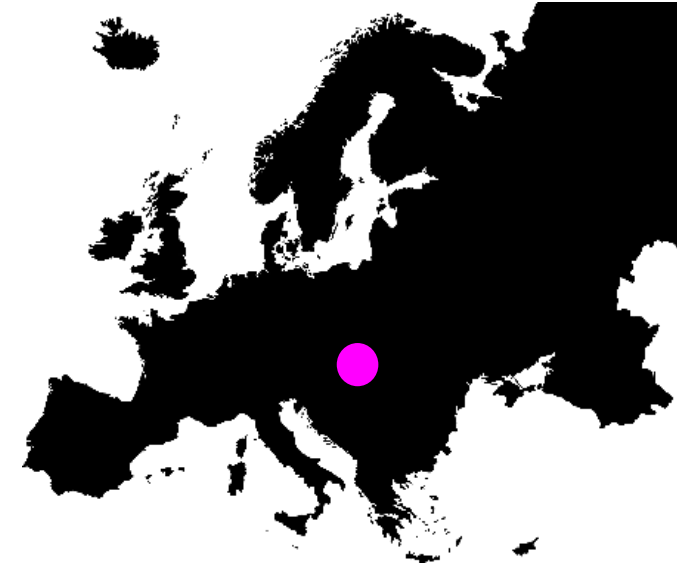


The growing issue of influenza infections in pigs and the related zoonotic risk

Are pigs overestimated as a source of zoonotic influenza viruses?



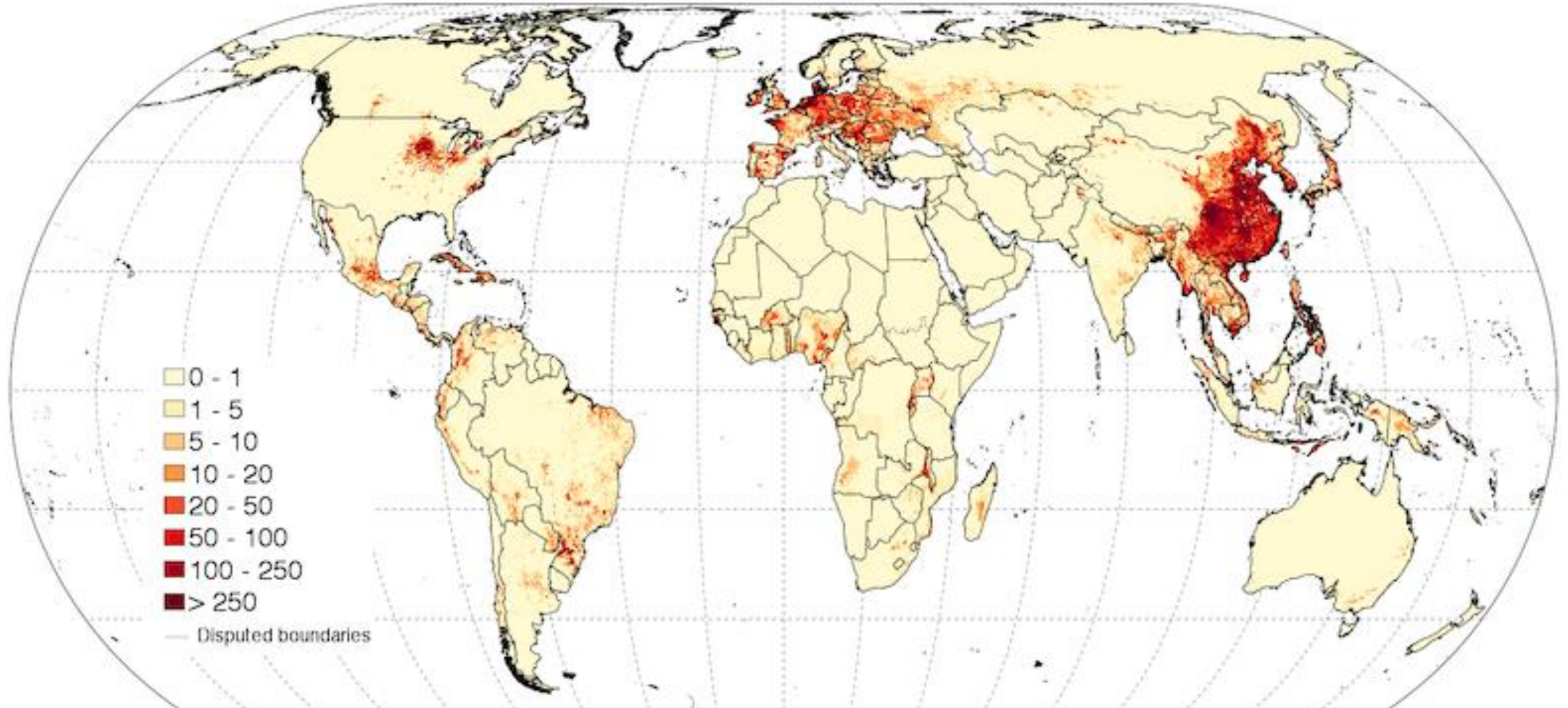
Graaf A, Hennig Ch, Beer M,
Harder T



May 12, 2022
Budapest, Hungary

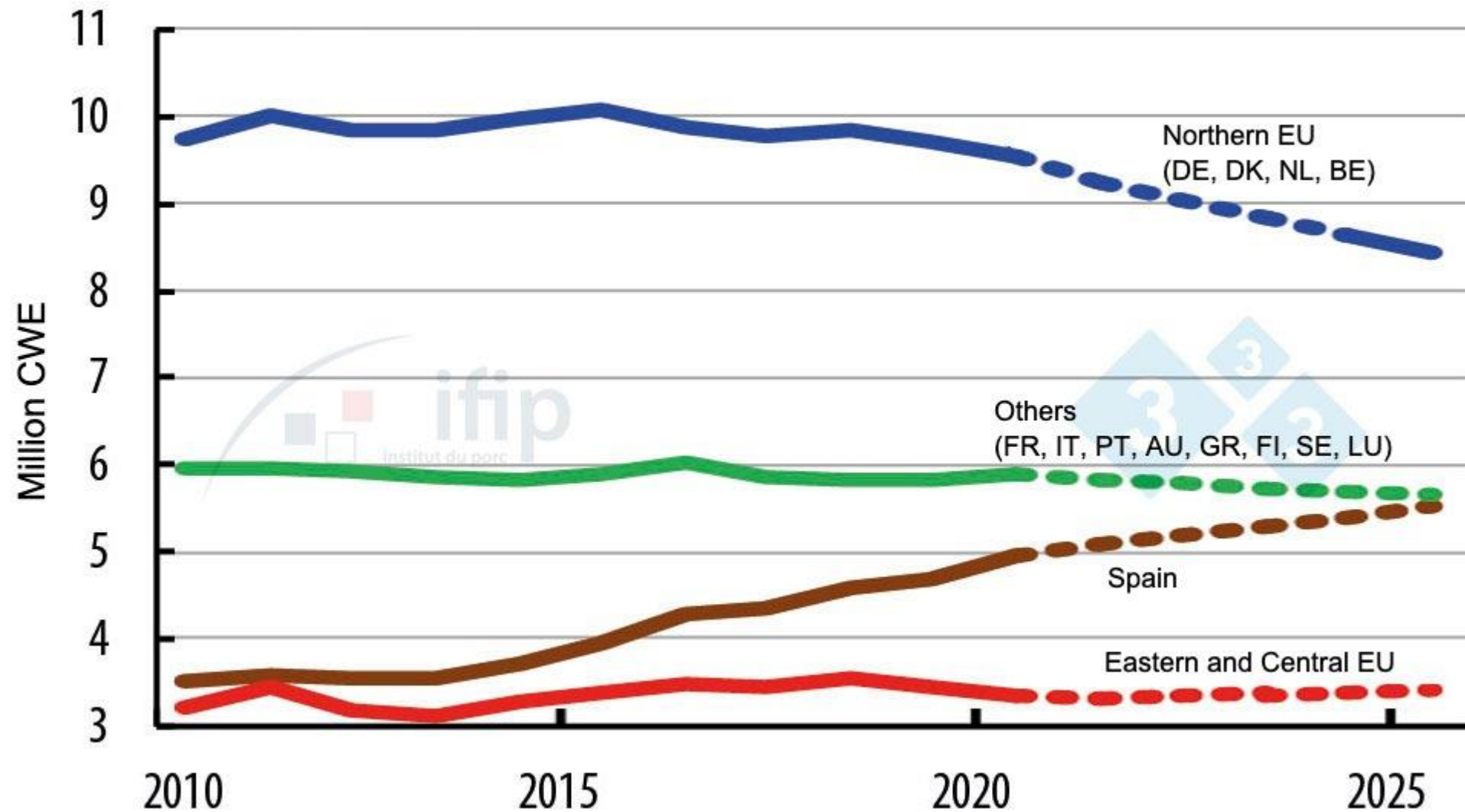
Host population density

Number of pigs per square kilometre in 2010



<https://www.fao.org>

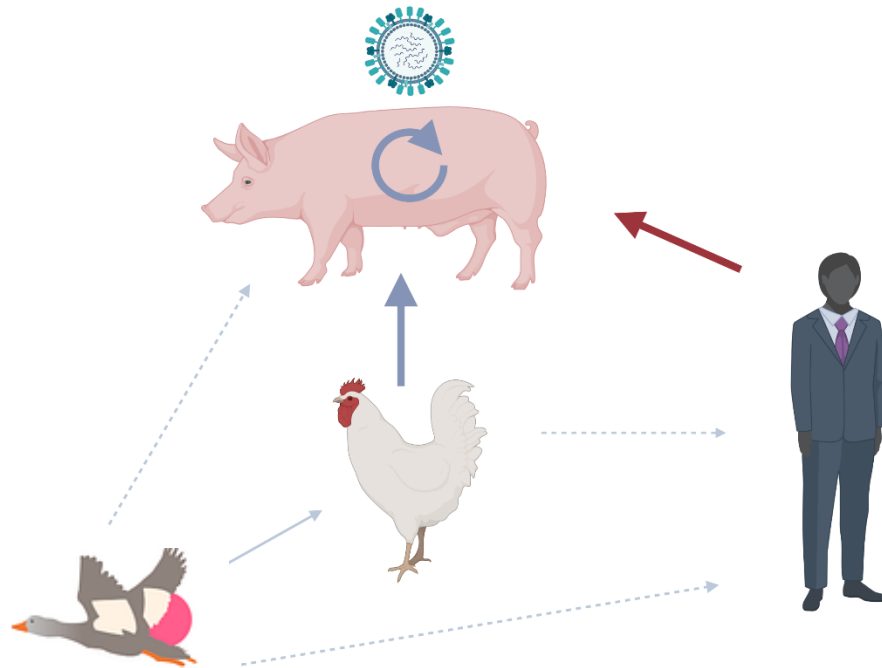
Expected stagnation of EU pig production



Data source: IFIP, 2022

Swine Influenza A Virus (swIAV)

- swIAV circulate globally and seasonally independent in pig populations
- Respiratory disease with high morbidity (80%), but low mortality (<1%) in swine

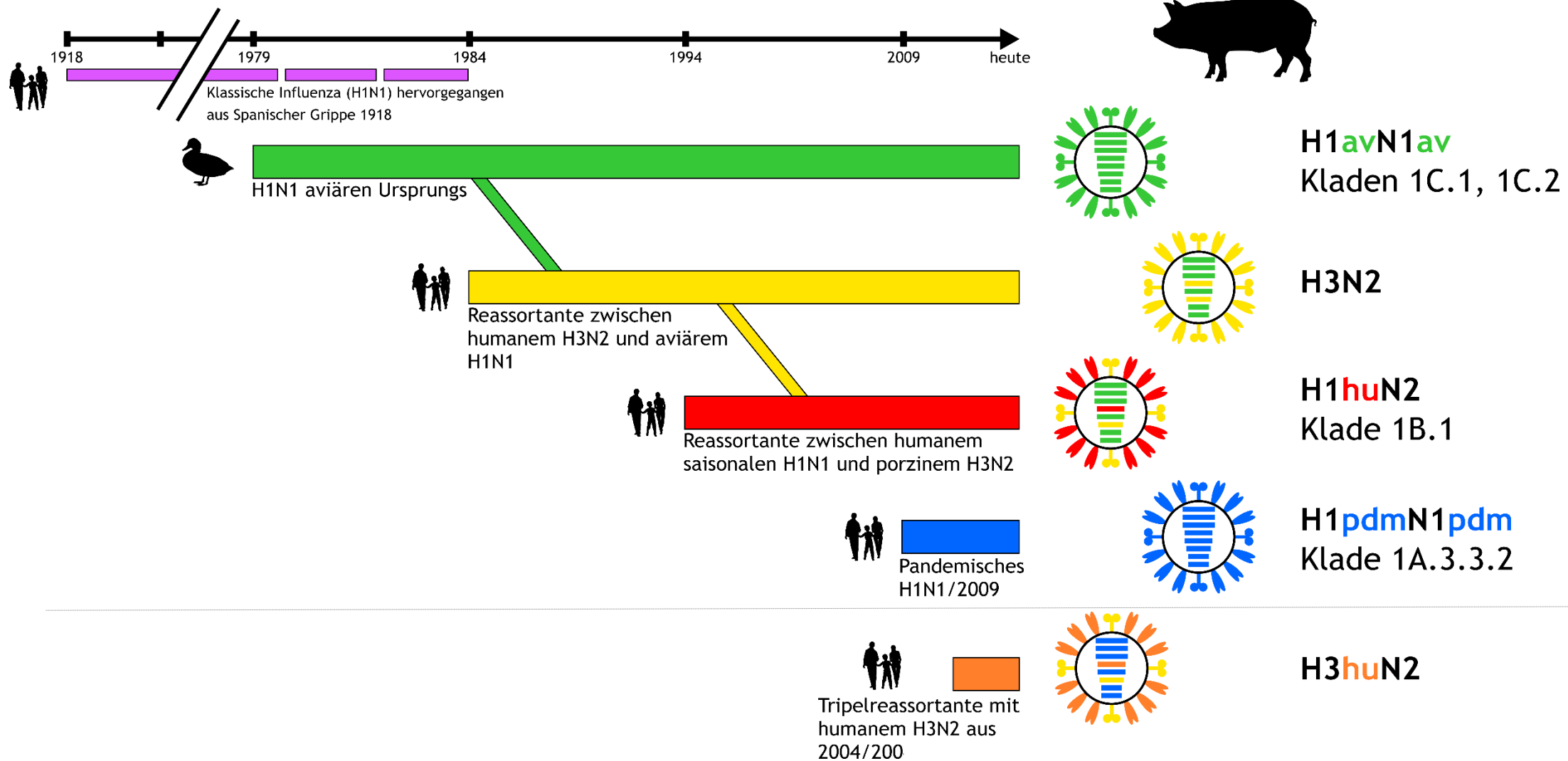


- Swine as a „**mixing vessel**“
- Co-infections of influenza viruses with avian, human or swine origin
- Transmission of IAV from human to swine possible at any time (**reverse zoonosis**)



Influenza in pigs - European situation

Four major reassortant swIAV lineages emerged and co-circulate in European domestic pig populations

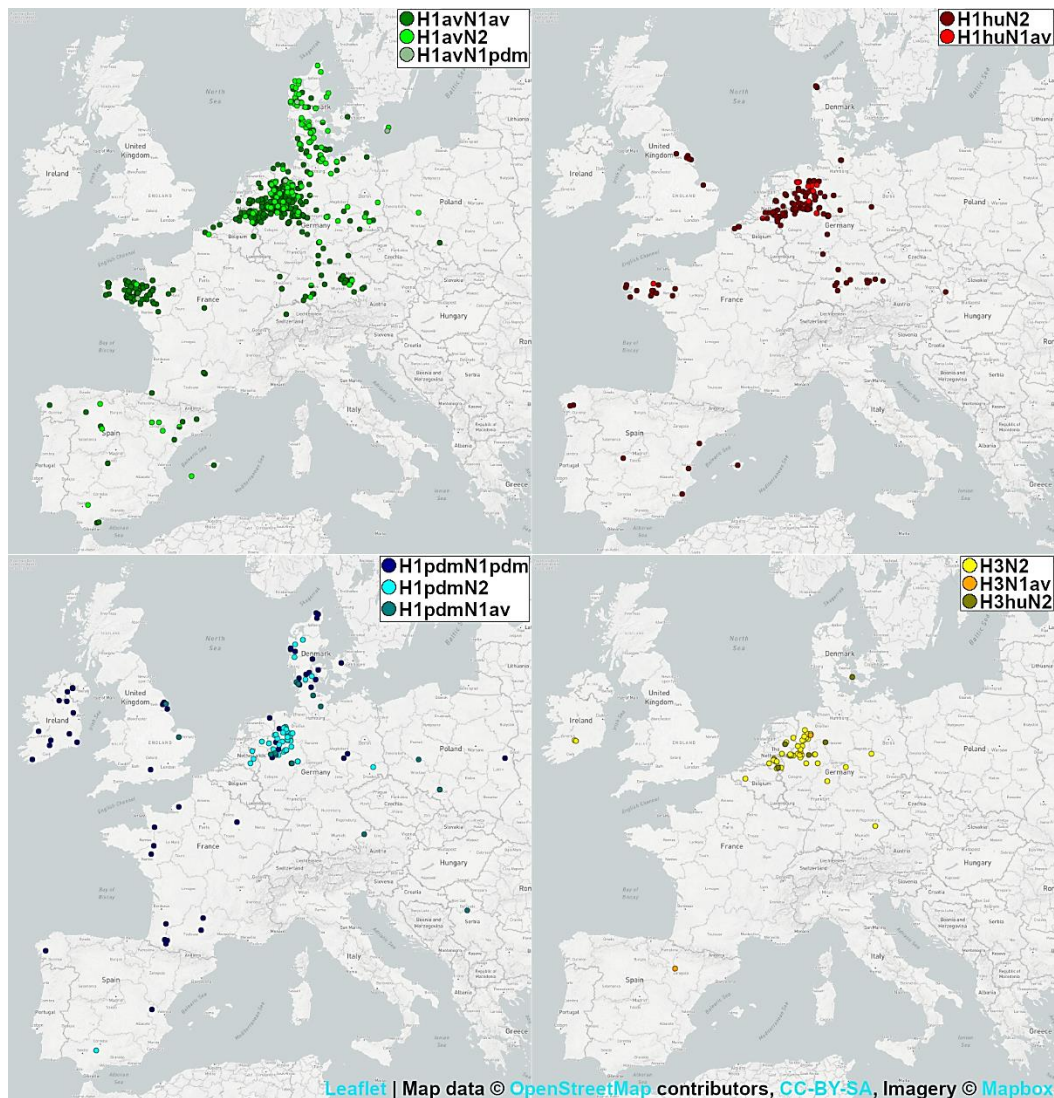




European swIAV surveillance, 2015-2019

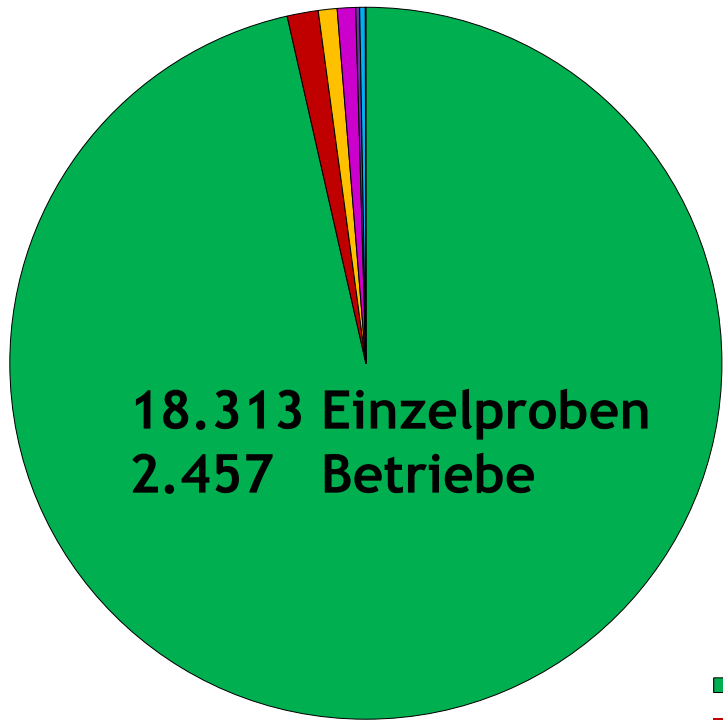
swIAV-subtypes are geographically restricted

