Animal &
Plant Health
Agency

Addressing future risk based AI surveillance challenges for Europe and application of relevant diagnostics

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APHA-Weybridge

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Virtual Meeting 23rd – 24th September 2020

Problem to be addressed

- Wild bird surveillance has successfully been used as an early warning tool for H5 HPAI
- Funding fatigue and EU programme has not greatly changed over many years
- Have the historical programmes enriched our understanding of virus and host ecology?
- Is the surveillance structured to provide true early warning?
- To address continuing threat we need to apply surveillance/develop tools to understand mechanisms of virus reassortment and how the ecology of the natural host, connectivity with poultry, shapes and influences virus selection and emergence

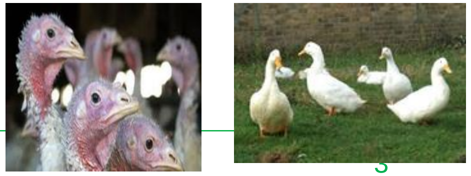
Spread of H5Nx (goose/Guangdong lineage) viruses to Europe

- Mediated via wild birds; migratory waterfowl
- Multiple epizootics

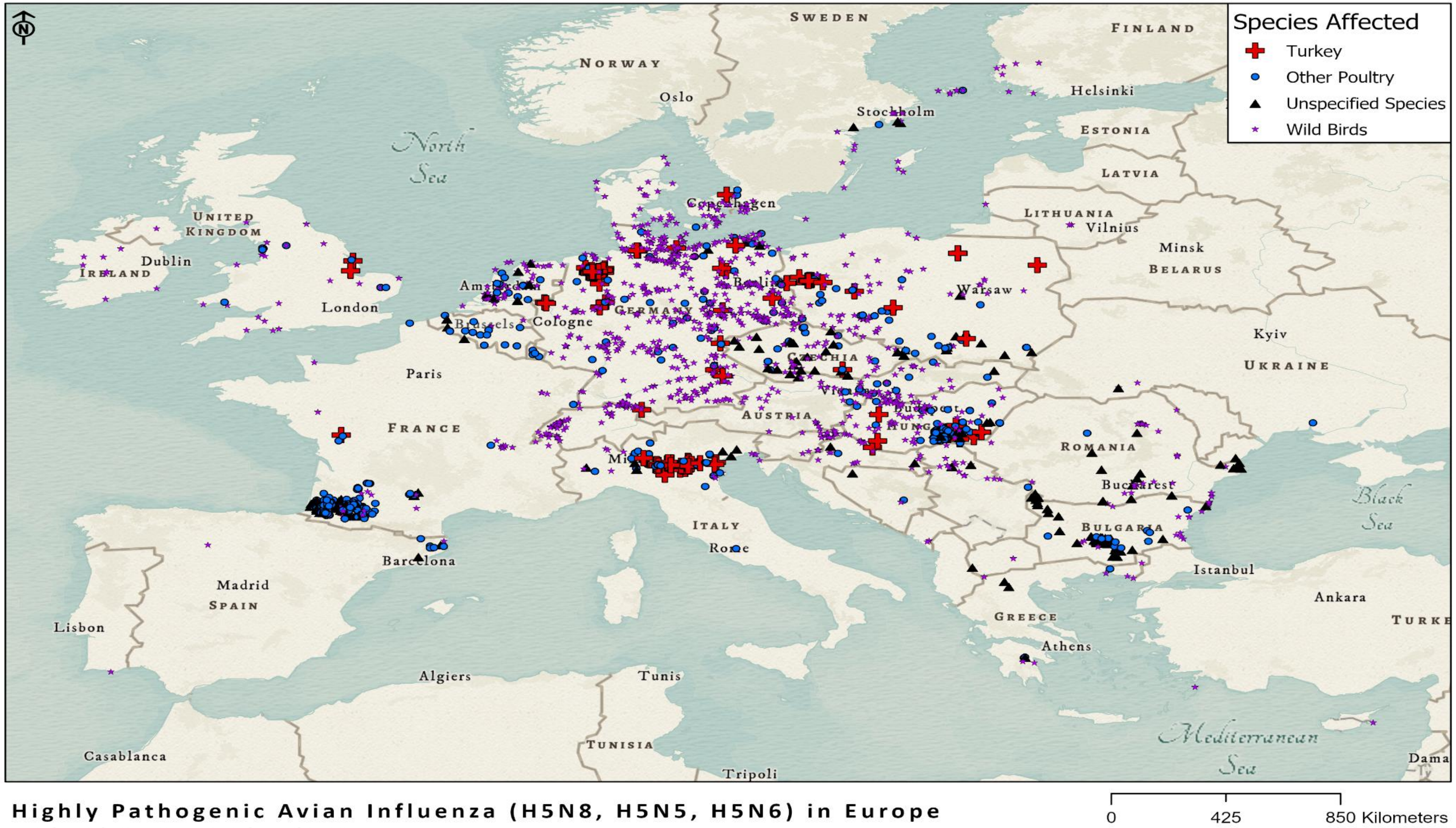
- 2005-2010: H5N1 pathogenic in wild birds; multiple poultry outbreaks
- 2014/2015 : H5N8 non pathogenic in wild birds; few poultry incursions
- 2016/2017: H5N8 (closely related virus) pathogenic in wild birds; large poultry epizootic

- 2017/2018: H5N6 (closely related in six genes to H5N8) less pathogenic in wild birds? Few poultry incursions

- 2017/2018: H5N8 (same virus as 16/17) ongoing minor epidemic in poultry in IT and BG
- 2020: H5N8 (reassorted 16/17 virus 6/8 genes): few wild bird cases; widespread poultry outbreaks



