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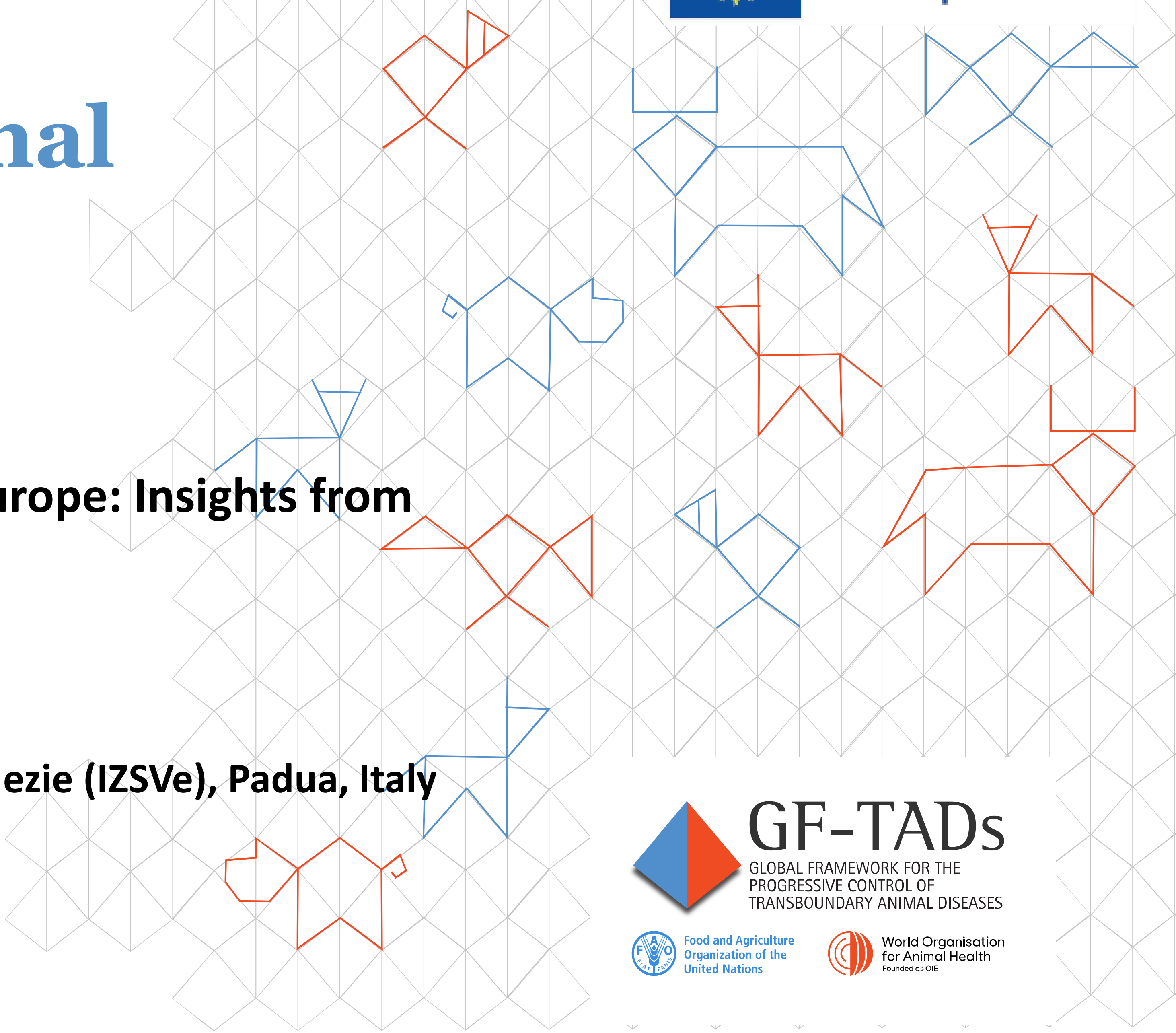

Genetic Evolution of HPAI Viruses in Europe: Insights from the 2024–2025 Epidemiological Year

Isabella Monne,
Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Padua, Italy

22-25/August/2025, Belgrade, Serbia

GF-TADs
GLOBAL FRAMEWORK FOR THE PROGRESSIVE CONTROL OF TRANSBOUNDARY ANIMAL DISEASES

Food and Agriculture Organization of the United Nations

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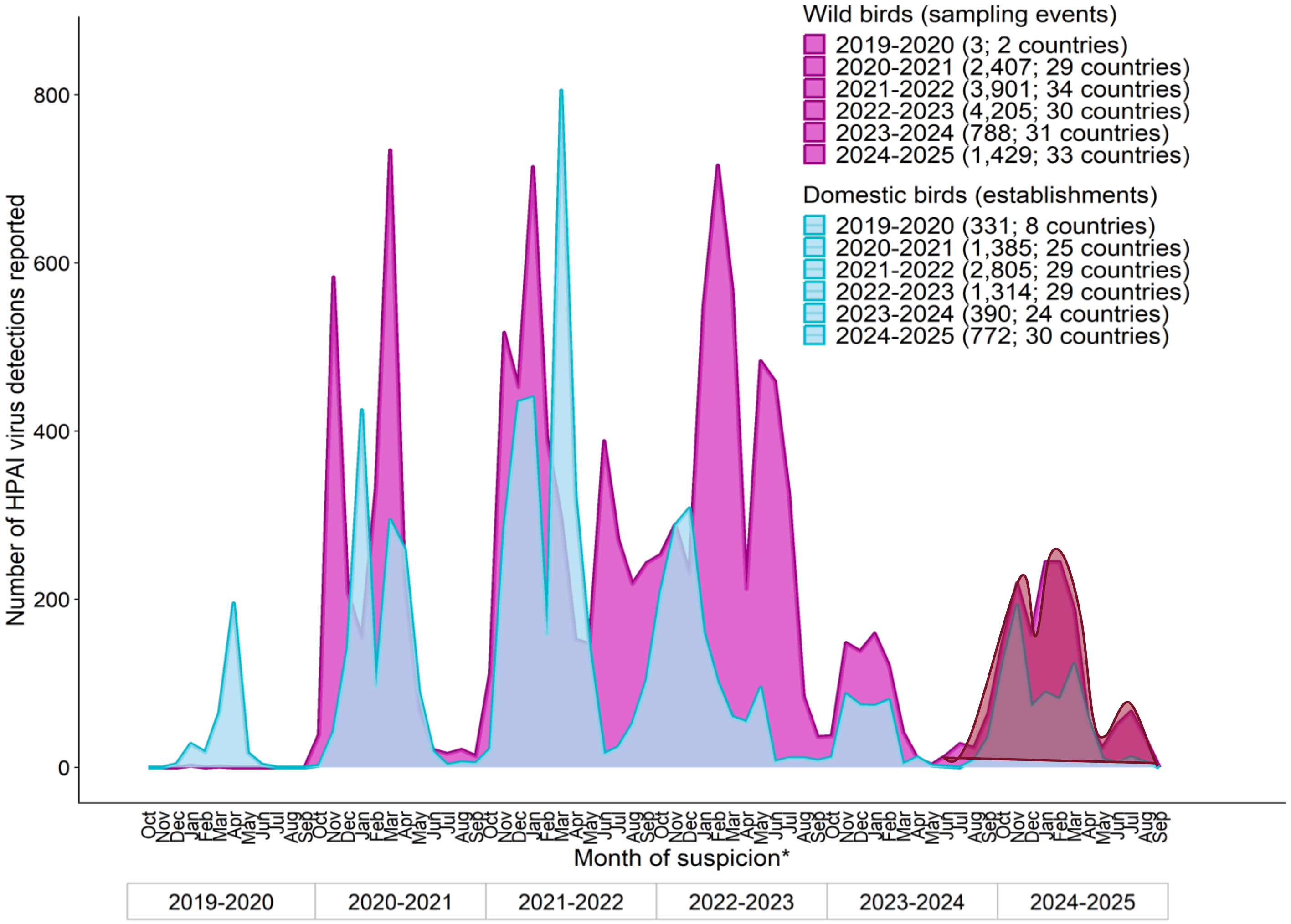
HPAI virus detections in wild and domestic birds (2019-2025)

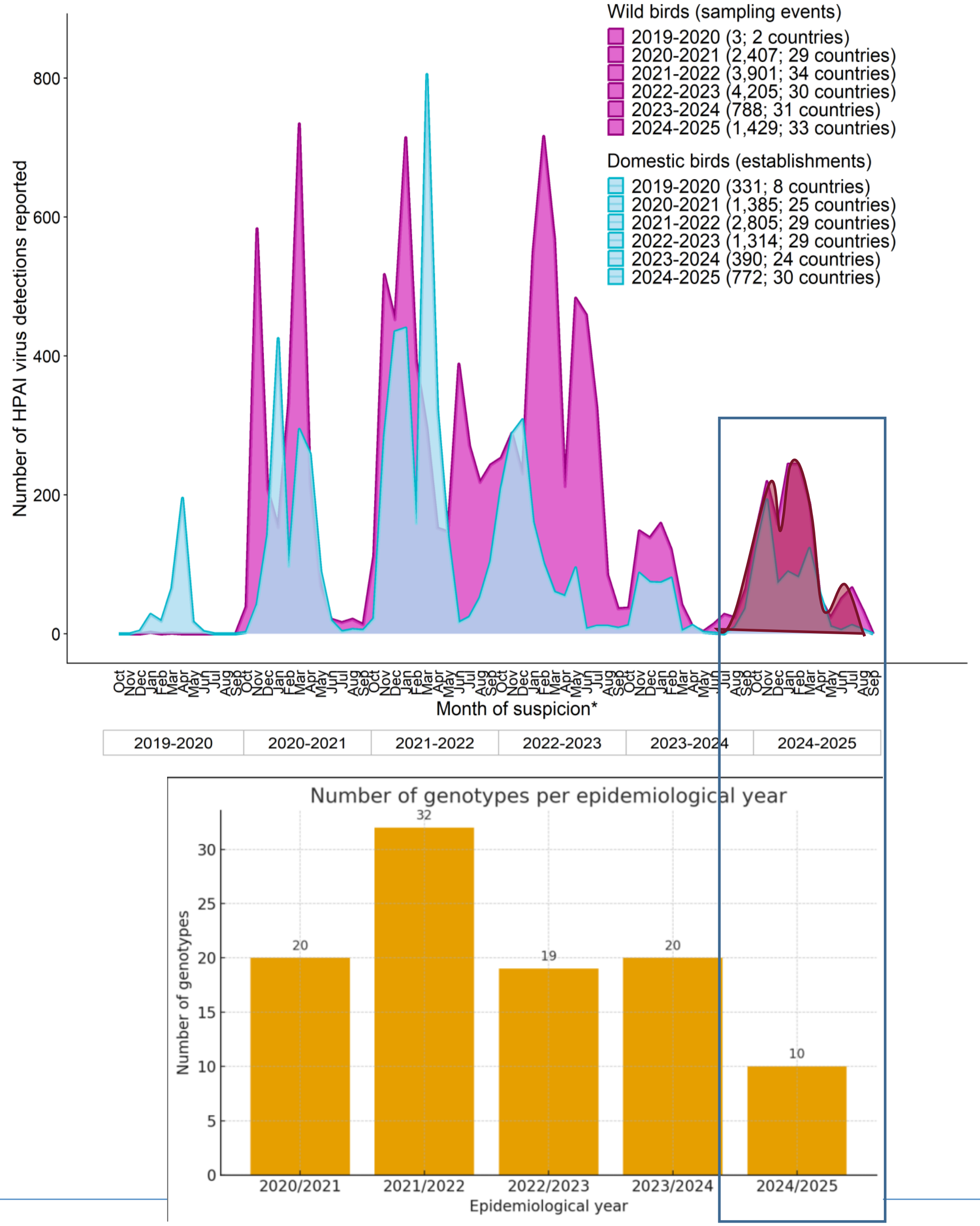


The overall number of HPAI H5Nx detections during this reporting period was higher than in the same period in the previous epidemiological year, in both domestic and wild birds.