- **H1avN1av** mainly in central Europe, not in UK and IRE
- **H1avN2** dominates in DK
- **H1pdmN1pdm** starts competing (very strong in UK)
- Increasing incidence of reassortants of H1pdm
- **H1pdmN2**
- Little known about eastern Europe



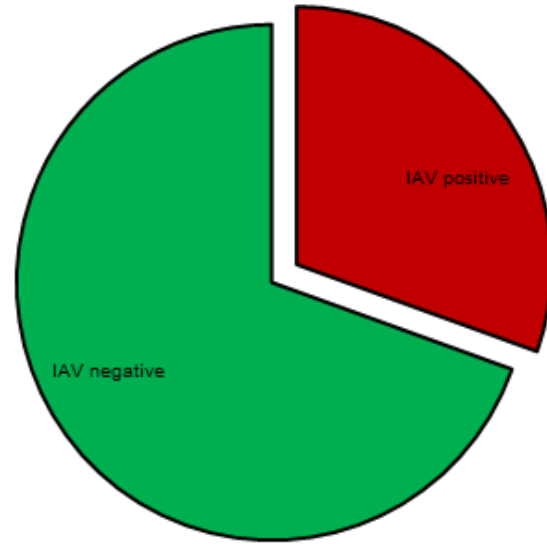
swIAVs are widespread in holdings with respiratory disease

■ swabs ■ lung-tissues ■ oral fluids ■ BALFs ■ TBSs ■ RNAs ■ isolates

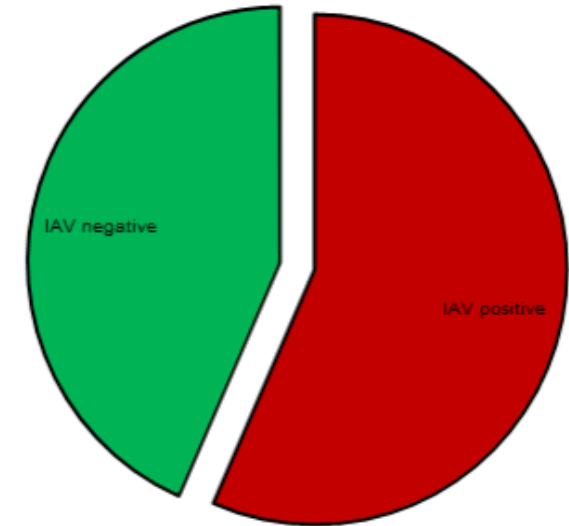


■ swabs
■ lung-tissues

Einzelproben

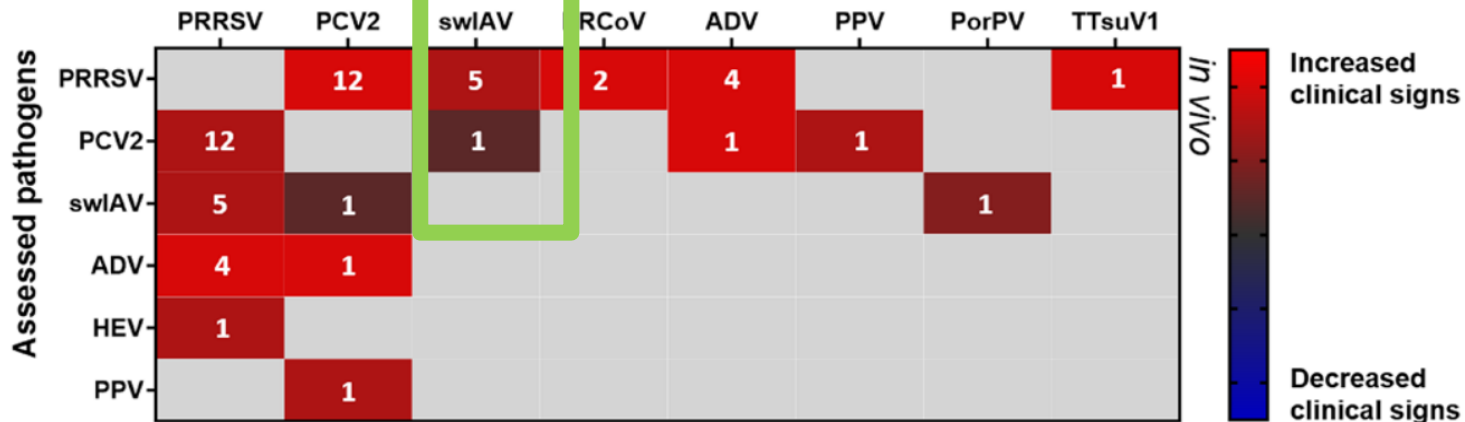
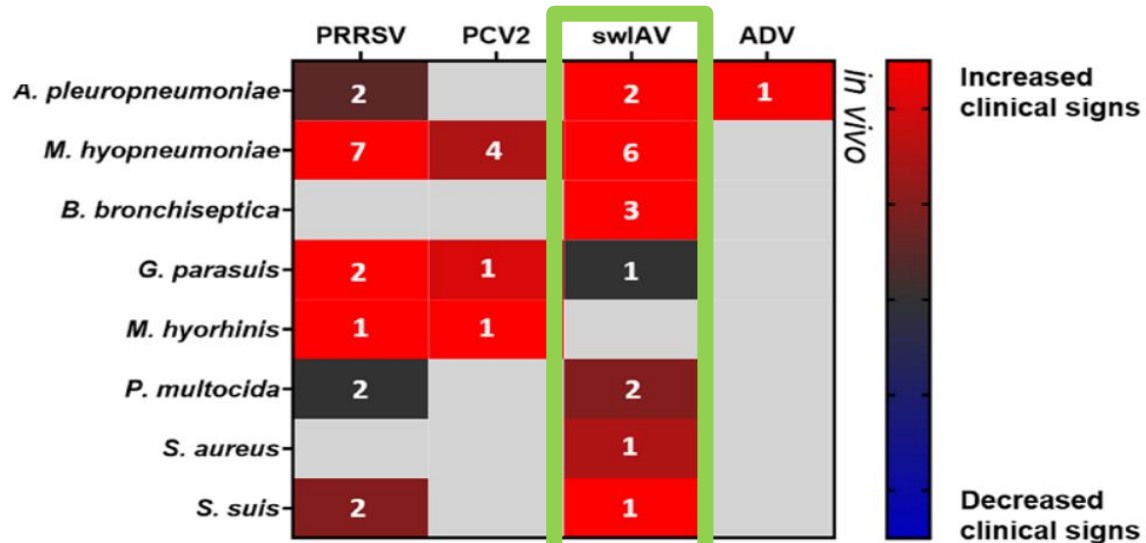


Betriebe



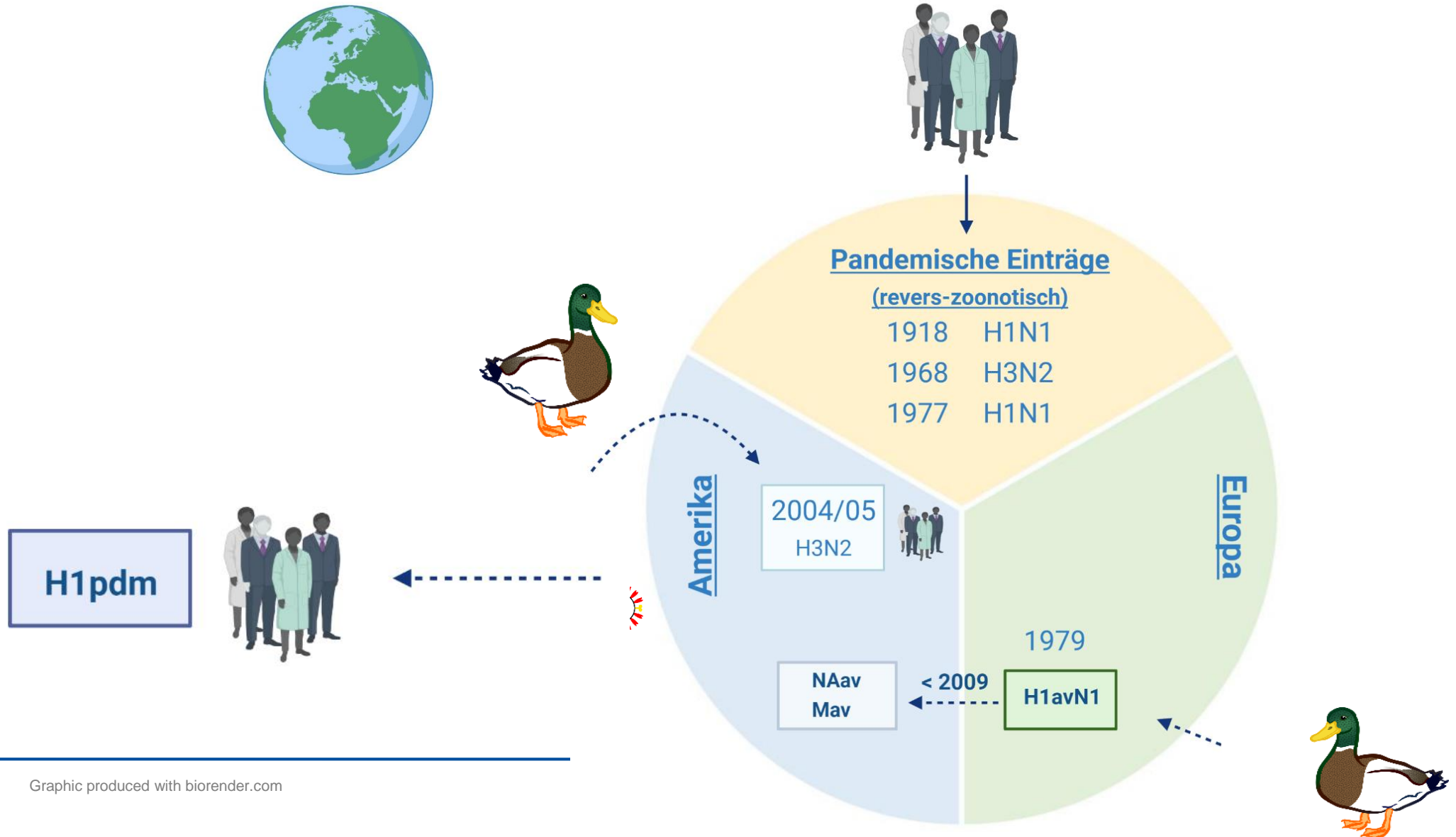
The PRDC pathogen network and potential new players

Assessed pathogens

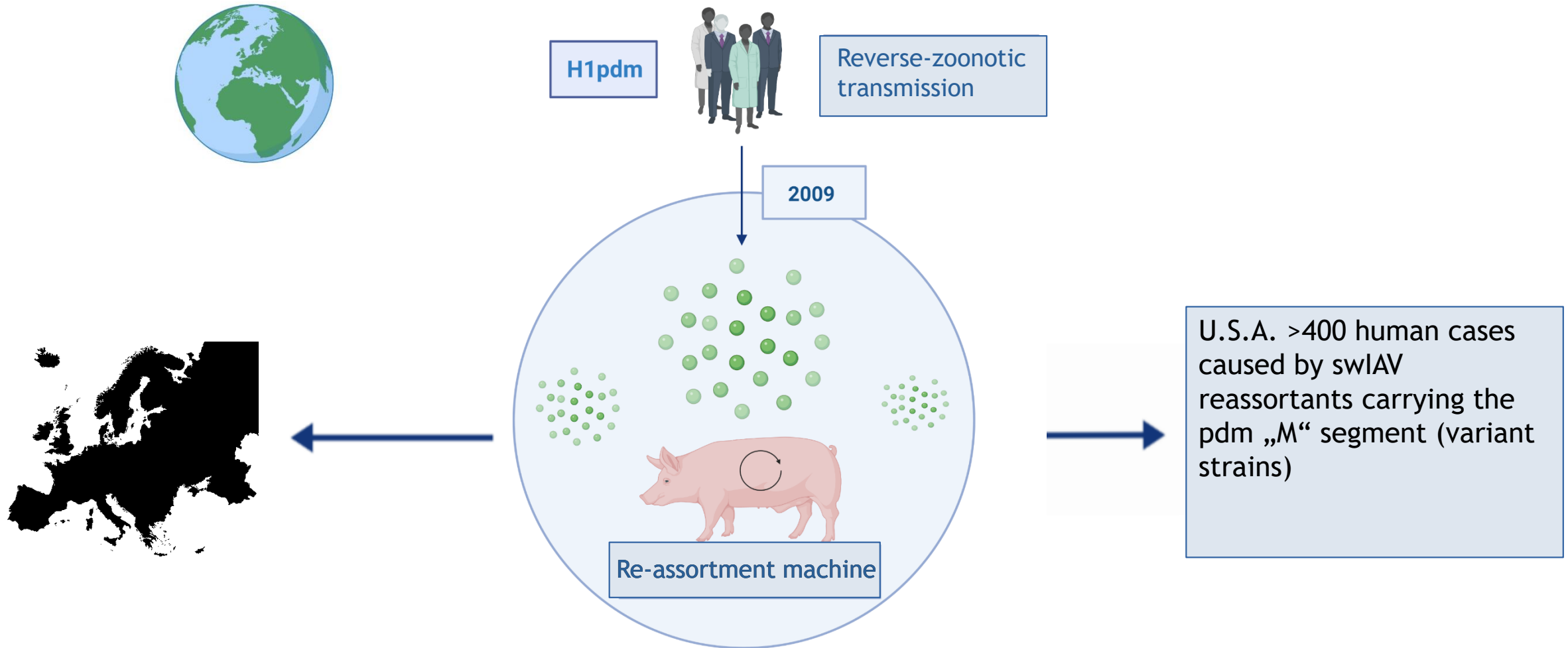


- Synergistic interactions enhance clinical signs
- New players on the field
 - Porcine respirovirus
 - Porcine ortho-pneumo-virus

A global perspective of swIAv epidemiology



Re-entering the cycle



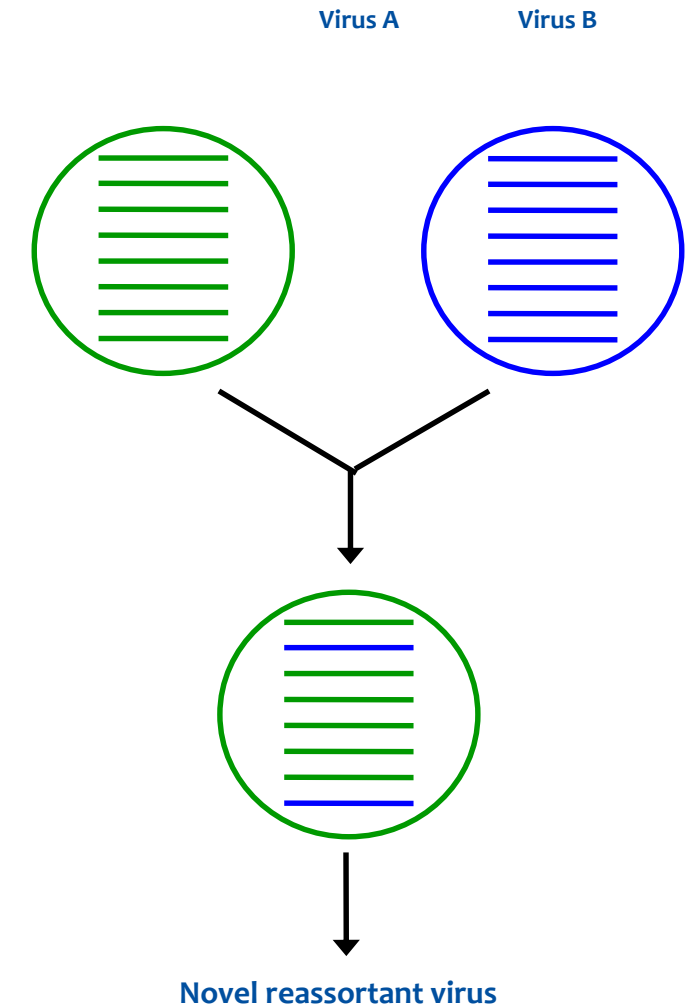
Antigenic drift and shift

- **Antigenic drift:**

- Accumulation of mutations in hemagglutinin protein (HA)
- Modified HA can evade host immunity

