



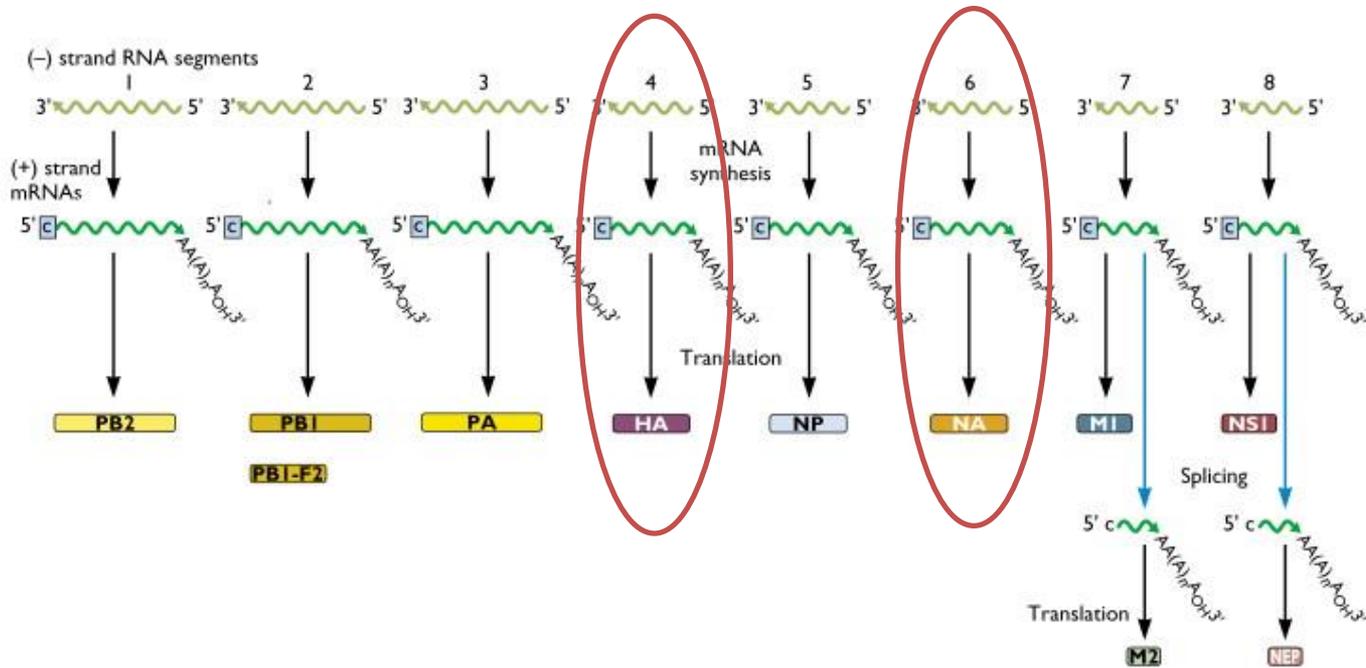
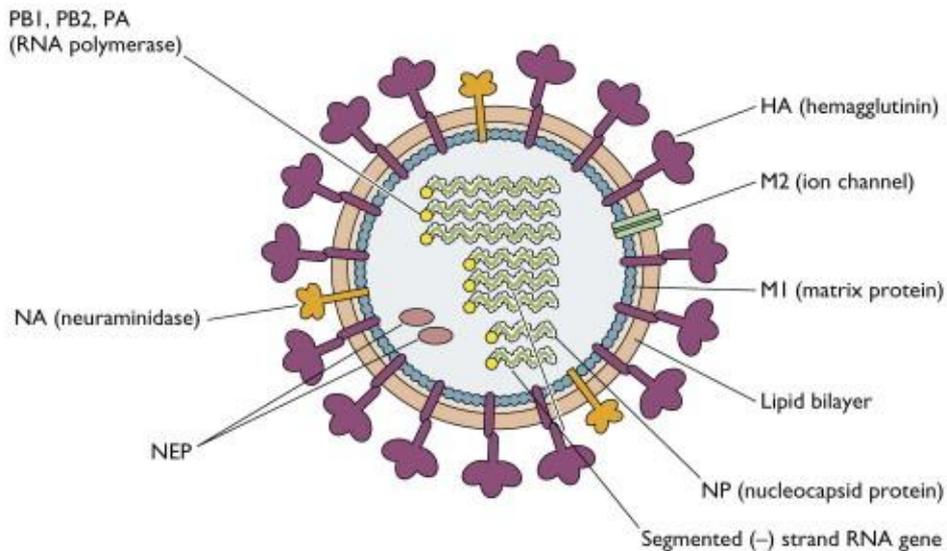
Virus influenzale del suino. Metodologie di studio e quadro epidemiologico



