One Health in Action: Lessons from HPAI

Isabella Monne

Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Padova, Italy

Regional Workshop

Accelerating the Operationalisation of the One Health Joint Plan of Action (OHJPA) in Veterinary Services in the European Region

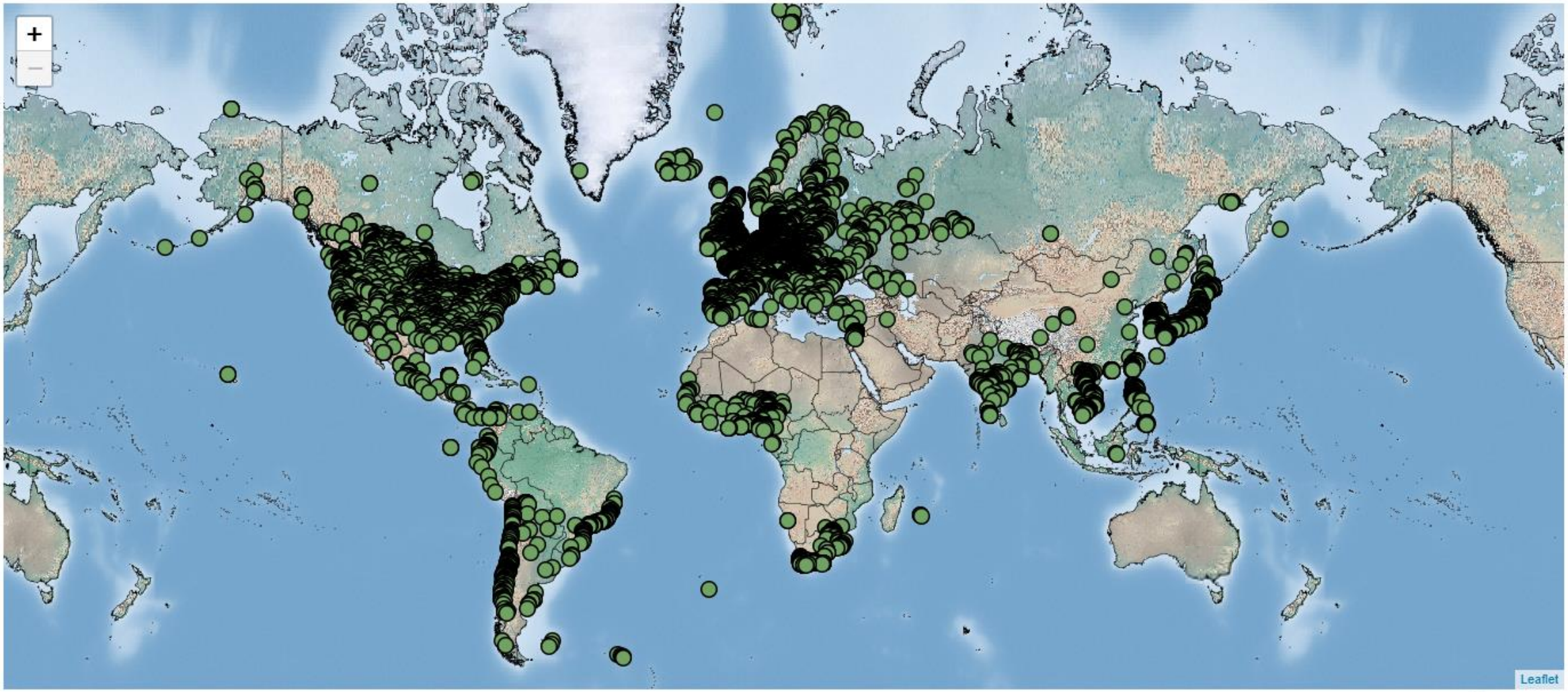
18-20 November 2025 - Athens, Greece

The evolution of avian influenza surveillance and control in a One Health perspective

- From poultry farms to the **most remote areas of the planet**
- Inclusion of **new species and new ecosystems**
- **Multidisciplinary** approach

HPAI (H5 of the gs/Gd/96 lineage, clade 2.3.4.4b)

The first documented panzootic caused by an HPAI virus since 1878, the year when a highly pathogenic form of influenza was first identified in poultry by Edoardo Perroncito.

