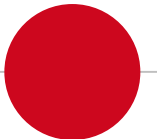


## HPAI in Europe: genetic characteristics

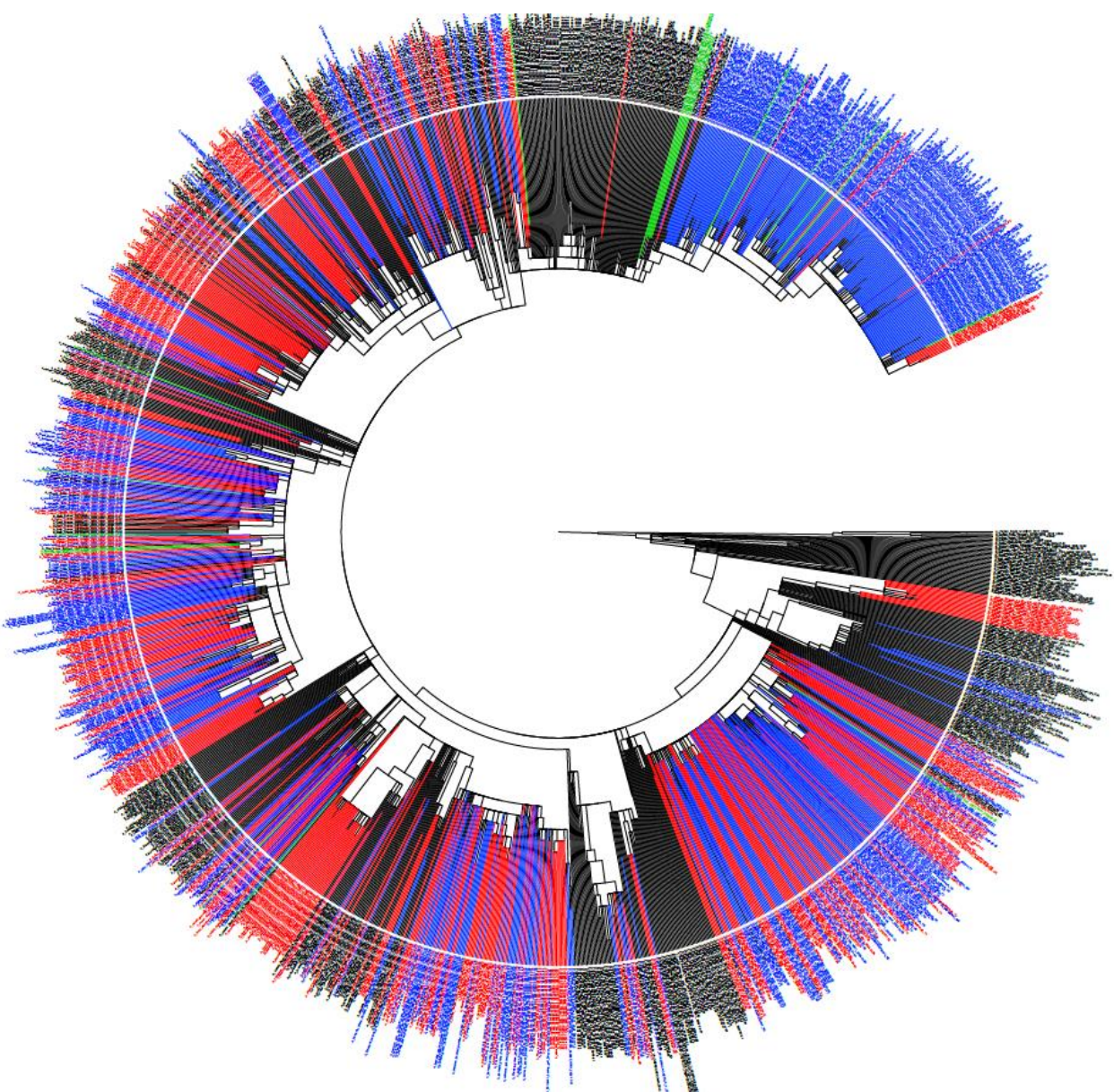
**Alice Fusaro on behalf of the EURL Team**

*Istituto Zooprofilattico Sperimentale delle Venezie (IZSve), Padua, Italy*

2 October 2023



# 2022-2023 epidemic wave in Europe



Complete genome sequences available since October 2022 → >1750 viruses from 24 European countries

