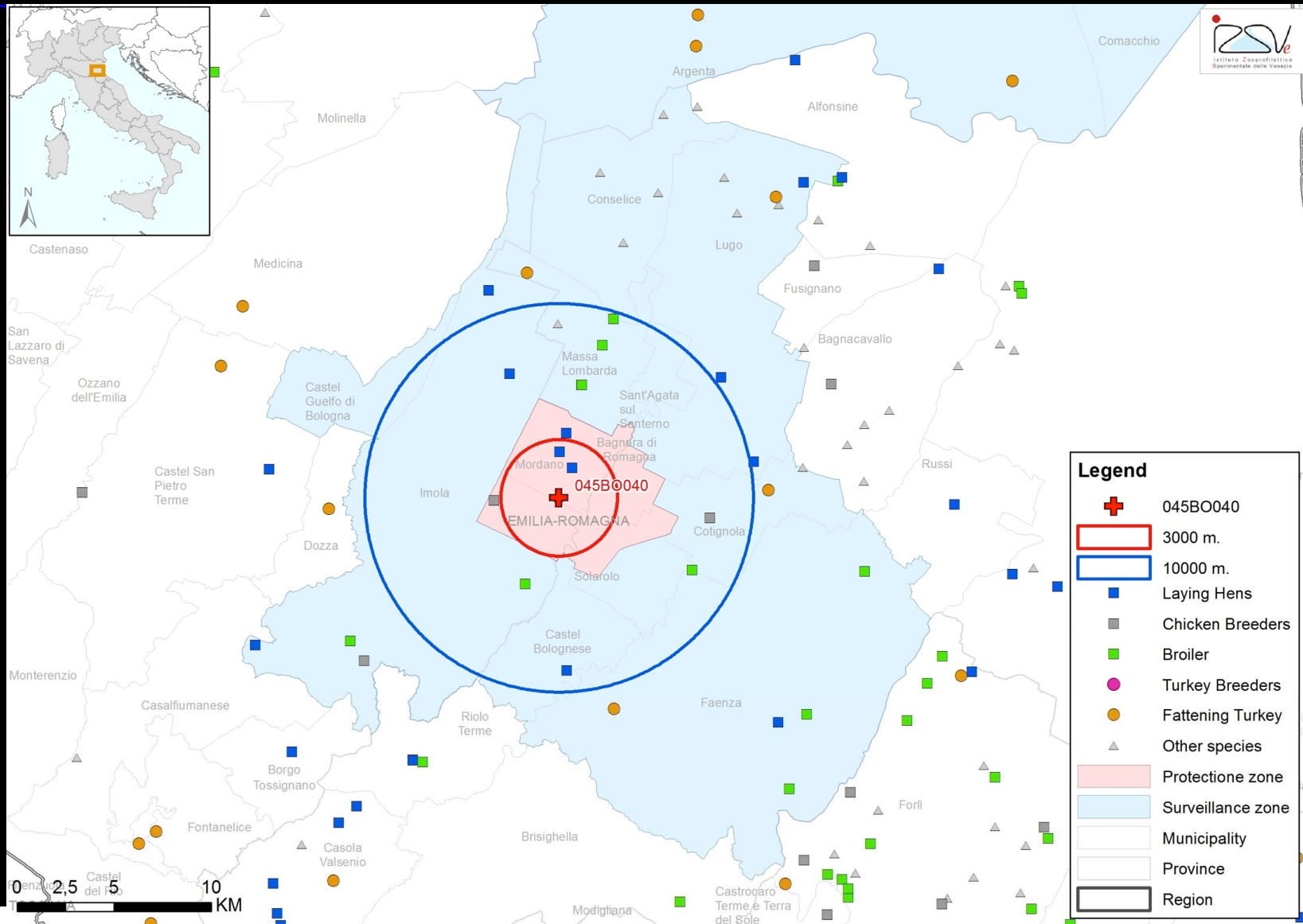
- A large layer operation tested H7 RT-PCR positive on samples collected on 19/08/2013
- RT-PCR positivity for H7N7 subtype at NRL on 20/08/2013
- H7N7 HPAI virus was detected only in one shed (n. 64)
- This farm was part of the same vertically integrated group and was epidemiologically linked to the first outbreak (several transports of table eggs to the packaging centre located inside the holding in the period at risk)



Second HPAI H7N7 outbreak

- Layer holding located in Mordano municipality, Bologna province (Emilia Romagna Region)
- 12 poultry sheds with no external areas
- Number of birds : 584934 laying hens
- A packaging centre that in the period at risk received table eggs from 50 layer farms of the same vertically integrated group
- Restriction measures enforced on 20/08/2013
- Protection and surveillance zones established on 21/08/2013
- Depopulation measures are on going