Avian influenza overview June–September 2025

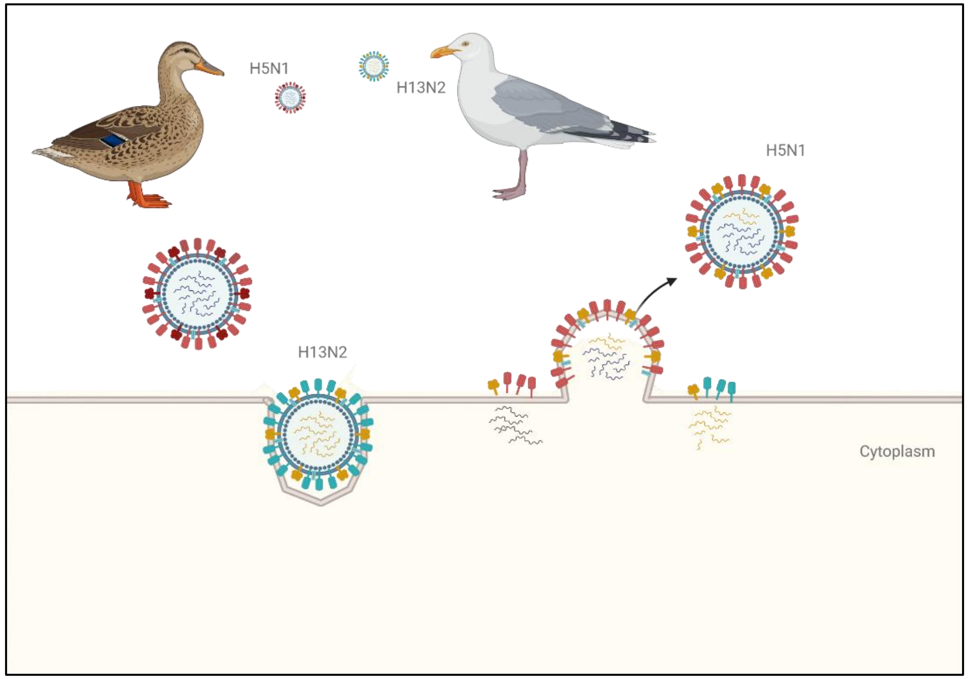
This figure is taken from the EFSA-ECDC-EURL ‘Avian influenza overview June-September 2025’ that will be published on 25 September 2025

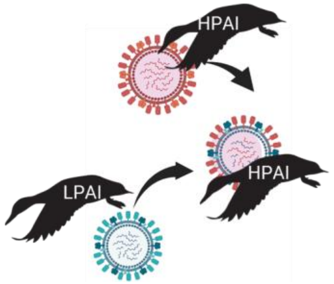
HPAI virus detections in wild and domestic birds (2019-2025)

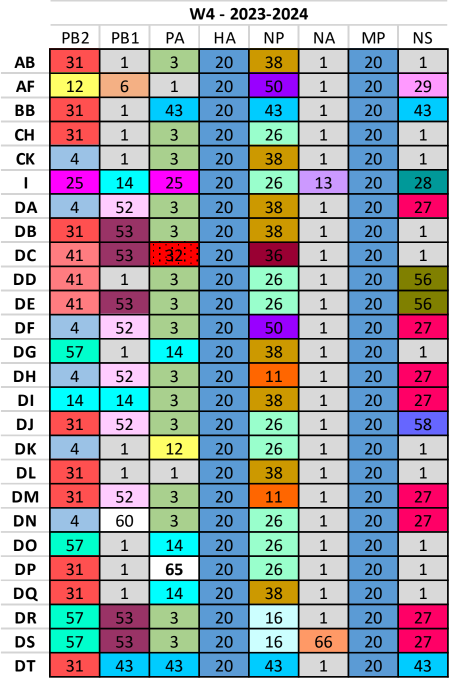


As expected, during this epidemiological year the H5Nx clade 2.3.4.4b virus has continued to evolve through:

- accumulation of mutations
- genetic reassortment







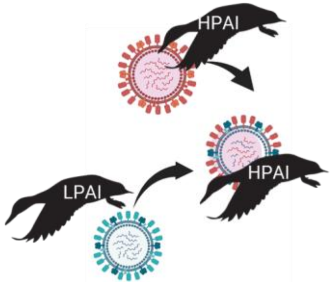
W4 - 2023-2024	PB2	PB1	PA	HA	NP	NA	MP	NS
AB	31	1	3	20	38	1	20	1
AF	12	6	1	20	50	1	20	29
BB	31	1	43	20	43	1	20	43
CH	31	1	3	20	26	1	20	1
CK	4	1	3	20	38	1	20	1
I	25	14	25	20	26	13	20	28
DA	4	52	3	20	38	1	20	27
DB	31	53	3	20	38	1	20	1
DC	41	53	32	20	36	1	20	1
DD	41	1	3	20	26	1	20	56
DE	41	53	3	20	26	1	20	56
DF	4	52	3	20	50	1	20	27
DG	57	1	14	20	38	1	20	1
DH	4	52	3	20	11	1	20	27
DI	14	14	3	20	38	1	20	27
DJ	31	52	3	20	26	1	20	58
DK	4	1	12	20	26	1	20	1
DL	31	1	1	20	38	1	20	1
DM	31	52	3	20	11	1	20	27
DN	4	60	3	20	26	1	20	27
DO	57	1	14	20	26	1	20	1
DP	31	1	**65**	20	26	1	20	1
DQ	31	1	14	20	38	1	20	1
DR	57	53	3	20	16	1	20	27
DS	57	53	3	20	16	66	20	27
DT	31	43	43	20	43	1	20	43

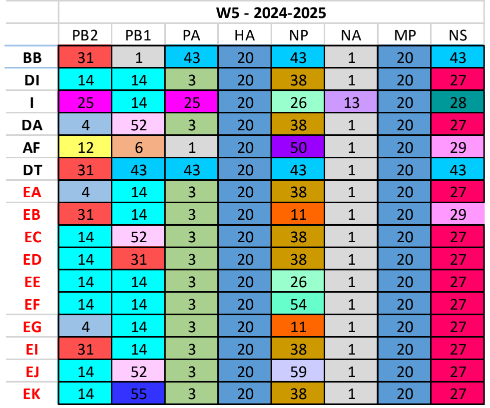
630 WGS from **24 European countries**

(October 2023 – September 2024)

20 new genotypes

Bottleneck effect?





W5 - 2024-2025	PB2	PB1	PA	HA	NP	NA	MP	NS
BB	31	1	43	20	43	1	20	43
DI	14	14	3	20	38	1	20	27
I	25	14	25	20	26	13	20	28
DA	4	52	3	20	38	1	20	27
AF	12	6	1	20	50	1	20	29
DT	31	43	43	20	43	1	20	43
EA	4	14	3	20	38	1	20	27
EB	31	14	3	20	11	1	20	29
EC	14	52	3	20	38	1	20	27
ED	14	31	3	20	38	1	20	27
EE	14	14	3	20	26	1	20	27
EF	14	14	3	20	54	1	20	27
EG	4	14	3	20	11	1	20	27
EI	31	14	3	20	38	1	20	27
EJ	14	52	3	20	59	1	20	27
EK	14	55	3	20	38	1	20	27

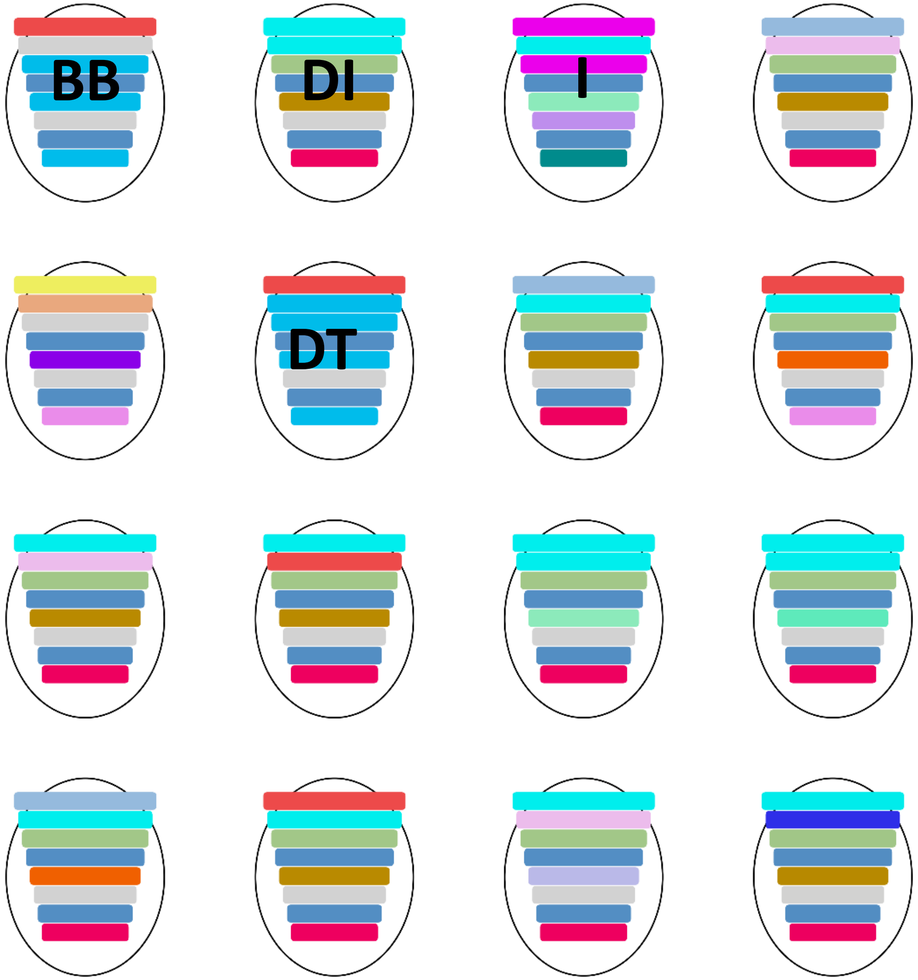
1500 WGS collected from **32 European countries**

(October 2024 – August 2025)

10 new genotypes

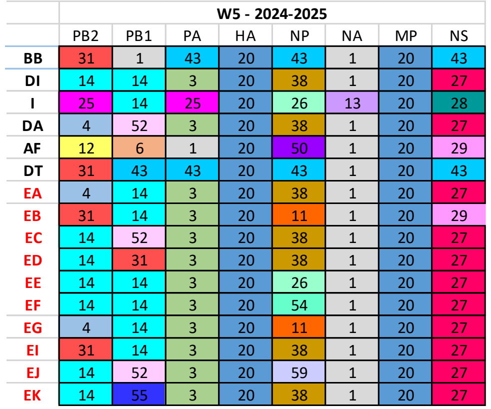
Fusaro A., Zecchin B., et al., Virus Evolution, 2024; https://doi.org/10.1093/ve/veae027

Genetic diversity, 2024-2025



The characterized viruses during the 2024-2025 epidemiological year belong to **four major genotypes, all of which had already been identified in previous incursion**:

- 3 H5N1 genotypes **EA-2022-BB, EA-2024-DI**, **EA-2024-DT**
- 1 H5N5 genotype (**EA-2021-I**)



	W5 - 2024-2025							
	PB2	PB1	PA	HA	NP	NA	MP	NS
BB	31	1	43	20	43	1	20	43
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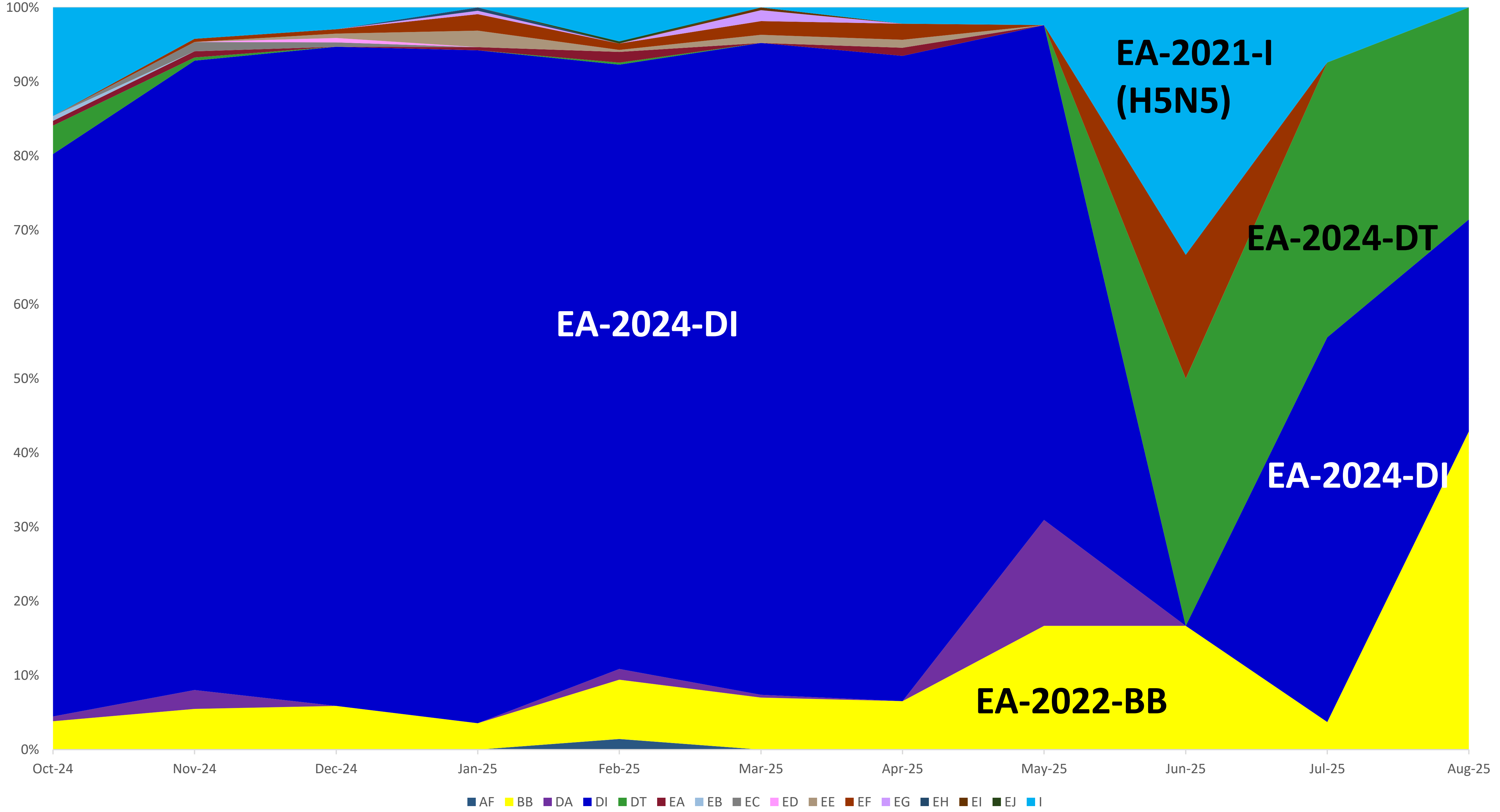
EA	4	14	3	20	38	1	20	27
EB	31	14	3	20	11	1	20	29
EC	14	52	3	20	38	1	20	27
ED	14	31	3	20	38	1	20	27
EE	14	14	3	20	26	1	20	27
EF	14	14	3	20	54	1	20	27
EG	4	14	3	20	11	1	20	27
EI	31	14	3	20	38	1	20	27
EJ	14	52	3	20	59	1	20	27
EK	14	55	3	20	38	1	20	27