## HPAI H5 viruses collected in Europe since October 2022

- HPAI H5 from wild birds
- HPAI H5 from domestic birds
- HPAI H5 from mammals



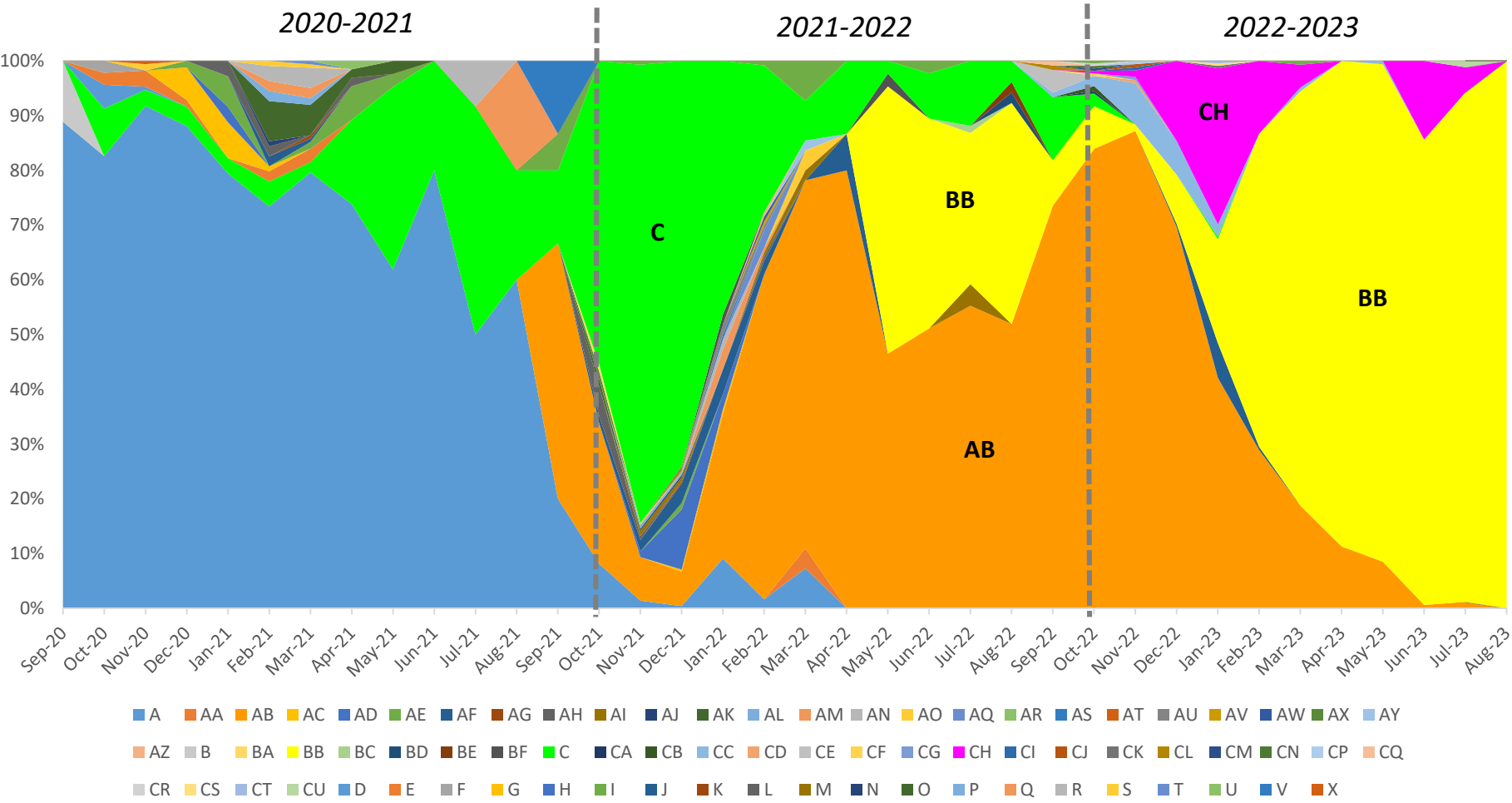
# Genetic diversity

October 2022 - August 2023

Subtype	PB2	PB1	PA	HA	NP	NA	MP	NS	Genotype	Representative European viruses	N. of viruses
H5N1									<b>BB</b>	H5N1 A/Herring_gull/France/22P015977/2022-like	<b>872</b>
									<b>AB</b>	H5N1 A/duck/Saratov/29-02/2021-like	<b>669</b>
									<b>CH</b>	H5N1 A/Eurasian_Wigeon/Netherlands/3/2022-like	<b>155</b>
									<b>CC</b>	H5N1 A/chicken/Germany-NI/AI04794/2022-like	35
									<b>AF</b>	H5N1 A/chicken/Italy/IZSLT122448_21VIR9218-1/2021-like	16
									<b>C</b>	H5N1 A/Eurasian_Wigeon/Netherlands/1/2020-like	8
									<b>CB</b>	H5N1 A/Mallard/Netherlands/19/2022-like	2
									<b>CU</b>	H5N1 A/pheasant/Italy/23VIR6483-3/2023-like	2
									<b>CA</b>	H5N1 A/Mallard/Netherlands/18/2022-like	1
									<b>CD</b>	H5N1 A/swan/Italy/22VIR10875-2/2022-like	1
									<b>CF</b>	H5N1 A/mallard/Italy/22VIR9762-1/2022-like	1
									<b>CG</b>	H5N1 A/flamingo/Italy/22VIR11494-1/2022-like	1
									<b>CI</b>	H5N1 A/Great_Cormorant/Netherlands/1/2022-like	1
									<b>CK</b>	H5N1 A/chicken/Poland/H05-N/2023-like	1
									<b>CM</b>	H5N1 A/Corvus_monedula/Belgium/11918_0006/2022-like	1
									<b>CN</b>	H5N1 A/great_egret/France/22P021741/2022-like	1
									<b>CP</b>	H5N1 A/Peregrine_falcon/Netherlands/4/2024-like	1
									<b>CR</b>	H5N1 A/pheasant/England/251536/2022-like	1
									<b>CS</b>	H5N1 A/mute_swan/Poland/MB021/2023-like	1
									<b>CT</b>	H5N1 A/Black-headed_gull/Netherlands/4/2023-like	1
H5N2									<b>CJ</b>	H5N2 A/Eurasian_Wigeon/Netherlands/6/2022-like	1
H5N5									<b>I</b>	H5N5	2