Second outbreak - Restriction zones



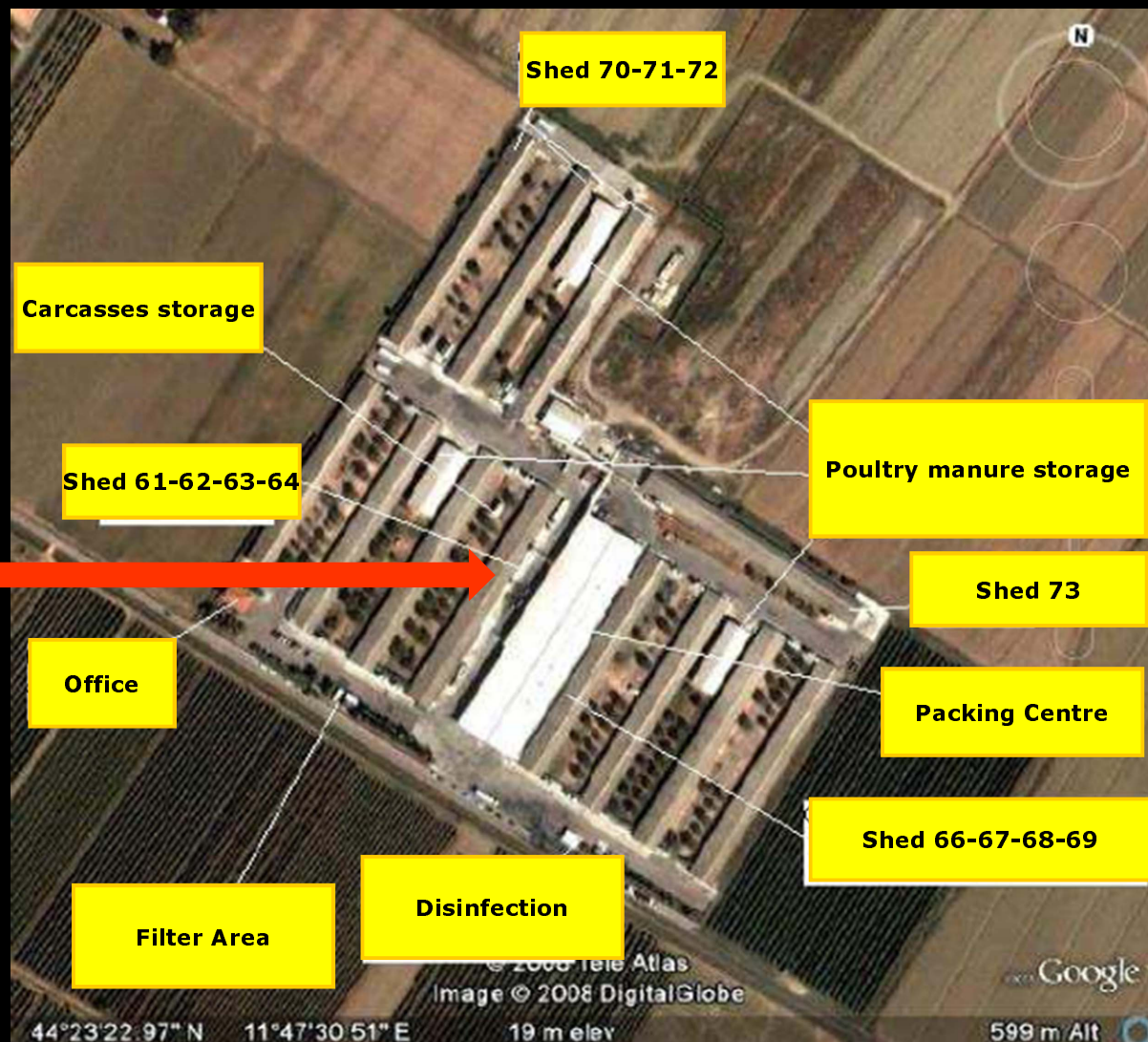
Second outbreak - Restriction zones

Poultry population size in 3 and 10 km radius

<i>3 km radius</i>		
Type of holding	N. of poultry farms	Number of birds
Meat turkey	0	0
Layer	2	106047
Broiler	0	0
<i>10 km</i>		
Type of holding	N. of poultry farms	Number of birds
Broiler breeder	2	117000
Layer	3	224384
Broiler	6	206000

