Monitoring the genetic evolution of HPAI in Europe: insights from the WOAH/FAO/EU Reference Laboratory at the IZSVe

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-Standing Group of Experts on High Pathogenicity Avian Influenza in Europe-

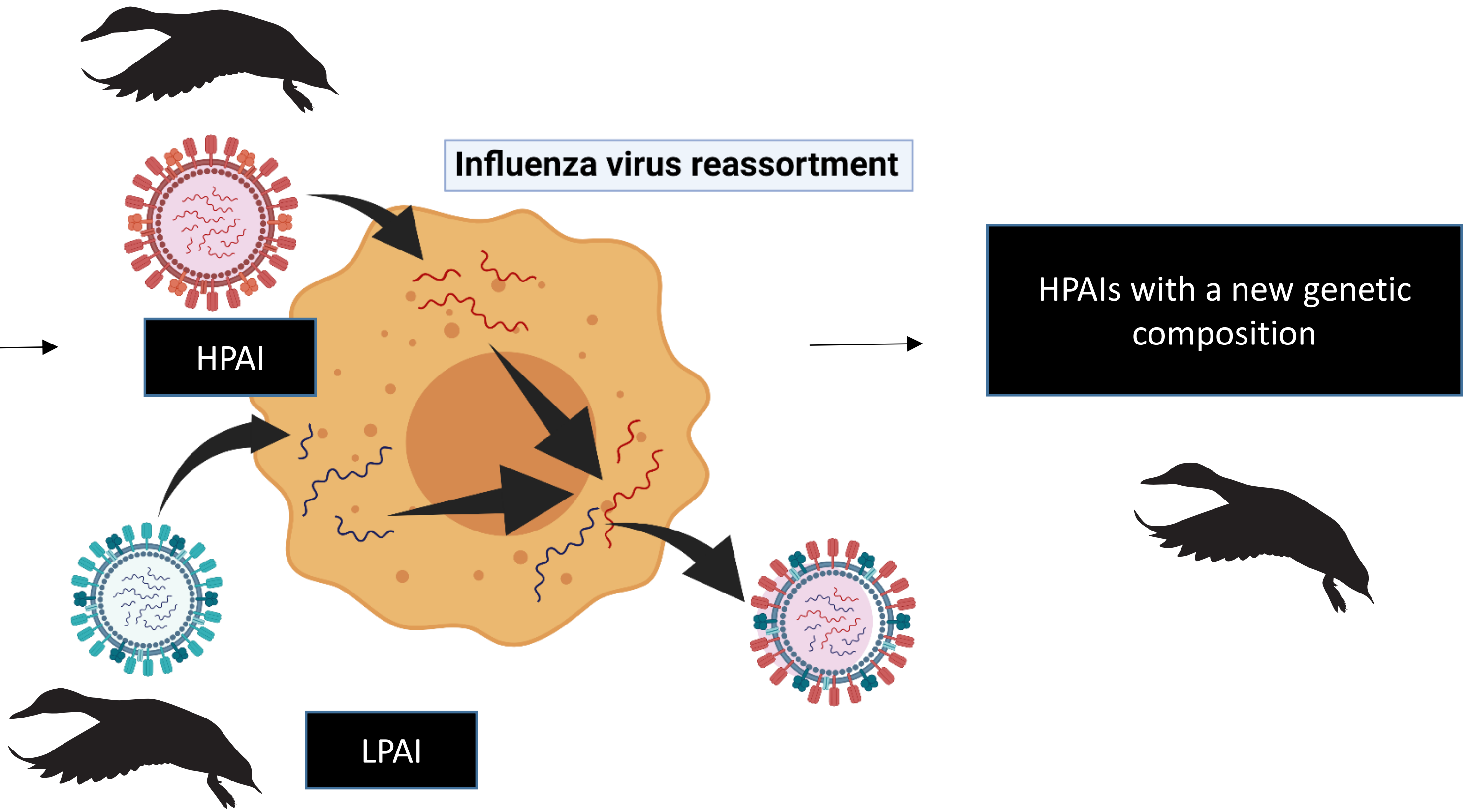
The **evolution** of HPAI viruses of clade 2.3.4.4b in Europe has **continued** throughout the 2023-2024 epidemiological year (October 2023-August 2024).

High pathogenic avian influenza A(H5) viruses of clade 2.3.4.4b in Europe

-Persistence of HPAI in wild birds and expasion of the avian host range

+

-Unprecedent ability to generate diversity through reassortment



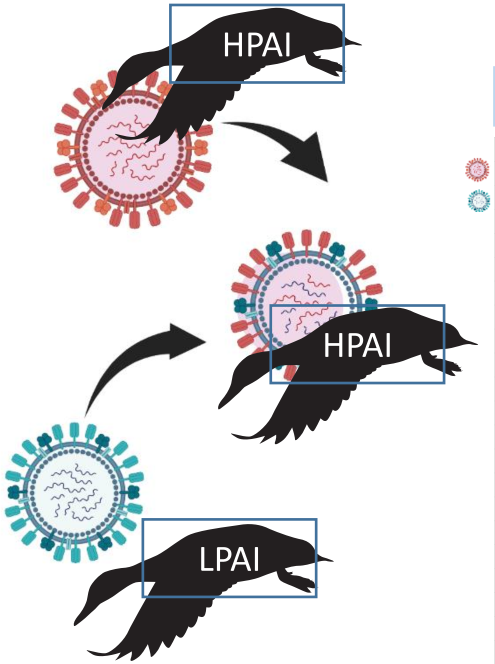
HPAI

LPAI

Influenza virus reassortment

HPAIs with a new genetic composition

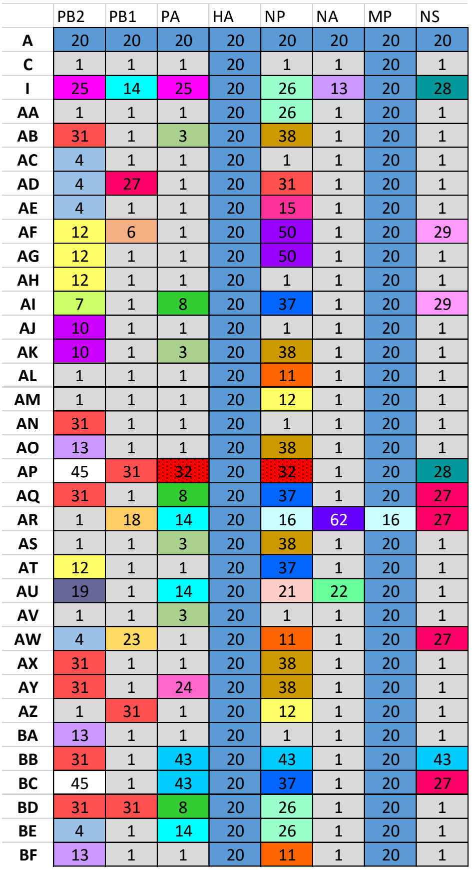
Europe: Evolution of H5 clade 2.3.4.4b reassortants



Epidemic wave: 2020-2021

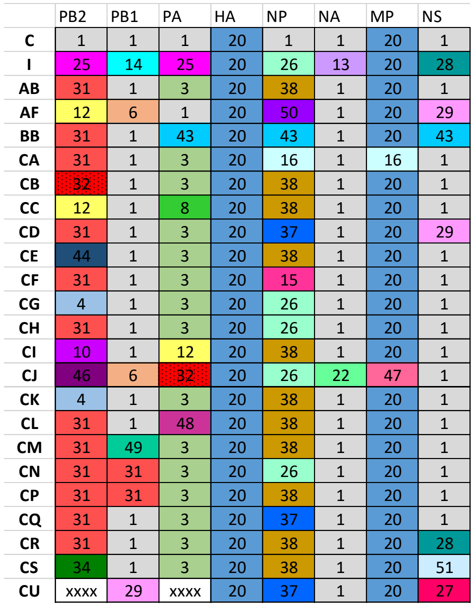
	PB2	PB1	PA	HA	NP	NA	MP	NS
A	20	20	20	20	20	20	20	20
B	35	28	1	20	1	30	20	20
C	1	1	1	20	1	1	20	1
D	20	20	20	20	20	27	20	20
E	20	20	29	20	20	27	20	20
F	27	20	29	20	37	27	20	20
G	31	31	32	20	12	17	20	1
H	20	31	29	20	27	63	20	20
I	25	14	25	20	26	13	20	28
J	31	23	5	20	26	2	20	28
L	25	1	1	20	1	13	20	1
M	20	20	14	20	20	20	20	20
N	12	33	20	20	36	64	20	20
O	20	29	14	20	20	20	20	20
P	31	23	3	20	26	2	20	28
Q	20	20	20	20	26	20	20	20
R	20	20	29	20	15	27	20	20
S	20	20	29	20	20	20	20	20
U	20	20	29	20	15	20	20	20
V	34	1	1	20	1	1	20	1
X	20	9	14	20	16	61	20	20

