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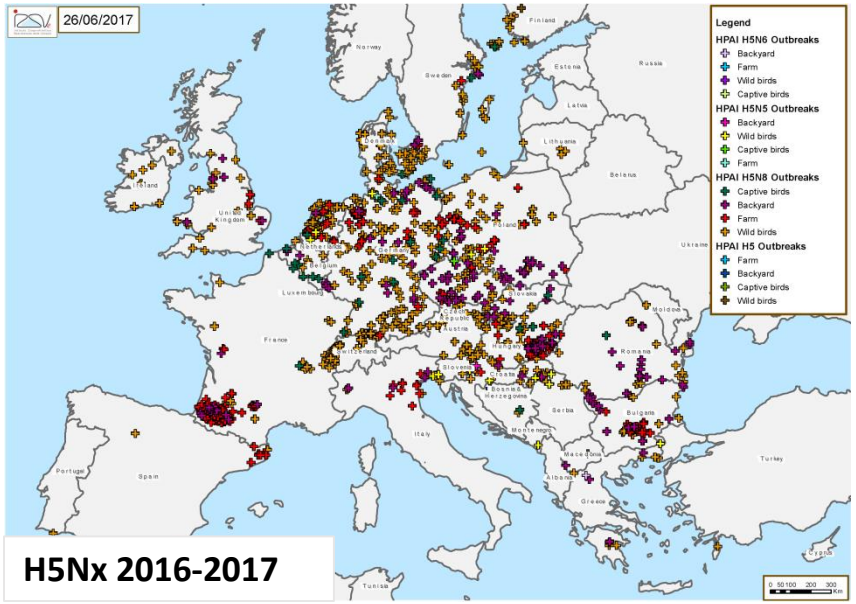
Update on the genetic characteristics of HPAI H5NX and analyses of the situation of HPAI in wild birds in Europe in 2020-2021

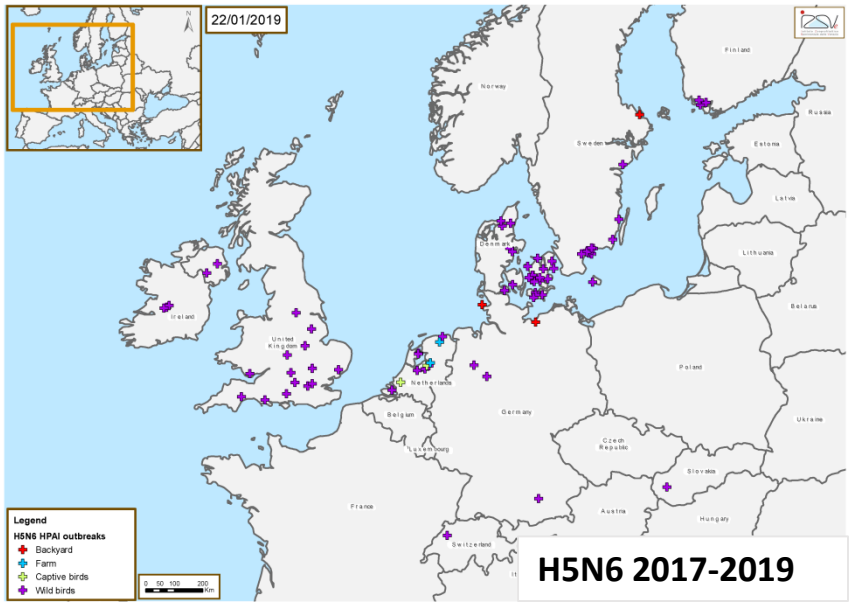
Alice Fusaro and Calogero Terregino – AI-ND EURL

PAFF meeting, 17 June 2021

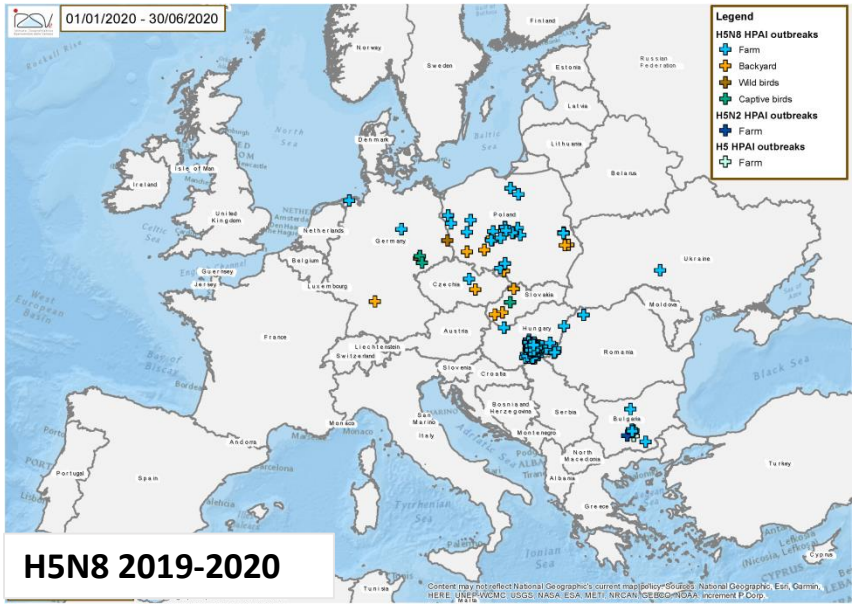
Multiple introductions of clade 2.3.4.4b in Europe



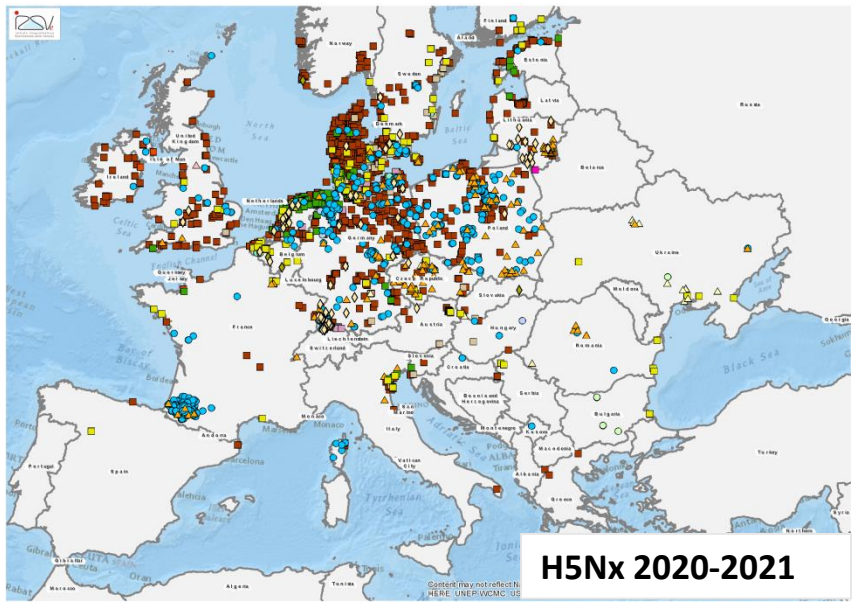
H5Nx 2016-2017



H5N6 2017-2019



H5N8 2019-2020



H5Nx 2020-2021

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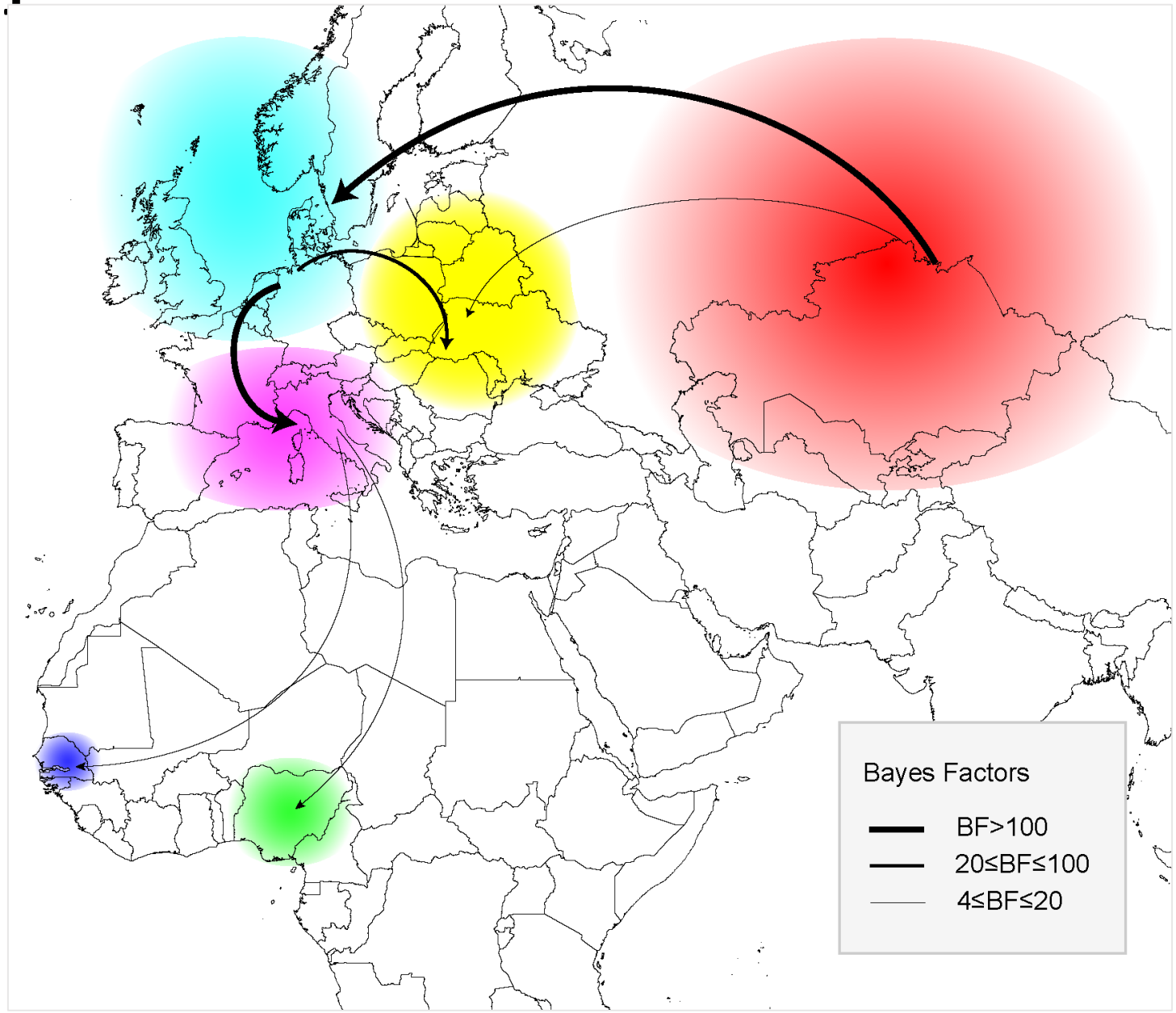
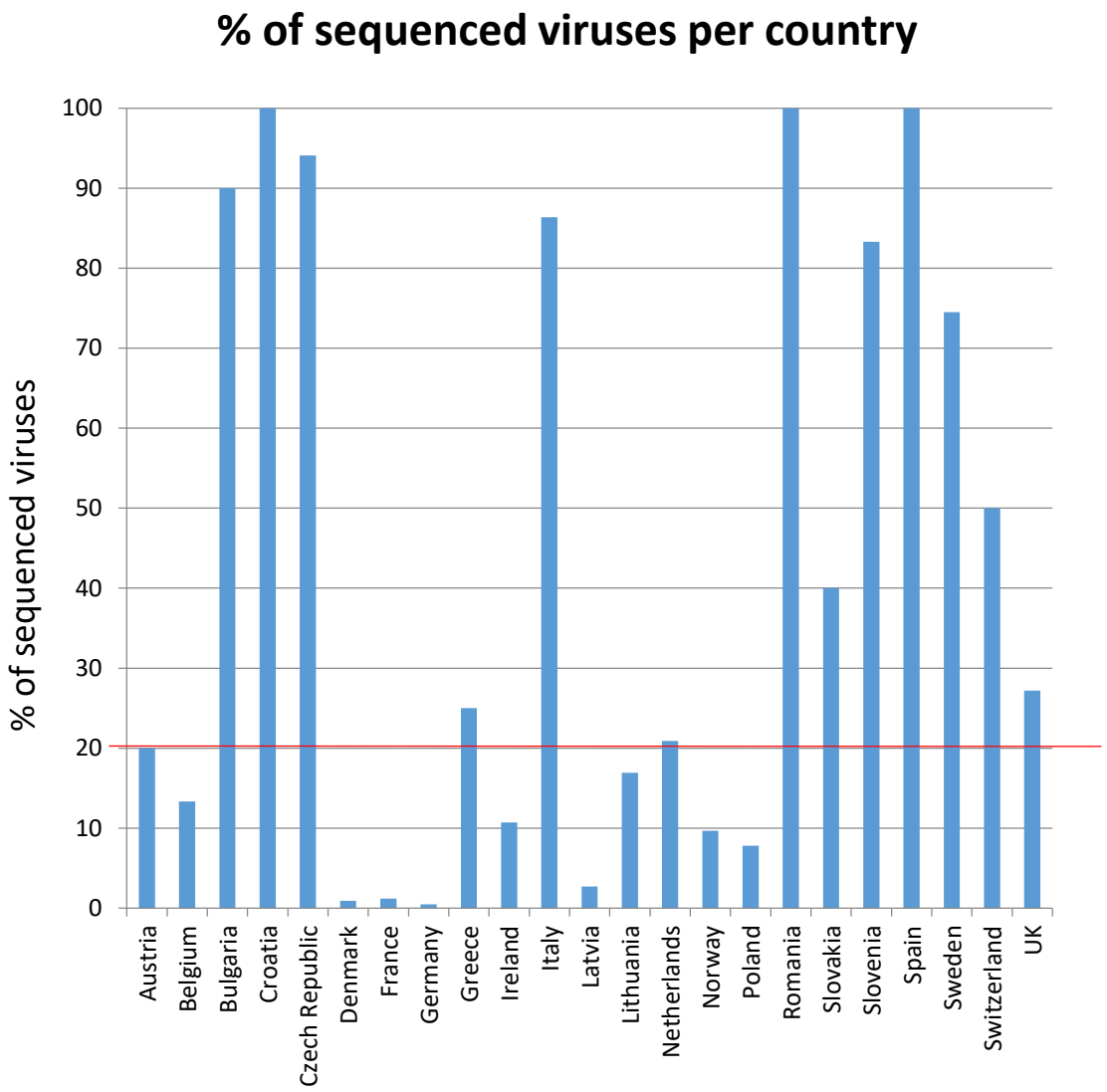
2