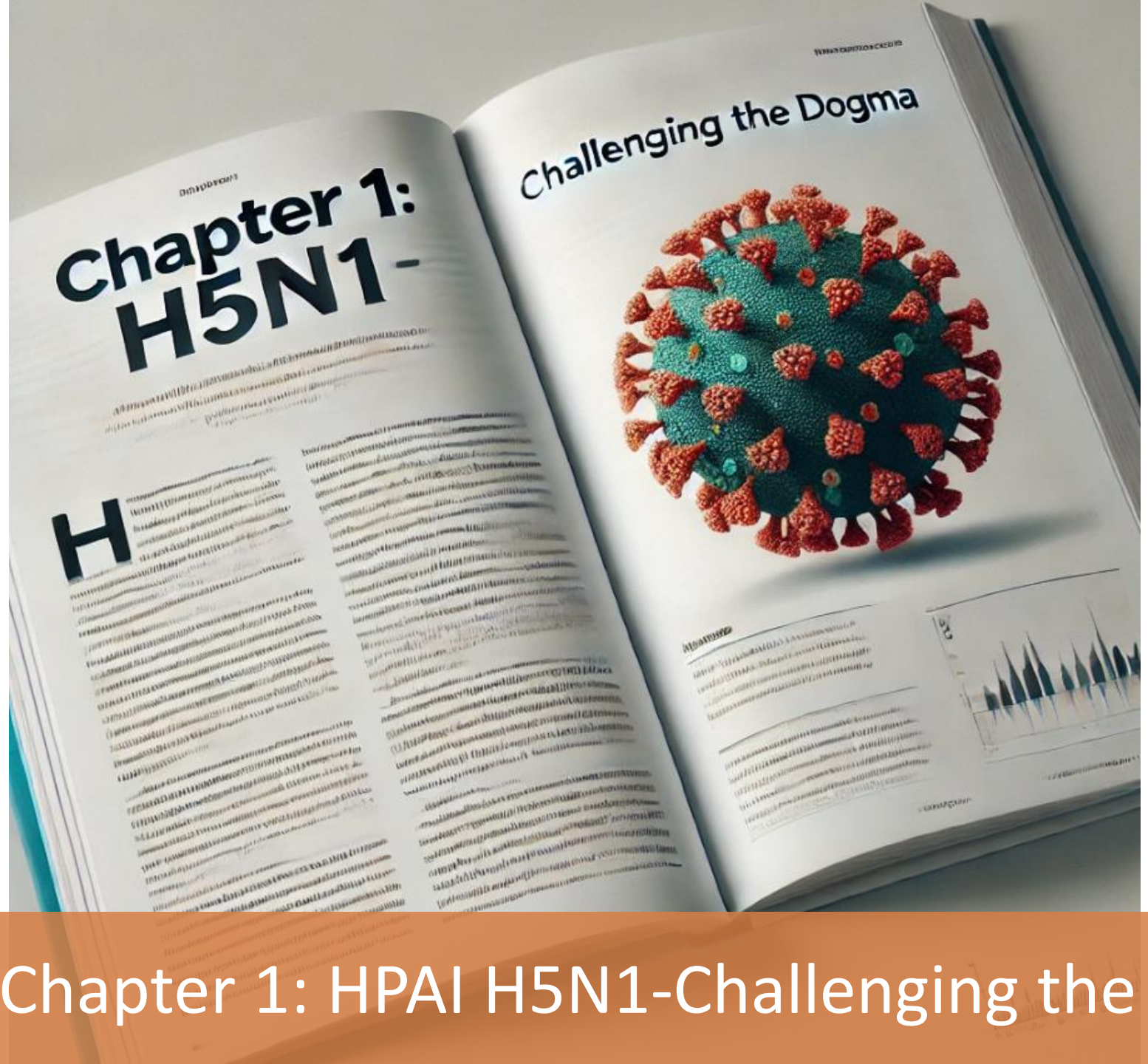


09/05/2025

2021-2025

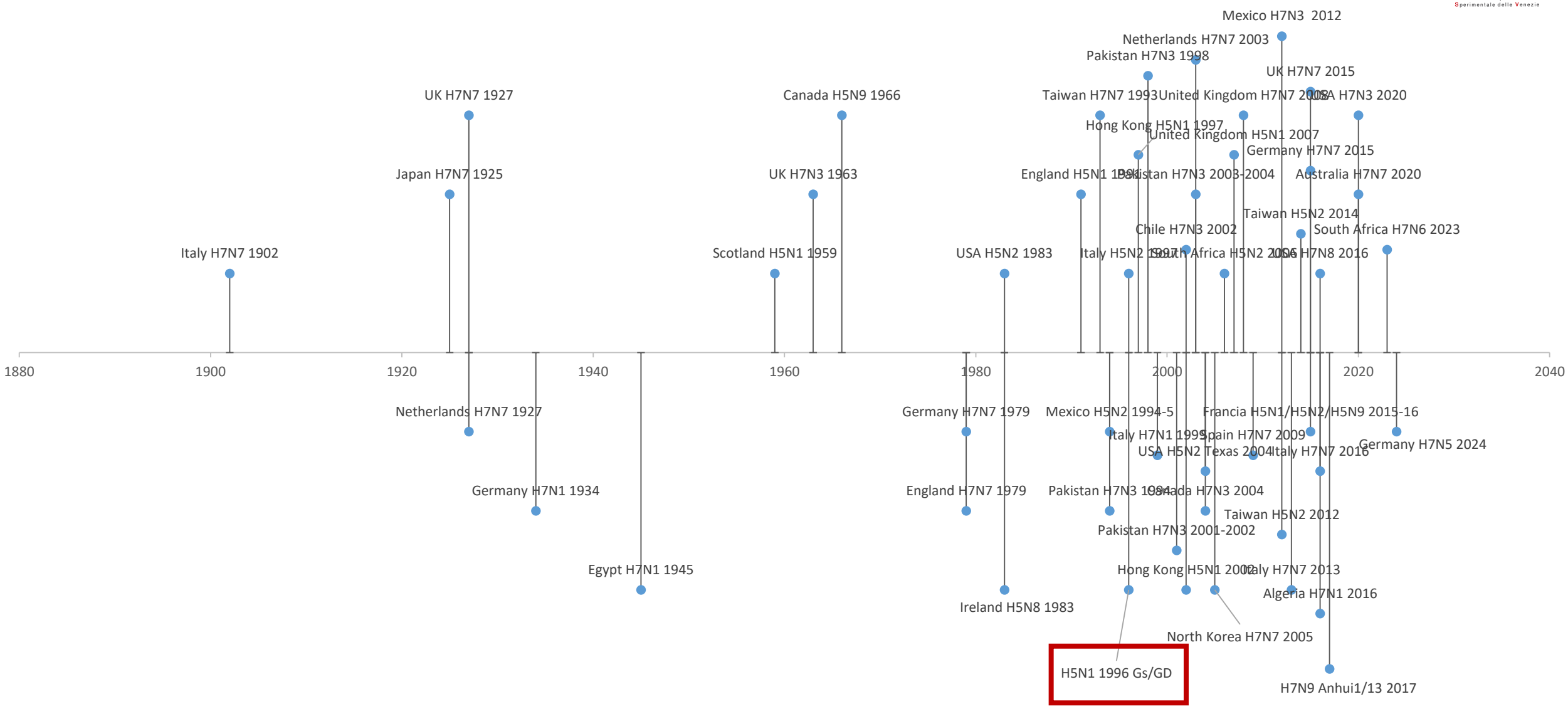
EMPRES
Global Animal Disease Information System


