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The ‘Big Picture’ internationally – the science of AI virus

Prof Ian Brown

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Animal and Plant Health Agency-Weybridge

A Joint Government/Poultry Industry Workshop

Lessons Learned in the 2020/21 AI ‘Season’

13TH September 2021

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Importance of International collaboration and global connections for early warning –threat detection

- **APHA's International Reference Laboratory for Avian influenza** virus maintains global links for **early warning of disease threat**
  - **H5N8 HPAI disease threat to Europe, Middle East/Africa and central Asia raised before European incursions**
  - **New strain related to 2016/17 European virus but distinct**

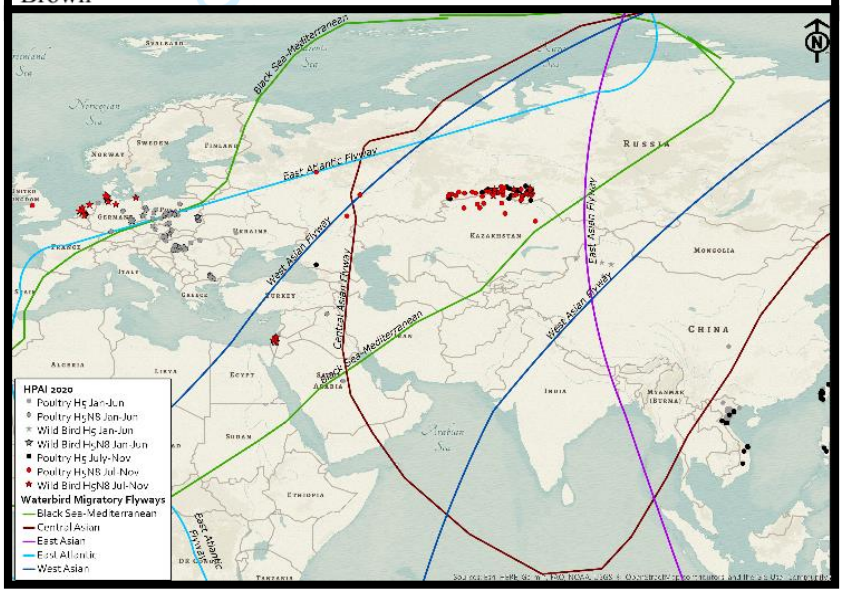
- International links Russia, Kazakhstan, and Iraq

- Integrated rapid genomic assessment of pathogens
- Assured relevant diagnostics

- Uncertain utility of current vaccines –poor match

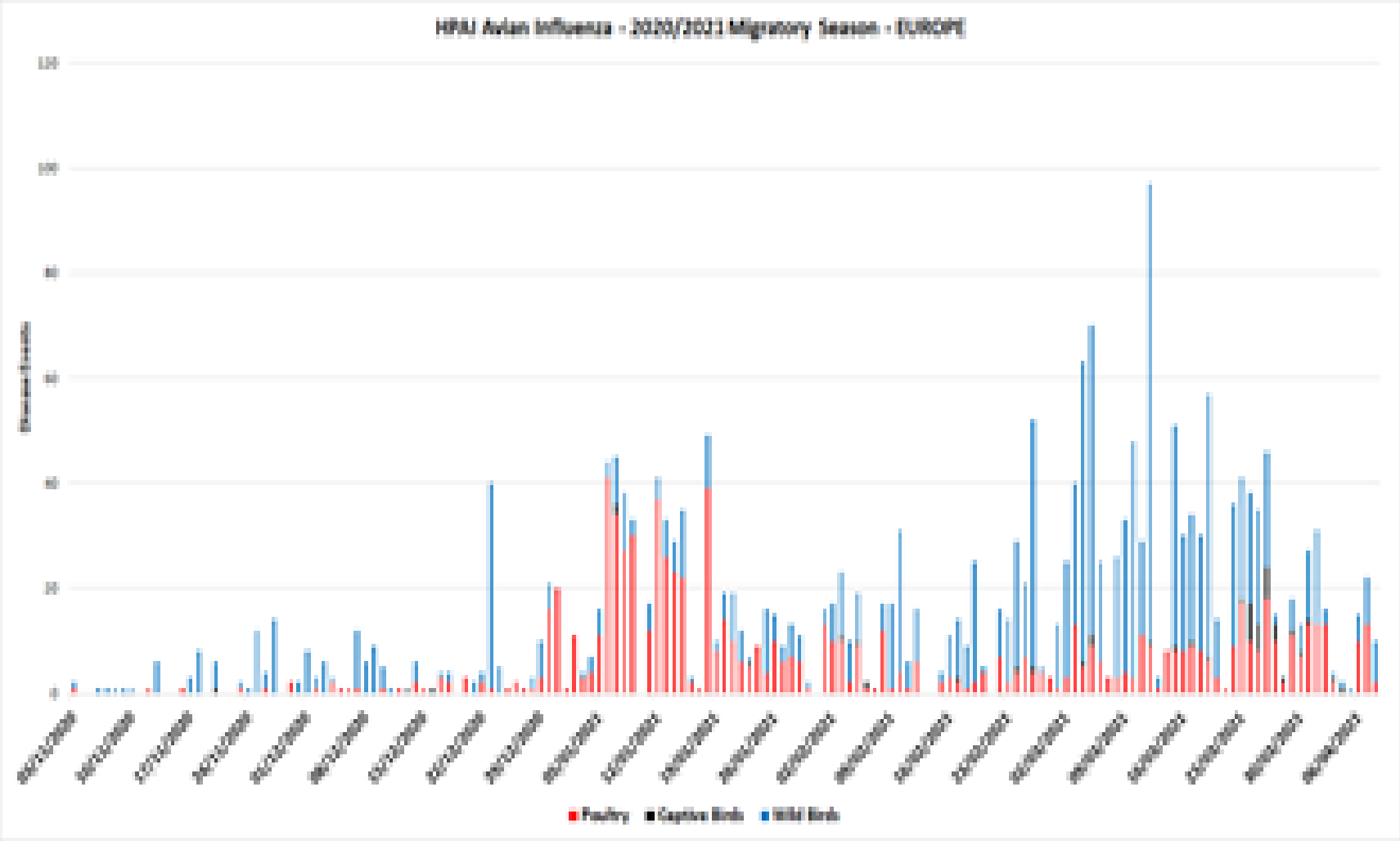
Emergence and spread of novel H5N8, H5N5 and H5N1 clade 2.3.4.4 highly pathogenic avian influenza: 2020

Nicola S Lewis[1,2]*, Ashley C Banyard[1], Elliot Whittard[1], Talgat Karibayev[3], Thamer AL Kafagi[4], Ilya Chvala[5], Alex Byrne[1], Saduakassova Meruyert (Akberovna)[6], Jacqueline King[8], Timm Harder[8], Christian Grund[8], Steve Essen[1], Scott M.Reid[1], Adam Brouwer[1], Nikolay G Zinyakov[5], AzimkhanTegzhanov[3], Victor Irza[5], Anne Pohlmann[8], Martin Beer[8], Ron AM Fouchier[7], Sultanov Akhmetzhan (Akievich)[6], Ian H Brown[1]



Lewis et al 2021; Emerging Microbes and Infections 10 (1) 148-151.

Epidemic curve of H5 HPAI outbreaks in Europe 2020/21



Recent cases in wild birds in the Netherlands

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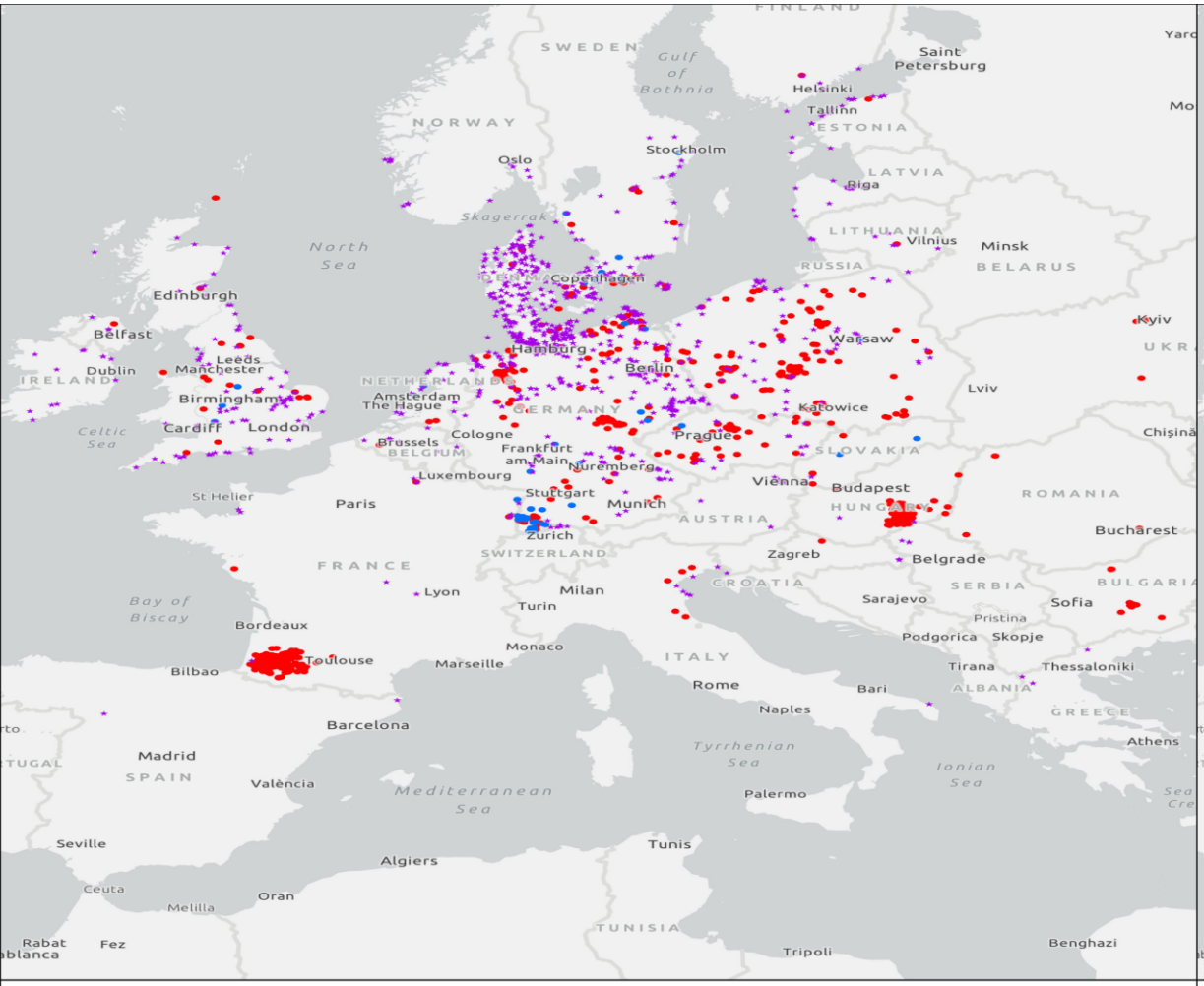
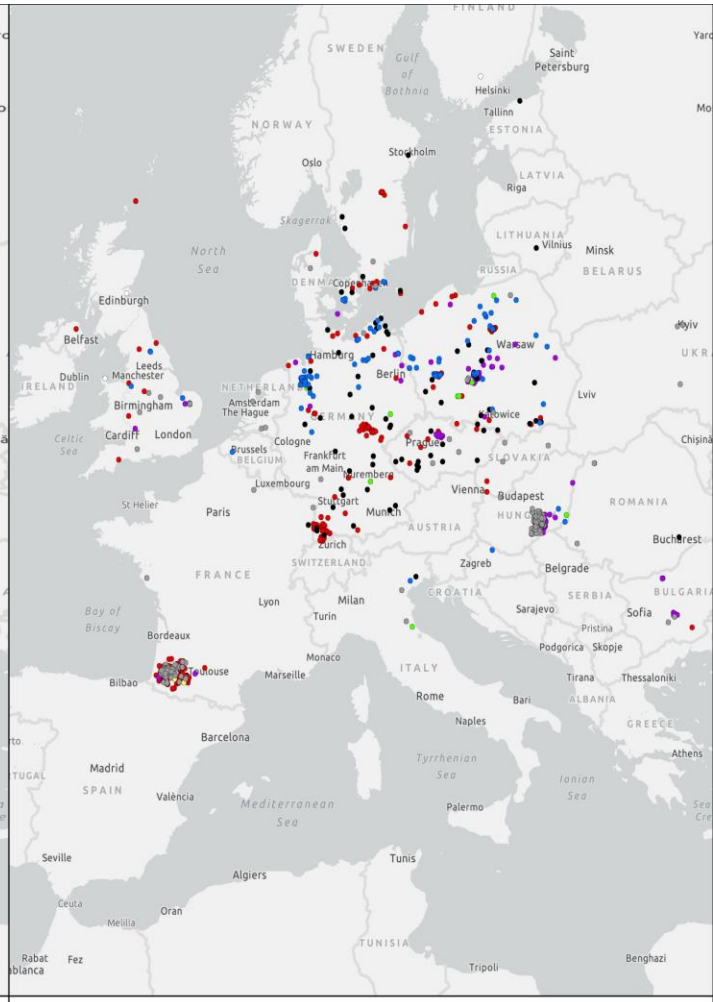