Origin and spatial spread of the HPAI H5NX, 2020-2021



Sequencing data

Country	N. sequenced viruses	N. of cases until 04/06/2021	% of sequenced viruses
Austria	6	30	20,0
Belgium	4	30	13,3
Bulgaria	9	10	90,0
Croatia	4	4	100,0
Czech Republic	64	68	94,1
Denmark	3	330	0,9
France	6	513	1,2
Germany	7	1546	0,5
Greece	1	4	25,0
Ireland	3	28	10,7
Italy	19	22	86,4
Latvia	1	37	2,7
Lithuania	11	65	16,9
Netherlands	23	110	20,9
Norway	3	31	9,7
Poland	35	448	7,8
Romania	19	19	100,0
Slovakia	4	10	40,0
Slovenia	5	6	83,3
Spain	4	4	100,0
Sweden	79	106	74,5
Switzerland	1	2	50,0
UK	31	114	27,2
TOT	**342**	**3537**	**10**



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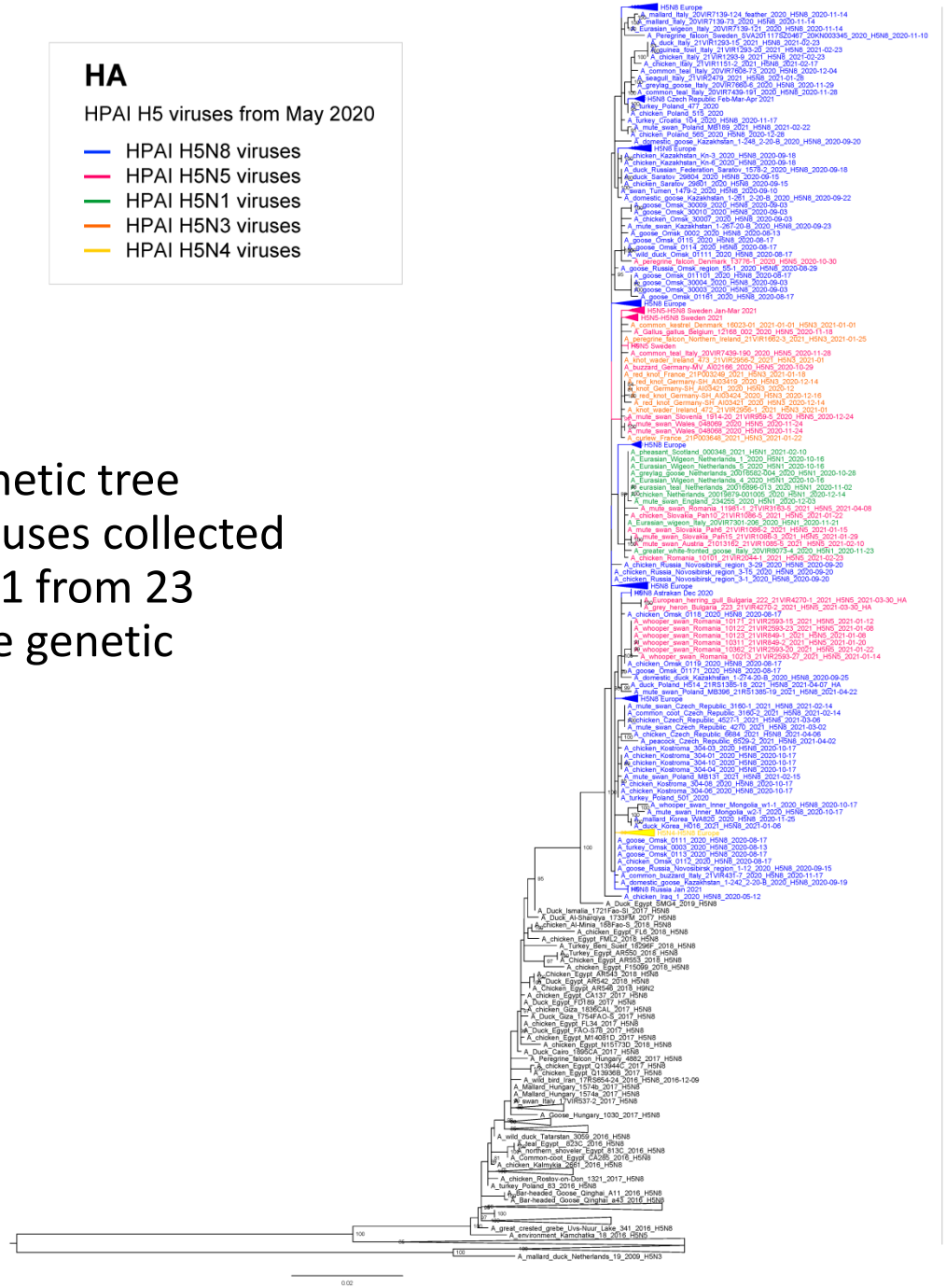

The topology of the HA phylogenetic tree shows that all the HPAI A(H5) viruses collected between July 2020 and May 2021 from 23 European countries form a single genetic group within clade **2.3.4.4b**

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17 genotypes generated from multiple reassortment events

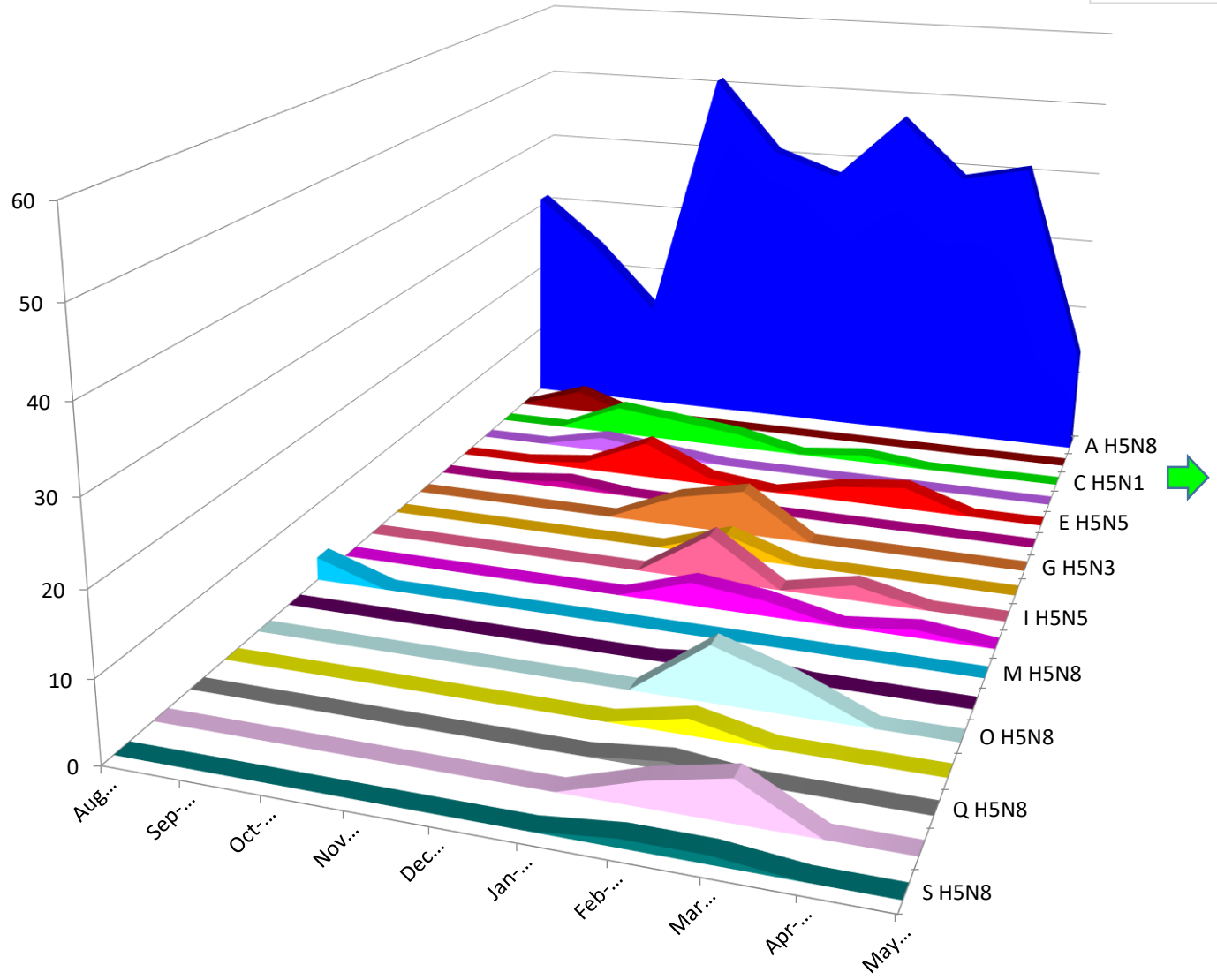
Subtype	Genotype	Gene segments							
		PB2	PB1	PA	HA	NP	NA	M	NS
H5N8	A								
	B								
	M								
	O								
	Q								
	S								
H5N1	C								
H5N5	D								
	E								
	F								
	H								
	I								
	L								
	N								
	R								
H5N3	G								
H5N4	P								

- HPAI Iraq/1/2020_H5N8
- Bangladesh/17D747/2016_H3N5
- Bangladesh/37607/2019_H10N3
- Belgium/10811/2019_H5N6
- Belgium/11025/2017_H11N1
- Belgium/7828/2018_H12N5
- Egypt/MB-D-487OP/2016_H7N3
- Egypt/P2-29/2017_H6N2
- Germany/2020_H5N8
- Hubei/ZYSYG3/2015_H6N2
- North-Kazakhstan/20/2018_H3N8
- Kurgan/1048/2018_H3N8
- Mongolia/451/2018_H4N1
- Mongolia/876/2019_H3N8
- HPAI Netherlands/1/2020_H5N1
- Netherlands/59/2015_H6N5
- Novosibirsk/964k/2018_H12N5
- Netherlands/1/2011_H3N8
- France/20P017917_2020_H5N3 (N3)
- Bangladesh/38920/2019_H7N4 (N4)

The information reported are based on the sequences available in GISAID or sent to EURL by NRLs or produced by EURL

Genotypes distribution per month

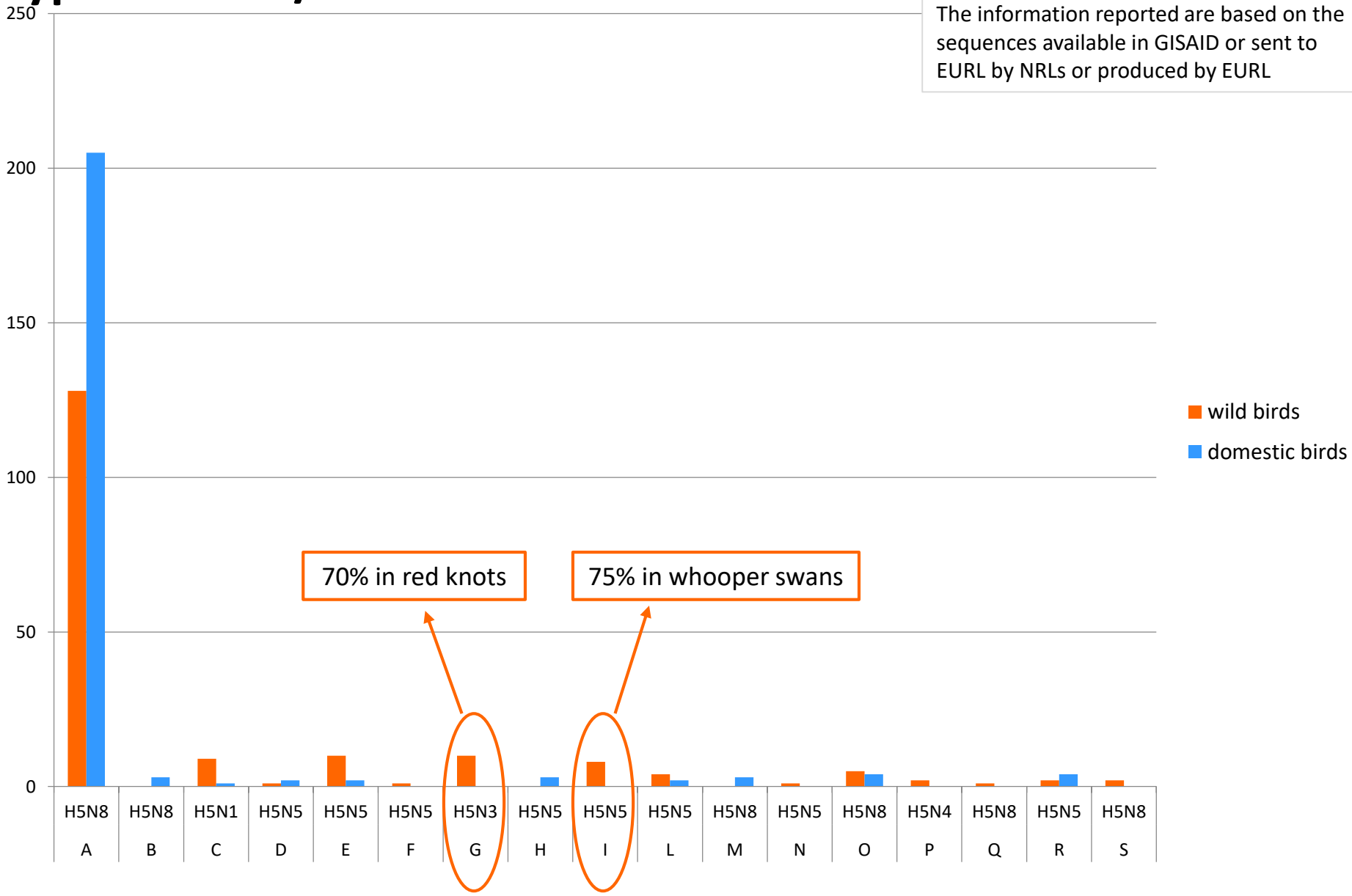
The information reported are based on the sequences available in GISAID or sent to EURL by NRLs or produced by EURL