Epidemic wave: 2021-2022



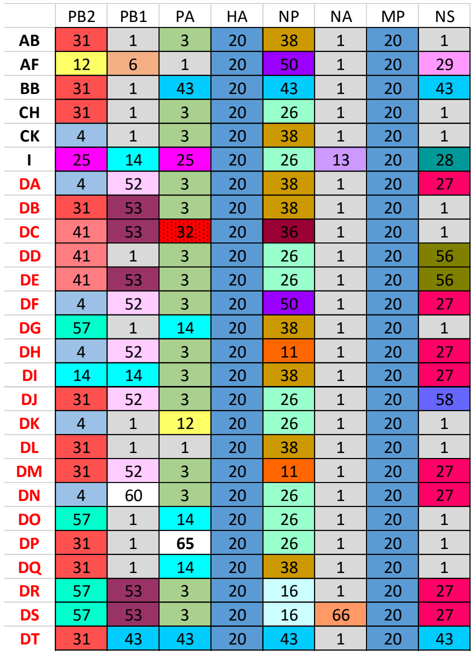
	PB2	PB1	PA	HA	NP	NA	MP	NS
A	20	20	20	20	20	20	20	20
C	1	1	1	20	1	1	20	1
I	25	14	25	20	26	13	20	28
AA	1	1	1	20	26	1	20	1
AB	31	1	3	20	38	1	20	1
AC	4	1	1	20	1	1	20	1
AD	4	27	1	20	31	1	20	1
AE	4	1	1	20	15	1	20	1
AF	12	6	1	20	50	1	20	29
AG	12	1	1	20	50	1	20	1
AH	12	1	1	20	1	1	20	1
AI	7	1	8	20	37	1	20	29
AJ	10	1	1	20	1	1	20	1
AK	10	1	3	20	38	1	20	1
AL	1	1	1	20	11	1	20	1
AM	1	1	1	20	12	1	20	1
AN	31	1	1	20	1	1	20	1
AO	13	1	1	20	38	1	20	1
AP	45	31	32	20	32	1	20	28
AQ	31	1	8	20	37	1	20	27
AR	1	18	14	20	16	62	16	27
AS	1	1	3	20	38	1	20	1
AT	12	1	1	20	37	1	20	1
AU	19	1	14	20	21	22	20	1
AV	1	1	3	20	1	1	20	1
AW	4	23	1	20	11	1	20	27
AX	31	1	1	20	38	1	20	1
AY	31	1	24	20	38	1	20	1
AZ	1	31	1	20	12	1	20	1
BA	13	1	1	20	1	1	20	1
BB	31	1	43	20	43	1	20	43
BC	45	1	43	20	37	1	20	27
BD	31	31	8	20	26	1	20	1
BE	4	1	14	20	26	1	20	1
BF	13	1	1	20	11	1	20	1

Epidemic wave: 2022-2023



	PB2	PB1	PA	HA	NP	NA	MP	NS
C	1	1	1	20	1	1	20	1
I	25	14	25	20	26	13	20	28
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
CA	31	1	3	20	16	1	16	1
CB	32	1	3	20	38	1	20	1
CC	12	1	8	20	38	1	20	1
CD	31	1	3	20	37	1	20	29
CE	44	1	3	20	38	1	20	1
CF	31	1	3	20	15	1	20	1
CG	4	1	3	20	26	1	20	1
CH	31	1	3	20	26	1	20	1
CI	10	1	12	20	38	1	20	1
CJ	46	6	32	20	26	22	47	1
CK	4	1	3	20	38	1	20	1
CL	31	1	48	20	38	1	20	1
CM	31	49	3	20	38	1	20	1
CN	31	31	3	20	26	1	20	1
CP	31	31	3	20	38	1	20	1
CQ	31	1	3	20	37	1	20	1
CR	31	1	3	20	38	1	20	28
CS	34	1	3	20	38	1	20	51
CU	xxxx	29	xxxx	20	37	1	20	27

Epidemic wave: 2023-2024

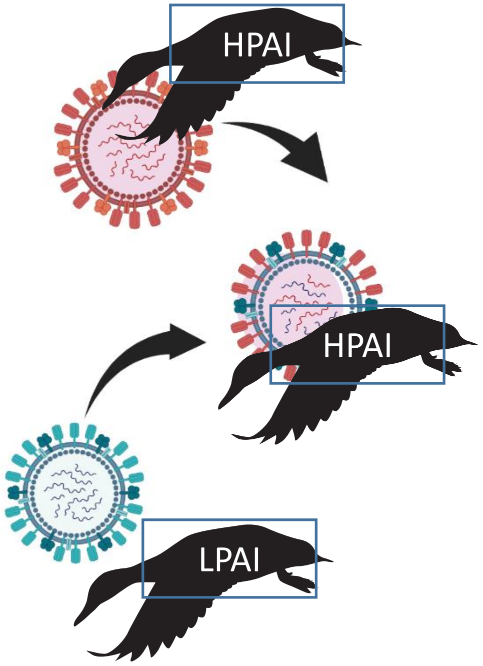


	PB2	PB1	PA	HA	NP	NA	MP	NS
AB	31	1	3	20	38	1	20	1
AF	12	6	1	20	50	1	20	29
BB	31	1	43	20	43	1	20	43
CH	31	1	3	20	26	1	20	1
CK	4	1	3	20	38	1	20	1
I	25	14	25	20	26	13	20	28
DA	4	52	3	20	38	1	20	27
DB	31	53	3	20	38	1	20	1
DC	41	53	32	20	36	1	20	1
DD	41	1	3	20	26	1	20	56
DE	41	53	3	20	26	1	20	56
DF	4	52	3	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

AB (H5N1-A/duck/Saratov/29-02V/2021-like)	EPI_ISL_5463797
AF (H5N1-A/chicken/Italy/IZSLT-122448_21VIR9218-1/2021-like)	EPI_ISL_7733644
BB (H5N1-A/Herring_gull/France/22P015977/2022-like)	EPI_ISL_13519451
CH (H5N1-A/Eurasian_Wigeon/Netherlands/3/2022-like	EPI_ISL_15925882
CK (H5N1 A/Cormorants/Sweden/SVA240102SZ0114/FB00004/M-2023-like)	EPI_ISL_18815224
I (H5N5-A/whooper_swan/Romania/10123_21VIR849-1/2021-like)	EPI_ISL_1665268
DA (H5N1-A/mute_swan/Slovenia/PER1486-23TA_23VIR10323-22/2023-like)	EPI_ISL_18612233
DB (H5N1- A/herring_gull/Germany-NI/2023AI08764/2023-like)	EPI_ISL_18526630
DC (H5N1-A/Common_Buzzard/Netherlands/23023642-002/2023-like)	EPI_ISL_18506031
DD (H5N1-A/Pheasant/England/113705/2023-like)	EPI_ISL_18538399
DE (H5N1-A/Chicken/Scotland/114176/2023-like)	EPI_ISL_18538402
DF (H5N1_A/hens/Bulgaria/309_24VIR2991-20/2024-like)	\
DG (H5N1-A/Gallus_gallus/Belgium/11307_0002/2023-like)	EPI_ISL_18607170
DH (H5N1-A/teal/Italy/23VIR11492-4/2023-like)	EPI_ISL_18812084
DI (H5N1-A/mute_swan/Poland/MB008-1/2024-like)	EPI_ISL_18787976
DJ (H5N1 A/Barnacle_Goose/Netherlands/24000825-002/2024-like)	EPI_ISL_18918831
DK (H5N1-A/barnacle_goose/Denmark/09539-1.02/2023-like)	\
DL (H5N1-A/Chicken/England/005435/2024-like)	EPI_ISL_18927384
DM (H5N1-A/cygnus-olor/Romania/16868_24VIR1002-1/2023-like)	EPI_ISL_18956171
DN (H5N1-A/peregrine-falcon/Cyprus/24VIR1053/2024-like)	EPI_ISL_18956184
DO (H5N1 A/fox/Germany-BW/2024AI00417/2024-like)	EPI_ISL_18937337
DP (H5N1 A/turkey/Poland/H40-T2/2024-like)	EPI_ISL_19033547
DQ (H5N1_A/chicken/Germany-MV/2024AI00860/2024-like)	EPI_ISL_19014079
DR (H5N1_A/Red_Knot/Germany-SH/2024AI01285/2024-like)	EPI_ISL_19014084
DS (H5N8_A/Red_Knot/Germany-SH/2024AI01485/2024-like)	EPI_ISL_19030161
DT (H5N1-A/gull/Spain/5061-23_24VIR4860-8/2023-like)	EPI_ISL_19207490

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

Europe: Evolution of H5 clade 2.3.4.4b reassortants



Epidemic wave: 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
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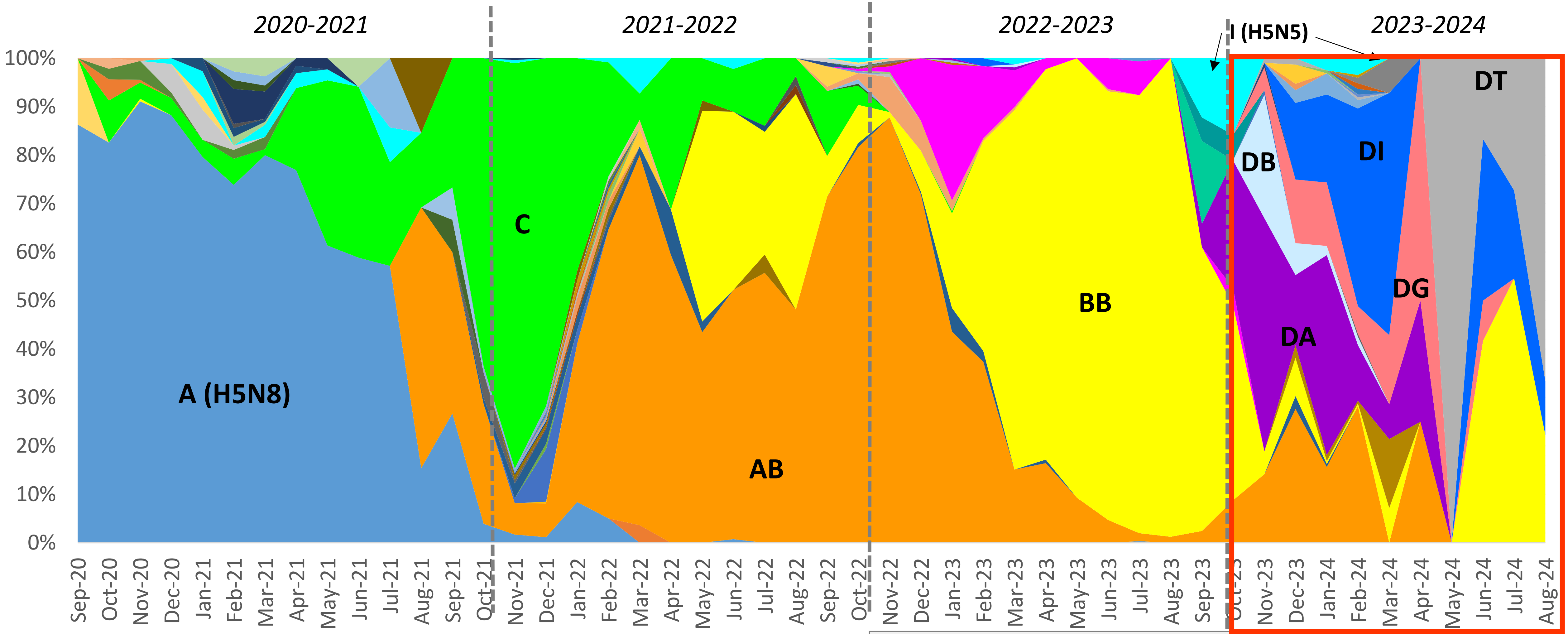
DP	31	1	**65**	20	26	1	20	1
DQ	31	1	14	20	38	1	20	1
DR	57	53	3	20	16	1	20	27
DS	57	53	3	20	16	66	20	27
DT	31	43	43	20	43	1	20	43