Highly Pathogenic Avian Influenza Events (Oct 2020-Apr 2021)

- Poultry
- Captive Birds
- Wild Birds



Highly Pathogenic Avian Influenza Events (Oct 2020-Apr 2021)

- Chicken
- Duck
- Fois Gras
- Goose
- Mixed
- Game Bird
- Turkey
- Unspecified

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Epidemiology of H5 HPAI epizootic 2020-21 clade 2.3.4.4b

- Primary incursions wild bird mediated
  - >1200 poultry outbreaks largest ever in Europe
- Secondary spread between poultry premises
  - Local production practices that increase spread risk
  - Fomite not airborne via people, equipment, vehicles
- Lethal outcomes high mortality
  - Some attenuation in disease in adult domestic ducks/geese
- Large virus diversity both at serotype and genetic level
  - **H5N8,** H5N5, H5N1, *H5N3, H5N4*
  - *Multiple genotypes within serotypes*
- Range of measures to control in Europe
  - Stamping out, control zones, housing orders, vaccination prohibited

Wild bird cases: H5N8 2016-17 Versus H5Nx 2020-21

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2016-17: H5N8

45 Birds
14 Species
16 Counties

Wigeons (12)
Mute swans (12)
Common buzzard (4)
Greylag geese (3)
Black-headed gulls (2)
Canada geese (2)
Pochard ducks (2)
Whooper swans (2)
White-fronted goose (1)
Mallard duck (1)
Peregrine falcon (1)
Cormorant (1)
Eurasian teal (1)
Kestrel (1)

2020-21: H5N8

Virus subtype	Number
H5N1	10
H5N5	6
H5N8	276
H5Nx	9
Grand Total	301

23 species

32 counties

	H5N1	H5N5	H5N8	H5Nx	Total
England	**10**	**1**	**258**	**7**	**276**
Black Swan			11		11
Brent Goose			6		6
Buzzard			3	1	4
Canada Goose	1		24		25
Great White Egret				1	1
Grey Heron			1		1
Greylag goose			8	2	10
Herring Gull			2		2
Kestrel			1		1
Lesser Black Backed Gull			1		1
Lesser Black headed Gull			1		1
Mixed Avian			1		1
Mute Swan	**8**	**1**	**155**	**1**	**165**
Peregrine Falcon			2		2
Pink footed goose			2		2
Shelduck			1		1
Sparrow Hawk			1		1
Unspecified (Goose/swan)			23		23
Whooper Swan			21	2	23
Wigeon			1		1
Common Buzzard	1		2		3
Red Kite			1		1
Wales		**5**	**1**		**6**
Little Grebe			1		1
Mute Swan		5			5
Scotland			**17**	**2**	**19**
Greylag goose			1		1
Mute Swan			7		7
Unspecified Swan			4		4
Whooper Swan			4		4
Knot				1	1
Red Knot			1	1	2
Total	**10**	**6**	**276**	**9**	**301**

Introducers grazing/dabbling ducks/migratory geese; rapid spread to indigenous species; heavy infection pressure in environment

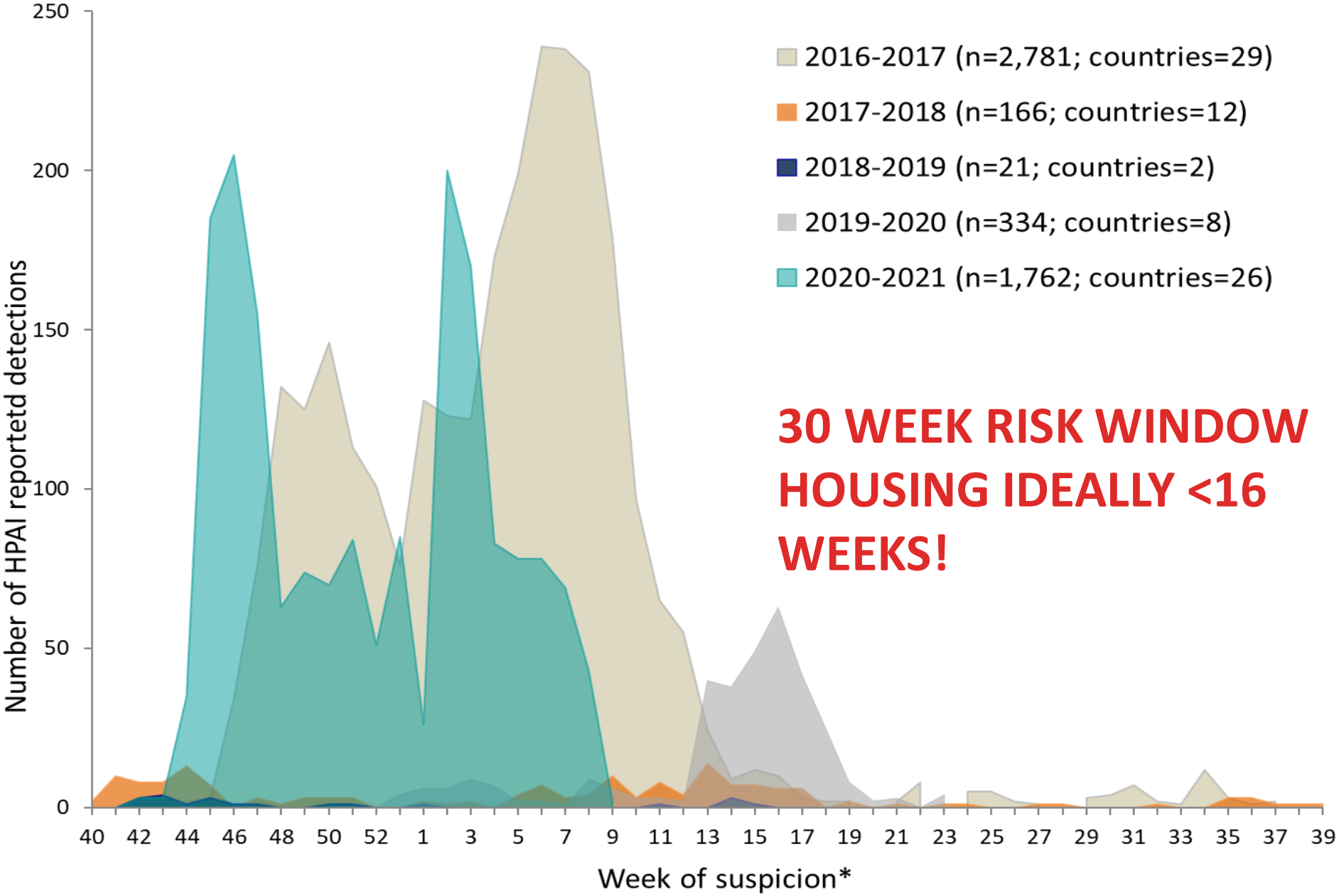
Role of sentinel and bridge species for risk assessment of poultry introductions?