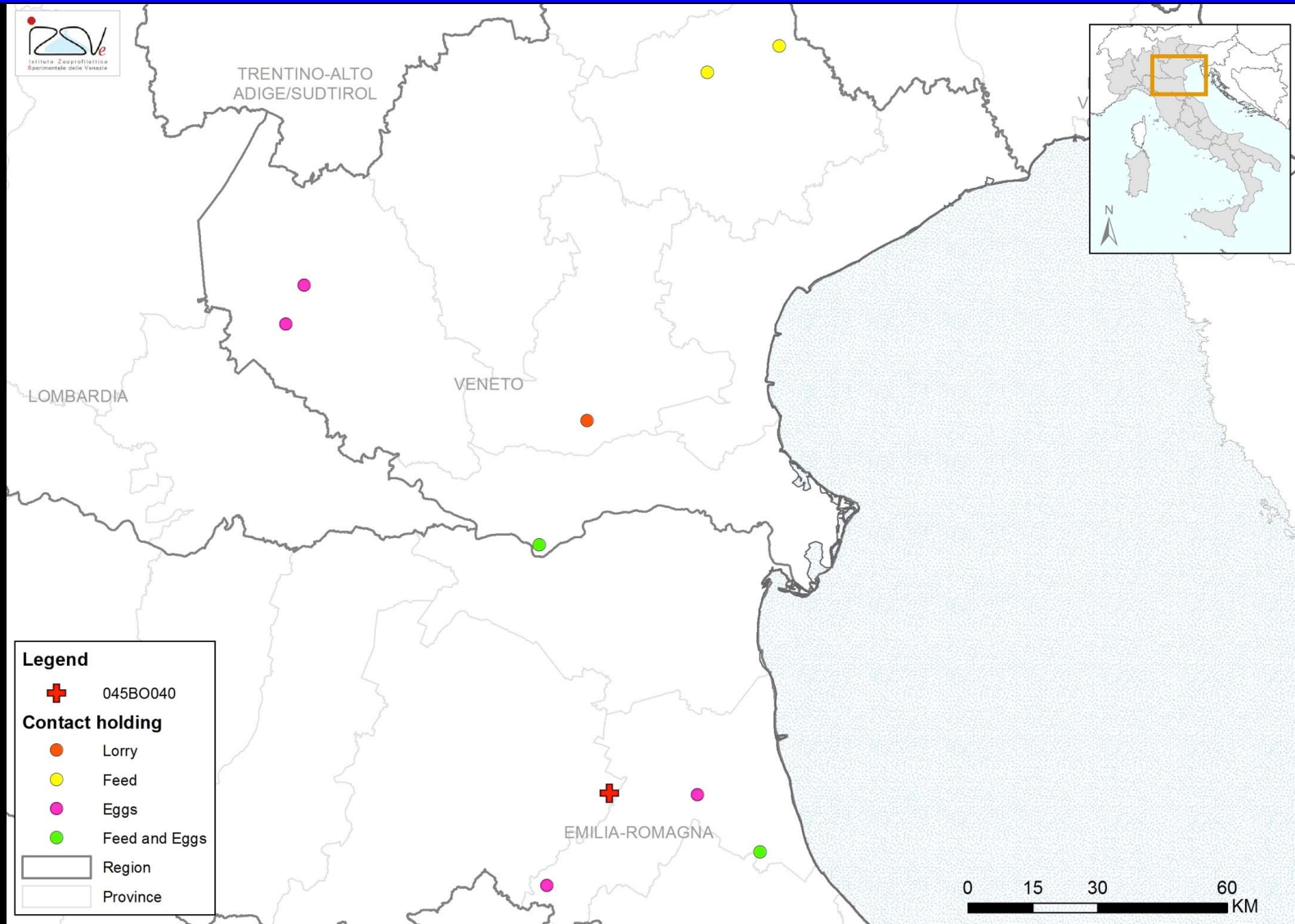
Second HPAI H7N7 outbreak

RT-PCR
positive
shed



Second outbreak -Results of epidemiological investigation

Contact holdings





Third outbreak - Restriction zones

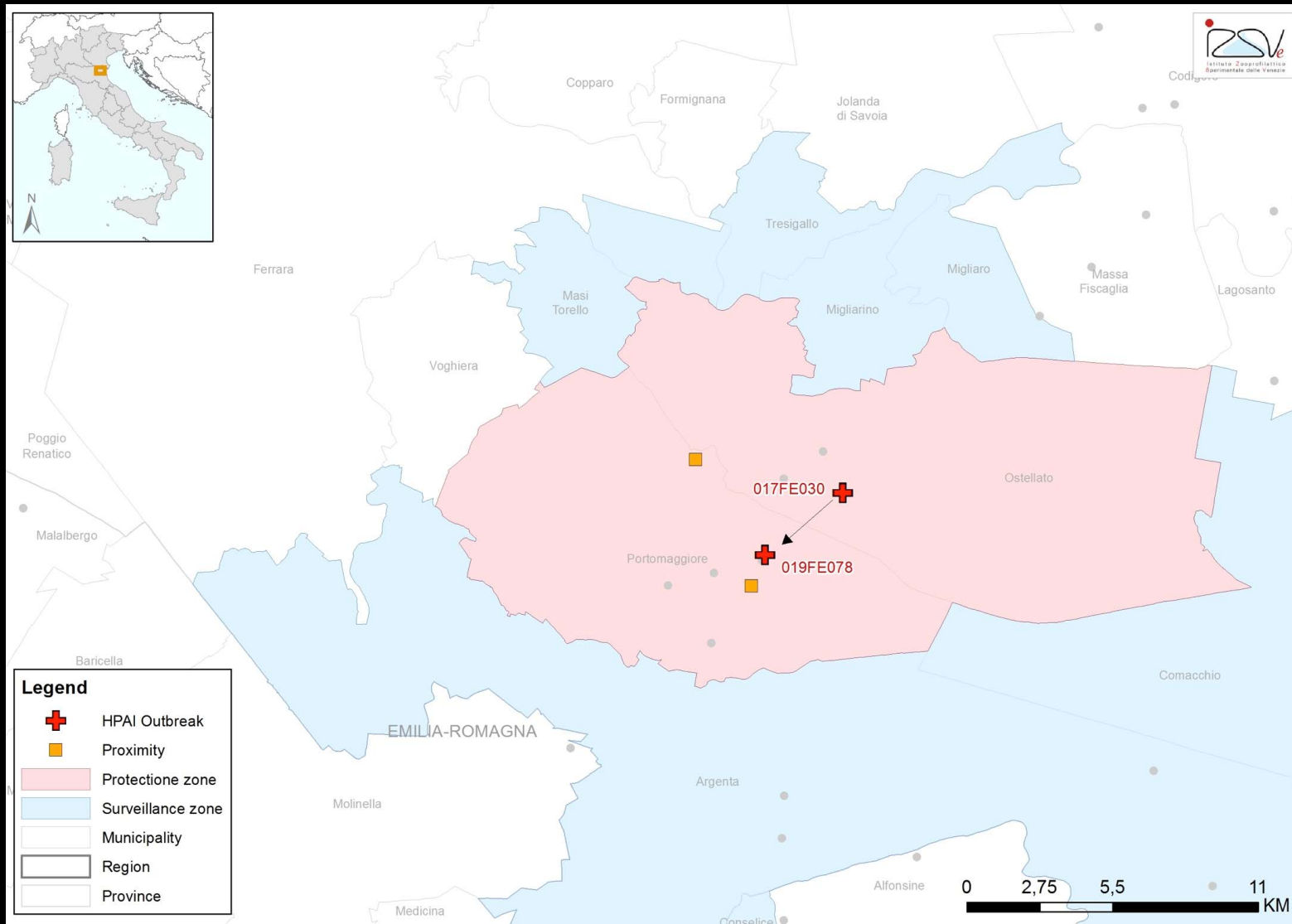
- A meat turkey farm – Portomaggiore municipality (Ferrara province)
- Located at 3.7 km from the 1st outbreak
- Number of birds : 19.850 female turkeys ready for slaughter
- Increased mortality rate on 21/08/2013
- RT-PCR H7 and positivity: 23/08/2013 (HPAI 24/08/2013)
- Depopulation measures completed on going

Third HPAI H7N7 outbreak



Data di acquisizione delle immagini: 5/24/2011 44°41'38.36"N 11°53'17.88"E elev -3 m alt 279 m