>95% of the characterized viruses belong to three major genotypes

# Temporal dynamics of the virus genotypes in Europe: 2020-2023



**H5N1-Genotype C**  
Eurasian wigeon/Netherlands-like

**H5N1-Genotype AB**  
Duck/Saratov-like

**H5N1-Genotype BB**  
Herring gull/France-like

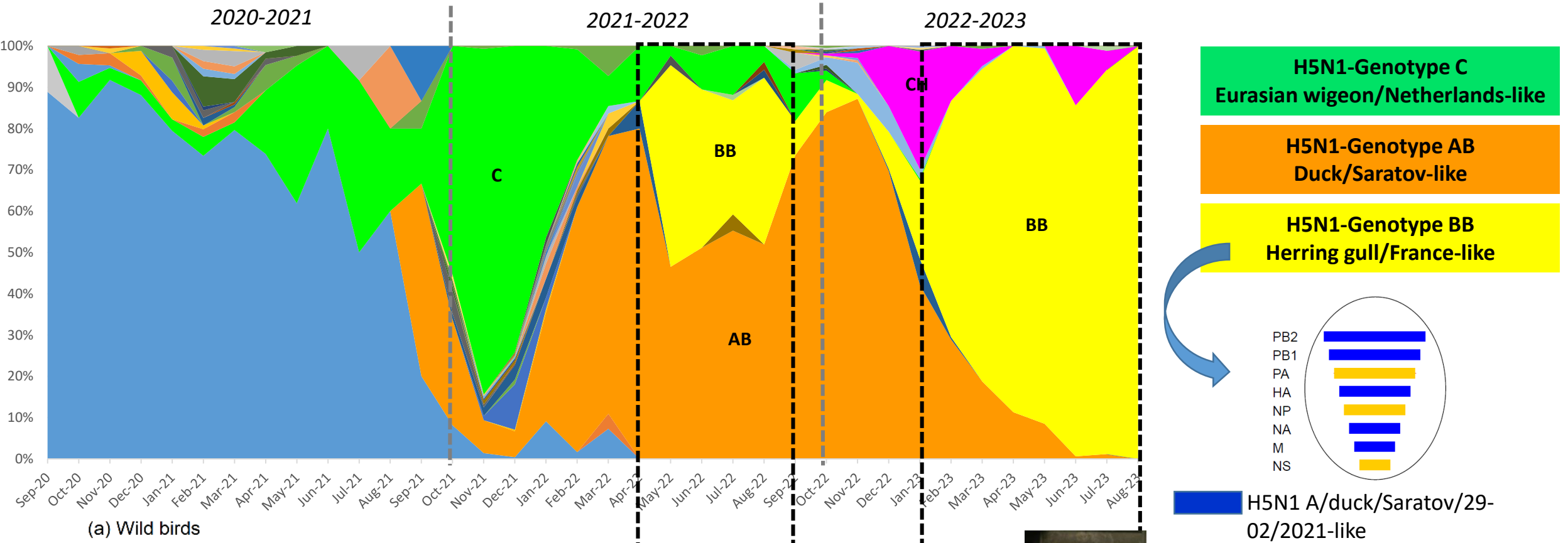
PB2  
PB1  
PA  
HA  
NP  
NA  
M  
NS

**H5N1 A/duck/Saratov/29-02/2021-like**

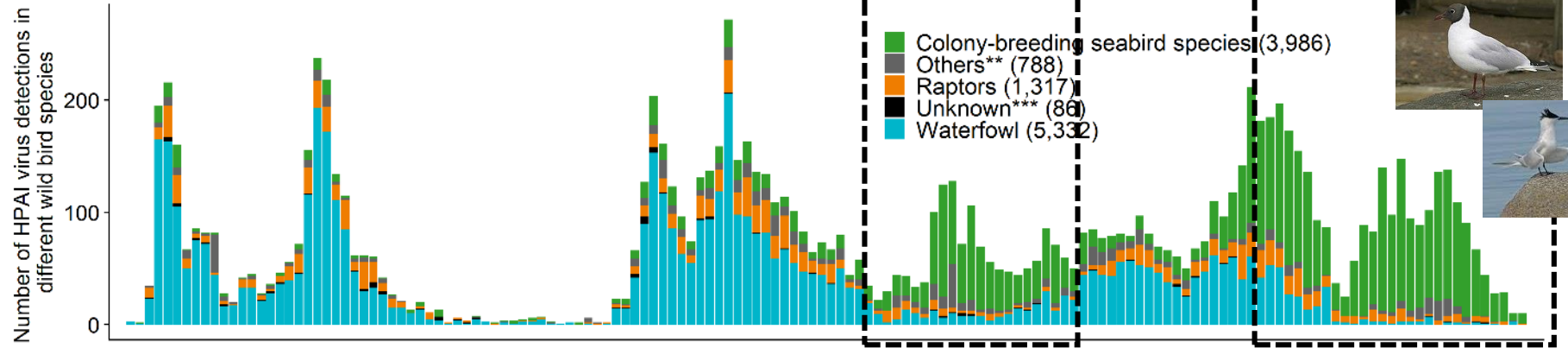
**gull-adapted H13 subtype**



# Temporal dynamics of the virus genotypes in Europe: 2020-2023



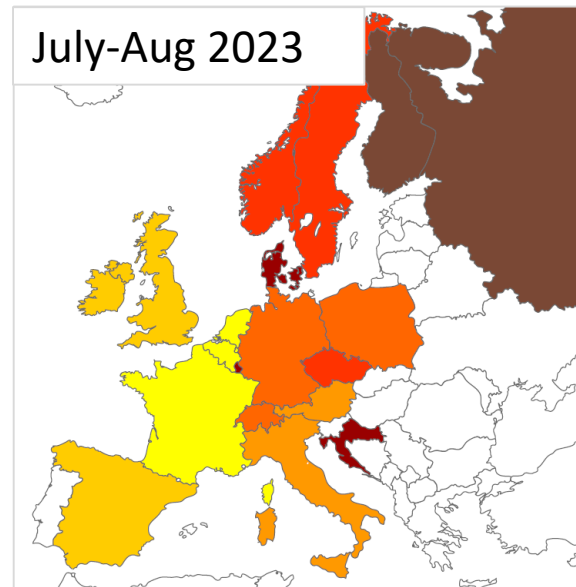
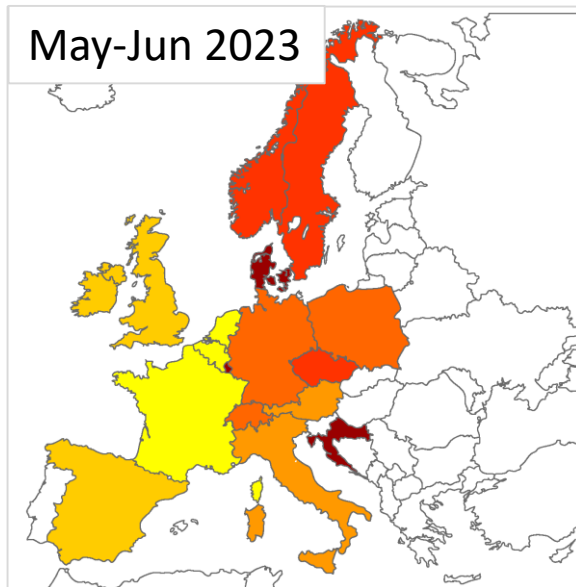
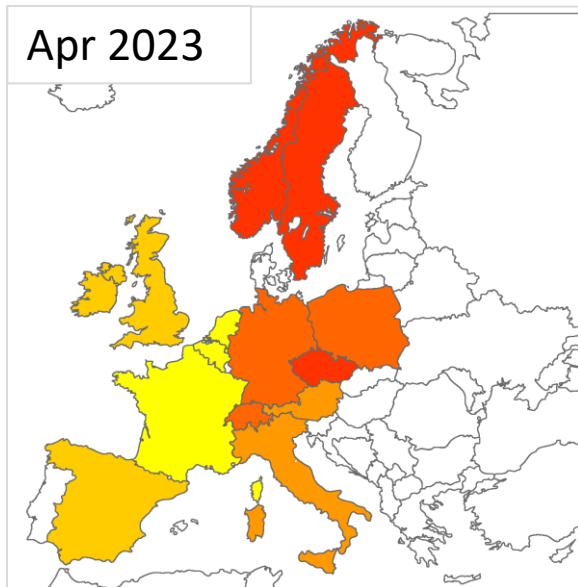
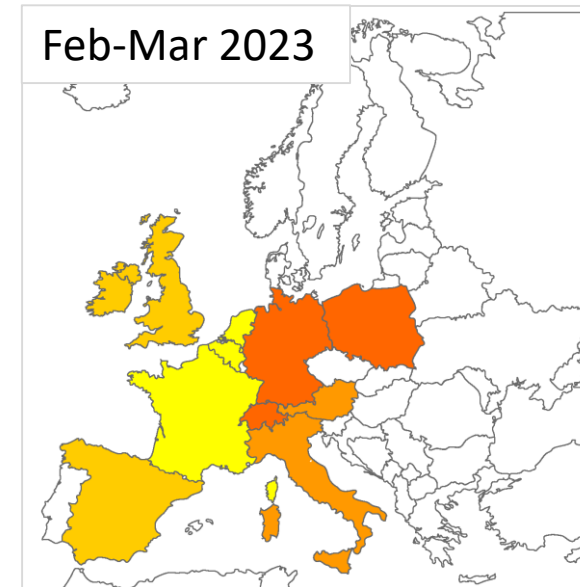
(a) Wild birds



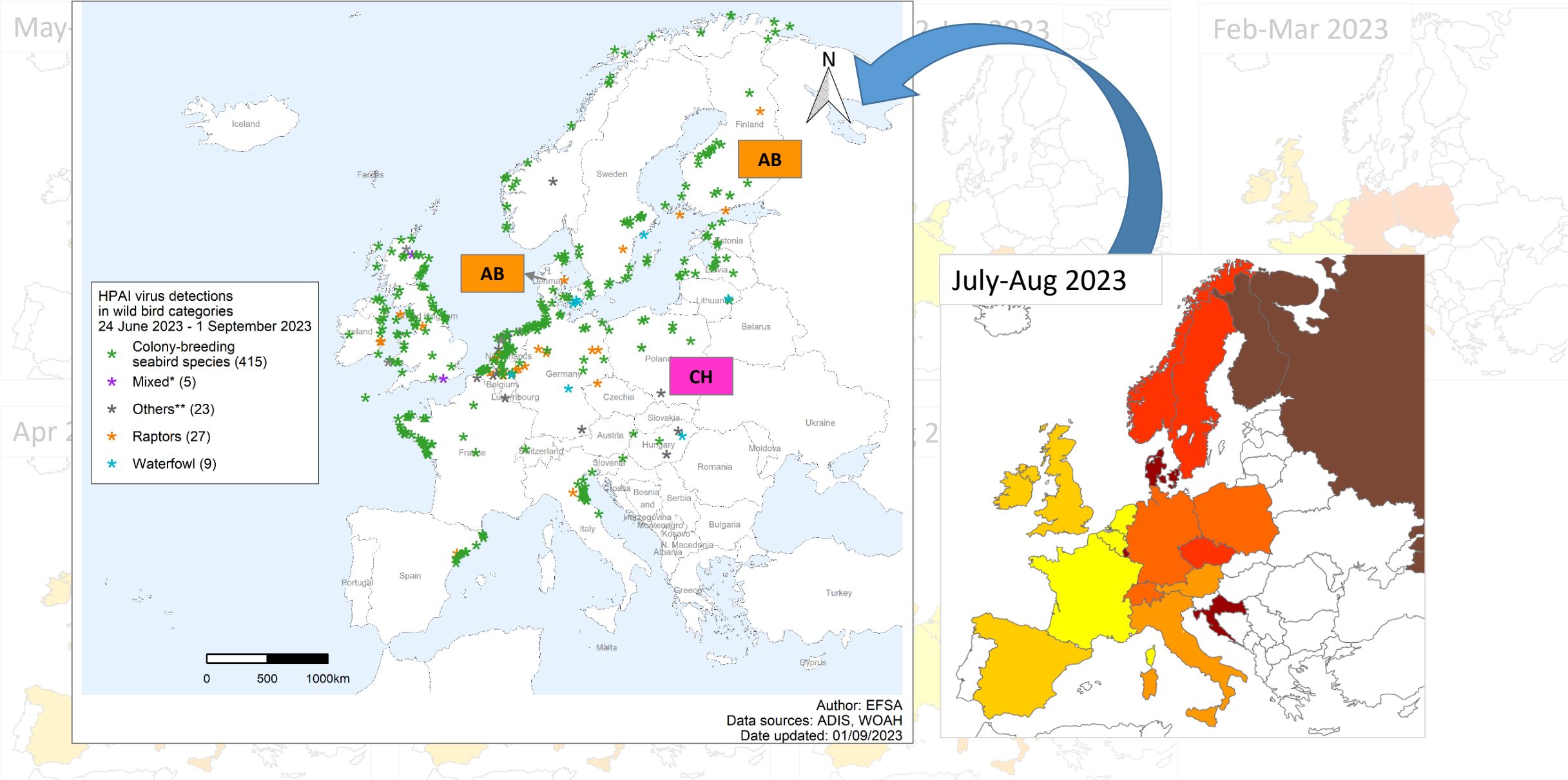
Blue: H5N1 A/duck/Saratov/29-02/2021-like  
 Yellow: gull-adapted H13 subtype

Source: EFSA, Scientific report: Avian influenza overview June-September 2023. EFSA Journal 2023