Fusaro A., Zecchin B., et al. High pathogenic avian influenza A(H5) viruses of clade 2.3.4.4b in Europe – why trends of virus evolution are more difficult to predict, Virus Evolution, 2024; https://doi.org/10.1093/ve/veae027

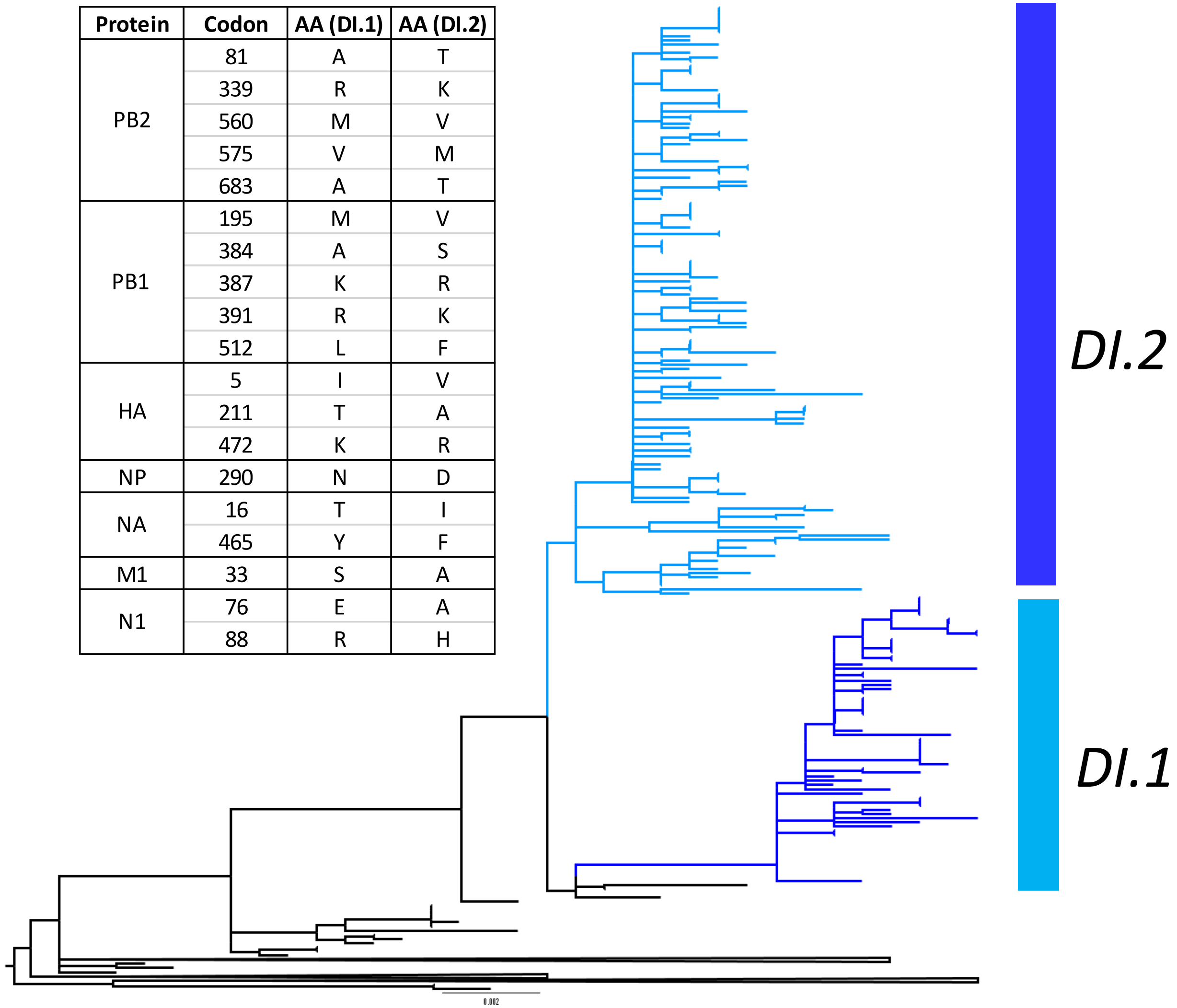
Temporal dynamics of the virus genotypes in Europe in wild and domestic birds: 2020-2025



Evolution of the genotype EA-2024-DI

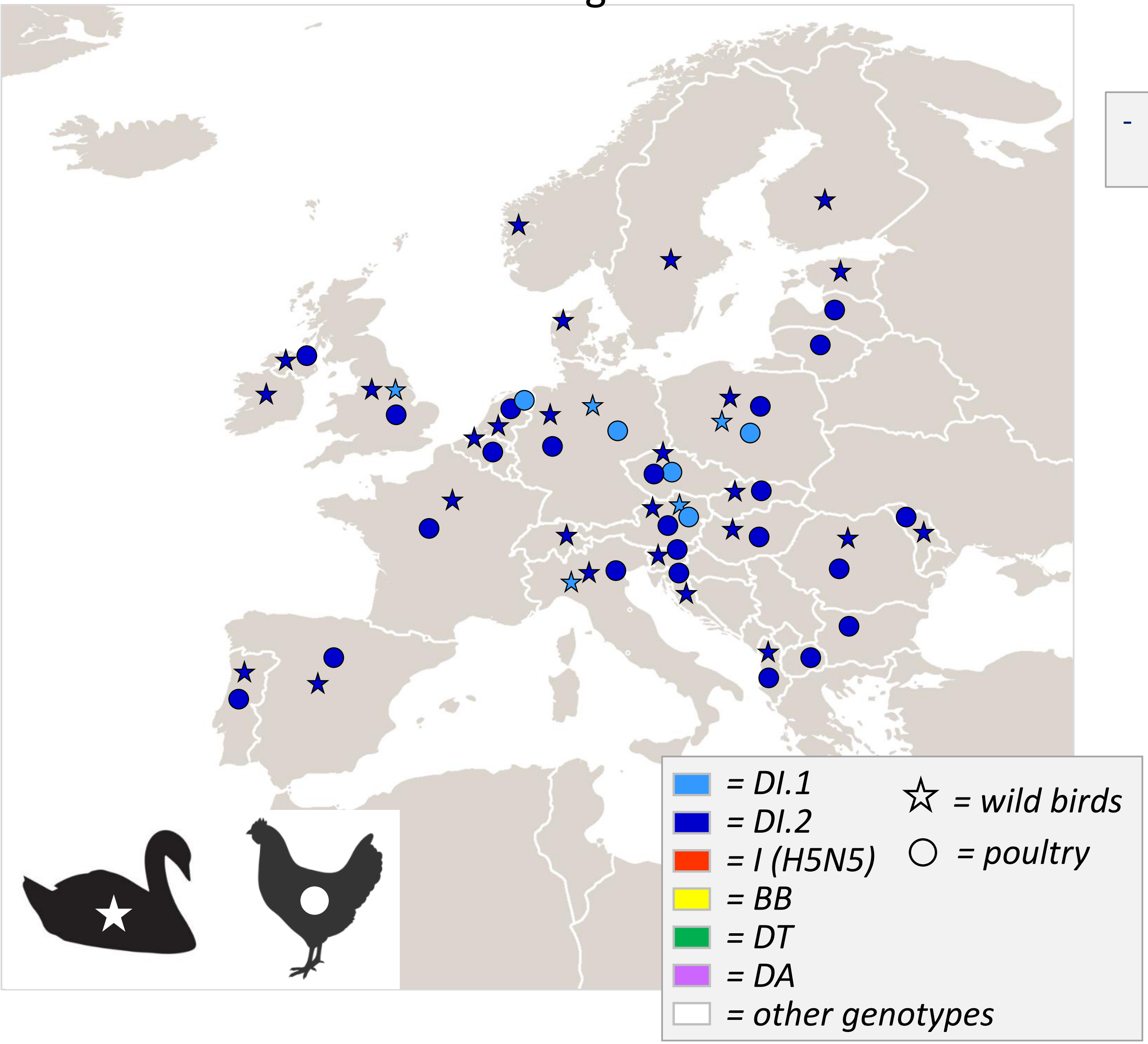
🟦 =DI.2
🟦 =DI.1

Protein	Codon	AA (DI.1)	AA (DI.2)
PB2	81	A	T
	339	R	K
	560	M	V
	575	V	M
	683	A	T
PB1	195	M	V
	384	A	S
	387	K	R
	391	R	K
	512	L	F
HA	5	I	V
	211	T	A
	472	K	R
NP	290	N	D
NA	16	T	I
	465	Y	F
M1	33	S	A
N1	76	E	A
	88	R	H



The genotypes distribution in birds in Europe

October 2024 – August 2025



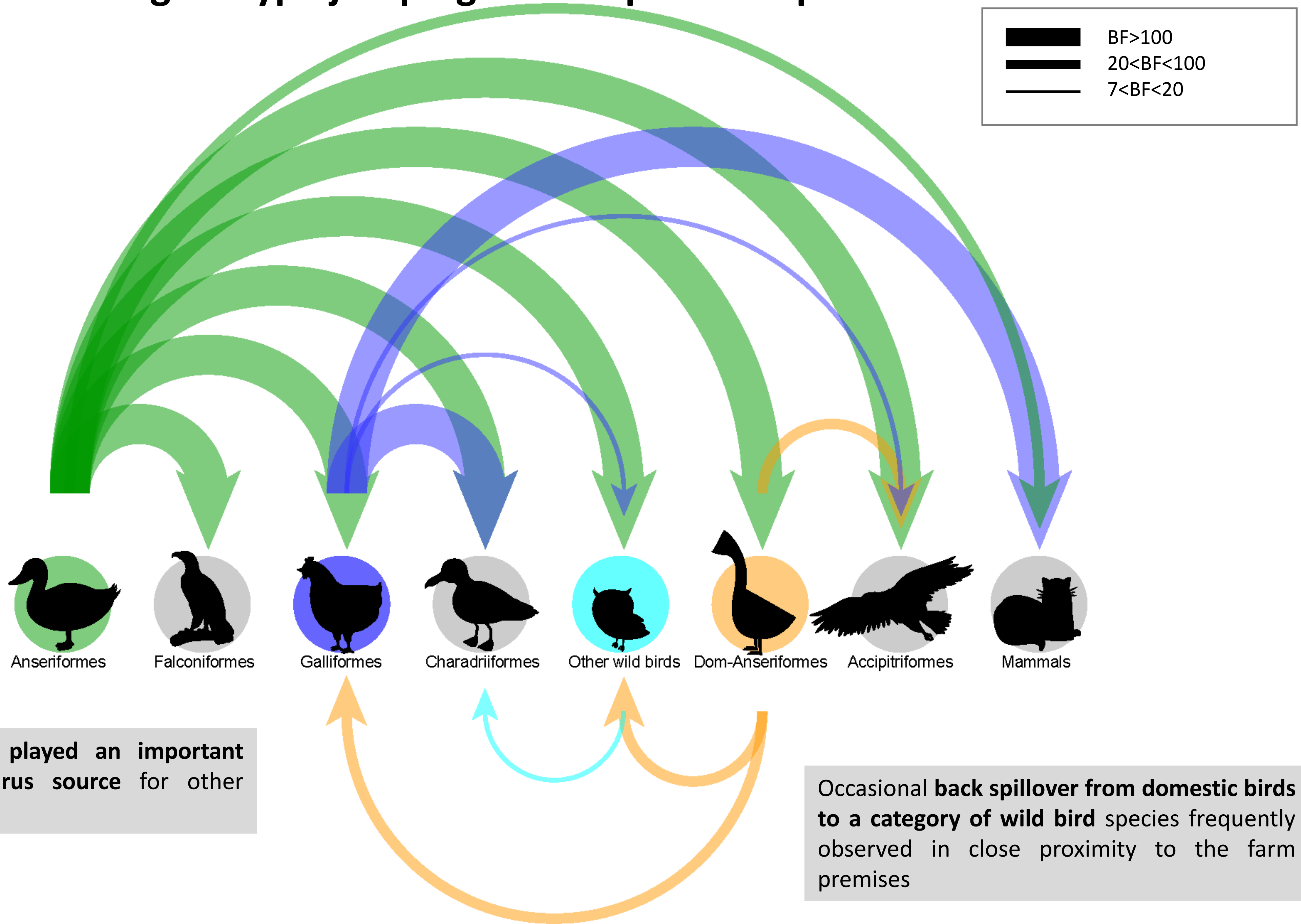
- **EA-2024-DI** genotype has mainly been detected among waterfowl and domestic birds across the entire Europe.