~ 650 complete genomes

from 25 distinct countries in the European region

26 distinct genotypes

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



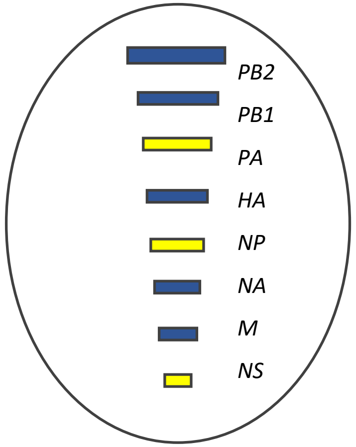
>93% of the characterized viruses during the 2023-2024 epidemiological year belong to **eight** major genotypes:

- 3 genotypes presistently circulating in Europe from previous epidemiological years (**AB, BB, I**)
- 5 new genotypes, which emerged in 2023-2024 from reassortment events (DA, DI, DG, DB, DT)

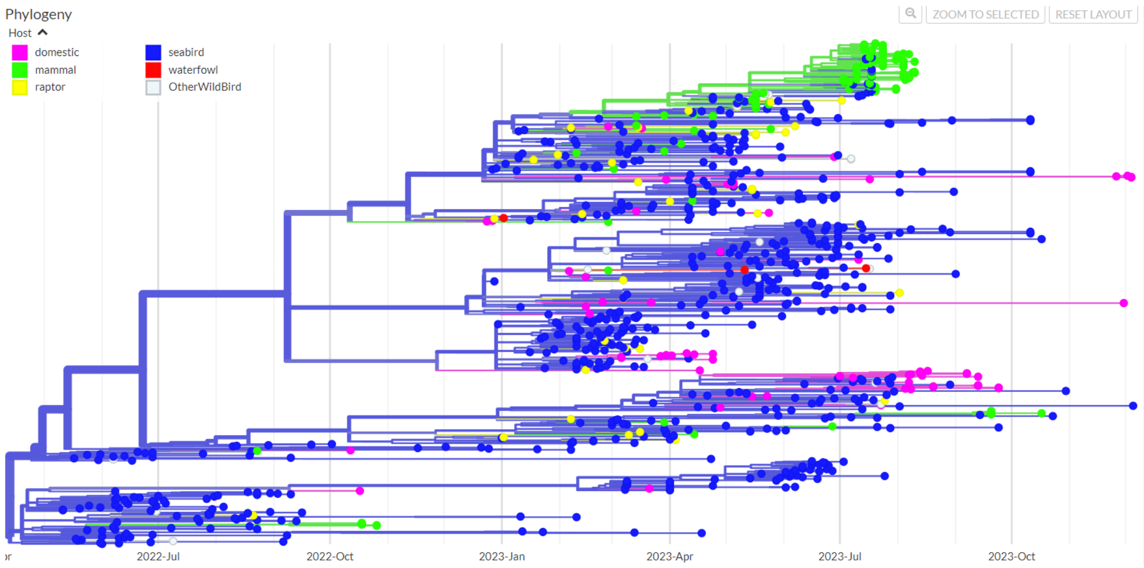
Why is it so crucial to understand the genotype of circulating viruses?

EA-2022-BB

- Emerged from reassortment with A(H13) LPAI viruses, a subtype primarily found in gulls, in spring 2022.
- Circulated extensively among **Laridae** in Europe from February to September 2023.
- Genotype EA-2022-BB was responsible of all the outbreaks reported in fur farms in Europe in 2022–2023 (Spain and Finland).



EA-2022-BB



From Nextstrain https://nextstrain.org/avian-flu/h5n1/ha/2y

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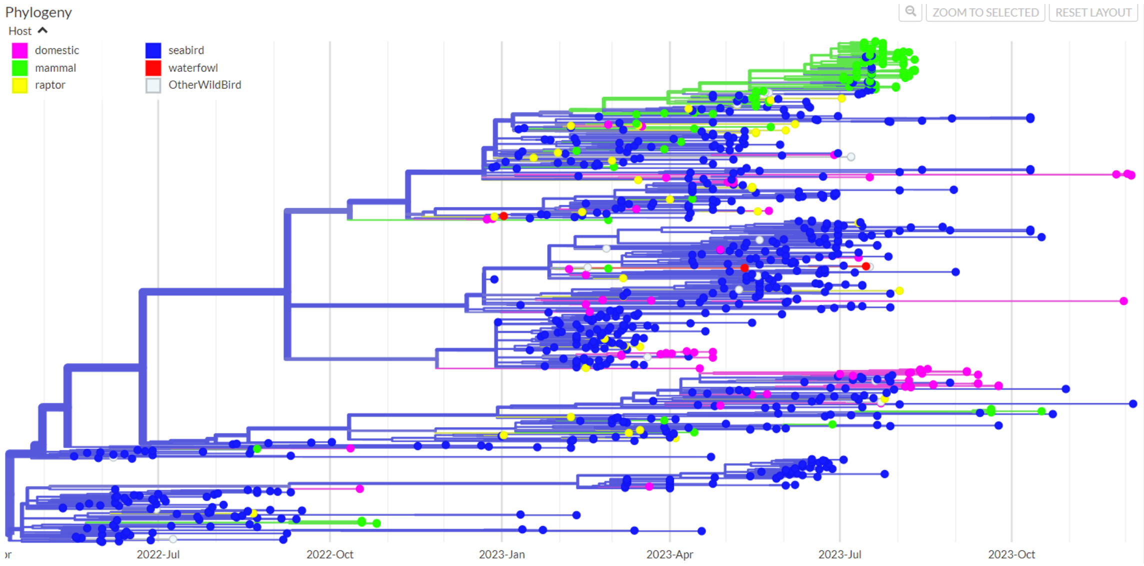
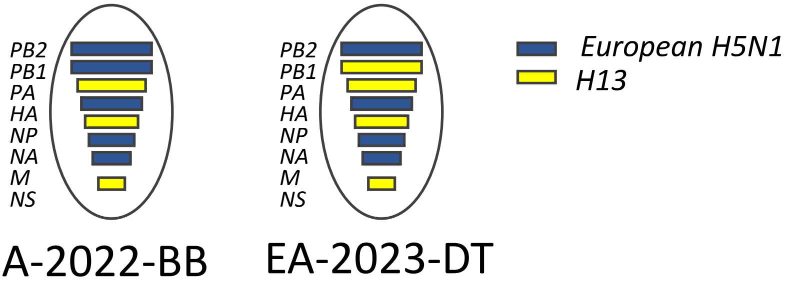


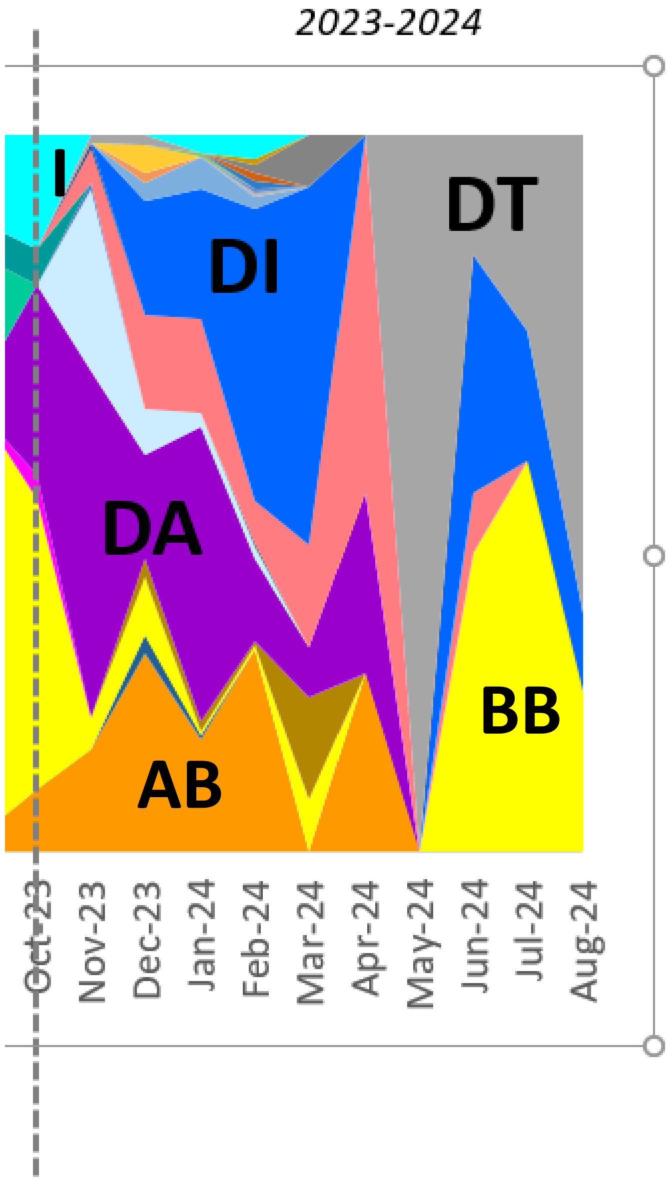
From Nextstrain https://nextstrain.org/avian-flu/h5n1/ha/2y

Temporal trend: EA-2022-BB (2023-2024)

- It has only been detected at low level in the first months of the current epidemiological year. However, **since June 2024, a rapid increase of the frequency** of the EA-2022-BB genotype has been observed along the **northwestern continental coast** of Europe, with most cases detected among Laridae.