Highly pathogenic avian influenza virus October 2016 to November 2017



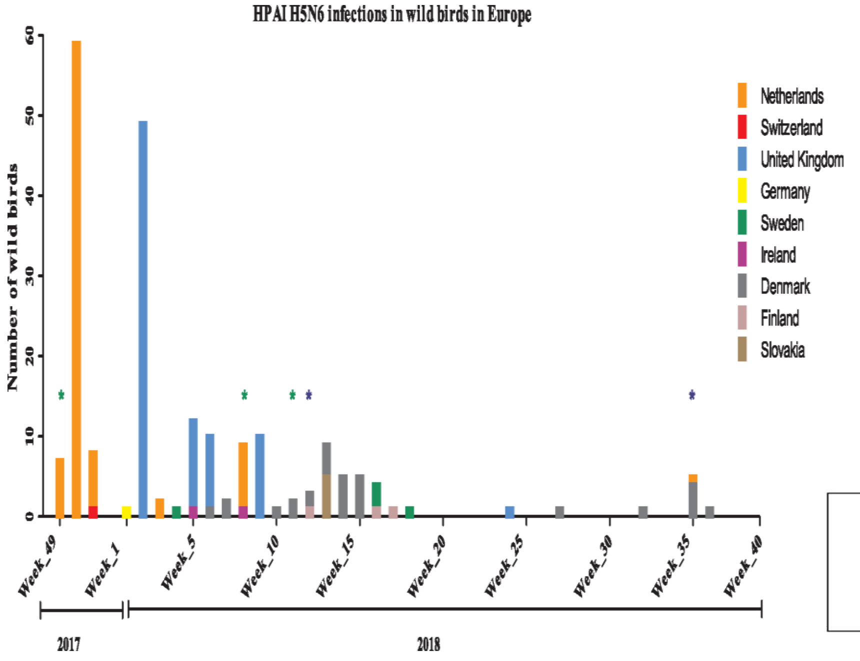
Highly Pathogenic Avian Influenza (H5N8, H5N5, H5N6) in Europe
19/10/2016 - 30/11/2017

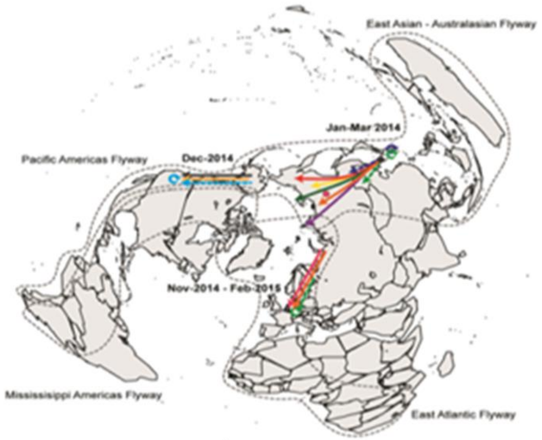
Closest genetic relatives:

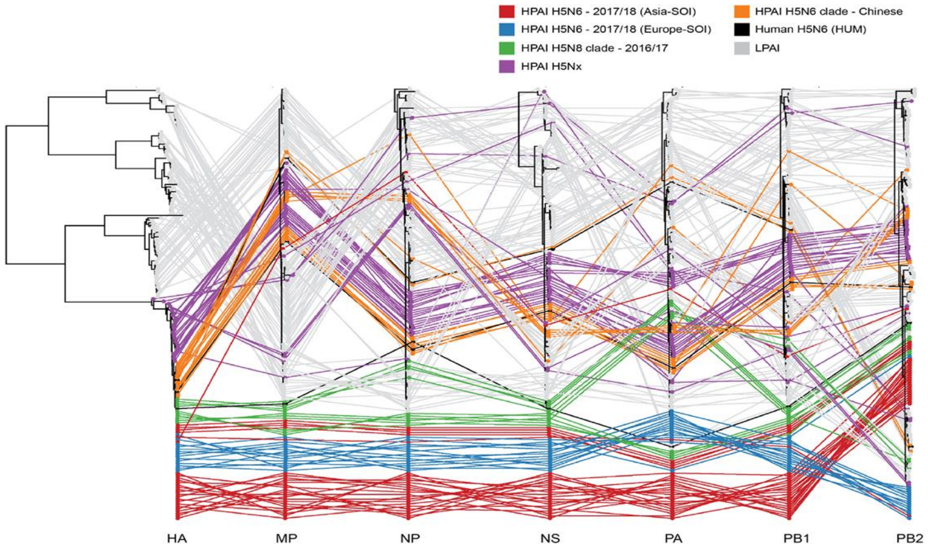
A/mute_swan/England/SA21_180652/2018(H5N6)

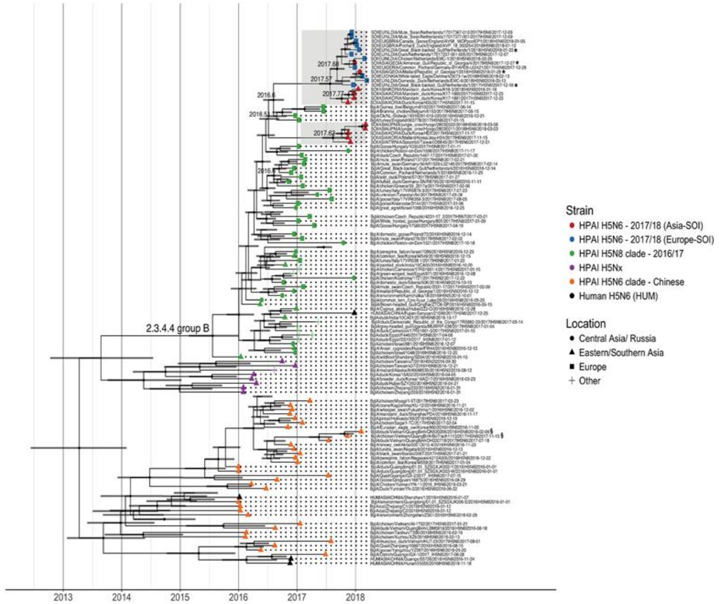
- PB2: H6N1|A/Mallard/Republic_of_Georgia/7/2015|MD|2015-10-10
- PB1:H5N8|A/mallard/Republic_of_Georgia/2/2016|MD|2016-12-9
- PA: H7N7|A/Mallard/Republic_of_Georgia/10/2016|MD|2016-08-30
- HA: H5N8|Russia|A/chicken/Chechnya/58/2017|2017-01-08
- NP:H5N8|A/mallard/Republic_of_Georgia/2/2016|MD|2016-12-9
- NA:H4N6|A/Domestic_Duck/Republic_of_Georgia/8/2016|OD|2016-09-14 (sentinel in the duck trap)
- MP: H5N8|Belgium|A/Cygnus_olor/Belgium/1567/2017|2017-02-21
- NS: H5N8|A/mallard/Republic_of_Georgia/2/2016|MD|2016-12-9