- **EA-2024-DI** genotype has been also detected in Republic of Georgia, Southern Russia and in Israel

🟦 = *DI.1*
🟦 = *DI.2*
🟥 = *I (H5N5)*
🟨 = *BB*
🟩 = *DT*
🟪 = *DA*
⬜ = *other genotypes*

☆ = *wild birds*
○ = *poultry*

Host transmission events: DI genotype jumping in multiple host species



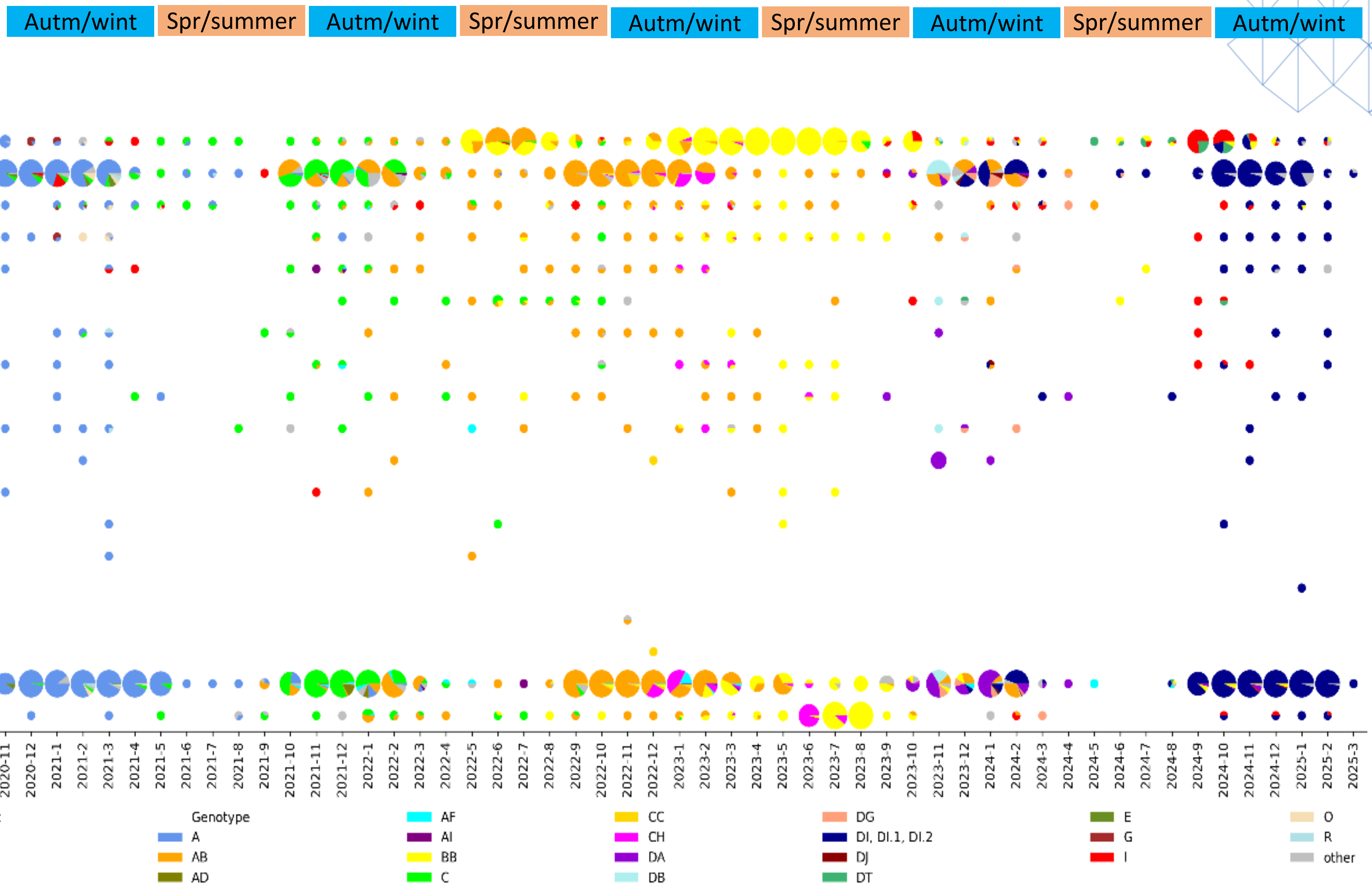
Anseriformes played an important role as a virus source for other species

Occasional **back spillover from domestic birds to a category of wild bird** species frequently observed in close proximity to the farm premises

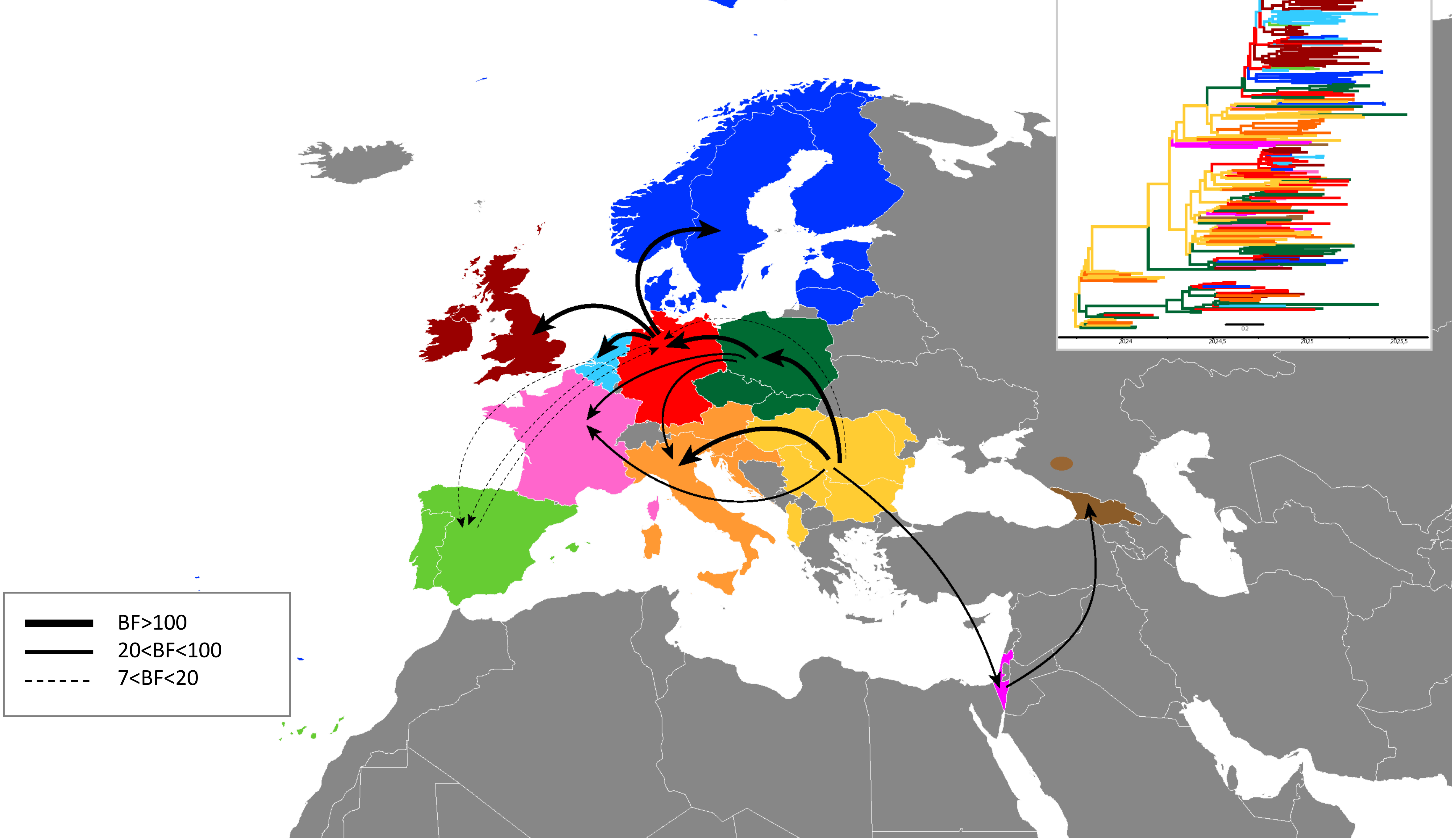
Host-Specific Genotype Patterns

What insights have we gained from analyzing the epidemic waves of recent years?

The genotypes circulating each month in most of the host orders mirrored the ones circulating in Anseriformes, suggesting that **Anseriformes played an important role as a reservoir and virus source** for other species.