Chiara Chiapponi-IZSLER SEZIONE DI PARMA
Mantova 01/03/2019

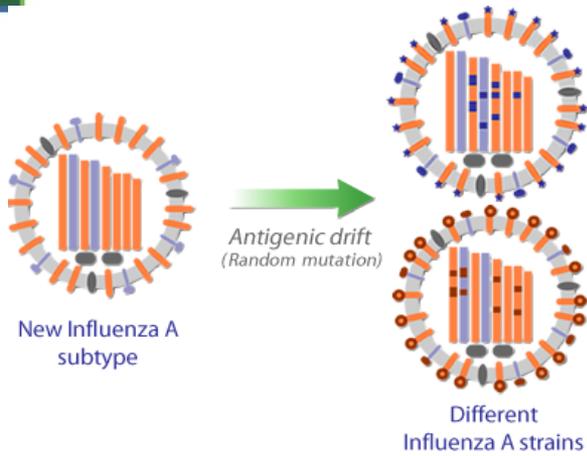


IAV

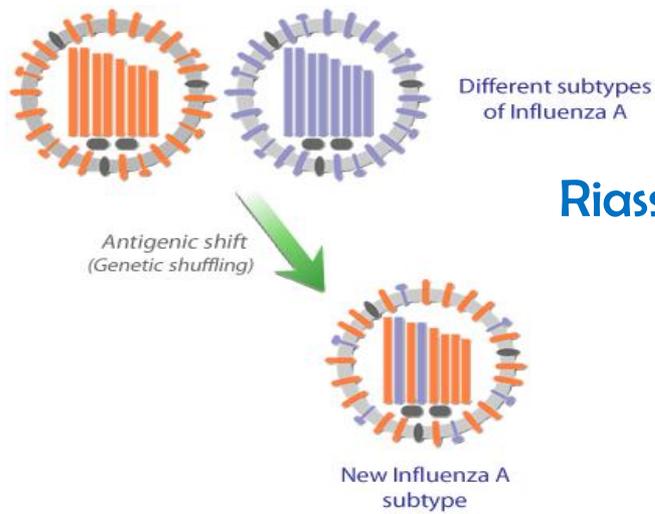




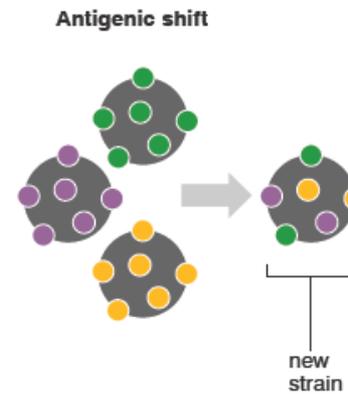
Virus dell'Influenza



Mutazione

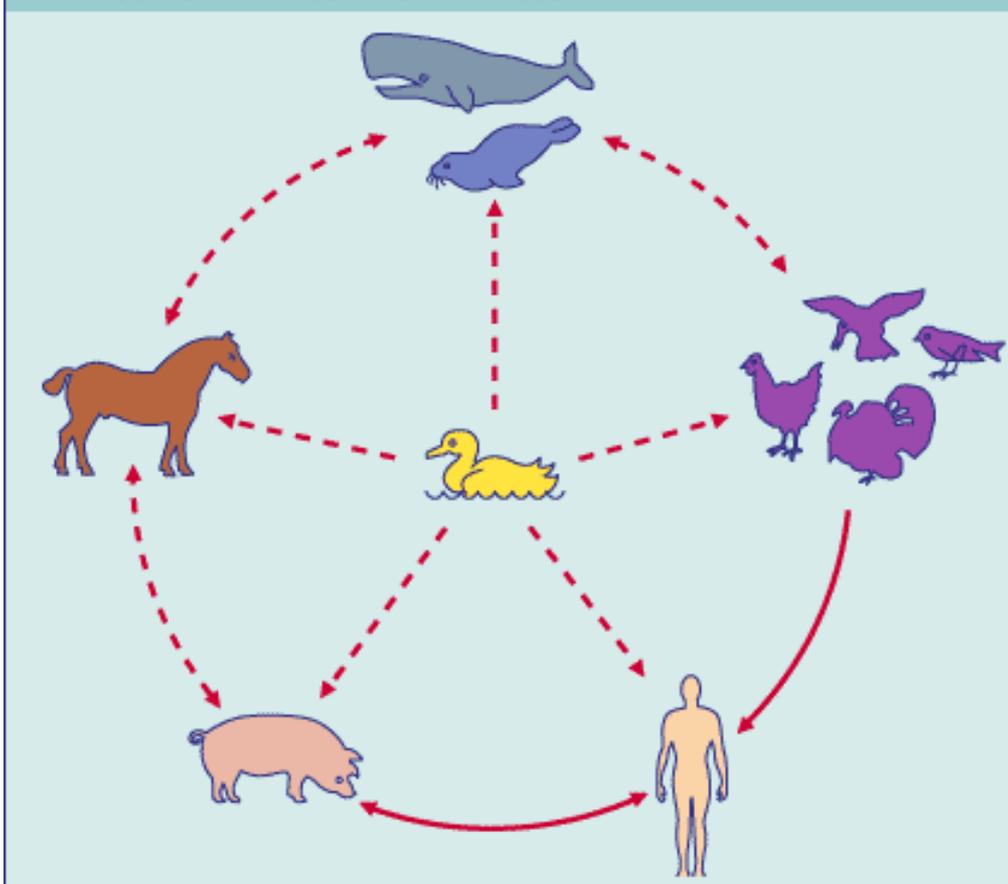


Riassortimento





The reservoir of influenza A viruses



Aviari (H1-H16) (N1-N9)

Uomo(H1N1-H3N2-H2N2)

Suino (H1N1-H1N2-H3N2)
(H3N1-H3N3- H9N2 H4N6)

Veterinary Science Tomorrow 2002

Esempio A/Swine/Italy/1521/98

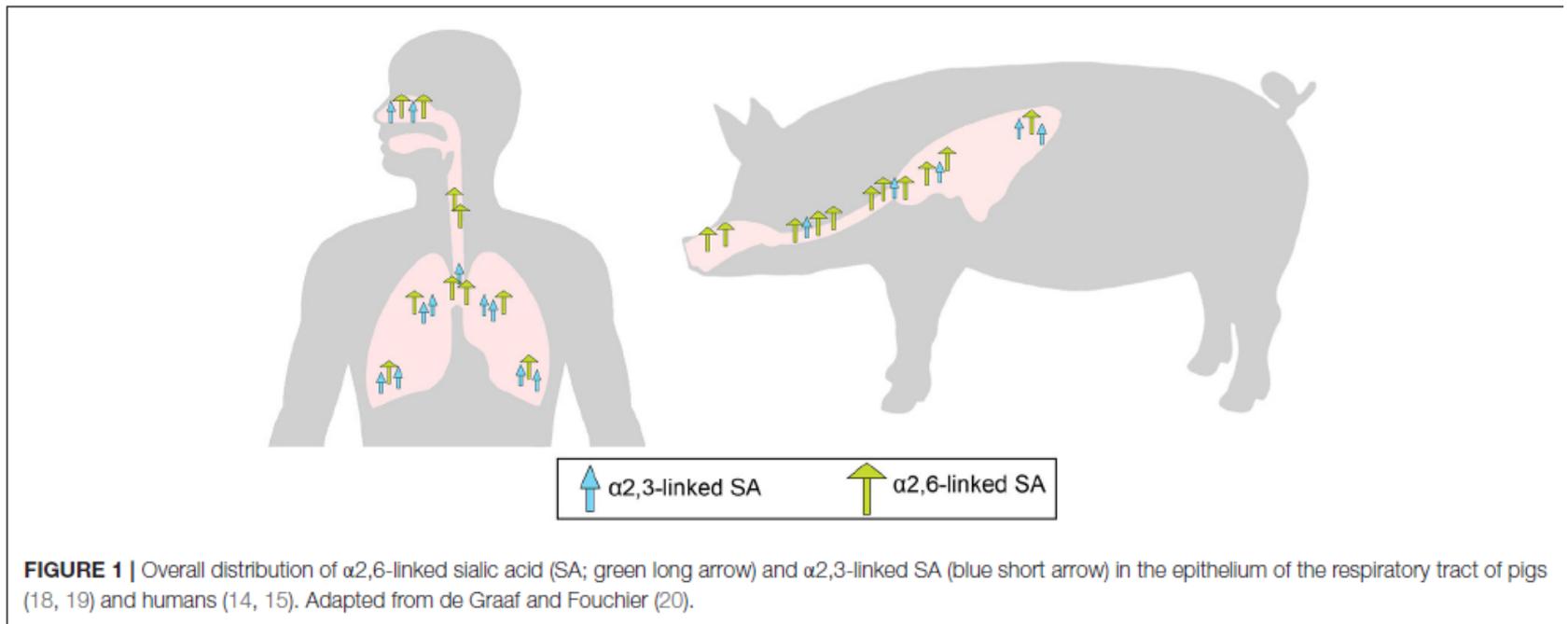
A/New York/55/04



La suscettibilità del suino verso i virus influenzali umani: determinanti

Rajao et al.

Human-Origin Influenza Viruses in Pig



Rajao DS, Vincent AL and Perez DR
(2019) Adaptation of Human Influenza
Viruses to Swine. *Front. Vet. Sci.* 5:347. doi: 10.3389/fvets.2018.00347



PATOGENESI

- Infezione limitata al **tratto respiratorio**
- Viremia evento rarissimo
- Sperimentalmente osservato replicazione virale nel tessuto polmonare ed escrezione virale da 1 a 6-7 gg p.i.



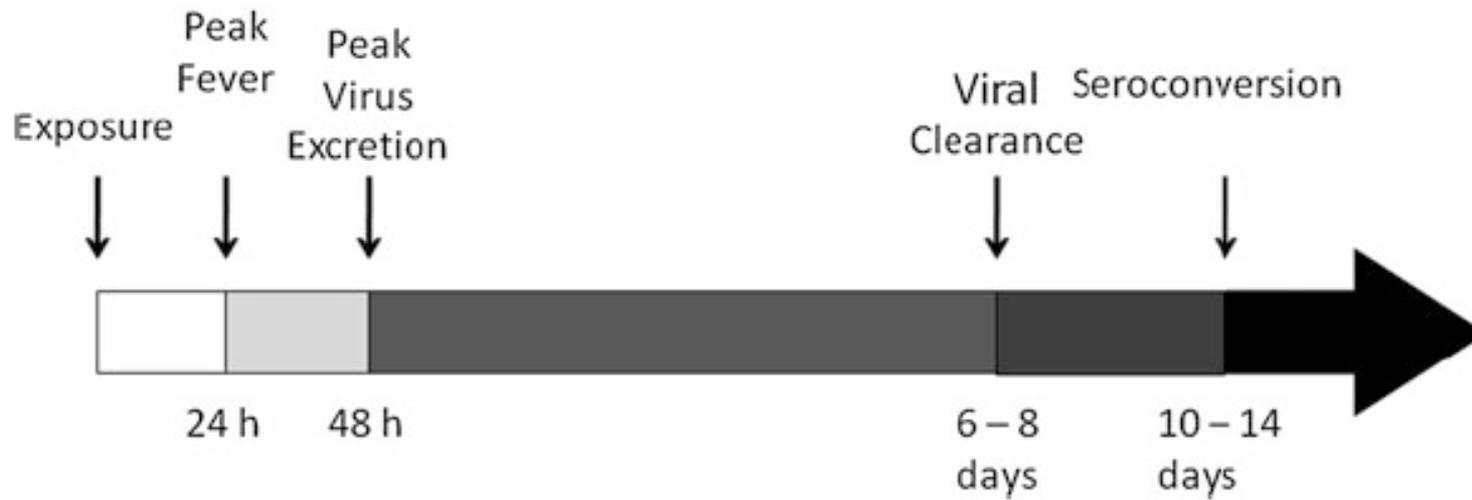
QUADRO CLINICO



- Rapida insorgenza dopo 1-3 gg incubazione
- Rialzo febbrile (40,5 -41,5 °C)
- Depressione, disappetenza, tachipnea, respirazione addominale, tosse
- Descritto calo fertilità in % variabile e aborti nelle tre settimane successive all'infezione
- Morbilità elevata, mortalità < 1%
- Gravità dell'andamento della malattia variabile
 - Stato immunitario allevamento
 - Più suscettibili suini 3-6 mesi
 - Concomitanza infezioni batteriche e/ o virali
 - Caratteristiche antigeniche sottotipo/i influenzali coinvolti