Created with mapchart.net



Chapter 1: HPAI H5N1-Challenging the dogma

H5 and H7 HPAI lineages



HPAI: Challenging dogma

Paradigm
«Wild waterbirds are natural reservoir of LPAI»

«New challenge:

Wild waterbirds as reservoir and victims of HPAI»

Picture from Shutterstock

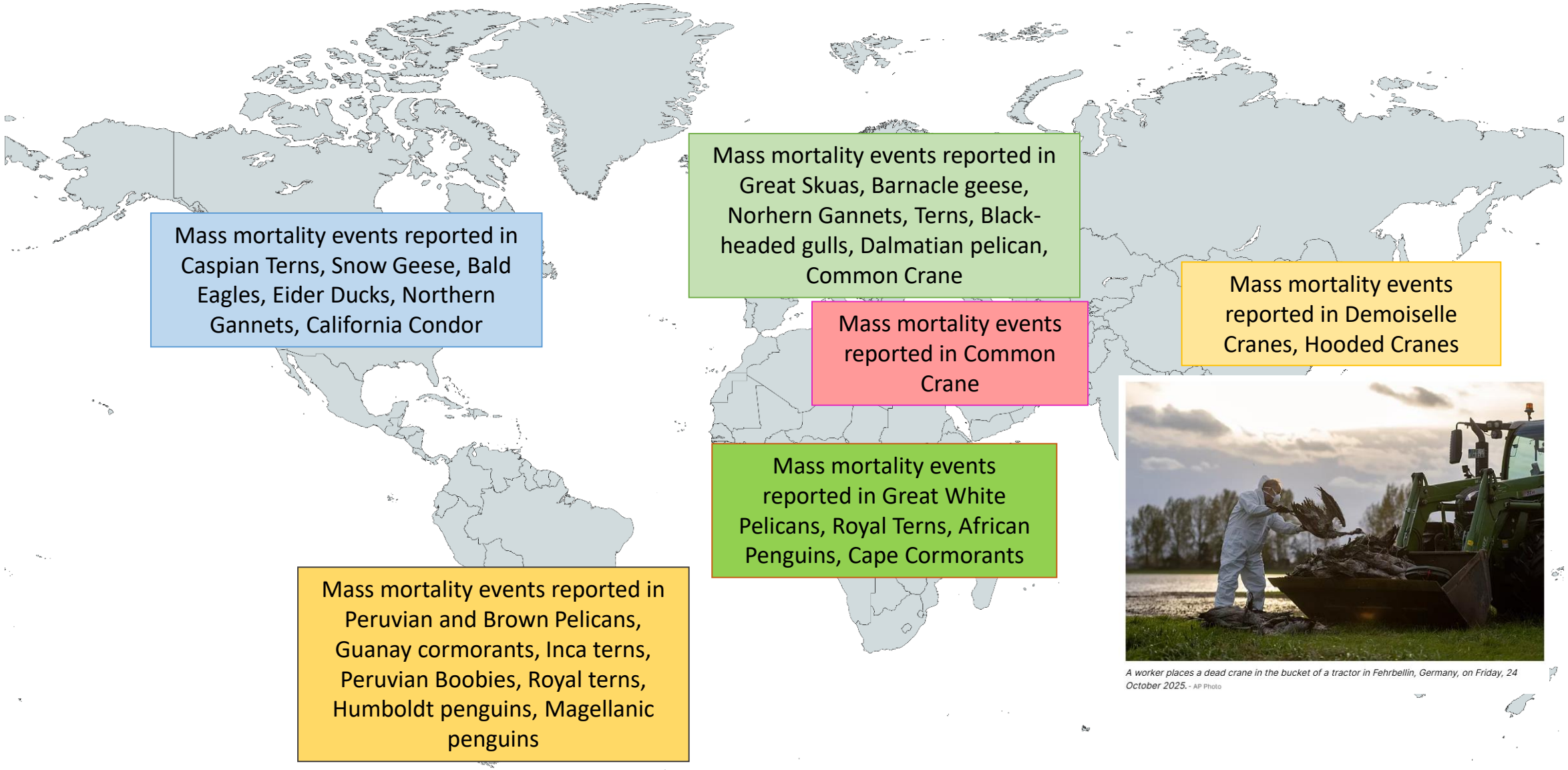
Unprecedented conservation impacts on wild birds between 2021-2025