- **Antigenic shift:**

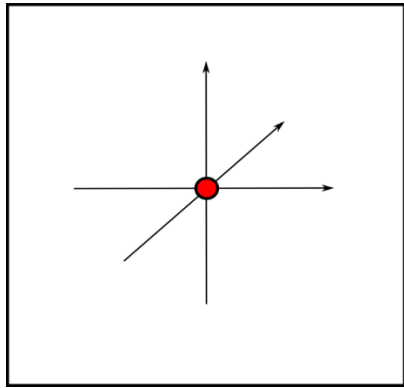
- Co-infection with different Influenza viruses leads to progeny with reassorted genomes
- exchange of viral genome segments



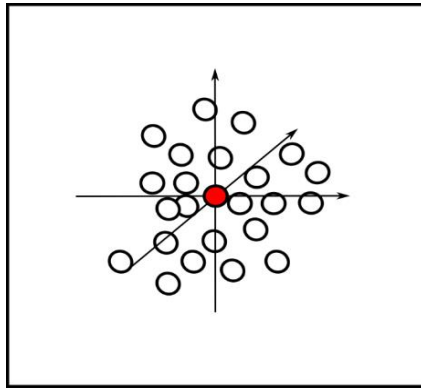
3)

Molecular mechanisms elucidate a continuing emergence of influenza viruses

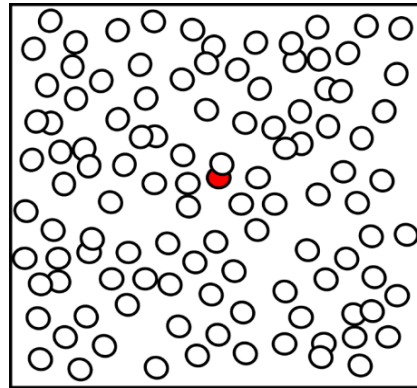
Missing correcting functions of the viral replication machinery (RdRp)



Clonal virus



Sloppy genome replication produces clouds of genetically similar but not identical virus genomes



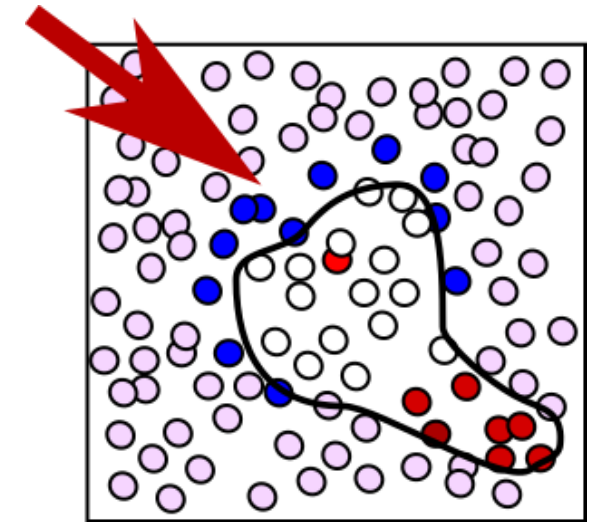
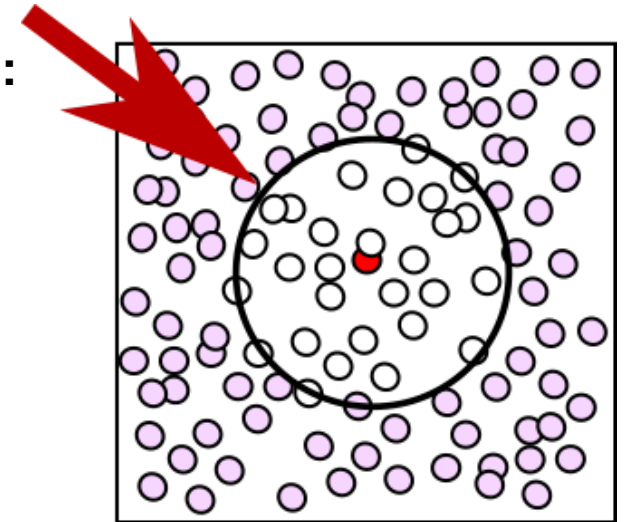
If sloppy replication would continue unrestrictedly

Fitness horizon:
Restricts the number of replications-competent variants

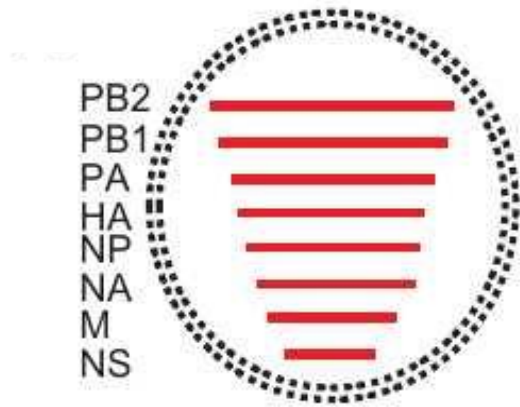
Selection pressure:
(e.g. immunity, host species jumps)

Selection pressure + fitness horizon =

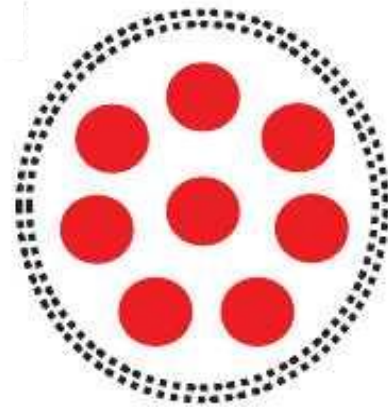
antigenic drift



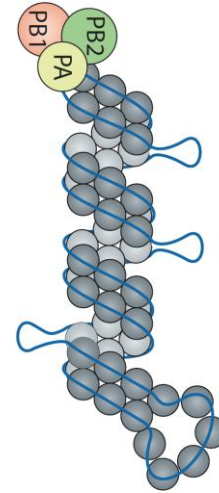
Segmented genome enables reassortments



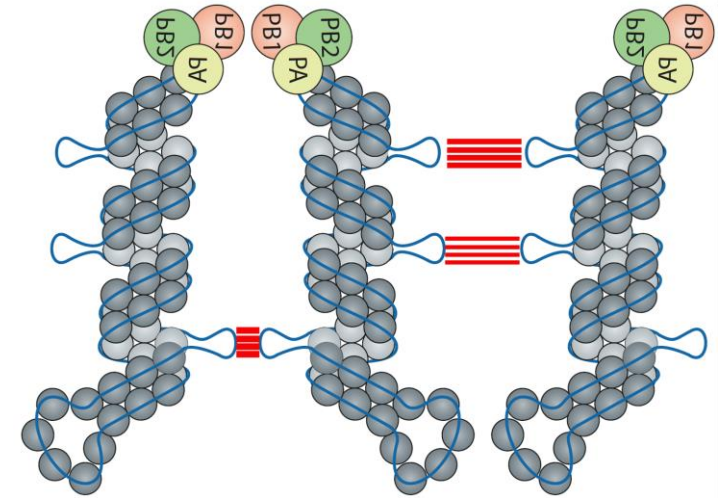
IAV virion



'7+1' arrangement

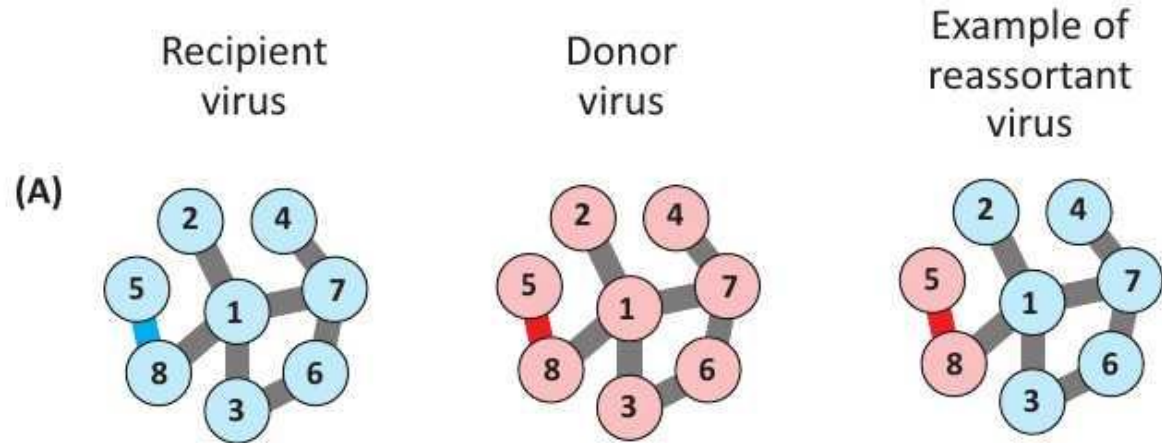


Irregularly packaged ribonucleocapsids allow the formation of RNA nooses



Intersegmental RNA:RNA pairings define „matching“ segments

Segmented genome enables reassortments

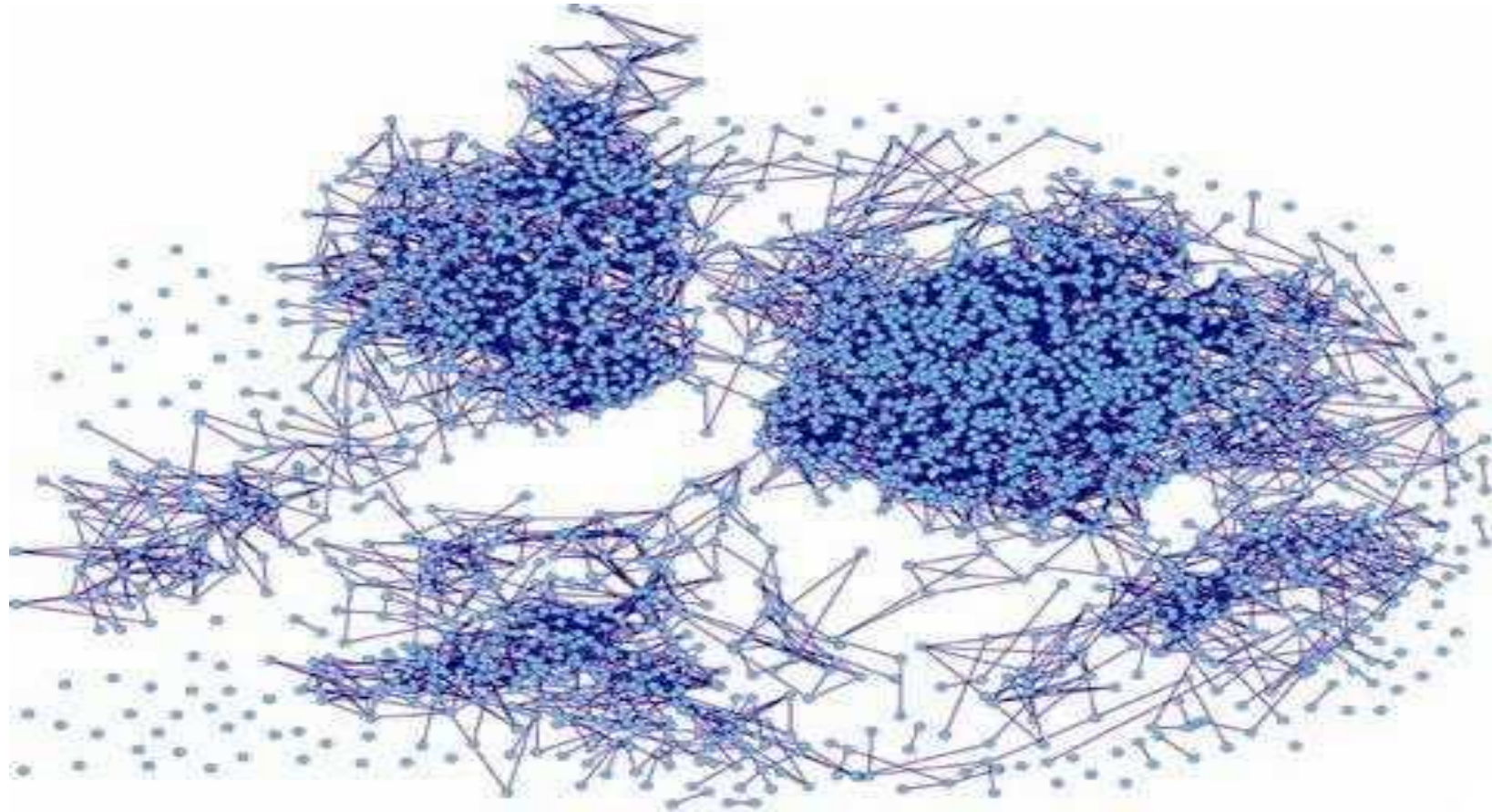


In case HA (no. 4) and/or NA (no. 6) are reassorting an

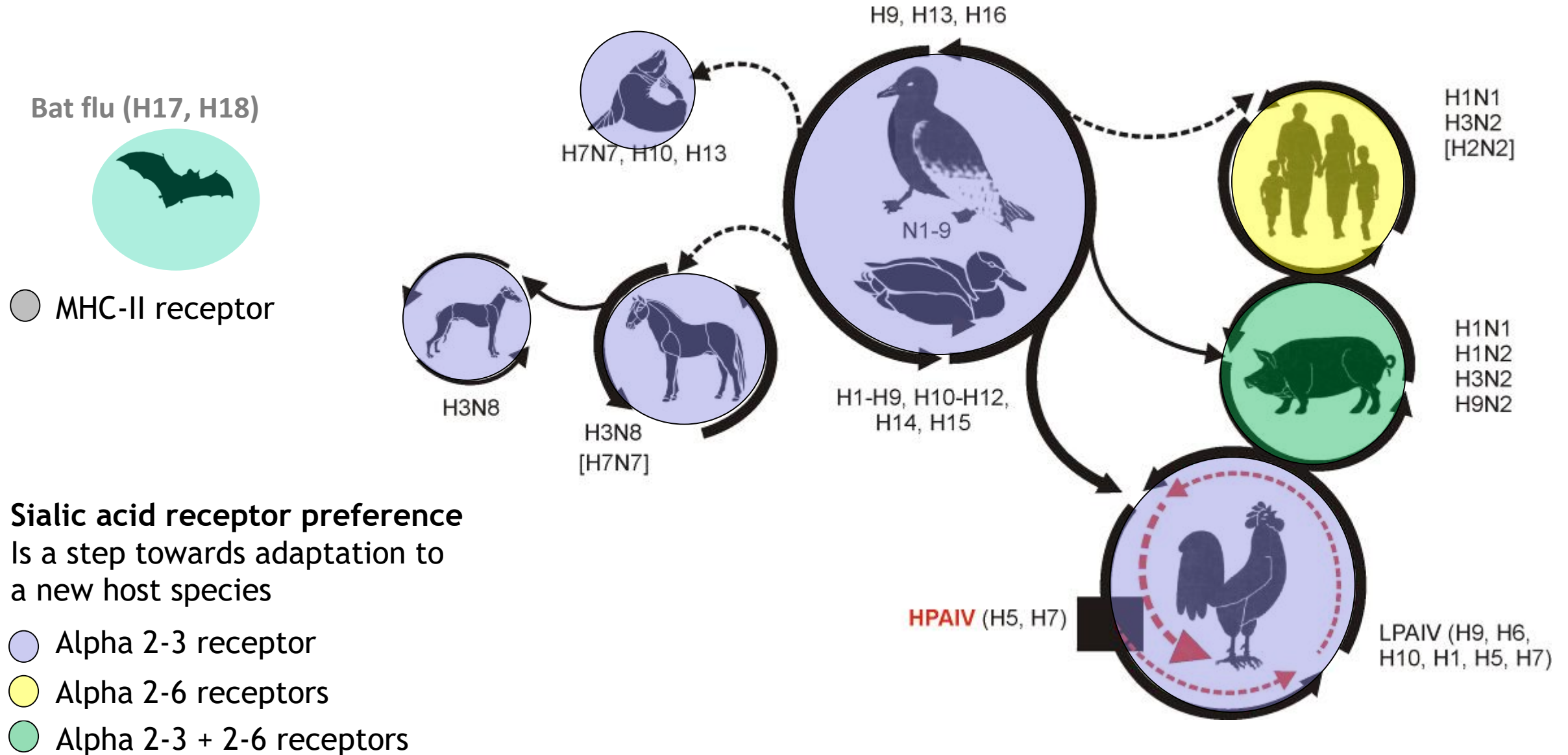
antigenic shift

occurs.