S. Detmer et al.



Jürgen A. Richt • Richard J. Webby
Editors
Swine Influenza



Diagnosi di laboratorio: unica possibilità per ottenere diagnosi di certezza

Campioni di elezione : tampone nasale , tessuto polmonare

Dimostrazione presenza RNA virale (PCR)

Isolamento virale : uova embrionate

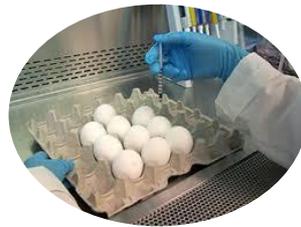
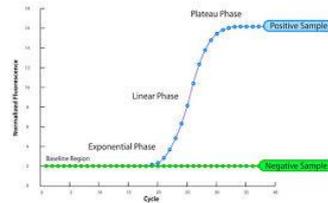
substrati cellulari MDCK, NSK, CACO2

possibilità di procedere ad identificazione

Indagine sierologica su sieri convalescenti(?) HI- ELISA NP



Screening: Real-time RT-PCR per IAV



pos

isolamento

tipizzazione

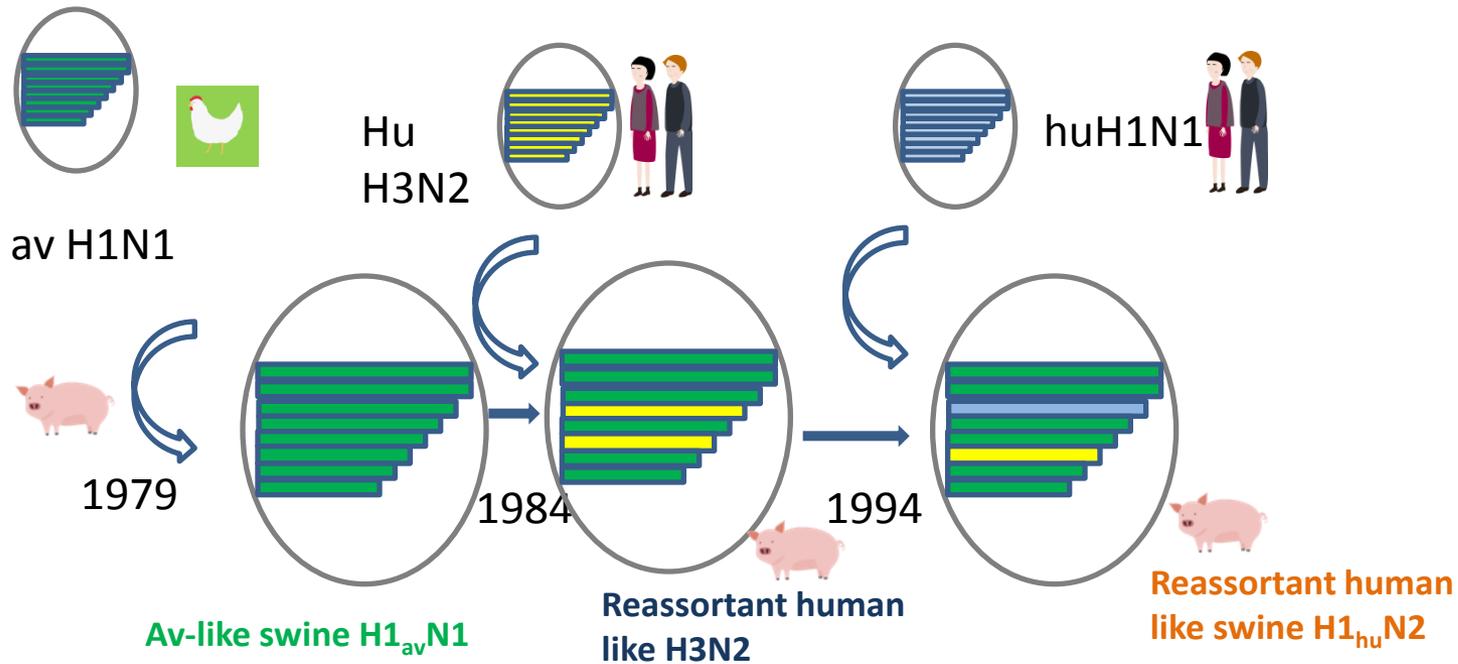
sequenziamento

H1N1
H1N1pdm
H1N2
H3N2

AATAGATAGCTGCCTGCATGCTAGCTAGCTAGCTTTGCTATAGCTAGCTGATCG



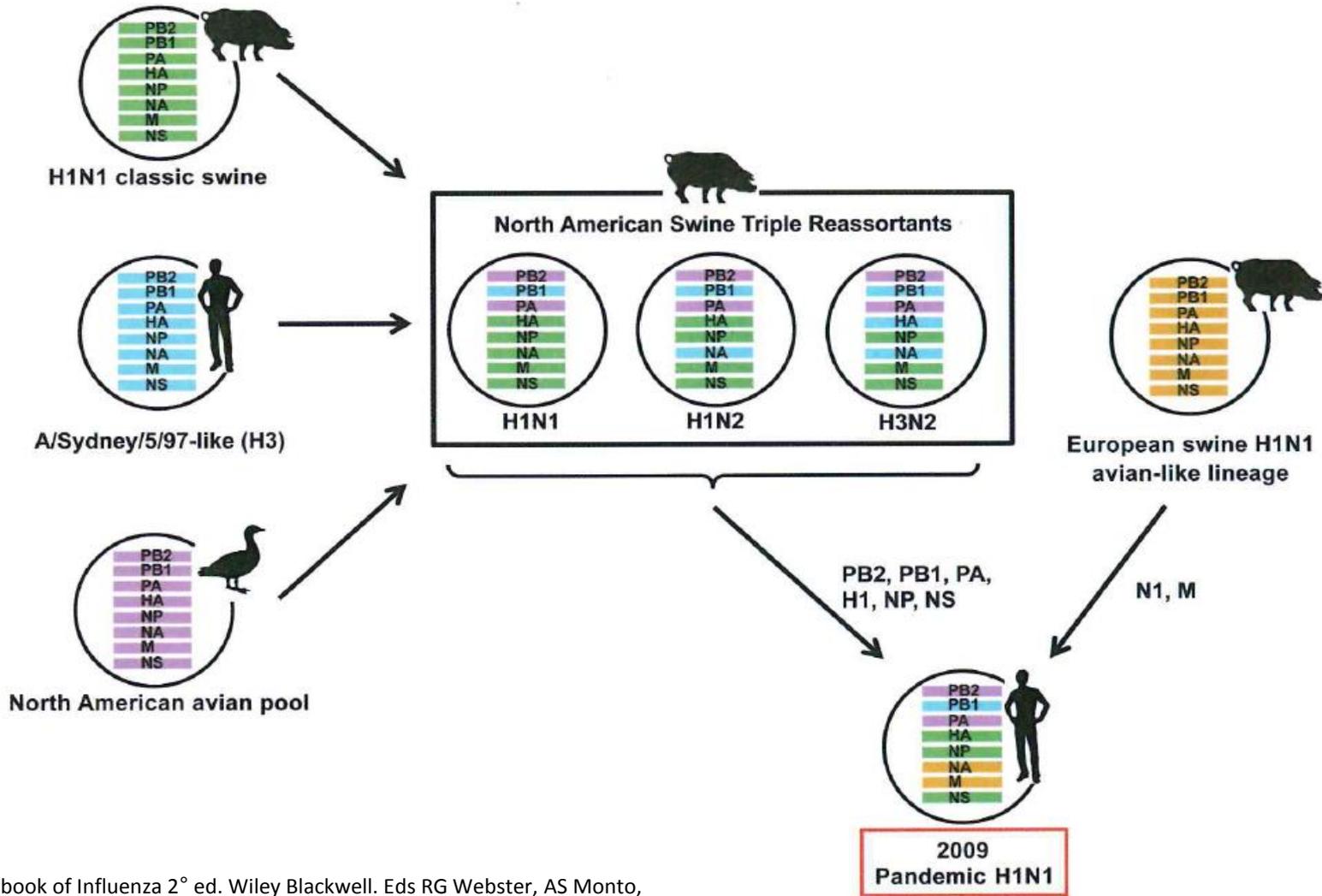
I sottotipi in Europa: genesi





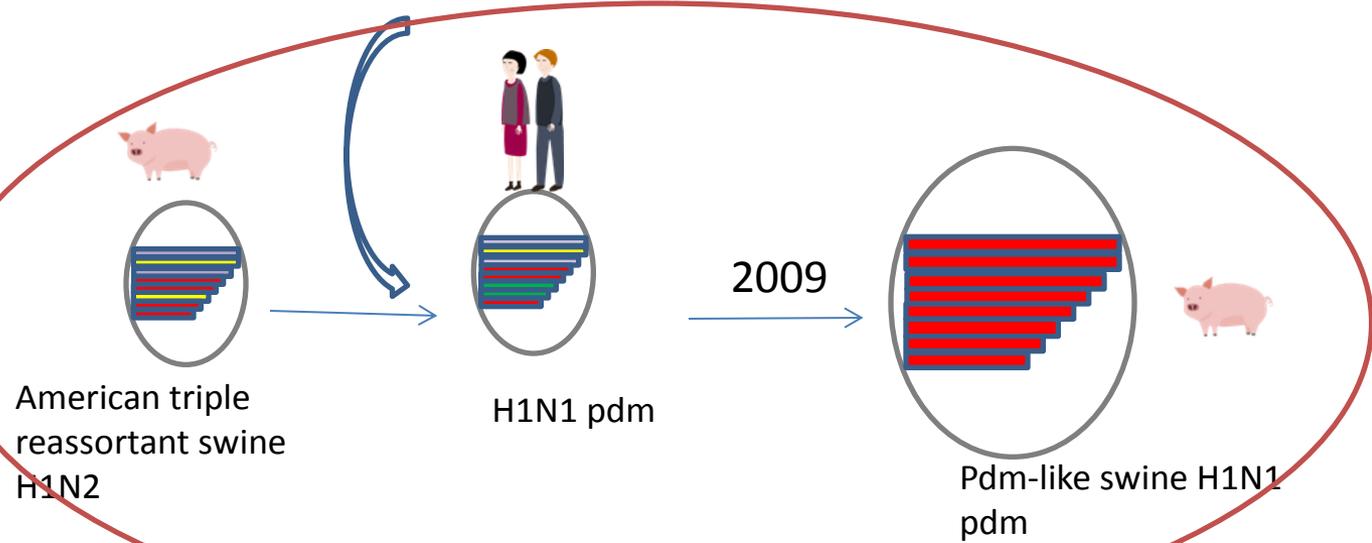
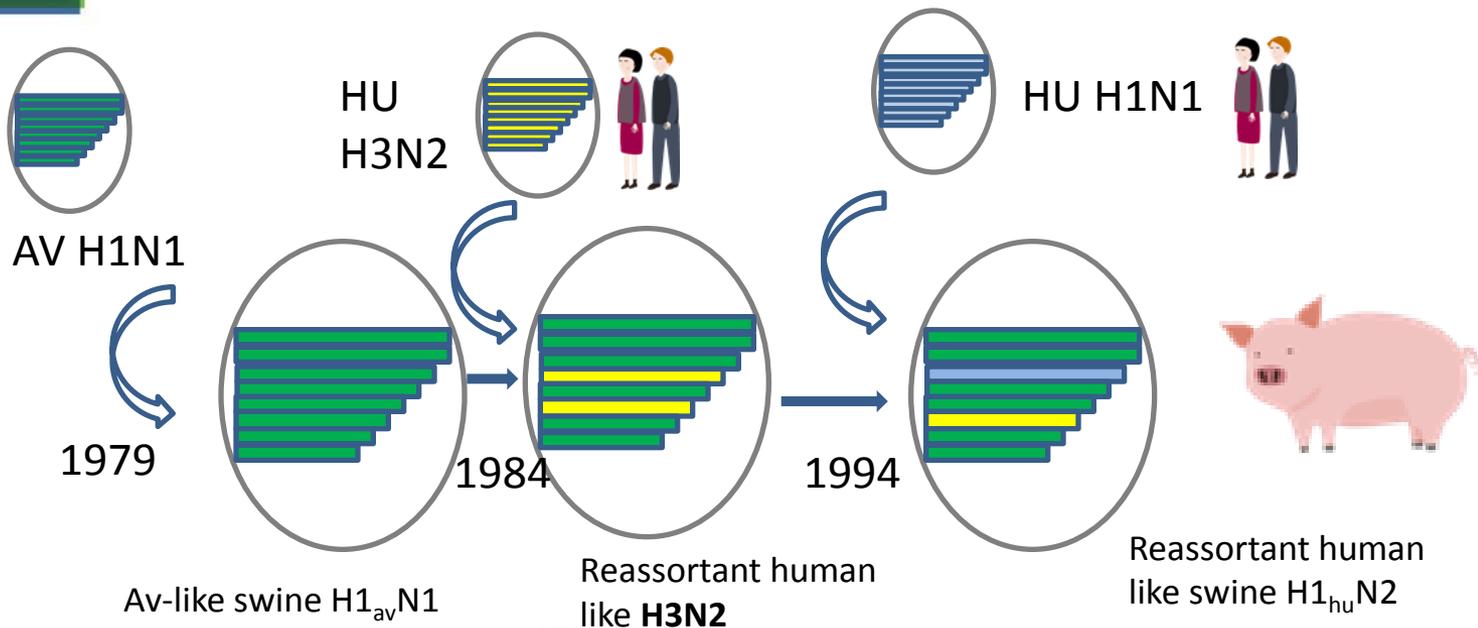
II virus H1N1PDM09