Lack of recent sequences from H5N1 cases

Genotypes in wild/domestic birds

The information reported are based on the sequences available in GISAID or sent to EURL by NRLs or produced by EURL



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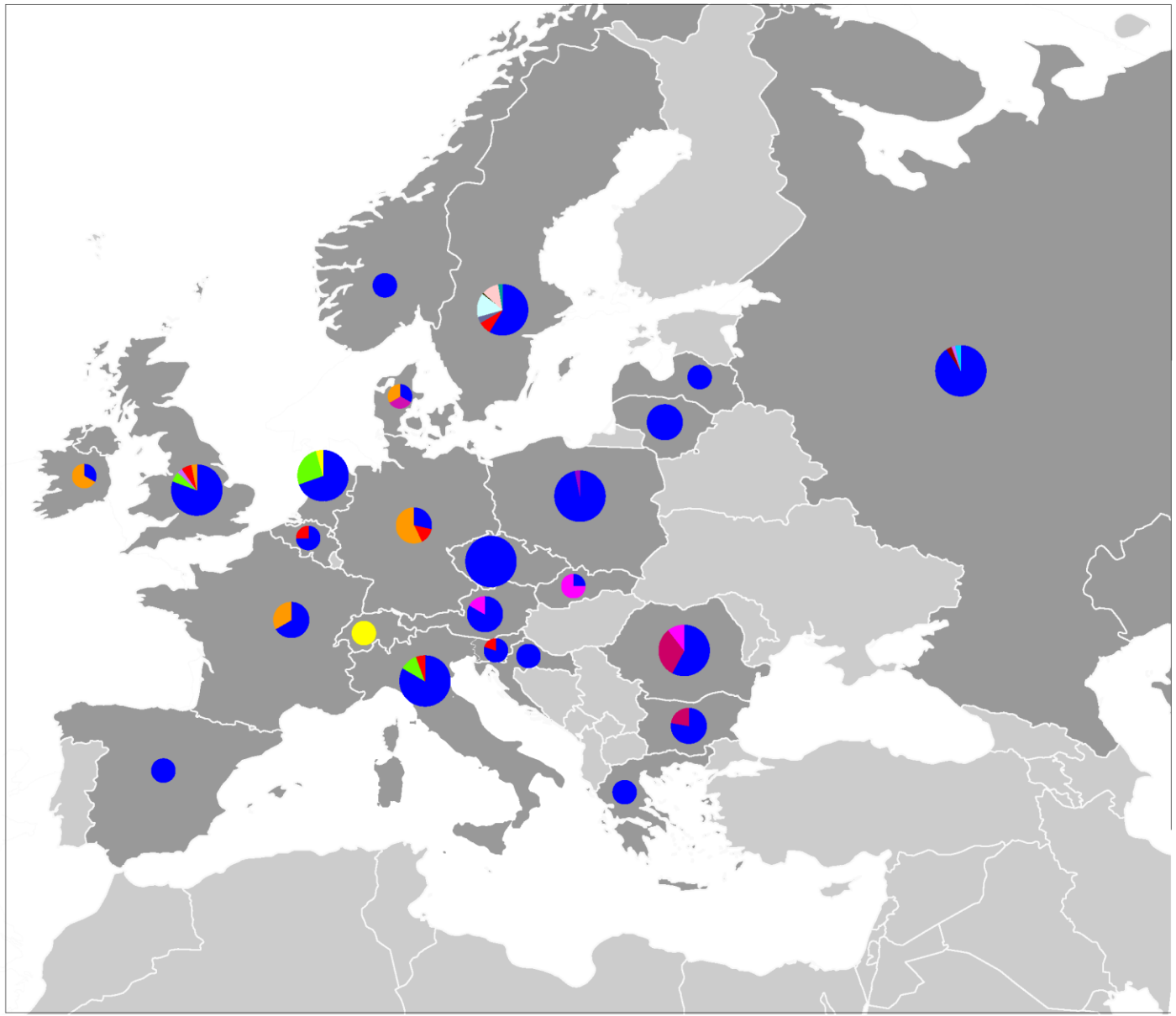
Geographic distribution of genotypes



Genotypes

- A
- B
- M
- O
- Q
- S

H5N8

- D
- E
- F
- H
- I
- L
- N
- R

H5N5

- C H5N1
- G H5N3
- P H5N4

Number of sequenced viruses

- 1≤N≤5
- 6≤N≤15
- N>16

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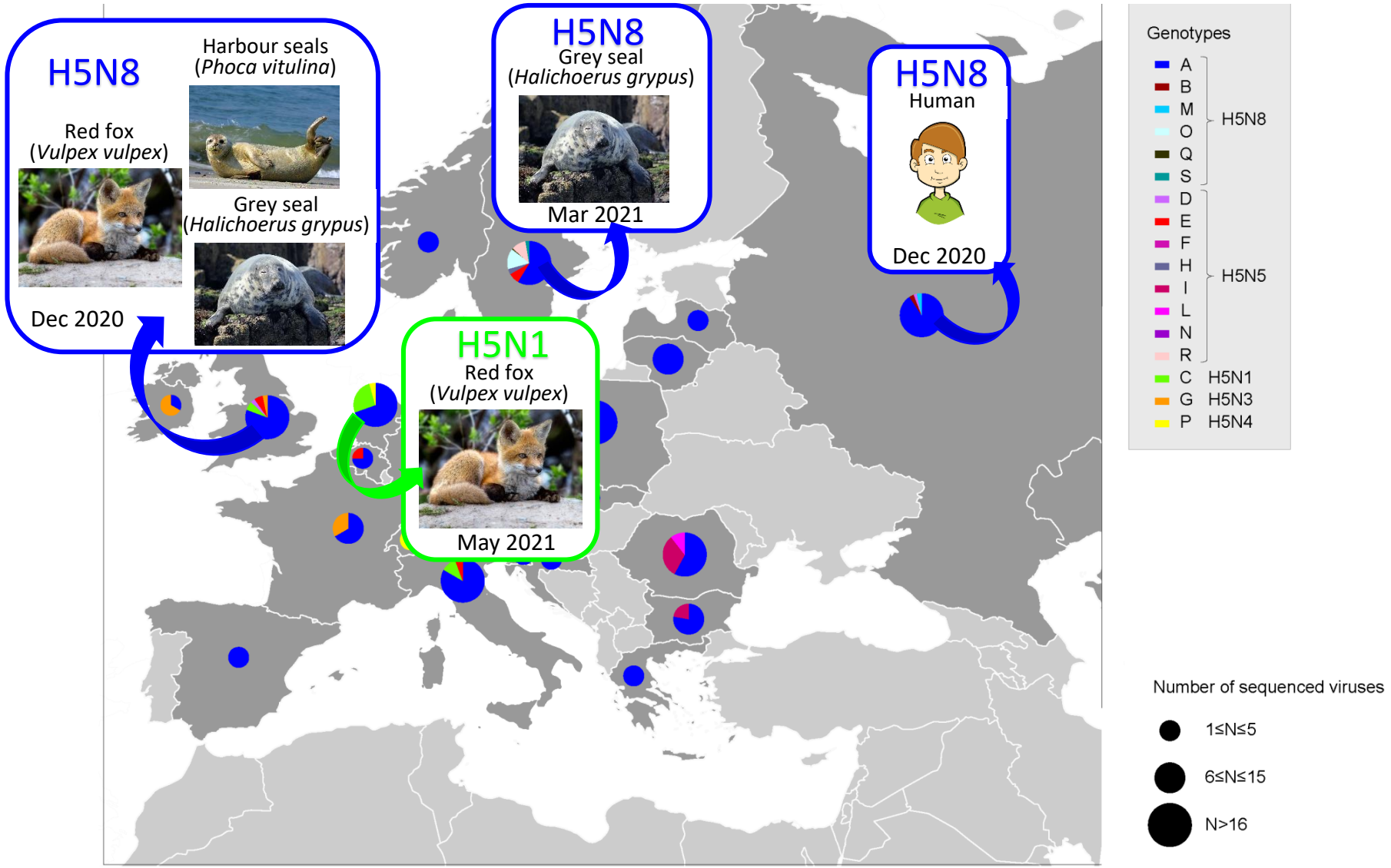
Geographic distribution of genotypes



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Mutations

Mutations recognized as associated with mammalian adaptation (PB2-M64T, PB2-E627K, PB2-D701N, PB1-S678N, have been only sporadically identified in the analysed viruses. Specifically, at the level of the proteins of the polymerase complex some European viruses contain mutations associated with an increase in polymerase activity and viral replication in mammalian cells and/or an increase in virulence in mice, such as:

PB2 D701N

Mutation associated with increased ability to replicate in mammalian cells and with an increased virulence in mice

- two HPAI **H5N8** viruses collected in **Poland** in May 2021 from **ducks and geese**

- HPAI **H5N8** viruses collected from wild **mammals** in **UK** in December 2020

PB2 E627K

Mutation recognized as one of the most important mammalian adaptive markers, correlated with increased replication and virulence in mammals

- one HPAI **H5N5** virus collected in **Romania** in February 2021 from **chickens**

- a HPAI **H5N8** virus collected from a **grey seal** in **Sweden** in March 2021

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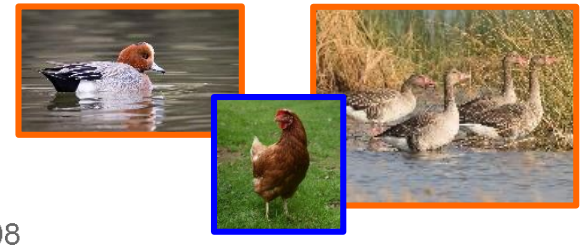
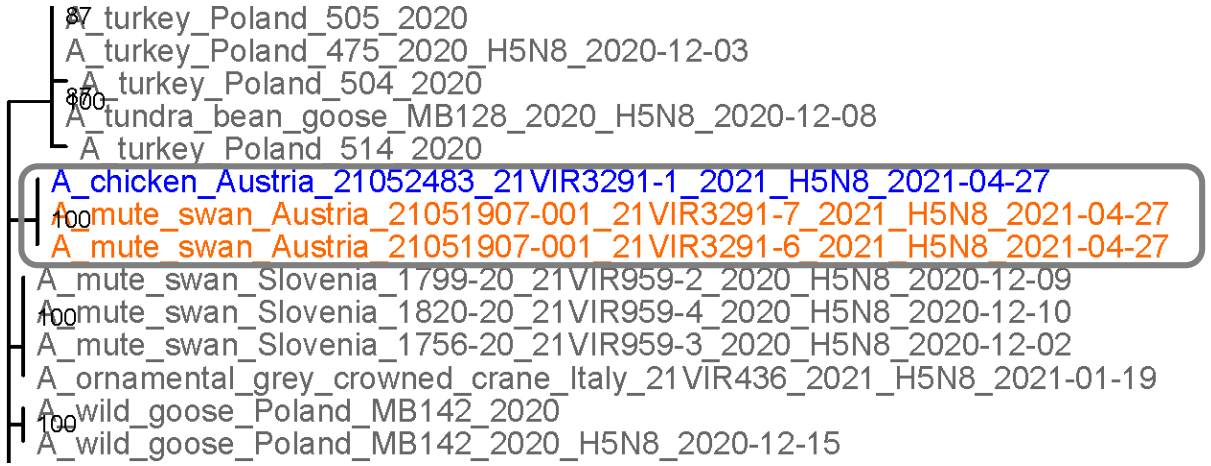
Liang et al., 2021; Herfst et al.; Manzoor et al., 2009; Kim et al., 2010; Suttie et al., 2019, Gabriel et al., 2005; Li et al., 2005; Czudai-Matwich et al., 2014

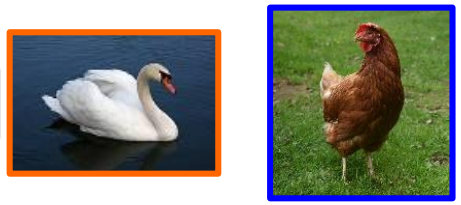
Genetic analyses to support epidemiological investigation