- Sentinel and bridge species at key habitats
  - Raptors
  - Scavengers (ie gulls, pigeons, crows)
  - Bridge species (ie farm dwellers- starlings, sparrows)

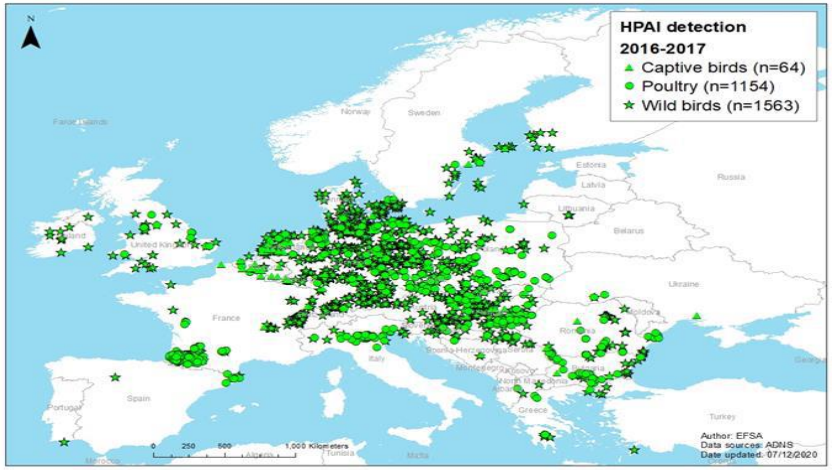


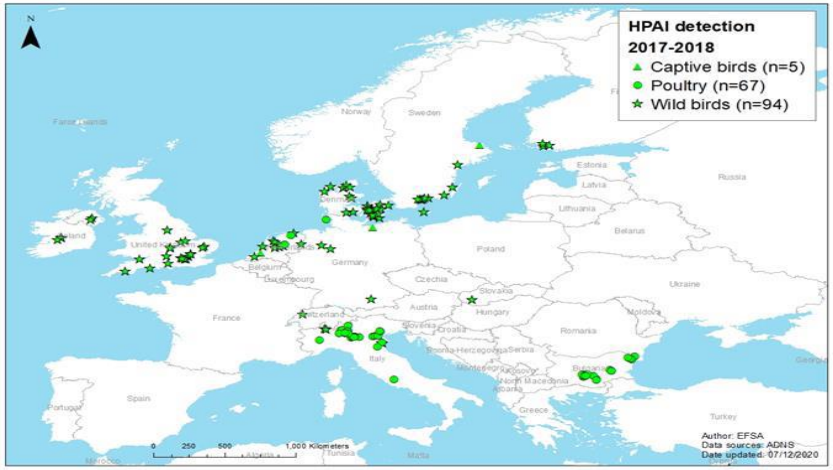


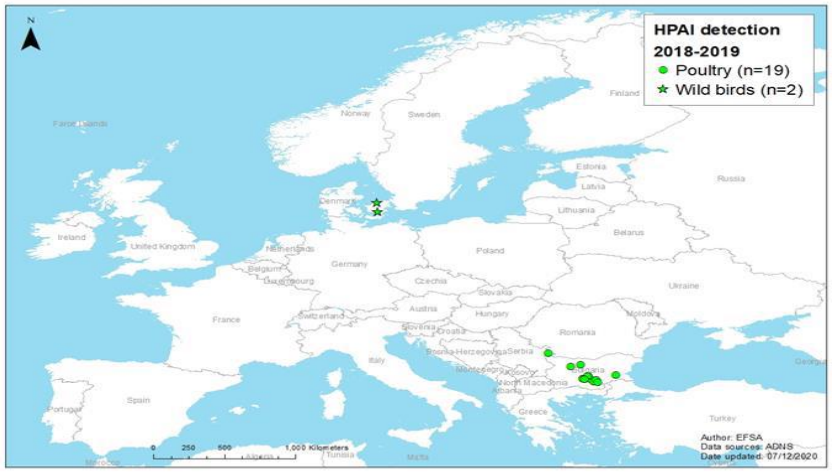
- Gap to investigate movement patterns of bridge species via telemetry
  - Small scale movements of bridge species in and around farms to better understand avian connectivity in an agricultural landscape

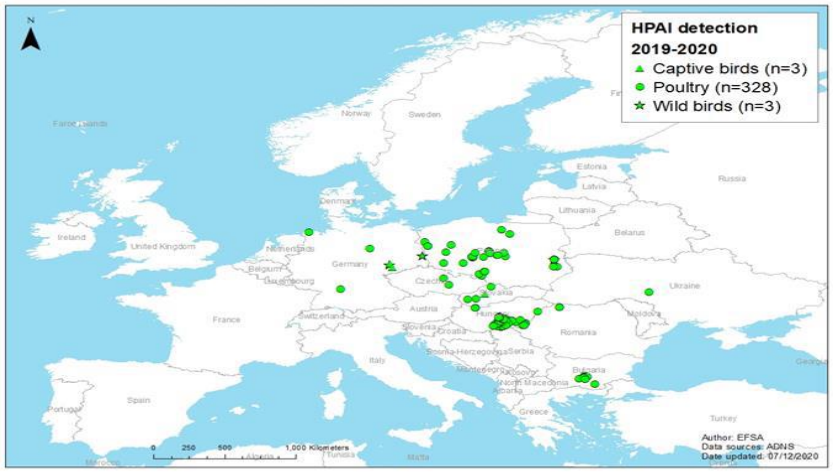


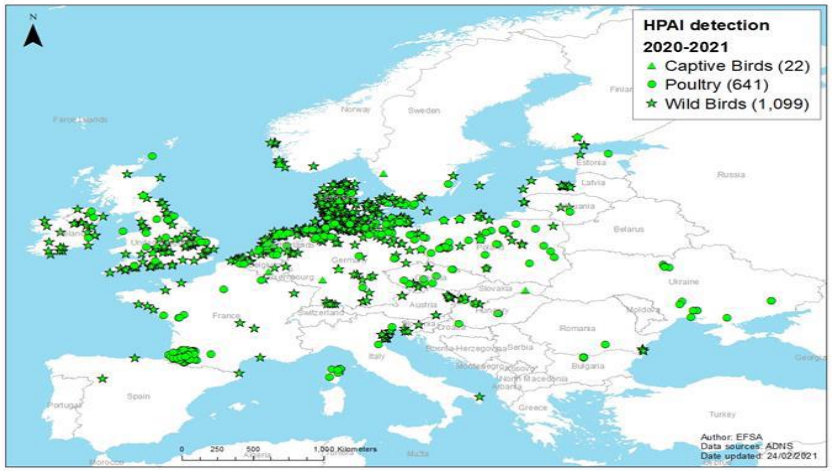
* When the date of suspicion is not available then the date of confirmation is used to assign the week of suspicion.
Data source: ADNS and OIE (23.02.2021), EFSA.


HPAI detection
2016-2017
Captive birds (n=64)
Poultry (n=1154)
Wild birds (n=1563)
Author: EFSA
Data sources: ADNS
Date updated: 07/12/2020


HPAI detection
2017-2018
Captive birds (n=5)
Poultry (n=67)
Wild birds (n=94)
Author: EFSA
Data sources: ADNS
Date updated: 07/12/2020

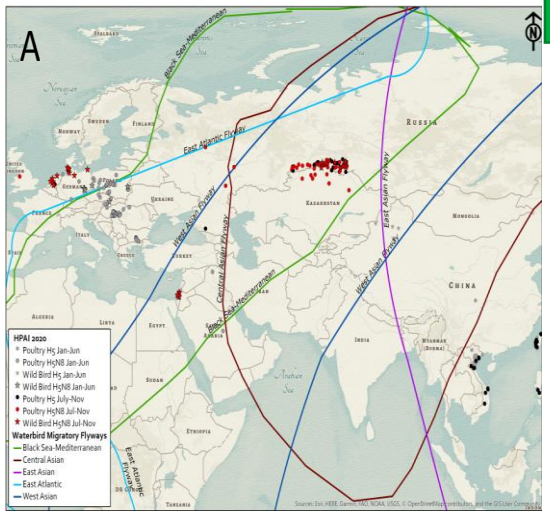

HPAI detection
2018-2019
Poultry (n=19)
Wild birds (n=2)
Author: EFSA
Data sources: ADNS
Date updated: 07/12/2020

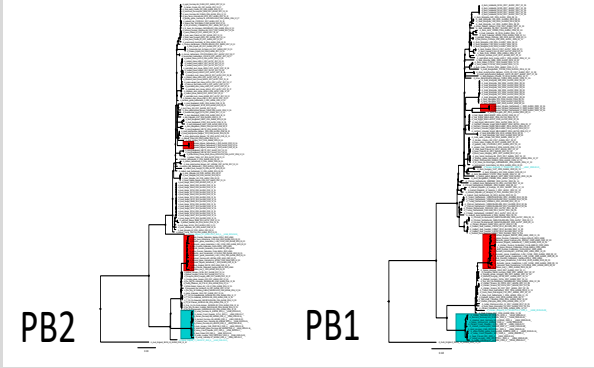

HPAI detection
2019-2020
Captive birds (n=3)
Poultry (n=328)
Wild birds (n=3)
Author: EFSA
Data sources: ADNS
Date updated: 07/12/2020

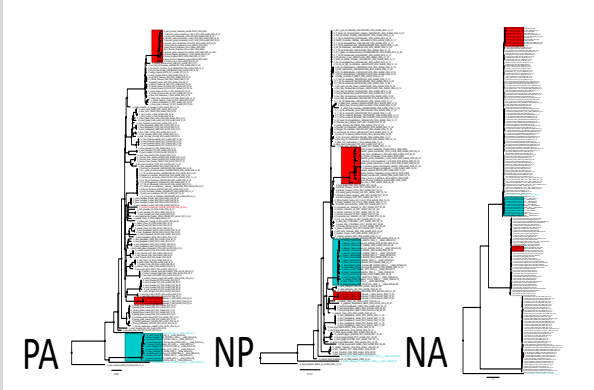

HPAI detection
2020-2021
Captive Birds (22)
Poultry (641)
Wild Birds (1,099)
Author: EFSA
Data sources: ADNS
Date updated: 24/02/2021

2021/22??

Lewis et al 2021; Emerging Microbes and Infections 10 (1) 148-151.







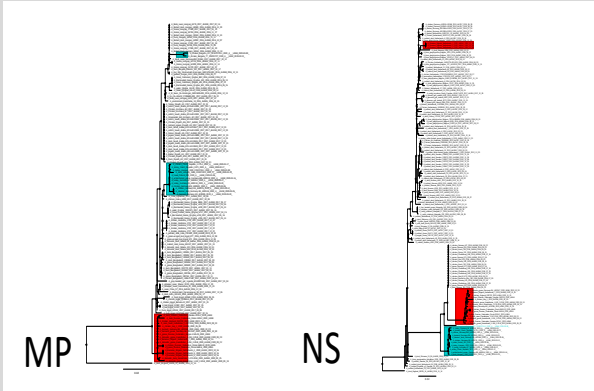


Figure 1A: *Map showing Highly Pathogenic Avian Influenza outbreaks in Europe, Western Asia and the Middle East in 2020. Simplified representations of waterbird migratory flyways are overlaid.* Location of outbreaks were obtained from Empres-i, the European animal disease notification system (ADNS) and from OIE immediate notification reports. Maps were generated using ArcGIS Pro 2.4.3.