EMERGENCE AND EVOLUTION OF PANDEMIC VIRUS STRAINS



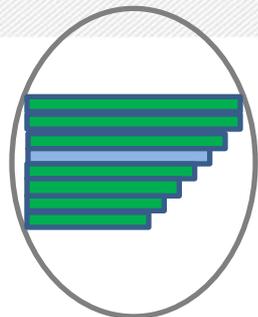


I sottotipi in Europa: dopo il 2009

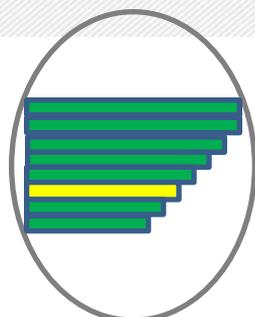




I sottotipi in Europa: dopo il 2009



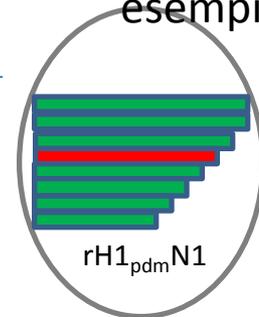
rH1_{hu}N1



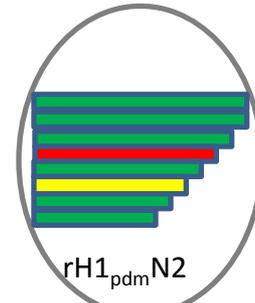
rH1_{av}N2

Riassortanti di seconda generazione

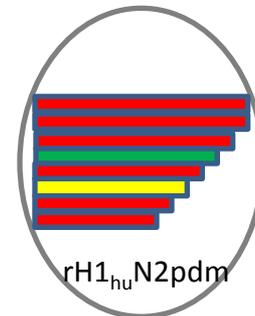
Nuovi riassortanti: esempi



rH1_{pdm}N1

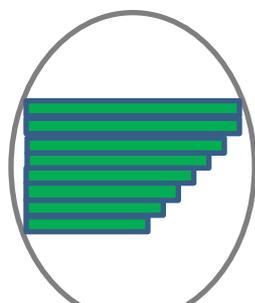


rH1_{pdm}N2

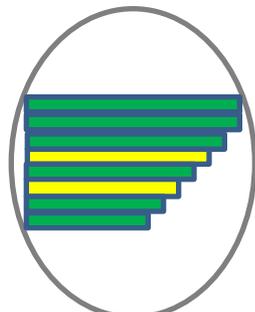


rH1_{hu}N2pdm

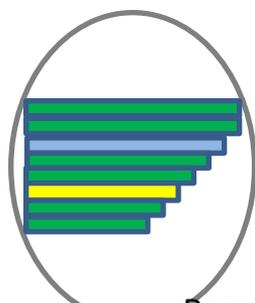
Ceppi endemici europei



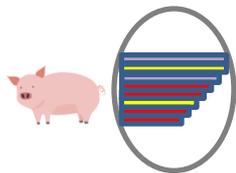
Av-like swine H1_{av}N1



Reassortant human like H3N2



Reassortant human like swine H1_{hu}N2

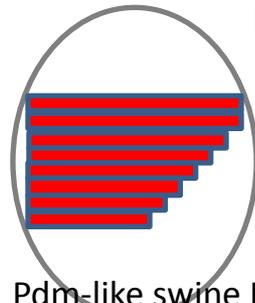


American triple reassortant swine H1N2



H1N1 pdm

2009



Pdm-like swine H1N1 pdm



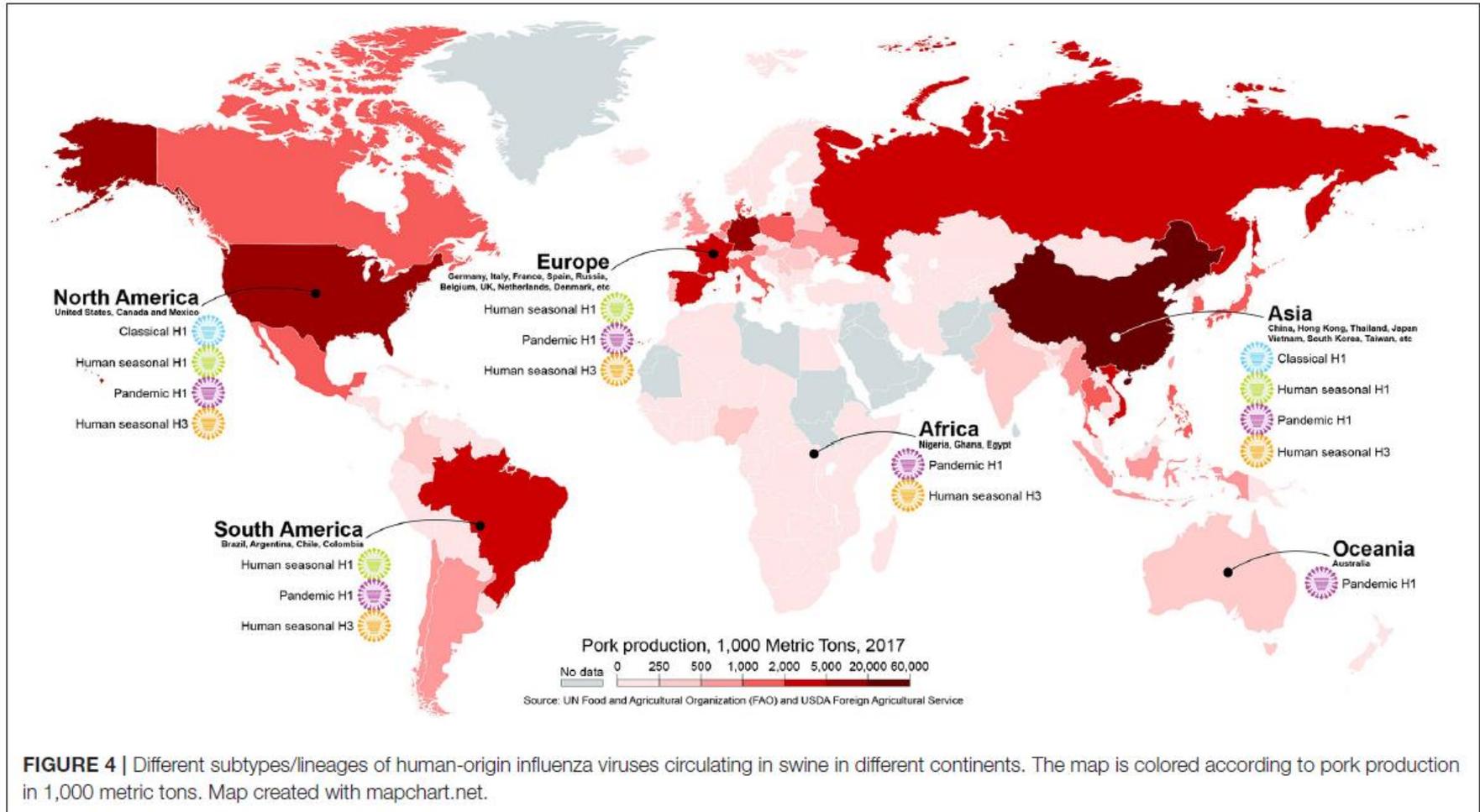
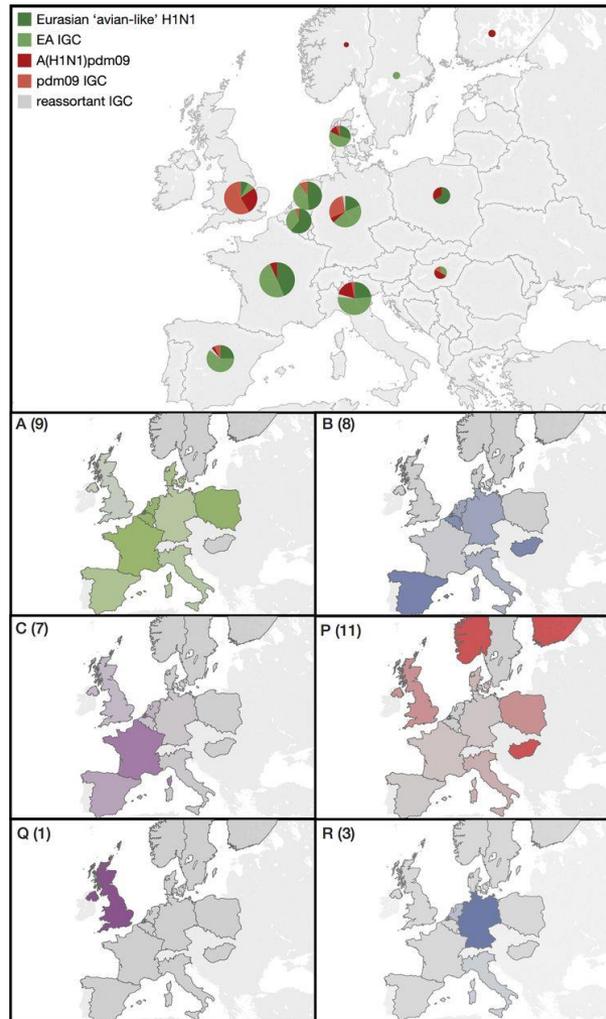


FIGURE 4 | Different subtypes/lineages of human-origin influenza viruses circulating in swine in different continents. The map is colored according to pork production in 1,000 metric tons. Map created with mapchart.net.