100% identical viruses





100% identical viruses



wild

domestic

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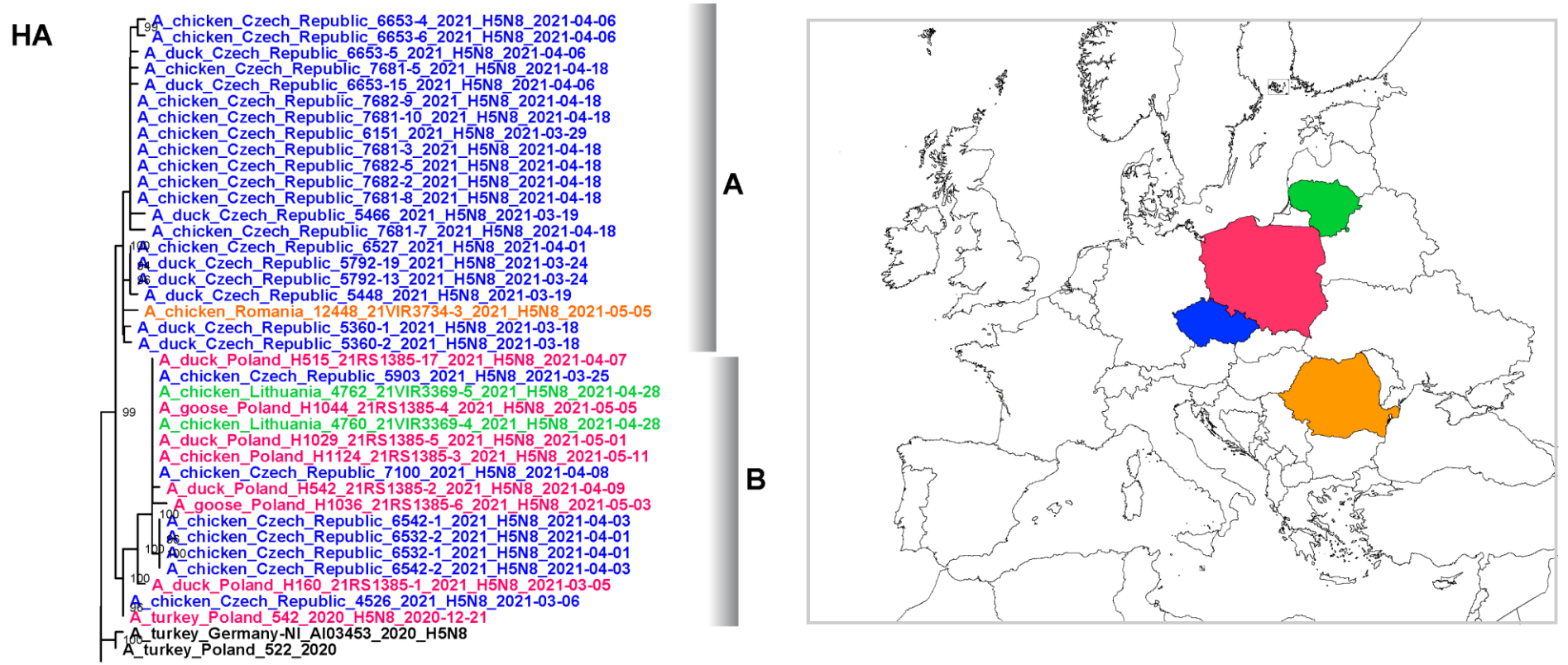
Genetic analyses to support epidemiological investigation



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Main observations and conclusion on genetic characteristics of HPAI H5NX in Europe in 2020/21

- The co-circulation in Europe and Central Asia of 17 different genotypes confirms the high propensity of this virus to undergo multiple reassortment events.
- The H5 viruses characterized to date retain a preference for avian-type receptors; however the reports of transmission events to human and wild mammals, the sporadic identification of mutations of mammal adaptation, as well as their high genetic variability, indicate the potential of this strain to represent a zoonotic risk.
- Ongoing outbreaks in wild and domestic birds that share the habitat with wild or domestic mammals, as well as the global diffusion of this clade also in geographic areas with limited surveillance, pose the risk of virus evolution and acquisition of mutations associated to mammal adaptation.
- Continued surveillance of avian influenza virus in wild and domestic birds combined with the timely generation and sharing of complete viral genome sequences are essential for:
    - Monitor virus evolution
    - Rapidly identify emergence of novel genotypes
    - Identify new virus introductions
    - Timely recognize any mutation resulting in changes in viral properties that are relevant for animal and public health
    - Support the epidemiological investigation in tracing the virus transmission among farms

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A(H5NX) HPAI in wild birds in Europe

FAO ALLERT – September 2016

Food and Agriculture Organization of the United Nations

empres watch

VOL 35 – SEPTEMBER 2016 EMPRES-ANIMALHEALTH@FAO.ORG | WWW.FAO.ORG/AG/EMPRES.HTML

H5N8 highly pathogenic avian influenza (HPAI) of clade 2.3.4.4 detected through surveillance of wild migratory birds in the Tyva Republic, the Russian Federation – potential for international spread

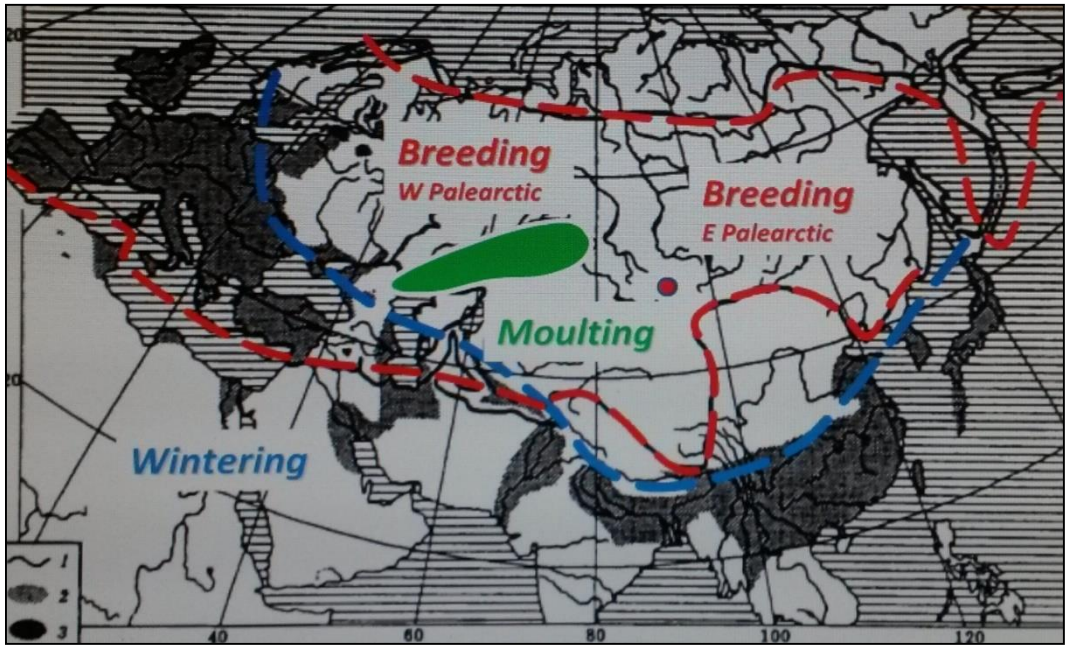
Contributors: *Les Sims, Sergei Khomenko, Akiko Kamata, Guillaume Belot, Jonathan Bastard, Elisa Palamara, Mirko Bruni, Sophie von Dobschuetz, Gwenaelle Dauphin, Eran Raizman, Juan Lubroth*

FAO ALLERT – September 2016

- **A Goose/Guangdong/96 lineage H5N8 highly pathogenic avian influenza (HPAI) virus of clade 2.3.4.4 was detected in migratory birds at Lake Ubsu-Nur** in the Tyva Republic of the Russian Federation, located along the Central Asian Flyway, **in early June 2016**
- In past seasons, the detection of HPAI viruses in this region of the Russian Federation **was followed by the detection of similar viruses in other locations**, especially to the west and south of the Tyva Republic. **All countries along this flyway** and those to the west in the former Soviet Republics, the Middle East, Eastern Europe and even Africa (especially West Africa) **should be on the alert for incursions of this virus**.

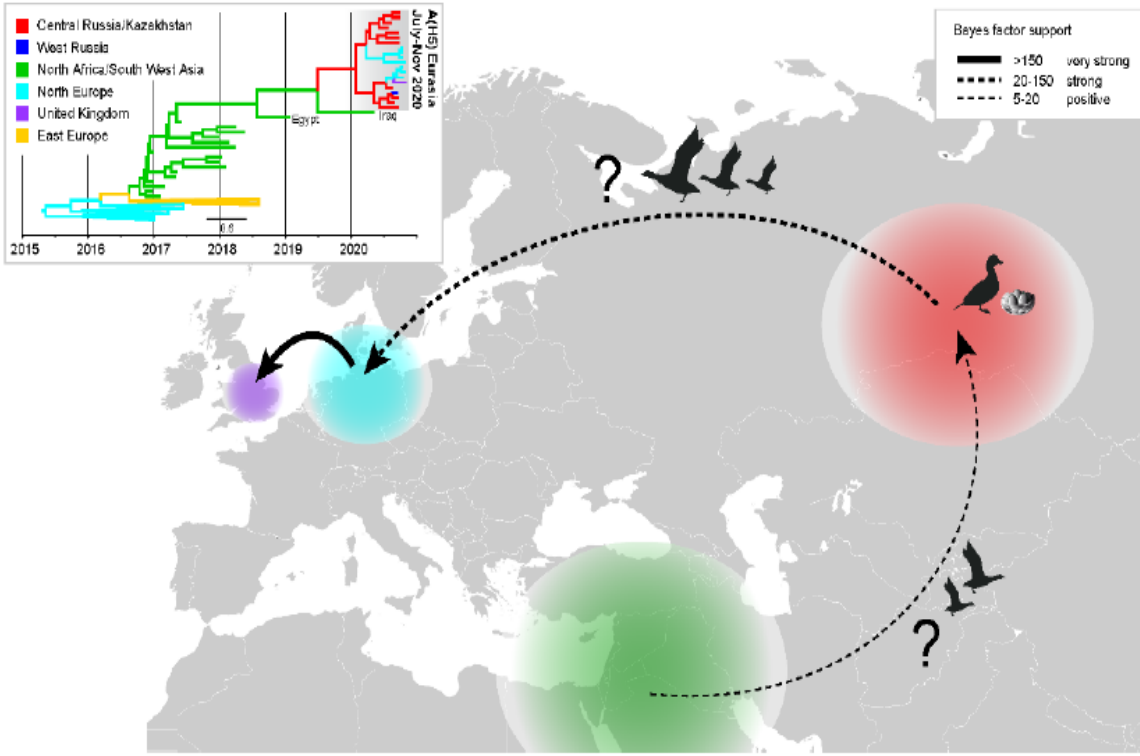
surveillance location at Ubsu-Nur Lake

Source: FAO



30 September 2020

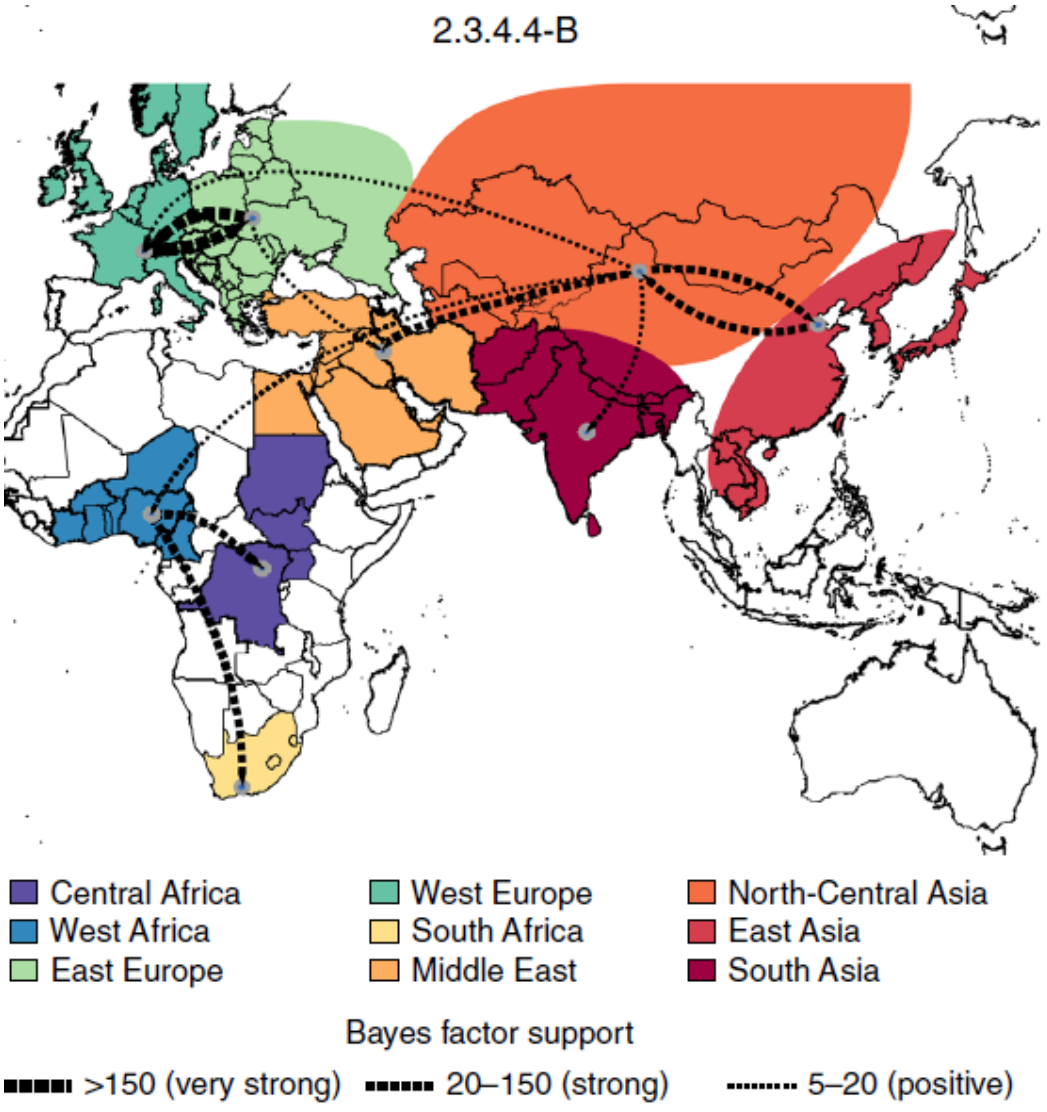
Avian influenza: EU on alert for new outbreaks