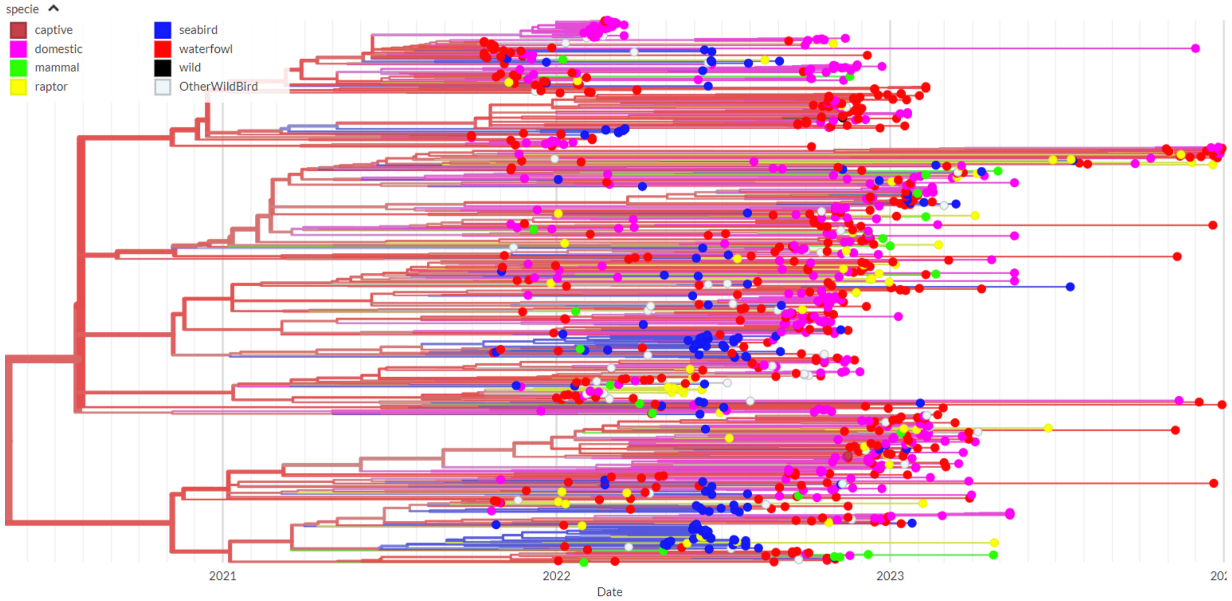
- A novel genotype (**EA-2023-DT**) originating from a reassortment event of the PB1 gene between the EA-2022-BB and a gull-adapted H13 subtype has been circulating among *Laridae* and *Sulidae* in Spain since November 2023 and has been identified during the summer season also in Portugal.





EA-2022-AB

- It was identified for the first time in September 2021 in the Russian Federation.
- In 2022 became the most frequent and widespread genotype in the European region.
- Primarily found in waterfowl (Anseriformes), was responsible of multiple outbreaks in domestic birds



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EA-2022-AB

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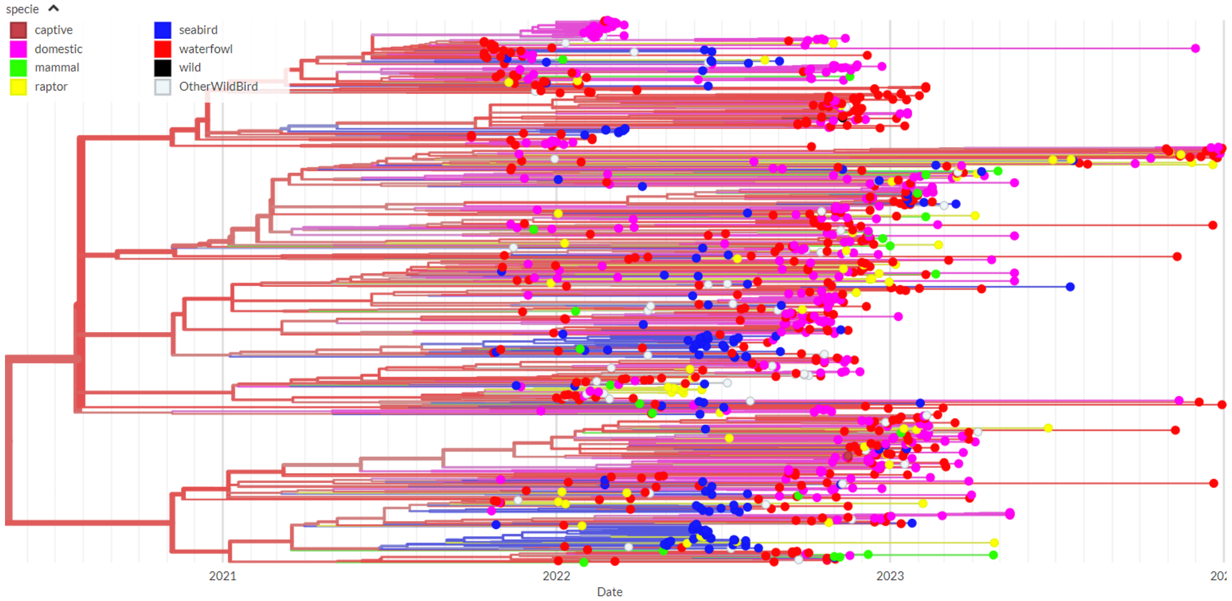
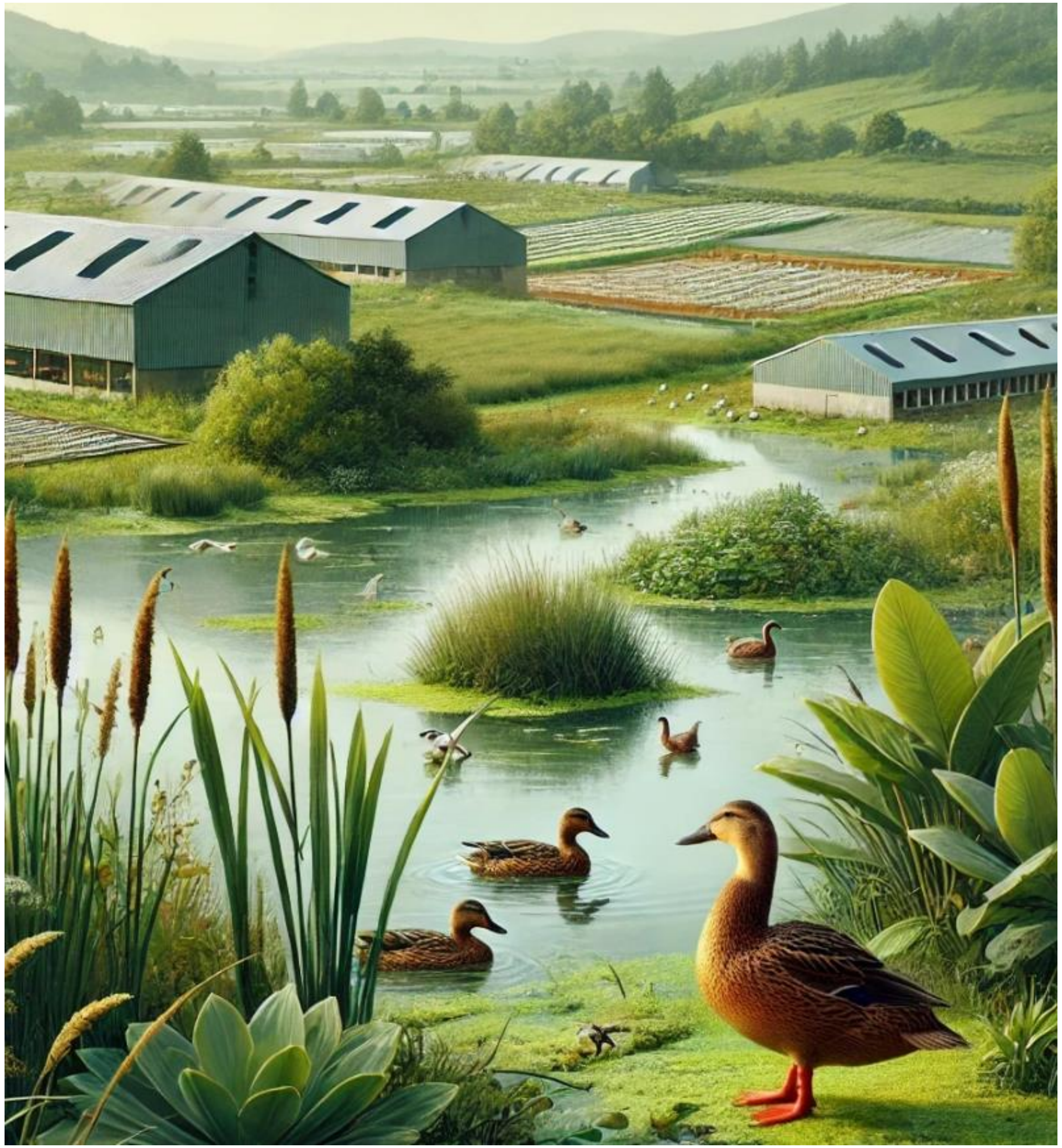
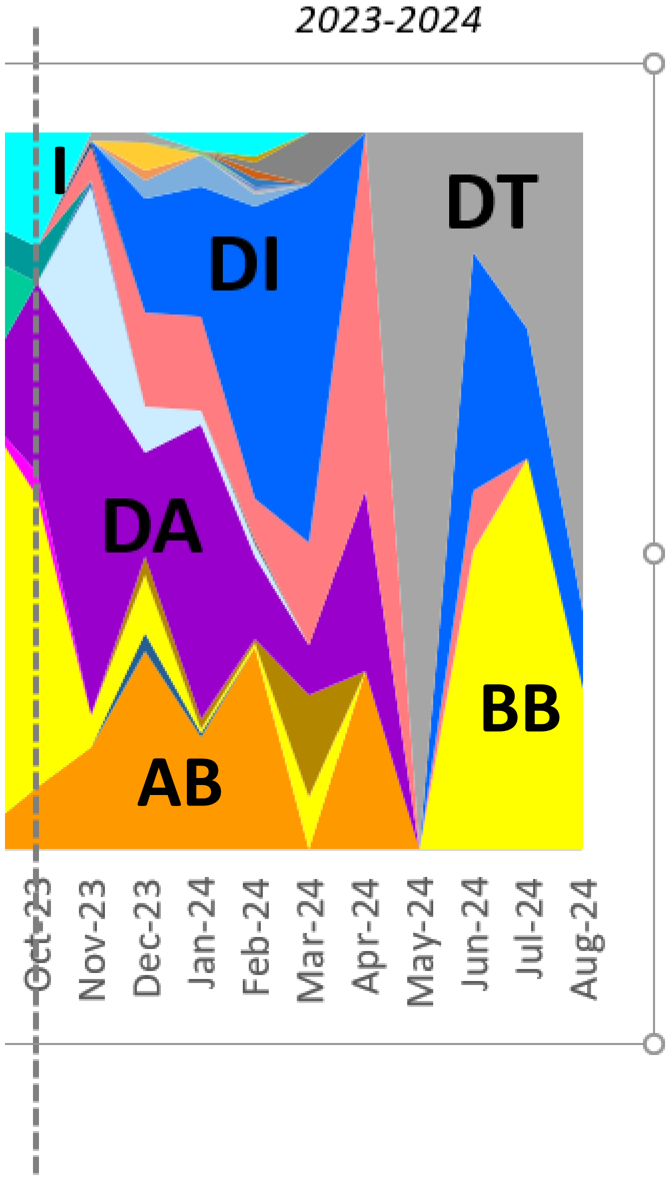