Genetic drift produces genome constellations



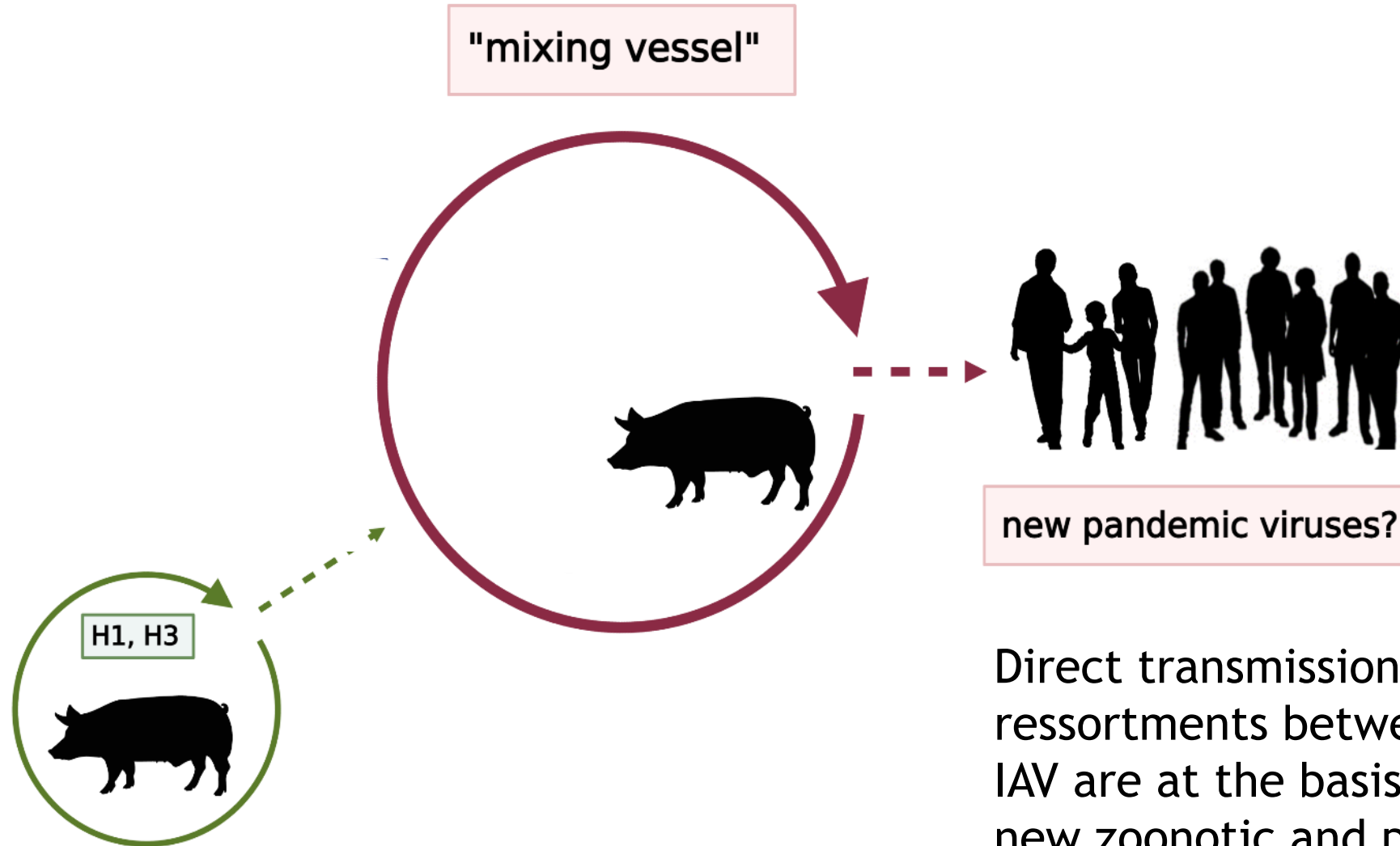
IAV quasispecies in the real world



Avian IAV in swine: Sporadic encounters

Representative Strain from Each Cluster ^a	Isolation Date ^b	Subtype	Country
A/swine/Hong Kong/10/1998	1998	H9N2	Hong Kong
A/swine/ShanDong/1/2003	2003	H9N2	China
A/swine/Guangxi/58/2005	2005	H9N2	China
A/swine/Jiangxi/1/2004	2004	H9N2	China
A/swine/Korea/S452/2004	2004	H9N2	Korea
A/swine/Guangxi/7/2007	1 February 2007	H9N2	China
A/swine/Henan/Y1/2009	2 July 2009	H9N2	China
A/swine/Shanghai/Y1/2009	13 October 2009	H9N2	China
A/swine/Guangdong/L1/2010	30 January 2010	H9N2	China
A/swine/Yantai/16/2012	21 September 2012	H9N2	China
A/swine/China/SPF_embryonated_ chicken_eggs/2015	18 May 2015	H9N2	China
A/swine/Shandong/TA009/2019	April 2019	H9N2	China

More than one „mixing vessel“



Direct transmission with adaptation or reassortments between human and other IAV are at the basis of the emergence of new zoonotic and pandemic IAV.

Why may swIAV diagnosis be useful?

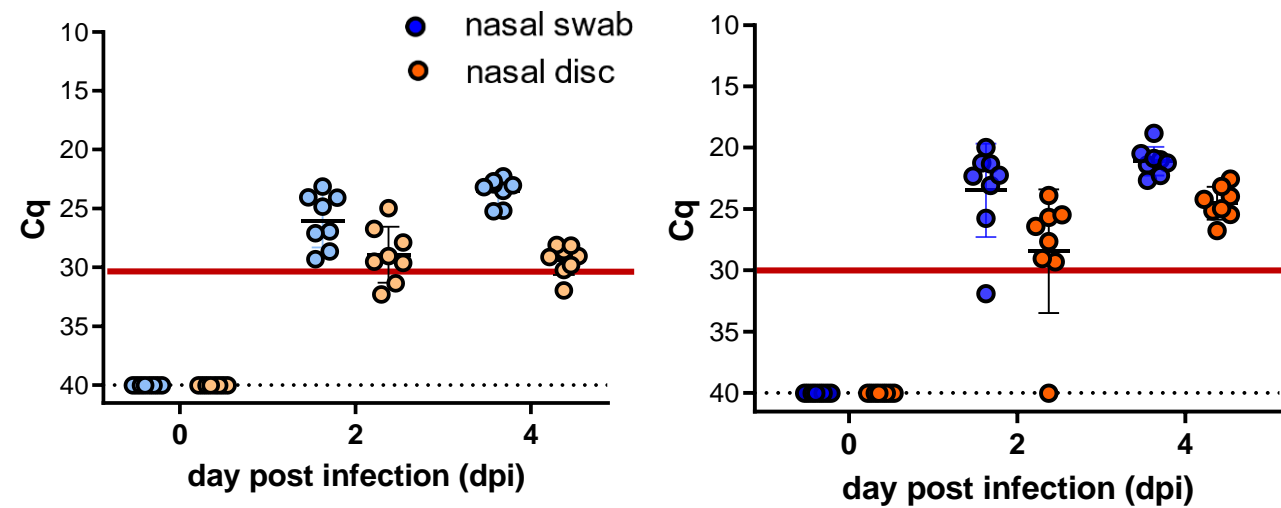
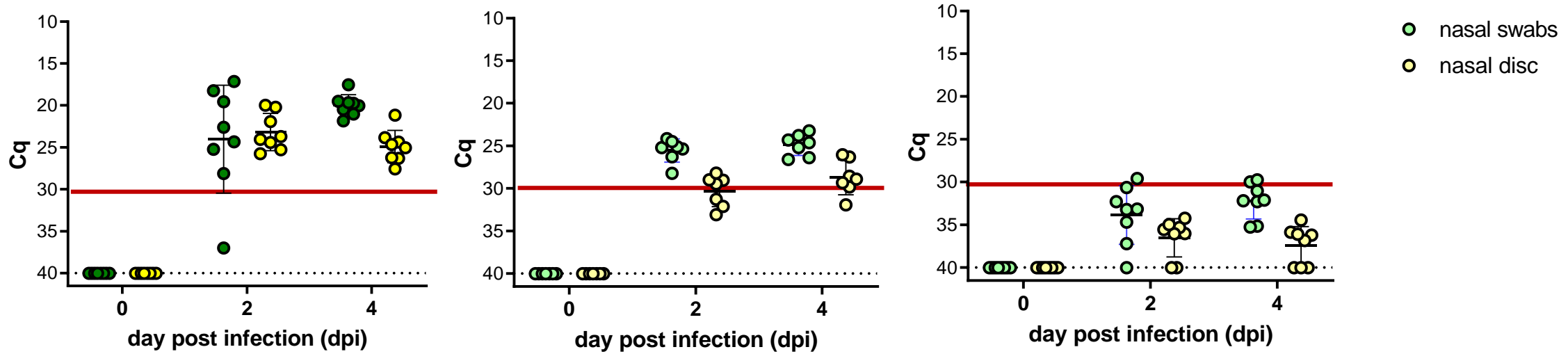
1. Does the holding have an influenza problem?

Generic influenza RT-qPCRs

2. Which subtypes are relevant?

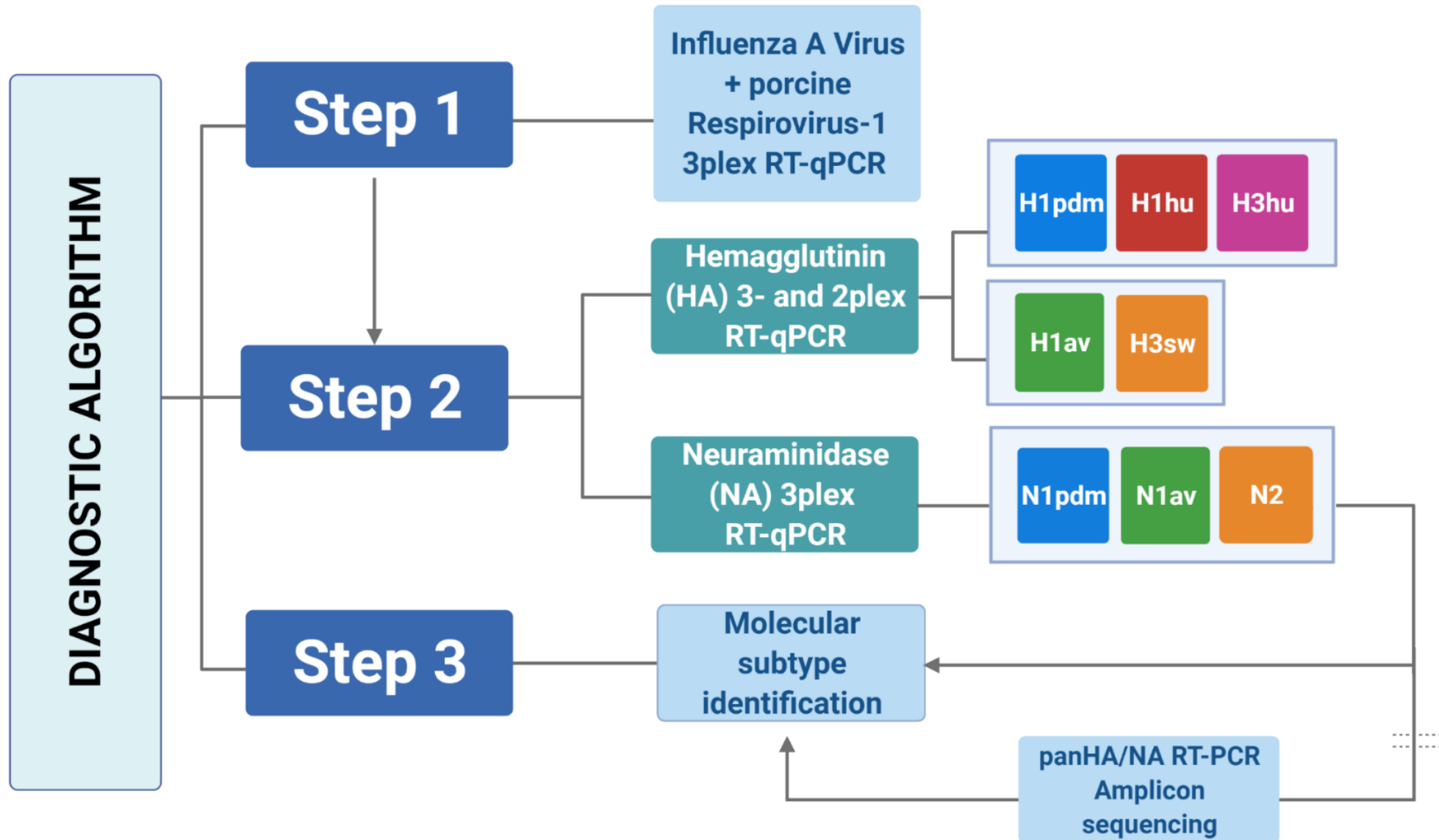
SwIAV subtyping RT-qPCRs

Nasal virus excretion

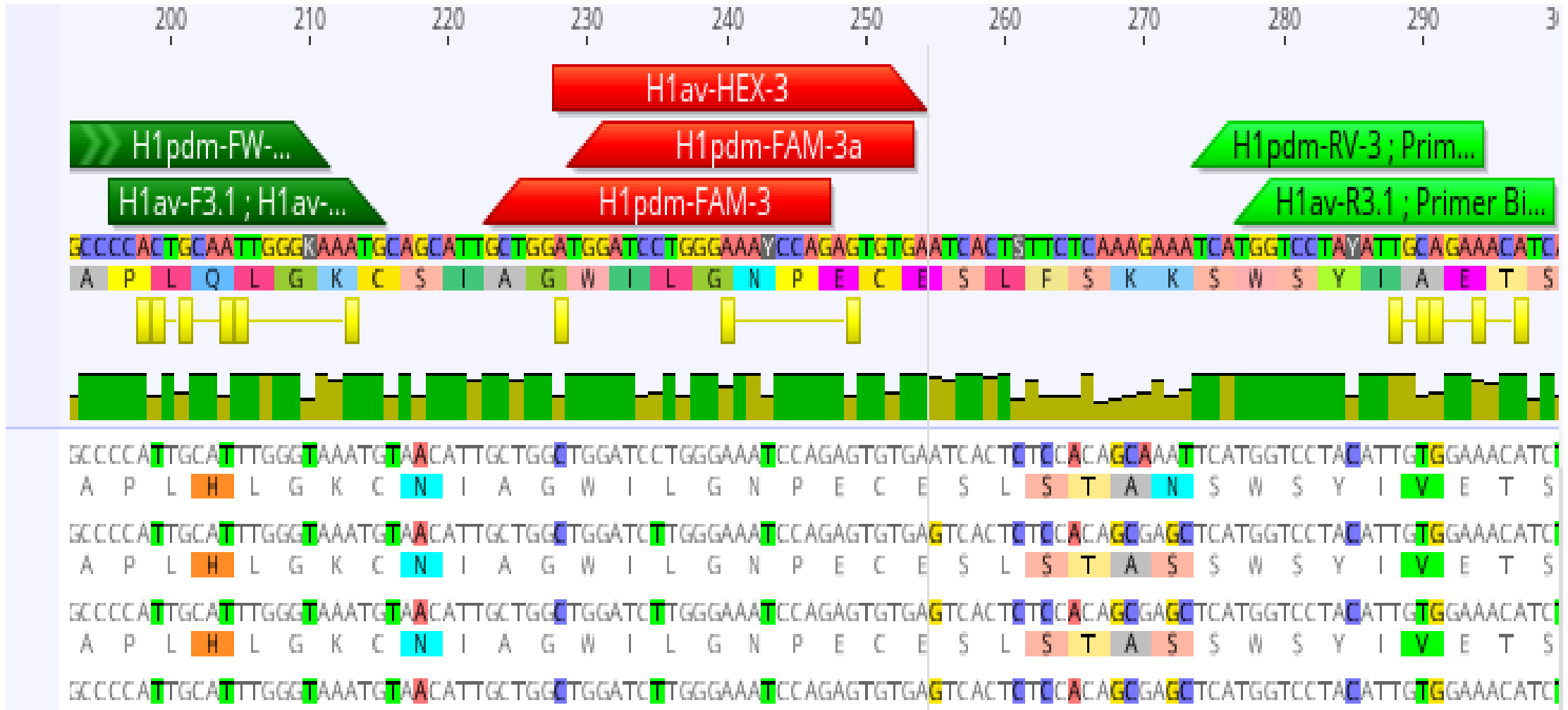


- Snout swabs work
- Quantitatively snout swabs are less sensitive by a factor of 10 compared to deep nasal swabs
- Qualitatively no difference
- Udder wipes, environmental samples?

swIAV surveillance - intricate diagnostic tools



swIAV subtyping requires frequent updating of PCRs



Why do we do swIAV diagnosis?

1. Does the holding have an influenza problem?

Generic influenza RT-qPCRs

2. Which subtypes are relevant?

SwIAV subtyping RT-qPCRs

3. Are the available vaccines effective in this holding?

Can not be effectively answered by molecular diagnostics!

- HA sequencing
- Antigenic cartography
- Vaccination-challenge experiments

Why do we do swIAV diagnosis?

1. Does the holding have an influenza problem?
 2. Which subtypes are relevant?
3. Are the available vaccines effective in this holding?
4. Do the relevant strains have zoonotic potential?