https://www.bbc.com/future/article/20240425-how-dangerous-is-bird-flu-spread-to-wildlife-and-humans

https://www.abc.net.au/news/2025-07-22/avian-influenza-not-the-cause-of-coral-bay-bird-deaths/105546660

Unprecedented conservation impacts on wild birds between 2021-2025

Istituto Zooprofilattico Sperimentale delle Venezie



A worker places a dead crane in the bucket of a tractor in Fehrbellin, Germany, on Friday, 24 October 2025. - AP Photo

HPAI H5N1: cascading across the food web

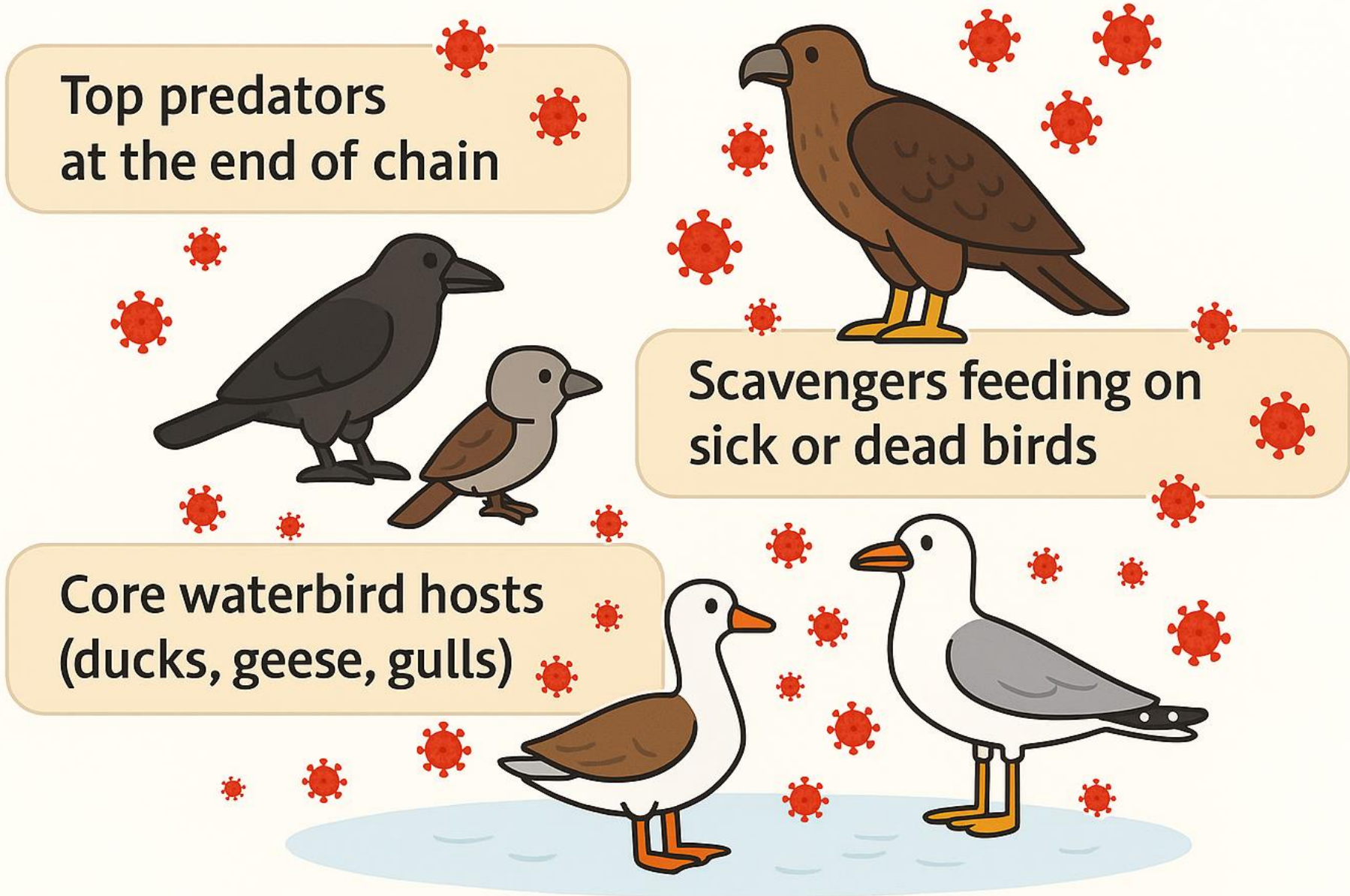


Illustration created using ChatGPT (OpenAI), based on the author's prompt.