Multiple seeding events with H5N6 in Asia and Europe 2017-18



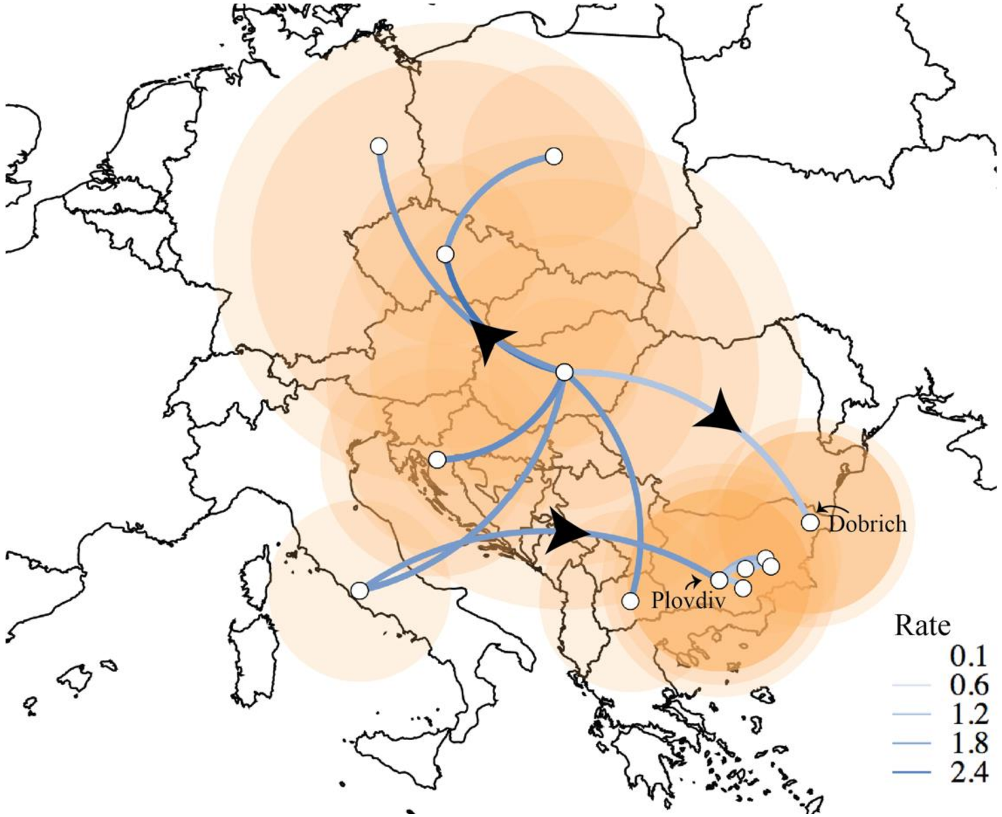


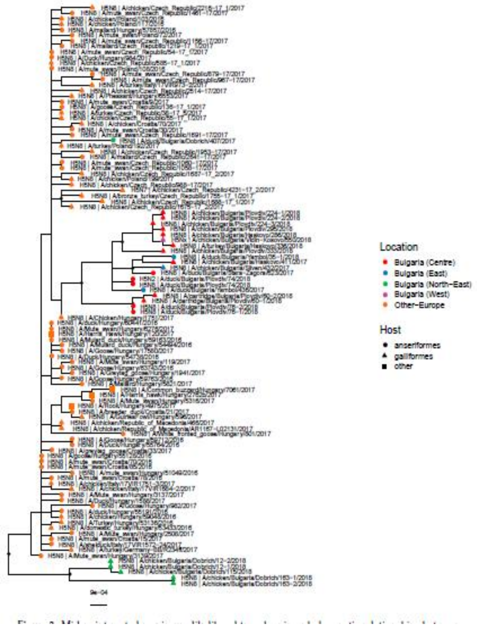


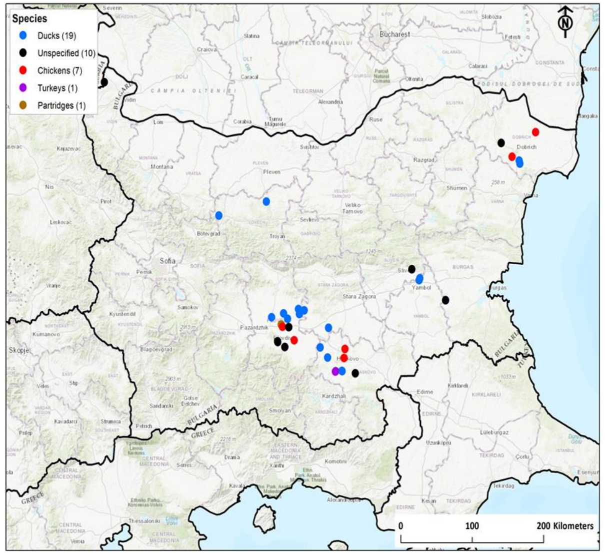


Poen et al; Virus Evol, Volume 5, Issue 1, January 2019, vez004, https://doi.org/10.1093/ve/vez004