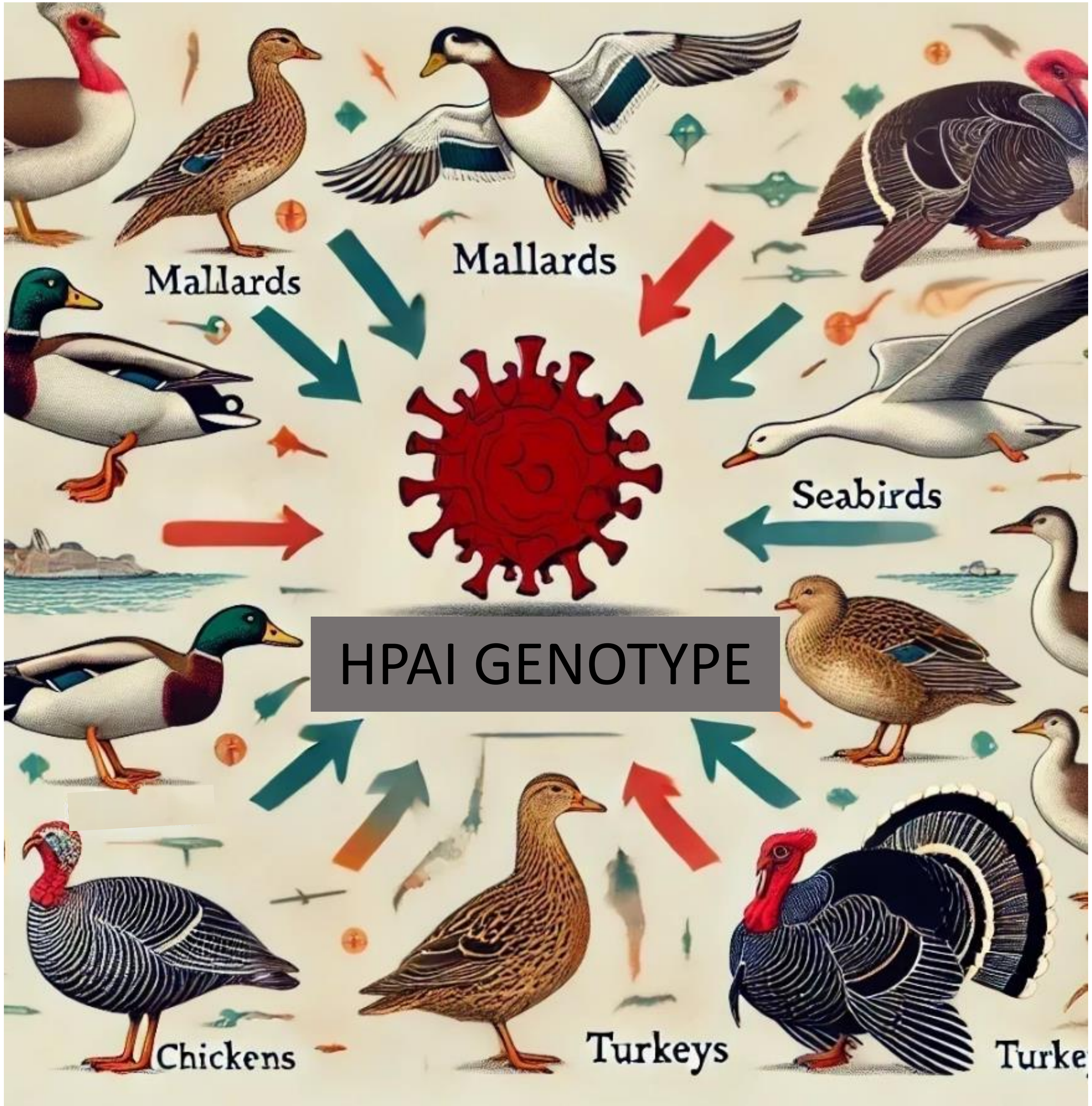
From Nextstrain https://nextstrain.org/avian-flu/h5n1/ha/2y



Temporal trend: EA-2022-AB (2023-2024)

Genotype AB was identified in Europe during the autumn and winter months, and its frequency progressively decreased until it disappeared by summer. This temporal trend was also observed in previous epidemic waves.

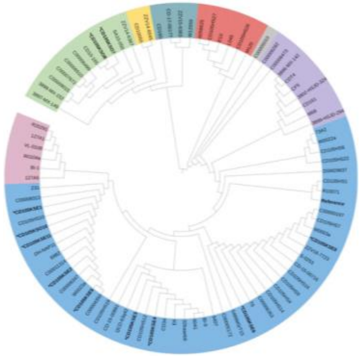




Genotype adaptation to a specific host affects viral spread in both time and space

Genotype prediction tool

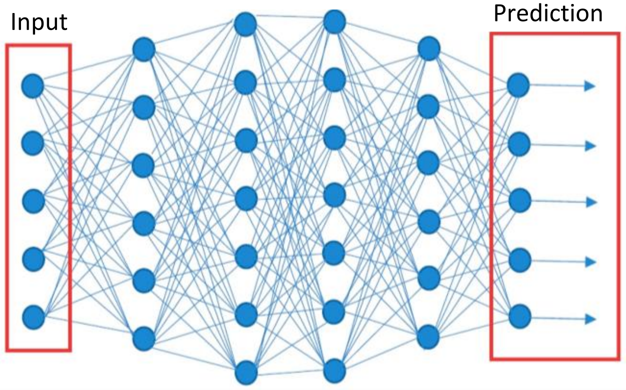
Definition of genotypes by experts through manual data curation