HPAI: Challenging dogma

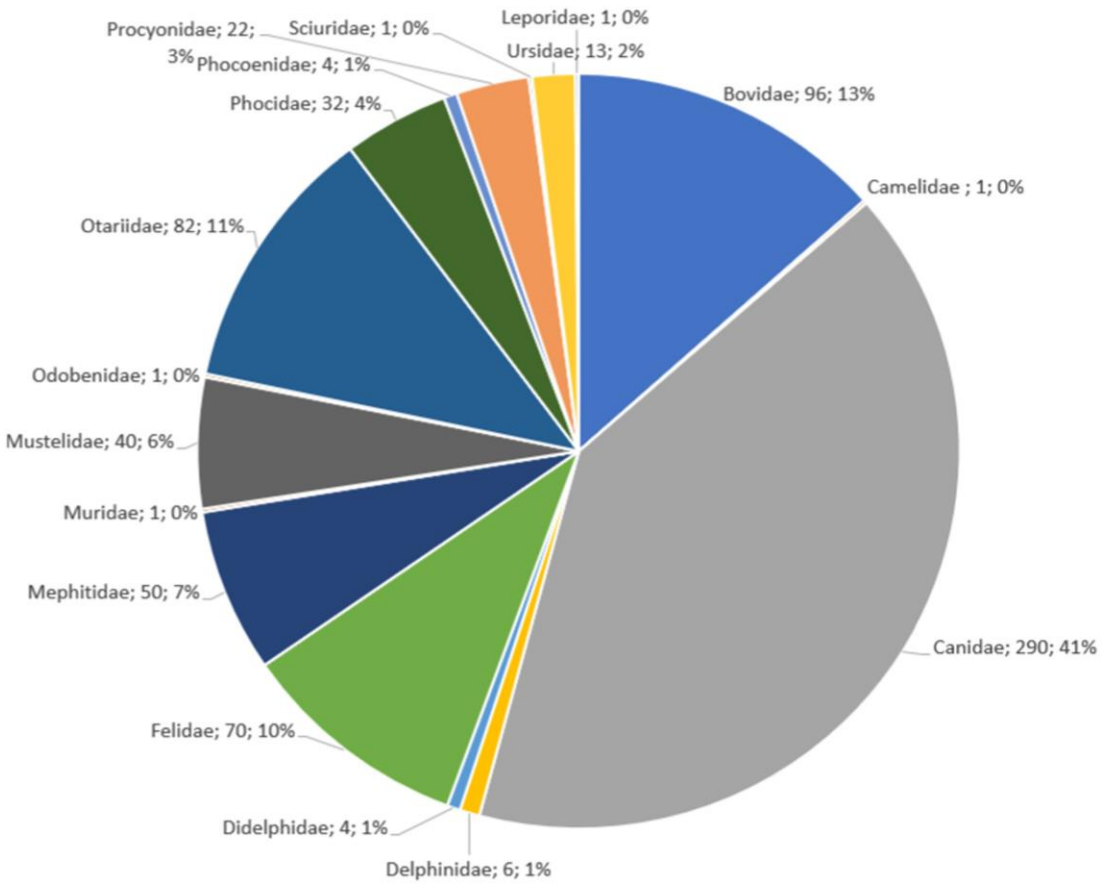
Paradigm «Sporadic HPAIV spillover into mammals»

«New challenge:

Infection of a broad range of mammals »

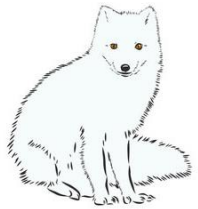
HPAI H5Nx: Unprecedented impacts on mammals

91 species affected



H5 HPAI shows features of a **host-range generalist** within birds, with expanding spillover to mammals

EFSA Journal, Volume: 23, Issue: 1, First published: 29 January 2025, DOI: (10.2903/j.efsa.2025.9191)



Fur farms in Spain (n. 1) and in Finland (n.76)

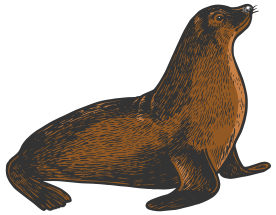
Livestock (1049 Dairy herds)

Clusters of infection in mammals caused by 2.3.4.4b H5 HPAI

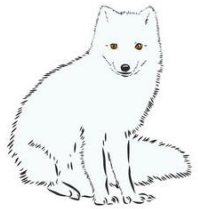
35 cats (+1 caracal and 1 dog) in Poland in distinct locations; 2 shelter cats in South Korea; 3 cats in Icelands; 2 in Italy; 2 in Belgium.

Multiple events in the USA linked to cases in Dirary herds and poultry.

Mass mortality event in a zoo in Vietnam (47 tigers, 3 lions, 1 leopard)



Thousands of sea lions and elephant seals in Latin America.