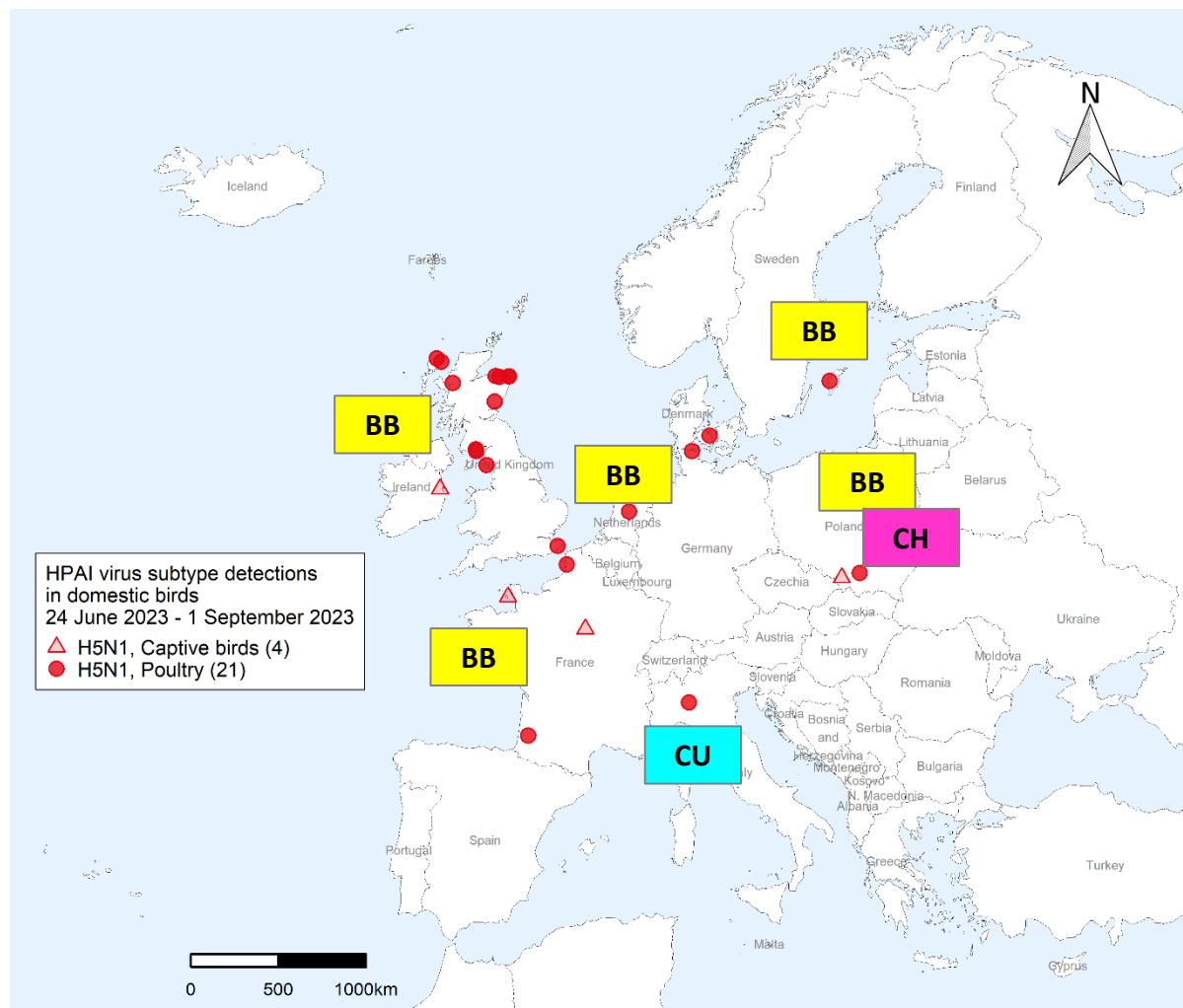
# The geographic expansion of the BB genotype



# The genotypes in wild birds in Europe, June-August 2023



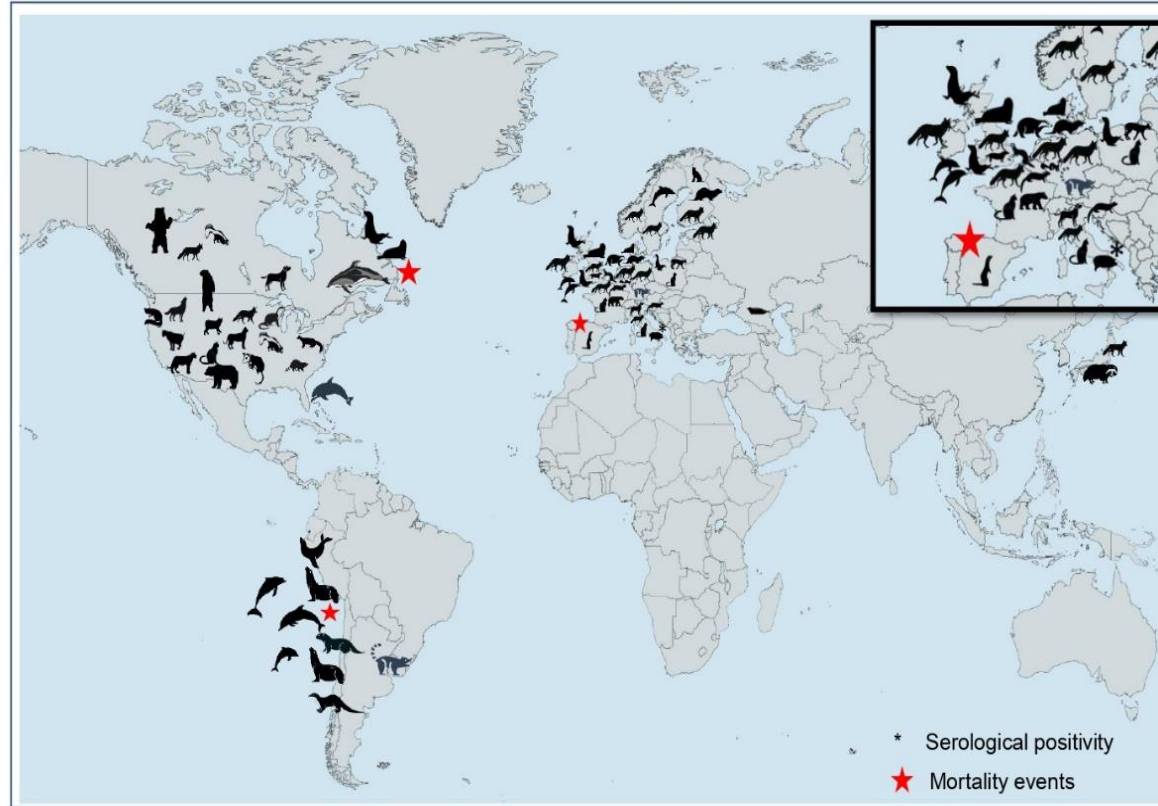
# The genotypes in domestic birds in Europe, June-August 2023



Author: EFSA  
Data sources: ADIS, WOAH  
Date updated: 01/09/2023



# HPAI H5 outbreaks in mammals



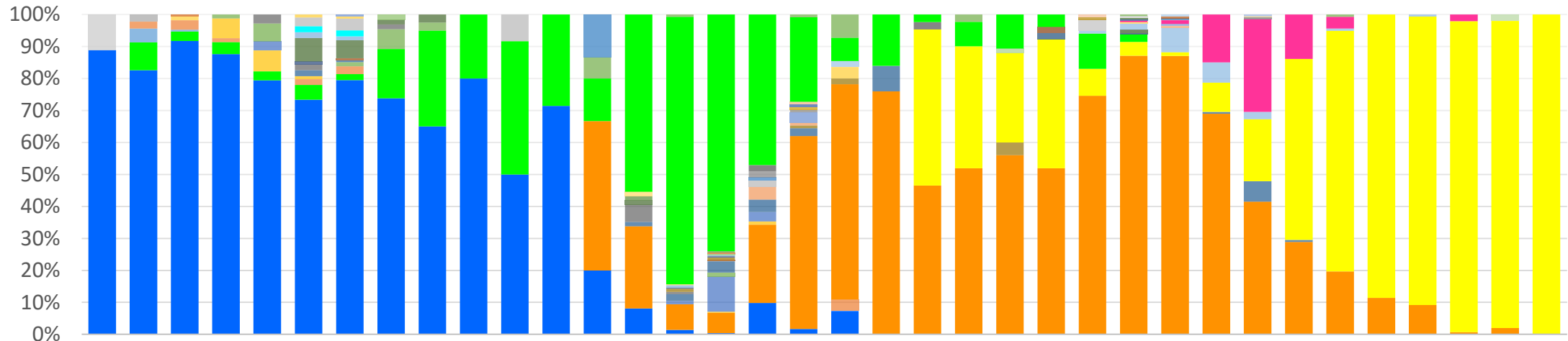
- |   |   |   |   |
|---|---|---|---|
| American black bear ( <i>Ursus americanus</i> )       | Caracal ( <i>Caracal caracal</i> )                  | Ferret ( <i>Mustela furo</i> )                          | Raccoon ( <i>Procyon lotor</i> )                          |
| American mink ( <i>Neogale vison</i> )                | Caspian seal ( <i>Pusa caspica</i> )                | Fisher cat ( <i>Pekania pennanti</i> )                  | Red fox ( <i>Vulpes vulpes</i> )                          |
| American pine marten ( <i>Martes americana</i> )      | Cat ( <i>Felis catus</i> )                          | Grey seal ( <i>Halichoerus grypus</i> )                 | Skunk ( <i>Mephitis mephitis</i> )                        |
| Amur leopard ( <i>Panthera pardus orientalis</i> )    | Chilean dolphin ( <i>Cephalorhynchus eutropia</i> ) | Harbour porpoise ( <i>Phocoena phocoena</i> )           | South American coati ( <i>Nasua nasua</i> )               |
| Amur tiger ( <i>Panthera tigris</i> )                 | Common dolphin ( <i>Delphinus delphis</i> )         | Harbour seal ( <i>Phoca vitulina</i> )                  | South America fur seal ( <i>Arctocephalus australis</i> ) |
| Asiatic black bear ( <i>Ursus thibetanus</i> )        | Coyote ( <i>Canis latrans</i> )                     | Japanese raccoon dog ( <i>Nyctereutes viverrinus</i> )  | South American bush dog ( <i>Speothos venaticus</i> )     |
| Beech marten ( <i>Martes foina</i> )                  | Dog ( <i>Canis lupus familiaris</i> )               | Kodiak grizzly bear ( <i>Ursus arctos horribilis</i> )  | South American sea lion ( <i>Otaria flavescens</i> )      |
| Bobcat ( <i>Lynx rufus</i> )                          | Eurasian badger ( <i>Meles meles</i> )              | Marine otter ( <i>Lontra felina</i> )                   | Southern river otter ( <i>Lontra provocax</i> )           |
| Bottlenose dolphin ( <i>Tursiops truncatus</i> )      | Eurasian lynx ( <i>Lynx lynx</i> )                  | Mountain lion ( <i>Puma concolor</i> )                  | Virginia opossum ( <i>Didelphis virginiana</i> )          |
| Brown bear ( <i>Ursus arctos</i> )                    | Eurasian otter ( <i>Lutra lutra</i> )               | North American river otter ( <i>Lontra canadensis</i> ) | White-sided dolphin ( <i>Lagenorhynchus acutus</i> )      |
| Burmeister's porpoise ( <i>Phocoena spinipinnis</i> ) | European polecat ( <i>Mustela putorius</i> )        | Pig ( <i>Sus scrofa</i> )                               |   |