Origin of third outbreak – Neighbourhood spread

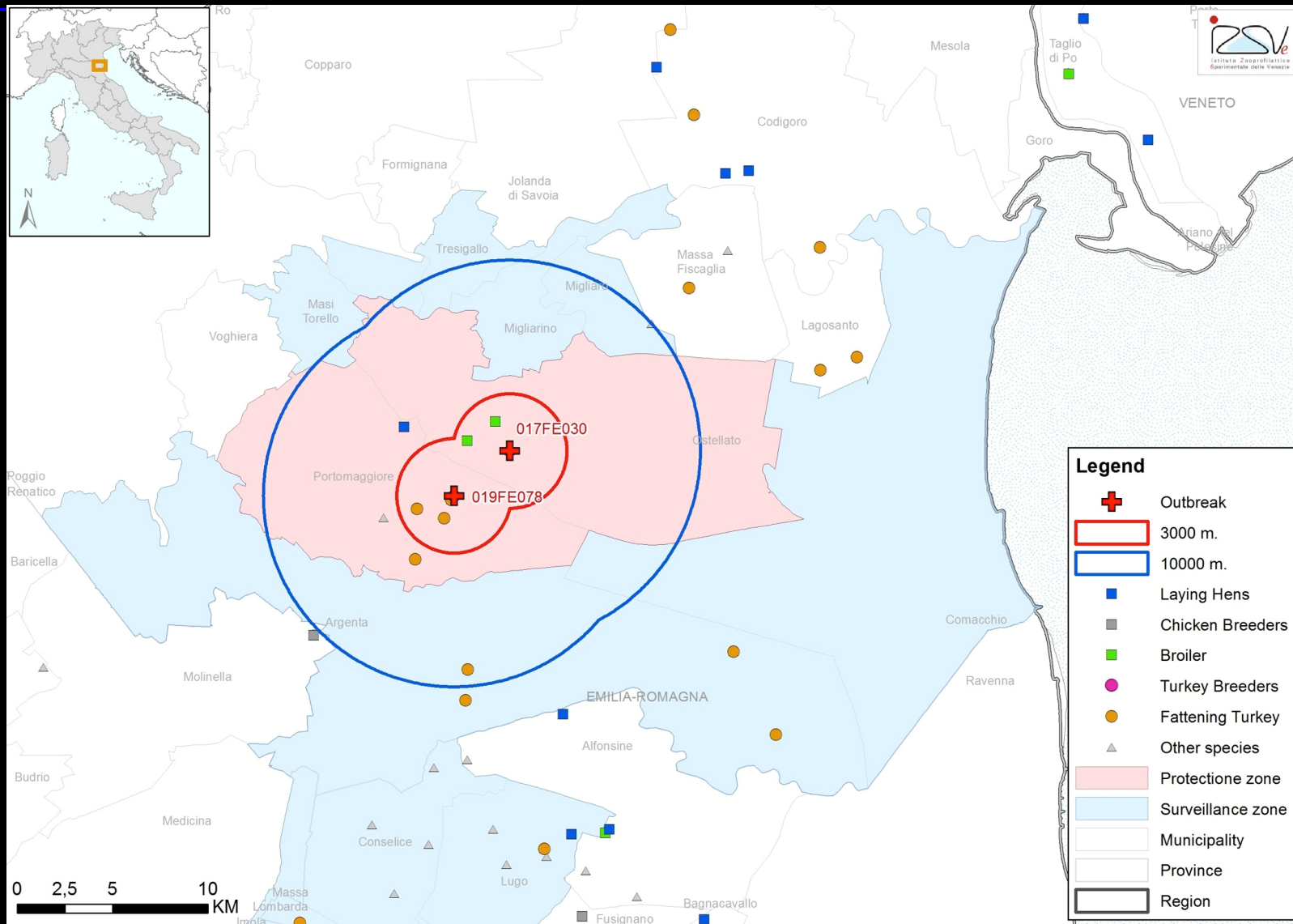




Third outbreak - Results of epidemiological investigation

The epidemiological investigation did not identify any risk contacts from or to other poultry holdings