Independent ‘local’ evolution in some settings





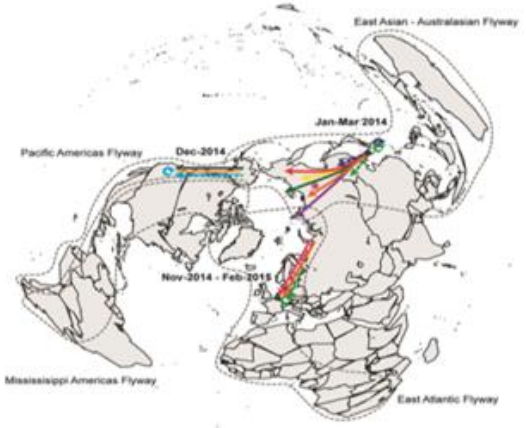


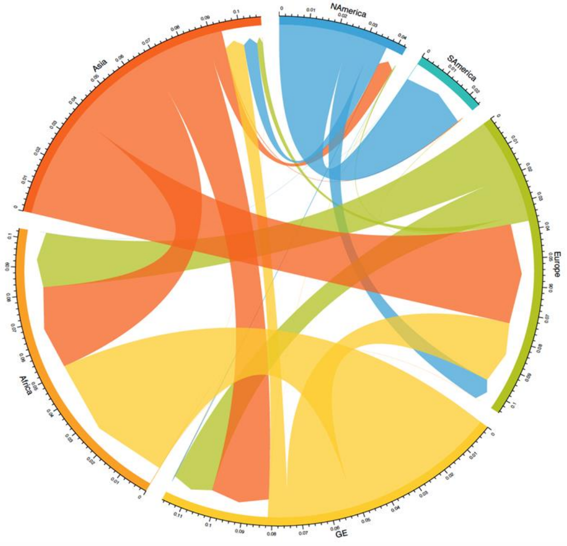
Venkatesh et al (2020) Regional transmission and reassortment of 2.3.4.4b highly pathogenic avian influenza (HPAI) viruses in Bulgarian poultry 2017/18; *Viruses; 12*(6), 605; https://doi.org/10.3390/v12060605

Evolution in the wild host reservoir; better understanding required

- Understand the role wild birds might play in disseminating influenza A viruses between geographic areas

- Characterize the evolutionary dynamics of avian influenza A viruses in places where bird population turnover is high due to frequent migratory events, and where there are overlapping host species distributions and annual life cycle stages





Role for migratory wild birds in the global spread of avian influenza H5N8 *Science* 14 Oct 2016: Vol. 354, Issue 6309, pp. 213-217

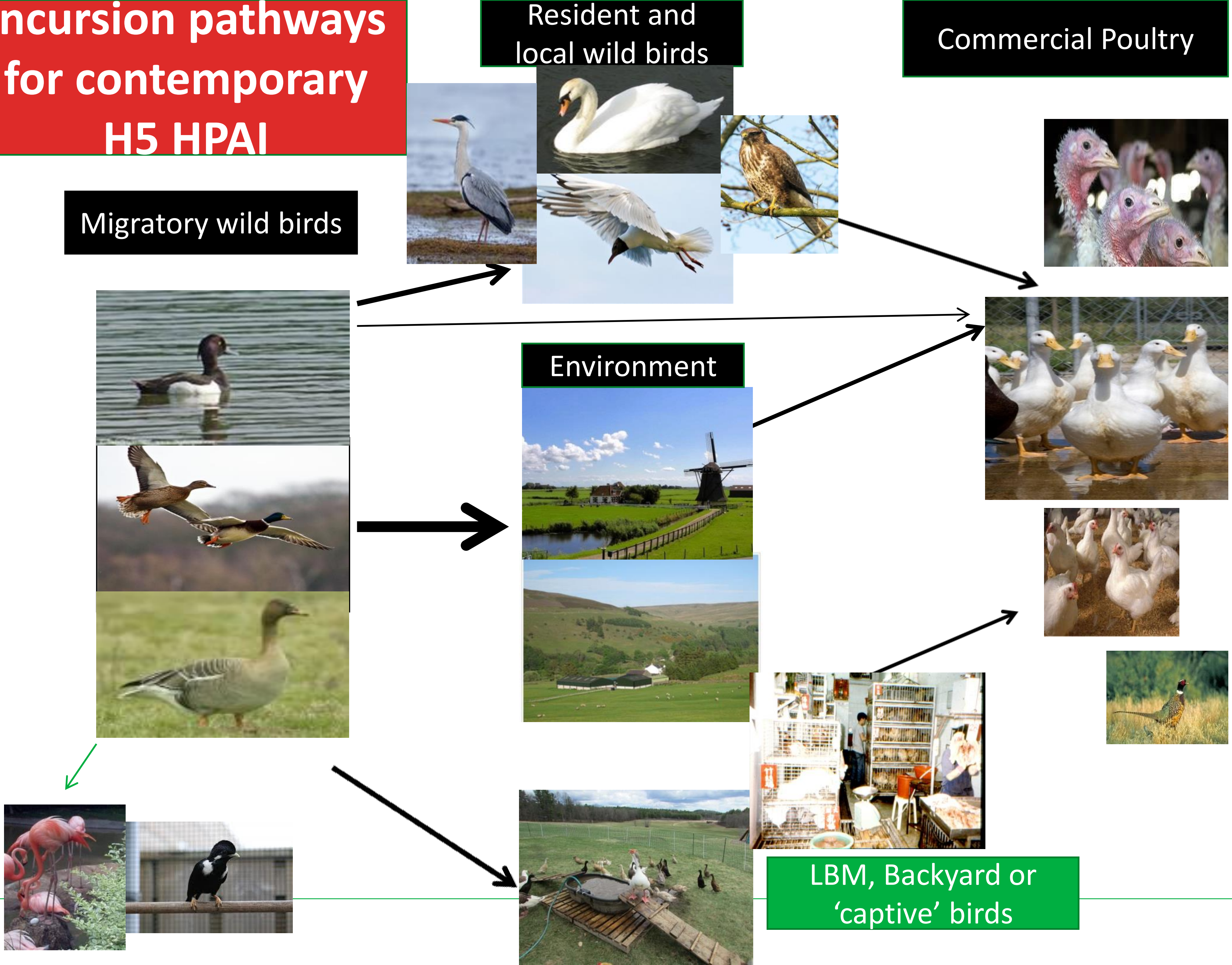
Requirements for effective early warning surveillance

- To assess the risk for Europe from emerging HPAI viruses and to reduce the potential for enzootic circulation
- Requirement for hypothesis-driven surveillance in wild bird vectors to identify HPAI virus at early warning sites.
- Develop and optimise novel detection and characterization strategies for avian influenza viruses.
- Focusing within Europe, new risk metrics for poultry farms are required to identify:
  - pathways for incursion
  - potential timing and geospatial location
  - informing proportionate risk mitigation measures.
- Goal: An early-warning system that will buy time for the poultry industry by 1-2 months; earlier detection of HPAIV incursions in Europe to enable rapid responses and enlarging the window for detection and intervention.