Frequencies of the swIAV genotypes across Europe 2009-2013



Simon J. Watson et al. *J. Virol.* 2015;89:9920-9931

Journal of Virology



23 diversi genotipi in Europa 2009-2013



	Internal segments						External segments		Isolates analysed	
	PB2	PB1	PA	NP	MP	NS	HA	NA	Count	Percentage
A	Green	Green	Green	Green	Green	Green	Green	Green	85	29
B	Green	Green	Green	Green	Green	Green	Blue	Blue	38	13
C	Green	Green	Green	Green	Green	Green	Purple	Purple	26	9
D	Green	Green	Green	Green	Green	Green	Green	Blue	13	5
E	Green	Green	Green	Green	Green	Green	Purple	Blue	11	4
F	Green	Green	Green	Green	Green	Green	Purple	Teal	8	3
G	Green	Green	Green	Green	Green	Green	Green	Purple	5	2
H	Green	Green	Green	Green	Green	Green	Purple	Green	3	1
I	Green	Green	Green	Green	Green	Green	Green	Light Purple	2	<1
J	Green	Green	Green	Green	Green	Green	Purple	Light Purple	2	<1
K	Green	Green	Green	Green	Green	Green	Red	Green	2	<1
L	Green	Green	Green	Green	Green	Green	Green	Teal	1	<1
M	Green	Green	Green	Green	Red	Green	Green	Green	1	<1
N	Green	Green	Green	Green	Red	Green	Blue	Blue	1	<1
O	Red	Green	Red	Red	Green	Green	Blue	Red	1	<1
P	Red	Red	Red	Red	Red	Red	Red	Red	35	12
Q	Red	Red	Red	Red	Red	Red	Purple	Purple	22	8
R	Red	Red	Red	Red	Red	Red	Red	Blue	13	5
S	Red	Red	Red	Red	Red	Red	Red	Green	3	1
T	Red	Red	Red	Red	Red	Red	Green	Blue	2	<1
U	Red	Red	Red	Red	Red	Red	Green	Green	2	<1
V	Red	Red	Red	Red	Red	Red	Blue	Blue	1	<1
W	Red	Red	Red	Red	Red	Red	Blue	Light Purple	1	<1
Undetermined									12	4
Total samples									290	100

Italia

H1N1

H3N2

H1N2

H1N2 r

H1N1 PDM

H1N2 r

Blue A/swine/Gent/1/1984-like H3N2

Purple A/swine/Scotland/410440/1994-like H₁_N2

Green Eurasian avian-like H₁_N1

Red A(H1N1)pdm09

Teal A/swine/Italy/4675/2003-like N2

Light Purple Human seasonal-like N2



Circolazione dei principali sottotipi in Europa dati ESNIP3 :2010-2013



Country	Number of subtyped viruses	Influenza A virus subtypes and lineages within subtypes						
		H1N1			H3N2	H1N2		Others
		H1 _{av} N1	rH1 _{hu} N1	H1N1pdm	H3N2	H1 _{hu} N2	rH1 _{av} N2	reass. pdm-like sw HxNy
Belgium	29	16	0	0	10	3	0	0
Denmark	254	68	0	79	0	0	89	18
Finland	4	1	0	3	0	0	0	0
France	350	240	4	7	1	88	10	0
Germany	874	536	6	40	88	94	23	87
Greece	3	0	0	0	3	0	0	0
Hungary	38	19	0	12	4	1	0	2
Israel	2	0	0	1	1	0	0	0
→ Italy	179	82 (45%)	2	10 (5%)	38 (21%)	39 (22%)	7	1
Netherlands	39	19	0	0	11	9	0	0
Poland	30	15	0	11	3	1	0	0
Slovakia	1	1	0	0	0	0	0	0
Spain	27	10	0	0	12	5	0	0
United-Kingdom	57	4	0	32	0	6	0	15
Total	1887	1011	12	195	171	246	129	123
%		53.6	0.6	10.3	9.1	13.0	6.8	6.5

doi:10.1371/journal.pone.0115815.t004

Simon G, Larsen LE, Dürrwald R, Foni E, Harder T, et al. (2014) European Surveillance Network for Influenza in Pigs: Surveillance Programs, Diagnostic Tools and Swine Influenza Virus Subtypes Identified in 14 European Countries from 2010 to 2013. PLOS ONE 9(12): e115815.

<https://doi.org/10.1371/journal.pone.0115815>

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0115815>



FREQUENZA CASI POSITIVI PER INFLUENZA A : 26%

Period	Number of subtyped viruses RT_PCR	H1N1		H3N2	H1N2
		H1N1 %	H1N1pdm (2009) %	Hu-like reass. sw H3N2 %	H1N2 %
2014	102	35,3	2,0	12,7	50,0
2015	108	37,0	11,1	10,2	41,7
2016	123	36,6	10,6	29,3	23,6
2017	175	42,9	1,1	14,9	41,1
2018	158	35,4	1,3	12,0	51,3
2014-2018	666	37,8	4,7	15,8	41,7



La situazione italiana: studio 2010-2015 , 104 virus sequenziati



Subtype	Genotype	HA	NA	PB1	PB2	PA	NP	M	NS	N. strains (%)	source
H1N1	1									29 (27.9%)	SWINE
H3N2	2									25 (24.0%)	
H1N2	3									21 (20.2%)	
H1N2	4									8 (7.7%)	
H1N1	5									6 (5.8%)	
H1N2	6									5 (4.8%)	
H1N2	7									3 (2.9%)	
H1N1	8									2 (1.9%)	
H1N2	9									2 (1.9%)	
H1N2	10									1 (1.0%)	
H1N1	11									1 (1.0%)	
H3N2	12									1 (1.0%)	
H1N1	5									32 (42%)	HUMAN
H3N2	13									44 (48%)	

Chiapponi C, et al

Zoonoses Public Health. 2018 Feb;65(1):114-123.

EA avian H1-A/swine/Italy/1513/1998 H1N1

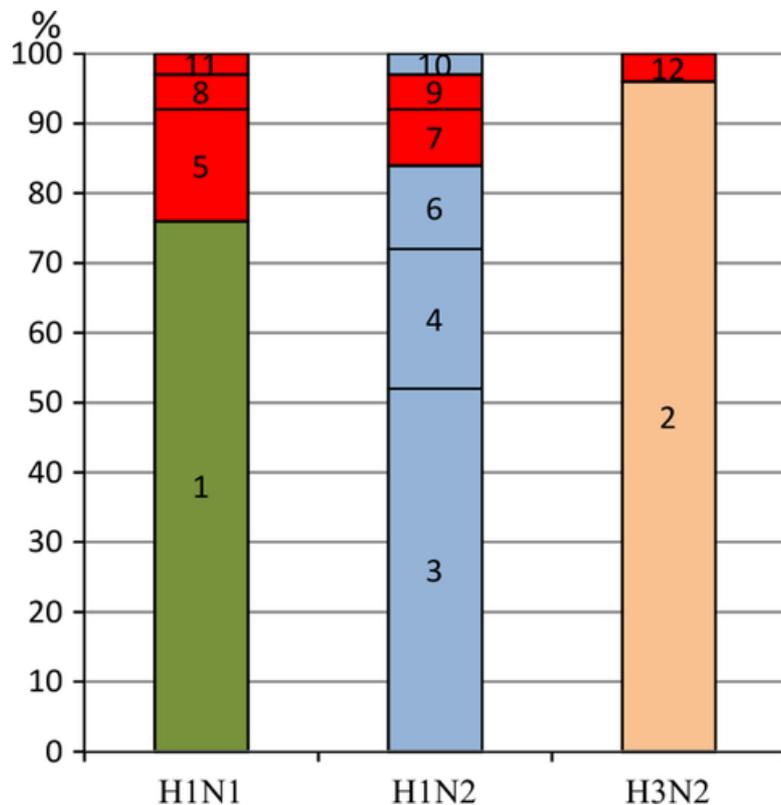
SW-H3N2-A/swine/Gent-1/1984 H3N2

HU-H3N2 A/Hong/Kong/280/1997 H3N2

A(H1N1)pdm09-A/California/04/2009 H1N1

HU H1-A/swine/Italy/4675/2003 H1N2

HU seasonal H3N2



Presenza di riassortanti pandemici tra i vari sottotipi in Italia.