Third outbreak - Restriction zones



Third outbreak - Restriction zones

Poultry population size in restriction zones

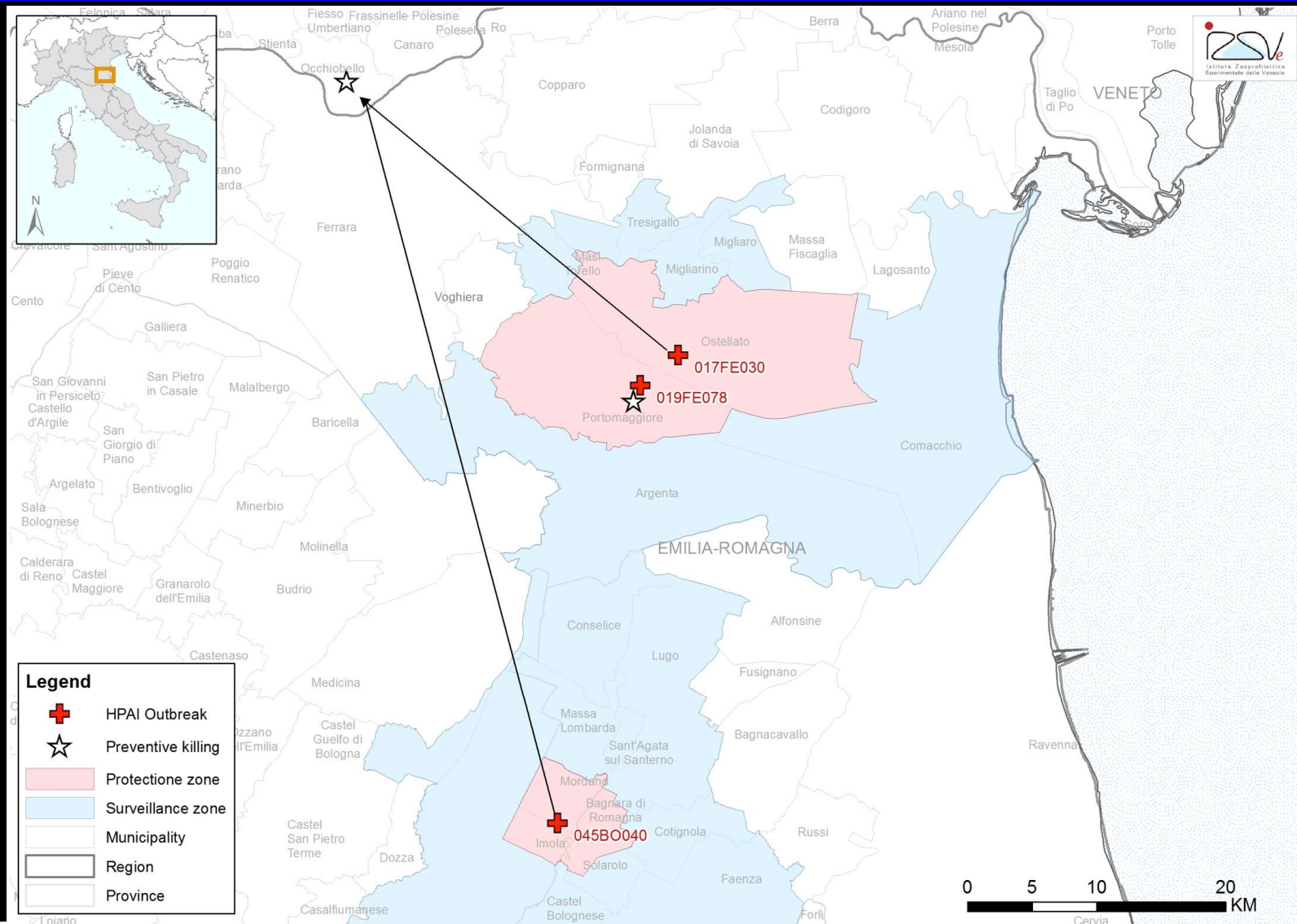
3 Km radius

Type of holding	N. of poultry farms	Number of birds
Meat turkey	3	149500
Layer	0	0
Broiler	1	220000

10 km

Type of holding	N. of poultry farms	Number of birds
Meat turkey	2	199000
Layer	1	17500
Brolier	1	158400