Source: EFSA, Scientific report: Avian influenza overview April-June 2023. EFSA Journal 2023

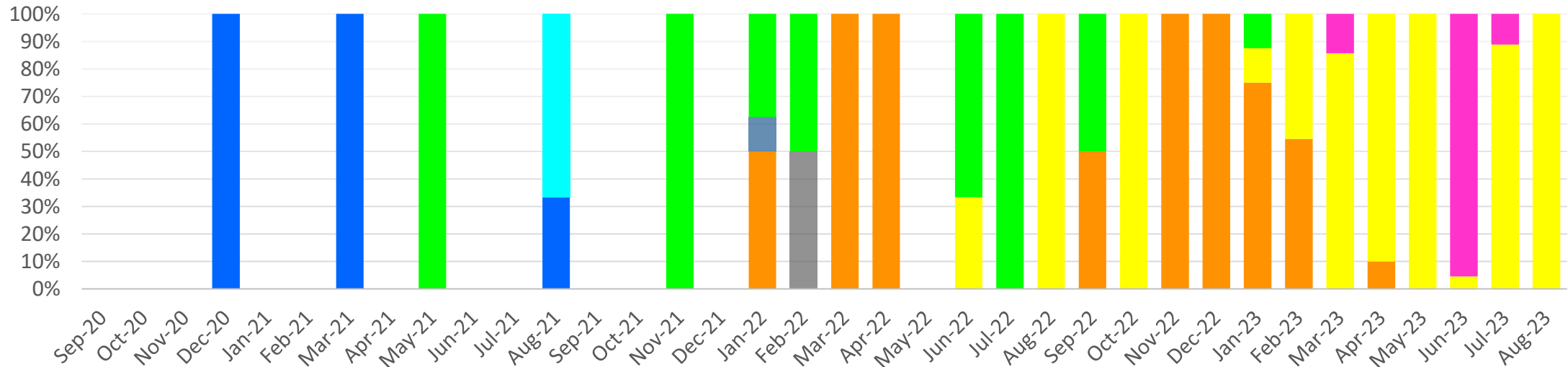


# Which genotypes infected mammalian species in Europe?

AVIAN

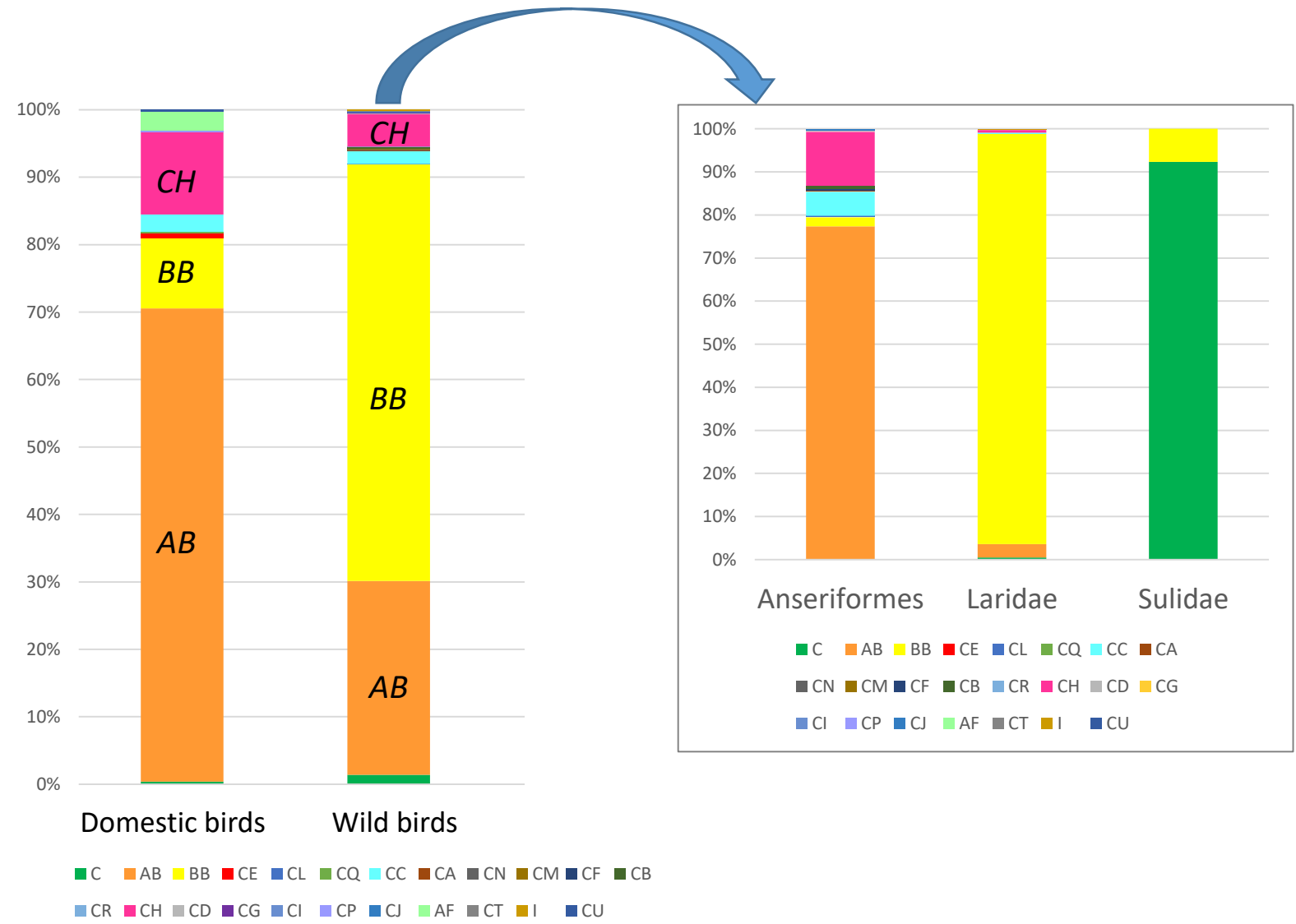


MAMMALS

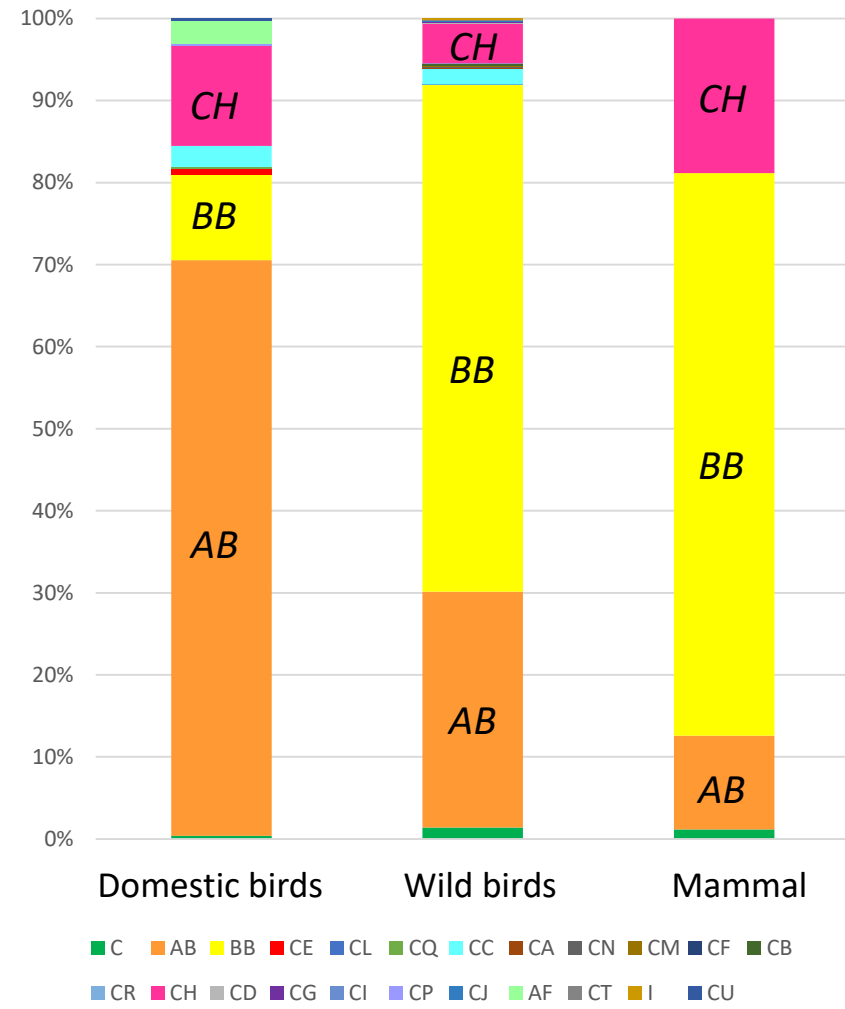


- A
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- AF
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- AK
- AL
- AM
- AN
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- AP
- AQ
- AR
- AS
- AT
- AU
- AV
- AW
- AX
- AY
- AZ
- B
- BA
- BB
- BC
- BD
- BE
- BF
- C
- CA
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- CC