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Sustained mammal to mammal transmission

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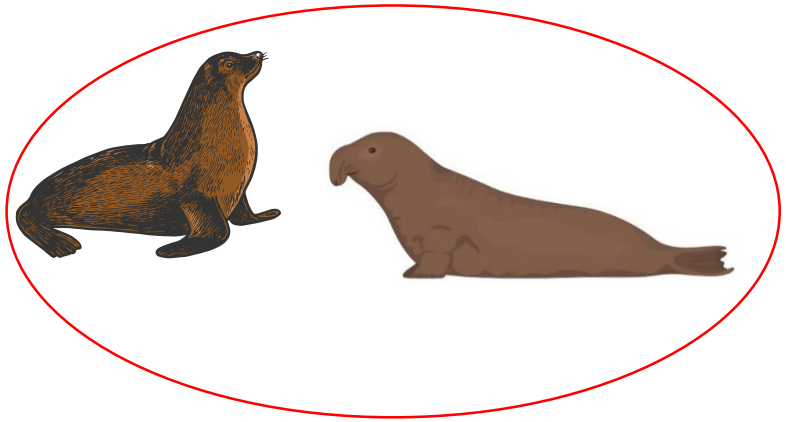

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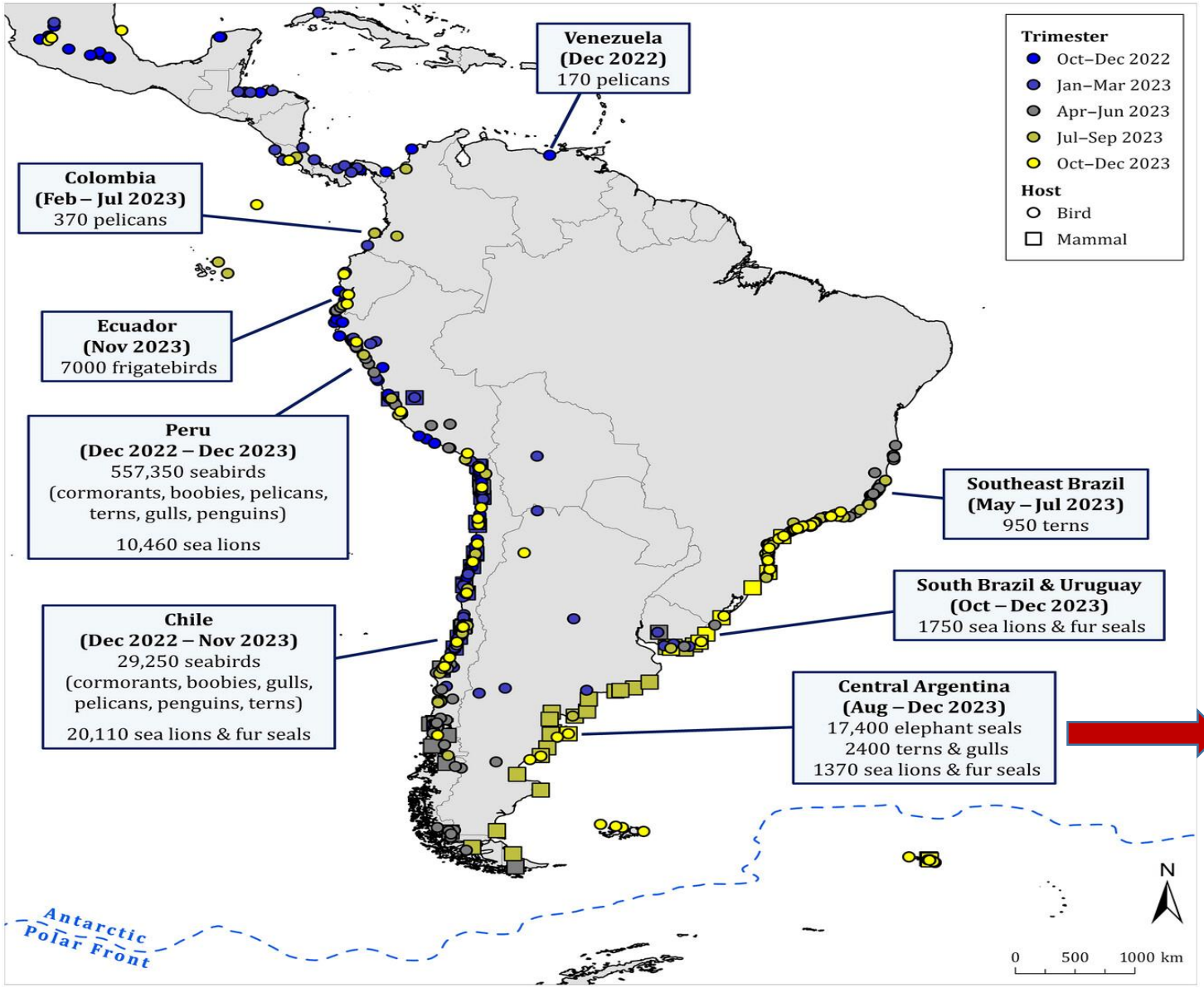
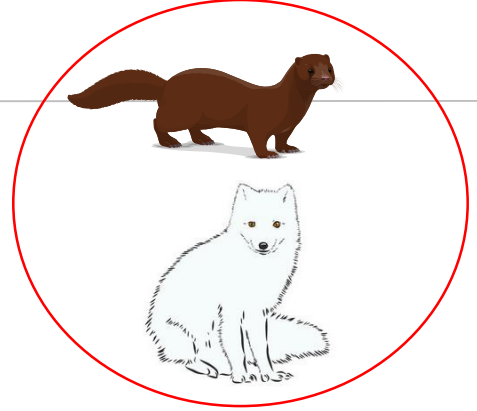