Pre-emptive killing

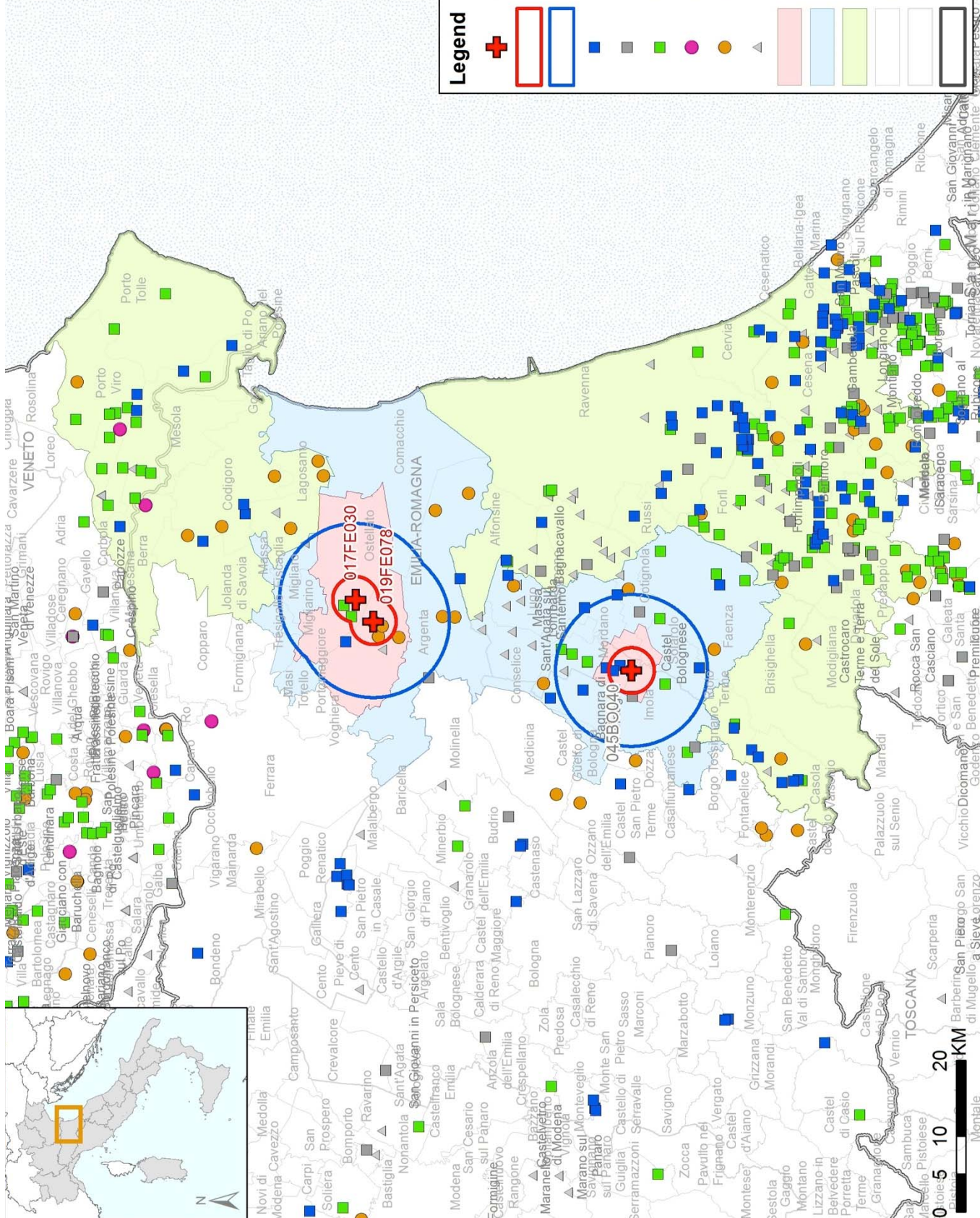


Further restricted zone – Art. 16 (4) 2005/94/EC

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



Legend

- + Outbreak
- 3000 m.
- 10000 m.
- Laying Hens
- Chicken Breeders
- Broiler
- Turkey Breeders
- Fattening Turkey
- ▲ Other species
- Protection zone
- Surveillance zone
- Restriction zone
- Municipality
- Province
- Region

0 5 10 20 KM

Poultry population size in restriction zones

protection zones

Type of holding	N. of poultry farms	Number of birds
Broiler breeder	1	95000
Meat turkey	5	265500
Layer	6	1128871
Broiler	2	378400