- CD
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- I
- J
- K
- L
- M
- N
- O
- P
- Q
- R
- S
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- V
- X

# ● Genotype distribution among different host categories in Europe, 2022-2023



# ● Genotype distribution among different host categories in Europe, 2022-2023



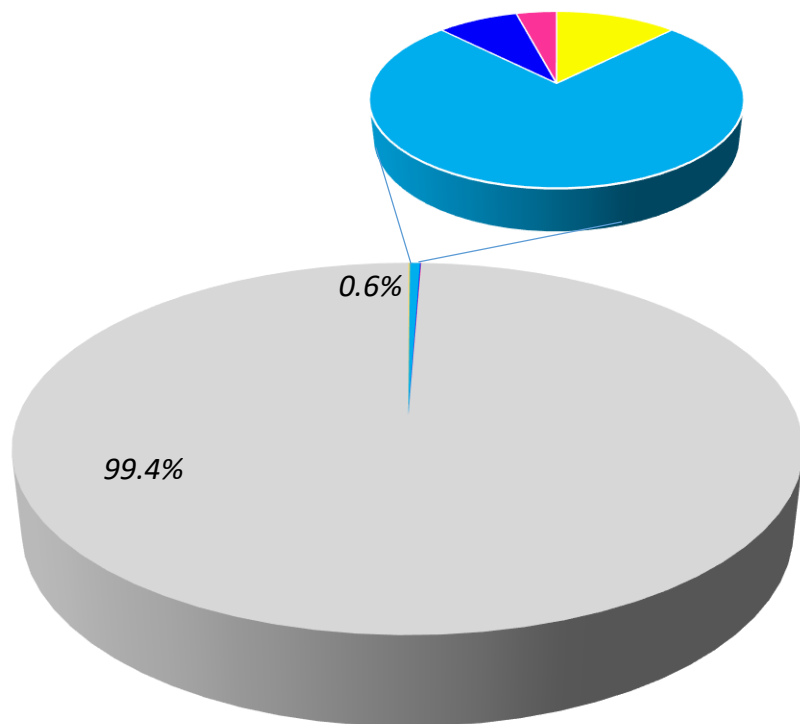
# Molecular markers of virus adaptation to mammals in Europe



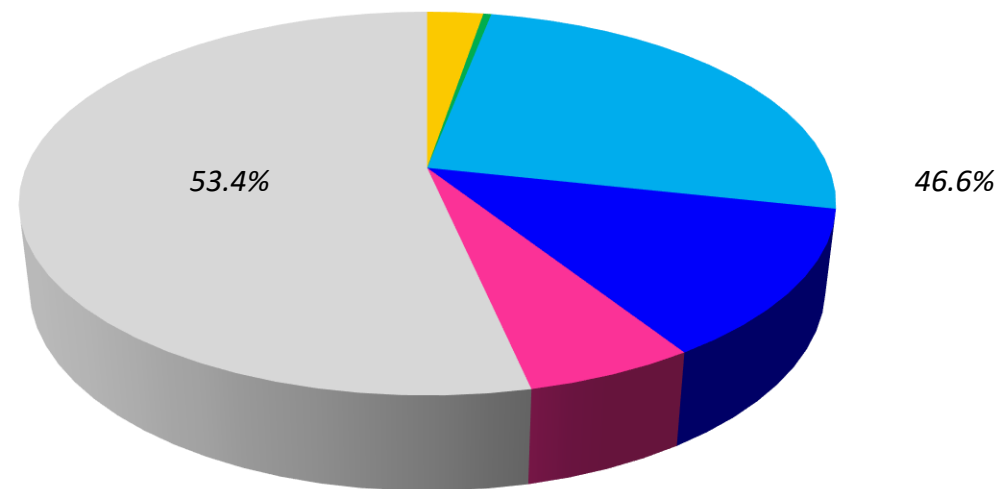
↑ *Increased zoonotic potential*



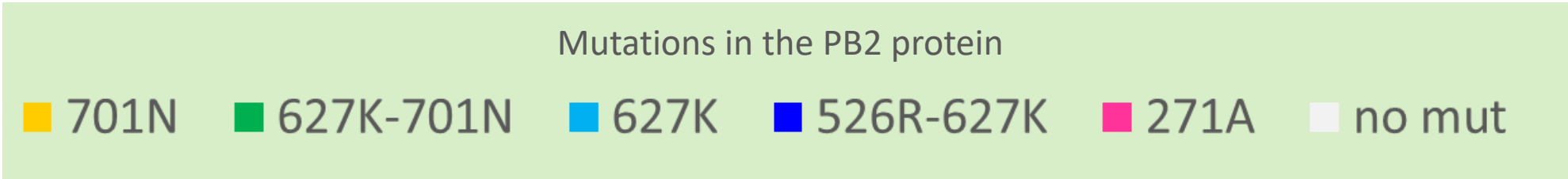
# Molecular markers of virus adaptation to mammals in Europe