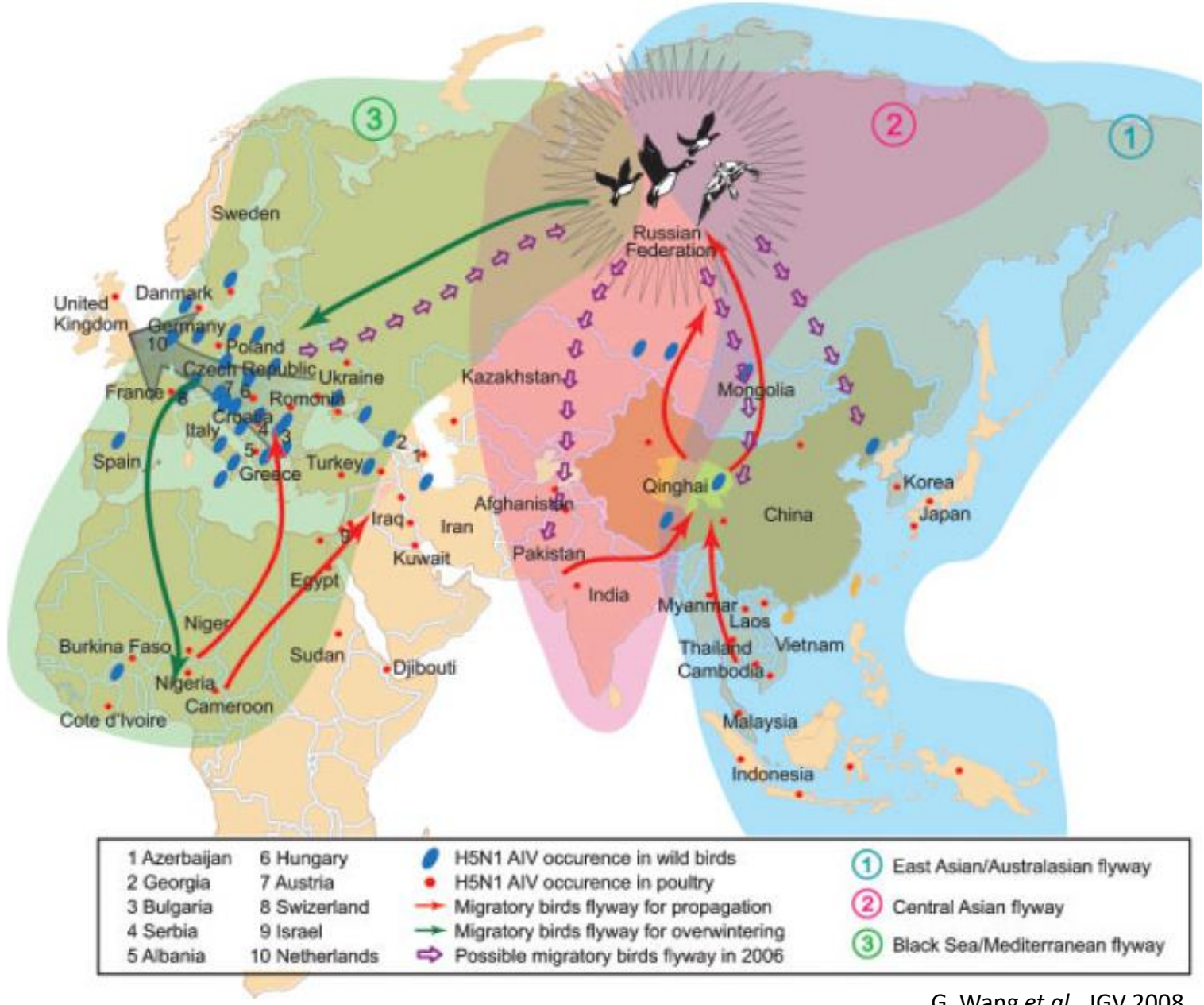
EU countries are being urged to step up surveillance and biosecurity measures to guard against possible new outbreaks of avian influenza this year.

The warning follows outbreaks of highly pathogenic avian influenza (HPAI) among wild and domestic birds in western Russia and Kazakhstan over the past few months. This region is a known autumn migration route for wild water birds heading to Europe.

Northern and eastern Europe are likely to be the most vulnerable to new outbreaks given past experience. When HPAI was detected in the same area of Russia in the summers of 2005 and 2016, epidemics followed in northern and eastern Europe. If the pattern is repeated this year, HPAI is expected to arrive in the same areas of Europe in autumn or winter. Subsequent spread to countries in southern and western Europe is also possible.



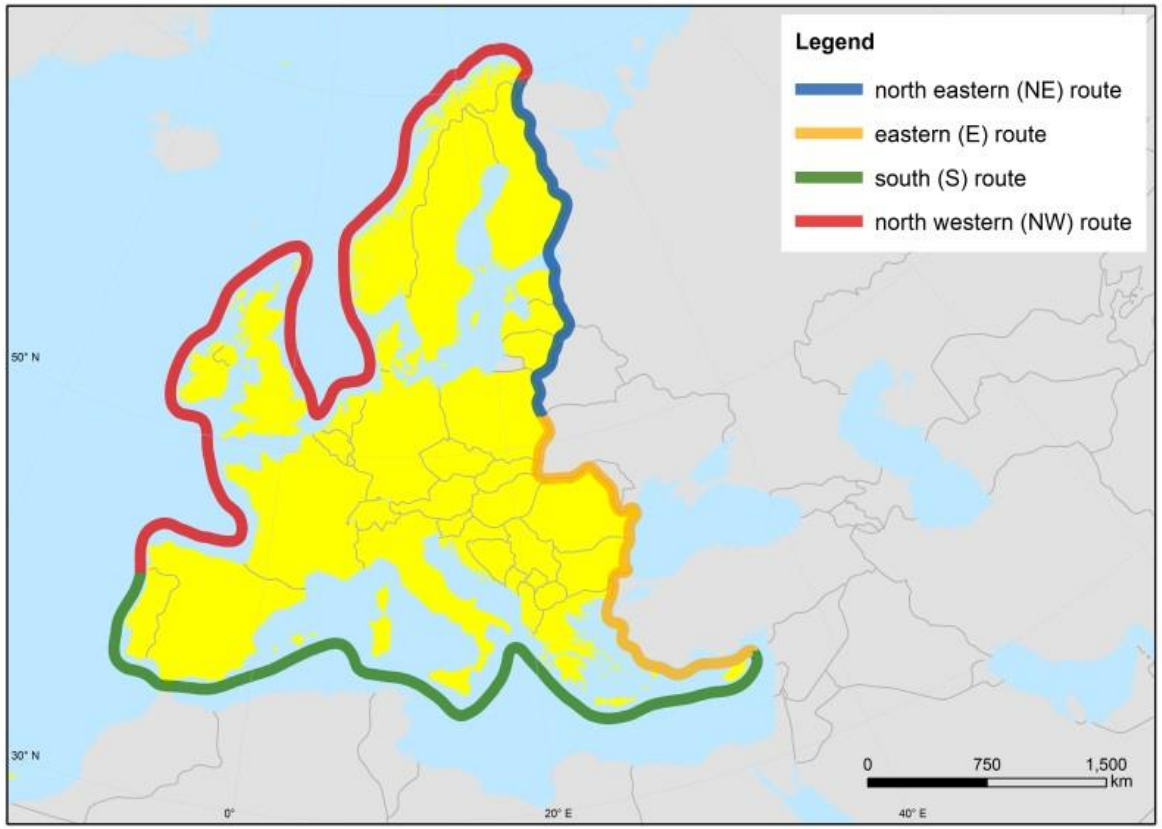
Fusaro *et al.*, NC 2019



G. Wang *et al.*, JGV 2008

AI introduction

- **Migratory water birds** represent the most likely pathway of AIV introduction into the EU
- Mainly via the **north eastern and eastern migratory routes**

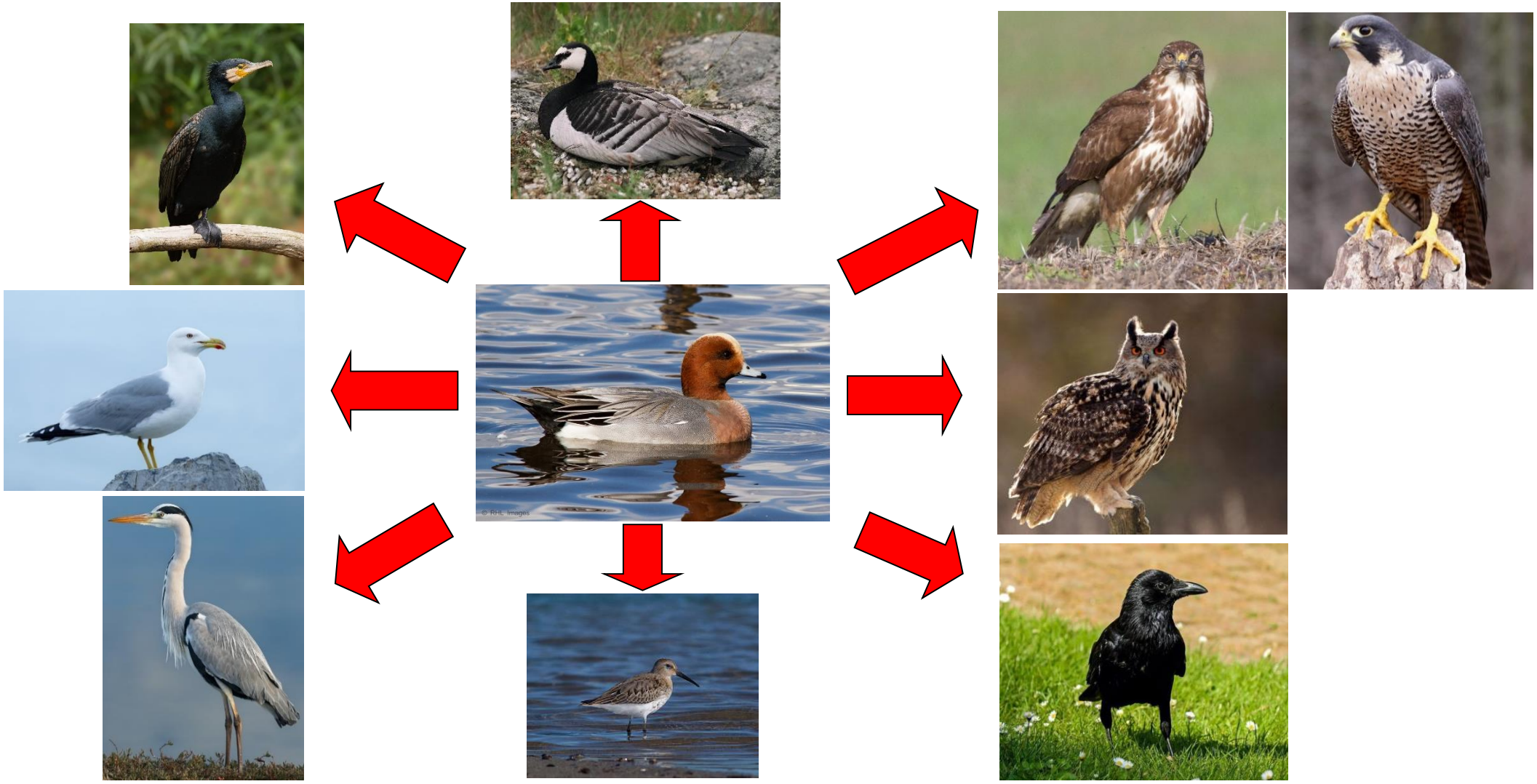


Clade	NE route	E route	S route	NW route
2.3.4.4	**Benchmark**	Slightly lower	Much lower	Much lower
2.2.1.2	Much lower	Much lower	Lower	Extremely low
2.3.2.1c	Similar	Similar	Lower	Extremely low

Source: EFSA

Circulation of new HPAI strains

High replication and spread of viruses in the environment with a prevalence of positivity up to 20-30% in some avian species