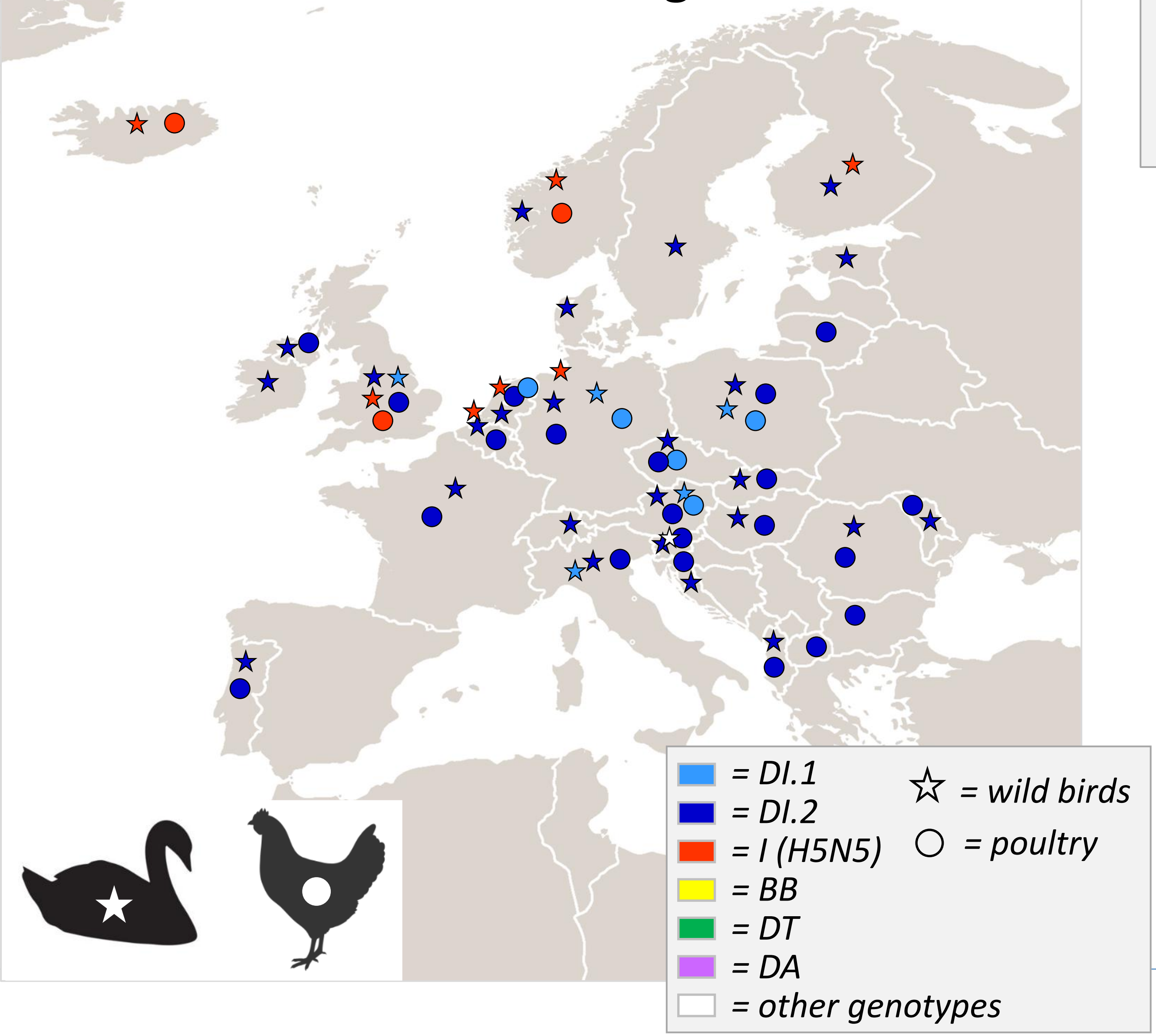


Dissemination dynamics of DI genotype



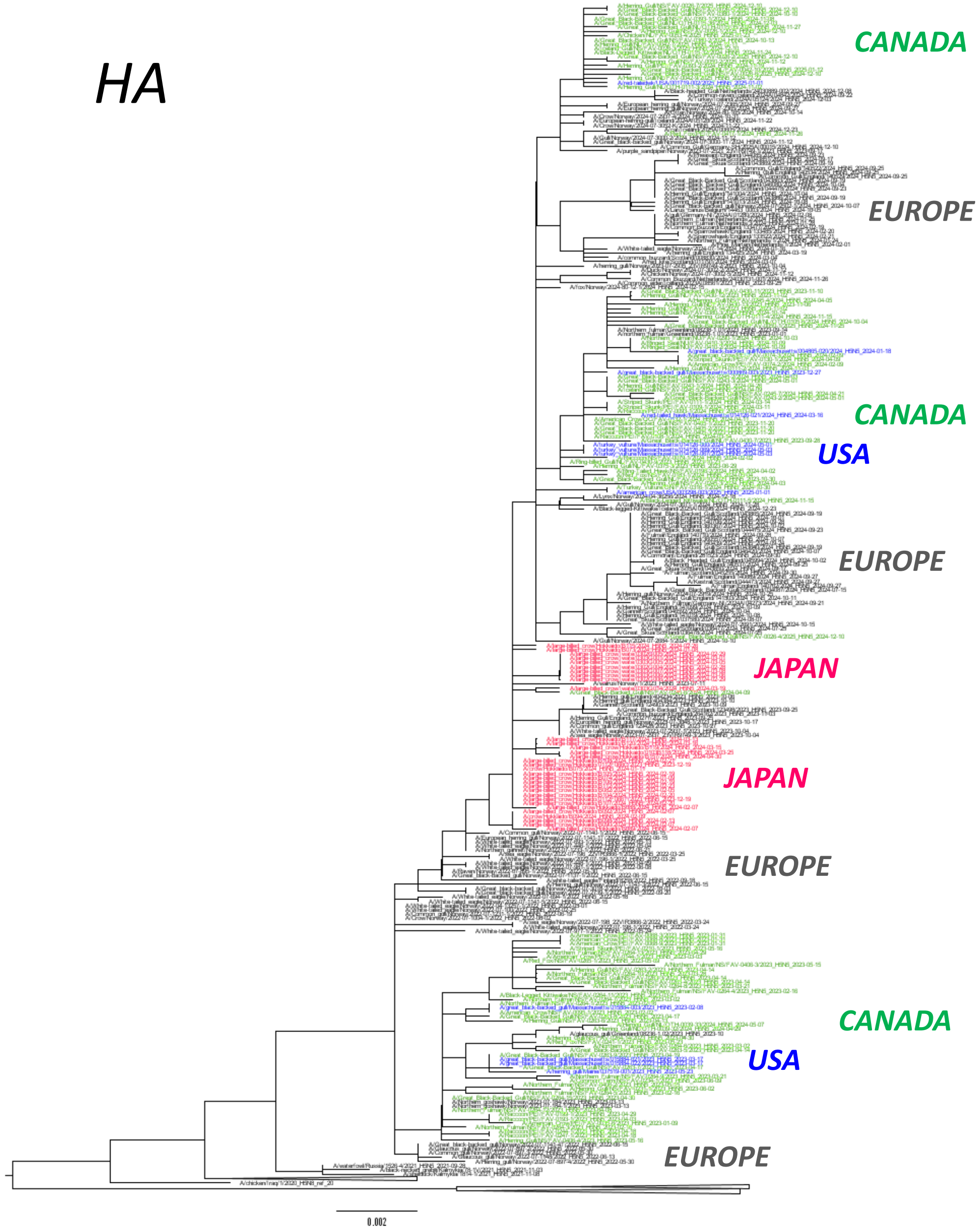
The genotypes distribution in birds in Europe

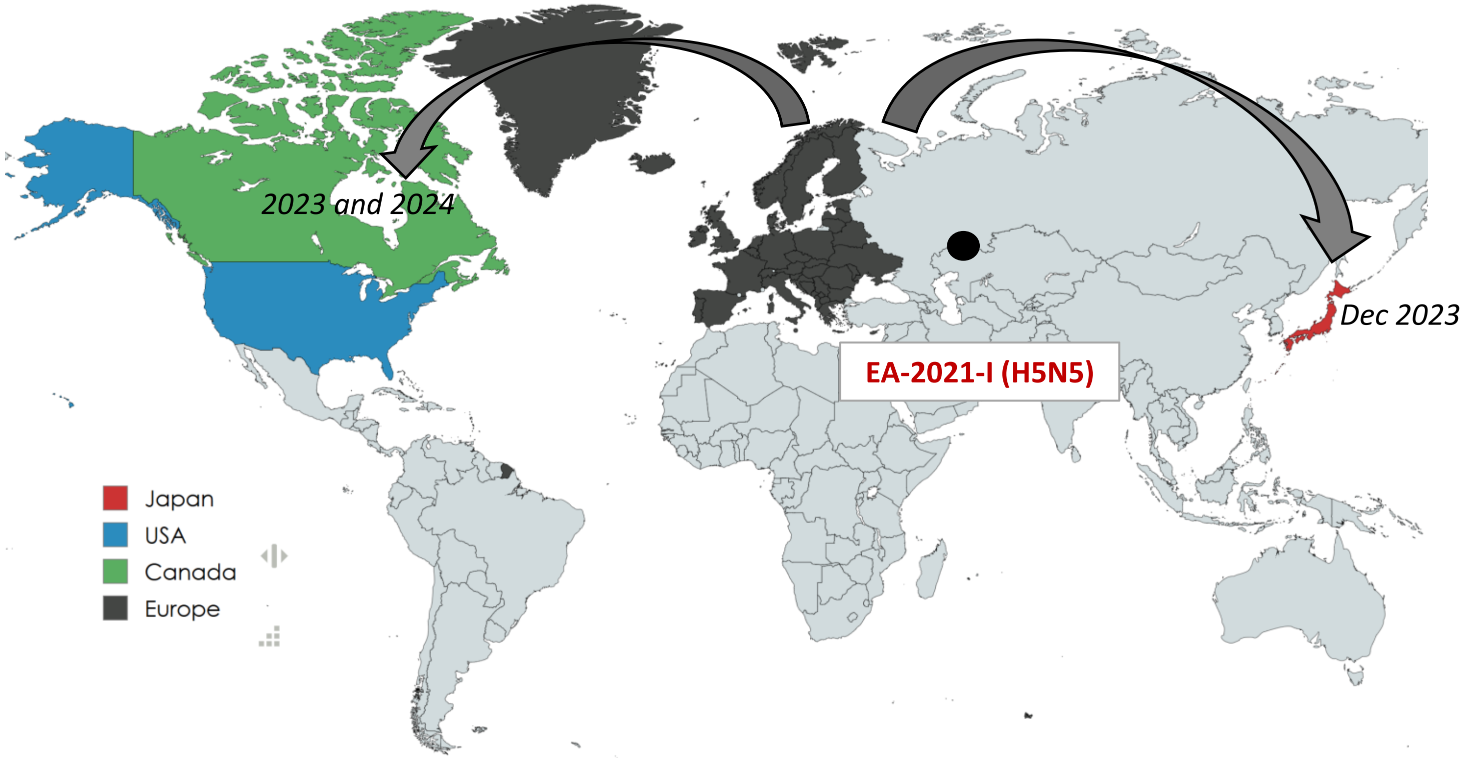
October 2024 – August 2025



- **EA-2021-I (H5N5)** persistently circulated in wild birds (mainly sea birds and raptors) in the UK, Norway and Iceland, with sporadically detection in Belgium, the Netherlands, Germany and Finland.

The geographic spread of the genotype EA-2021-I





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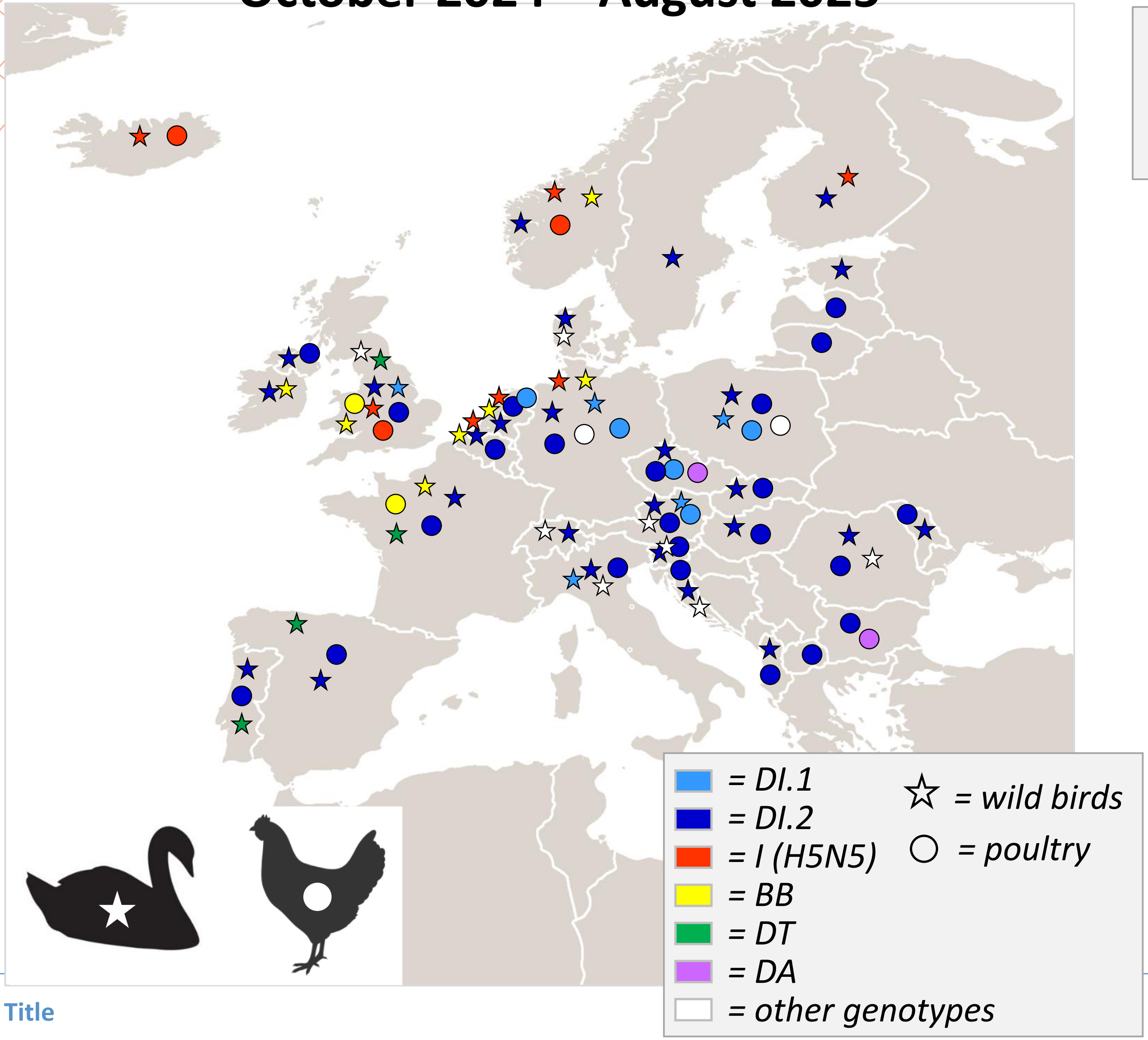
The genotypes distribution in birds in Europe

October 2024 – August 2025



- **EA-2022-BB and its descendent EA-2024-DT (gull associated genotypes)** has been mostly circulating among Laridae along the European coasts of the UK, France, Belgium, the Netherlands, Norway, Portugal and Spain.