dataset

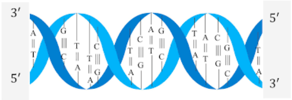
Machine learning

DATASET



model

Prediction tool



MODEL

NEW DATA

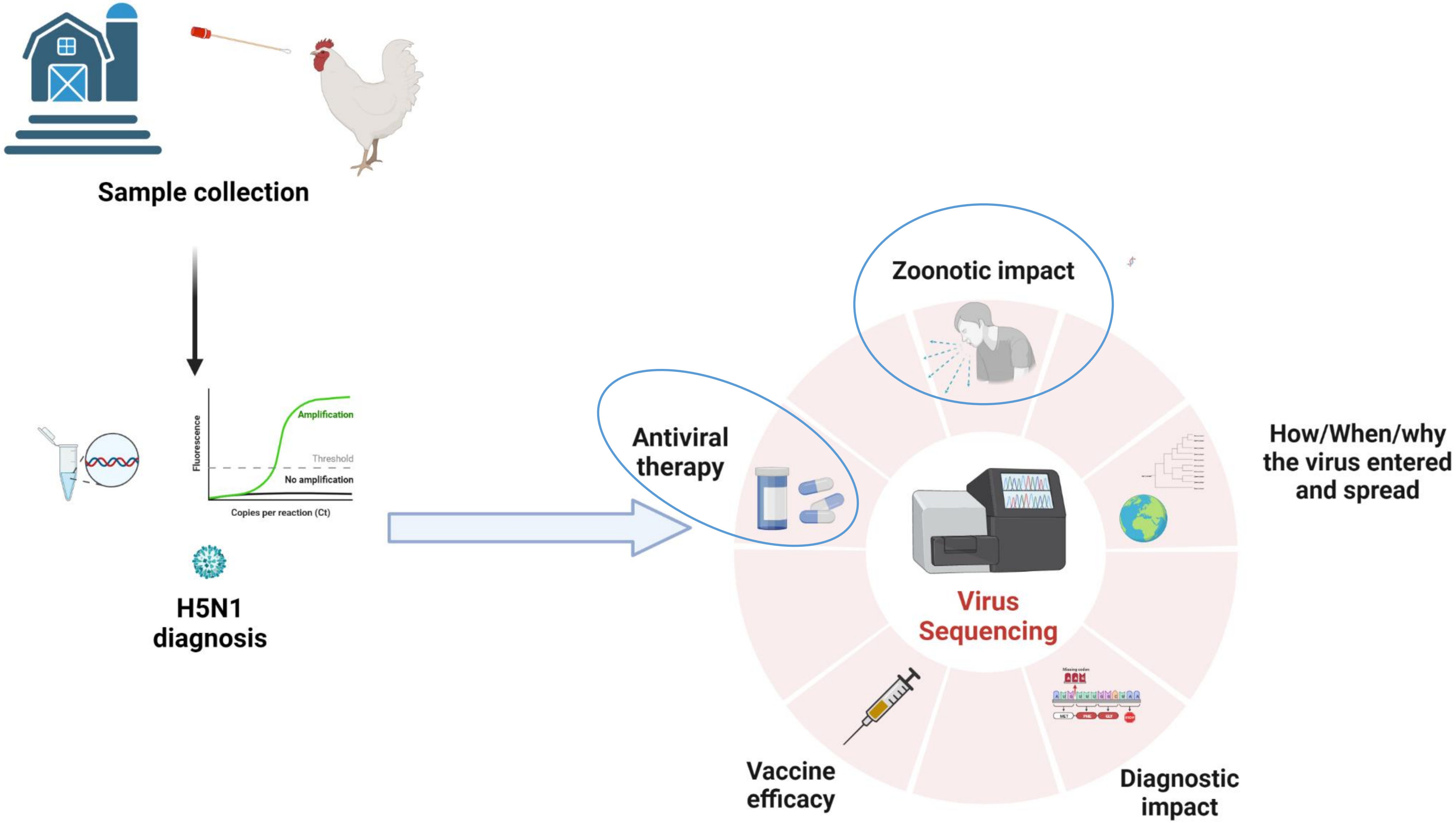
PREDICTION

Genotype definition on novel data in an automatic fashion

Performances: **97%** of new data correctly assigned, novel genotypes (i.e. not present in the dataset) recognized

Soon available for downloading on **github**

The benefits of genomic surveillance



Sample collection

H5N1 diagnosis

Virus Sequencing

Zoonotic impact

How/When/why the virus entered and spread

Diagnostic impact

Vaccine efficacy

Antiviral therapy

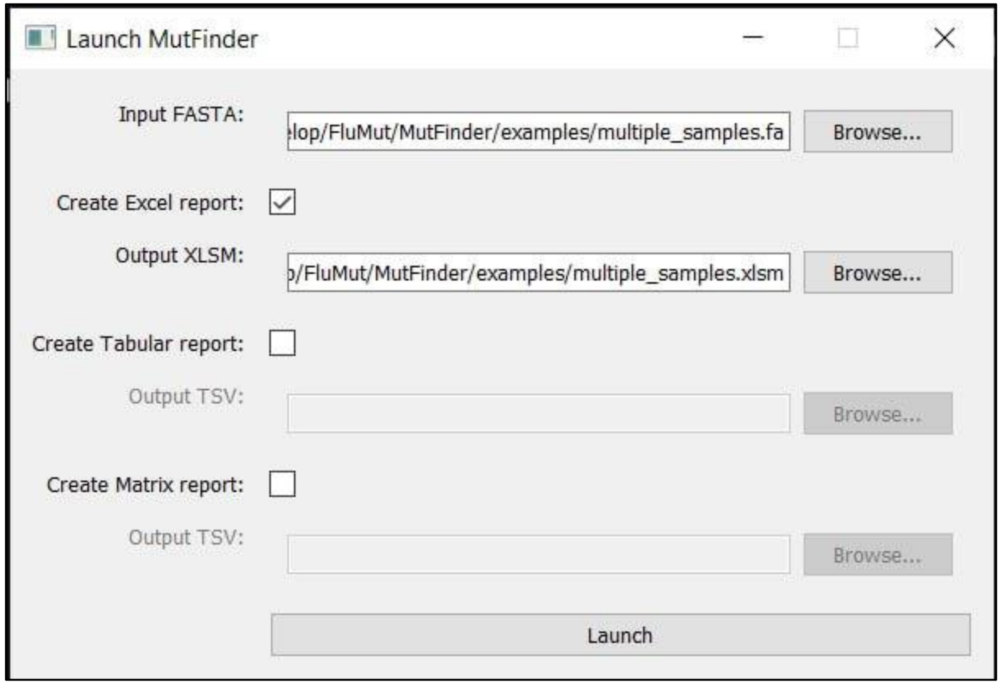
Created with BioRender.com

NUCLEOTIDE SEQUENCES

Development of a new molecular tool to automatically identify in the AIV genomes determinants involved in the crossing of the host species barrier (avian-mammals) (available in Github)

FLU
MUT

MARKERS OF INTEREST



Input FASTA: lop/FluMut/MutFinder/examples/multiple_samples.fa

Create Excel report: ☑

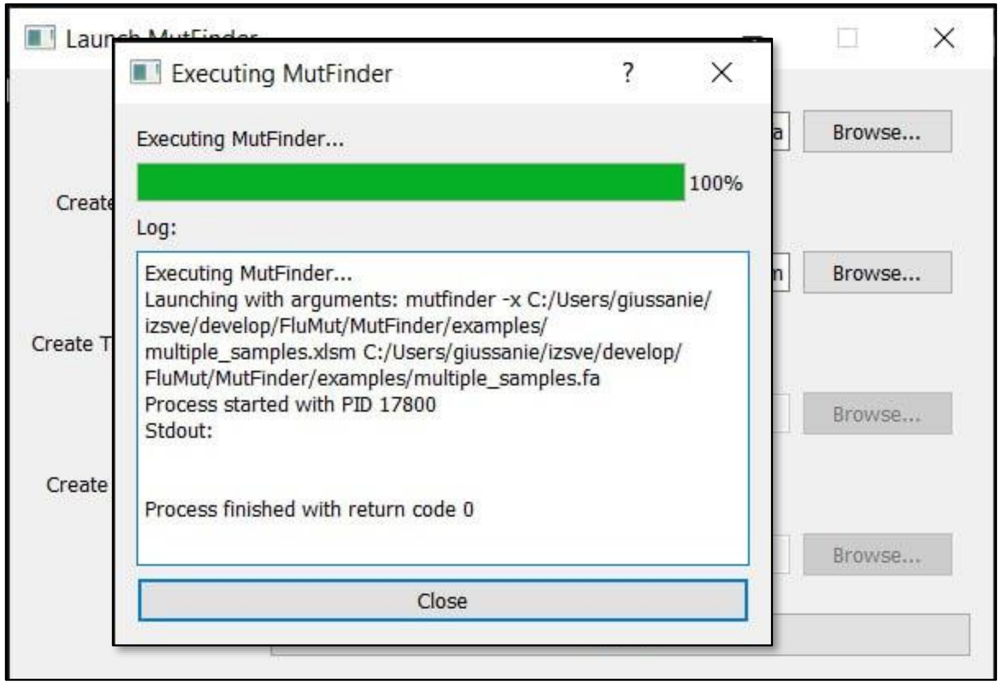
Output XLSM: /FluMut/MutFinder/examples/multiple_samples.xlsm

Create Tabular report: ☐

Output TSV:

Create Matrix report: ☐

Output TSV:



Executing MutFinder...

100%

Log:

```
Executing MutFinder...
Launching with arguments: mutfinder -x C:/Users/giussanie/
izsve/develop/FluMut/MutFinder/examples/
multiple_samples.xlsm C:/Users/giussanie/izsve/develop/
FluMut/MutFinder/examples/multiple_samples.fa
Process started with PID 17800
Stdout:


Process finished with return code 0
```

More information:

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

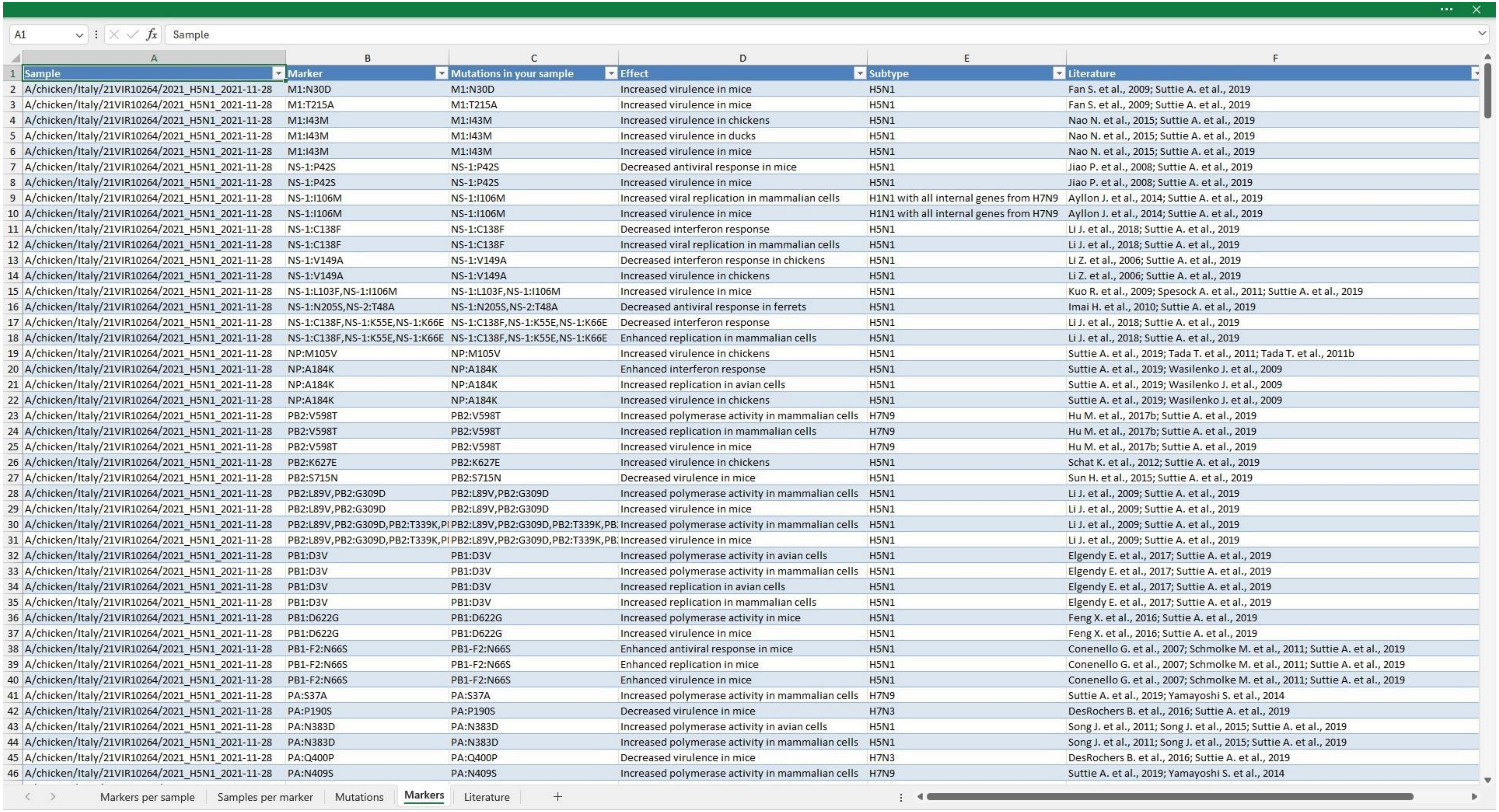
Curated and customizable database

- Molecular marker definitions
- Phenotypic effects
- Bibliography & literature references

MARKERS OF INTEREST

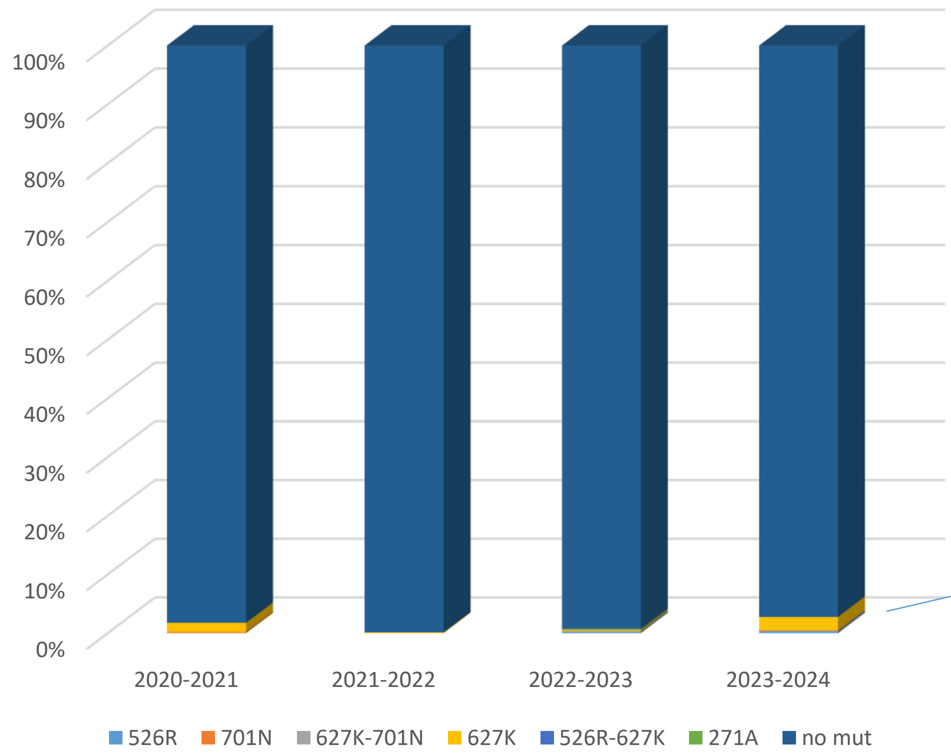
- Zoonotic potential
- Virulence alterations in host categories
- Resistance alterations to antiviral drugs

More information:
https://github.com/izsvenezie-virology/FluMut



	A	B	C	D	E	F
1	Sample	Marker	Mutations in your sample	Effect	Subtype	Literature
2	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	M1:N30D	M1:N30D	Increased virulence in mice	H5N1	Fan S. et al., 2009; Suttie A. et al., 2019
3	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	M1:T215A	M1:T215A	Increased virulence in mice	H5N1	Fan S. et al., 2009; Suttie A. et al., 2019
4	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	M1:I43M	M1:I43M	Increased virulence in chickens	H5N1	Nao N. et al., 2015; Suttie A. et al., 2019
5	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	M1:I43M	M1:I43M	Increased virulence in ducks	H5N1	Nao N. et al., 2015; Suttie A. et al., 2019
6	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	M1:I43M	M1:I43M	Increased virulence in mice	H5N1	Nao N. et al., 2015; Suttie A. et al., 2019
7	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NS-1:P42S	NS-1:P42S	Decreased antiviral response in mice	H5N1	Jiao P. et al., 2008; Suttie A. et al., 2019
8	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NS-1:P42S	NS-1:P42S	Increased virulence in mice	H5N1	Jiao P. et al., 2008; Suttie A. et al., 2019
9	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NS-1:I106M	NS-1:I106M	Increased viral replication in mammalian cells	H1N1 with all internal genes from H7N9	Ayllon J. et al., 2014; Suttie A. et al., 2019
10	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NS-1:I106M	NS-1:I106M	Increased virulence in mice	H1N1 with all internal genes from H7N9	Ayllon J. et al., 2014; Suttie A. et al., 2019
11	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NS-1:C138F	NS-1:C138F	Decreased interferon response	H5N1	Li J. et al., 2018; Suttie A. et al., 2019
12	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NS-1:C138F	NS-1:C138F	Increased viral replication in mammalian cells	H5N1	Li J. et al., 2018; Suttie A. et al., 2019
13	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NS-1:V149A	NS-1:V149A	Decreased interferon response in chickens	H5N1	Li Z. et al., 2006; Suttie A. et al., 2019
14	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NS-1:V149A	NS-1:V149A	Increased virulence in chickens	H5N1	Li Z. et al., 2006; Suttie A. et al., 2019
15	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NS-1:L103F,NS-1:I106M	NS-1:L103F,NS-1:I106M	Increased virulence in mice	H5N1	Kuo R. et al., 2009; Spesock A. et al., 2011; Suttie A. et al., 2019
16	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NS-1:N205S,NS-2:T48A	NS-1:N205S,NS-2:T48A	Decreased antiviral response in ferrets	H5N1	Imai H. et al., 2010; Suttie A. et al., 2019
17	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NS-1:C138F,NS-1:K55E,NS-1:K66E	NS-1:C138F,NS-1:K55E,NS-1:K66E	Decreased interferon response	H5N1	Li J. et al., 2018; Suttie A. et al., 2019
18	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NS-1:C138F,NS-1:K55E,NS-1:K66E	NS-1:C138F,NS-1:K55E,NS-1:K66E	Enhanced replication in mammalian cells	H5N1	Li J. et al., 2018; Suttie A. et al., 2019
19	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NP:M105V	NP:M105V	Increased virulence in chickens	H5N1	Suttie A. et al., 2019; Tada T. et al., 2011; Tada T. et al., 2011b
20	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NP:A184K	NP:A184K	Enhanced interferon response	H5N1	Suttie A. et al., 2019; Wasilenko J. et al., 2009
21	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NP:A184K	NP:A184K	Increased replication in avian cells	H5N1	Suttie A. et al., 2019; Wasilenko J. et al., 2009
22	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	NP:A184K	NP:A184K	Increased virulence in chickens	H5N1	Suttie A. et al., 2019; Wasilenko J. et al., 2009
23	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB2:V598T	PB2:V598T	Increased polymerase activity in mammalian cells	H7N9	Hu M. et al., 2017b; Suttie A. et al., 2019
24	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB2:V598T	PB2:V598T	Increased replication in mammalian cells	H7N9	Hu M. et al., 2017b; Suttie A. et al., 2019
25	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB2:V598T	PB2:V598T	Increased virulence in mice	H7N9	Hu M. et al., 2017b; Suttie A. et al., 2019
26	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB2:K627E	PB2:K627E	Increased virulence in chickens	H5N1	Schat K. et al., 2012; Suttie A. et al., 2019
27	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB2:S715N	PB2:S715N	Decreased virulence in mice	H5N1	Sun H. et al., 2015; Suttie A. et al., 2019
28	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB2:L89V,PB2:G309D	PB2:L89V,PB2:G309D	Increased polymerase activity in mammalian cells	H5N1	Li J. et al., 2009; Suttie A. et al., 2019
29	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB2:L89V,PB2:G309D	PB2:L89V,PB2:G309D	Increased virulence in mice	H5N1	Li J. et al., 2009; Suttie A. et al., 2019
30	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB2:L89V,PB2:G309D,PB2:T339K,P	PB2:L89V,PB2:G309D,PB2:T339K,PB	Increased polymerase activity in mammalian cells	H5N1	Li J. et al., 2009; Suttie A. et al., 2019
31	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB2:L89V,PB2:G309D,PB2:T339K,P	PB2:L89V,PB2:G309D,PB2:T339K,PB	Increased virulence in mice	H5N1	Li J. et al., 2009; Suttie A. et al., 2019
32	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB1:D3V	PB1:D3V	Increased polymerase activity in avian cells	H5N1	Elgendy E. et al., 2017; Suttie A. et al., 2019
33	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB1:D3V	PB1:D3V	Increased polymerase activity in mammalian cells	H5N1	Elgendy E. et al., 2017; Suttie A. et al., 2019
34	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB1:D3V	PB1:D3V	Increased replication in avian cells	H5N1	Elgendy E. et al., 2017; Suttie A. et al., 2019
35	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB1:D3V	PB1:D3V	Increased replication in mammalian cells	H5N1	Elgendy E. et al., 2017; Suttie A. et al., 2019
36	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB1:D622G	PB1:D622G	Increased polymerase activity in mice	H5N1	Feng X. et al., 2016; Suttie A. et al., 2019
37	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB1:D622G	PB1:D622G	Increased virulence in mice	H5N1	Feng X. et al., 2016; Suttie A. et al., 2019
38	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB1-F2:N66S	PB1-F2:N66S	Enhanced antiviral response in mice	H5N1	Conenello G. et al., 2007; Schmolke M. et al., 2011; Suttie A. et al., 2019
39	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB1-F2:N66S	PB1-F2:N66S	Enhanced replication in mice	H5N1	Conenello G. et al., 2007; Schmolke M. et al., 2011; Suttie A. et al., 2019
40	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PB1-F2:N66S	PB1-F2:N66S	Enhanced virulence in mice	H5N1	Conenello G. et al., 2007; Schmolke M. et al., 2011; Suttie A. et al., 2019
41	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PA:S37A	PA:S37A	Increased polymerase activity in mammalian cells	H7N9	Suttie A. et al., 2019; Yamayoshi S. et al., 2014
42	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PA:P190S	PA:P190S	Decreased virulence in mice	H7N3	DesRochers B. et al., 2016; Suttie A. et al., 2019
43	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PA:N383D	PA:N383D	Increased polymerase activity in avian cells	H5N1	Song J. et al., 2011; Song J. et al., 2015; Suttie A. et al., 2019
44	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PA:N383D	PA:N383D	Increased polymerase activity in mammalian cells	H5N1	Song J. et al., 2011; Song J. et al., 2015; Suttie A. et al., 2019
45	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PA:Q400P	PA:Q400P	Decreased virulence in mice	H7N3	DesRochers B. et al., 2016; Suttie A. et al., 2019
46	A/chicken/Italy/21VIR10264/2021_H5N1_2021-11-28	PA:N409S	PA:N409S	Increased polymerase activity in mammalian cells	H7N9	Suttie A. et al., 2019; Yamayoshi S. et al., 2014