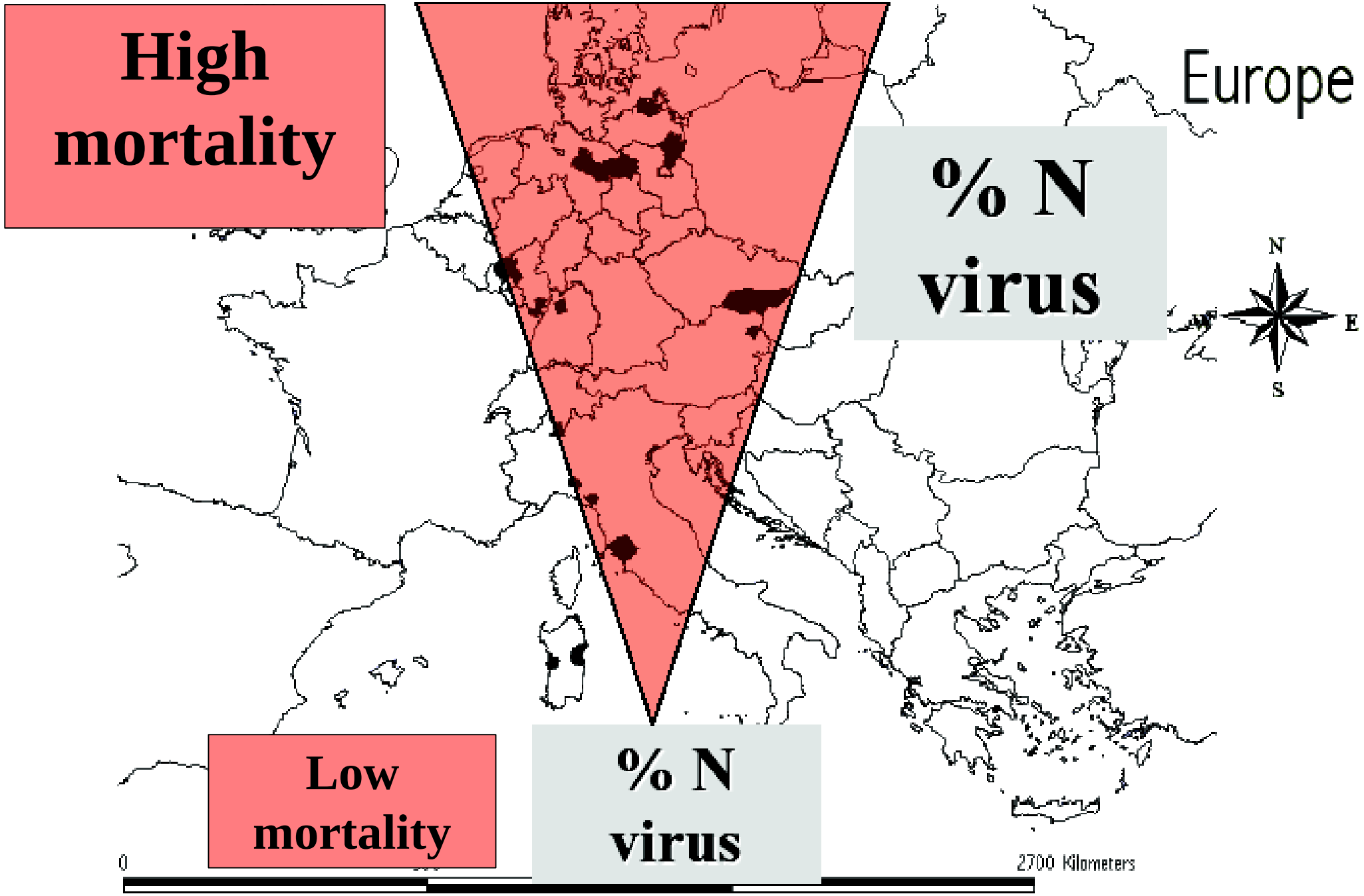
Involvement of mammal species

Table 1. Wild birds species involved in the HPAI epidemic, by order and number of cases.

Common name	Scientific name	Order	H5	H5N1	H5N2	H5N3	H5N4	H5N5	H5N8	H7N7	Total
Mute swan	*(Cygnus olor)*	*Anseriformes*	155	2	1			20	528	2**	**708**
Barnacle goose	*(Branta leucopsis)*	*Anseriformes*	6	28				6	346		**386**
Greylag goose	*(Anser anser)*	*Anseriformes*	17	18		1	3	3	338		**380**
Swan ns	*(Cygnus spp)*	*Anseriformes*	5				2	19	241		**267**
Common buzzard	*(Buteo buteo)*	*Accipitriformes*	4	3		1	2	5	130		**145**
Whooper swan	*(Cygnus cygnus)*	*Anseriformes*	26					5	99		**130**
Red knot	*(Calidris canutus)*	*Charadriiformes*				108		1			**109**
Anseriformes ns	*(Anseriformes)*	*Anseriformes*		71			1		25		**97**
Eurasian wigeon	*(Mareca penelope)*	*Anseriformes*	27	11				1	49*		**88**
Common pheasant	*(Phasianus colchicus)*	*Galliformes*							68		**68**
Mallard duck	*(Anas platyrhynchos)*	*Anseriformes*	12				2	1	47		**62**
Accipitriformes ns	*(Accipitriformes)*	*Accipitriformes*		5				3	48		**56**
Eurasian teal	*(Anas crecca)*	*Anseriformes*	28	2				1	12		**43**
Canada goose	*(Branta canadensis)*	*Anseriformes*	4	1				1	35		**41**
European herring gull	*(Larus argentatus)*	*Charadriiformes*	3	2			1	2	32		**40**
Gull ns	*(Laridae)*	*Charadriiformes*	3	7			2	2	26		**40**
Peregrine falcon	*(Falco peregrinus)*	*Falconiformes*	1			2	1	2	29		**35**

- **> 80** species affected
- **13** Orders
- Mostly **Anseriformes**
- **Swans and geese** are the most involved wild birds

https://www.izsvenezie.it/temi/malattie-patogeni/influenza-aviaria/situazione-epidemiologica-hpai-europa/


High mortality
Europe
% N virus
N
E
S
Low mortality
% N virus
0
2700 Kilometers

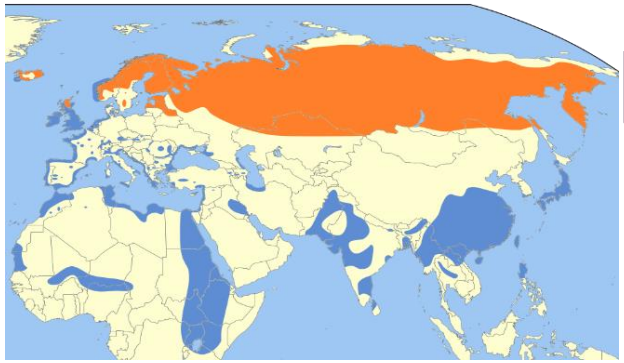
Arrival of clinically healthy infected wild birds

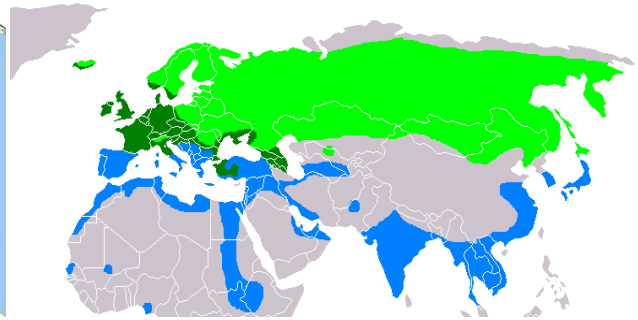


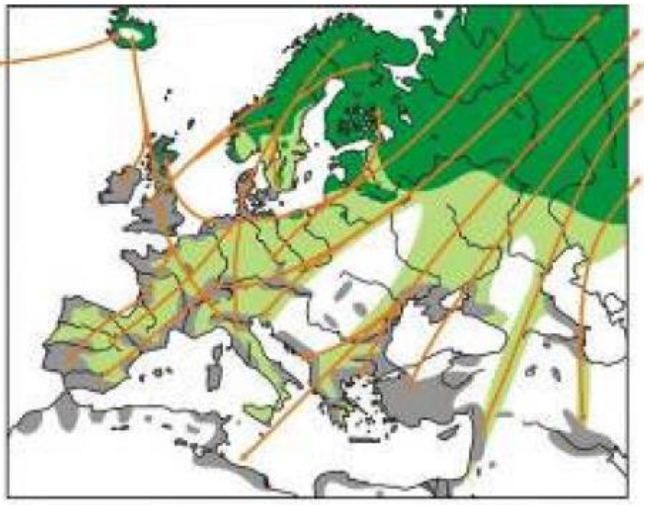


Species	H5*	H5N1	H5N5	H5N8	Total
Common teal (*Anas crecca*)	28	2	1	19	50
Eurasian wigeon (*Mareca penelope*)	17	5		16	38
Mallard duck (*Anas platyrhynchos*)	1			4	5
Northern shoveler (*Spatula clypeata*)	2			1	3
Northern pintail (*Anas acuta*)	1				1
Greylag goose (*Anser anser*)		1			1
Greater white-fronted goose *(Anser albifrons)*				1	1
Common buzzard (*Buteo buteo*)				2	2
Mediterranean gull *(Ichthyaetus melanocephalus)*				1	1
Total	49	8	1	44	102

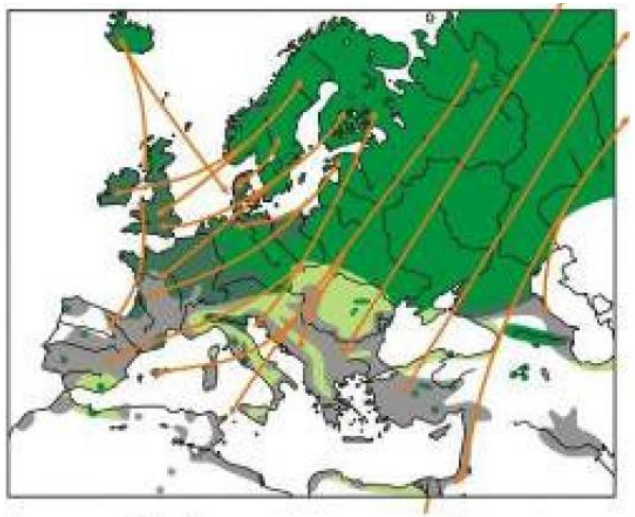
Dabbling ducks as (silent) carriers of HPAIVs