Assessing zoonotic potential of swIAVs

1. Resistance against huMxA

2. Aerosol-driven transmission

3. Escape from human population immunity

Cell Host & Microbe

CellPress

Resource

Surveillance of European Domestic Pig Populations Identifies an Emerging Reservoir of Potentially Zoonotic Swine Influenza A Viruses

Dinah Henritzi,^{1,10} Philipp Peter Petric,^{2,3,4,10} Nicola Sarah Lewis,^{5,6} Annika Graaf,¹ Alberto Pessia,⁷ Elke Starick,¹ Anja Breithaupt,⁸ Günter Strebellow,¹ Christine Luttermann,⁹ Larissa Mareike Kristin Parker,^{2,4} Charlotte Schröder,⁸ Bärbel Hammerschmidt,⁴ Georg Herrler,¹⁰ Elisabeth große Beilage,¹¹ Daniel Stadlbauer,¹² Viviana Simon,^{12,13} Florian Krammer,¹² Silke Wacheck,¹⁴ Stefan Pesch,¹⁵ Martin Schwemmler,^{2,4} Martin Beer,^{2,4} and Timm Clemens Harder^{1,10,*}



FRIEDRICH-LOEFFLER-INSTITUT

FLI

Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health



HuMxA resistance of swIAV

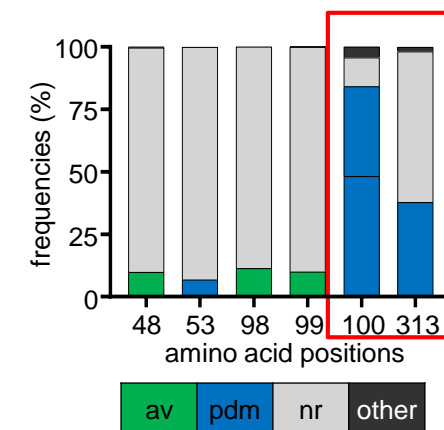
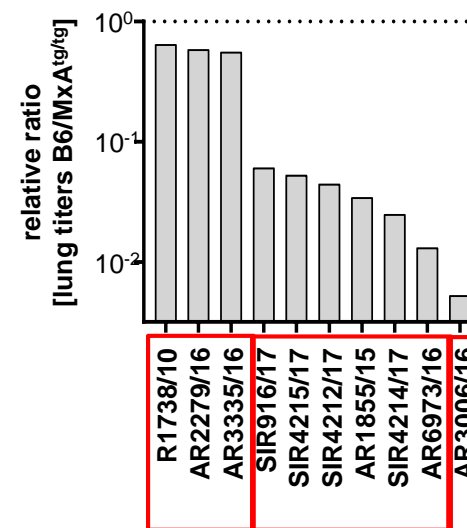
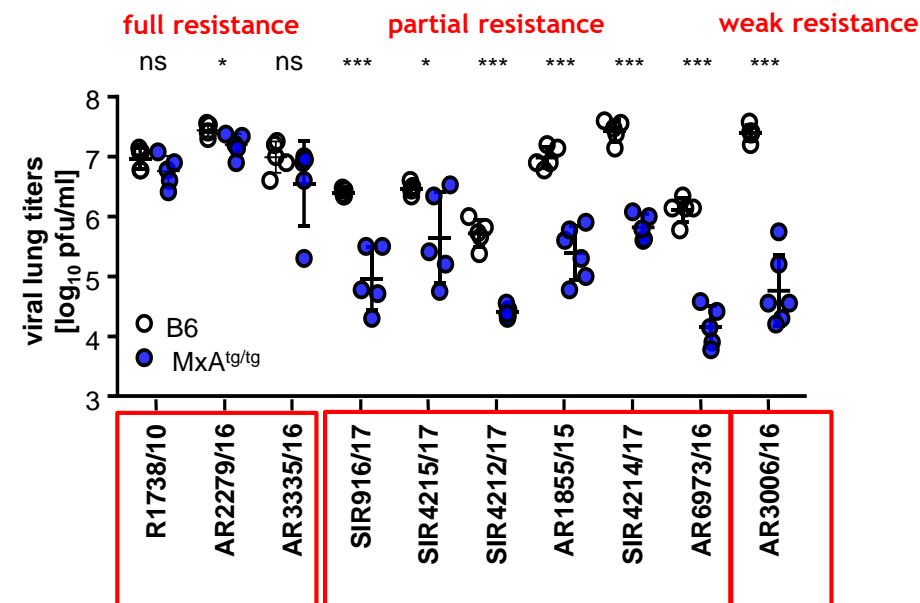


- MxA lends innate resistance against IAV replication in the human host
- MxA resistance is encoded on the NP protein:
 - Q48, K98, K99 in avian origin NP
 - D53, I100, V313 in pandemic H1/2009

(Dornfeld et al., 2018)

Virus ID	HA	NA	PB2	PB1	PA	NP	M	NS
R1738/10	av	av	av	av	av	av	av	av
AR2279/16	pdm	pdm	pdm	pdm	pdm	pdm	pdm	pdm
AR3335/16	av	av	av	av	av	av	av	av
SIR916/17	hu	N2g	av	av	av	av	av	av
SIR4215/17	av	av	av	av	av	av	av	av
SIR4212/17	av	av	av	av	av	av	pdm	av
AR1855/15	pdm	pdm	pdm	pdm	pdm	pdm	pdm	pdm
SIR4214/17	av	N2g	pdm	pdm	pdm	pdm	pdm	pdm
AR6973/16	av	N2g	av	av	av	av	av	av
AR3006/16	av	N2g	pdm	pdm	pdm	pdm	av	av

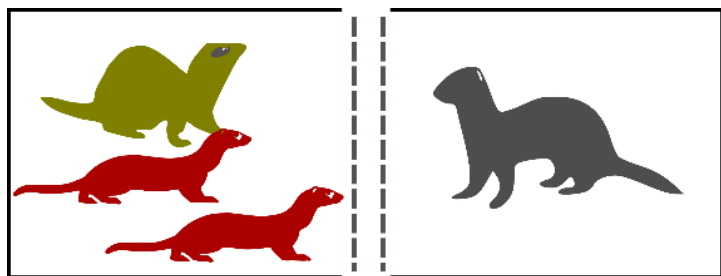
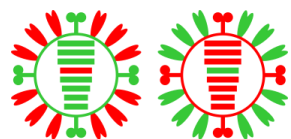
	48	53	98	99	100	313
R1738/10	Q	E	K	K	R	F
AR2279/16	K	D	R	R	I	V
AR3335/16	Q	E	R	K	R	F
SIR916/17	Q	E	K	K	R	F
SIR4215/17	Q	E	K	K	R	F
SIR4212/17	Q	E	K	K	R	F
AR1855/15	K	E	R	R	I	V
SIR4214/17	K	E	R	R	I	V
AR6973/16	Q	E	K	K	R	F
AR3006/16	K	E	R	R	I	V



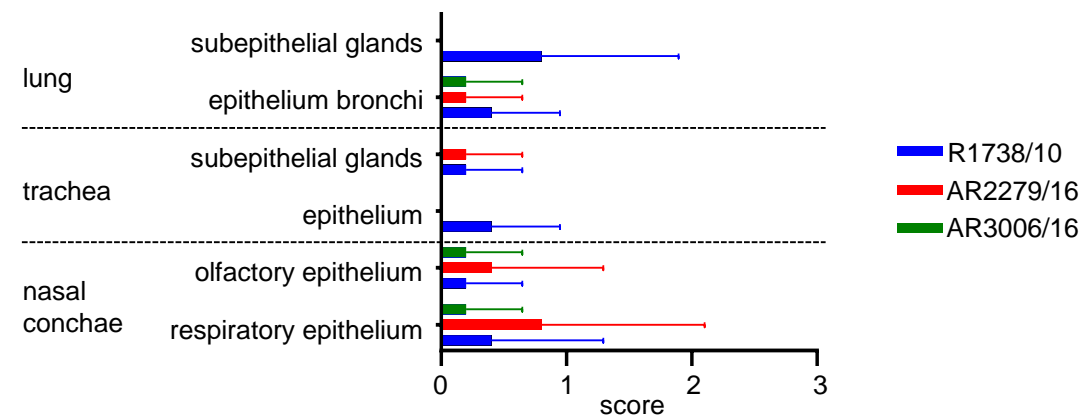
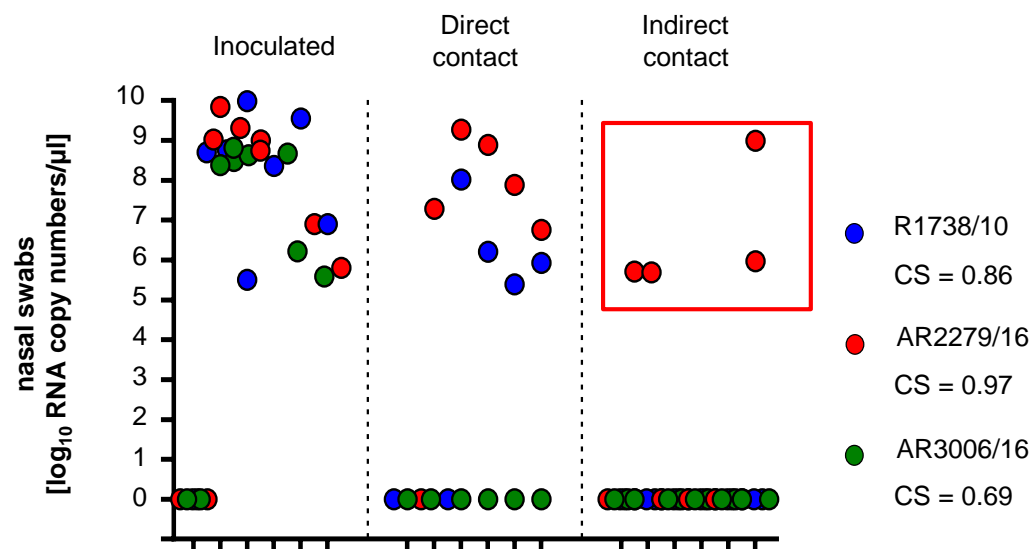


swIAV transmission in the ferret model

swIAV

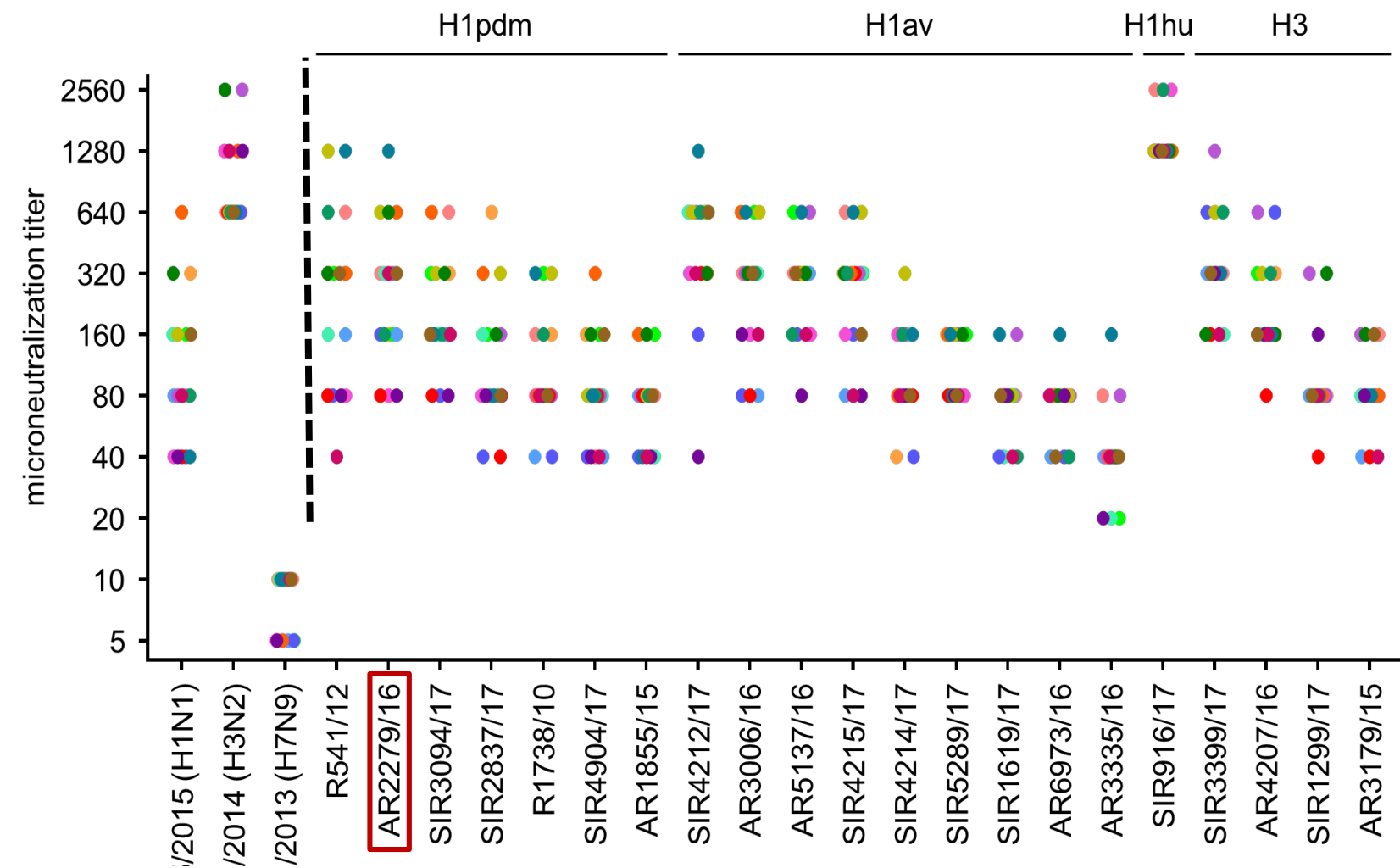


Direct contact and aerosol-driven transmission in ferrets





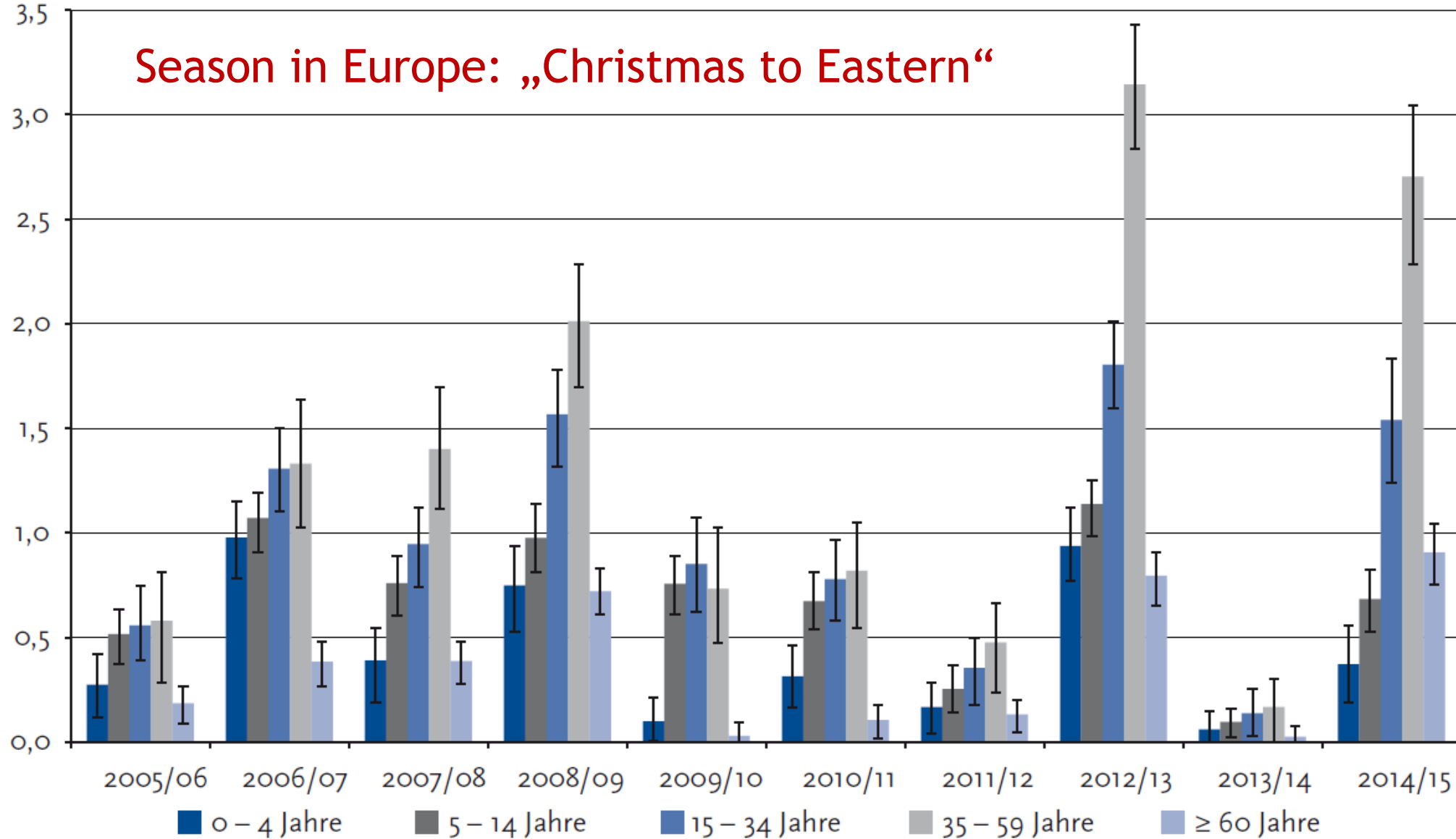
Reactivity of adult human sera against swIAV