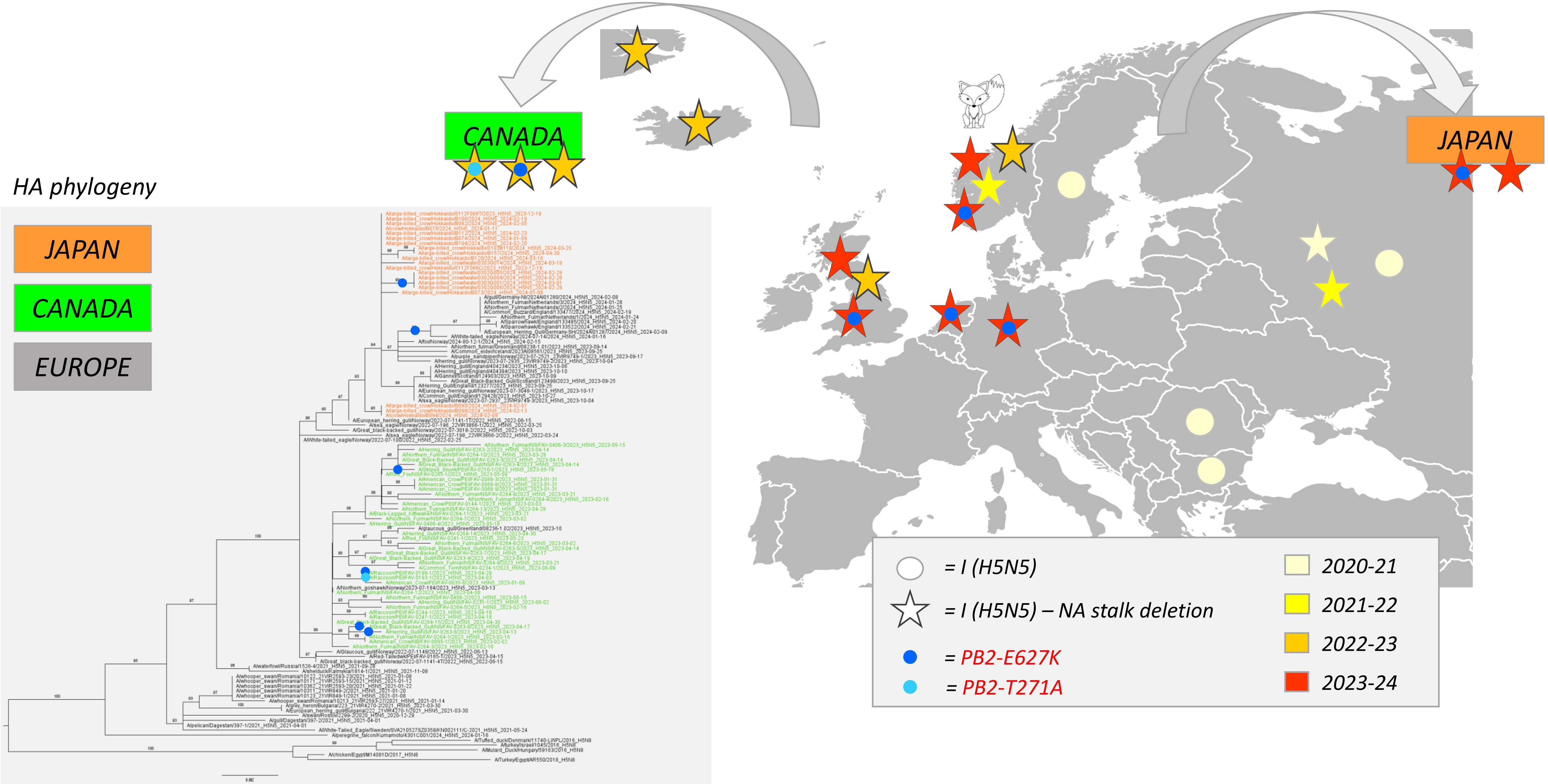
Temporal trends: Markers of adaptation in PB2 gene in avian species



☆ = *Wild birds, PB2-E627K*

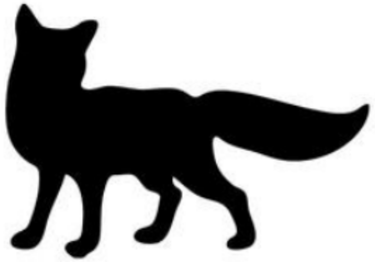
🟦 *I genotype (H5N5)*

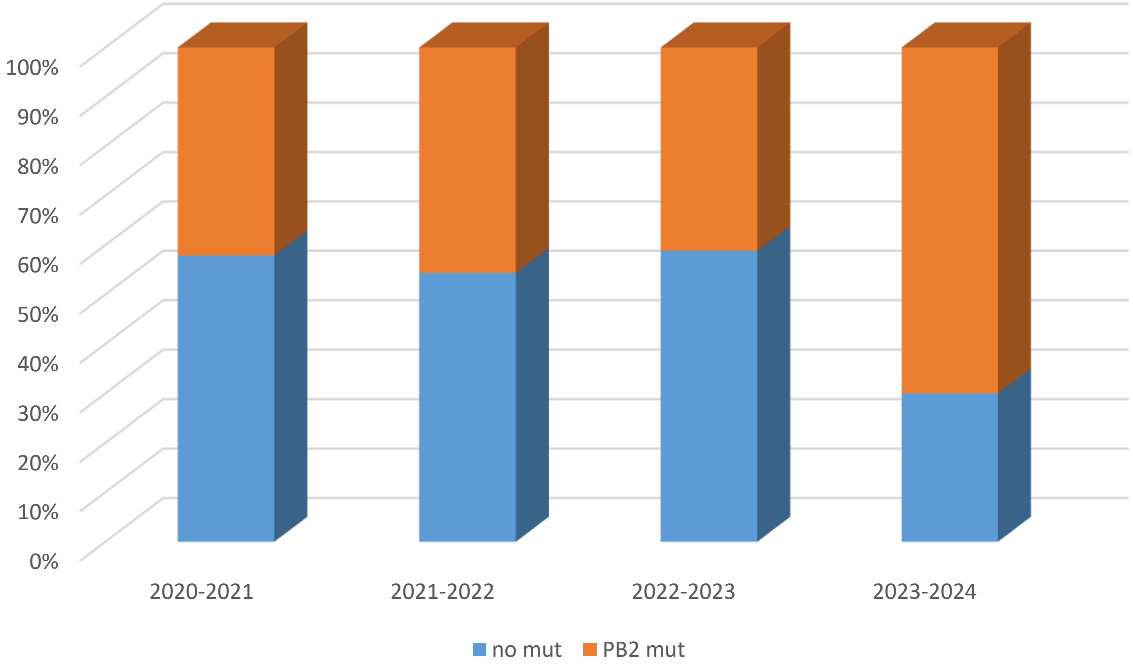
Evolution and spread of genotype EA-2021-I A(H5N5)



○	= I (H5N5)	🟨	2020-21
☆	= I (H5N5) – NA stalk deletion	🟨	2021-22
●	= PB2-E627K	🟧	2022-23
●	= PB2-T271A	🟥	2023-24

Temporal trends: Markers of adaptation in PB2 gene in mammals





Take home messages

- Genetic data are instrumental **to track the virus spread and identify novel incursions** of viruses which may represent a threat for human or animal health.
- Real time genome surveillance is essential also to promptly **identify viruses with mutations that can increase their zoonotic potential**.
- Laboratories must be equipped with or have **access to genetic sequencing platforms.**
- The **timely sharing** of genetic sequences and epidemiological data is essential for coordinating response measures and improving predictive capacity at the regional and global levels.

Guidance for genomic monitoring of AIV can be found here: https://www.izsvenezie.com/documents/reference-laboratories/avian-influenza/useful-resources/guidance-representative-genomic-avian-influenza-virus.pdf

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