Figure 1B:

Maximum likelihood phylogenetic trees of the HA, and PB2, PB1, PA, NP, NA, MP and NS genes.

May-November 2.3.4.4 emergent viruses are highlighted in red.

January-April 2.3.4.4 viruses are highlighted in blue

Relationships among the Eurasian 2020 H5 HPAI strains were inferred by adding the novel whole genome sequence data to data available on GisAID Epiflu downloaded on 5th November 2020. These data were aligned with MAFFT v7.407, manually curated and phylogenetic trees inferred using IQTree and ultrafast bootstrap node support. Trees were drawn using FigTree v1.4.4, rooted by A/duck/England/36226/2014 except for NA which was mid-point rooted, and nodes places in ascending order.

Where do these Clade 2.3.4.4b viruses come from; why different?

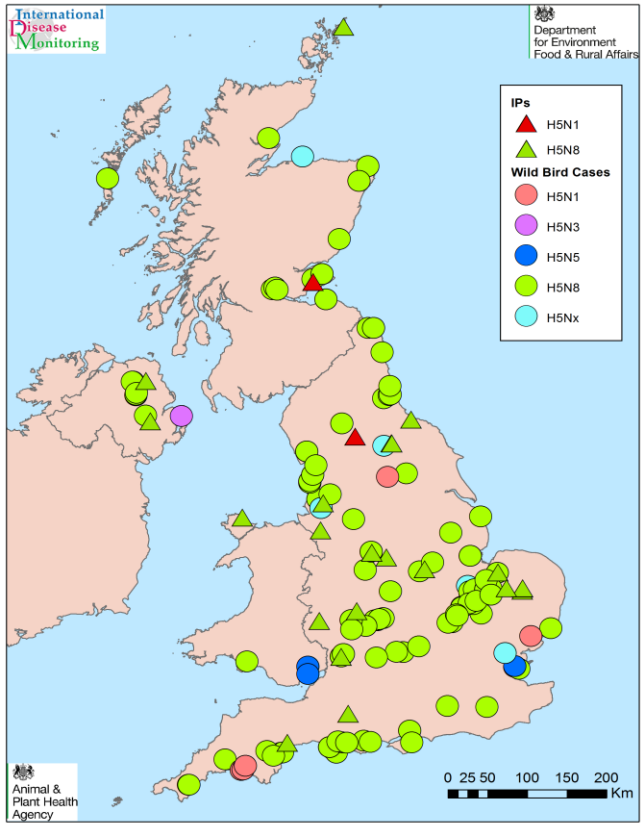
- H5N8 (late 2020)
- All genes match to those of a virus from poultry in Middle East 2018-9
  - East African flyway link?
- New genotypes now being detected
- Pathogenicity hot –IVPI 2.98

- H5N5
  - Retain at least 5 genes including HA of H5N8(late 20)
  - 3 genotypes so far

- H5N1
  - 2/8 genes from H5N8 (HA and M)

UK bespoke control measures to reduce risk to poultry

- **Continuous risk assessment to inform**
- *UK prevention order to restrict access (indirect or direct) of wild birds to poultry*
- *Housing of all poultry by order in December 2020 in force till 3/4/21*
- *Industry cooperation vital*
- Reduction in poultry cases?? N =24



UK HPAI IPs and Wild Bird Cases (as of 16/04/21)

Wild birds 317 detections

H5N8 2020-21 cases – observations

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- Biosecurity is key- attention to detail.
- Farm records management and visitors book essential.
- Infection pressure unprecedented in wild birds (RISK level changes)
- Geese over represented. Mute swans effective sentinels.
- H5N8 and H5N5 seen in wild birds – same genetic backbone. Same risk to human health (very low) and to poultry (High).
- Clinical presentations largely similar to classic HPAI in poultry.
- Infection of farmed duck species gave mild infection and recovery in older birds - younger birds succumbed but with some survival:

Pathogenicity and transmission in poultry

Highly pathogenic avian influenza virus H5N6 (clade 2.3.4.4b) has a preferable host tropism for waterfowl reflected in its inefficient transmission to terrestrial poultry A.H. Seekings, C.J. Warren, S.S. Thomas, S. Mahmood, J. James, A.M.P. Byrne, S. Watson, C. Bianco, A. Nunez, I.H. Brown, S.M. Brookes, M.J. Slomka,* Virology (2021) 559:74-85

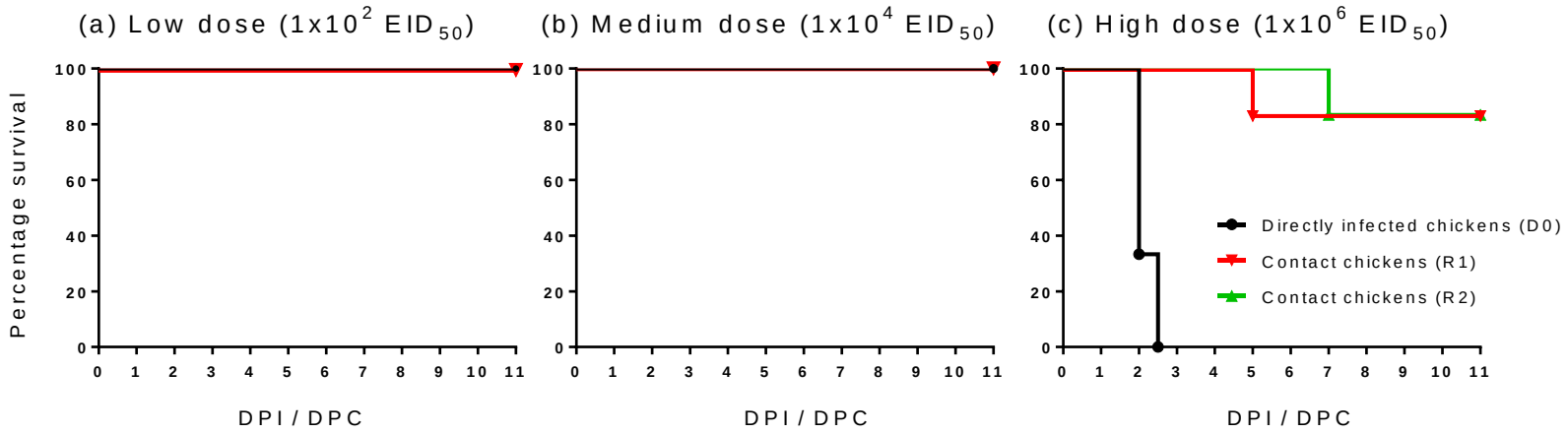
https://doi.org/10.1016/j.virol.2021.03.010

- Each outbreak has different characteristics
  - Host
  - Virus strain

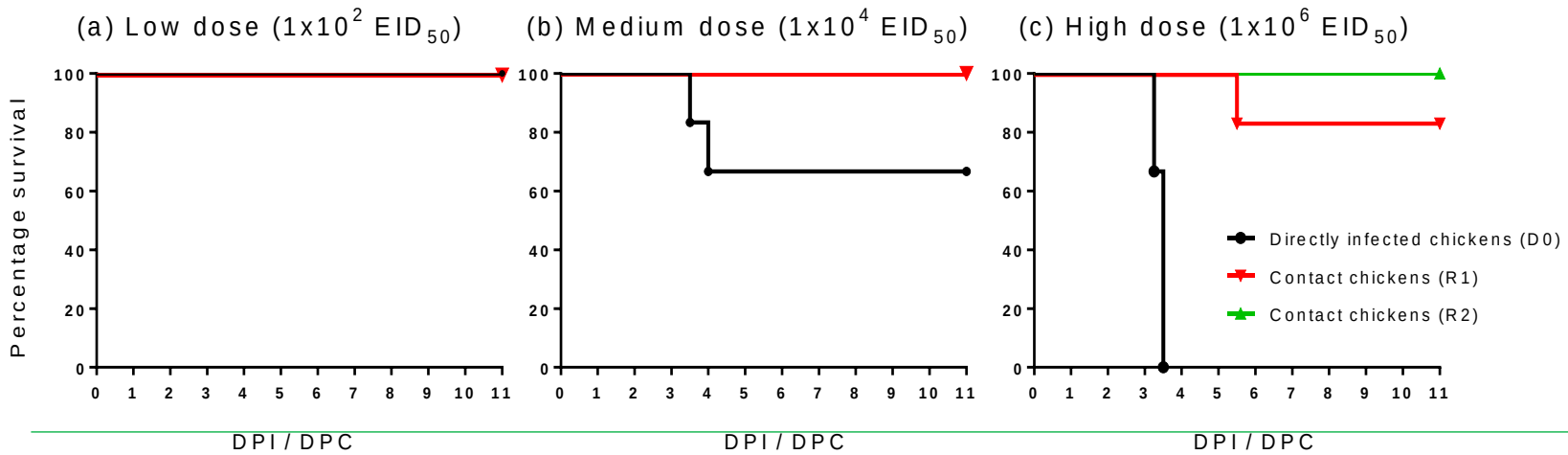
- APHA conduct during outbreak research to gather evidence for informing policy

Survival in chicken layers infected with different doses of HPAI

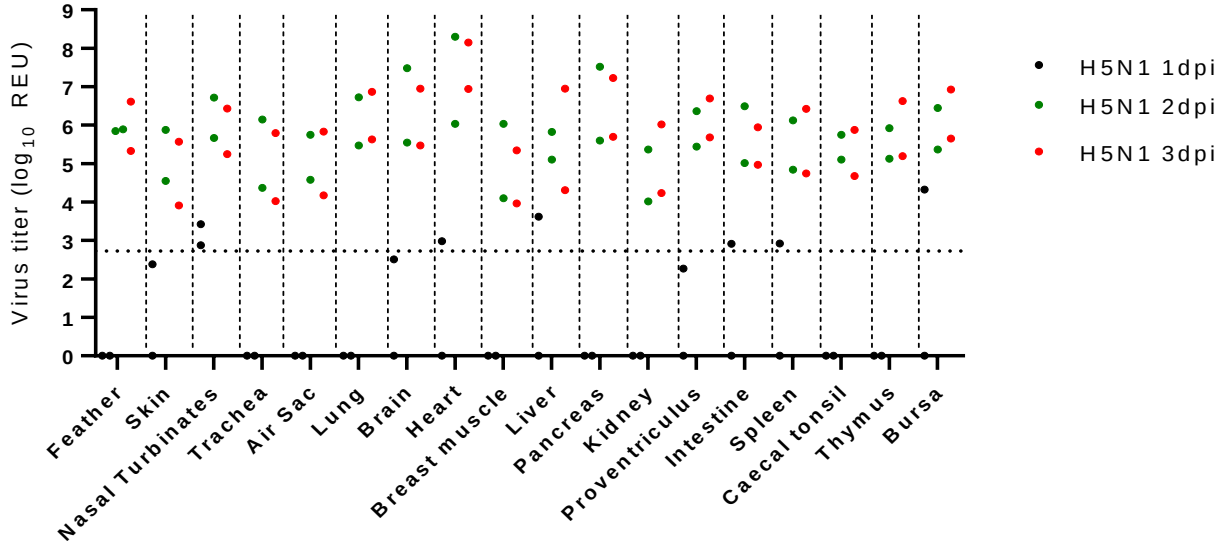
H5N1-2020: A/mute swan/England/SA14-234255/2020