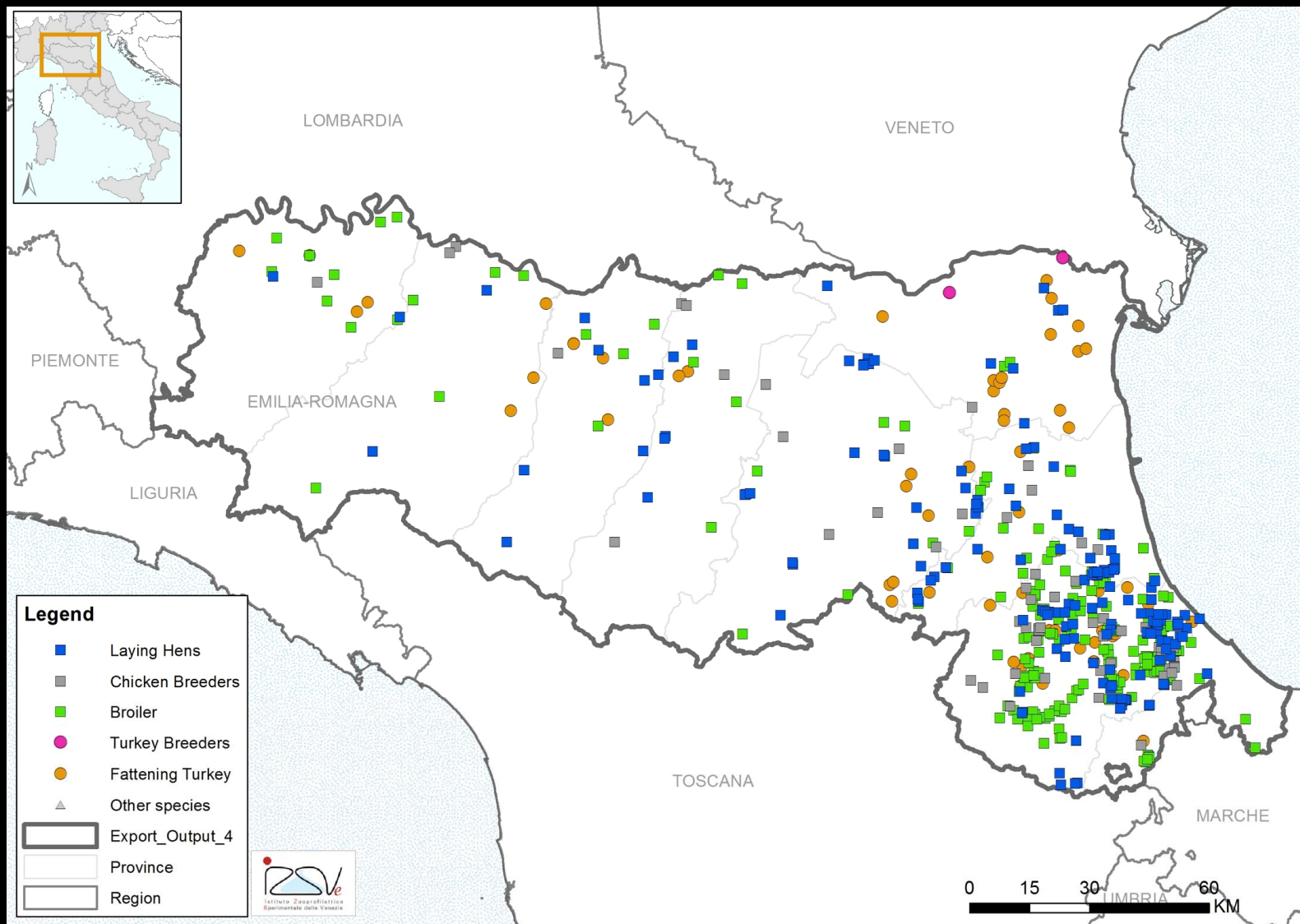
surveillance zones

Type of holding	N. of poultry farms	Number of birds
Broiler breeder	3	59000
Meat turkey	8	436500
Layer	8	376500
Broiler	10	395000

further restricted zones

Type of holding	N. of poultry farms	Number of birds
Broiler breeder	24	1039307
Meat turkey	26	1079800
Turkey breeder	2	18000
Layer	94	9591818
Broiler	97	7484753

Emilia Romagna Region – Industrial poultry holdings





Emilia Romagna Region – Poultry population size

EMILIA ROMAGNA

Type of holding	N. of poultry farms	Number of birds
Broiler breeder	58	2327666
Meat Turkey	67	2568184
Turkey breeder	2	27000
Layer	167	12877153
Broiler	232	15175773
	526	32975776



Provisions for the whole Emilia Romagna Region

The movement of

- live poultry and
- table eggs (originating directly from a layer farm)

OUT of the territory of Emilia Romagna region shall be authorized by the competent authority

Poultry companies must guarantee the complete separation of production activities taking place in Emilia Romagna from those in the other Italian regions



Provisions enforced in the whole Country

- Pre-moving testing: layers and pullets shall be officially inspected and virologically and serologically tested (30 swabs and 30 blood samples) before loading
- Table eggs shall not be delivered directly from a layer operation to a packaging centre or a thermal-treatment plant located within a poultry farm
- Additional biosecurity measures shall be applied on industrial poultry farms

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



Phylogeny – HA gene

A/chicken/Italy/13VIR4527-11/2013 (H7N7) HA gene sequence clusters with:

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



Phylogeny – HA gene

A/chicken/Italy/13VIR4527-11/2013 (H7N7) HA gene sequence

- 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)

H7N7 Italy 2013

99 A/chicken/Italy/13VIR4527-1/2013
A/chicken/Italy/13VIR4603/2013
A/chicken/Italy/13VIR4541-34/2013

[n/Netherlands/12014794/2012](#)

A/swan/Czech Republic/5416/2011
 A/chicken/Netherlands/11004875/11
 A/chicken/Neth/1100832/11
 95 A/chicken/Germany/R180/2011
 A/chicken/Netherlands/1101326/2011

– A/chicken/Germany-NI/R874/2010

7 47 501/4109

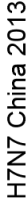
H7
T H7N1
007 H7

https://doi.org/10.1007/978-94-007-1111-1_11



Italian HPAI H7N7 virus, 2013 (A/ck/Italy/13VIR4527-11/13)

Italian HPAI H7N7 virus, Ferrara province, 2013 (A/ck/Italy/13VIR4527-11/13)
Sequence length A/ck/Italy/13VIR4527-11/13: 1368 nt





Phylogeny – HA gene

Molecular features – summary

- 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 A/chicken/Italy/13VIR4527-11/2013 (H7N7)
- No mutations associated with resistance towards adamantanes
- No mutations associated with an increased virulence or modified host range (i.e. PB2 627)



Whole genome analysis

Viruses showing the highest similarity with A/chicken/Italy/13VIR4527-11/2013
(excluding A/Anas crecca/Italy/13VIR1292-304/2013)

Gene	Virus	Subtype	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 Republic/14333-1K/2011	H3N8	99.1%
M	A/mallard/Germany/R193/09	H1N1	99.3%
NS	A/mallard/Sweden/2009	H6N2	99.1%