

LA NOSTRA
ESPERIENZA,
LA VOSTRA
SICUREZZA.

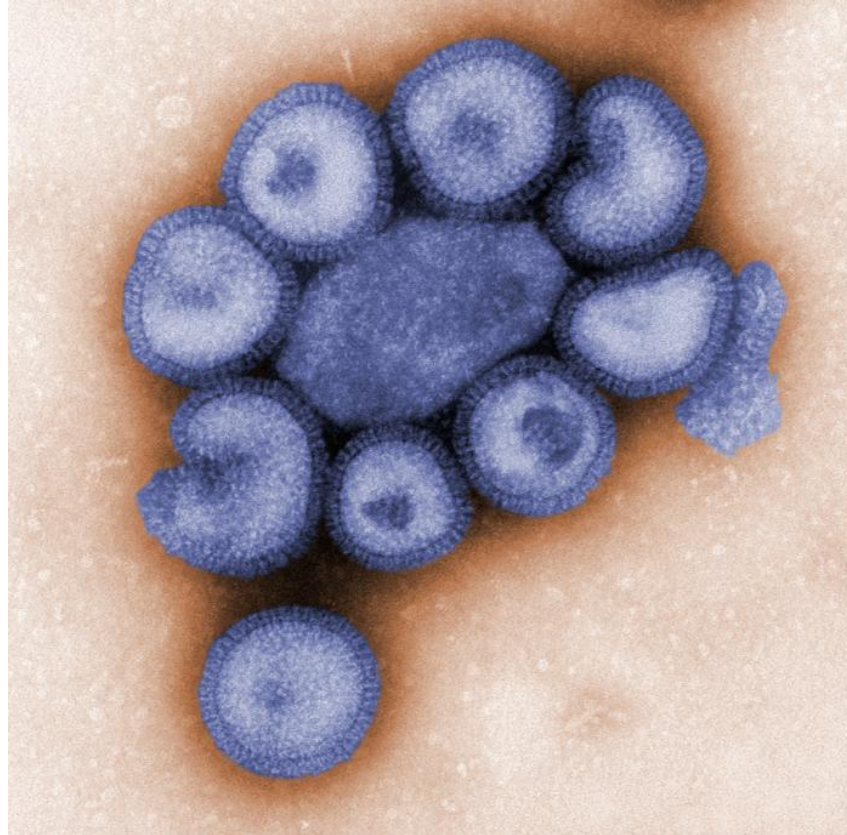
Il mondo animale come resevoir di ceppi influenzali epidemici e pandemici (past and future)



Ana Moreno



Influenza tipo A





Influenza aviare: una “nuova” emergenza?



NON è una malattia nuova



Segnalata da un veterinario Perroncito, nel 1878



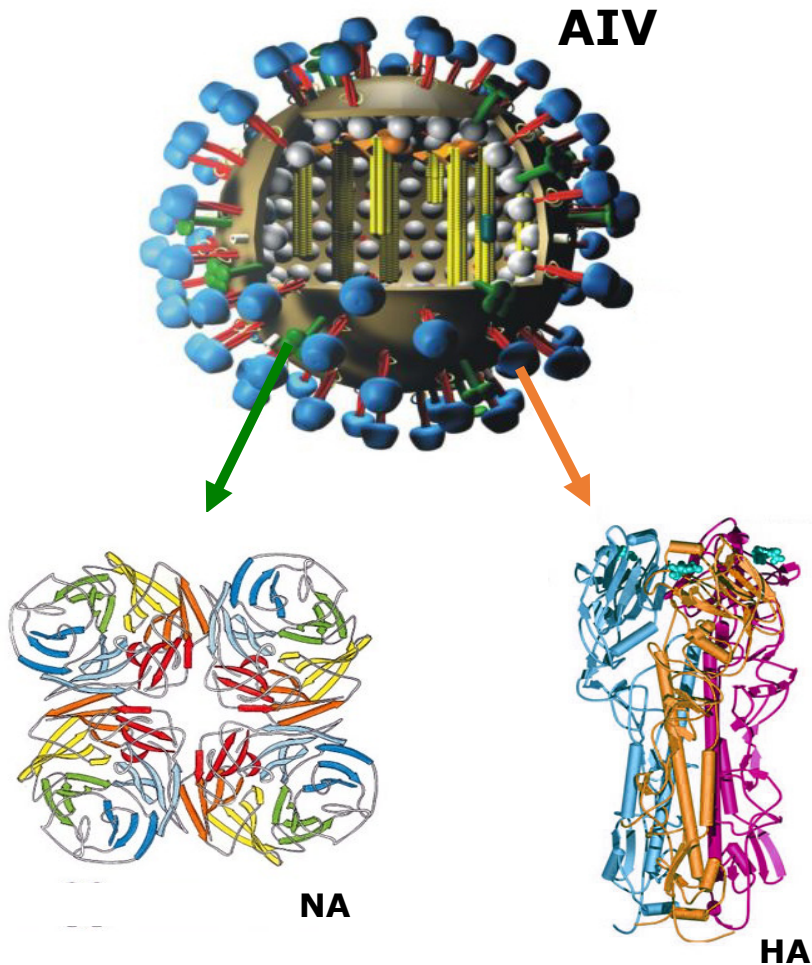
Identificazione dell'agente causale come un microrganismo filtrabile (1902 Centanni e Savonuzzi)



A/Chicken/Brescia/1902 H7N7



Conosciuta ad inizio secolo scorso come peste lombarda (Hutyra & Marek)



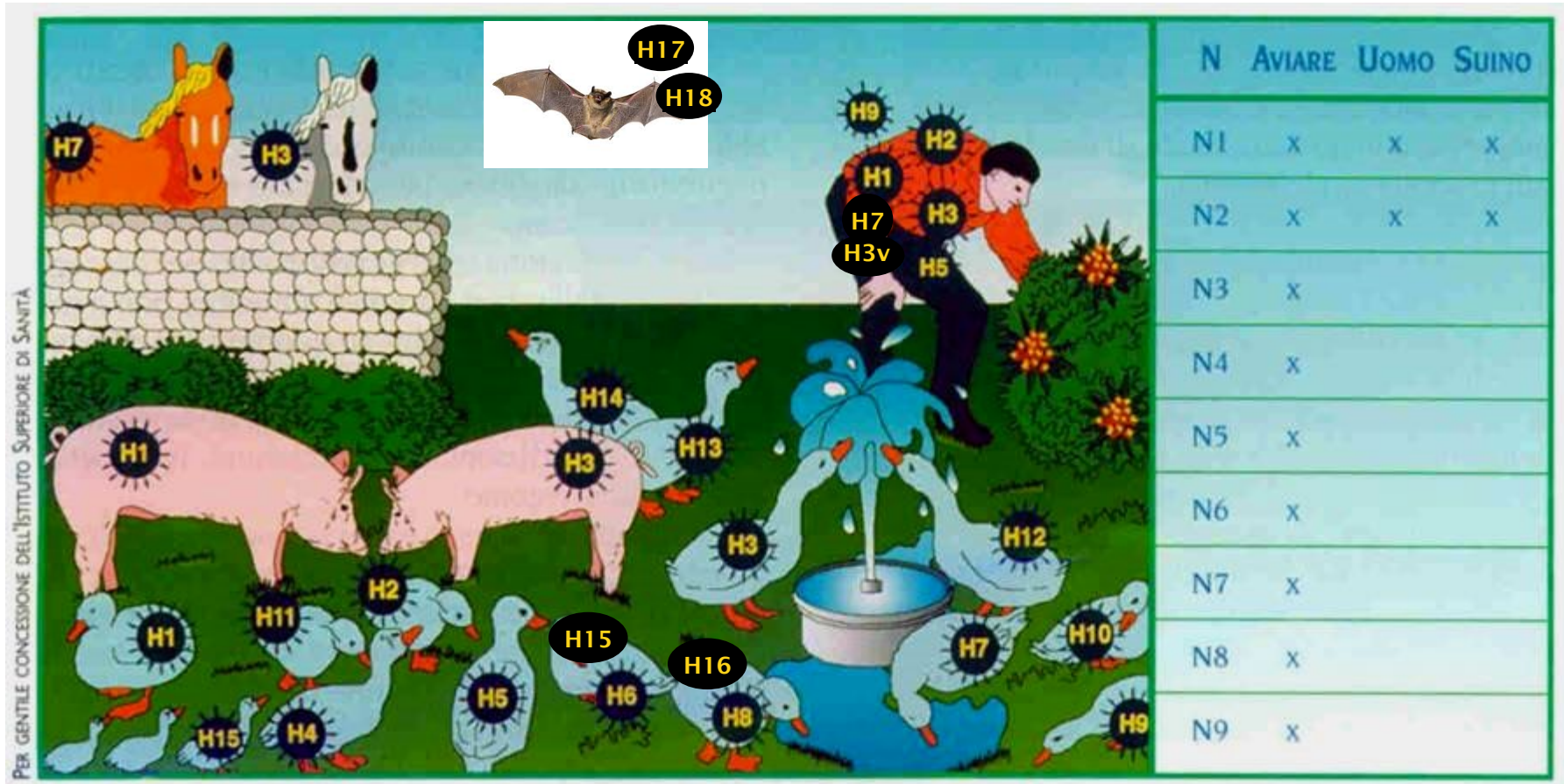
- *Orthomyxoviridae*
- *Influenzavirus* tipo A
- Sottotipi
 - Haemagglutinin (16) 17 e 18 nei pipistrelli
 - Neuraminidase (9) 10 e 11 nei pipistrelli

Combinazioni multipli HA-NA



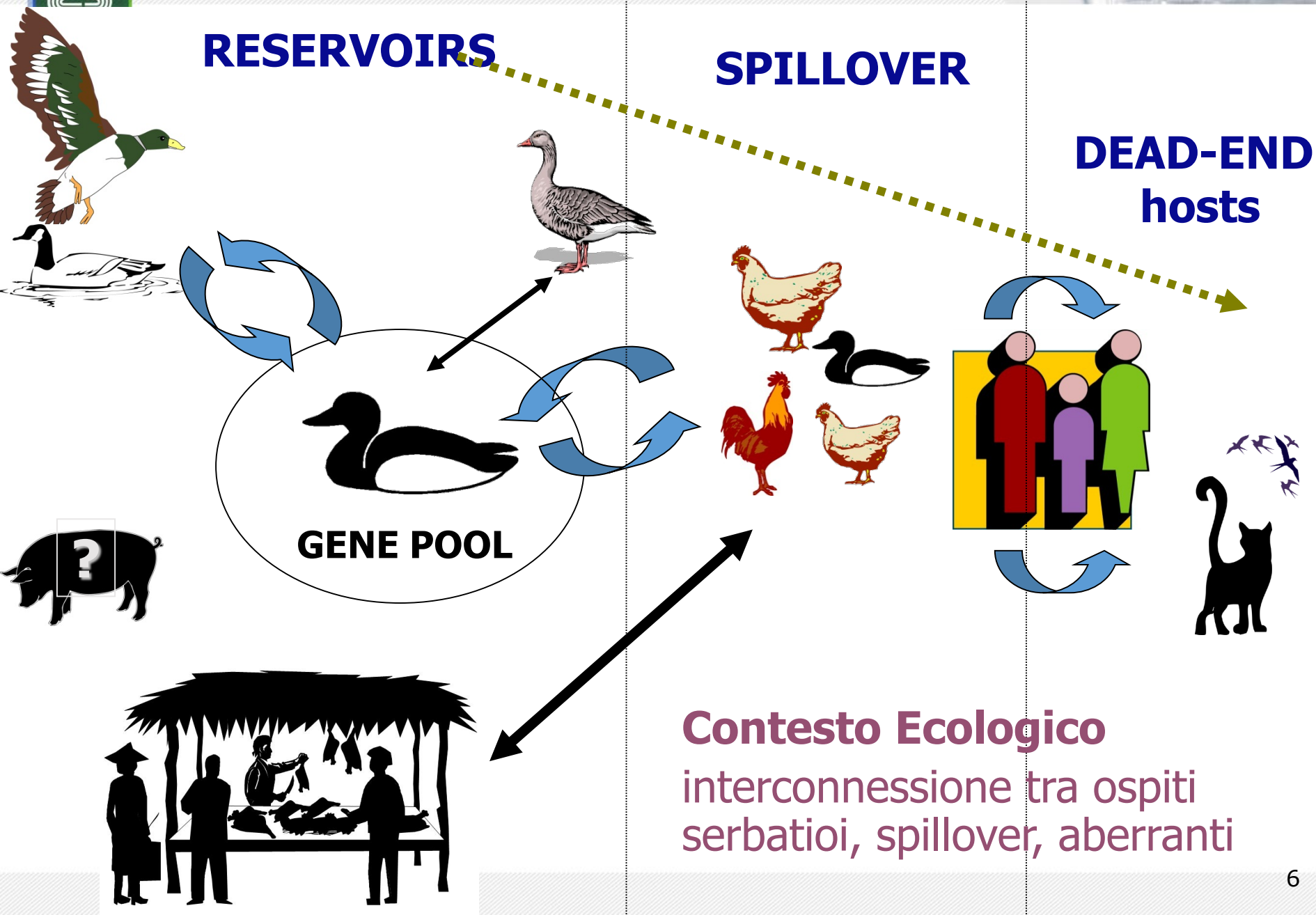
Specie sensibili ai virus dell'influenza A

Sensibilità ad H e N





Ecologia dei virus influenza A



Ospite reservoir



Anseriformi: anatre, oche, cigni



Caradriiformi: gabbiani, rondini marine, trampolieri



Mantiene l'infezione



Non contrae la malattia o solo in forma lieve



Animali giovani

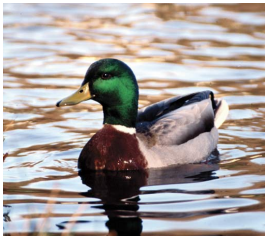


Ciclo oro-fecale



Flussi migratori

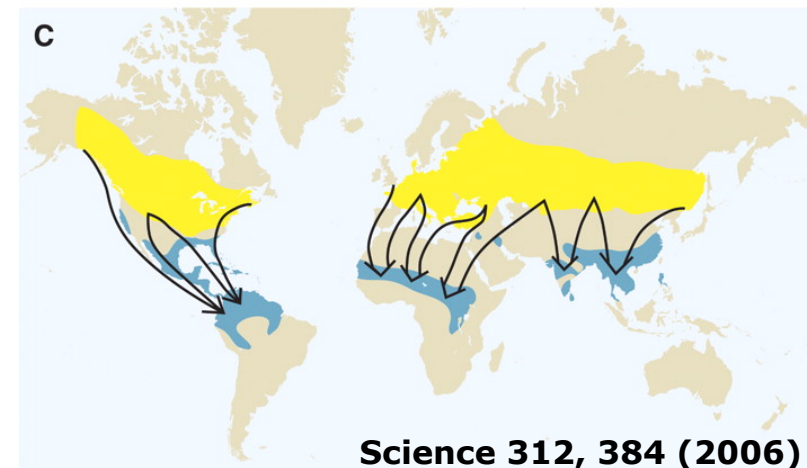
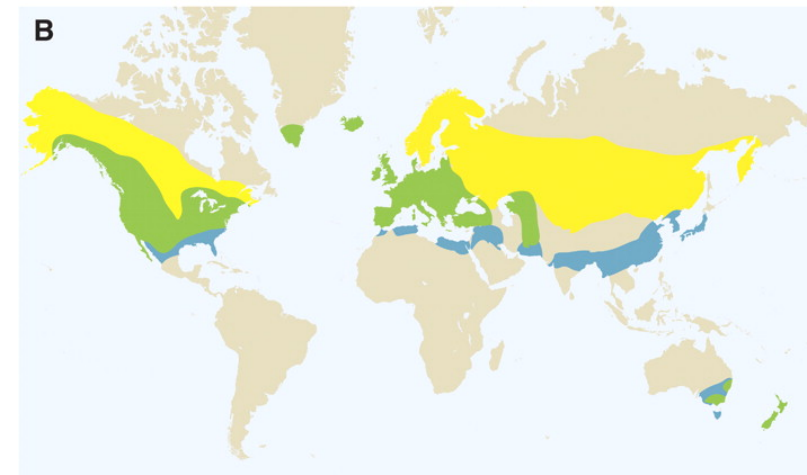
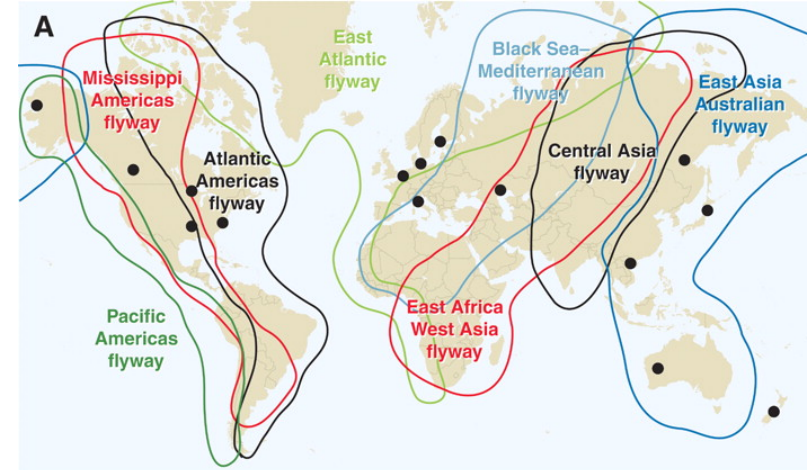
**Flussi migratori di
A. platyrhincos, *A. querquedula*
in Eurasia ed Africa ed *A.
discors* in America**