HPAI Evolution and Zoonotic Risk: Where Do We Stand?

Avian Flu: Death Threat | **Oct. 17, 2005**

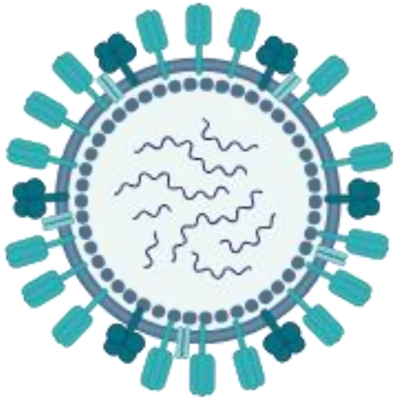
NEWS EXPLAINER | 27 January 2025

Will bird flu spark a human pandemic? Scientists say the risk is rising

H5N1 is adapting to new mammalian hosts, raising the possibility of the virus spreading between humans.

By Max Kozlov

Avian influenza virus preferences

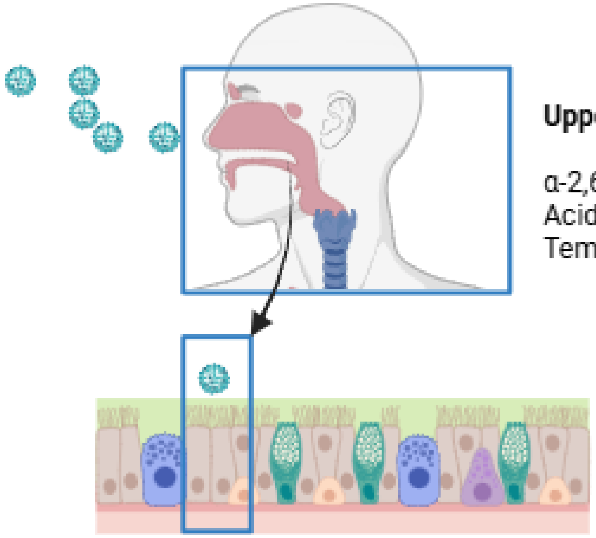


BINDING: AIV HAs bind α2-3-linked SA

pH STABILITY: Limited acid stability of the AIV HAs

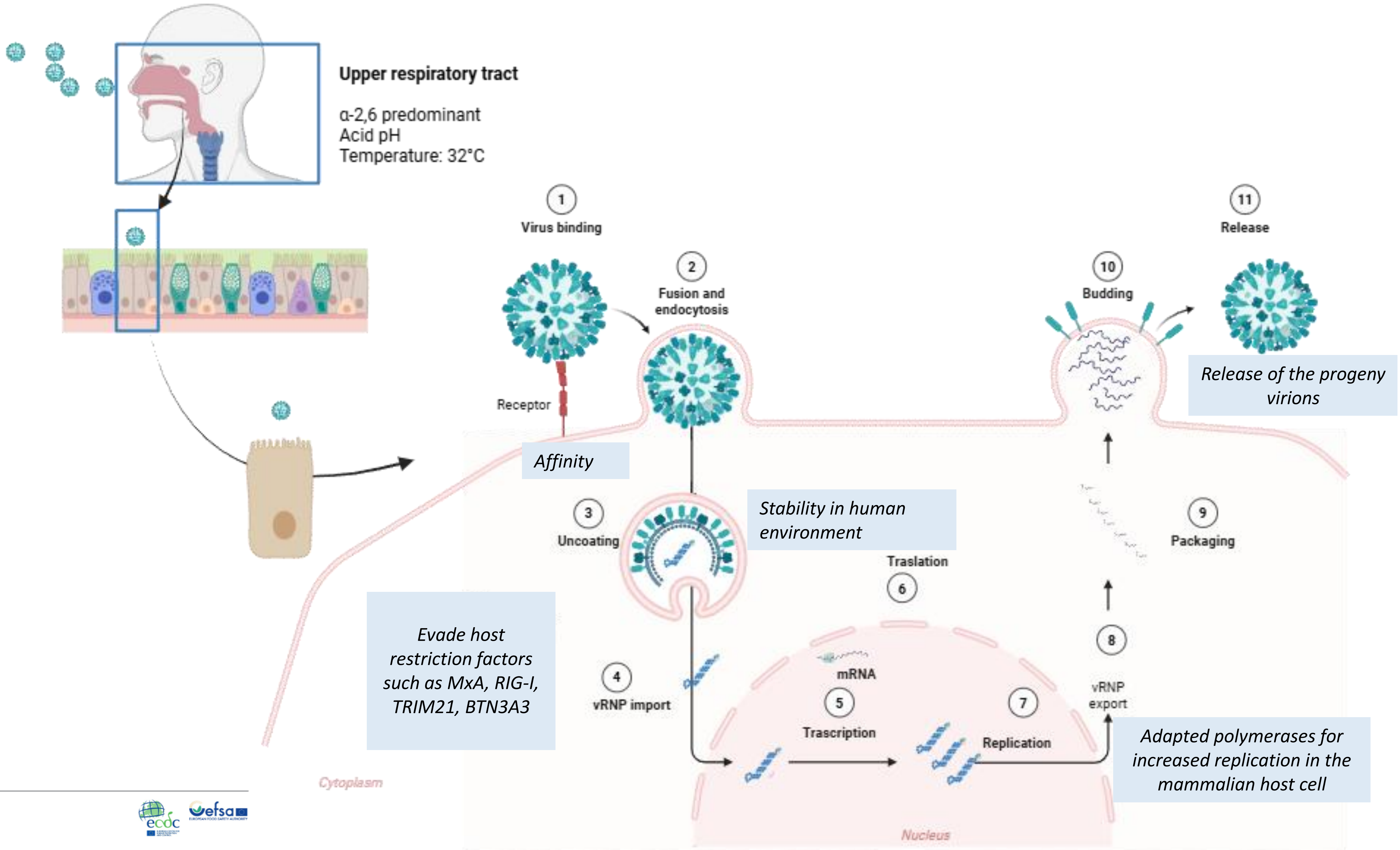
Temperature: AIV polymerases more active at high temperatures (i.e. 37 and 42 °C)

Created in https://BioRender.com



Upper respiratory tract

α-2,6 predominant
Acid pH
Temperature: 32°C



Affinity

Stability in human environment

Evade host restriction factors such as MxA, RIG-I, TRIM21, BTN3A3

Adapted polymerases for increased replication in the mammalian host cell

Release of the progeny virions

Adopted: 12 December 2024
DOI: 10.2903/j.efsa.2025.9191

SCIENTIFIC OPINION

Preparedness, prevention and control related to zoonotic avian influenza

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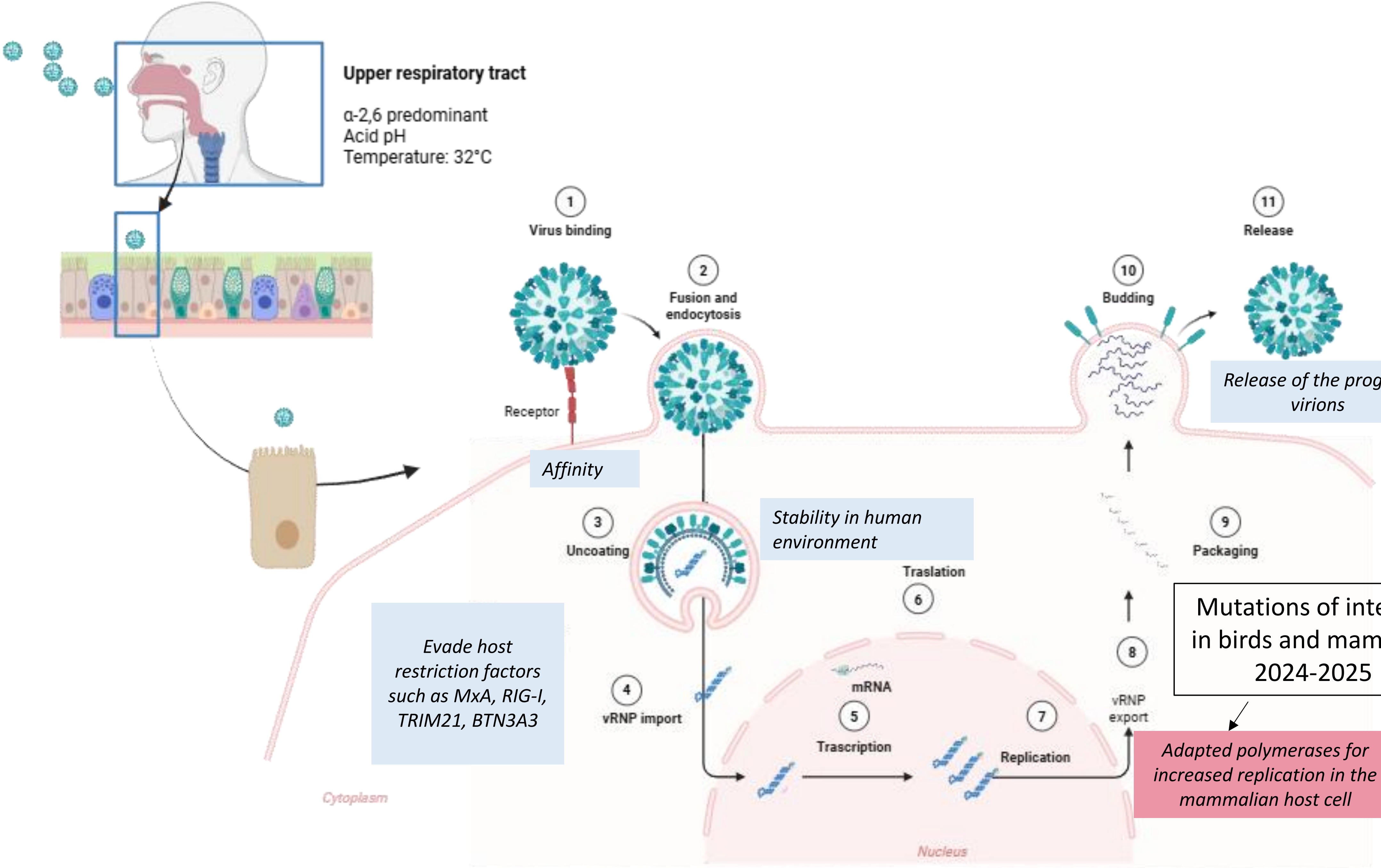

Affinity

Stability in human environment

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Release of the progeny virions