Percentages of genotypes 1–12 among H1N1, H1N2 and H3N2 subtypes of swIAVs. Genotypes with at least one A(H1N1)pdm09-derived gene are shown in red. Genotypes with endemic swIAV genes only are in green (H1N1), blue (H1N2) and orange (H3N2)

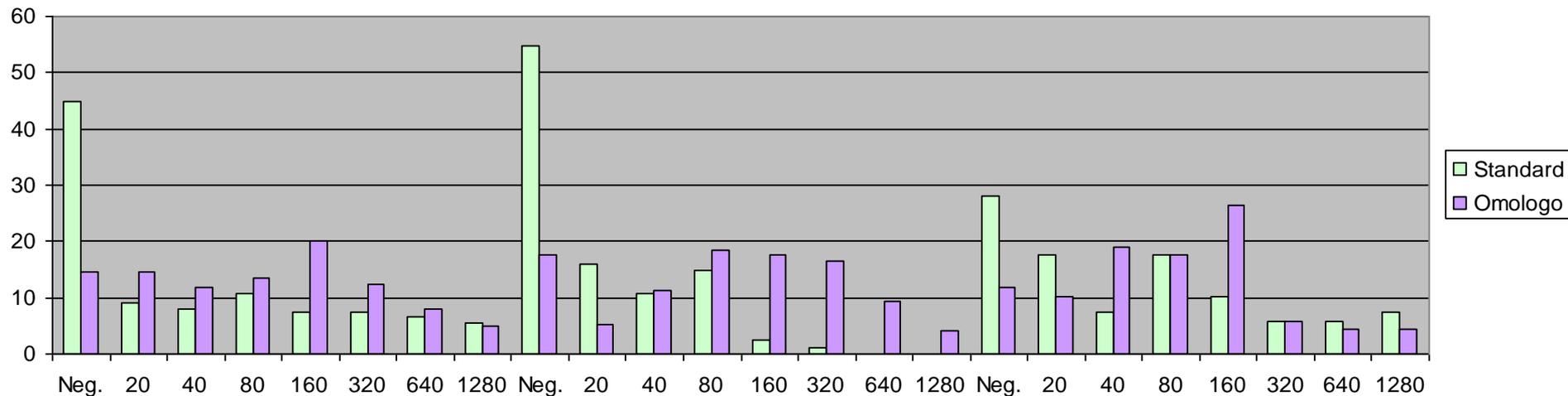


Risposta anticorpale, nei confronti di virus influenzale di referenza e ceppo omologo isolato in allevamento, in sieri convalescenti

H1N1

H1N2

H3N2

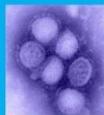


Barigazzi G. et al VIRVET 2009



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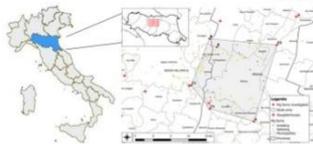
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HIGH GENETIC VARIABILITY OF SWINE INFLUENZA VIRUS IN A CONFINED AREA OF NORTHERN ITALY



Introduction



Swine influenza A virus (SIAV) circulates actively in the Italian pig population and subtypes H1avN1, H1huN2, H3N2 and H1N1pdm are mainly detected, but also reassortant (r) strains are occasionally isolated. In a confined area (388 Km²), where 83 pig farms were located, 3 pig farms were examined for SIAV presence during respiratory outbreaks.

Methods

37 sets of clinical samples (n.113) were collected from 3 different pig farms: A, B, C.

Farm A: Farrow to feeder - 800 sows - No flu vaccination
Nov 2013-Oct 2014: n. 15 samplings (34 samples) from piglets (25kg)

Farm B: Finishing pig farm site 2 (6-50 Kg) site 3 (since 50 Kg to slaughtering)
No flu vaccination
Jul 2013 and Dic 2014: n. 8 samplings (46 samples)

Farm C: Farrow to finish C1, Gilts production C2, Finishing pig farm C3

- 2009 (C1 e C2): n. 3 samplings (6 samples)
- 2011 (C1): n. 6 samplings (18 samples)
- 2012 (C2): n. 2 samplings (6 samples)
- 2014 (C3): n. 2 samplings (3 samples)



IAV RT-PCR screening → viral isolation → genetic and antigenic characterization → Miseq Illumina sequencing

Results and conclusions

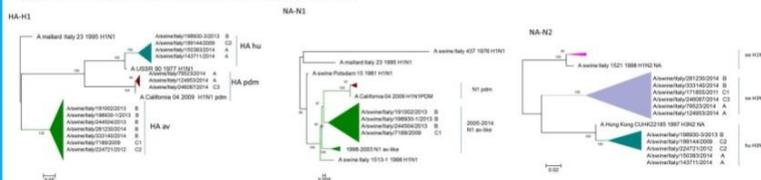
A: 4 H1N2 SIAVs were obtained. Genetic studies characterized 2 H1N2r pdm strains (German Papenburg lineage) and 2 H1N2r pdm strains showing H1N1pdm internal genes but HA and NA clustering with H1huN2hu Italian SIAV.

B: 8 SIAVs were isolated. Four strains were characterized as H1avN1, 2 strains as H1huN2hu while 2 strains showed to be r strains H1avN2sw.

C: 10 SIAVs belonging to different genetic lineages were detected: H1avN1, H1huN2hu, H3N2, H1avN2r and also H1N2r strain clustering with the German lineage.



Figure 1: Condensed phylogenetic trees of HA-H1, NA-N1 e NA-N2 genes from the alignment of sequences of the study and sequences of SIAVs from Genbank and IZSLER database.



A very high variability of gene constellation of SIAVs was described in three pig farms: 2, 3, and 5 SIAV lineages were detected respectively. The study underlines the increasing genetic diversity of Italian SIAVs also considering a limited number of herds (n. 3) in a confined area without animal introduction operations. These differences cannot be explained easily, the area is regarded as major node of trading network of swine and pork, this feature must be considered. Continue surveillance of SIAV infections to provide a baseline for updating and interpretation of the role of SIAV in zoonotic events and also to focus on relatedness to currently used vaccines should be further reinforced.



Materiali e Metodi



Tre unità produttive della filiera suina delle province di Modena e Reggio Emilia

A: Ciclo aperto - 900 scrofe - rimonta interna – commercializza suinetti 30 Kg
No vaccinazione influenza

Suinetti 20-25 Kg **Nov 2013-Ott 2014** : n. 15 conferimenti (34 campioni)

B: Produzione suino pesante- Sito 2 (6-50 Kg) Sito 3 (da 50 Kg alla macellazione)

Da unico fornitore introduzione suinetti a flusso continuo

No vaccinazione influenza

Ingrasso **Lug 2013** Magronaggio **Dic 2014**: n. 8 conferimenti (46 campioni)

C: **C1** ciclo chiuso 750 scrofe, **C2** unità produzione scrofette, **C3** finissaggio C2