 Aree di riproduzione

 Aree specie presenti tutto l'anno

 Aree di svernamento



Science 312, 384 (2006)



Ospite spillover



Pollame domestico



Suscettibile all'infezione se esposto



Trasmette l'infezione ad altri ospiti



Non mantiene la disseminazione virale per lungo tempo



Una malattia più grave dei reservoir



Ciclo respiratorio



Ospite aberrante

- ☼ S'infetta raramente
- ☼ di solito contrae una malattia grave
- ☼ influenti nella epidemiologia della malattia, ma possono esserne gravemente colpiti



Infezione H5N1:

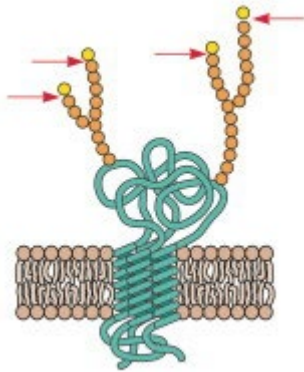
uomo, cane, gatto,
tigre, leopardo



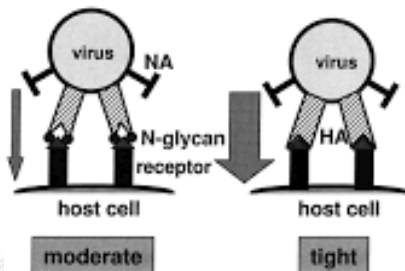
Interazione virus - ospite



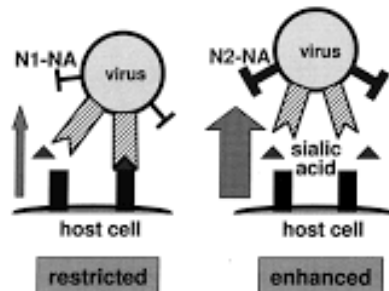
- Host-cell Receptor
- HA activity and receptor binding site
- NA activity
- HA-NA balance



Binding



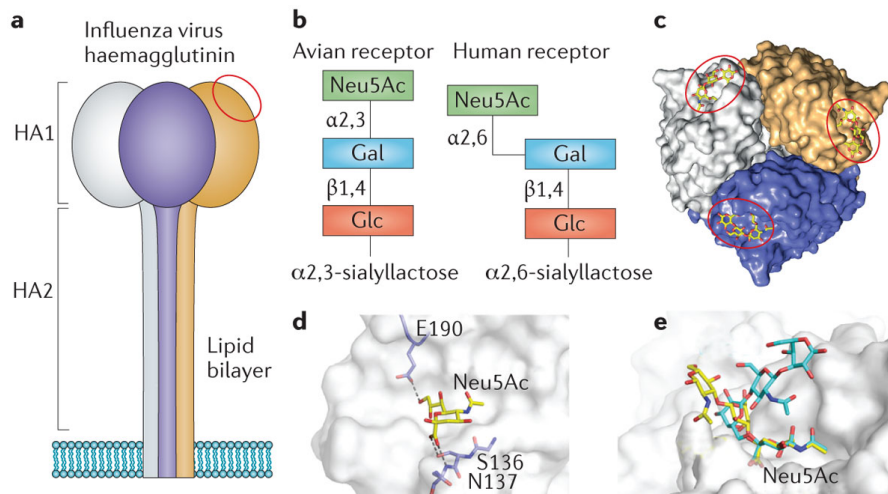
Release



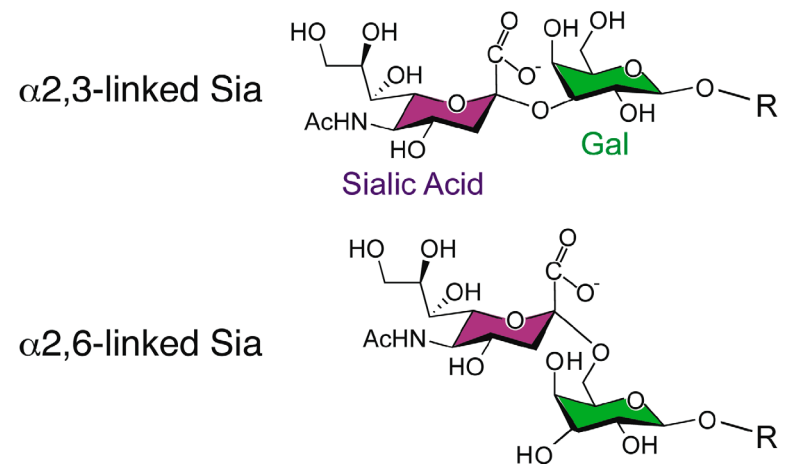


Host cell receptors

- 2 tipi di recettori cellulari:
- Legame tra acido sialico e galattosio
- Due possibili tipi di legame: alfa (2,3) e alfa (2,6), a seconda che il carbonio in posizione 2 dell'acido sialico si leghi al carbonio in posizione 3 o 6 del galattosio.



Nature Reviews | Microbiology

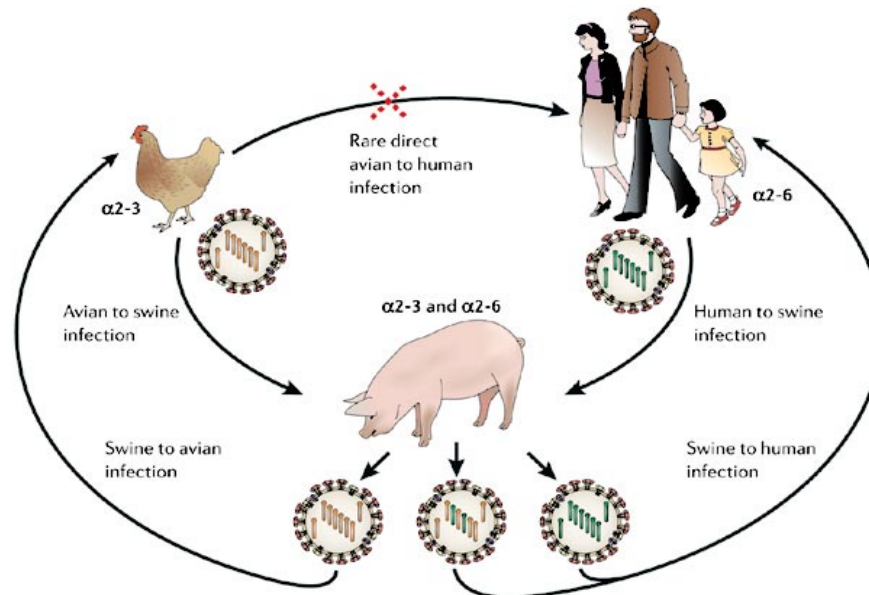




Virus influenzale A: recettori



- Il passaggio dei virus influenzali aviari all'uomo necessita di un passaggio intermedio nel suino
- 2 tipi di recettori:
 - ✓ $SA\alpha 2,3Gal$ – tipici dei volatili
 - ✓ $SA\alpha 2,6Gal$ – tipici dell'uomo
 - ✓ Suino – due tipi di recettori (mixing vessel)



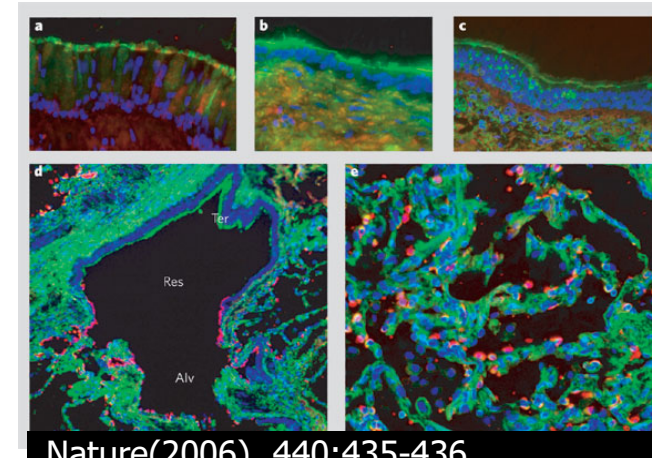


Distribuzione dei recettori nei tessuti ospiti



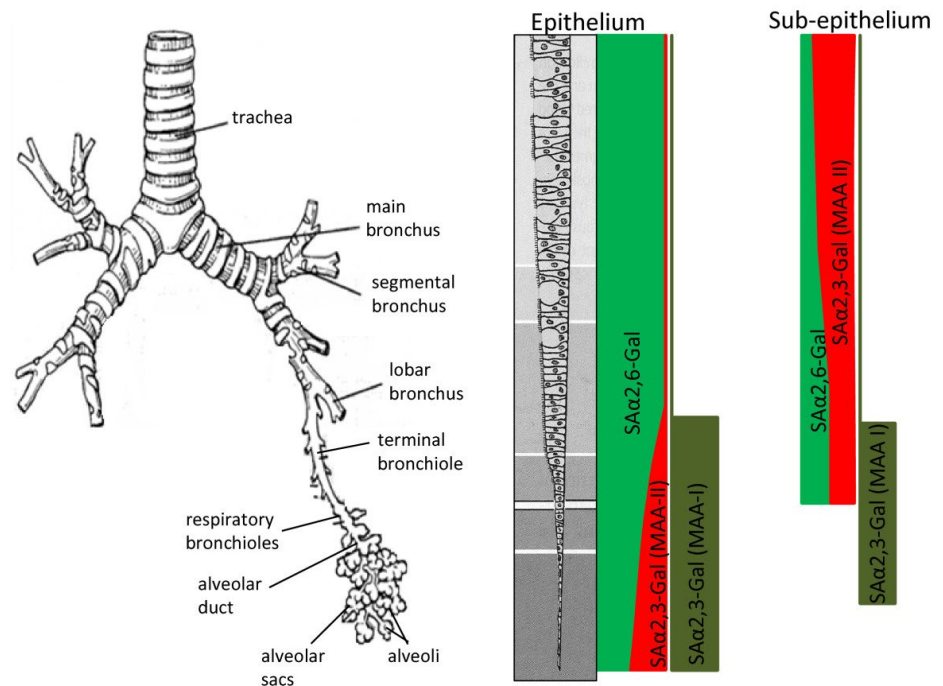
Prove Istochimica
(lectine):
Sambucus nigra - SA
 $\alpha 2,6$ Gal (verde)
Maackia amurensis- SA
 $\alpha 2,3$ Gal (rosso)

- Recettori SA $\alpha 2,6$ Gal -
faringe, trachea, bronchi
- Recettori SA $\alpha 2,3$ Gal –
bronchioli, alveoli
- N-acetylneuraminic acid
(Neu5Ac)



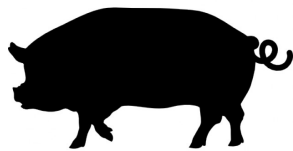
Nature(2006) 440:435-436

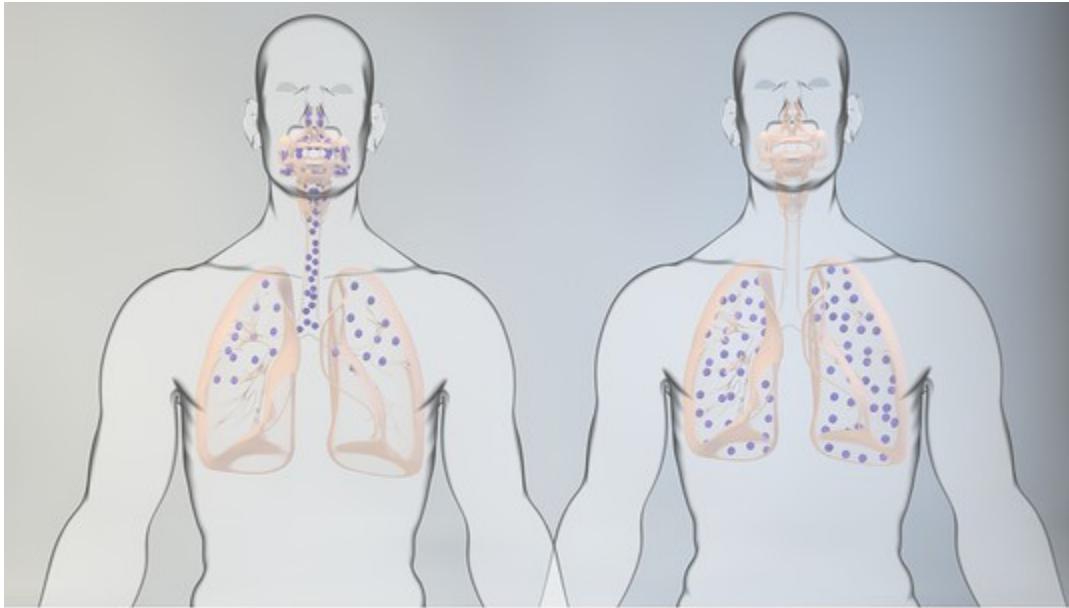
Relative receptor expression trend



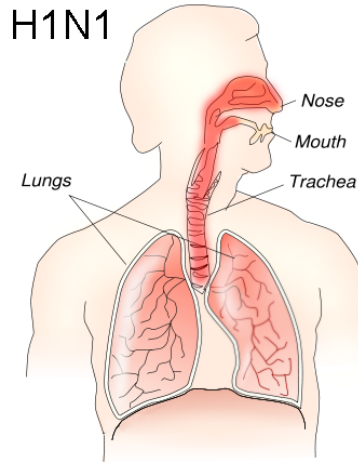
presenta la stessa distribuzione
di recettori dall'uomo
(Nelli et al, 2010)

N-glycolyneuraminic acid
(Neu5Gc)