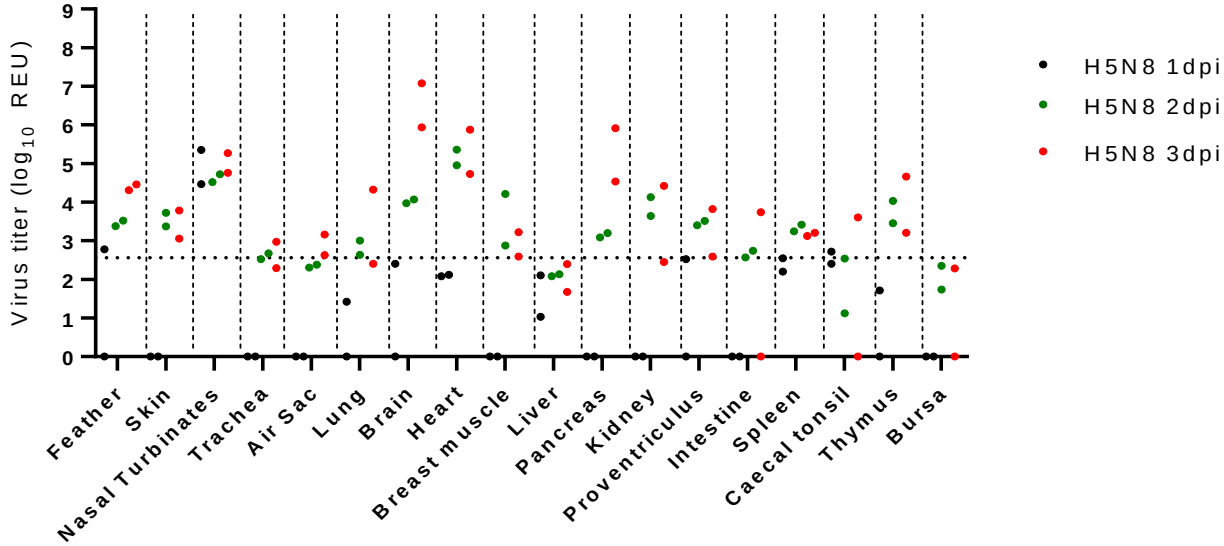


H5N8-2020: A/chicken/England/030786/2020



High dose time course tissue distribution in chicken layers





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Is the H5Nx HPAI epidemic the worst in Europe?

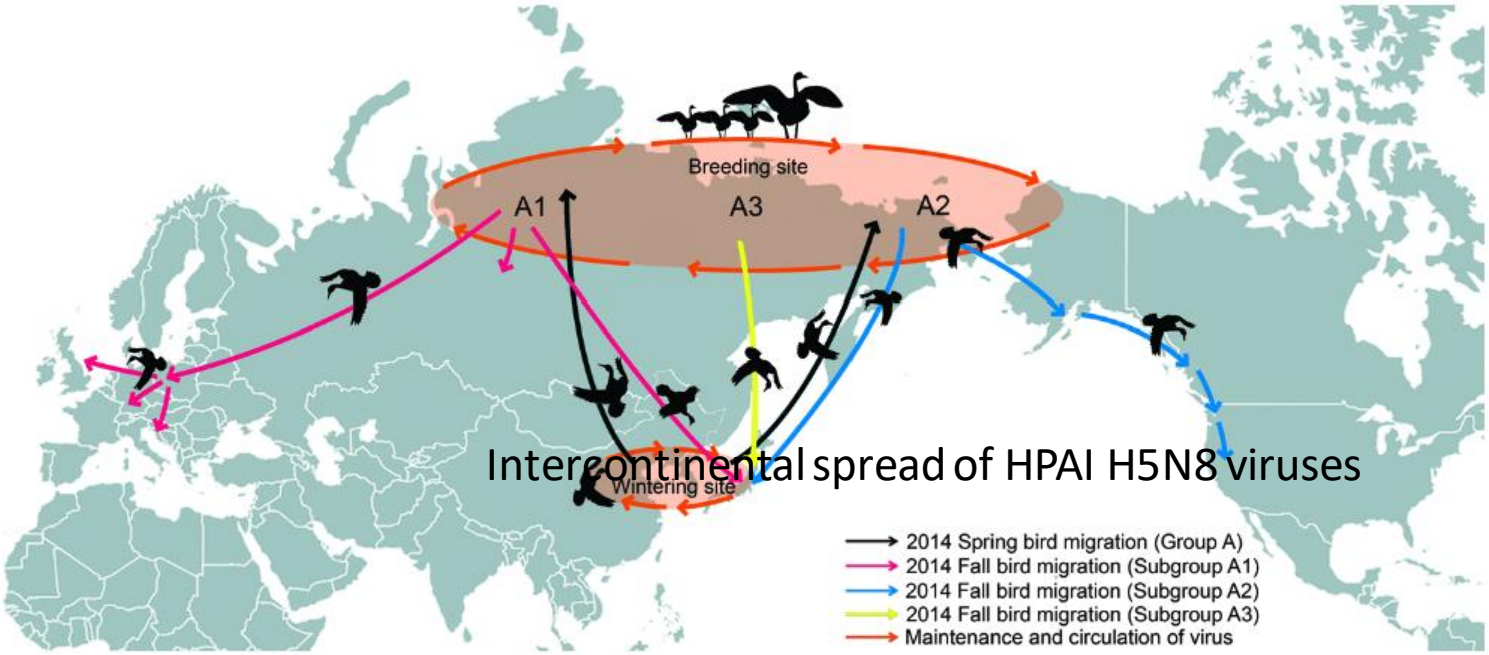
HPAI epizootics in Europe since 2000

	2005/2006 (H5N1)	2014/2015 (H5N8)	2015/2016 (H5N1, N2, N9)	2016-2018 (H5N8, N5, N6)	2019-2020 (H5N8)	2020-21 (H5N8, N1-N5)
No of countries	15	5	1	28	8	26
Poultry outbreaks	230	13	81	1188	328	967
Wild bird detections	479	5*	0	1583	3	1141
Captive Birds	0	1	0	64**	3	43
Total events	**709**	**19**	**81**	**2835**	**334**	**2151++**

"The 2020-2021 epidemic with a total of 3555 reported HPAI detections and around 22 400 000 affected poultry birds in 28 European countries appears to be one of the largest and most devastating HPAI epidemics ever occurred in Europe''. EFSA report May 21

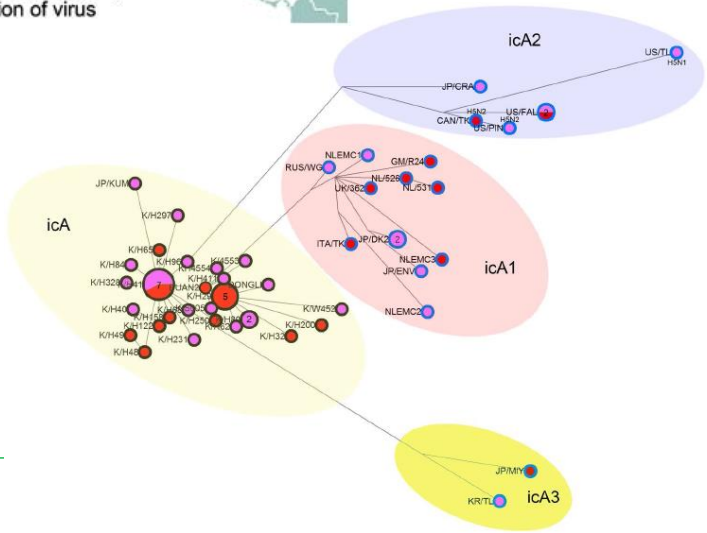
Can we expect annual new incursions to Europe??