What is the threat 2020, 2021, 2020??

OFFLU microreact map until 02/09/2020. Empres-i data

Incursion pathways for contemporary H5 HPAI



Resident and local wild birds

Commercial Poultry

Migratory wild birds

Environment

LBM, Backyard or ‘captive’ birds

Impacts of more ‘intelligent’ early warning systems/causal understanding

Knowledge of key vectors and timing will enable the

- application of risk based surveillance that will enable

i) implementation of risk based measures earlier to prevent introductions to poultry

ii) Intelligent regional application of measures according to risk and type of production system.

- Integrate evidence-based studies to move beyond pre-existing surveillance with its demonstrable limitations to incorporate
- cross-cutting methods in ecology, epidemiology, population disease dynamics and evolution,
- risk mitigation investigations that will influence farming practice and policy, resulting in better disease prevention.

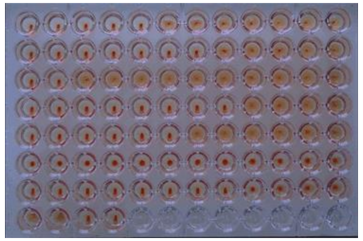
Novel detection strategies for AIV surveillance

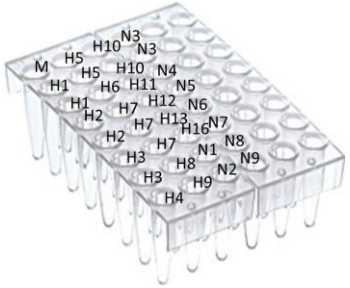
- Serology for wild bird application

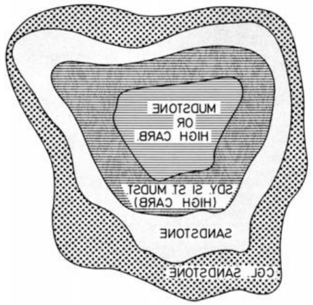
- Rapid sub- and pathotyping of viruses

- Gene sequencing and accurate host speciation

- Environmental sampling







Outputs

i) the presence (and frequency) e.g. of hemagglutinin sequences of HPAI viruses

ii) detect other relevant viral genetic traits

iii) investigate bird intensity and diversity and AIV diversity and evolution at temporally-relevant times.

Identification of key sites and species for early-warning system at eastern front of Europe

Identify key sites

- Identify regions bordering the eastern part of the Europe that are most suitable for environmental sampling and developing active surveillance sites ie wetlands in Ukraine, Georgia and Lithuania

Data sources on waterfowl distribution and migration:

i) distribution and staging data on key host dabbling ducks on the eastern front of the Europe (west of the Ural mountains);

ii) connectivity data from ringing recoveries, from ducks ringed and reported to national ringing schemes

iii) telemetry data from long-distance migrant ducks

- Exploit opportunities for passive surveillance

Identify key species

- Apply alternative techniques for environmental sampling combined with DNA barcoding for host species identification
- Serology on bird species under-represented in past surveillance programs (“targeted sampling”, revised list of target species EFSA 2017).

Identification of sentinel and bridge species for risk assessment of poultry introductions

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

- 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

Poultry industry risk-mitigation and policy

- Metadata analyses
    - AIV infection data collected from vector
    - Wild bird species’ temporal and spatial distributions within Europe

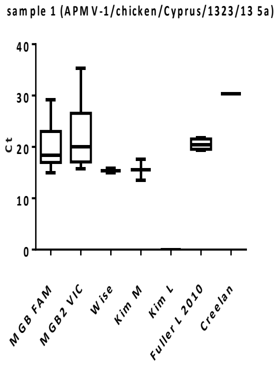
    - Determine high risk areas and high risk time windows for the poultry industry

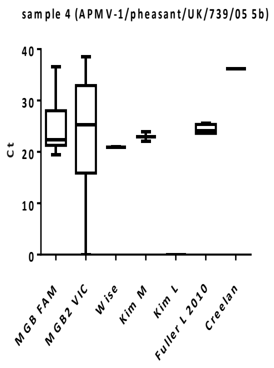
- Risk assessments of farming practices and mitigations

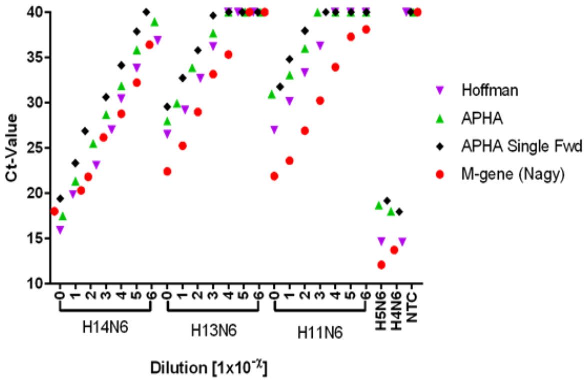
- Identify and evaluate (in terms of acceptance and effectiveness) potential key interventions against AIV incursion from wild birds in different poultry farming systems.
    - multiple countries, including with no-intervention controls

Continual programme of ongoing diagnostic test fitness for purpose- confidence in international assays

- Influenza viruses are highly variable and evolving and any assay should be continually assessed for validity
- All virus strains received through our international activities are tested in our recommended standard tests to ensure detection
- As new strains emerge we develop new tools if required for rapid detection/characterisation


sample 1 (APMV-1/chicken/Cyprus/1323/13 5a)


sample 4 (APMV-1/pheasant/UK/739/05 5b)