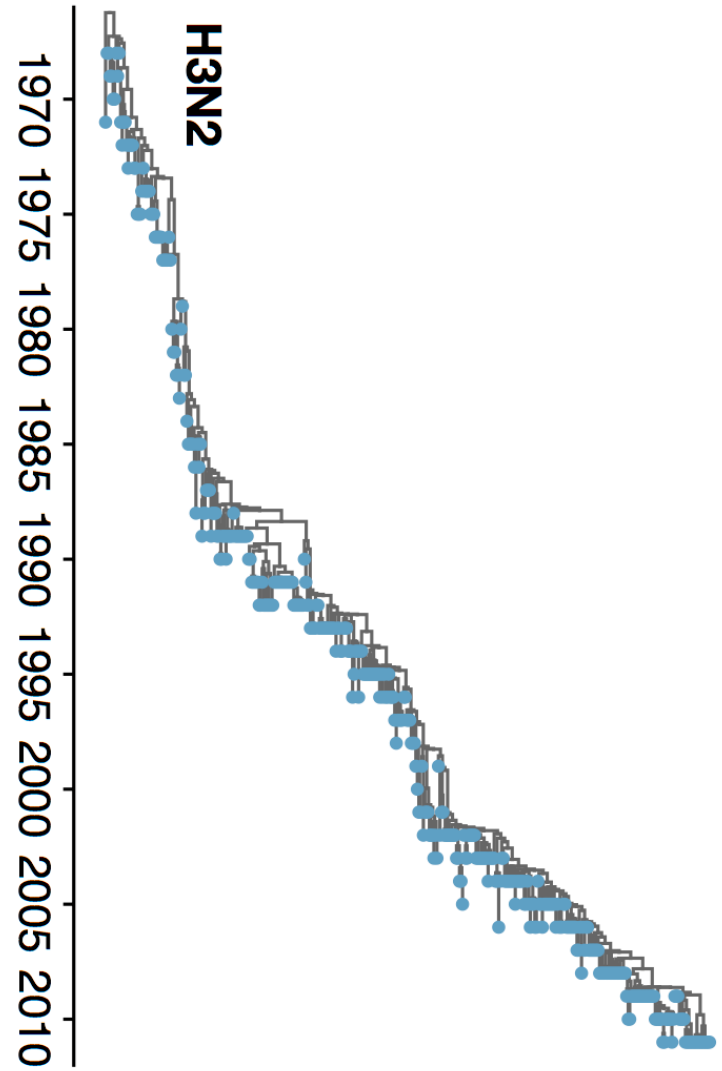
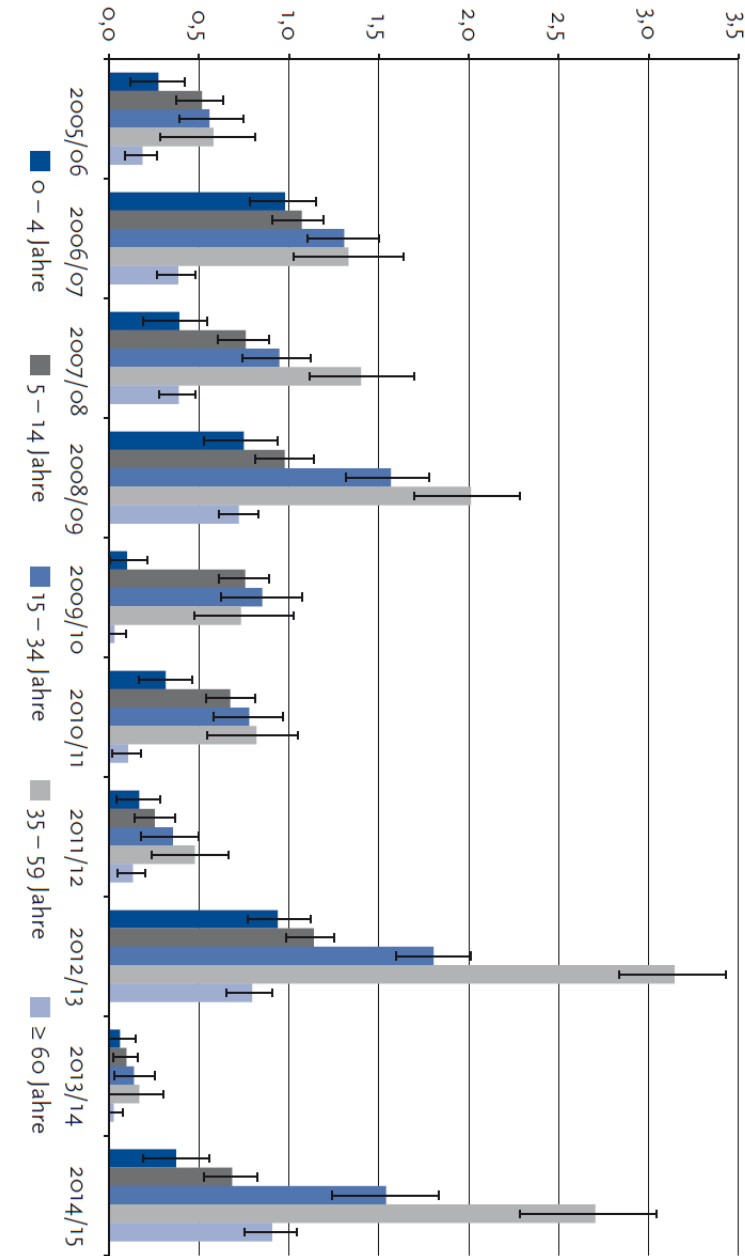
➤ How would children's sera have reacted?



Seasonality of human influenza

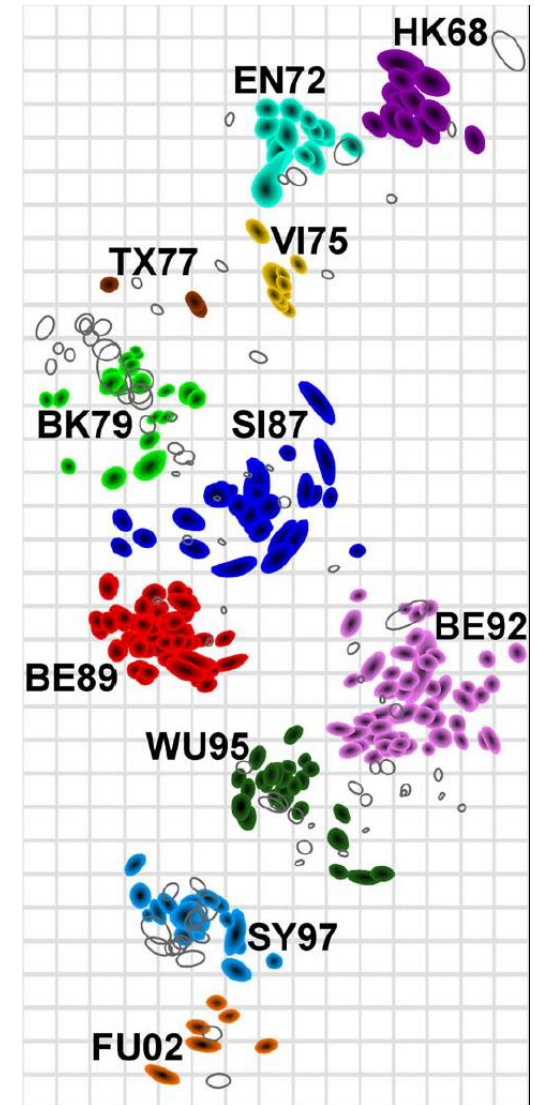


The main cause of seasonality: Antigenic drift



Phylogenetic analyses, HA H3

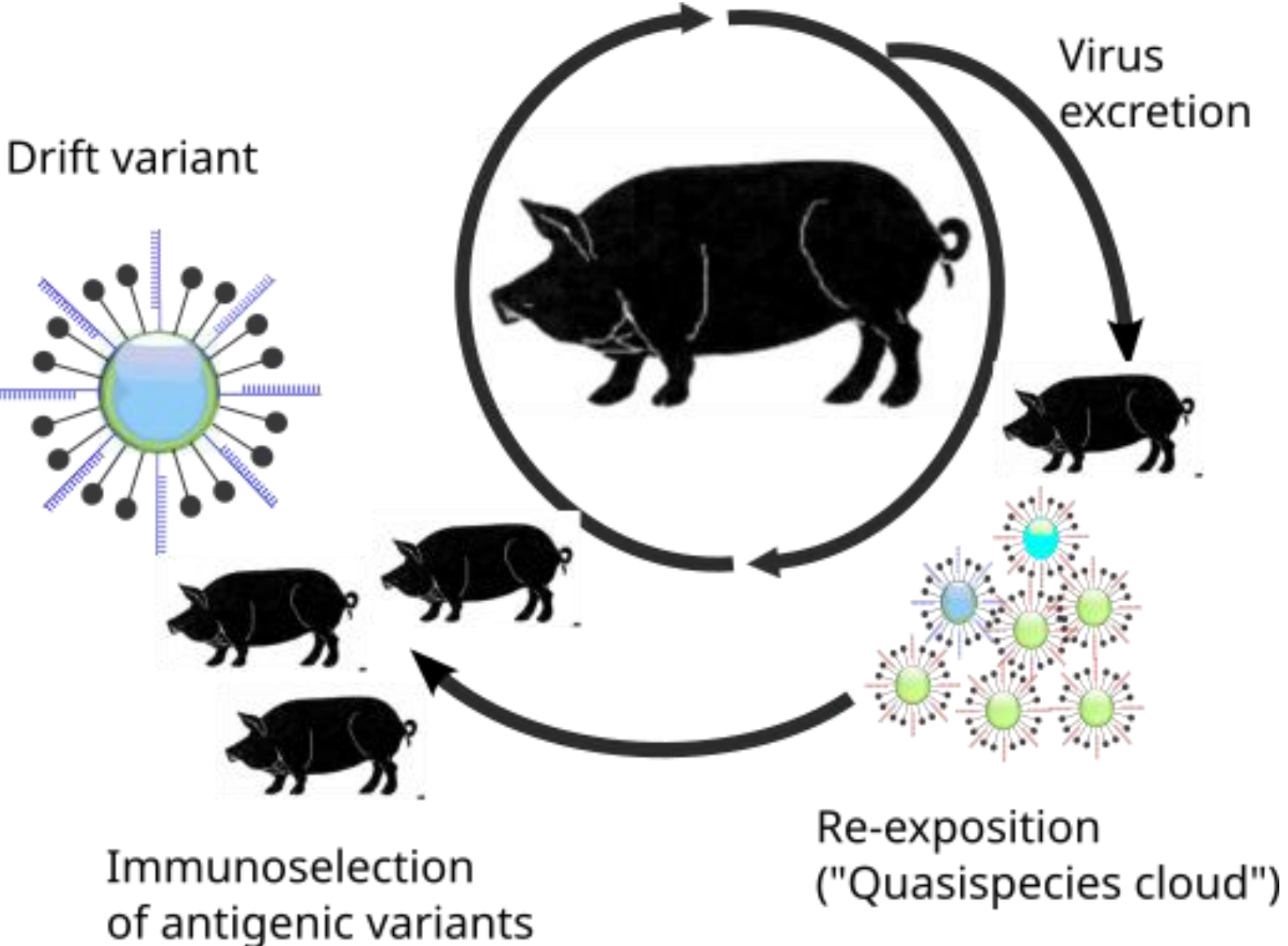
<https://bedford.io/projects/phylogenetics-lecture/flu.html#/11>



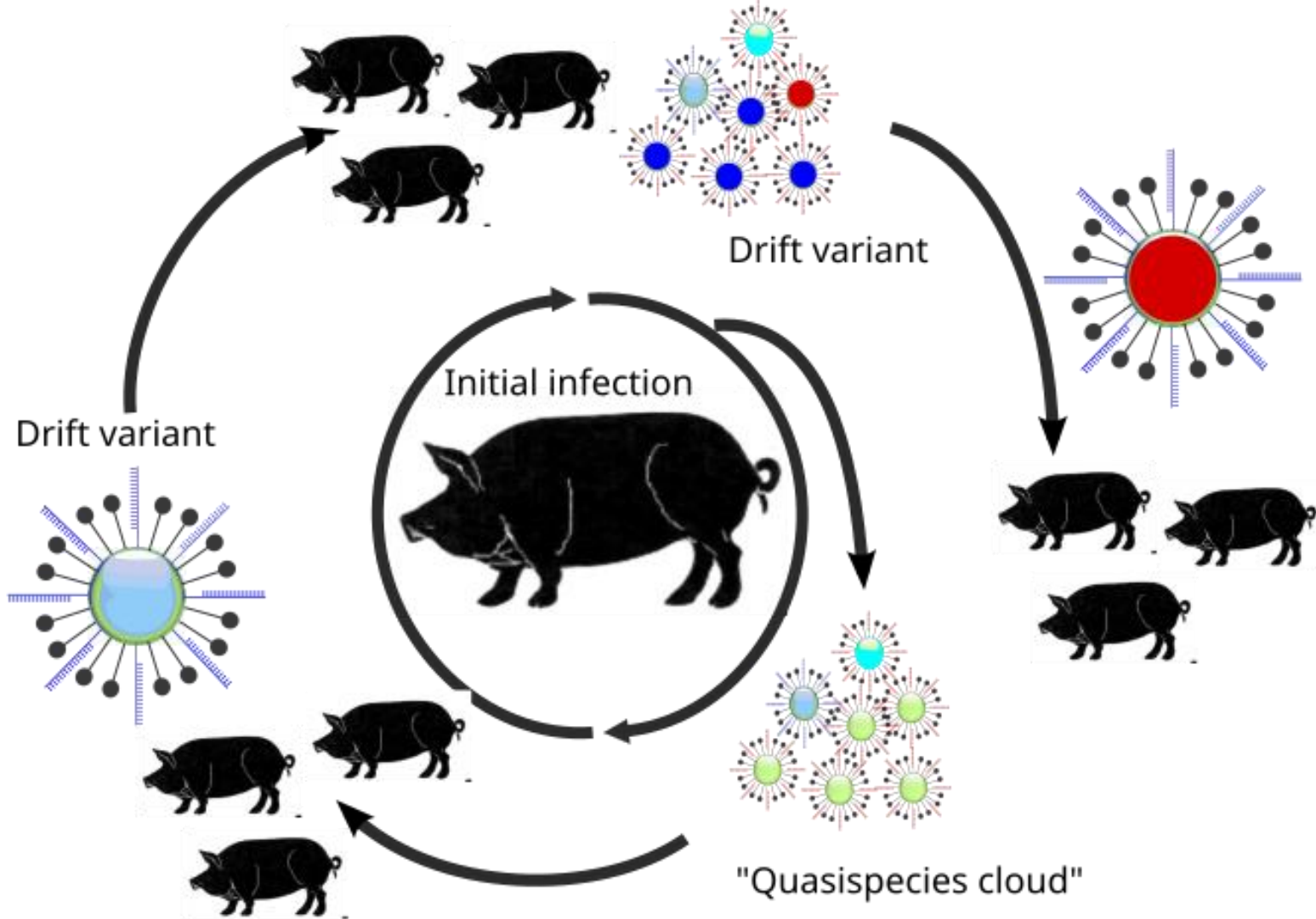
Antigenic cartography, HA H3

ad_cm_ai_nrl-annual-meetings-14_pres-10.pdf

Endemic swIAV infections in holdings foster antigenic drift



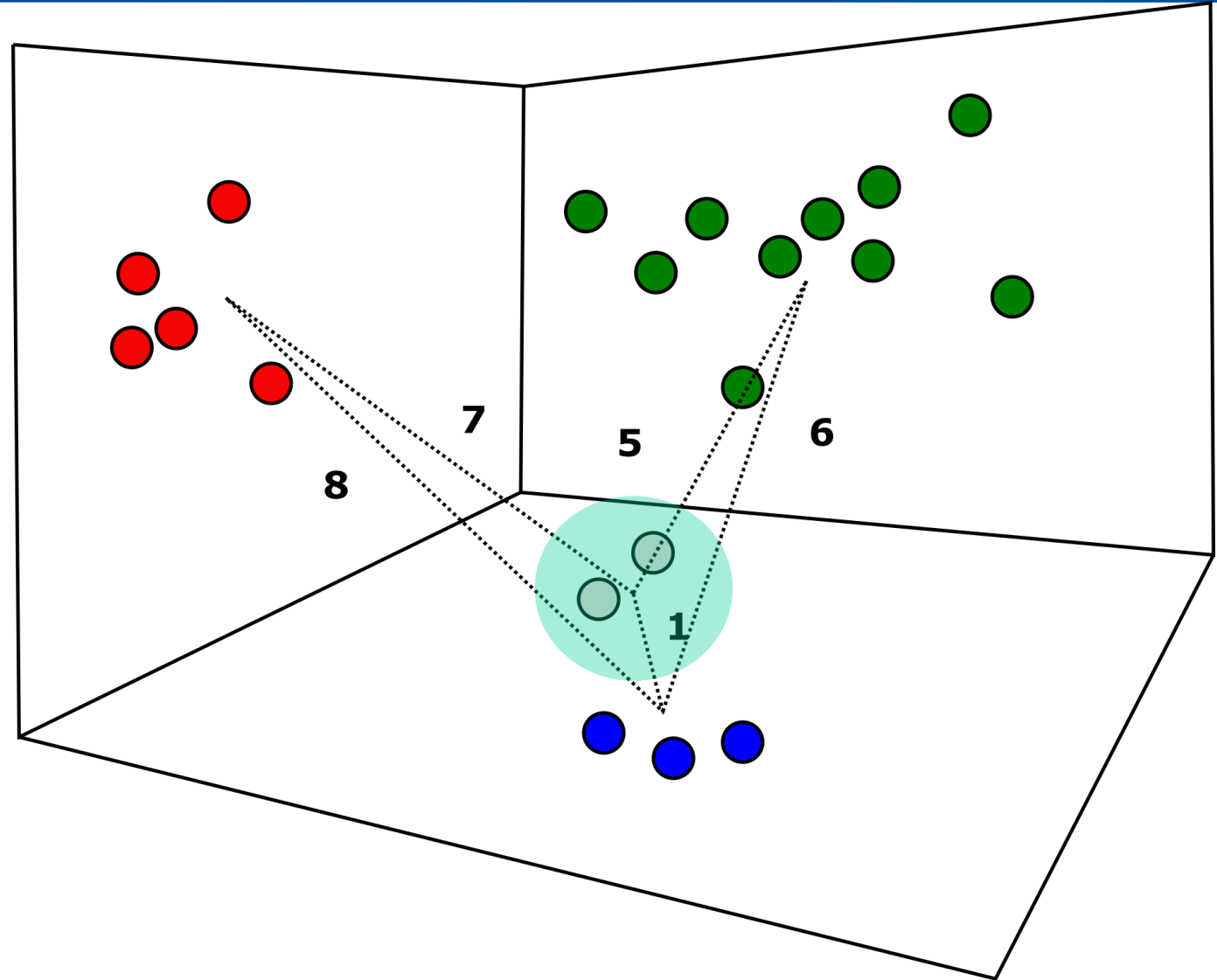
Endemic swIAV infections in holdings foster antigenic drift