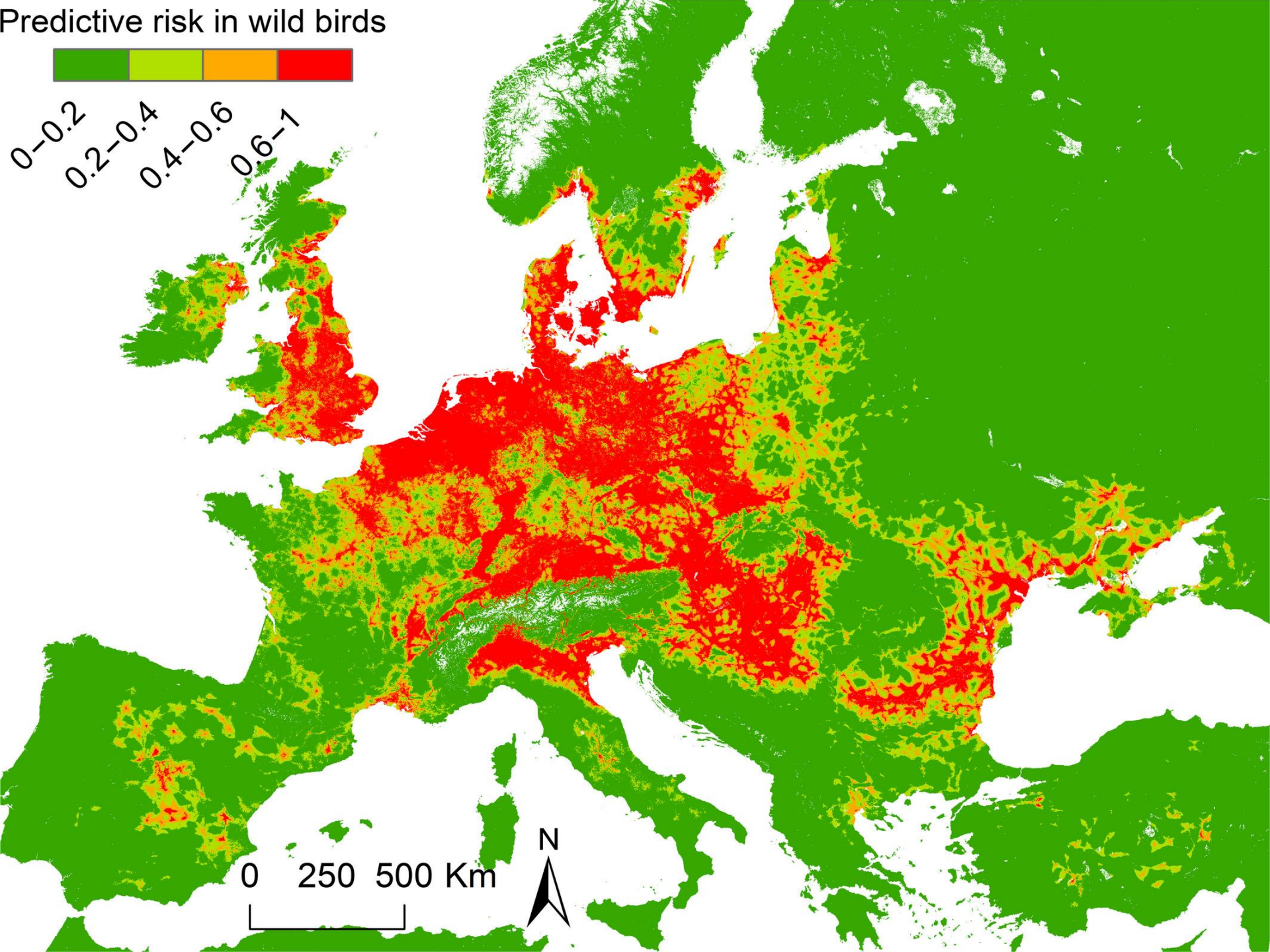
Intercontinental spread of HPAI H5N8 and H5Nx viruses



Lee et al. (2015)

Journal of Virology




Predictive risk in wild birds
0–0.2
0.2–0.4
0.4–0.6
0.6–1
0 250 500 Km
N

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Challenges for the future

Maintain high biosecurity/hygiene standards as part of normal practice

The virus or other pathogen will find the weakest link!!

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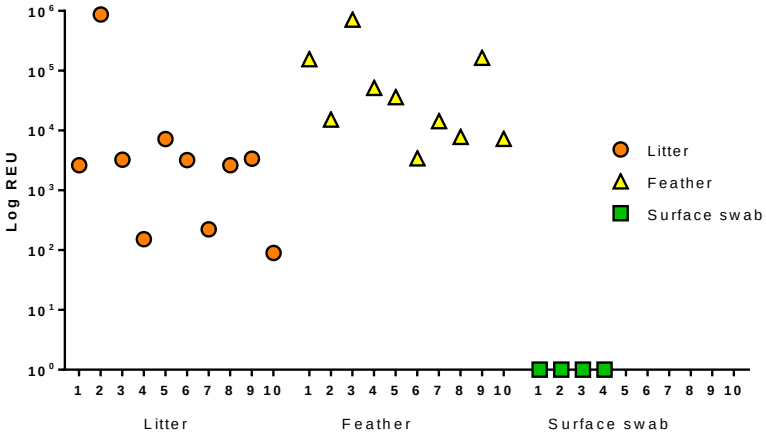
Entry pathways

- Virus source for poultry: wild bird origin

- Persistence of virus in the environment near, on and around farm
  - Virus survival in environment : >50 days/4ºC
  - Current H5N8 HPAI strains **20˚C - 8.4 days** /log of virus

DT value in days (unless specified) for log10 reduction in virus									
infectivity (* half-life in seconds) over time measured at 20°C									
Virus	Temp °C	Faeces	Litter	pH5*	pH7.2	pH9	Distilled water	Salted water	UVB Exposed
H7N1	20	0.83	<5min#	34.76sec*	18.46	8	21.28	14.42	2.63hrs
H5N1	20	4.41	<10min#	28.74sec*	15.17	3.61	52	52	2.78hrs
	*half-life in seconds	# complete loss of infectivity							



- Mode of introduction?: indirect via fomite
  - Including indoor units!

Assessment of virus survival for H5N8-2020 (APHA unpublished data)

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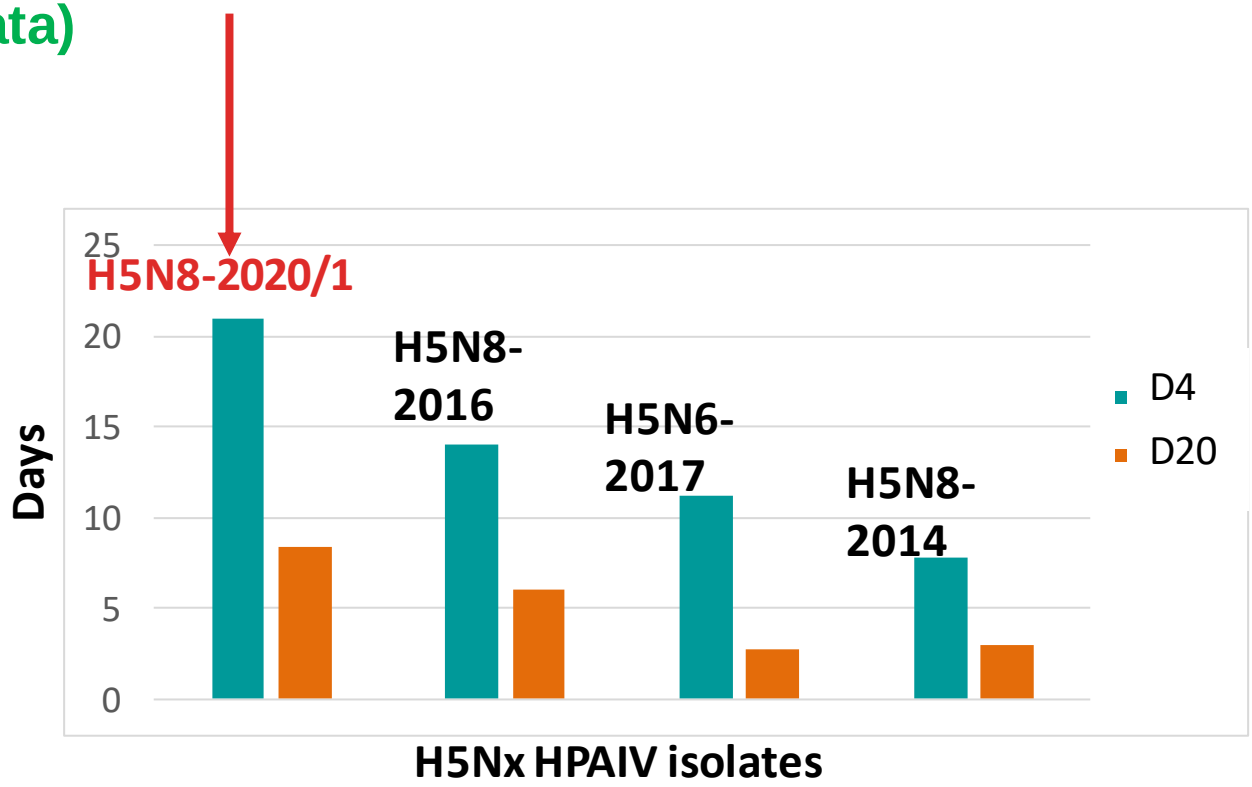
- **H5N8-2020 at 4˚C - 21.0 days/log of virus**
- **H5N8-2020 at 20˚C - 8.4 days** /log of virus
- H5N8-2020 at 30˚C - TBD



Conclusions

- *Compared to H5N8-2016 HPAIV data show H5N8-2020 HPAIV can survive 50% longer at 4˚C, and 40% longer at 20˚C*

AVIAN INFLUENZA BIOSECURITY GUIDE

YOU CAN MAKE A DIFFERENCE

NFU

British Free Range Egg Producers Association BFREPA

BIOSECURITY

Preventive measures applied at the farm, external environment, and entry points to the chicken house.

It reduces the risk of introducing pathogens that could affect the health of the flock, and could help stop the spread of avian influenza.

Prevent the spread of avian influenza

Important whole site considerations:

- **All** staff and guests should have training on farm procedures before starting work.
- **Only** clean vehicles with disinfected wheels and wheel arches can enter or exit (or where access is not essential they should be left at site perimeter).

- **Strictly** limit and control access to poultry flocks
- **Keep** visitors to a minimum and ensure you keep a visitor book
- **Maintain** active rodent controls
- **Minimise** contact between poultry and wild birds
- **Create** and regularly update biosecurity and AI contingency plans

Anything intended for use inside the shed such as bedding and feedstuffs should be safely stored on the outside and not open to the environment including wild birds and their faeces

TOOLS AND EQUIPMENT

Must Haves:

- Dedicated tools for each house.
- **Do** - Disinfect tools and equipment regularly and always before entering the house.
- **Do** - Protect tools from contamination by vermin or wild bird droppings.

Reduce the number of personnel coming on site, and of those that do, restrict access to limit possible contamination

INTERNAL ZONE — The ante-room/annex has two zones using a barrier (30cm high): Internal to the chicken house and external.

HOUSE ENTRY & EXIT PROCEDURE

The internal zone has everything you need to perform your work without crossing back to the external zone. — EXTERNAL ZONE

OUTSIDE | ANTE-ROOM / ANNEX EXTERNAL | ANTE-ROOM / ANNEX INTERNAL | CHICKENS | ANTE-ROOM / ANNEX INTERNAL | ANTE-ROOM / ANNEX EXTERNAL

- Use the foot dip provided (DEFRA/WG/SG approved disinfectant, recommended concentration). Replenish regularly in accordance with manufacturers instructions.
- Enter the house ante-room / annex and close the door behind you.
- Remove your outdoor footwear.
- Cross the barrier into the internal zone.
- Put on your indoor footwear and protective clothing (with exclusive use for that house). Incl. hats, gloves and RPE.
- Sanitise your hands - you are ready to go in.
- Clean any gross debris from your boots.
- Remove your indoor footwear protective clothing, hats, gloves etc and leave in the internal zone.
- Cross the barrier into the external zone of the ante-room / annex and put your outdoor footwear on.
- Sanitise your hands.
- Use the foot dip and leave the ante-room / annex.
- Close the door behind you.