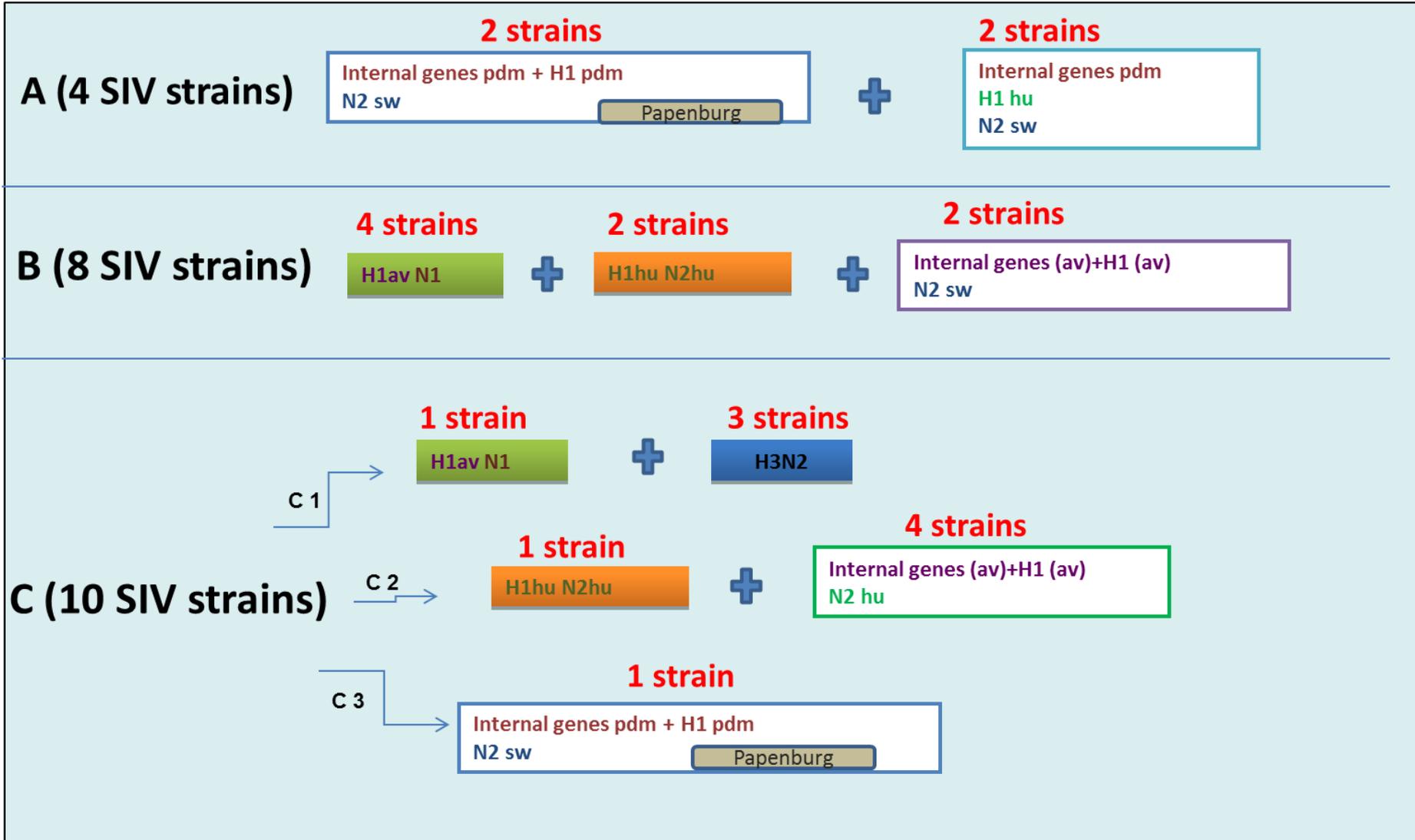
2009 (C1 e C2): n. 3 conferimenti (6 campioni)

2010 vaccinazione in scrofe **C1**

2011 (C1): n. 6 conferimenti (18 campioni)

2012 (C2): n. 2 conferimenti (6 campioni)

2014 (C3): n. 2 conferimenti (3 campioni)





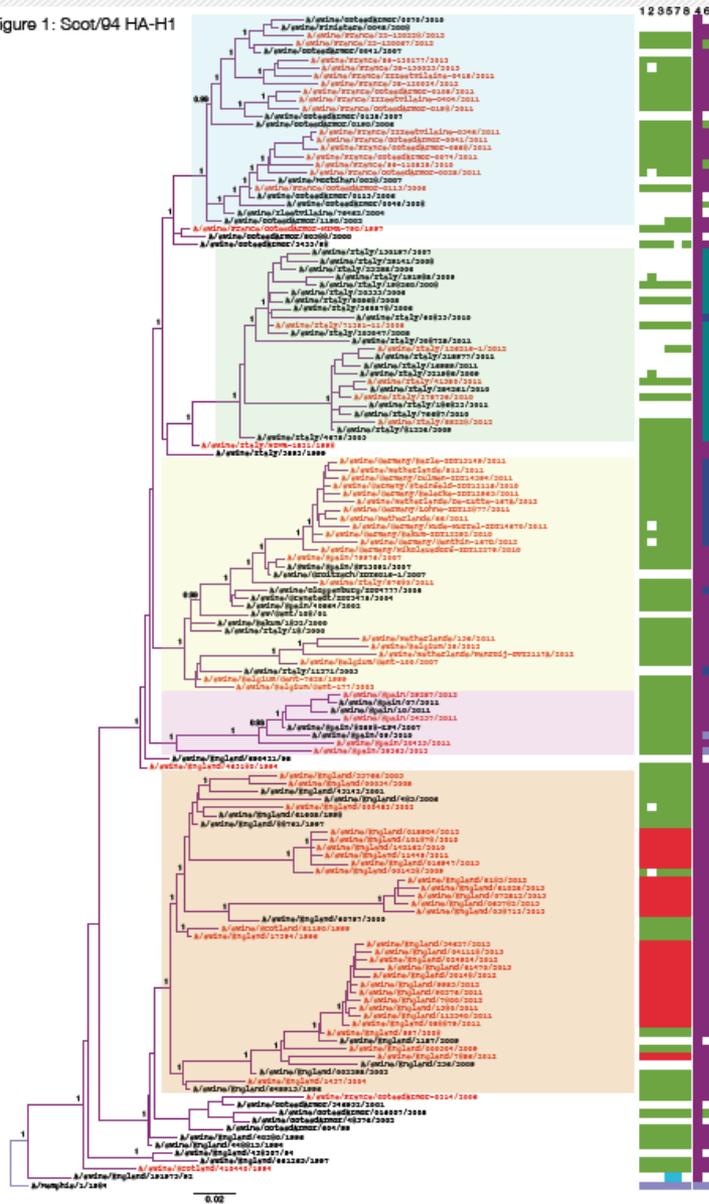
virus	farm	Date of sampling	PB1, PB2, PA, NP, M, NS	HA	NA	subtype
<i>A/swine/Italy/79523/2014</i>	A	Mar 2014	PDM	PDM	SW-H3N2	H1pdmN2sw
<i>A/swine/Italy/124953/2014</i>	A	May 2014	PDM	PDM	SW-H3N2	H1pdmN2sw
<i>A/swine/Italy/143711/2014</i>	A	May 2014	PDM	HU-LIKE	HU-H3N2	H1huN2hu r pdm
<i>A/swine/Italy/150383/2014</i>	A	Jun 2014	PDM	HU-LIKE	HU-H3N2	H1huN2hu r pdm
<i>A/swine/Italy/191002/2013</i>	B	Jul 2013	AV-LIKE	AV-LIKE	AV-LIKE	H1avN1
<i>A/swine/Italy/198930-1/2013</i>	B	Aug 2013	AV-LIKE	AV-LIKE	AV-LIKE	H1avN1
<i>A/swine/Italy/198930-3/2013</i>	B	Aug 2013	AV-LIKE	HU-LIKE	HU-H3N2	H1huN2hu
<i>A/swine/Italy/244504/2013</i>	B	Sept 2013	AV-LIKE	AV-LIKE	AV-LIKE	H1avN1
<i>A/swine/Italy/281230/2014</i>	B	Oct 2014	AV-LIKE	AV-LIKE	SW-H3N2	H1avN2sw
<i>A/swine/Italy/333140/2014</i>	B	Dic 2014	AV-LIKE	AV-LIKE	SW-H3N2	H1avN2sw
<i>A/swine/Italy/7189/2009</i>	C1	Jan 2009	AV-LIKE	AV-LIKE	AV-LIKE	H1avN1
<i>A/swine/Italy/199144/2009</i>	C2	Aug 2009	AV-LIKE	HU-LIKE	HU-H3N2	H1huN2hu
<i>A/swine/Italy/171855/2011</i>	C1	Jul 2011	AV-LIKE	SW-H3	SW-H3N2	H3N2
<i>A/swine/Italy/224721/2012</i>	C2	Sept 2012	AV-LIKE	AV-LIKE	HU-H3N2	H1avN2hu
<i>A/swine/Italy/246087/2014</i>	C3	Sept 2014	PDM	PDM	SW-H3N2	H1pdmN2sw



- Ristretto ambito territoriale (allevamenti delimitano 388 Km²)
(massima distanza 26 Km)
- Numero limitato di Aziende considerate **(n.5)**
- Allevamento A ,C1, C2 assenza introduzione suini
- Numero limitato di campioni esaminati **(n.113)**
- Consistente numero di virus influenzale isolati **(n.35)**
- Elevata variabilità genotipica degli isolati **(n. 7 lineaggi)**
- Allevamento A e C3 circolazione lineaggio Papenburg -
GERMANIA **(n.2)**



Supplementary Figure 1: Soot/04 HA-H1



FRANCE

← ITALY

GERMANY

SPAIN

ENGLAND



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GRAZIE PER L'ATTENZIONE!!