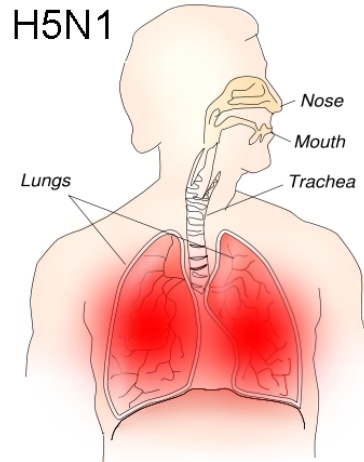


H1N1

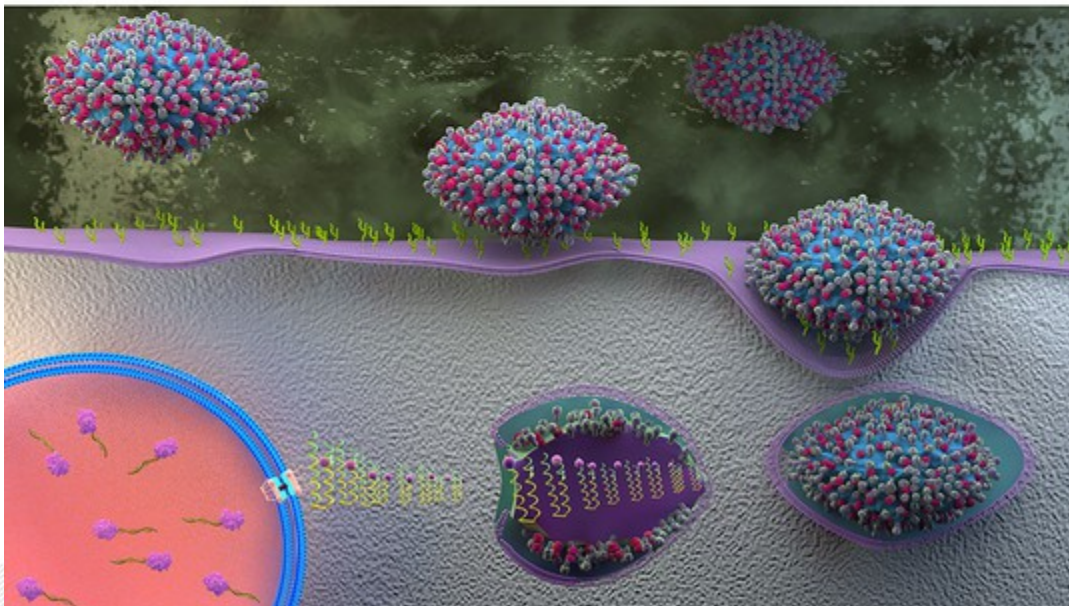


Easily spread
Rarely fatal

H5N1



Spreads slowly
Often fatal



H5N1HPAI, H7N9 LPAI in
Asia (2003-2016) -
Passaggio diretto dai
volatili all'uomo



Distribuzione dei recettori nei tessuti ospiti

Anatra



Recettori $SA\alpha 2,3Gal$ – trachea, intestino, rene



Recettori $SA\alpha 2,6Gal$ - rene

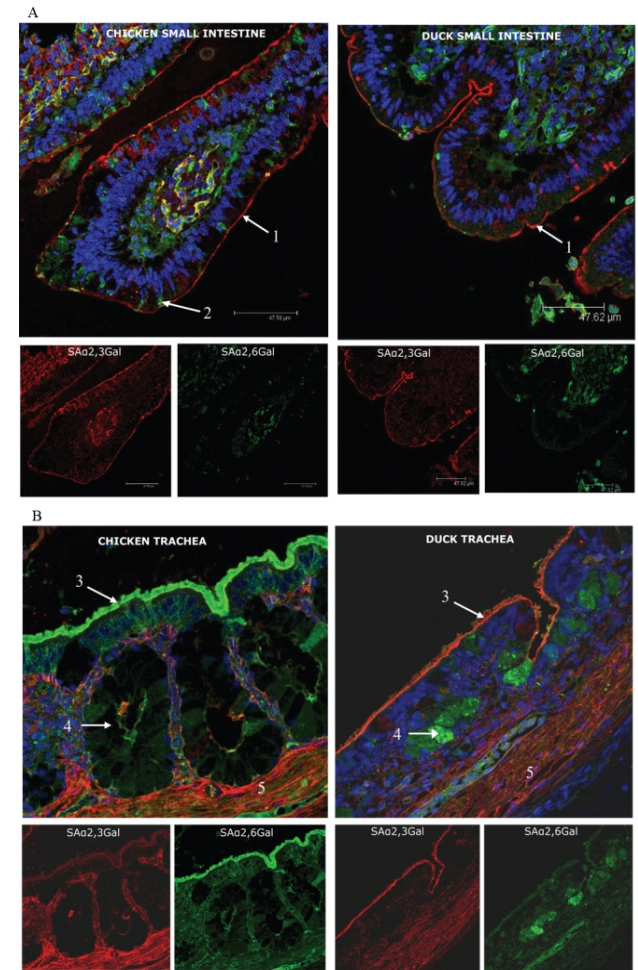
Pollo



Recettori $SA\alpha 2,6Gal$ – trachea, rene



Recettori $SA\alpha 2,3Gal$ – intestino





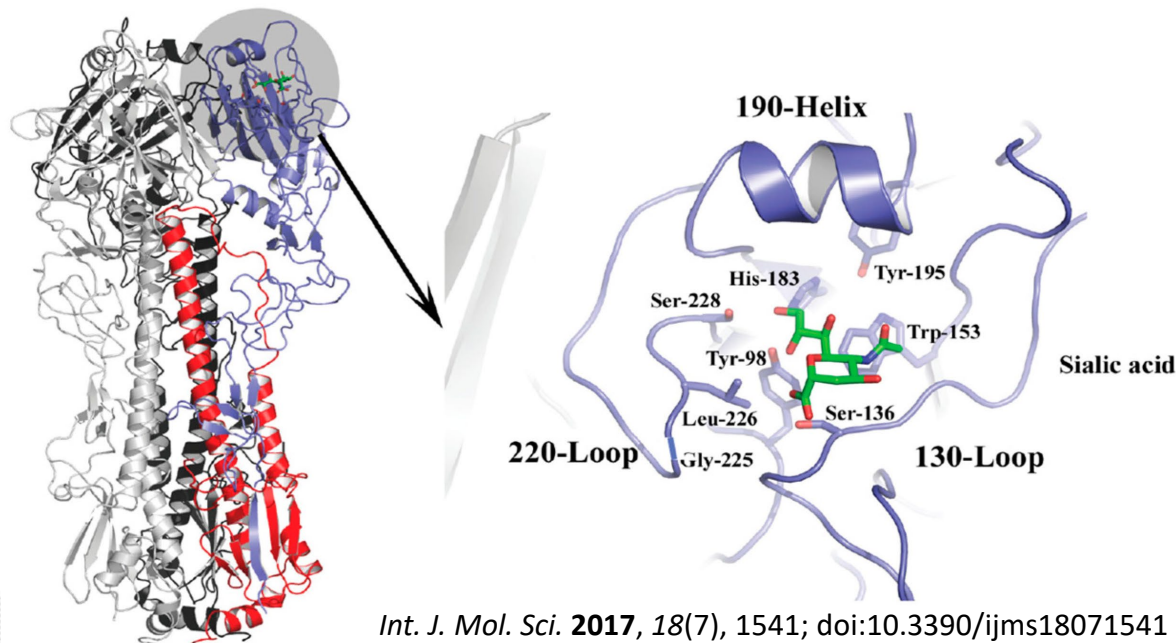
J Mol Genet Med(2009) 3:143-151



HA e sito recettoriale

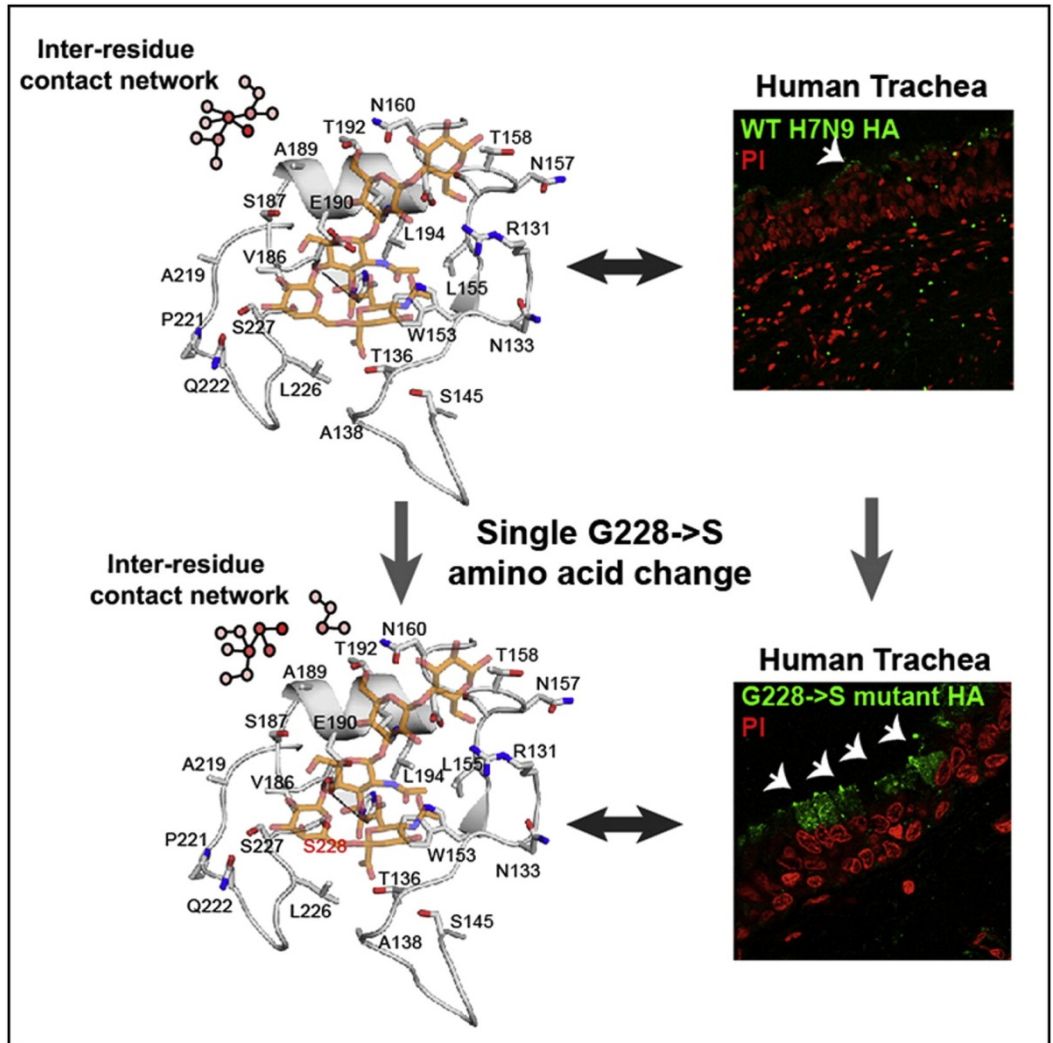
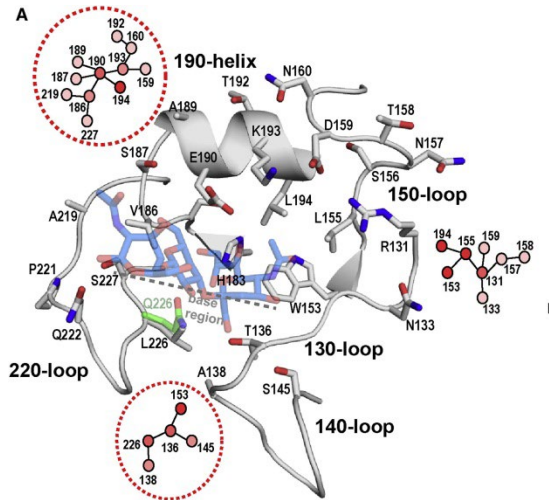


- Affinità tra proteina HA e recettore cellulare
- 2 tipi di recettori: SA α 2,3Gal – Virus aviari, equini
SA α 2,6Gal – Virus umani, suini
- Sito Recettoriale: 3 elementi strutturali: 130 loop, 220 loop e 190 helix
- Specificità recettori - mutazioni aa nella HA
 -  Leu226, Ser228 SA α 2,6Gal
 - Gln226, Gly228 SA α 2,3Gal (Vilnes et al, J Virol, 1998)
 -  E190D e D225G modificano specificità di recettore (SA α 2,6Gal)





Influenza A Virus H7N9 Hemagglutinin



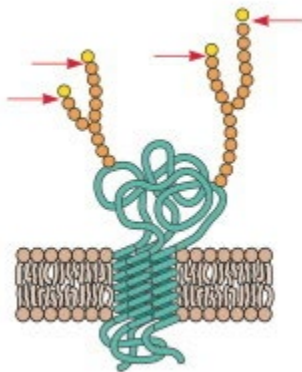


HA – NA balance

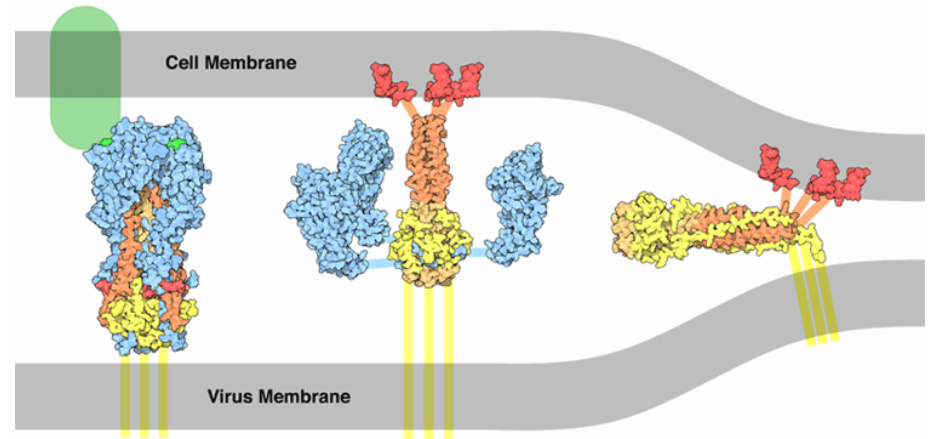


Necessaria per una efficiente trasmissione virale

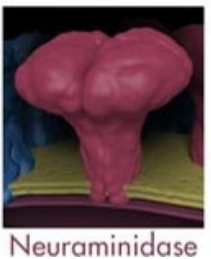
Utilizzano lo stesso recettore cellulare



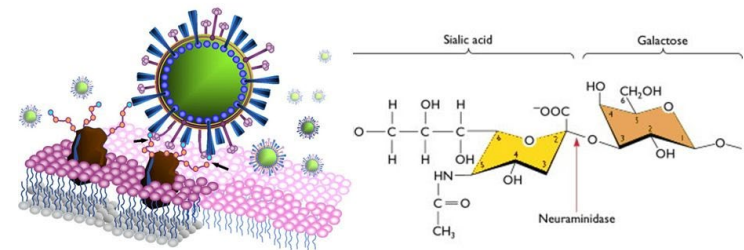
ENTRATA



Neuraminidase (NA) assists in viral exit

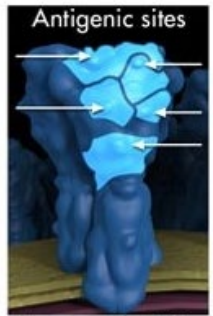


USCITA





HA – NA balance

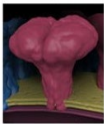


Hemagglutinin

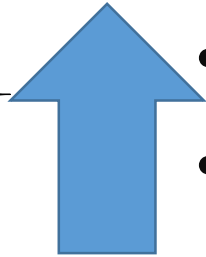
Neuraminidase



emagglutinin

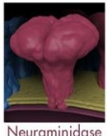


Neuraminidase



Efficient transmission:

- High HA avidity and high NA activity
- Low HA avidity and low NA activity



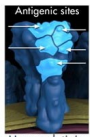
Neuraminidase

Hemagglutini

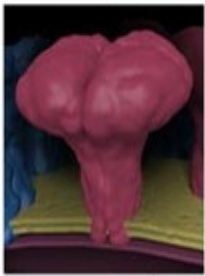


Not efficient transmission:

- Low HA avidity and high NA activity
- High HA avidity and low NA activity



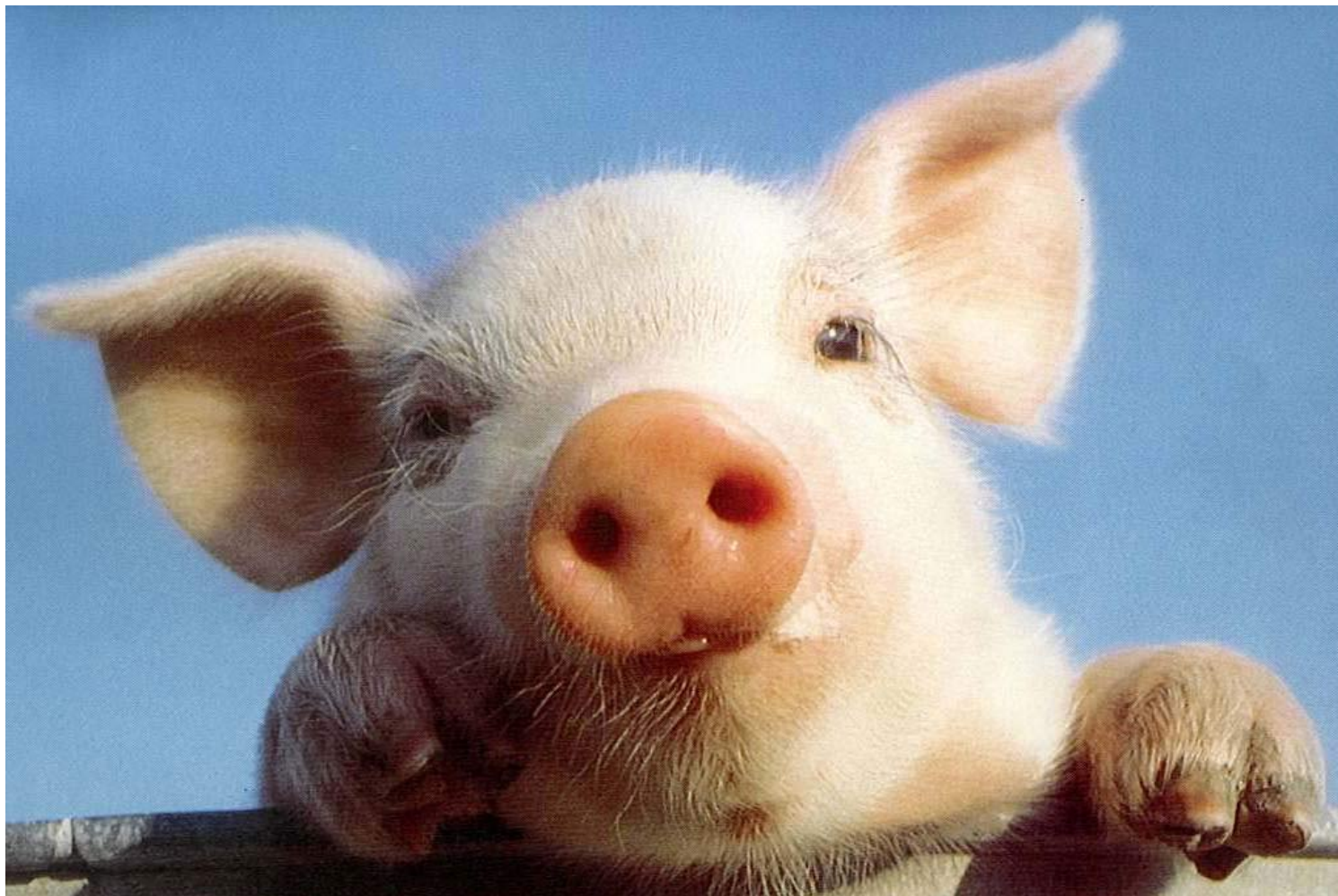
Hemagglutinin



Neuraminidase



Influenza suina



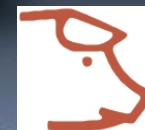


SIV, AIV: due lineaggi

Euroasiatico

Americano

H1N1, H3N2, H1N2 SIV



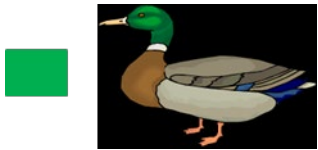
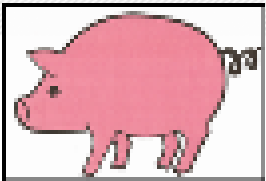
unico lineaggio



H1N1, H2N2, H3N2, H1N1pdm



European SVIs



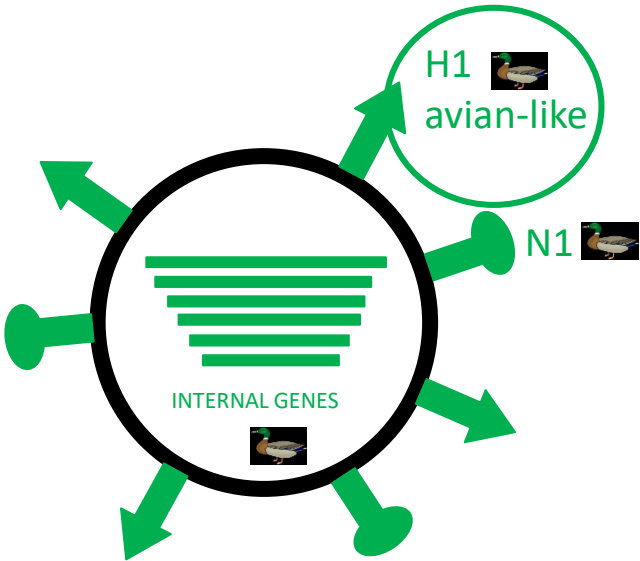
H1N1



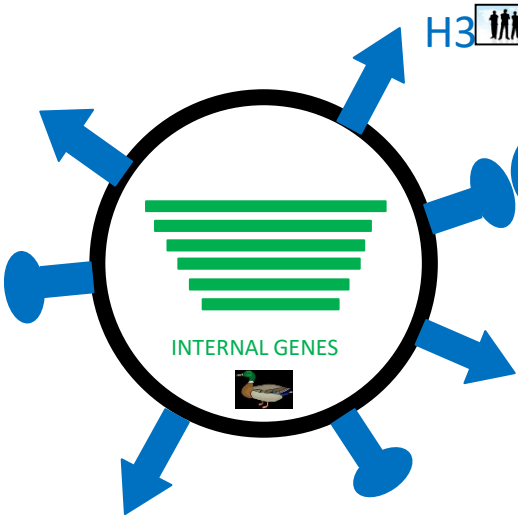
H3N2



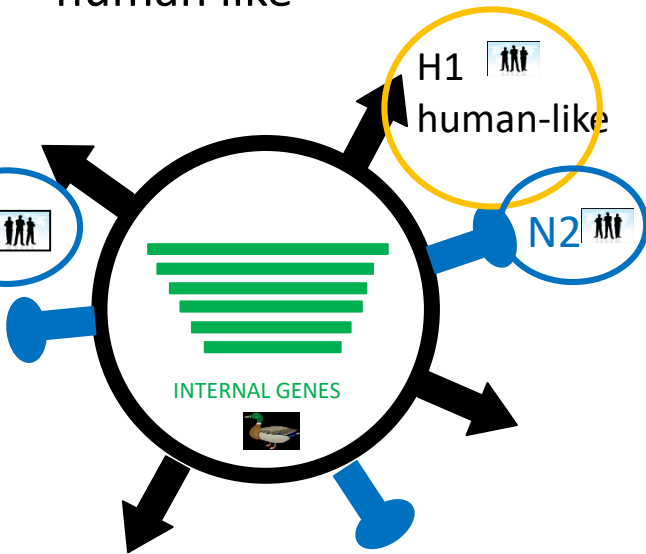
H1N1 avian like

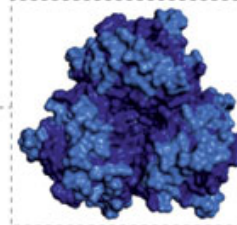


H3N2 reassortant human like



H1N2 reassortant human like

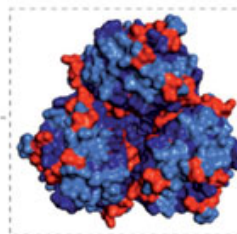




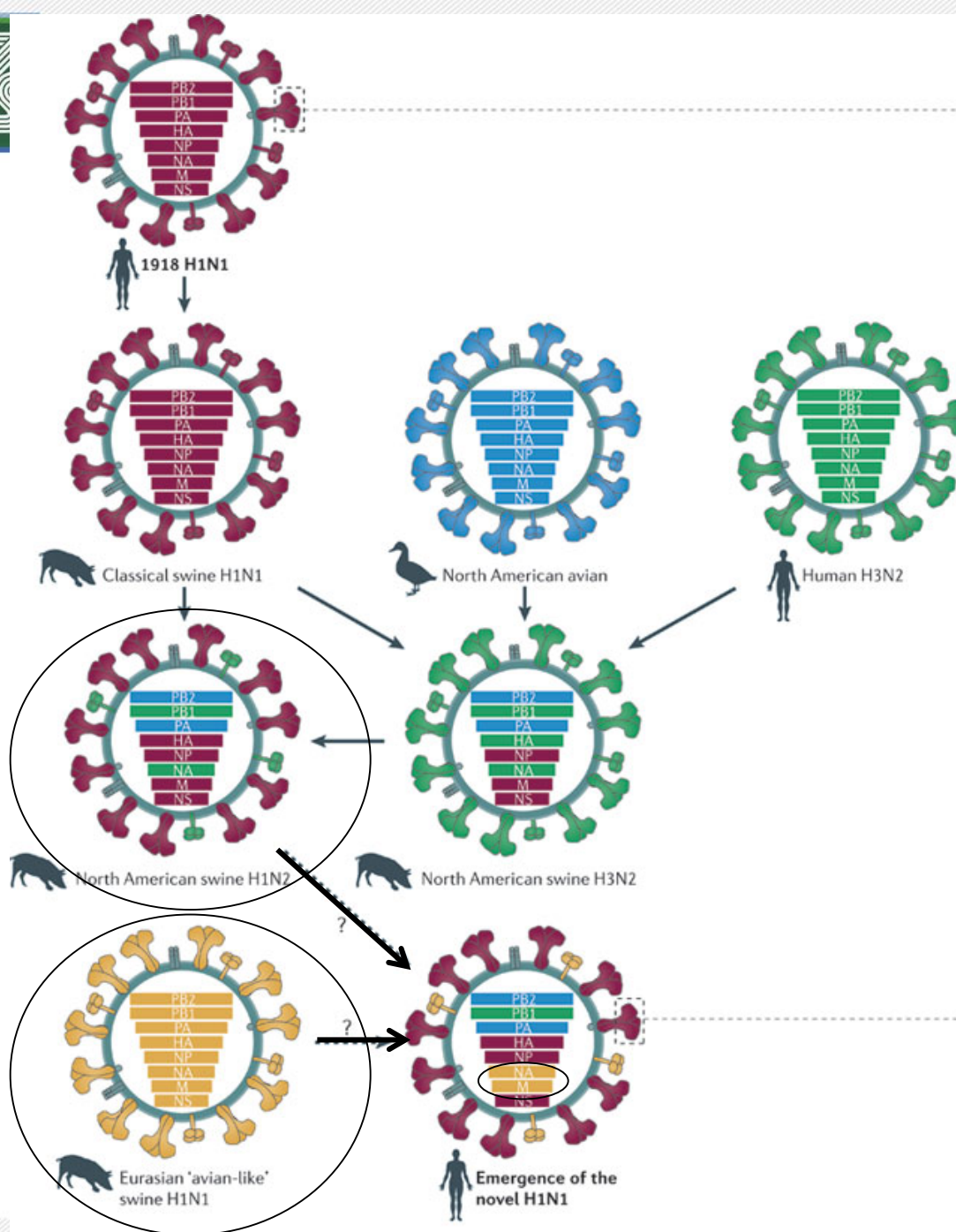
1918 H1N1 HA

2009H1N1 pdm

Antigenically similar HA
owing to lack of selection
pressure in pigs or drift in
pig-specific antigen sites



2009 H1N1 HA





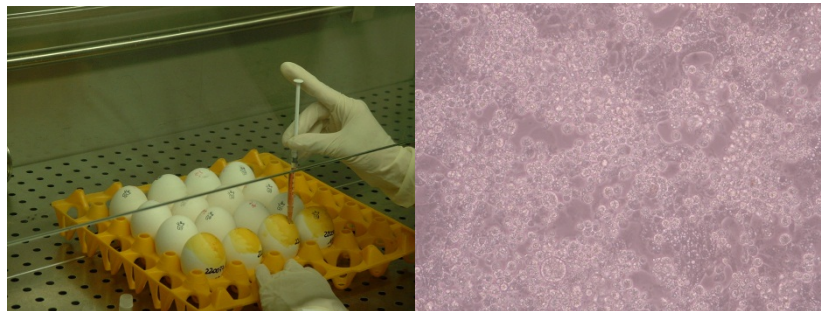
Piano di monitoraggio SIV dal 1998- 2018



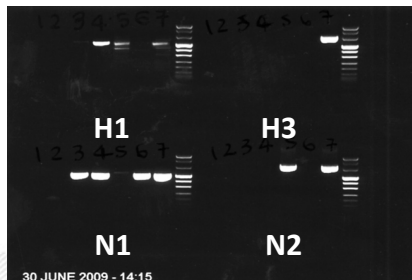
Campionamento: suini con forme respiratorie



Isolamento virale: UEP, colture cellulari (MDCK, Caco-2)

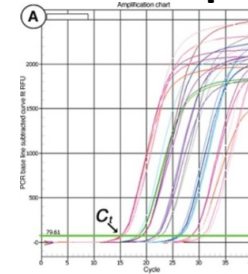


Tipizzazione genomica: multiplex RT-PCR (HA, NA) Chiapponi et al, J Virol met, 184:117-120, 2012

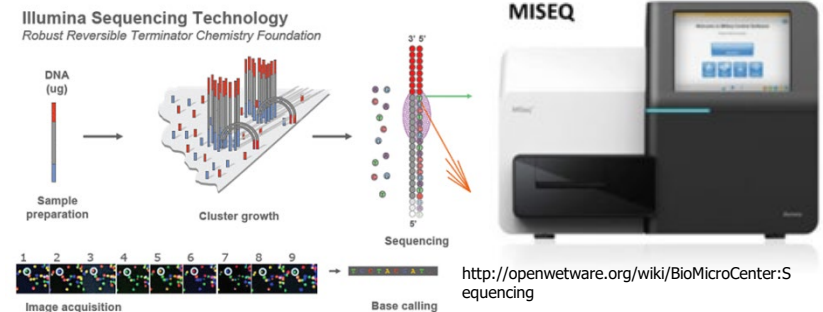


Screening: Real time RT-PCR (gene M) Spackman et al, J Clin Microbiol, 2002

Real time RT-PCR CDC per 2009 H1N1 pdm



Sequenziamento genoma completo: piattaforma NGS



Analisi filogenetica

- 1- EU SwIAV sequences obtained from the Influenza Research Database (<http://www.viprbrc.org/>)
- 2- Multiple sequence alignment using the MUSCLE algorithm.
- 3- Maximum likelihood phylogenetic tree performed using IQtree software and Model finder to determine the best model according to BIC



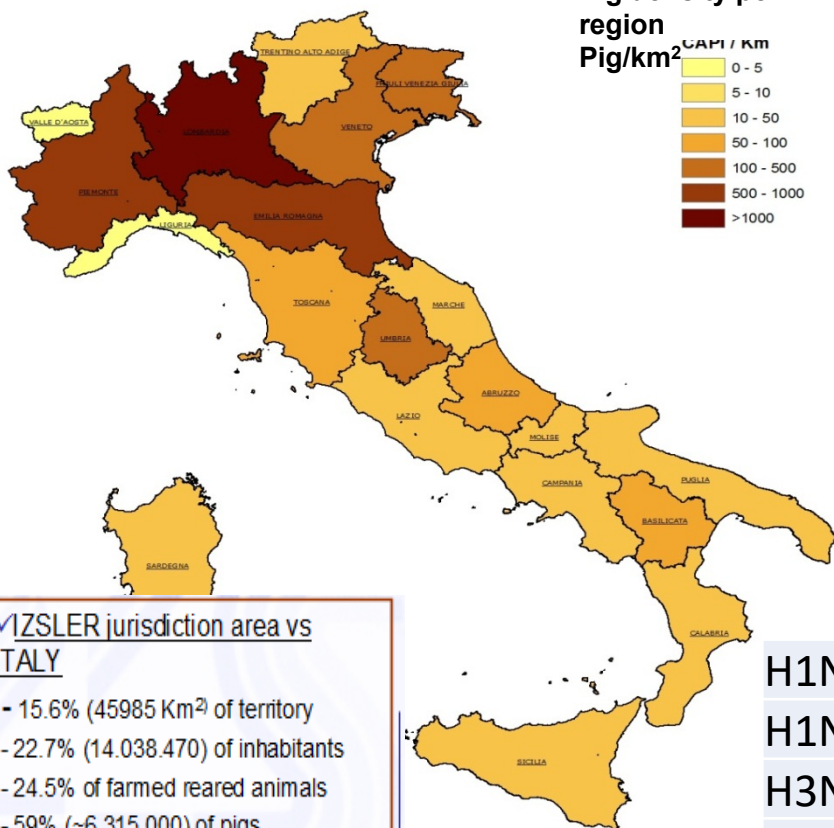
Distribuzione geografica degli allevamenti suini e dei ceppi SIV isolati nel periodo 1998-2018