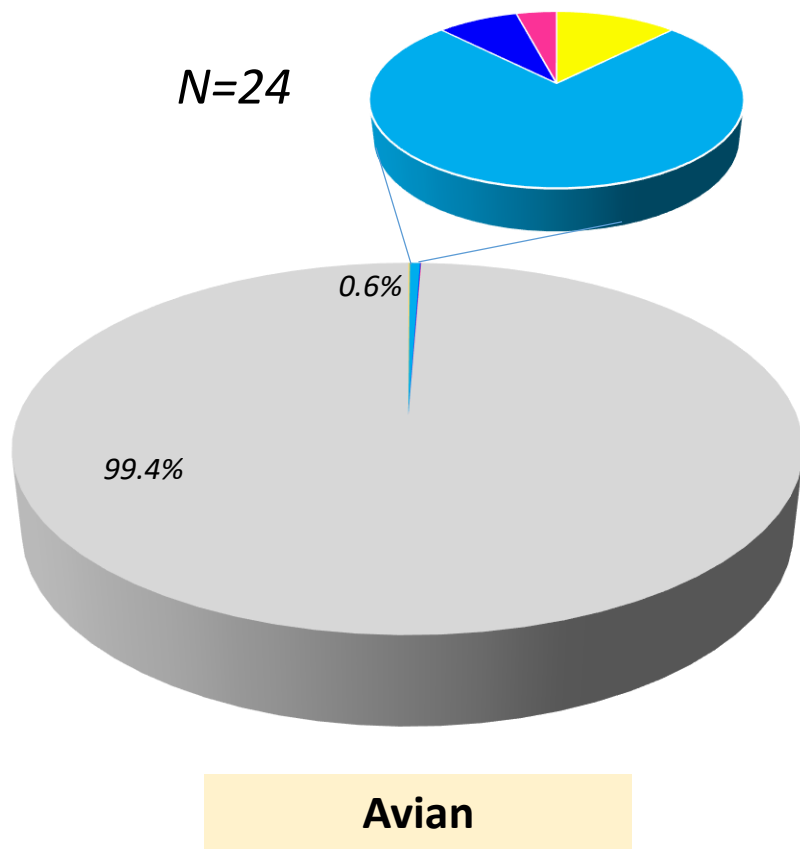
Avian



Mammals



# Molecular markers of virus adaptation to mammals in Europe



● 50% (N=12 H5N8 viruses) from a local transmission among poultry farms in Germany, March 2021. [PB2-627K]



● N=2 in H5 viruses identified in rheas in zoos in Germany (2021) and Spain (2022). [PB2-627K]



● N=4 in H5 viruses possibly linked to mammalian infections:

PB2-271A, N=1, Genotype BB, Italy 2023



PB2-701N, N=1, Genotype AB, Belgium 2022



PB2-627K, N=1, Genotype CH, Poland 2023



PB2-526R-627K, N=1, Genotype CH, Poland 2023

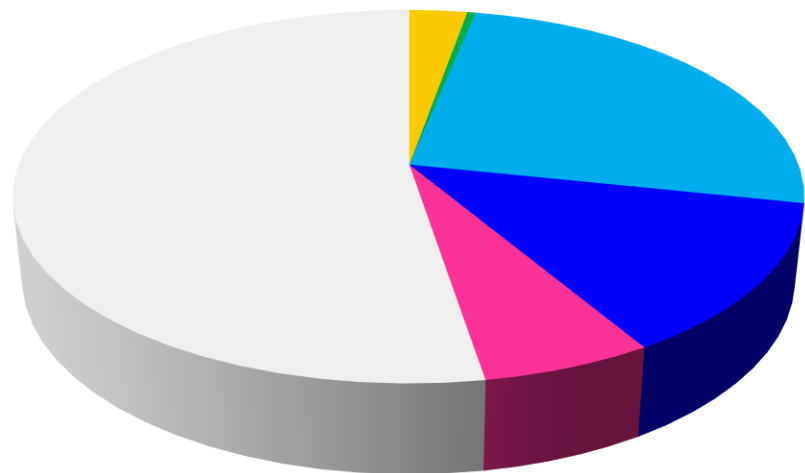


Mutations in the PB2 protein

■ 701N   
 ■ 627K-701N   
 ■ 627K   
 ■ 526R-627K   
 ■ 271A   
 ■ no mut

# Molecular markers of virus adaptation to mammals in Europe

## Mammals



149



13

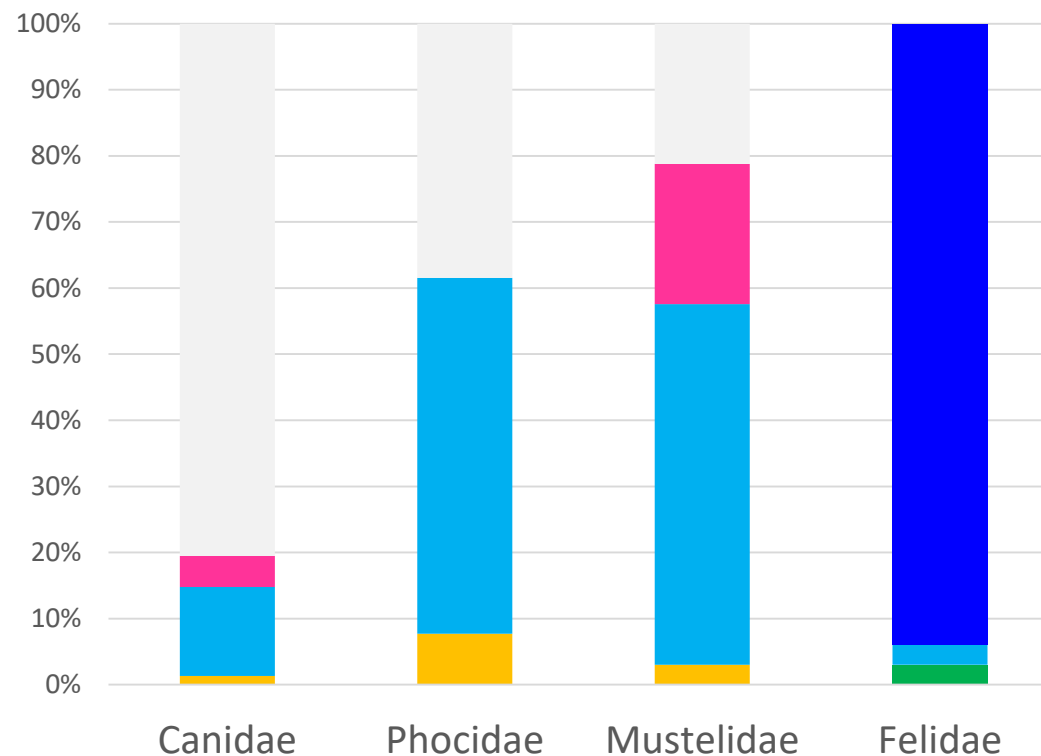


33



33

N. sequences



Mutations in the PB2 protein

■ 701N   
 ■ 627K-701N   
 ■ 627K   
 ■ 526R-627K   
 ■ 271A   
 ■ no mut

# Highly pathogenic avian influenza A (H5N1) in marine mammals and seabirds in Peru

Received: 8 March 2023

Mariana Leguía <sup>1,2</sup> ✉, Alejandra Garcia-Glaessner <sup>1,2</sup>, Breno Muñoz-Saavedra<sup>1,2</sup>, Diana Juárez<sup>1,2</sup>, Patricia Barrera <sup>1,2</sup>, Carlos Calvo-Mac <sup>2</sup>, Javier Jara<sup>3</sup>, Walter Silva<sup>3</sup>, Karl Ploog<sup>3</sup>, Lady Amaro <sup>3</sup>, Paulo Colchao-Claux <sup>4</sup>, Christine K. Johnson <sup>2,5</sup>, Marcela M. Uhart <sup>2,5</sup>, Martha I. Nelson <sup>6</sup> & Jesus Lescano <sup>3</sup>

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Journal of Comparative Pathology

journal homepage: [www.elsevier.com/locate/jcpa](http://www.elsevier.com/locate/jcpa)

Infectious disease

## Naturally occurring highly pathogenic avian influenza virus H5N1 clade 2.3.4.4b infection in three domestic cats in North America during 2023

Sarah J. Sillman<sup>\*</sup>, Mary Drozd, Duan Loy, Seth P. Harris

Journal of Infection  
Volume 87, Issue 4, October 2023, Pages e70–e72



Letter to the Editor

## Emergence of a novel reassortant H5N6 subtype highly pathogenic avian influenza virus in farmed dogs in China

Xin-Yan Yao, Chun-Yang Lian, Zhi-Hang Lv, Xue-Lian Zhang, Jian-Wei Shao

On This Page

## Cross-species transmission and PB2 mammalian adaptations of highly pathogenic avian influenza A/H5N1 viruses in Chile

Catalina Pardo-Roa<sup>#1,2</sup>, Martha I. Nelson<sup>#3</sup>, Naomi Ariyama<sup>#4</sup>, Carolina Aguayo<sup>5</sup>, Leonardo I.Almonacid<sup>6,7</sup>, Gabriela Muñoz<sup>5</sup>, Carlos Navarro<sup>8</sup>, Claudia Avila<sup>5</sup>, Mauricio Ulloa<sup>8,9</sup>, RodolfoReyes<sup>9</sup>, Eugenia Fuentes Luppichini<sup>2</sup>, Christian MathCarmen Gloria González<sup>5</sup>, Hugo Araya<sup>5</sup>, Jorge FJohow<sup>\*5</sup>, Rafael A. Medina<sup>\*2,11,12</sup>, Victor Neira<sup>\*4</sup>.