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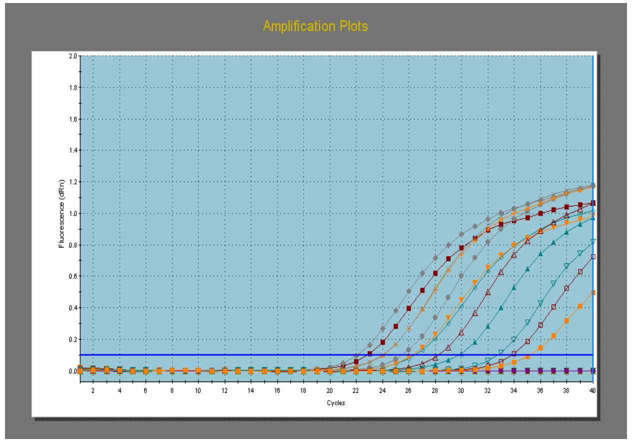
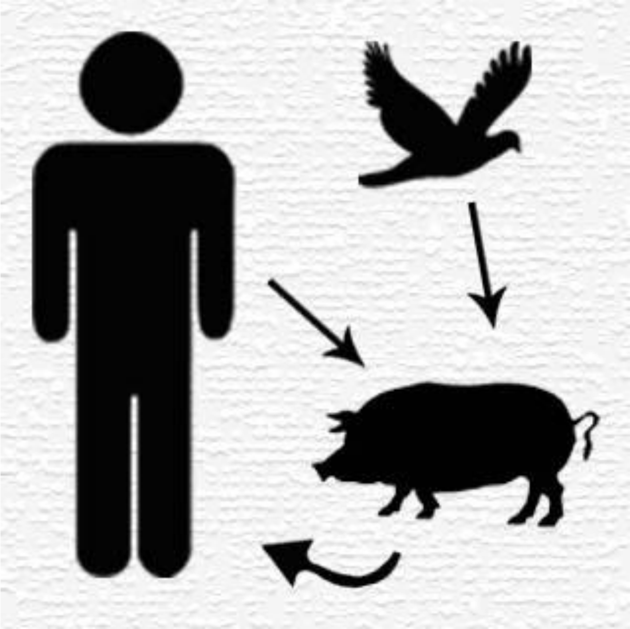
UKAS
TESTING

Nagy v2 M-gene RRT-PCR

- Alex Nagy (Czech AI/ND NRL) presented at Padova, June 2019
- Roche’s subsequent withdrawal of the UPL104 proprietary probe
    - *Crucial to the original Nagy v1 M-gene RRT-PCR*
- Alex’s new v2 M-gene RRT-PCR:
    - Retains same primers as v1 assay
        - Simple remedy!
    - New v2 MGB-probe:
        - **NB:** Extensive pan-IAV bioinformatics considered M-gene global diversity in its design
    - Ensures “One Health” application:
        - AIV
        - SwIV
        - Human seasonal IAVs
        - Other mammalian IAVs
- v2 M-gene RRT-PCR protocol and validation data:
    - Alex’s preprint available online:
        - https://doi.org/10.1101/2020.06.29.171306
    - See Marek Slomka’s posting 2/9/20 on the IZSVe – EURL website





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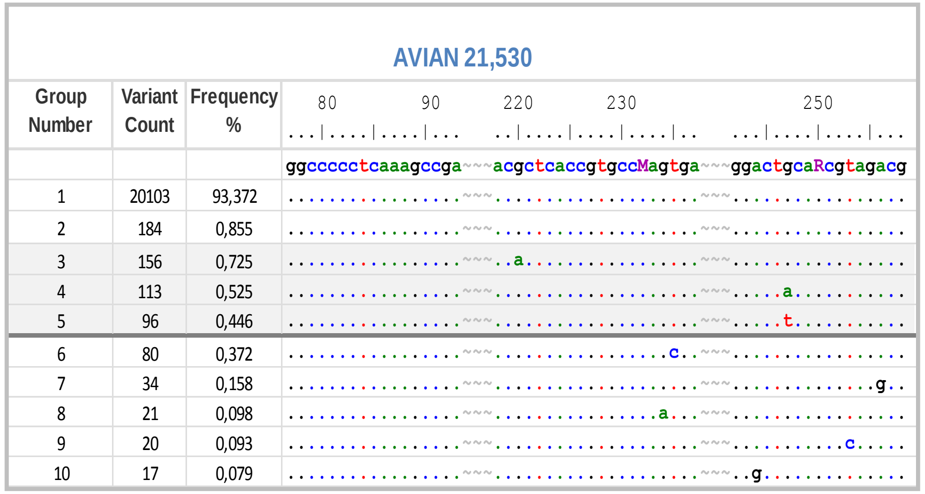
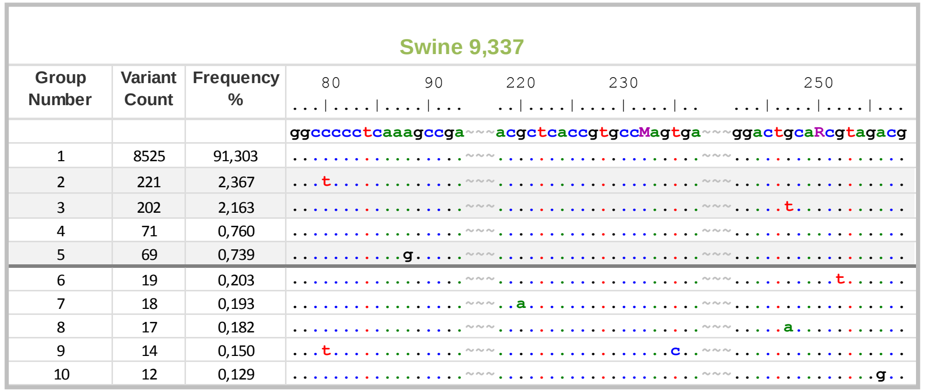
Nagy v2 M-gene RRT-PCR: IAV bioinformatics