Mutations of interest in birds and mammals 2024-2025

Adapted polymerases for increased replication in the mammalian host cell

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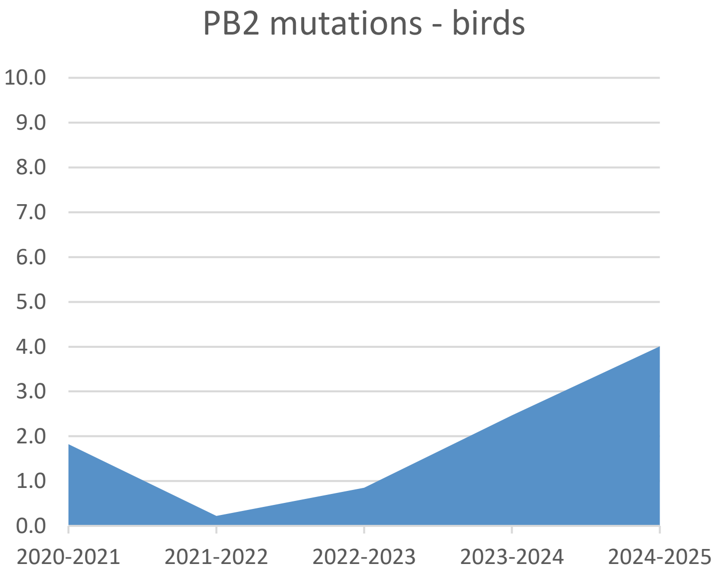
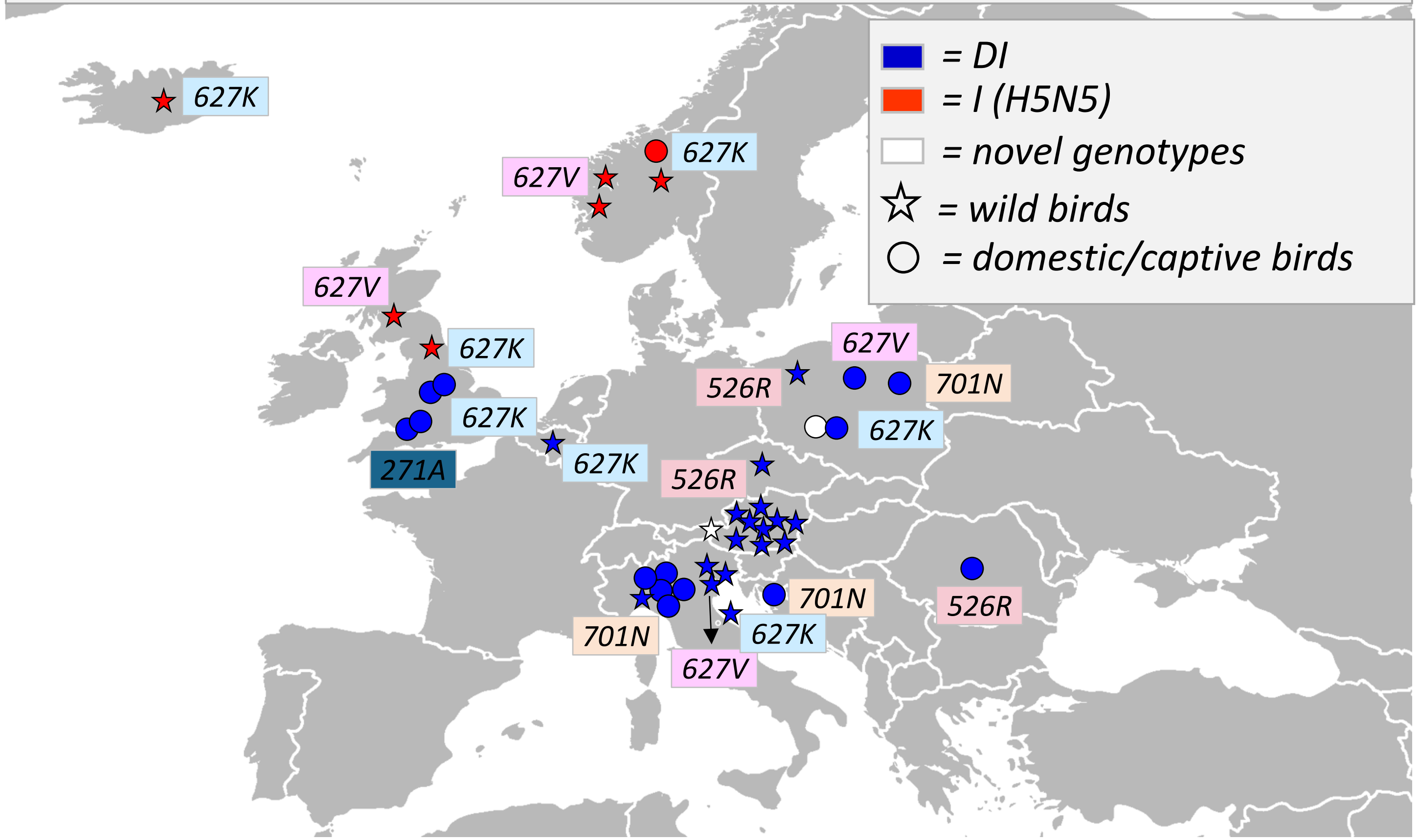
Mutations of interest in birds, October 2024-May 2025

- 65 HPAI H5 viruses collected from birds contain mutations in the PB2 protein associated with **adaptation to mammals** (E627K/V, D701N or K526R):
    - 43 A(H5N1) viruses collected from wild and domestic birds from eight different countries
    - 22 A(H5N5) viruses (genotype EA-2021-I) collected from wild and domestic birds in the United Kingdom, Norway and Iceland.

- An **increase in the relative frequency of detection of mammalian adaptive mutations in birds** was observed, in the 2024–2025 epidemiological year.

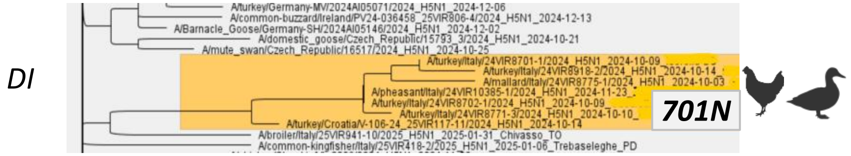


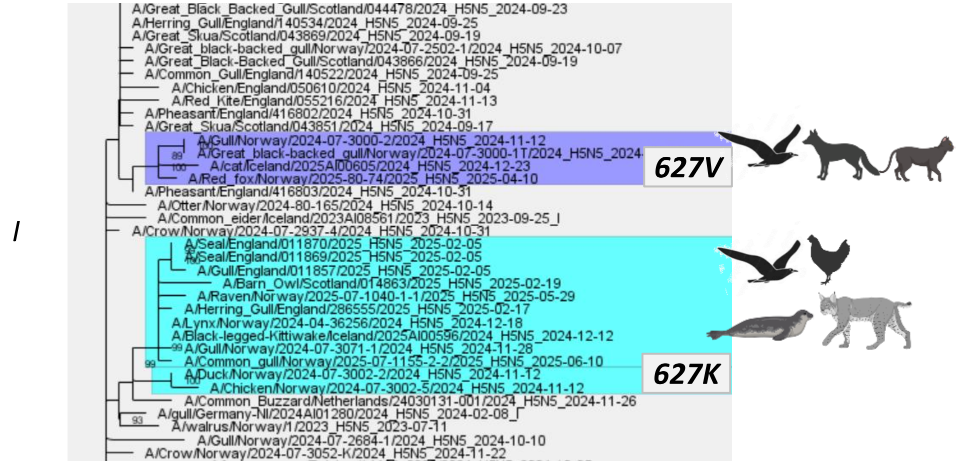
Geographic distribution in Europe of H5Nx viruses with molecular markers of mammalian adaptation PB2 E627K/V, K526R and D701N, 2024-2025

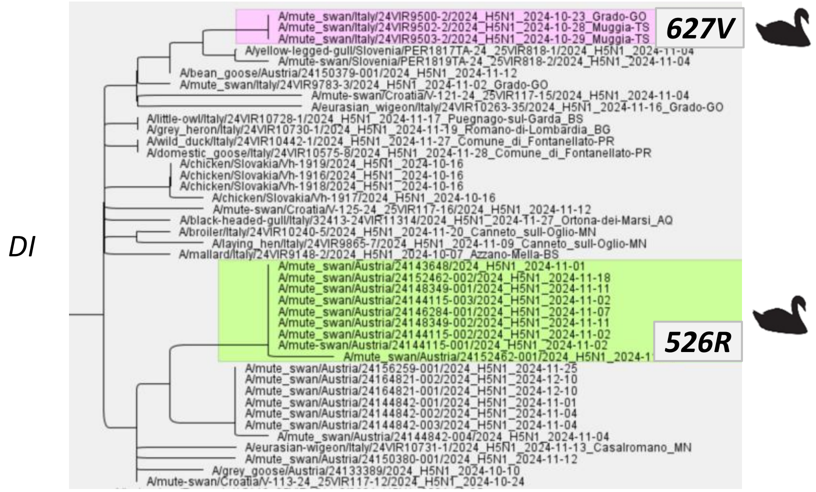


https://izsvenezie-virology.github.io/FluMut

Mutations of interest in birds, October 2024-May 2025

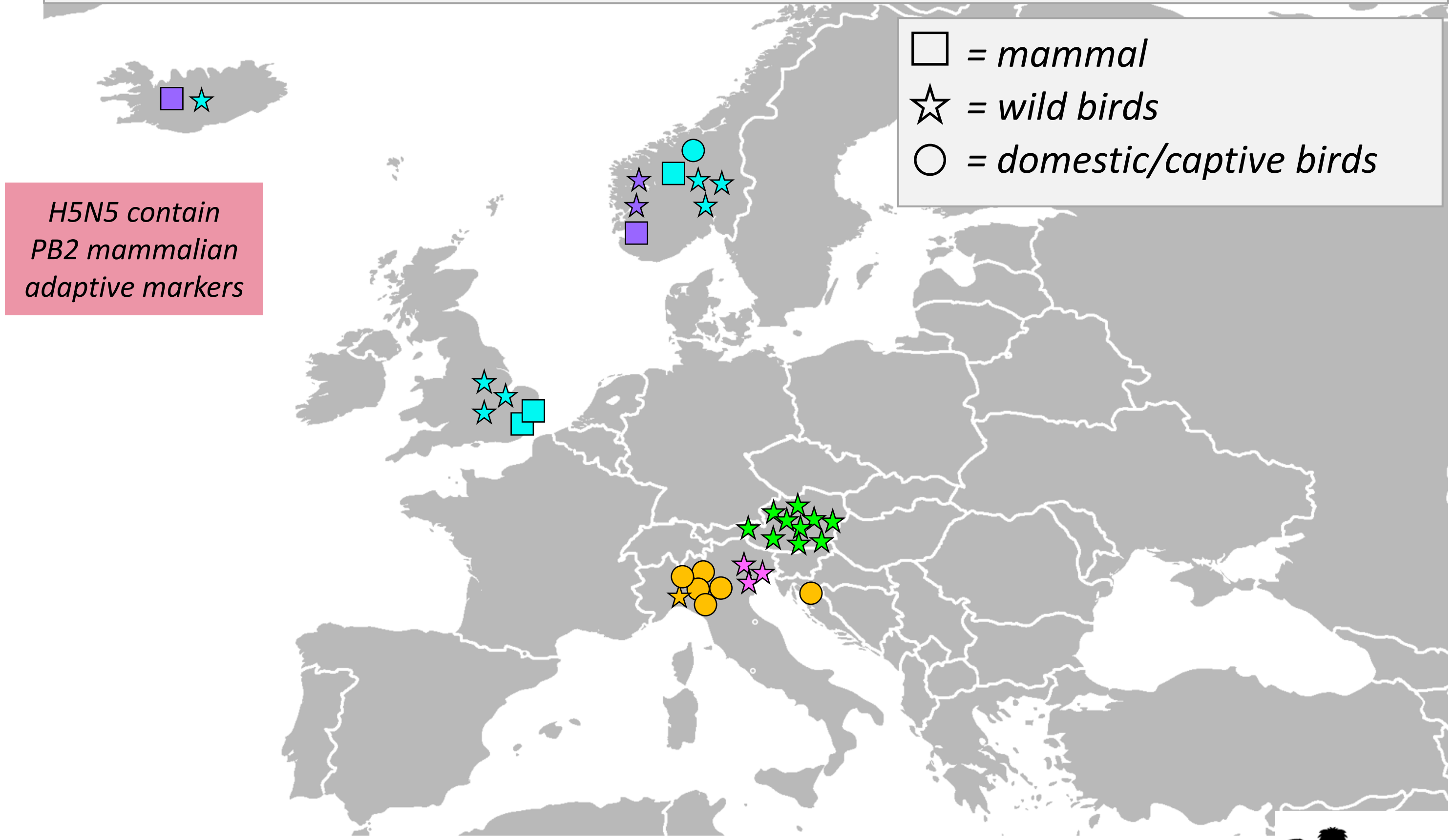






Geographic distribution in Europe of H5Nx viruses with molecular markers of mammalian adaptation PB2 E627K/V, K526R and D701N, 2024-2025