IN → OUT

LIMIT THE ATTRACTIVENESS OF THE SITE TO WILD BIRDS - INCLUDING LOCHS, STANDING WATER, GRAZING AREAS AND SPILT FOOD

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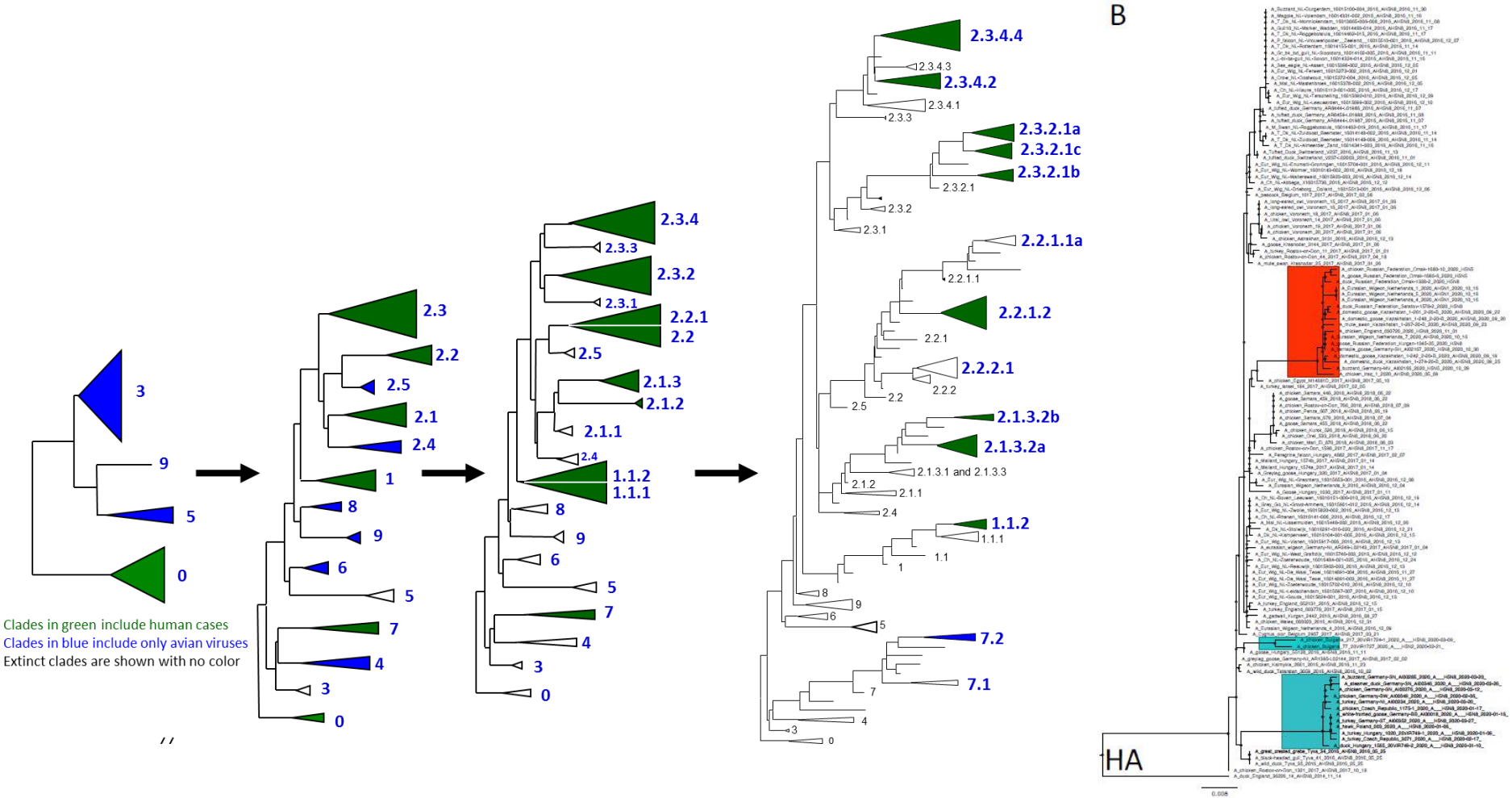
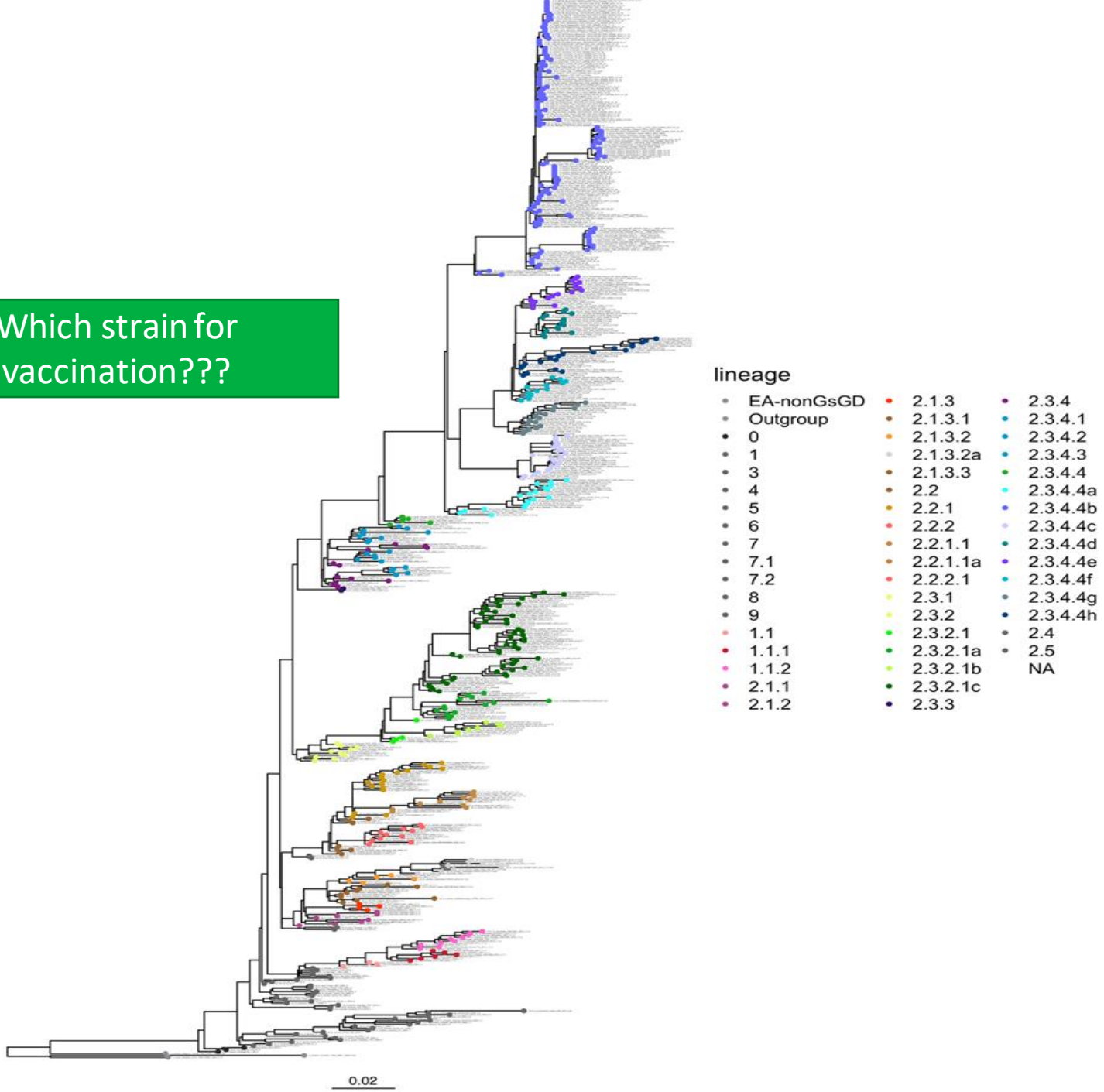
Evolution of H5 HPAI viruses



Lewis et al. 2020, in press

Which strain for vaccination???



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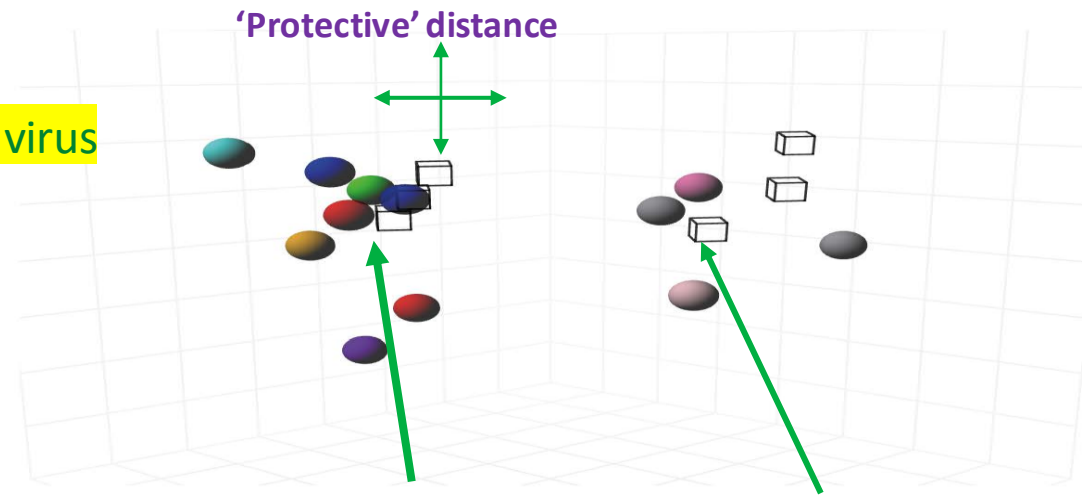
Vaccines must be antigenically matched to circulating strains for efficacy

- Antigenically matched vaccines to field strains
  - can prevent disease, reduce shedding of virus, but may not completely prevent transmission to naïve birds

- Poorly matched vaccines to field strains
  - Reduce disease signs, may partially reduce virus shedding but will not stop transmission between birds/flocks
  - Without active monitoring of vaccinated flocks may at worst enable silent spread