AVIAN 21,530

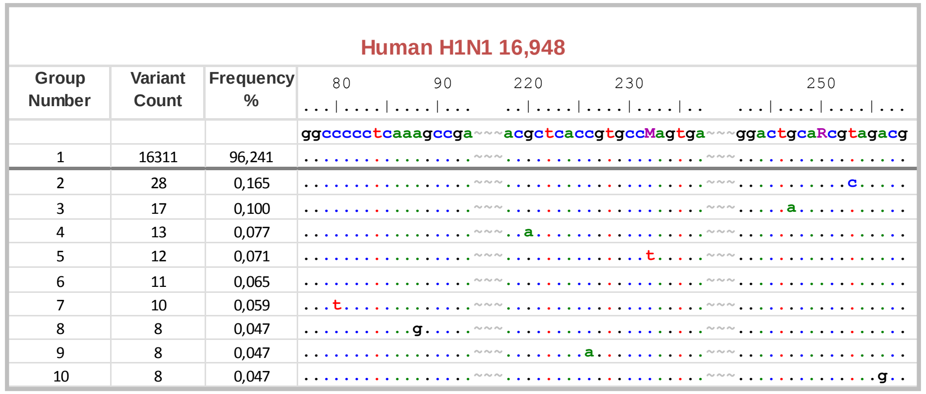
Group Number	Variant Count	Frequency %	`80 90 220 230 250` `...|....|....|...   ..|....|....|....|..   ...|....|....|...`
			`ggccccctcaaagccga~~~acgctcaccgtgccMagtga~~~ggactgcaRcgtagacg`
1	20103	93,372	`.................~~~....................~~~.................`
2	184	0,855	`.................~~~....................~~~.................`
3	156	0,725	`.................~~~..a.................~~~.................`
4	113	0,525	`.................~~~....................~~~.....a...........`
5	96	0,446	`.................~~~....................~~~.....t...........`
6	80	0,372	`.................~~~.................c..~~~.................`
7	34	0,158	`.................~~~....................~~~..............g..`
8	21	0,098	`.................~~~................a...~~~.................`
9	20	0,093	`.................~~~....................~~~...........c.....`
10	17	0,079	`.................~~~....................~~~..g..............`



Swine 9,337

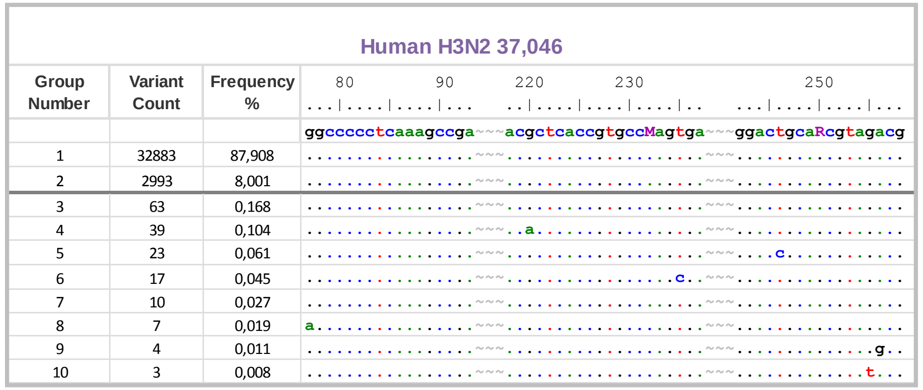
Group Number	Variant Count	Frequency %	`80 90 220 230 250` `...|....|....|...   ..|....|....|....|..   ...|....|....|...`
			`ggccccctcaaagccga~~~acgctcaccgtgccMagtga~~~ggactgcaRcgtagacg`
1	8525	91,303	`.................~~~....................~~~.................`
2	221	2,367	`...t.............~~~....................~~~.................`
3	202	2,163	`.................~~~....................~~~.....t...........`
4	71	0,760	`.................~~~....................~~~.................`
5	69	0,739	`...........g.....~~~....................~~~.................`
6	19	0,203	`.................~~~....................~~~..........t......`
7	18	0,193	`.................~~~..a.................~~~.................`
8	17	0,182	`.................~~~....................~~~.....a...........`
9	14	0,150	`...t.............~~~.................c..~~~.................`
10	12	0,129	`.................~~~....................~~~..............g..`

Adapted from Nagy *et al*:
https://doi.org/10.1101/2020.06.29.171306



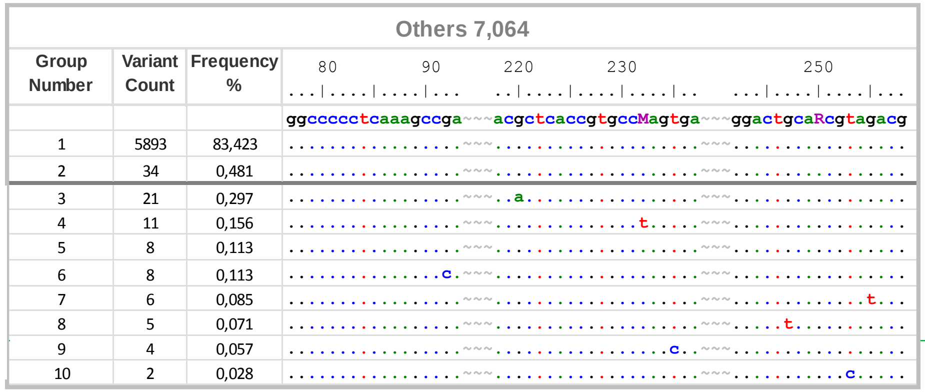
Human H1N1 16,948

Group Number	Variant Count	Frequency %	`80 90 220 230 250` `...|....|....|...   ..|....|....|....|..   ...|....|....|...`
			`ggccccctcaaagccga~~~acgctcaccgtgccMagtga~~~ggactgcaRcgtagacg`
1	16311	96,241	`.................~~~....................~~~.................`
2	28	0,165	`.................~~~....................~~~...........c.....`
3	17	0,100	`.................~~~....................~~~.....a...........`
4	13	0,077	`.................~~~..a.................~~~.................`
5	12	0,071	`.................~~~..............t.....~~~.................`
6	11	0,065	`.................~~~....................~~~.................`
7	10	0,059	`...t.............~~~....................~~~.................`
8	8	0,047	`...........g.....~~~....................~~~.................`
9	8	0,047	`.................~~~........a...........~~~.................`
10	8	0,047	`.................~~~....................~~~..............g..`