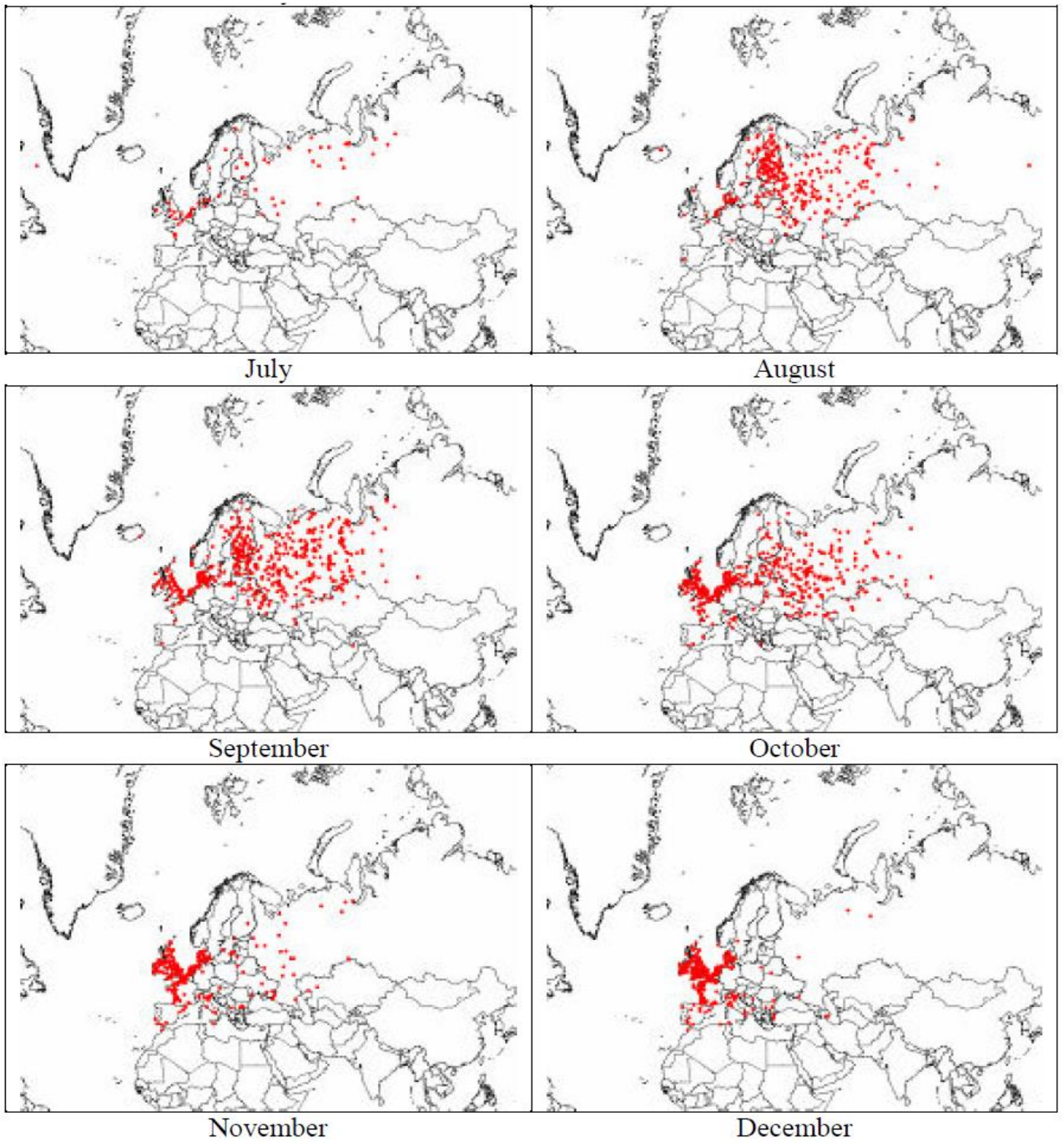




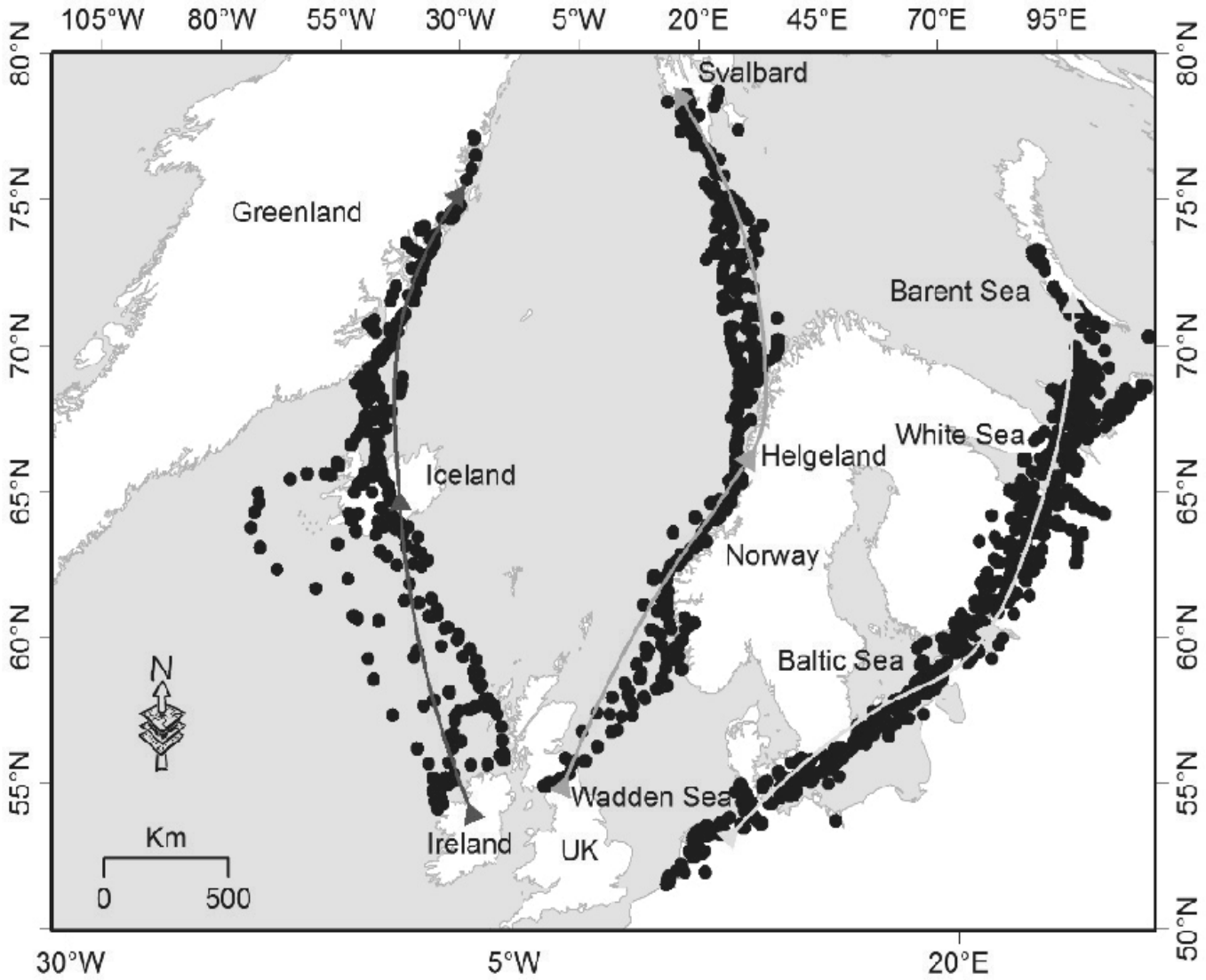
Eurasian Wigeon *Anas penelope*
Distribution Map: Bauer, Bezzel & Fiedler 2006, AULA-Verlag



Common Teal *Anas crecca*
Distribution Map: Bauer, Bezzel & Fiedler 2006, AULA-Verlag

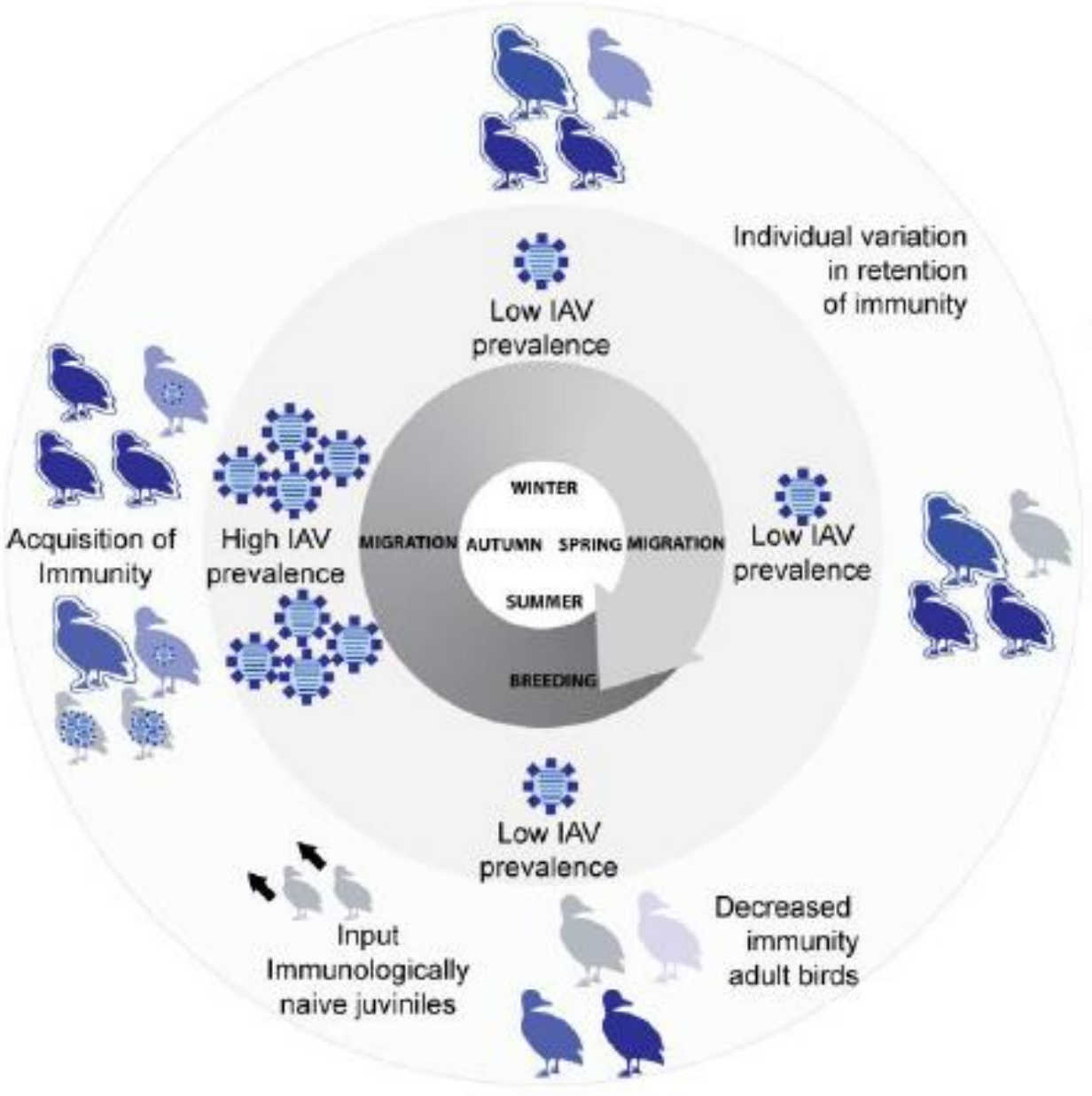


Interaction between resistant and highly susceptible species in wintering quarters



What is happening now?

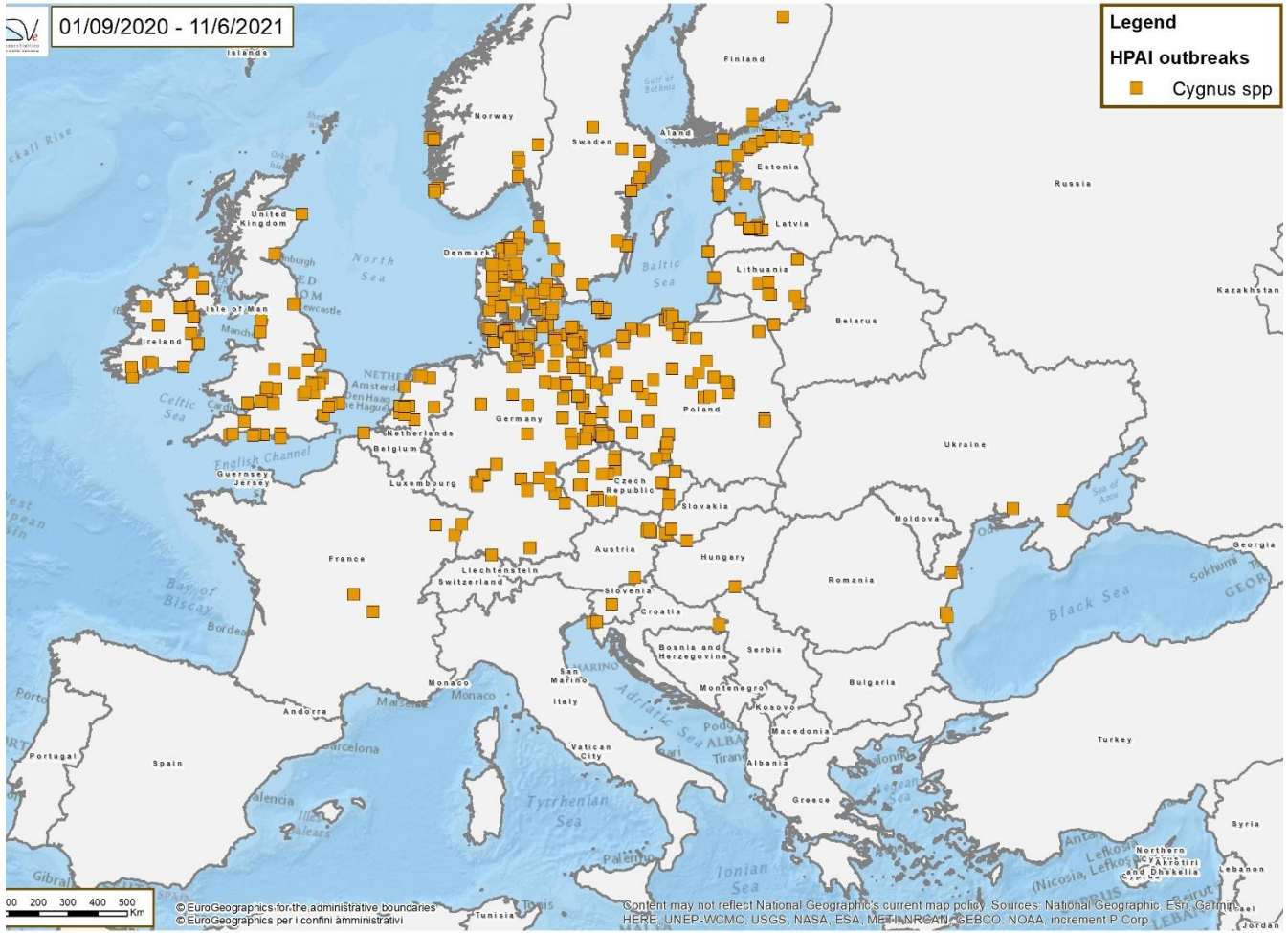
The longer persistence of HPAI in wild birds compared to the one observed in previous years may indicate an involvement of juveniles of several species of wild birds as well as an increasing risk for mammals