-species extinction
-breakdown of predatory–prey interactions,
-modification of nutrient cycling

Conservation Biology, First published: 31 May 2025, DOI: (10.1111/cobi.70052)



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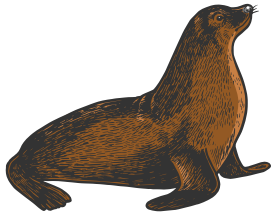

Thousands of sea lions and elephant seals in Latin America.

Article

Spillover of highly pathogenic avian influenza H5N1 virus to dairy cattle

https://doi.org/10.1038/s41586-024-07849-4

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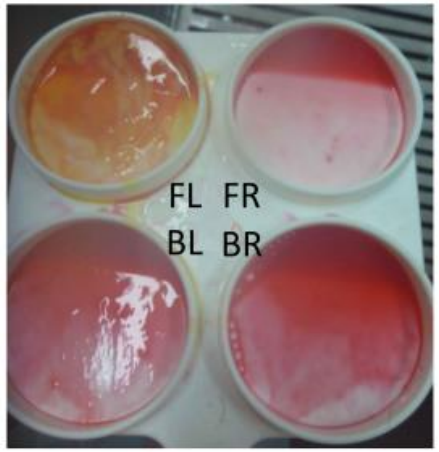
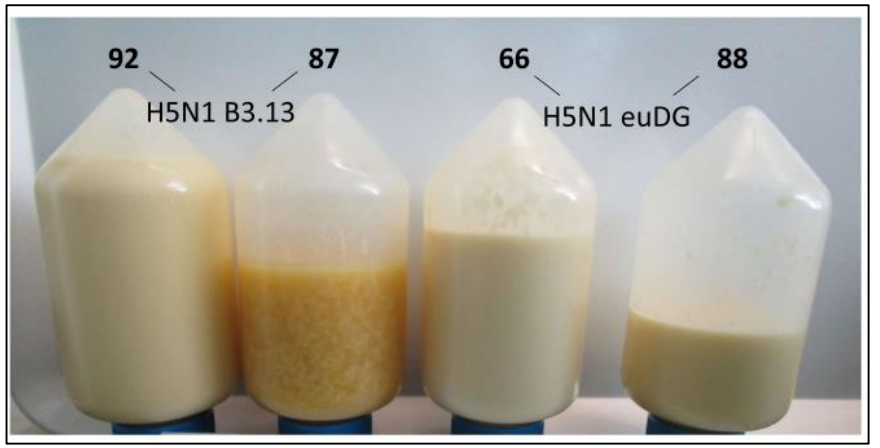
Open access

Leonardo C. Caserta[1,6], Elisha A. Frye[1,6], Salman L. Butt[1,6], Melissa Laverack[1], Mohammed Nooruzzaman[1], Lina M. Covaleda[1], Alexis C. Thompson[2], Melanie Prarat Koscielny[2], Brittany Cronk[1], Ashley Johnson[2], Katie Kleinhenz[2], Erin E. Edwards[4], Gabriel Gomez[4], Gavin Hitchener[1], Mathias Martins[4], Darrell R. Kapczynski[5], David L. Suarez[5], Ellen Ruth Alexander Morris[4], Terry Hensley[4], John S. Beeby[1], Manigandan Lejeune[1], Amy K. Swinford[4], François Elvinger[1], Kiril M. Dimitrov[4,5] & Diego G. Diel[1,6]