Human H3N2 37,046

Group Number	Variant Count	Frequency %	`80 90 220 230 250` `...|....|....|...   ..|....|....|....|..   ...|....|....|...`
			`ggccccctcaaagccga~~~acgctcaccgtgccMagtga~~~ggactgcaRcgtagacg`
1	32883	87,908	`.................~~~....................~~~.................`
2	2993	8,001	`.................~~~....................~~~.................`
3	63	0,168	`.................~~~....................~~~.................`
4	39	0,104	`.................~~~..a.................~~~.................`
5	23	0,061	`.................~~~....................~~~....c............`
6	17	0,045	`.................~~~.................c..~~~.................`
7	10	0,027	`.................~~~....................~~~.................`
8	7	0,019	`a................~~~....................~~~.................`
9	4	0,011	`.................~~~....................~~~..............g..`
10	3	0,008	`.................~~~....................~~~.............t...`



Others 7,064

Group Number	Variant Count	Frequency %	`80 90 220 230 250` `...|....|....|...   ..|....|....|....|..   ...|....|....|...`
			`ggccccctcaaagccga~~~acgctcaccgtgccMagtga~~~ggactgcaRcgtagacg`
1	5893	83,423	`.................~~~....................~~~.................`
2	34	0,481	`.................~~~....................~~~.................`
3	21	0,297	`.................~~~..a.................~~~.................`
4	11	0,156	`.................~~~..............t.....~~~.................`
5	8	0,113	`.................~~~....................~~~.................`
6	8	0,113	`...............c.~~~....................~~~.................`
7	6	0,085	`.................~~~....................~~~.............t...`
8	5	0,071	`.................~~~....................~~~.....t...........`
9	4	0,057	`.................~~~.................c..~~~.................`
10	2	0,028	`.................~~~....................~~~...........c.....`

Nagy v2 M-gene RRT-PCR: ‘wet’ validation

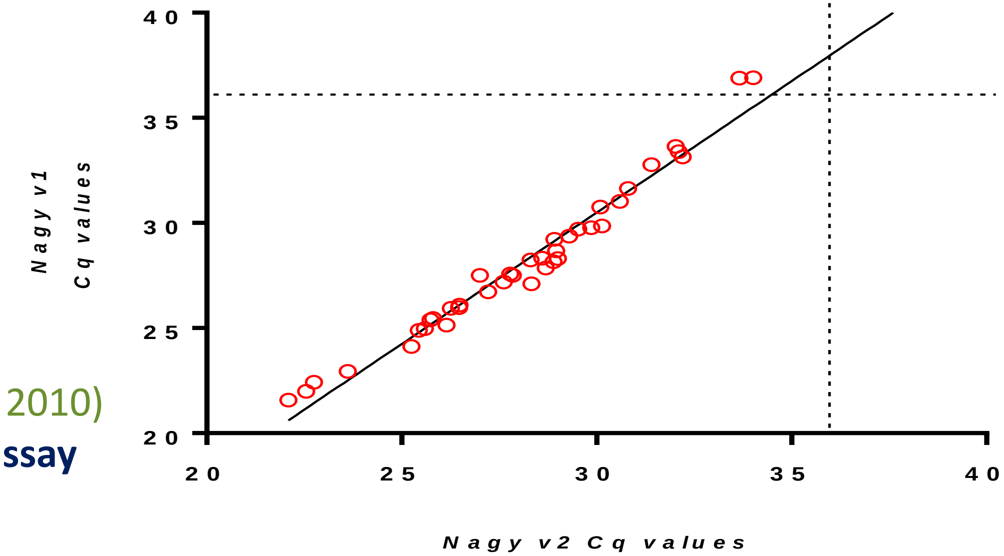
APHA diverse IAV viral isolates:
- AIVs n=77
  - *Includes current Gs/Gd clades*
- SwIVs (European) n=54
- Human seasonal IAVs n=6
- NDV / AOAV-1 n=15
- IBV n=6

Avian testing:
- Nagy v1
- Nagy v2
- Spackman

Swine / human testing:
- Nagy v1
- Nagy v2
- ‘Perfect Match’ M-gene assay (Slomka *et al* 2010)

Fitness for purpose demonstrated for the v2 assay

Clinical specimens (n=86) sourced at APHA:
- AIVs n=79
- SwIVs n=7
- Tested by three M-gene RRT-PCRs (as left)
- Example of swabs (n=39) from UK H7N7 (2015) HPAIV outbreak:



Equivalent relative sensitivity v1 v v2
Improved specificity/spectrum v2 v v1 or ‘Spackman’

Multicentre collaborative effort

Additional IAVs & clinical specimens provided by:
- Alex’s own lab (including Public Health [human] origin)
- Slovak & Hungarian colleagues

Summary

- New approaches required for truly early warning systems through wild bird surveillance
- Improved knowledge of reservoir host, ecology, environment and relevant interfaces with poultry required
- Technology improvements offer new opportunities
- New improved well validated M gene available for use
- We challenge funders and competent authorities to consider supporting new opportunities for designing improved wild bird surveillance!

Acknowledgements:

Co-creators of concept

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Ron Fouchier, Erasmus MC, NL
Martin Beer and Timm Harder, FLI, DE
Jonas Waldenstrom, Linneuniversitetet, SE

M gene Nagy 2 development

Alexander Nagy and colleagues

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Natalie McGinn
James Seekings
Susan Collins
Rowena Hansen
Elliott Whittard
Adam Brouwer
Ash Banyard
Richard Ellis, Bioinformatics, APHA

Annual Report
on surveillance
for avian influenza in
poultry and in wild birds in
Member States of the
European Union
in 2017

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https://science.vla.gov.uk/fluglobalnet/

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