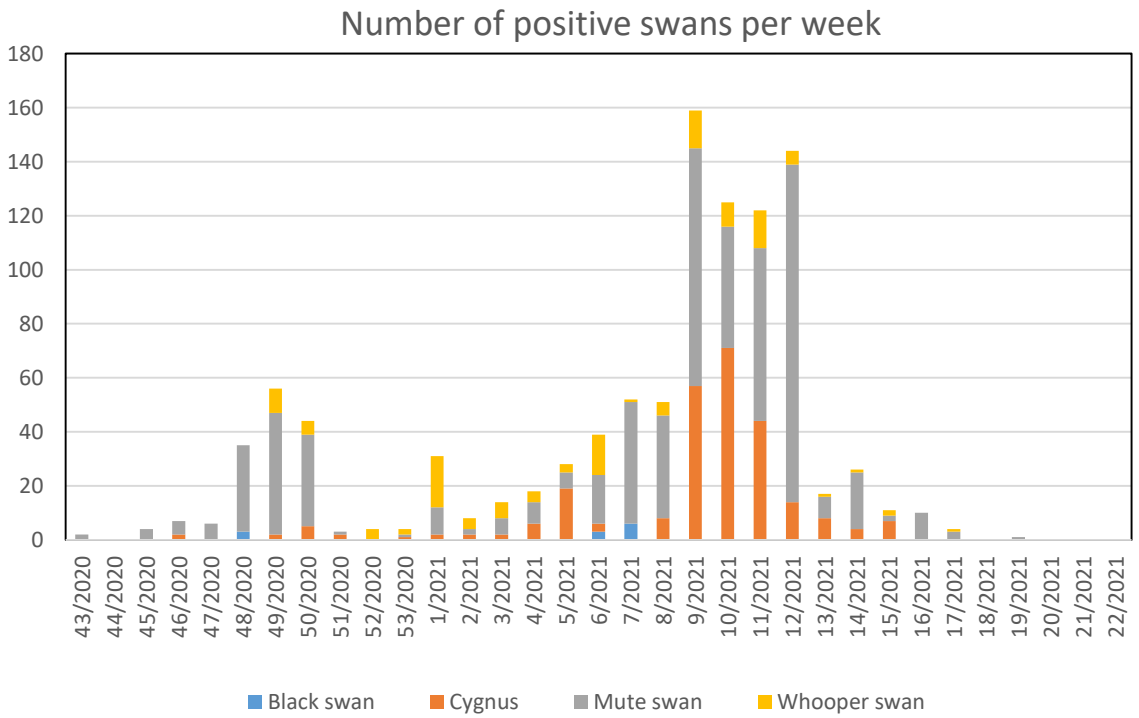


Source: EFSA

Interaction between resistant and highly susceptible species in breeding sites

Interaction between resistant and highly susceptible species in breeding sites



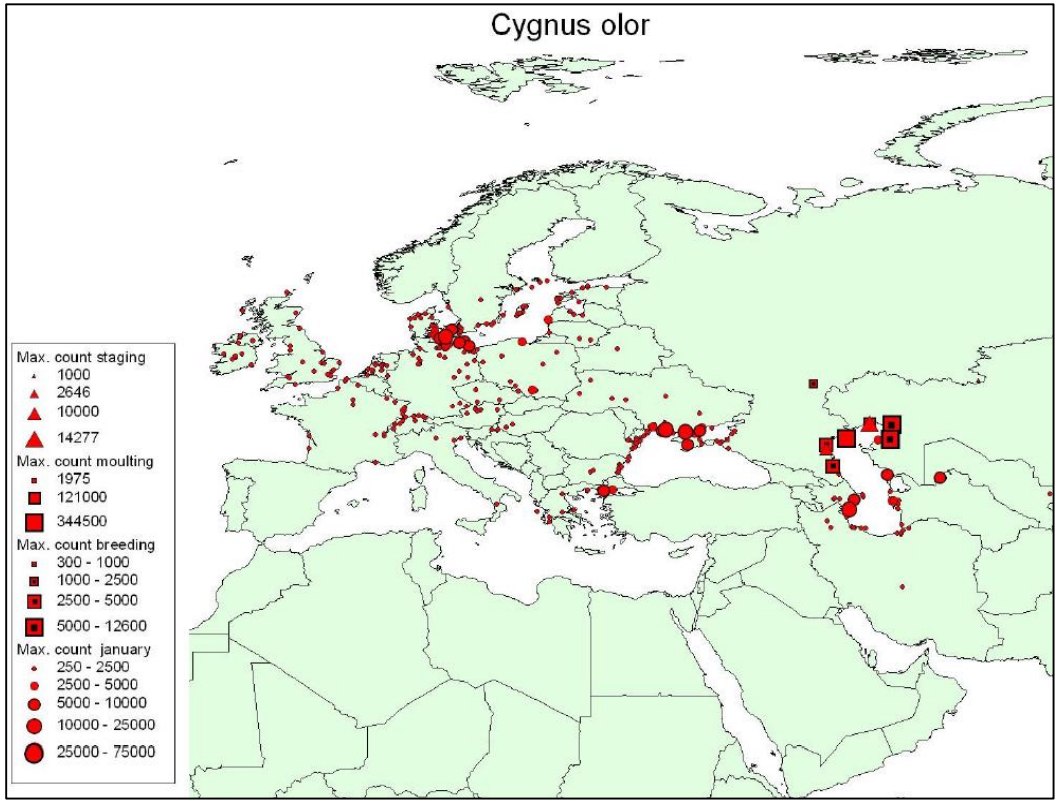


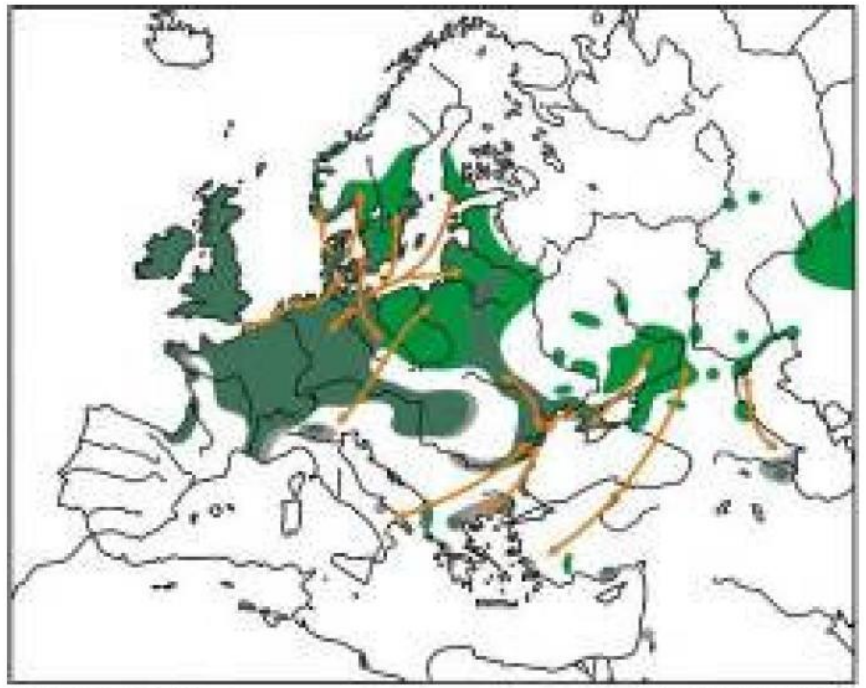
Interaction between resistant and highly susceptible species in breeding sites

The mute swan was the most affected species, particularly in the early spring when high risk wild bird species colonize the breeding sites

The European Mute swan population is largely sedentary, except in the southern and southern eastern Europe where birds is more migratory during winter, but movements are strongly influenced by the severity of winter weather.

Considering that in spring there are no significant movements of swan population that are preparing the breeding season, **it is probable that the disease was spread by different species and that the swans**, being highly susceptible to HPAI and conspicuous when they die, **acted as sentinel of the disease**





Mute Swan *Cygnus olor*

Distribution Map: Bauer, Bezzel & Fiedler 2006, AULA-Verlag

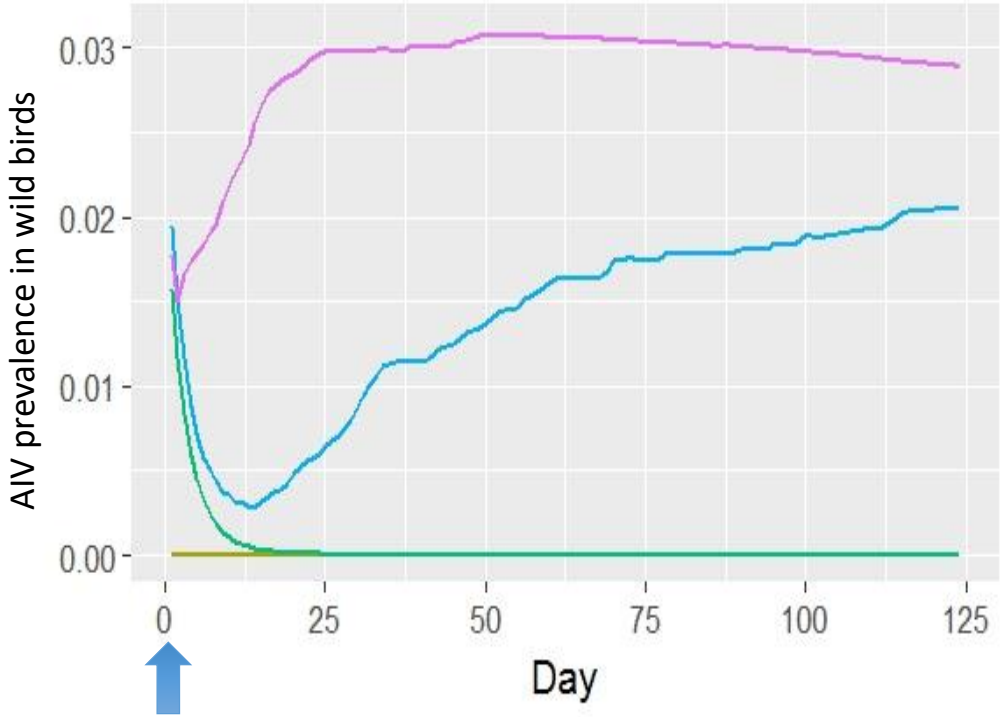
What should we expect?

Highly sensitive wild bird species (swans, gulls, terns, cormorant, etc.) and mammals with habitat overlapping that of infected wild waterfowl or feeding on wild waterfowl carcasses (diurnal and nocturnal raptors) are at high risk of HPAI infection and death

The persistent presence of HPAI A(H5) viruses in wild birds and in the environment holds high the risk of avian influenza incursions with the potential further spread among establishments, primarily in areas with high poultry densities and near to high concentrations of high risk wild bird species

AI introduction

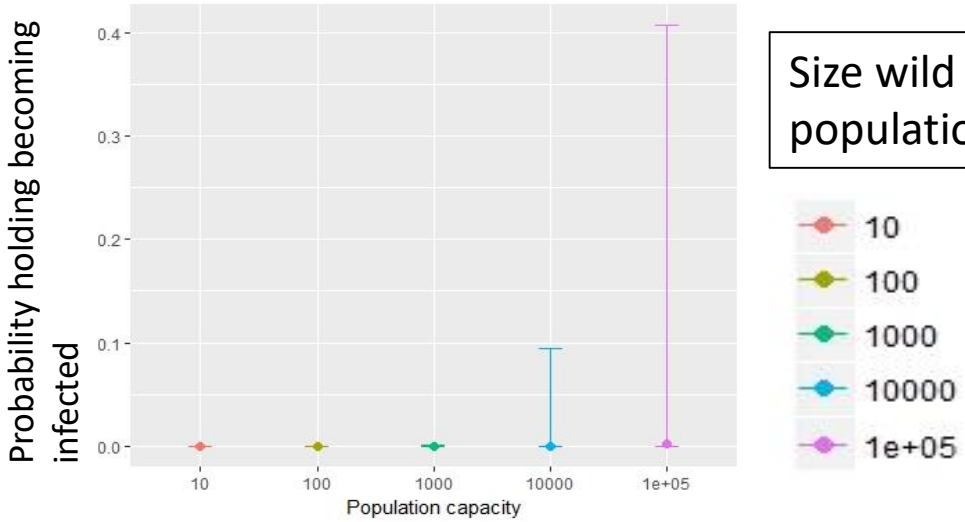
According to a mathematical model, **AIV amplification and spread** are proportional to the capacity of **wild bird populations**



Source: EFSA

AI introduction

The **AIV prevalence in water birds** as well as the **size and composition of the wild bird reservoir** determine the probability of a holding to become infected



Reduction by implementing/improve biosecurity!

Size wild bird population

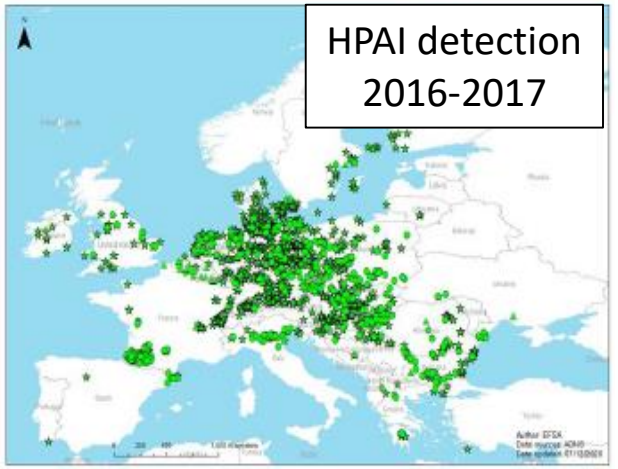
Source: EFSA

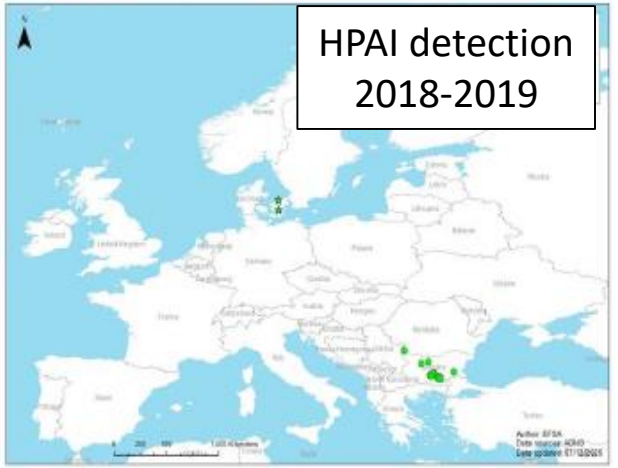
Near future

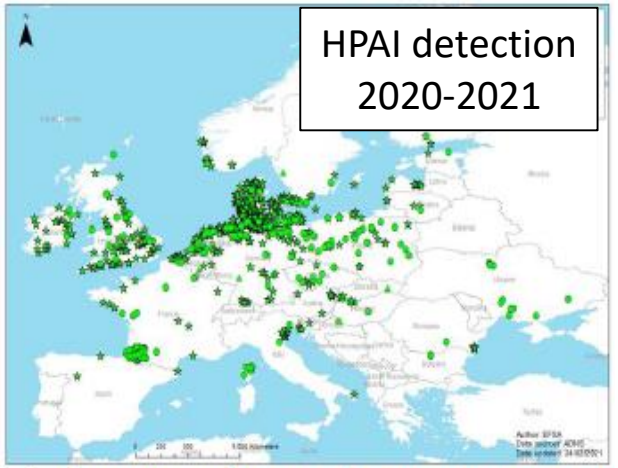
The high circulation of HPAIVs is persisting this year. This leads to think of a high circulation in the migratory populations and of possible new waves in the next fall and winter seasons in the European countries

A possible reduction in circulation could occur due to the cyclic nature of the disease in the wild (herd immunity acquired in the previous months?)

Which of these two scenarios will arise is a matter that cannot be predicted at the moment







Grazie dell’attenzione!

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