Swine density (breeders and fattening pigs)

Pig density per region
Pig/km²

CAPI / Km

- 0 - 5
- 5 - 10
- 10 - 50
- 50 - 100
- 100 - 500
- 500 - 1000
- >1000



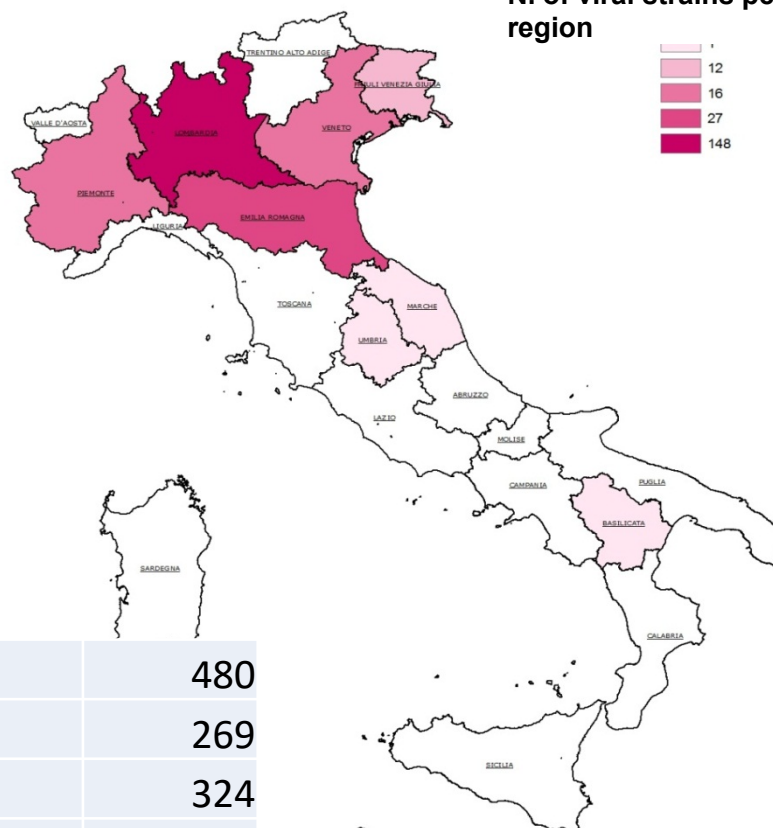
✓IZSLER jurisdiction area vs ITALY

- 15.6% (45985 Km²) of territory
- 22.7% (14.038.470) of inhabitants
- 24.5% of farmed reared animals
- 59% (~6.315.000) of pigs
- c.a. 40% of the GNP

Geographical distribution of swine influenza strains (SIVs)

N. of viral strains per region

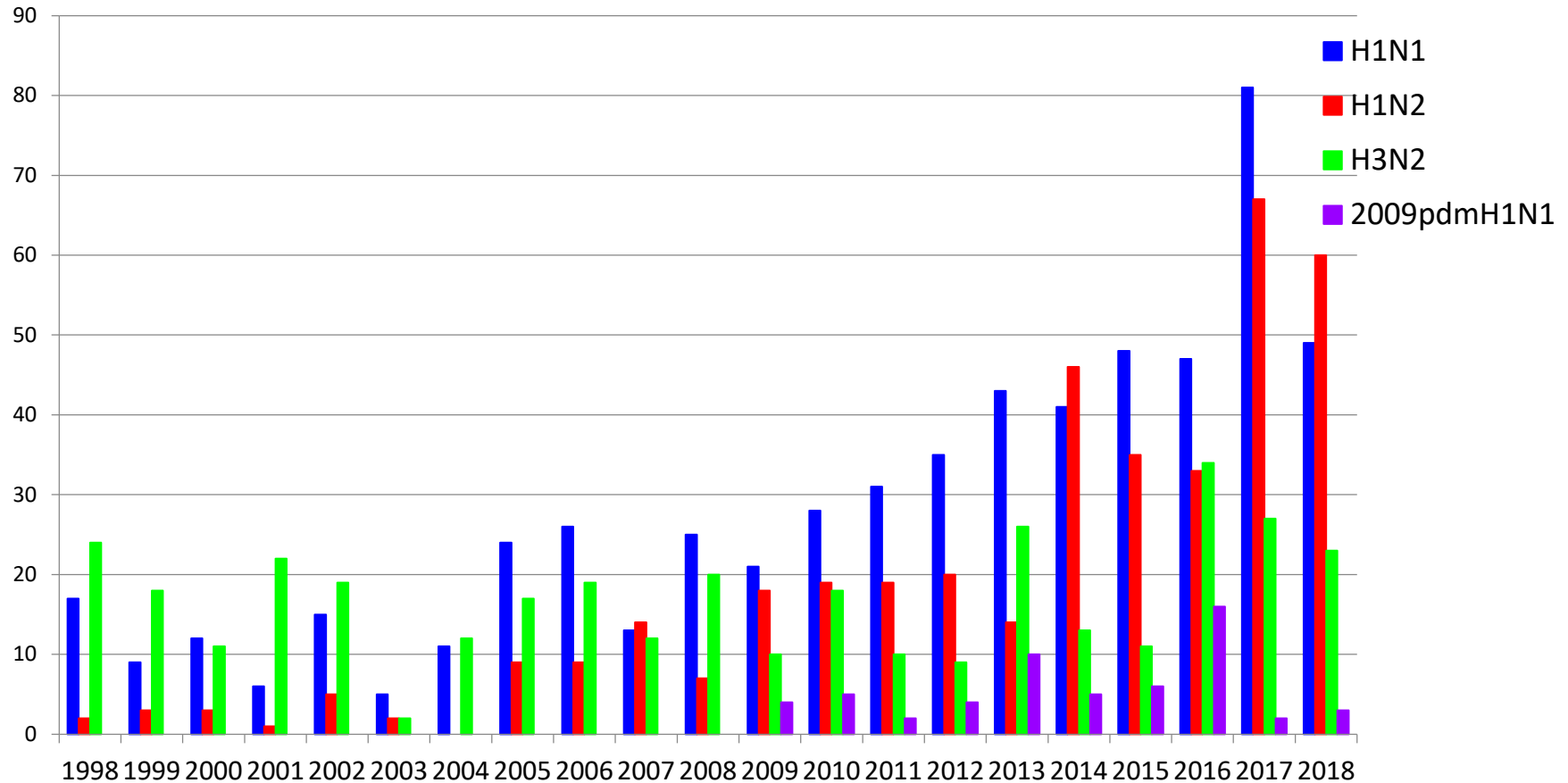
- 1
- 12
- 16
- 27
- 148



H1N1	480
H1N2	269
H3N2	324
H1N1pdm	53
total	1126



Ceppi SIV isolati nel periodo 1998-2018 divisi per anno e per sottotipo





Diversità genetica



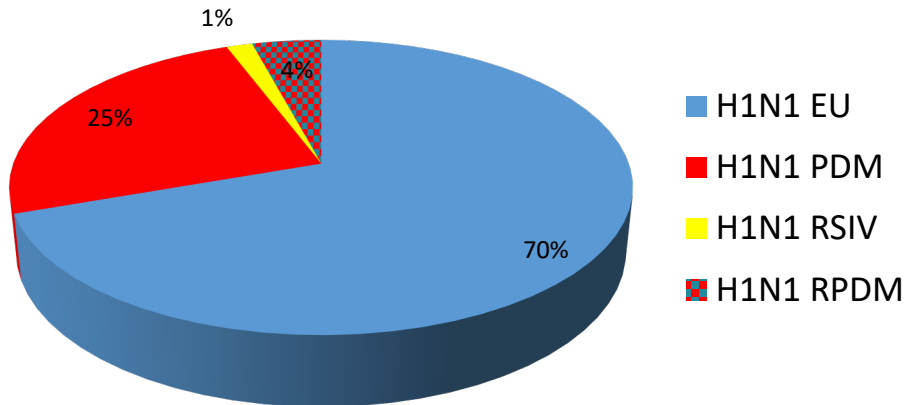
Subtype	Global H1clas	Genotype	Number of strains	PB2	PB1	PA	N	M	NS	HA	NA	Gene combination
H1N1	1C.2.1	1	133									EU Avian- like H1av N1
	1A.3.3.2	5	47									A(H1N1)pdm2009
	1C.2.1	8	5									EU Avian- like H1av N1 Rpdmd2009
	1B.1.2.2	16	3									EU Avian- like A/Sw/Italy/4675/2003-like H1hu N1
	1A.3.3.2	11	1									EU Avian- like RH1pdm2009 N1
	1C.2.1	17	1									EU Avian- like H1av N1 Rpdmd2009internal genes(IG)
	1A.3.3.2	23	1									A(H1N1)pdm2009- R EU avian like IG
H1N2	1B.1.2.1	21	9									EU A/Sw/Scotland/410440/94-like H1hu N2
	1B.1.2.2	3	74									EU A/Sw/Italy/4675/2003-like H1hu RN2hu
	1C.2.1	4	28									EU Avian- like H1av RN2sw
	1C.2.1	6	13									EU Avian- like H1av RN2hu
	1B.1.2.2	7	13									EU A/Sw/Italy/4675/2003-like H1huRN2 Rpdmd2009IG
	1A.3.3.2	18	8									A(H1N1)pdm2009 RN2sw
	1C.2.1	15	4									EU A/Sw/Scotland/410440/94-like RH1av N2
	1B.1.2.2	10	4									EU A/Sw/Italy/4675/2003-like H1hu RN2sw
	1.B.1.2.1	24	1									EU A/Sw/Scotland/410440/94-like H1hu RN2
	1C.2.1	18	2									EU H1av RN2sw pdm2009 IG
	1C.2.1	22	4									EU H1av RN2sw pdm2009 IG
	1B.1.2.2	14	2									EU A/Sw/Italy/4675/2003-like H1hu RN2Scot-94
	1.B.1.2.1	19	3									EU A/Sw/Scotland/410440/94-like H1hu RN2sw
	1C.2.1	20	1									EU Avian- like H1av RN2Scot-94 Rpdmd2009
	1C.2.1	25	1									EU Avian- like H1av RN2sw Rpdmd2009
H3N2		2	85									EU A/Sw/Gent/1/1984 -like H3N2
		12	1									EU A/Sw/Gent/1/1984 -like H3N2 Rpdmd2009
		tot	444									



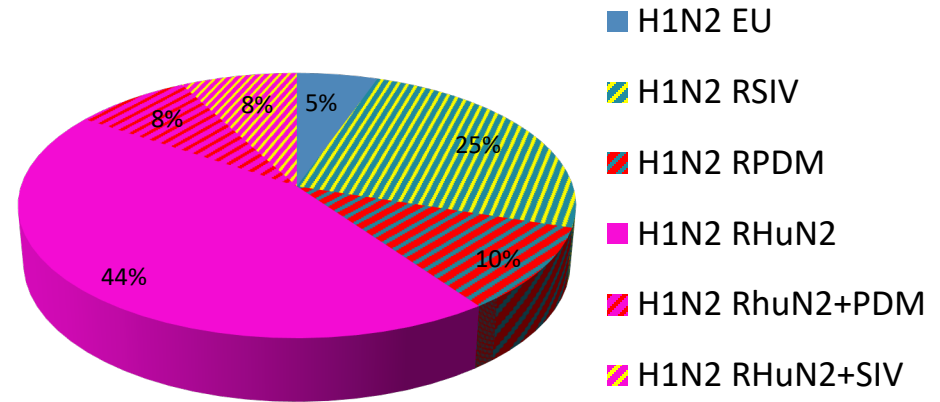
Eventi di riassortimento – combinazioni di geni 1998-2018 (444 ceppi)