Map of vaccine antibody match to field virus

- A/DUCK/POTSDAM/84 H5N6
- A/CHICKEN/SCOTLAND/59 H5N1
- A/ANHUI/1/2005 H5N1
- A/TURKEY/TURKEY/1/2005 H5N1
- A/SICHUAN/26221/2014 H5N6
- A/DUCK/ENGLAND/36254/2014 H5N8
- A/DUCK/HYOGO/1/2016 H5N6
- A/TURKEY/HUNGARY/53433/2016 H5N8
- A/MUTE SWAN/CROATIA/102/2016 H5N5
- A/TURKEY/ITALY/17VIR576-11/2017 H5N8
- A/CHICKEN/ENGLAND/030786/2020 H5N8
- A/CHICKEN/ENGLAND/030547/2020 H5N8



Current strains

2005/6 vaccines

Lewis et al 2021 in press Vaccine

Impact of poor vaccine match

- Influenza makes copying errors and some fix in changed amino acids
  - Can effect antigenicity of virus
- Viruses with antigenic changes that escape vaccinal immunity
- These viruses may be selected for in a vaccinated population
- Likely very small proportion are ‘fit’
- The long evolutionary branches on recent viruses support maintenance in poultry populations

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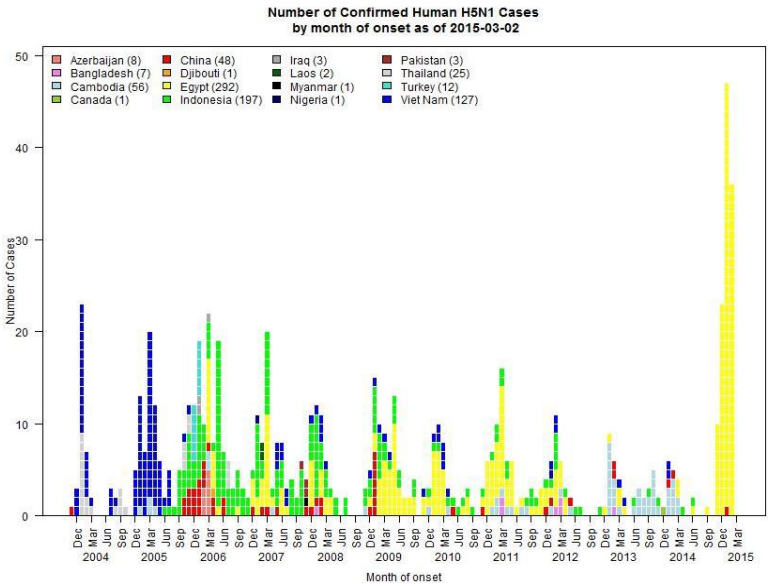
Zoonotic threat

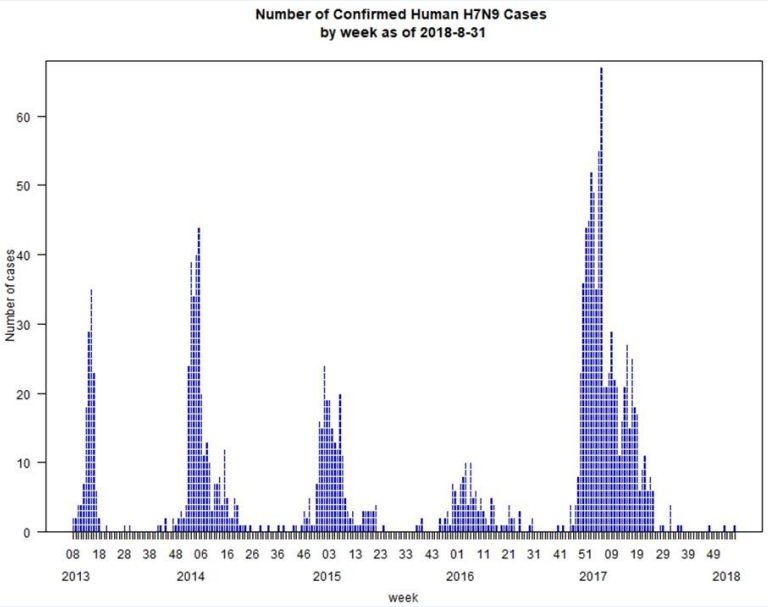
Zoonotic spillover events



H5N1 HPAIV
WHO 23.10.20:
16 countries 455/861 (cfr 53%)

7 cases of human H5N8 infection in Astrakan region in Dec 20
Workers on infected poultry farm c1m birds
PCR and antibody positive
Genetically virus largely identical to poultry virus
No reported transmission between humans

These are first ever reported H5N8 infections in humans



H7N9 LPAIV
FAO 04.12.2019:
3 countries (all cases epidemiologically linked to China)
616/1568 (cfr 39%)

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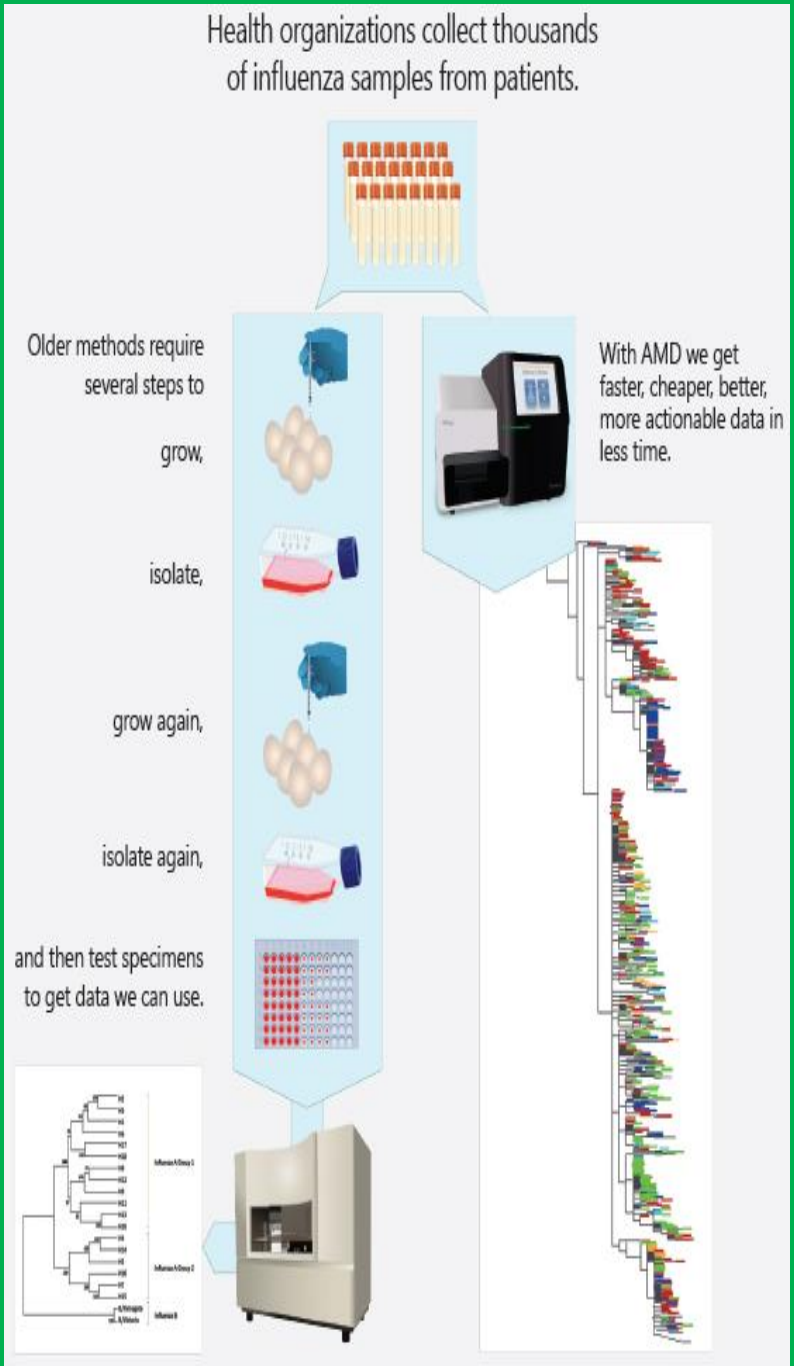
Zoonotic risk and food safety

- **No evidence** of H5N8 viruses infecting humans
- Infection and therefore establishment within community deemed very low
- **No resistance** to antivirals detected in sequence
- Full genome sequence has been assessed for humanising mutations according to a global mutation database (CDC) that have been associated with the ability to infect, replicate in and caused disease in humans- **No humanising mutations detected**- threat essentially =2016 H5N8
- **FSA advice properly cooked foods safe to eat**

Table 1. A/chicken/England/030685/2020 H5N8: Genetic changes that may increase virulence, signal adaptation to mammalian species or alter susceptibility to existing antivirals.

Protein	Amino acid position/motif	Phenotypic consequences	Reference
PB2	I292V [Same as 2016]	Increased polymerase activity in mammalian cell line, increased virulence in mice	Gao et al. 2019; Xiao et al. 2016
	V598T [Same as 2016]	Increased polymerase activity and replication in mammalian cells, increased virulence in mice	Hu et al. 2017
	K627E [Same as 2016]	Increased virulence in chickens	Schat et al. 2012
	S715N [Same as 2016]	Decreased virulence in mice	Sun et al. 2015
	L89V, G308D [Same as 2016]	Increased polymerase activity in mammalian cell line and increased virulence in mice	Li et al. 2009
	L89V, G309D, T339K, R477G, I495V, K627E, A676T [Same as 2016]	Increased polymerase activity in mammalian cell line and increased virulence in mice	Li et al. 2009



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Conclusions/lessons learnt

- A new H5 HPAI virus(es) emerged in the Middle East and spread to Central Asia (including Russia) during 2020
- Wider spread to Europe and Africa; largest European epidemic with HPAI still ongoing
- Incursions to new areas mediated by wild birds but secondary transmission between poultry farms
- Internationally harmonised PCR best tool for diagnosis
- Virus can be maintained in environment for long periods; farm biosecurity needs strengthening
- Traditional vaccines based on 2005/6 H5N1 viruses may have limited efficacy for these current viruses
- Zoonotic risk considered low but case amongst poultry workers on farm in Astrakan region
- Prospects for immediate control/future threat remain challenging!
- Current activity suggests possible threat this winter; batten down the hatches!

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Emergence of H5N8;EMI 2020

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Thank you for your attention

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