Immune-driven selection of antigenically altered "fitter" virions

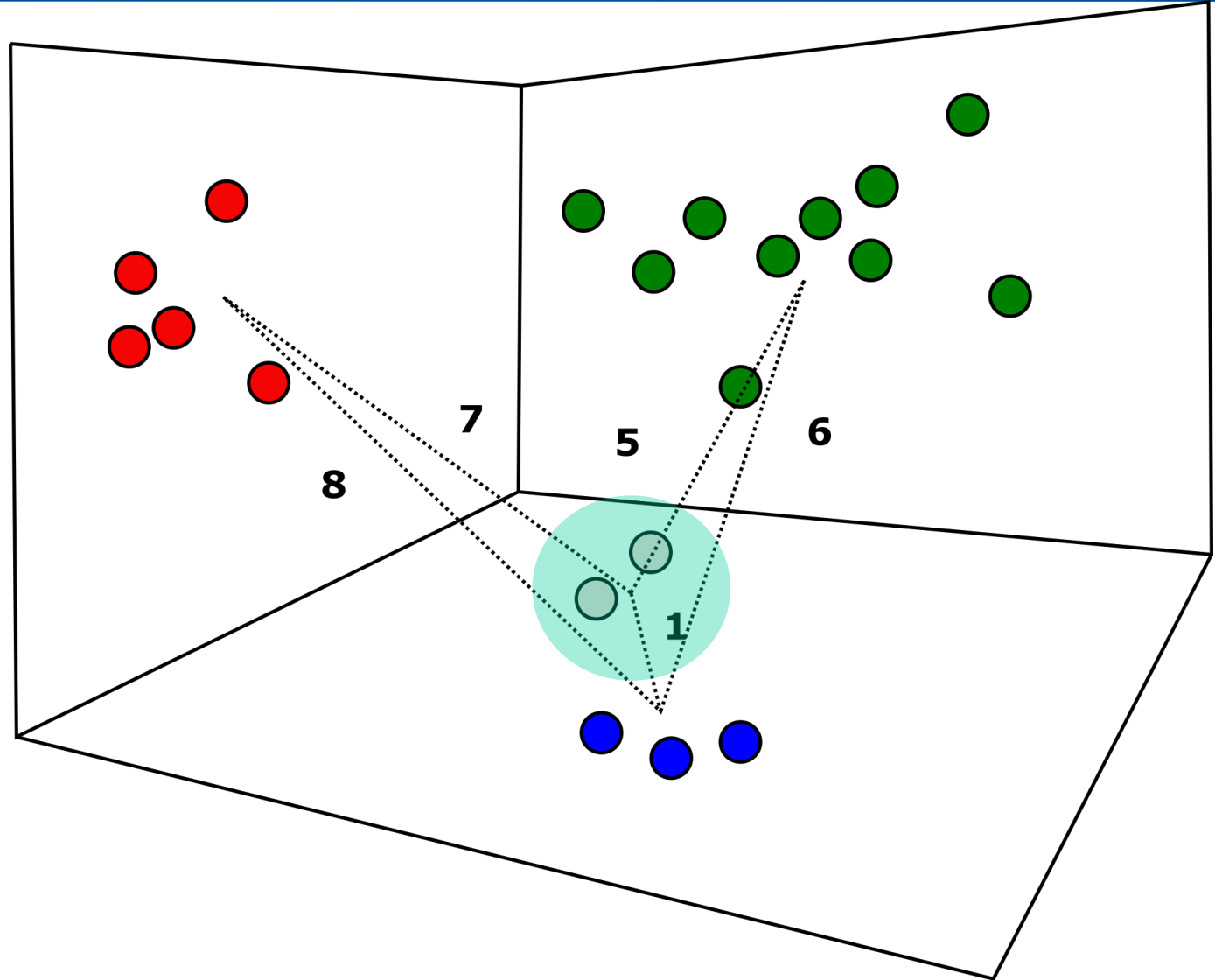
How to find the best possible vaccine virus?

- Field and vaccine virus must show a sufficiently high **antigenic match**.
- **Antigenic cartography** depicts antigenic matches graphically.
- **Antigenic distances** can be quantified.
- **Distances > 2 units** signal less favourable antigenic matches.



Overcoming poor antigenic matches

- The aim is to **broaden protective immunity** in a way that all relevant strains are covered:
- Use of **potent adjuvants**
- Use of **heterologous boosting schemes**
- **Exchange/update** of vaccine strains (local, regional vaccines)
- Use of **universal vaccines**



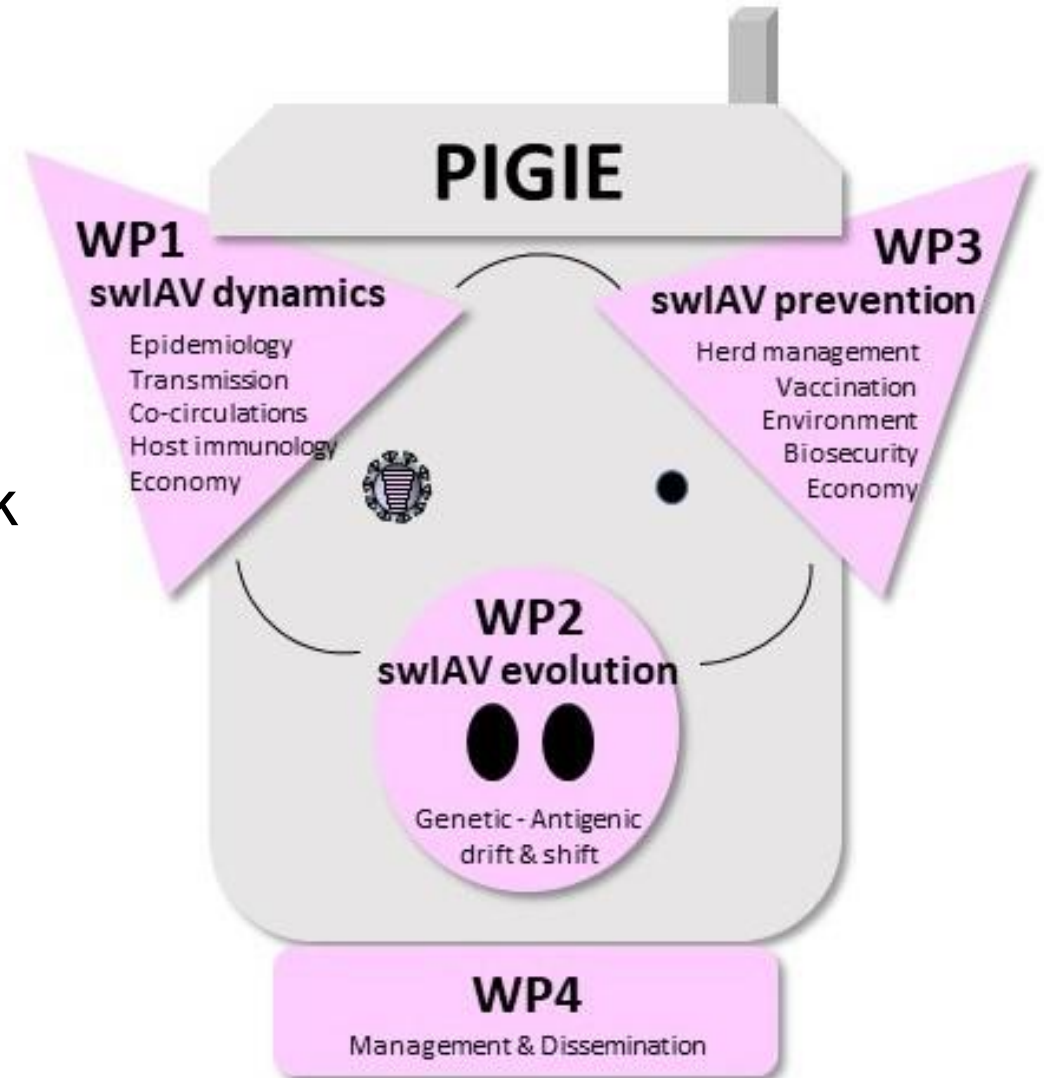
PIGIE - new EU project on endemic swIAV infections



April 2021
to
March 2024

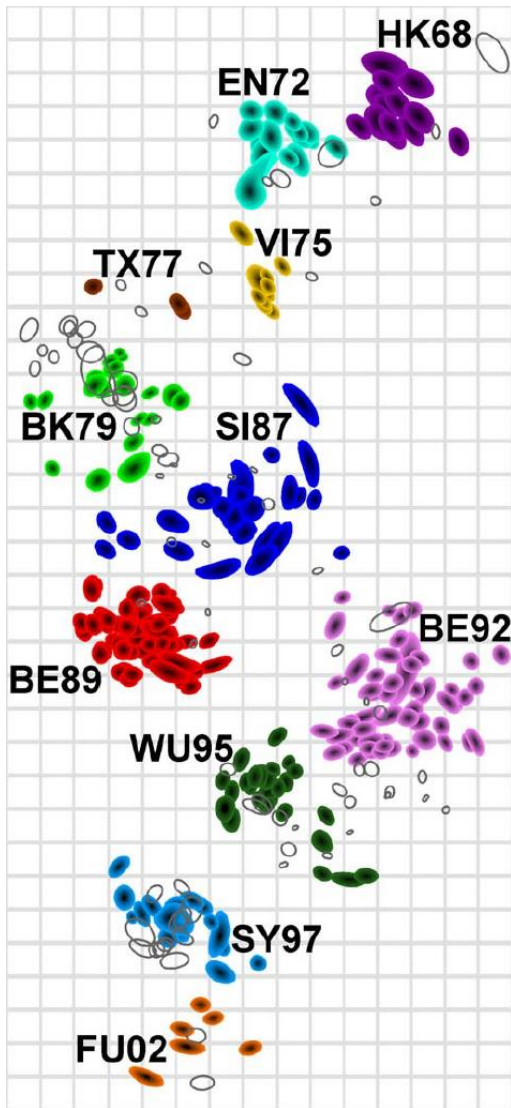
- Dr. G. Simon, ANSES, France (coordinator)
- Prof. L. Larsen, Univ Copenhagen, Denmark
- Dr. C. Chiapponi, IZSLER, Parma, Italy
- Dr. H. Everett, APHA, Weybridge, UK
- Prof. E. Mateo de Antonio, Univ Barcelona, Spain
- Prof. T. Harder, FLI, Prof. E. große Beilage, TiHo, Germany

Figure 1. Organization of PIGIE's activities into WPs and their interrelationships (PIGIE = Pig Influenza Genetics, Intervention and Epidemiology)



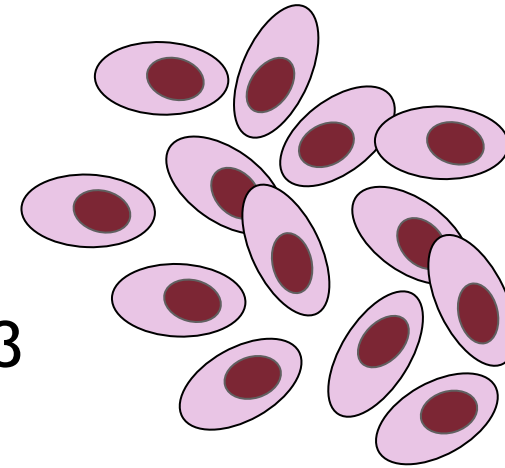
„Frozen evolution“ in swIAV may also be dangerous

Souza CK et al. J Virol.
2022; 96: e0137421



Reverse zoonotic transmission
to pigs

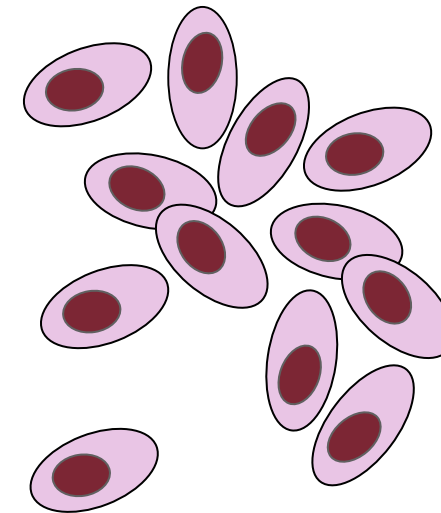
1993



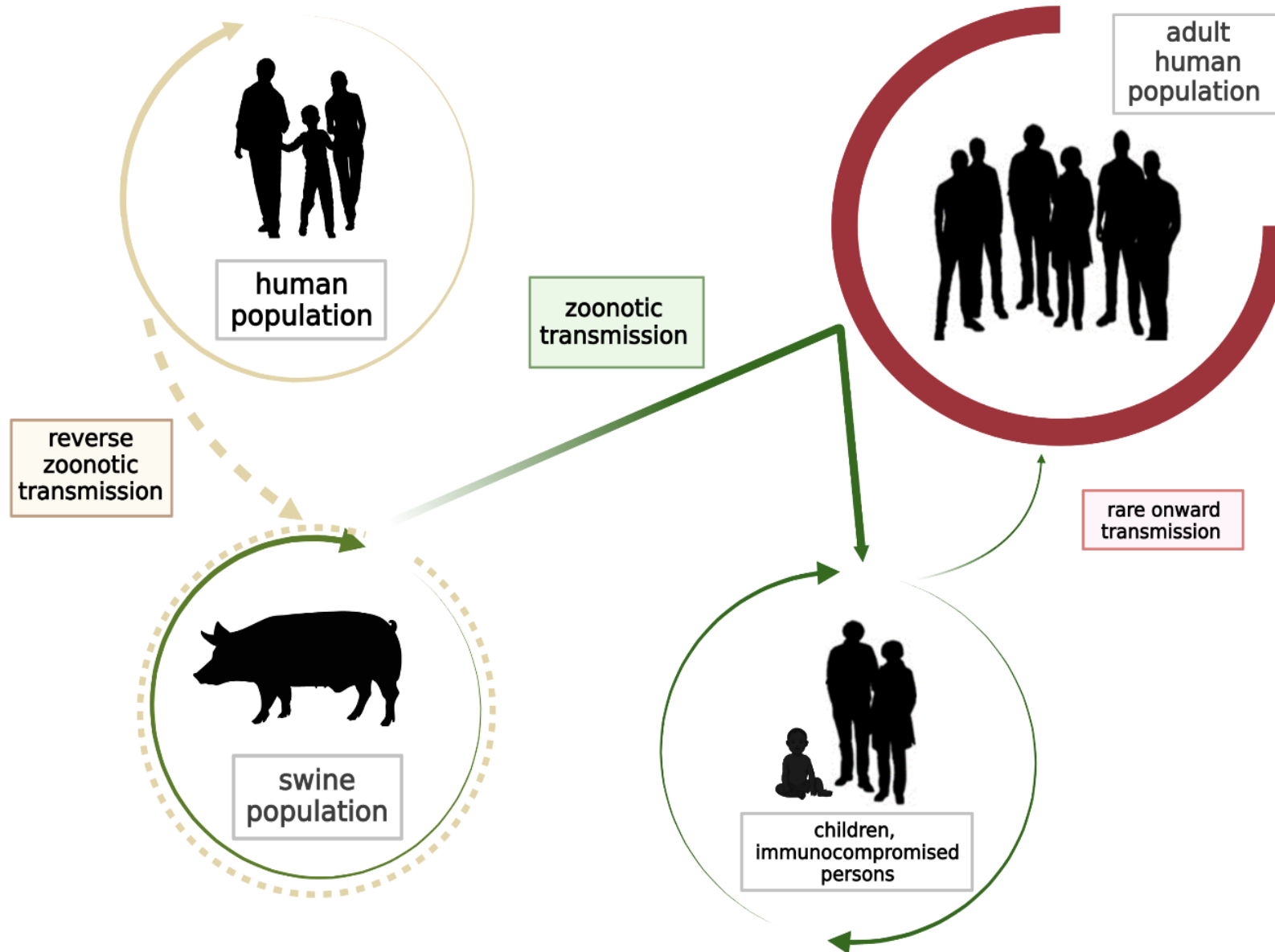
Zoonotic transmission to men

2020

Problem for human population born
after 1995: No sufficient
population immunity, because
of on-going antigen drift in human
but not in porcine population!



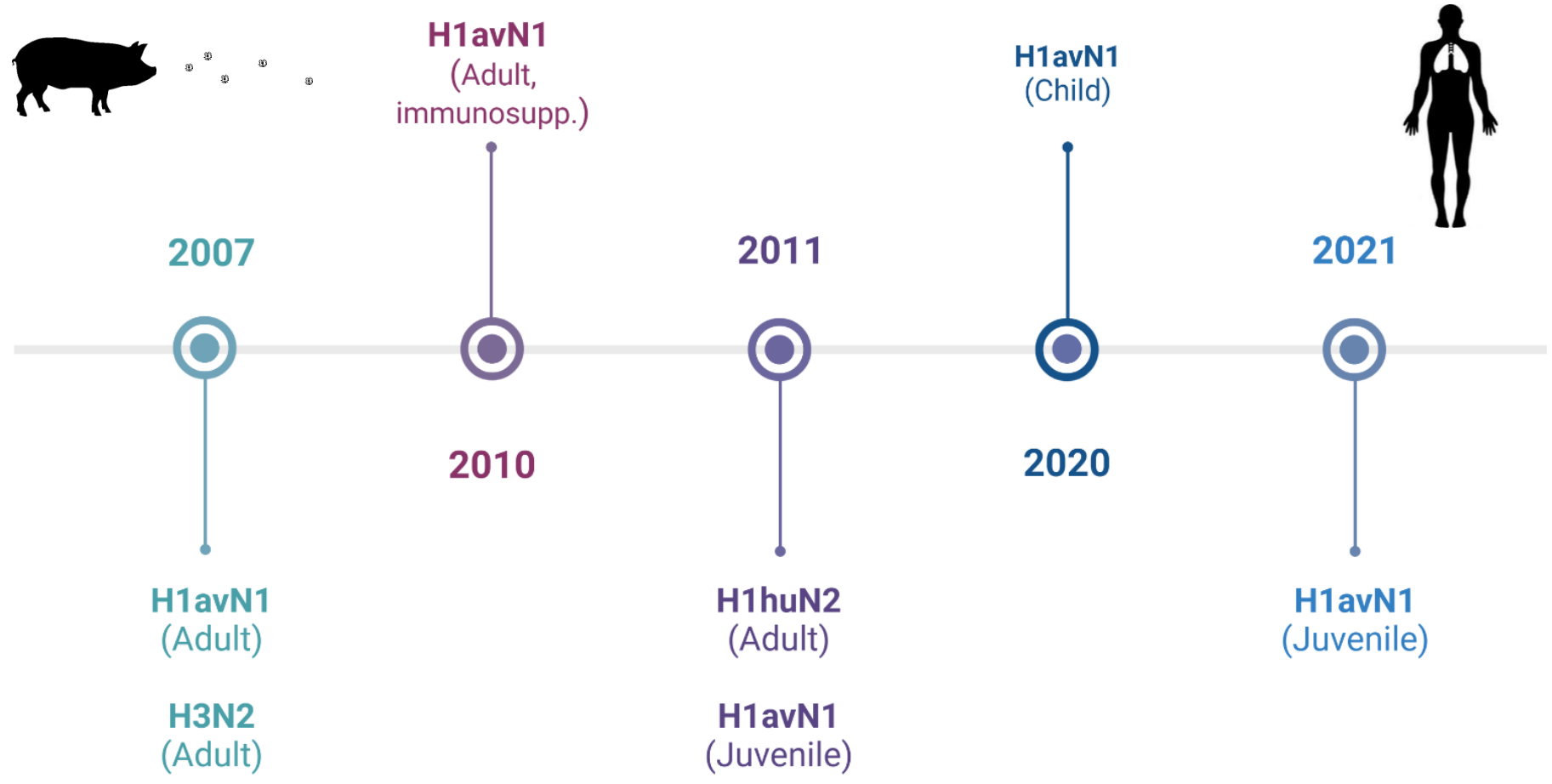
Potentially susceptible human populations



Children and/or immuno-compromised people may play an important role as a first line of susceptibles for zoonotic swIAV.