## Highly Pathogenic Avian Influenza A(H5N1) Virus Outbreak in New England Seals, United States

Wendy Puryear<sup>1</sup>, Kaitlin Sawatzki<sup>1</sup>, Nichola Hill, Alexa Foss, Jonathon J. Stone, Lynda Doughty, Dominique Walk, Katie Gilbert, Maureen Murray, Elena Cox, Priya Patel, Zak Mertz, Stephanie Ellis, Jennifer Taylor, Deborah Fauquier, Ainsley Smith, Robert A. DiGiovanni Jr., Adriana van de Guchte, Ana Silvia Gonzalez-Reiche, Zain Khalil, Harm van Bakel, Mia K. Torchetti, Kristina Lantz, Julianna B. Lenoch, Jonathan Runstadler



Journal of Comparative Pathology

journal homepage: [www.elsevier.com/locate/jcpa](http://www.elsevier.com/locate/jcpa)

Infectious disease

## Naturally occurring highly pathogenic avian influenza virus H5N1 clade 2.3.4.4b infection in three domestic cats in North America during 2023

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Emerging Microbes &amp; Infections



Emerging Microbes &amp; Infections

ISSN: (Print) (Online) Journal homepage: <https://www.tandfonline.com/loi/temi20>

## Characterization of neurotropic HPAI H5N1 viruses with novel genome constellations and mammalian adaptive mutations in free-living mesocarnivores in Canada

Tamiru N. Alkie, Sherri Cox, Carissa Embury-Hyatt, Brian Stevens, Neil P. Margo J. Pybus, Wanhong Xu, Tamiko Hisanaga, Matthew Suderman, Jan Koziuk, Peter Kruczkiewicz, Hoang Hai Nguyen, Mathew Fisher, Oliver L. Cassidy N. G. Erdelyan, Ori Hochman, Davor Ojkic, Carmencita Yason, Maria Bravo-Araya, Laura Bourque, Trent K. Bollinger, Catherine Soos, Jolene Giacinti, Jennifer Provencher, Sarah Ogilvie, Amanda Clark, Roby MacPhee, Glen J. Parsons, Hazel Eaglesome, Sayrah Gilbert, Kelsey Sabo, Richard Davis, Alexandra Jerao, Matthew Ginn, Megan E.B. Jones & Yohannes Berhane

## EMERGING INFECTIOUS DISEASES®

EID Journal &gt; Volume 29 &gt; Early Release &gt; Main Article

Disclaimer: Early release articles are not considered as final versions. Any changes will be reflected in the online version in the month the

Volume 29, Number 10—October 2023

Dispatch

## Influenza A(H5N1) Virus Infections in 2 Free-Ranging Black Bears (*Ursus americanus*), Quebec, Canada

Benjamin T. Jakobek, Yohannes Berhane, Marie-Soleil Nadeau, Carissa Embury-Hyatt, Oliver Lung, Wanhong Xu, and Stéphane Laird



Journal of Infection

Volume 87, Issue 4, October 2023, Pages e70-e72



Letter to the Editor

## Emergence of a novel reassortant H5N6 subtype highly pathogenic avian influenza virus in farmed dogs in China

Xin-Yan Yao, Chun-Yang Lian, Zhi-Hang Lv, Xue-Lian Zhang, Jie

Genetic analysis revealed that the HA proteolytic cleavage site of GX30/H5N6 possessed six basic amino acids motif (RERRRKR/GLF), indicating potential for increased pathogenicity.<sup>9</sup> Notably, the Q226L substitution, a human-like biomarker on the receptor-binding site (RBS), was observed in GX30/H5N6, suggesting a binding preference for human-like receptors (sialic acid alpha2,6-galactose). Additionally, six amino acid changes (137A, 158N, 160A, 192I, 222Q, and 227R), which could enhance binding to human-like receptors, were also observed within the RBS of the HA protein ([Table S1](#)). Like most



# Epidemiologic, Clinical, and Genetic Characteristics of Human Infections with Influenza A(H5N6) Viruses, China

Wenfei Zhu,<sup>1</sup> Xiyan Li,<sup>1</sup> Jie Dong,<sup>1</sup> Hong Bo, Jia Liu, Jiaying Yang, Ye Zhang, Hejiang Wei, Weijuan Huang, Xiang Zhao, Tao Chen, Jing Yang, Zi Li, Xiaoxu Zeng, Chao Li, Jing Tang, Li Xin, Rongbao Gao, Liqi Liu, Min Tan, Yuelong Shu, Lei Yang, Dayan Wang

**Table 4.** Mammalian adaptation–related molecular markers of the human and nonhuman A(H5N6) viruses, China

Protein	Biologic effect	Mutations	Amino acids	Human viruses	Nonhuman viruses
HA*	Altered receptor specificity	T192I	T	22	1,238
			A	2	24
			I	18	36
			K	0	2
	Altered receptor specificity	Q226L	Q	39	1,302
			L	2	0
			Q/R	1	0
	Altered receptor specificity	S227N/R	S	6	136
			G	3	31
			H	1	2
			H/R	2	0
			Q	0	92
			R	30	1,036
Altered receptor specificity	G228S	C	0	4	
		G	42	1,302	

## Take home messages

- Most of the currently circulating viruses in birds in Europe belong to the BB genotype. However, we cannot exclude bias due to the fact that the highest number of H5N1 viruses collected and genetically characterized are from *Laridae*.
- To date, no key mutations associated to the switch in the virus binding preference from avian to human-type receptors have been identified in the H5 collected in Europe. However, matter of concern is the detection of a mutation that could change the receptor binding preference from avian to human in a H5N6 virus of clade 2.3.4.4b collected in farmed dogs in China.
- Molecular markers of mammalian adaptation in the PB2 protein can be rapidly acquired by the virus during infection in mammalian species. Viruses containing such mutations may have a greater zoonotic potential.
- Surveillance and real-time genetic characterization is highly recommended to promptly identify viruses with mutations that can increase their zoonotic potential.
- Asymptomatic H5 infections in mammalian species, including pets, has been demonstrated (Moreno et al., 2023; Chestakova et al. 2023). This raises concerns over the possibility of subclinical infections with emerging viruses with increased zoonotic potential in animals in close contact with humans.



# Acknowledgements

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## IZSVE - EURL

Isabella Monne, Bianca Zecchin, Ambra Pastori, Edoardo Giussani, Alessia Schivo, Annalisa Salviato, Silvia Ormelli, Maria Varotto, Francesco Bonfante, Calogero Terregino

**The authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu™ Database**

Bianca Zecchin



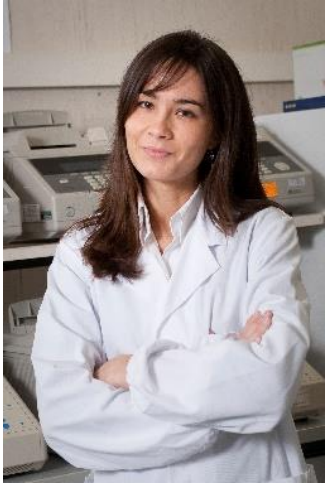
Ambra Pastori



Alessia Schivo



Isabella Monne



Annalisa Salviato



*Thank you  
for your attention*



Alice Fusaro



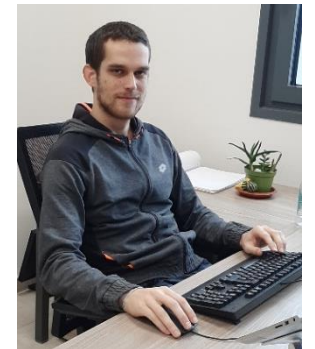
Silvia Ormelli



Maria Varotto



Elisa Palumbo



Edoardo Giussani

# Are mutations associated to resistance toward antiviral drugs increasing?

Mutations associated with antiviral resistance have been occasionally identified in the circulating strains. Specifically, about 3% of the characterised viruses contain mutations associated with reduced inhibition by neuraminidase inhibitors (NAI) and 1.7% possess mutations which can cause increased resistance to amantadine and rimantadine.