H5N5 contain PB2 mammalian adaptive markers



https://izsvenezie-virology.github.io/FluMut

FLU MUT

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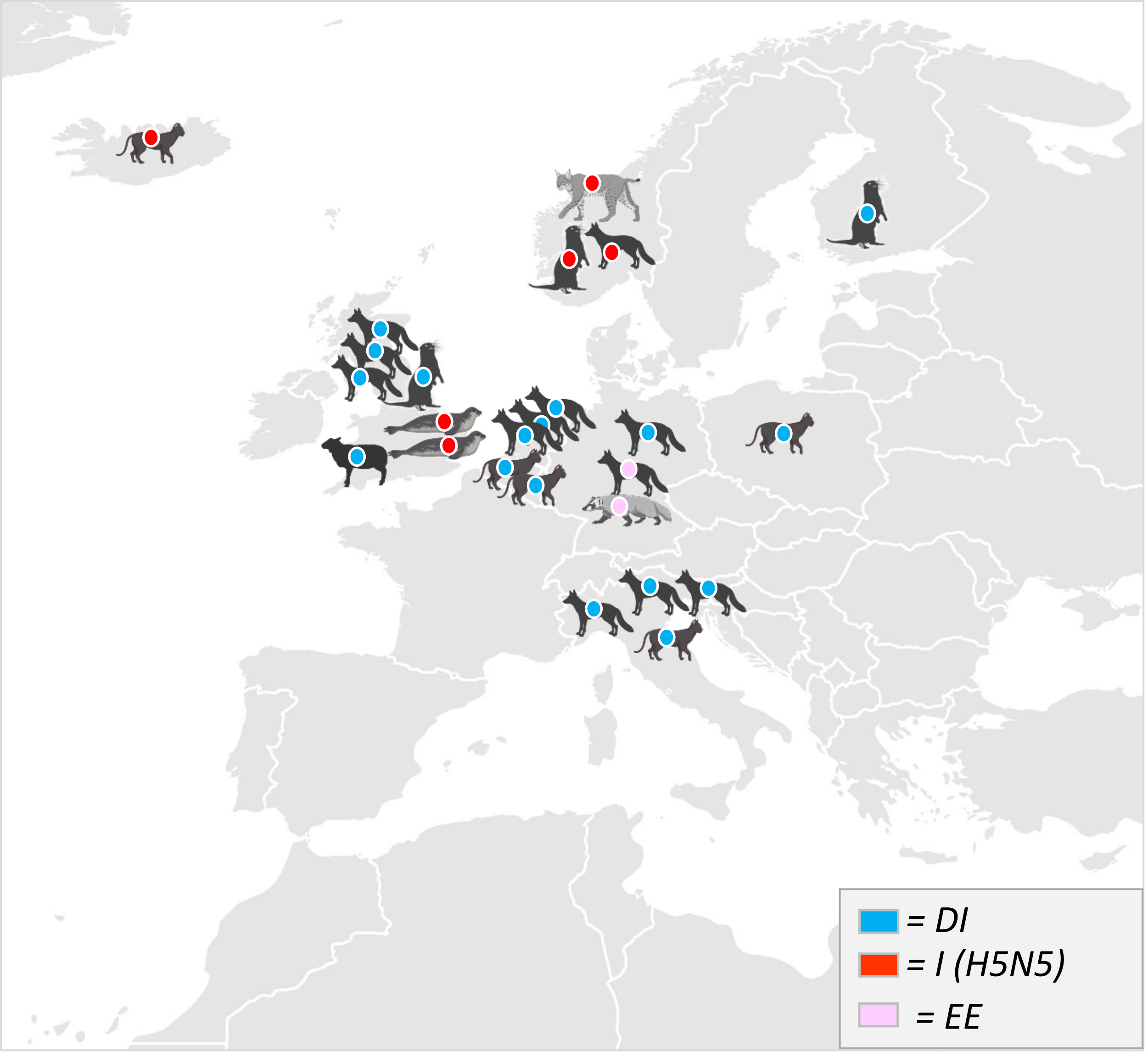
Genetic characteristics of HPAI A(H5Nx) viruses circulating in mammals in Europe October 2024 – May 2025

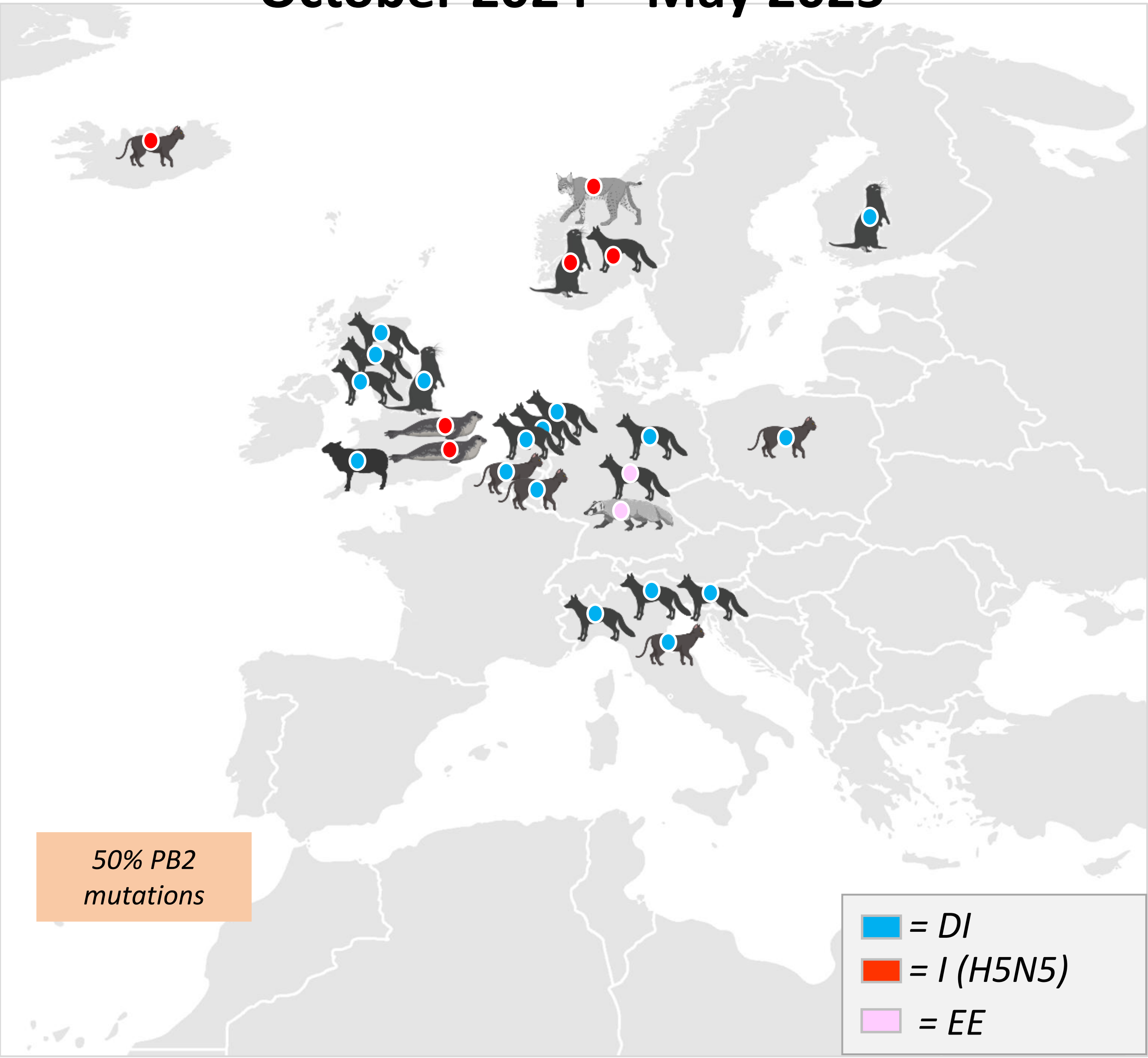


"...when a virus infects mammal hosts that are in close contact with humans"

- Based on genetic investigations, cases in domestic mammals have almost always resulted from exposure to infected poultry (e.g. cats in Italy, Poland, and Belgium, and a sheep in England). However, infections have also been reported in domestic cats likely exposed to infected wild birds (Iceland).

Genetic characteristics of HPAI A(H5Nx) viruses circulating in mammals in Europe

October 2024 – May 2025



- Since October 2024, about 50% of the characterised viruses from mammals in Europe contain at least one of the adaptive markers associated with an increased virulence and replication in mammals in the PB2 protein

Conclusions

This epidemic wave has been characterized by a drastic reduction in the number of emerging genotypes and by the predominant circulation of genotypes that had already emerged in previous epidemiological years (DI, I, BB, DT genotypes).

From a zoonotic risk perspective, we observed an increasing prevalence of polymerase mutations in viruses detected in both domestic and wild birds. This may suggest spillover events into wild mammals that current surveillance systems are unable to capture.

The detection of genotypes carrying adaptive mutations also in domestic animals, such as cats, underlines the importance of monitoring the infection status of these species when potentially exposed to infected birds.

Genetic analyses (2020–present) suggest that waterfowl (order Anseriformes) may play a key role in introducing HPAI into domestic birds. As autumn migration begins and large winter aggregations form across Europe, the spillover risk to domestic birds increases—therefore it is reccomended to intensify surveillance and biosecurity around wetlands and along migration corridors.

HPAI evolution is **complex**, and **complexity makes predictions difficult**.

Promoting access to genetic data as an integral part of the diagnostic workflow, and fostering global—not just regional—data sharing and capacity building, is therefore crucial

Acknowledgements

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Special thanks to all colleagues from the **European National Reference Laboratories and WOAH/FAO Reference Laboratories/Centres for sharing genetic sequences and epidemiological data**

IZSVe - EURL

Alice Fusaro, Bianca Zecchin, Lara Cavicchio, Marta Dianati, Enrico Savegnago, Edoardo Giussani, Alessandro Sartori, Alessia Schivo, Annalisa Salviato, Silvia Ormelli, Maria Varotto, Gianpiero Zamperin, Calogero Terregino

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European Commission within the framework of the activities foreseen by the **European Union Reference Laboratory for Avian Influenza and Newcastle Disease**.

EURL for AI and NDV

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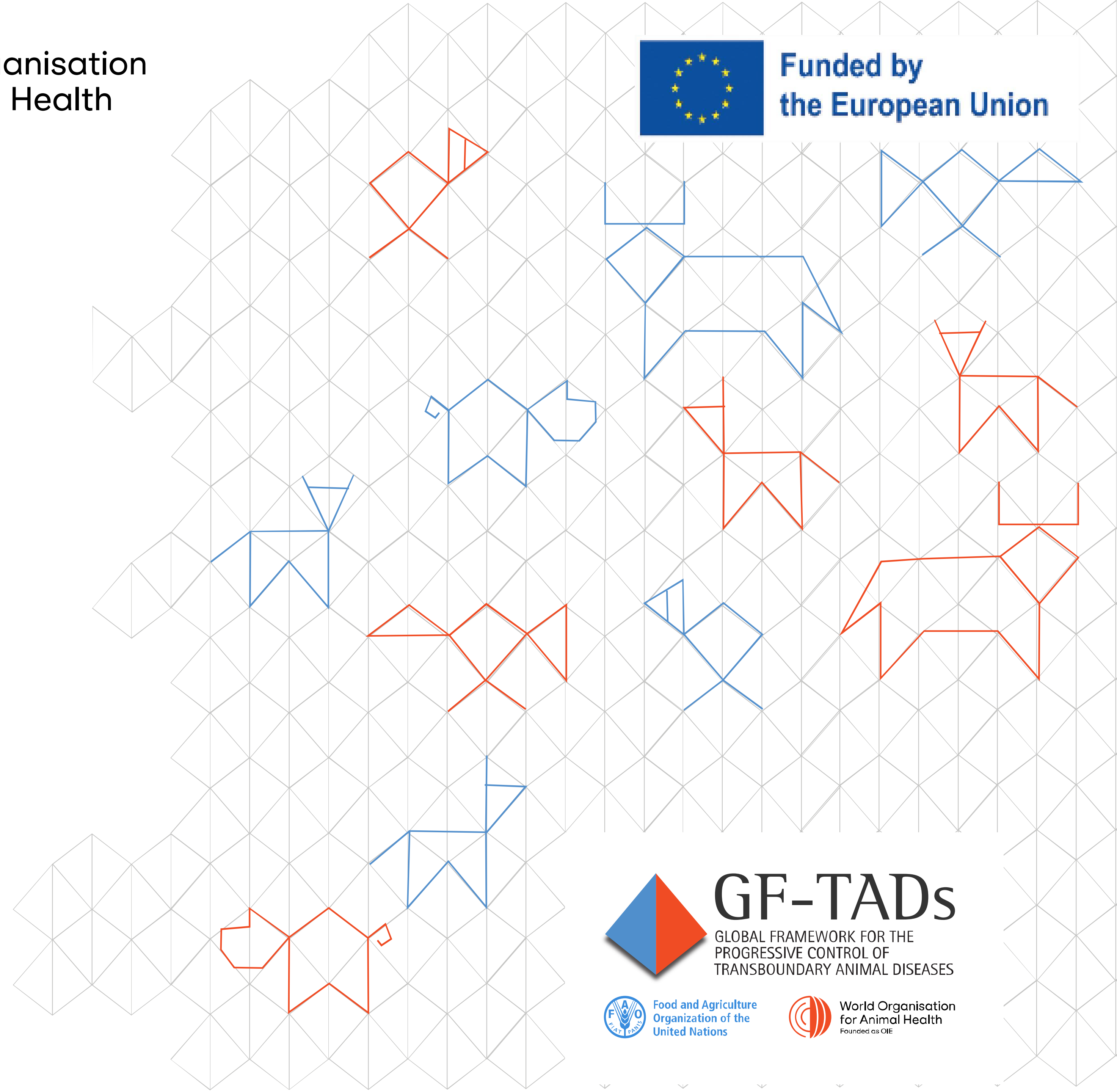
THANK YOU



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