Human-Swine interface in Germany

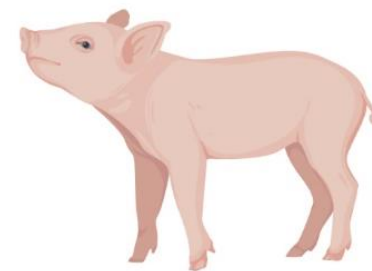
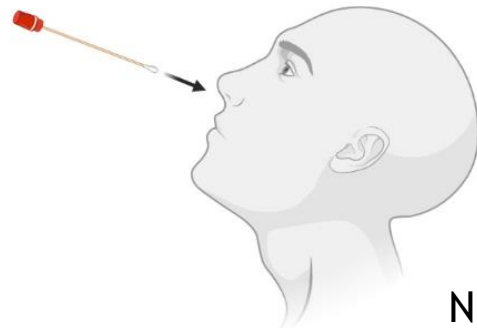
How frequent are zoonotic and reverse-zoonotic transmissions of influenza viruses effectively?





Active-passive swIAV surveillance: Testing nasal swabs of pigs and men

Sampling material
being sent on
request



Nasal swabbing of staff and pigs at the same day

Repeated sampling requested:
Monthly, quarterly or when fresh respiratory
symptoms are detected



Free transport to FLI

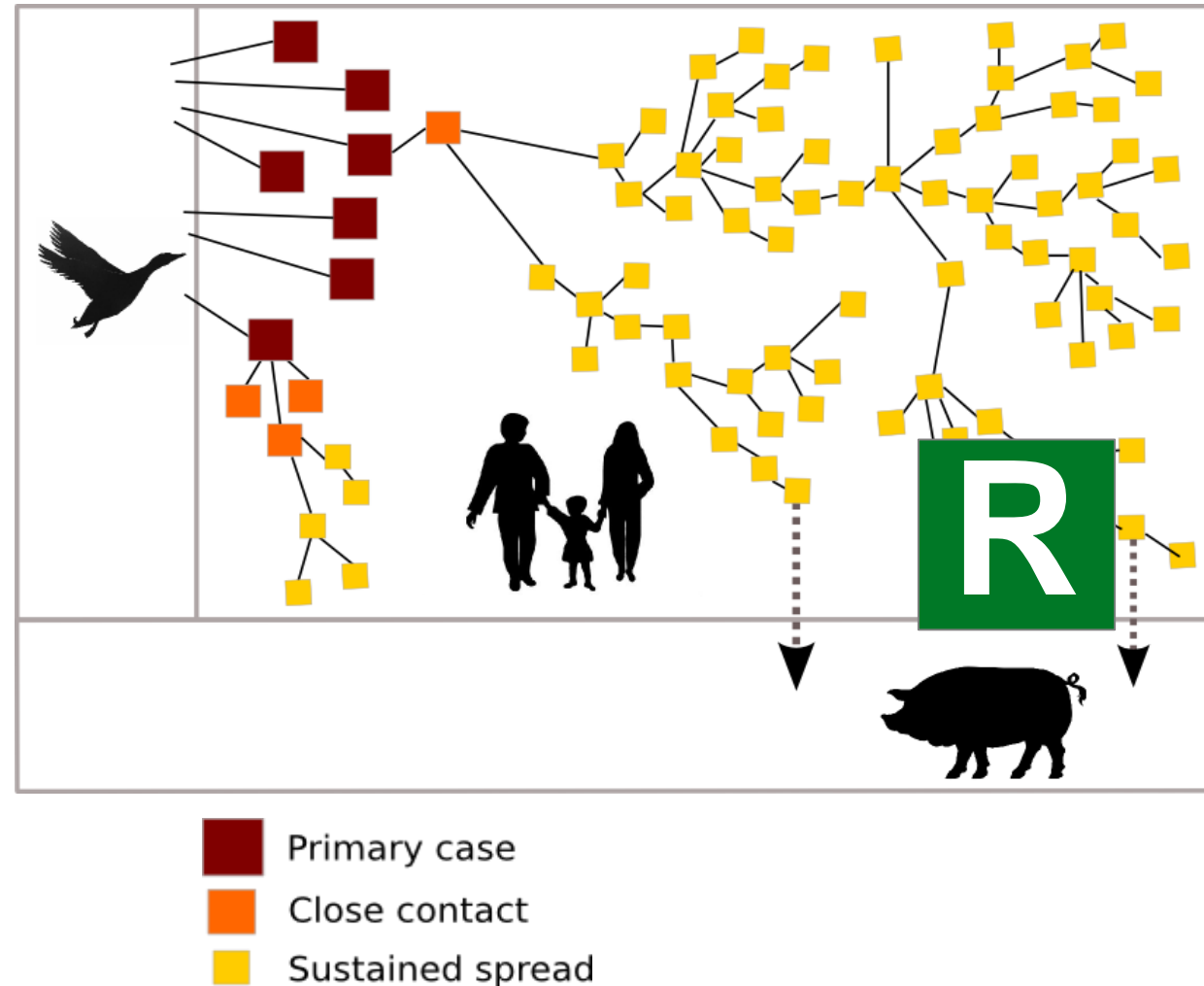


Free testing for swIAV



Zoonotic pressure of swIAV - the risks

- There is an **intimate interface** between pigs and men.
- Pigs as *mixing vessels* can be infected with IAV of different species and they do act as **reassortment machines**.
- Most swIAV originated from **revers zoonotic transmissions** of human IAV.
- Changing herd structures create new reservoirs fostering **endemic circulation** and **on-going evolution** of swIAV
- Genetic building blocks of pre-pandemic IAV circulate in swine populations



Zoonotic pressure of swIAV - keep cool but act

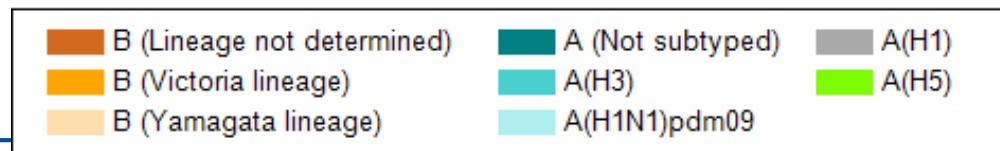
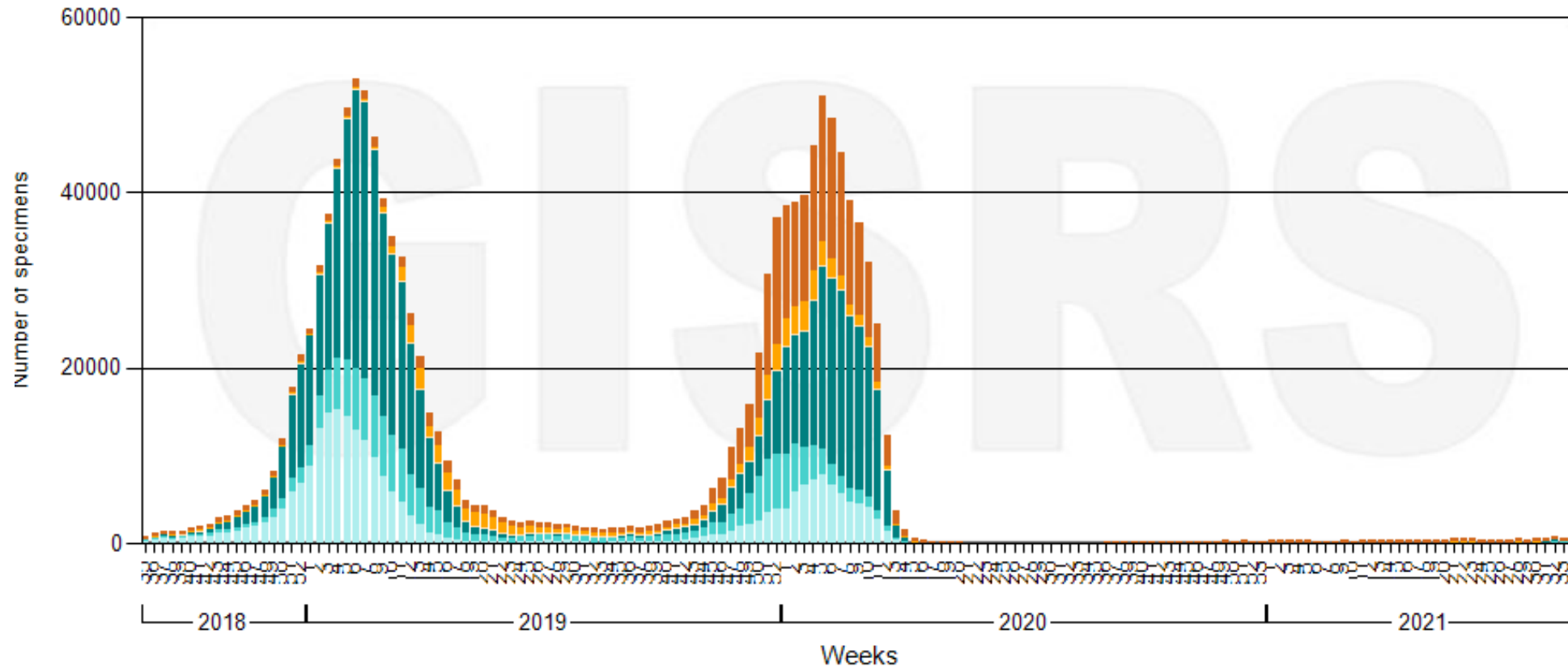
- swIAV can be controlled by **biosecurity, herd management and vaccination**



<https://www.pinterest.fr/pin/14988611233910470/>

What happened to human seasonal flu during COVID?

Number of specimens positive for influenza by subtype



Data from: All sites

Re-newed emergence of human seasonal flu after lifting COVID restrictions

Comparison of number of influenza detections by subtype

data as of:
23.08.2021 06:30:04



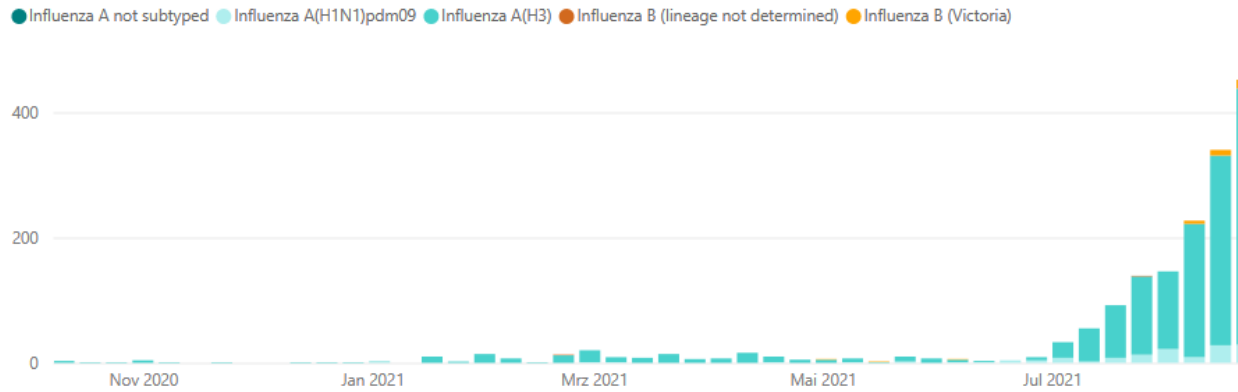
Select time range (by date or using the slider)

09.10.2020 22.08.2021

- Alle auswählen
- Influenza A not subtyped
- Influenza A(H1)
- Influenza A(H1N1)pdm09
- Influenza A(H3)
- Influenza B (lineage not determined)
- Influenza B (Victoria)
- Influenza B (Yamagata)

Select Zone/Country/Area/Territory

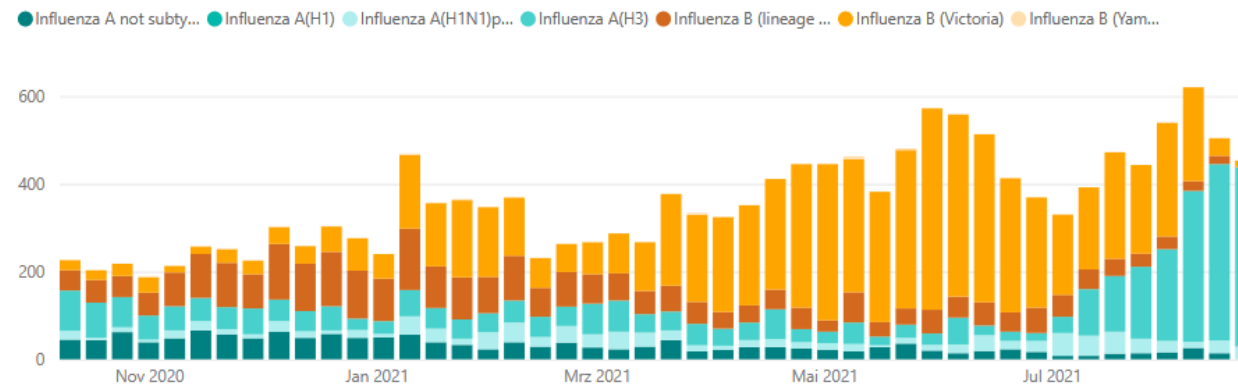
- Oceania Melanesia Polynesia
- South West Europe
- South-East Asia
- Southern Africa
- Southern Asia
 - Afghanistan
 - Bangladesh
 - Bhutan
 - India
 - Iran (Islamic Republic of)
 - Maldives
 - Nepal



selected countries
India

Select Zone/Country/Area/Territory

- Select All
- Central America and Caribbean
- Central Asia
- Eastern Africa
- Eastern Asia
- Eastern Europe
- Middle Africa
- North America
- Northern Africa
- Northern Europe
- Oceania Melanesia Polynesia
- South West Europe



selected countries

- Afghanistan, Albania, Algeria, Andorra, Angola, Antigua and Barbuda, Argentina, Armenia, Australia, Austria, Azerbaijan, Bahamas, Bahrain, Bangladesh, Barbados, Belarus, Belgium, Belize, Benin, Bhutan, Bolivia (Plurinational State of), Bosnia and Herzegovina, Botswana, Brazil, Brunei Darussalam, Bulgaria, Burkina Faso, Burundi, Cambodia, Cameroon, Canada, Cabo Verde, Central African Republic, Chad, Chile, China, Colombia, Comoros, Congo, Cook Islands, Costa Rica, Croatia, Cuba, Cyprus, Czechia, Côte d'Ivoire, Democratic People's Republic of Korea, Democratic Republic of the Congo, Djibouti, Dominica, Dominican Republic, Ecuador, Egypt, El Salvador, Equatorial Guinea, Eritrea, Estonia, Ethiopia, Fiji, Finland, France, Gabon, Gambia, Georgia, Germany, Ghana, Greece, Grenada, Guatemala, Guinea, Guinea-Bissau, Haiti, Honduras, Hungary, Iceland, India, Indonesia, Iraq, Ireland, Israel, Italy, Jamaica, Japan, Jordan, Kazakhstan, Kenya, Kiribati, Korea, Democratic Republic of the Congo, Kuwait, Kyrgyzstan, Laos, Latvia, Lebanon, Lesotho, Liberia, Lithuania, Luxembourg, Madagascar, Malawi, Malaysia, Maldives, Mali, Malta, Marshall Islands, Mauritania, Mauritius, Mexico, Micronesia (Federated States of), Moldova, Monaco, Mongolia, Montenegro, Morocco, Mozambique, Myanmar, Namibia, Nauru, Nepal, Netherlands, New Caledonia, New Zealand, Nicaragua, Niger, Nigeria, North Macedonia, Norway, Oman, Pakistan, Palau, Panama, Papua New Guinea, Paraguay, Peru, Philippines, Poland, Portugal, Qatar, Romania, Rwanda, Saint Kitts and Nevis, Saint Lucia, Saint Vincent and the Grenadines, Samoa, San Marino, Saudi Arabia, Senegal, Serbia, Seychelles, Sierra Leone, Singapore, Slovakia, Slovenia, South Africa, South Korea, South Sudan, Spain, Sri Lanka, Sudan, Suriname, Sweden, Switzerland, Taiwan, Tajikistan, Tanzania, Thailand, Timor-Leste, Togo, Tonga, Trinidad and Tobago, Tunisia, Turkey, Turkmenistan, Tuvalu, Uganda, Ukraine, United Arab Emirates, United Kingdom, United States of America, Uruguay, Uzbekistan, Vanuatu, Venezuela, Viet Nam, West Bank and Gaza, Yemen, Zambia, Zimbabwe

Data source: www.who.int/flunet

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Zoonotic pressure of swIAV - keep cool but act

- swIAV can be controlled by **biosecurity, herd management and vaccination**
- **Other mixing vessels exist, other (avian) IAV have zoonotic potential (highly pathogenic AIV, H9N2)**
- Pigs have proven to be involved in 1 out of 5 human influenza **pandemics**
- **Vaccines** may need improvement (local, regional, universal types)
- **Vaccination schemes and application pathways** may need updating (targeting local immunity of suckling piglets and endemically infected herds)



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