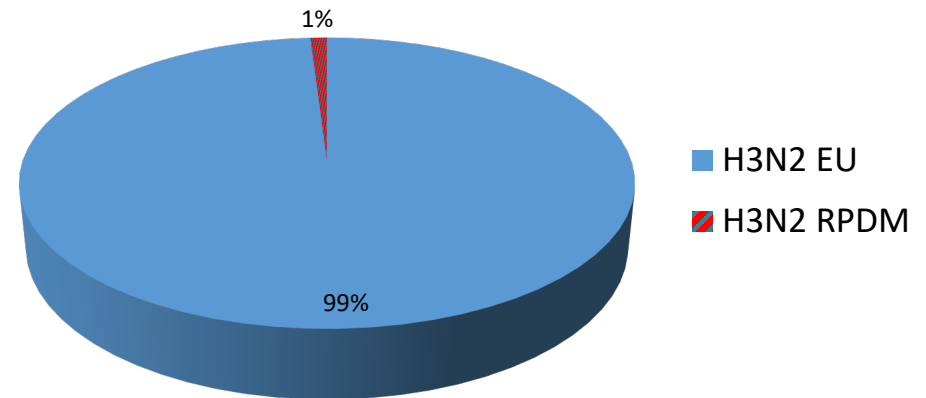
H1N1



H1N2



H3N2



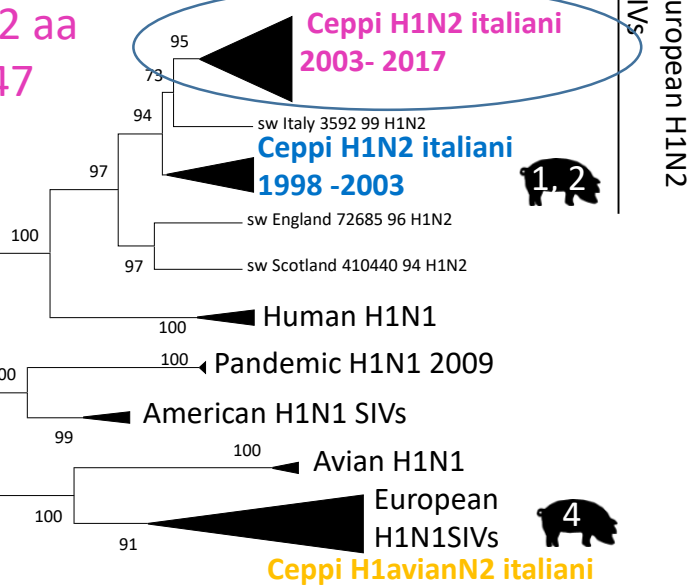


H1N2 Analisi filogenetica dei geni HA e NA



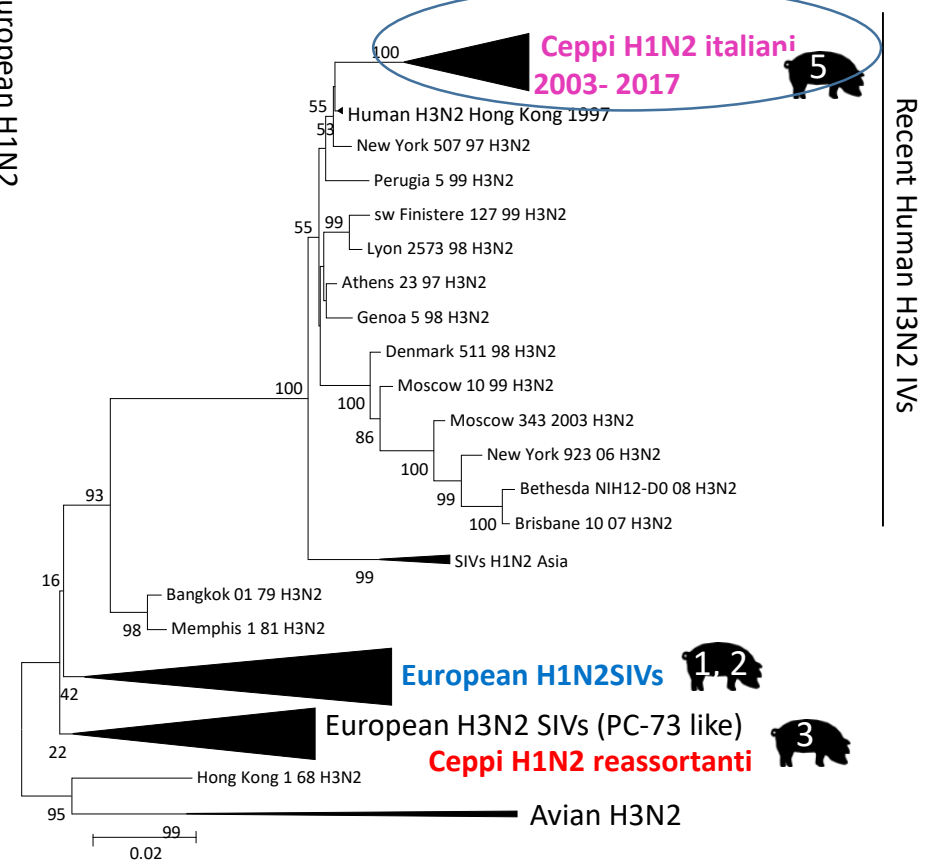
HA gene

Delezione 2 aa
pos 146-147
nella HA



0.05

NA gene



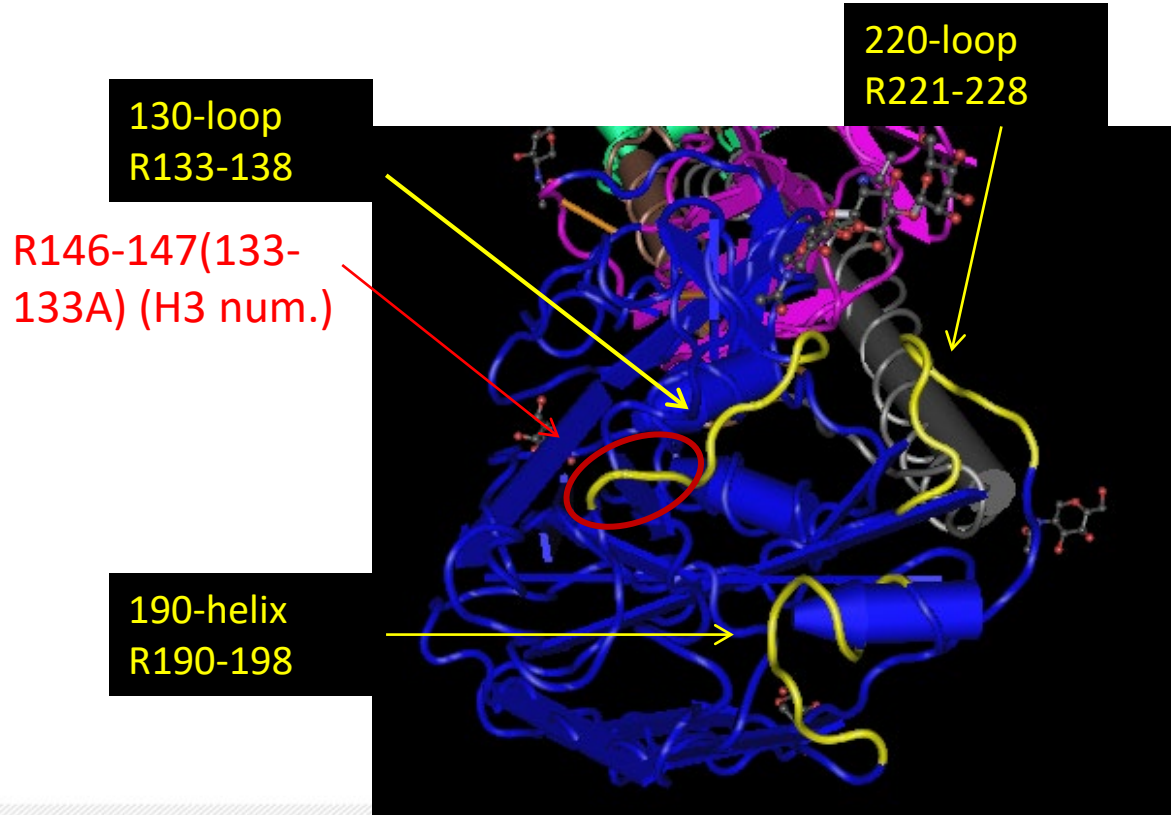
0.02



3D-MMS HA monomero



Receptor binding domain (Ha et al., 2002)





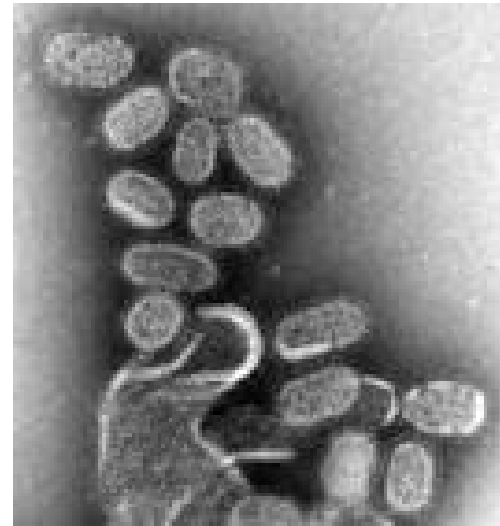
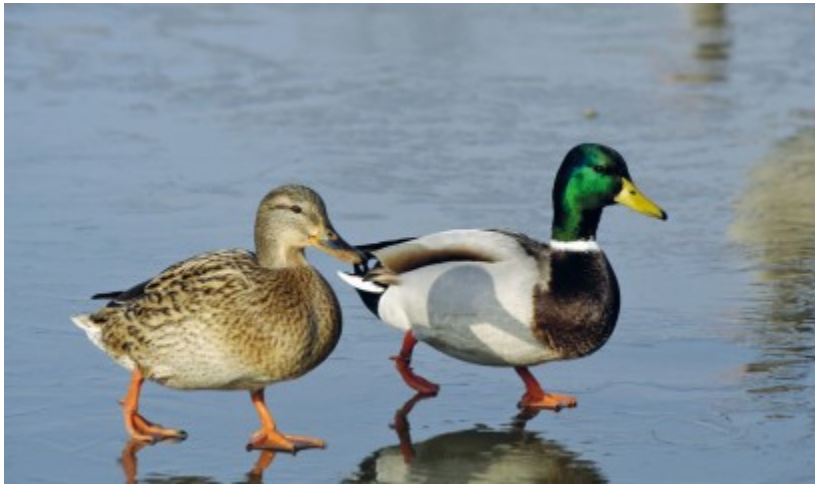
Eventi di riassortimento



- Sequenziamento genoma completo di 444 ceppi (1998-2018)
- Eventi di riassortimento multipli tra ceppi umani e suini
- Non coinvolti ceppi aviari
- Diversi pattern di riassortimento
- H1N2 sottotipo più sottoposto a fenomeni di riassortimento
- H3N2 più stabile
- Recente comparsa di diversi fen riassortimento tra ceppi H1N1, H1N2 e ceppi H1N1pdm



Influenza aviare





Influenza aviare: due malattie



Distinte per:

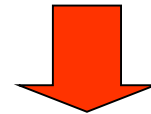
- Sintomatologia clinica indotta nei volatili
- Caratteristiche degli stipiti virali:



Influenza aviaria a
bassa patogenicità
(LPAI)



Influenza aviaria ad
alta patogenicità
(HPAI)



H5, H7



Virus a bassa patogenicità



Possiedono un solo aminoacido basico nel sito di “*cleavage*”

Es. sequenze aminoacidiche H7:

-PEIPKGR*GLF-,

-PENPKGR*GLF-

Virus ad alta patogenicità

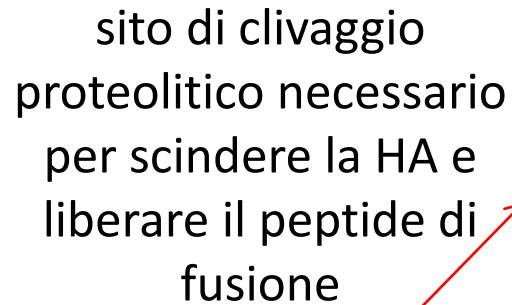


Possiedono più aminoacidi basici nel sito di “*cleavage*”

Es. sequenze aminoacidiche H7:

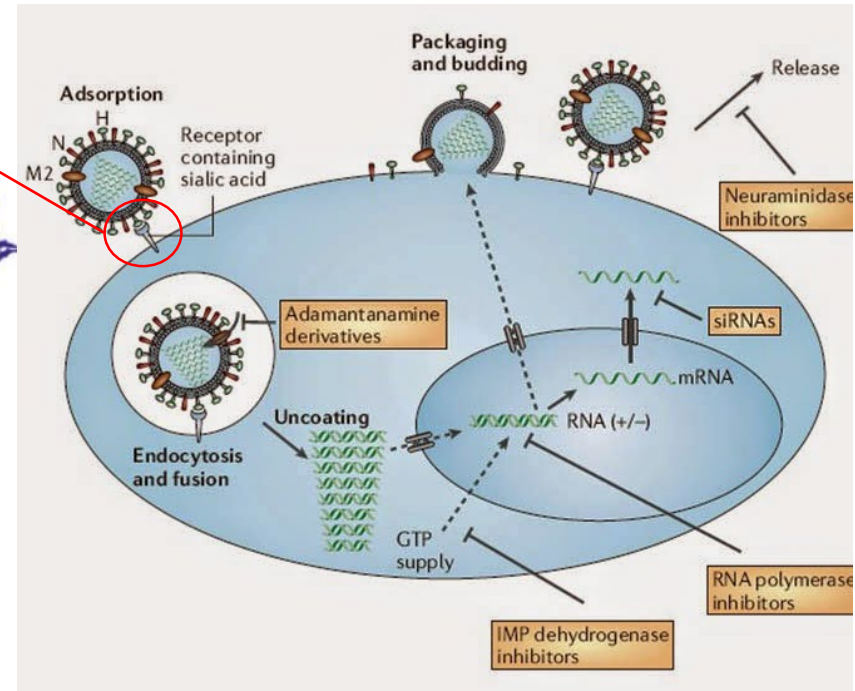
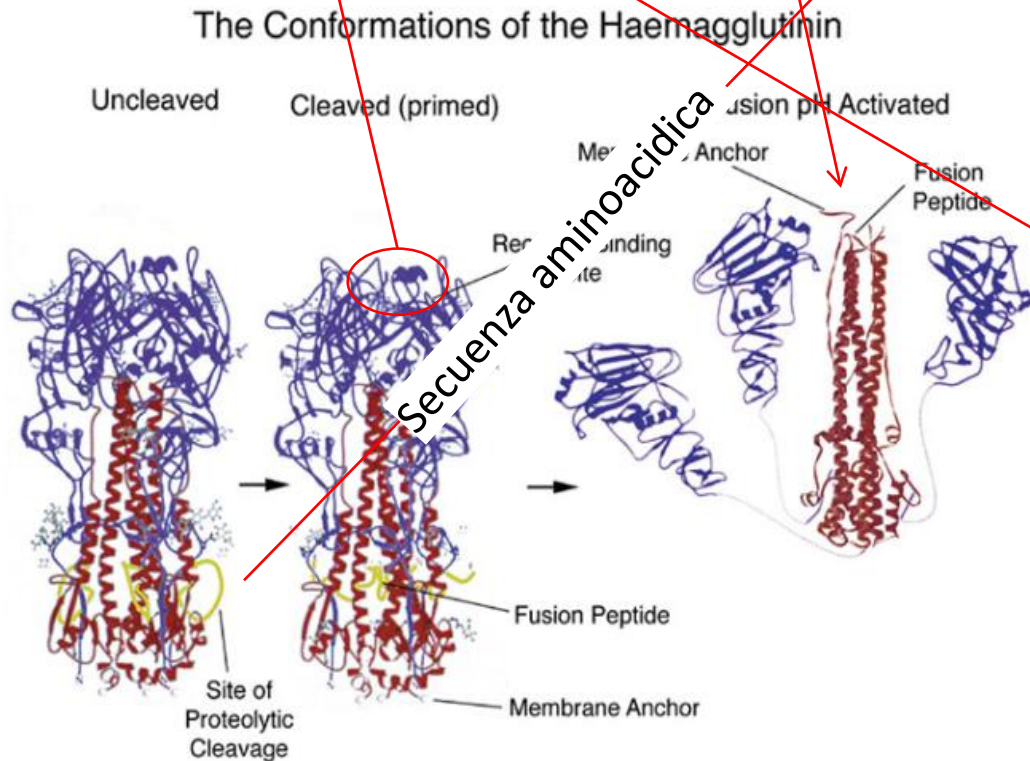
-PEIPKKKKR*GLF-, -
PETPKRKRKR*GLF-,

-PEIPKKREKR*GLF-, -
PETPKRRRR*GLF-



BASSA: enzimi di tipo tripsinico
nelle mucose respiratorie e
digerenti

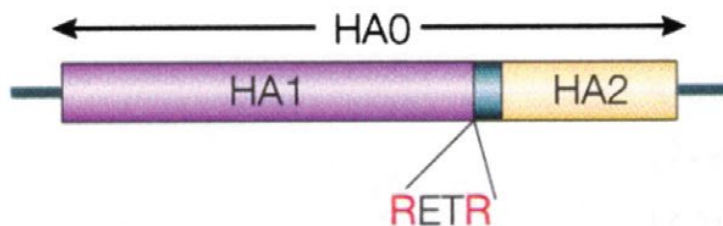
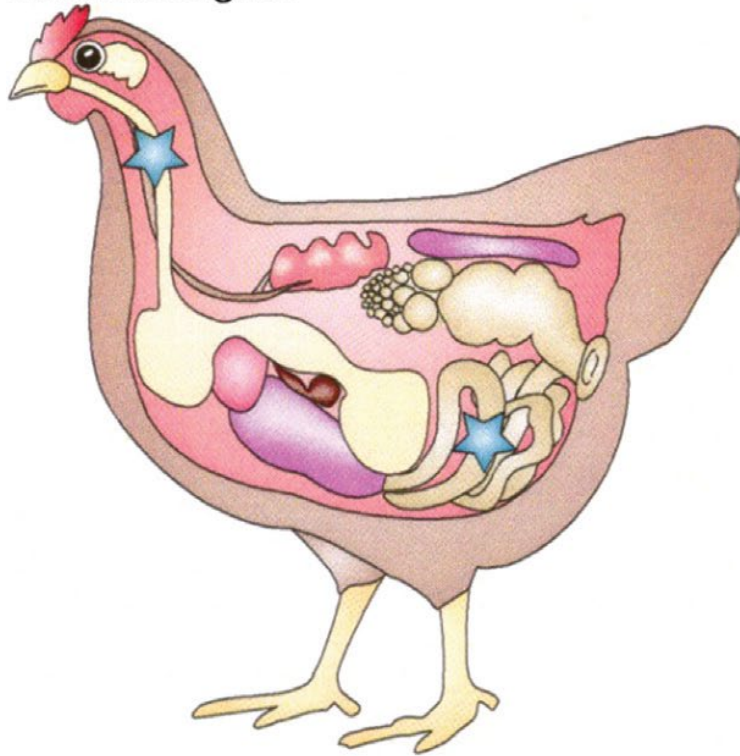
ALTA: enzimi presenti in tutti i tessuti





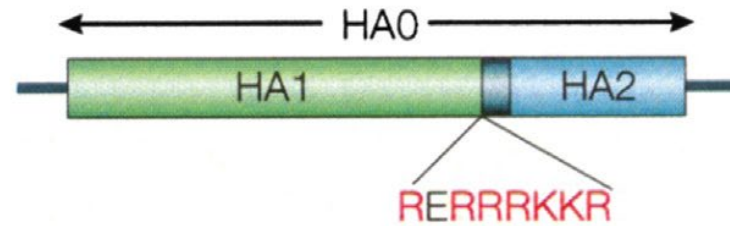
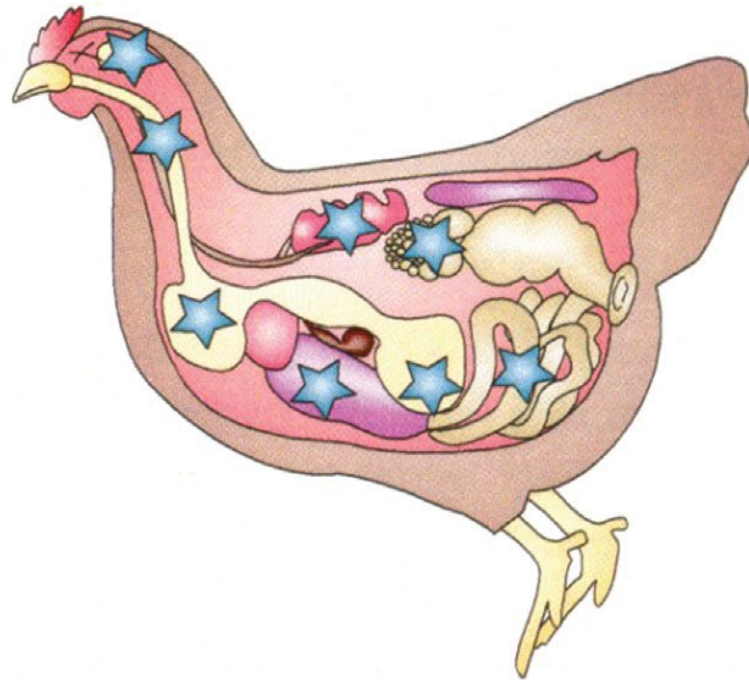
LPAI

Proteases localized in respiratory and intestinal organs



HPAI

Ubiquitous proteases

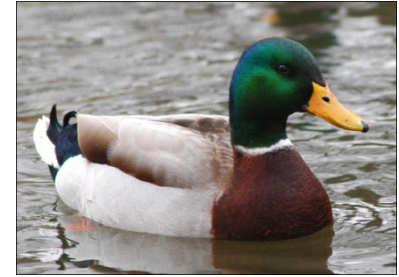




Virus influenzali H5, H7



- Uccelli acquatici selvatici: virus a bassa patogenicità
- Pollame domestico: adattamento dei virus influenzali
 - HA addizione siti di glicosilazione
 - NA delezione aminoacidi
- Virus H5 e H7: variazione della patogenicità (drift antigenico)



Influenza aviaria a bassa virulenza (H5 e H7) LPAI

Influenza aviaria ad alta virulenza (HPAI)





Disposizioni legislative



DIR 2005/94/CEE

Influenza aviaria - infezione del pollame o altri volatili causata da virus influenzale A:

- sottotipi H5, H7
- IVPI > 1,2 nei pulcini di 6 sett



Manuale OIE maggio 2012

- **Notifiable Avian Influenza Virus (NAI)**
HPAIV, tutti AIV H5 e H7
- **LPAIV – tutti i virus non NAI**





H7N1 LPAI – 1999 (tacchini)





H7N1 HPAI -1999-2000 (tacchini)





HPAIV H5N8





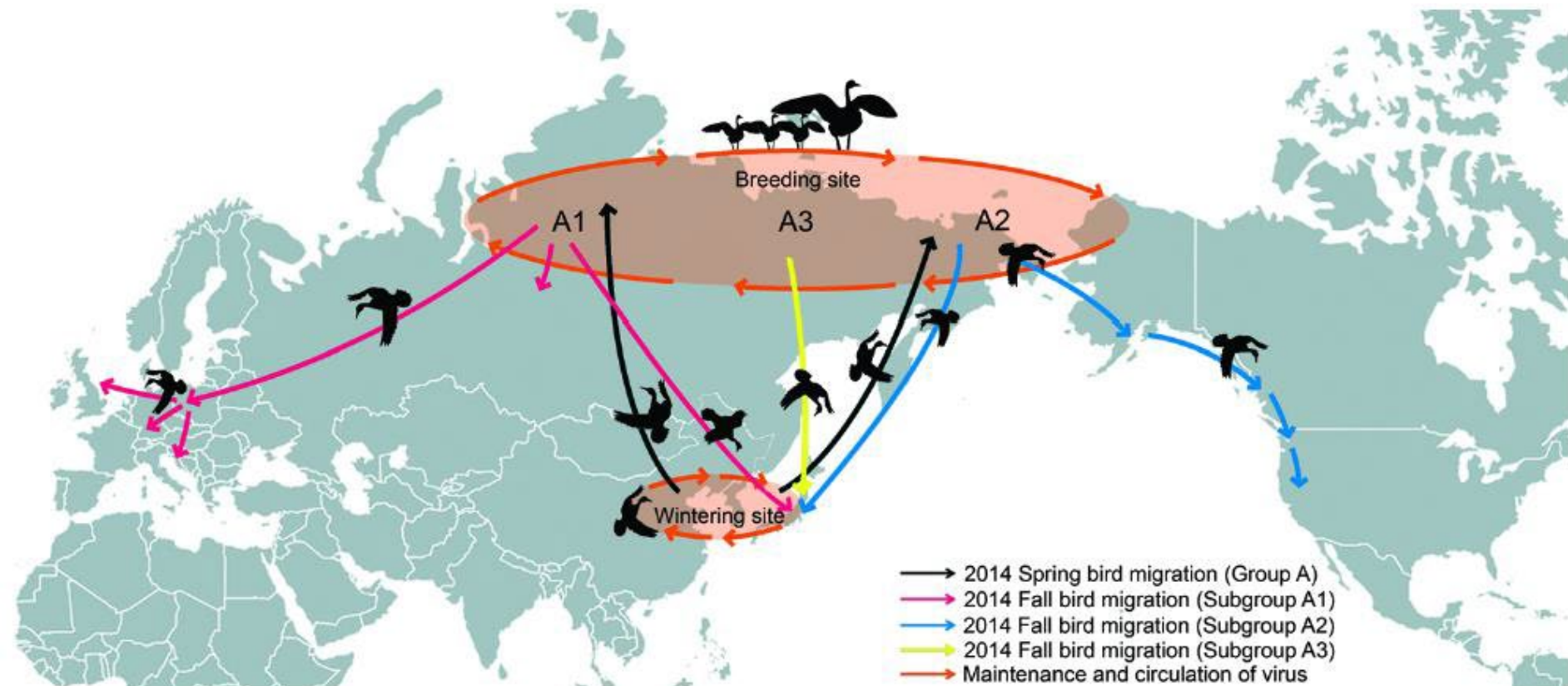
HPAIV H5N8



- January 2014, novel reassortant HPAI viruses of subtype H5N8
- Asian-lineage subtype H5 highly pathogenic avian influenza (HPAI) clade 2.3.4.4, as is the case for A(H5N6) and A(H5N1) viruses from Asia
- The viruses have been detected in captured and apparently healthy wild migratory birds and dead wild birds as well as in domestic chickens, geese and ducks
- Isolated from free-living wild birds of the orders Accipitriforme, Anseriforme, Charadriiforme, Falconiforme and Gruiforme
- Low to moderate virulence in mammals



Indicative transmission routes of HPAI A(H5N8) through birds migrating into Europe

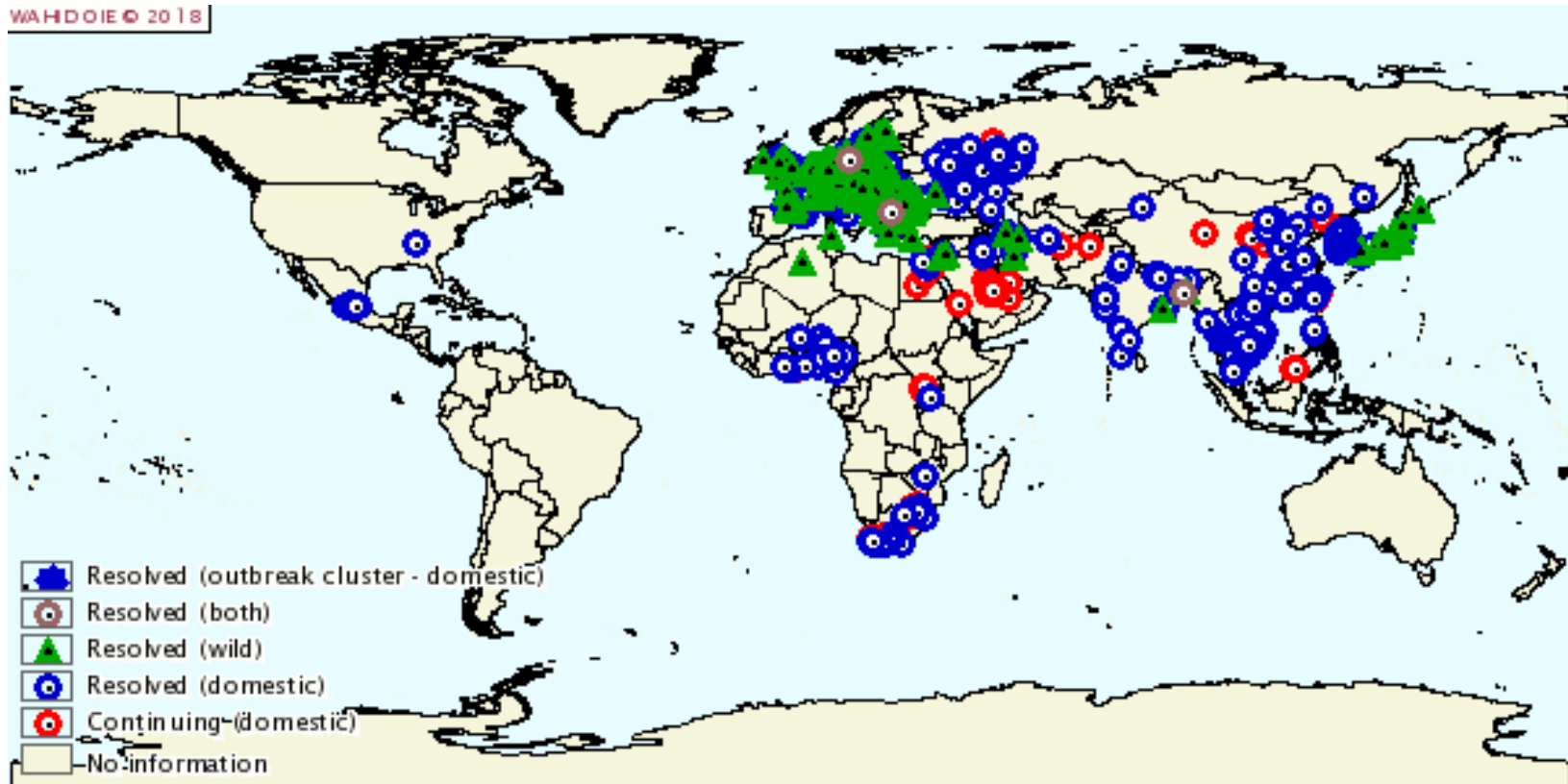




HPAIV 1-01-2017/10-2018



WHA/DOIE © 2018





05/02/2018

Legenda

Positività HPAI H5N5

- Allevamento Industriale
- Allevamento Rurale
- Selvatico
- In cattività

Positività HPAI H5N8

- Allevamento Industriale
- Allevamento Rurale
- Selvatico
- In cattività

Positività HPAI H5

- Allevamento Rurale
- Selvatico

0 50 100 200 300 Km

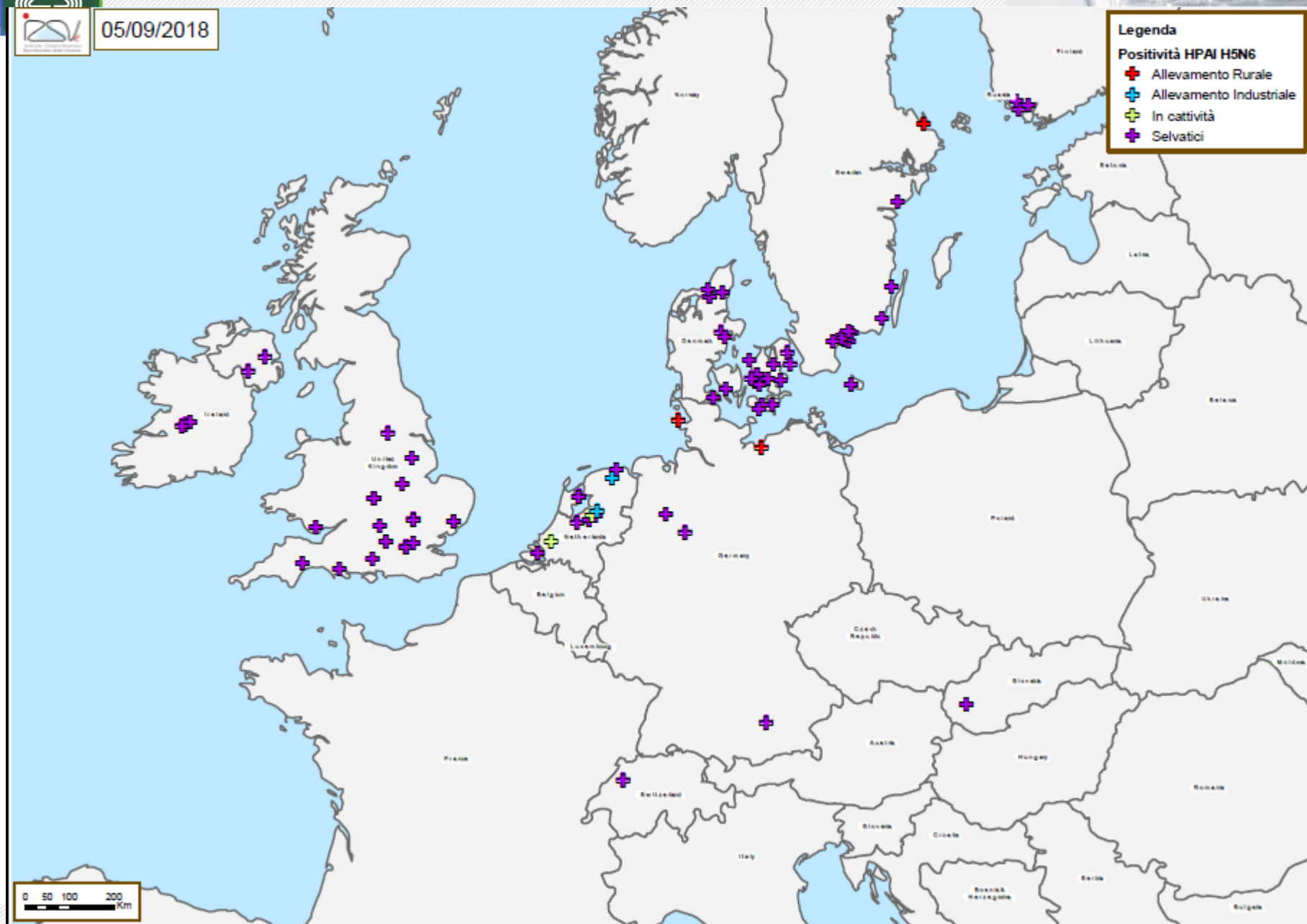


05/09/2018

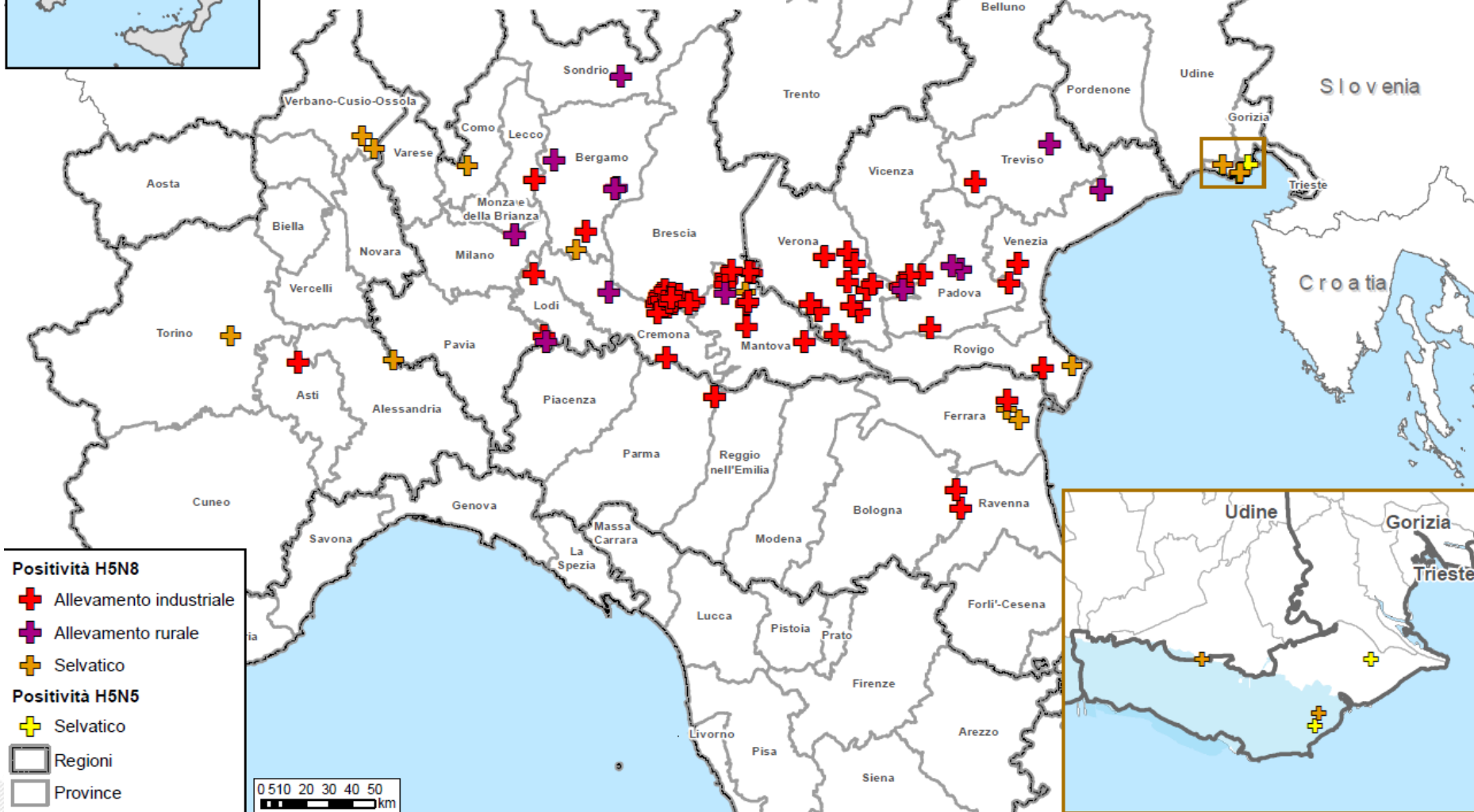
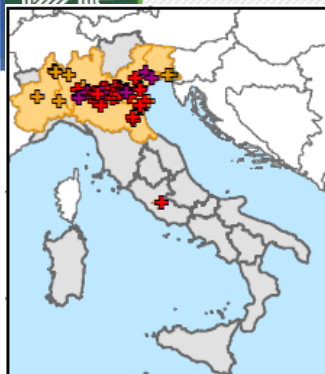
Legenda

Positività HPAI H5N6

- Allevamento Rurale
- Allevamento Industriale
- In cattività
- Selvatici



83 focolai: 47 Lombardia
27 Veneto
5 Emilia Romagna
2 Piemonte
1 Lazio
1 FVG





Avian influenza – human cases



How Infected Backyard Poultry Could Spread Bird Flu to People

Human Infections with Bird Flu Viruses Rare But Possible

1 Direct Contact

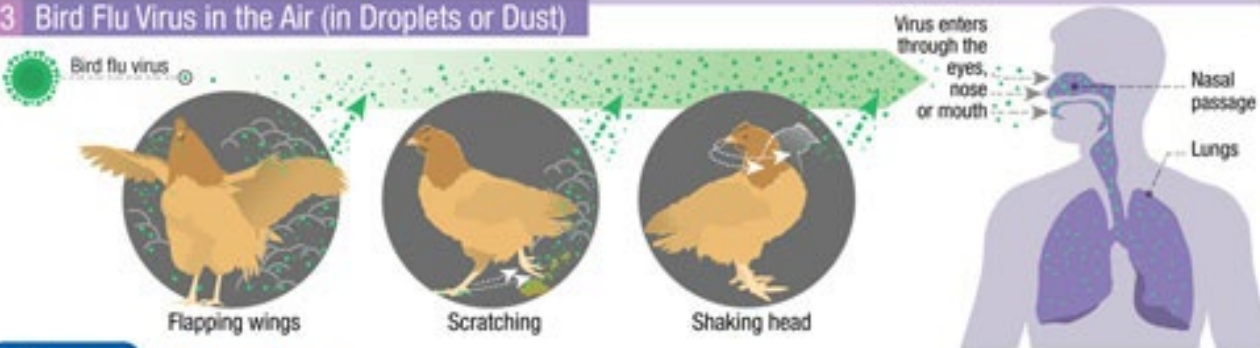
(Most Common)



2 Contaminated Surfaces



3 Bird Flu Virus in the Air (in Droplets or Dust)



U.S. Department of
Health and Human Services
Centers for Disease
Control and Prevention

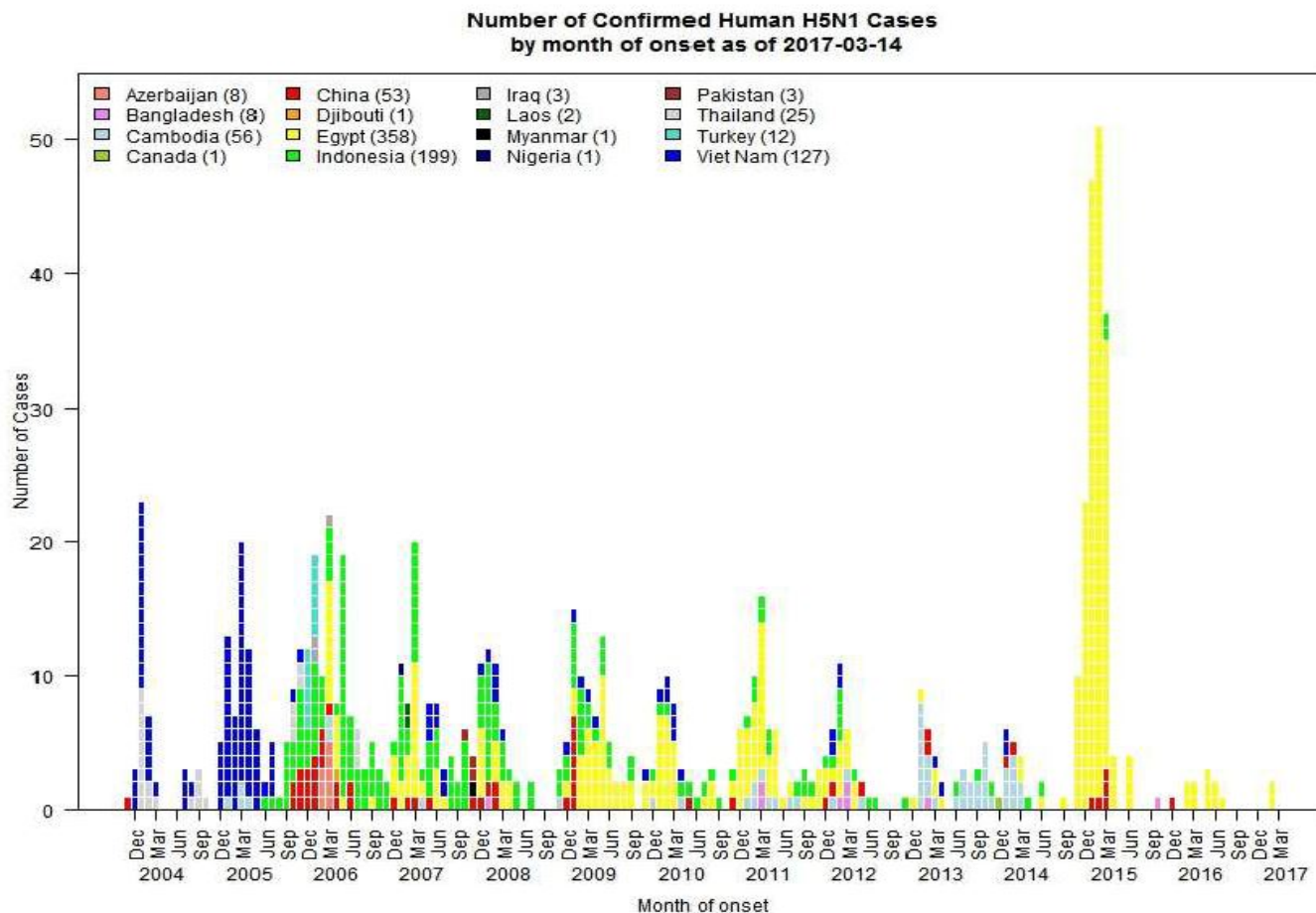
www.cdc.gov/flu/avianflu/avian-in-humans.htm

50091150



Casi Umani – dati WHO

- **H5N1**
- Dal 2003 a aprile 2017: 858 casi confermati con 453 morti in 16 paesi
- Da Gennaio 2018: Nessun nuovo caso
- **H5N6**: Dal 2014 19 casi con 6 morti





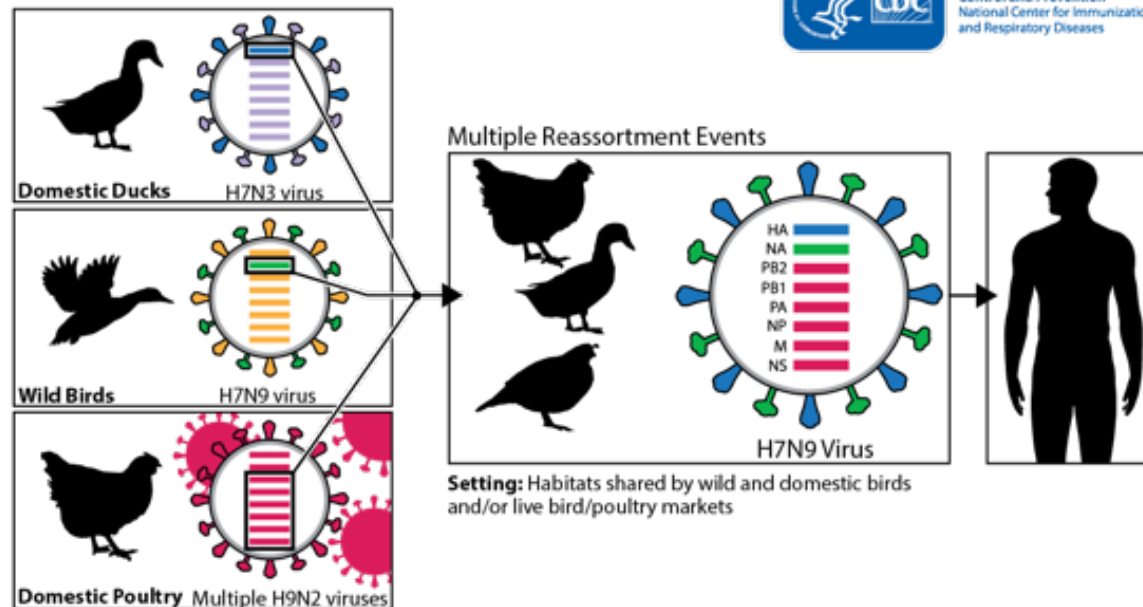
H7N9

- Dal 2013 a ottobre 2018: 1567 casi confermati, 615 morti (dati WHO)
- Nessun caso da settembre 2018

Genetic Evolution of H7N9 Virus in China, 2013



Centers for Disease
Control and Prevention
National Center for Immunization
and Respiratory Diseases



The eight genes of the H7N9 virus are closely related to avian influenza viruses found in domestic ducks, wild birds and domestic poultry in Asia. The virus likely emerged from "reassortment," a process in which two or more influenza viruses co-infect a single host and exchange genes. This can result in the creation of a new influenza virus. Experts think multiple reassortment events led to the creation of the H7N9 virus. These events may have occurred in habitats shared by wild and domestic birds and/or in live bird/poultry markets, where different species of birds are bought and sold for food. As the above diagram shows, the H7N9 virus likely obtained its HA (hemagglutinin) gene from domestic ducks, its NA (neuraminidase) gene from wild birds, and its six remaining genes from multiple related H9N2 influenza viruses in domestic poultry.

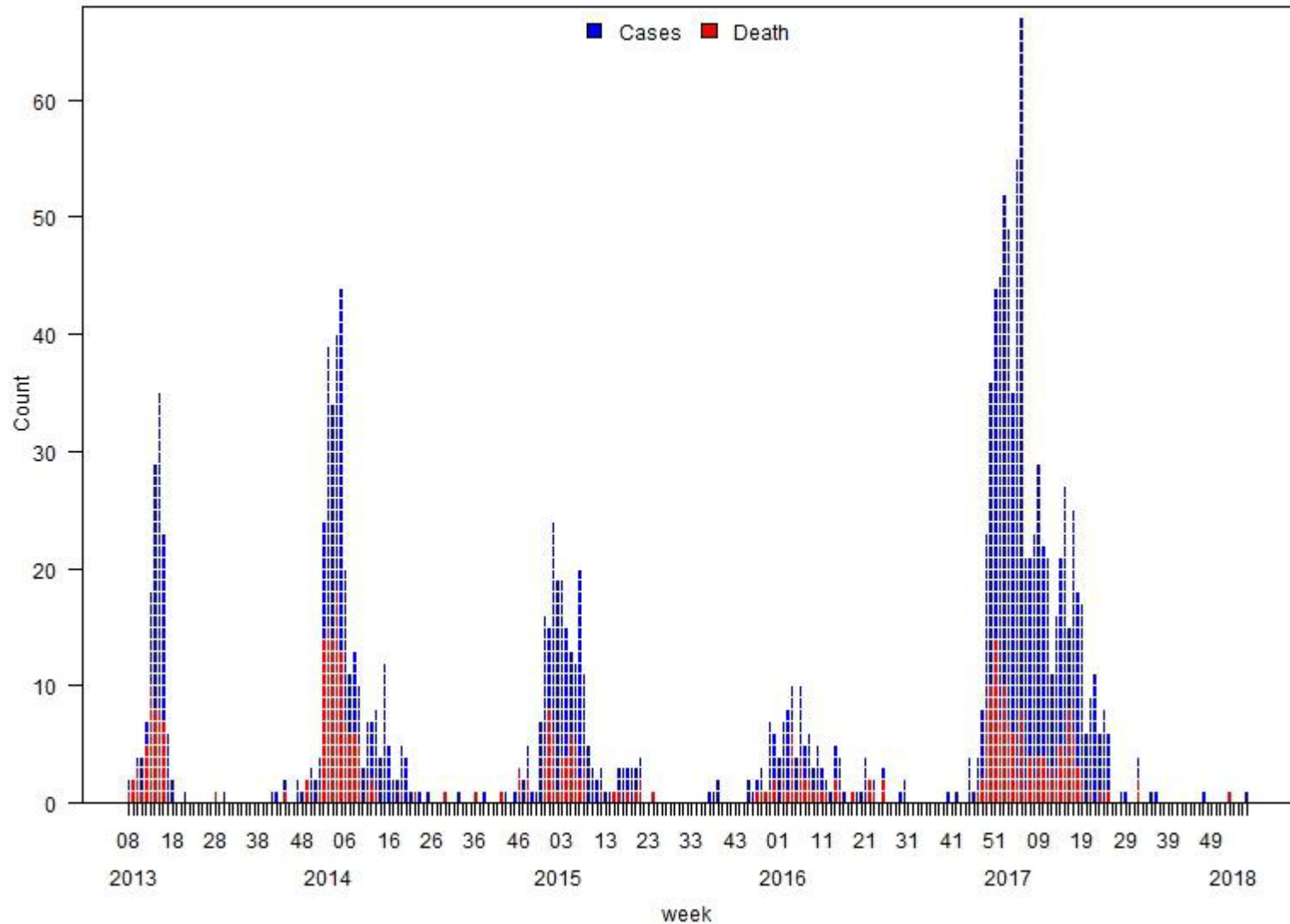


H7N9 casi umani





Epidemiological curve of avian influenza A(H7N9) cases in humans by week of onset, 2013-2018.





Live bird markets – mixing species





Cultural practices





Specific farming systems





Gastronomic culture: raw duck blood





Conclusioni



- Influenza aviare sottotipi H5, H7:
 - HPAI e LPAI
 - denuncia obbligatoria
 - Abbattimento di tutti i soggetti sani e infetti presenti nella azienda infetta
- Influenza suina:
 - Eventi di riassortimento multipli
 - Coinvolgimento di ceppi suini e umani ma non aviari

Grazie per l'attenzione



Sezione Diagnostica Brescia
Sezione di Mantova
Sezione di Parma
Sezione di Reggio Emilia
Sezione di Forlì



ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DELLA LOMBARDIA E DELL'EMILIA ROMAGNA
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DI MILANO



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