Choose time period: Total Outbreak

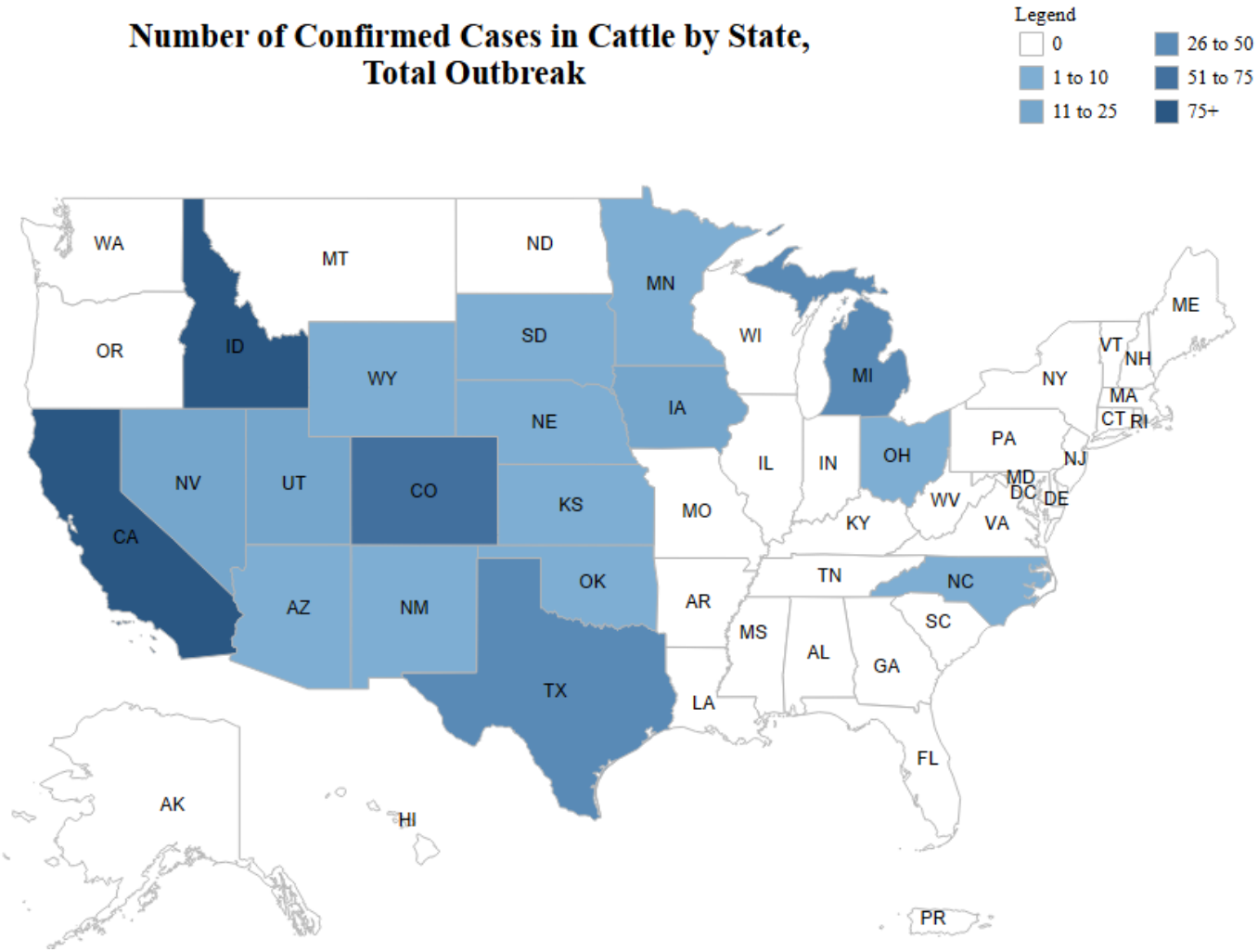
Choose species: Cattle

Situational Update

Click for International Exports

In the Total Outbreak, in Cattle, there were:
1,082 Confirmed Cases in **18** States

Number of Confirmed Cases in Cattle by State, Total Outbreak



https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/hpai-confirmed-cases-livestock Accessed 16/11/2025

JOURNAL ARTICLE ACCEPTED MANUSCRIPT

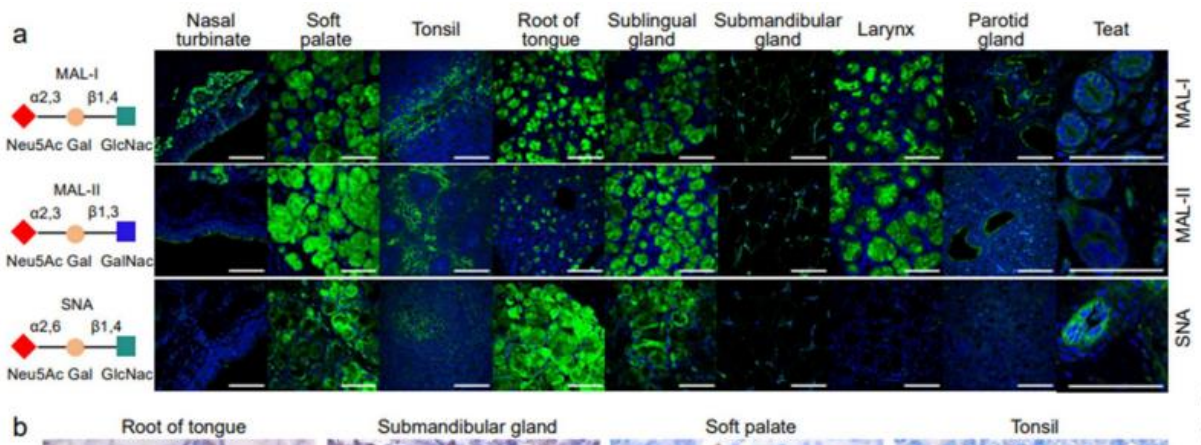
H5N1 virus invades the mammary glands of dairy cattle through "mouth-to-teat" transmission

Jianzhong Shi , Huihui Kong , Pengfei Cui , Guohua Deng , Xianying Zeng , Yongping Jiang , Xijun He , Xianfeng Zhang , Lei Chen , Yichao Zhuang ... Show more
Author Notes

National Science Review, nwaf262, https://doi.org/10.1093/nsr/nwaf262
Published: 01 July 2025

Abstract

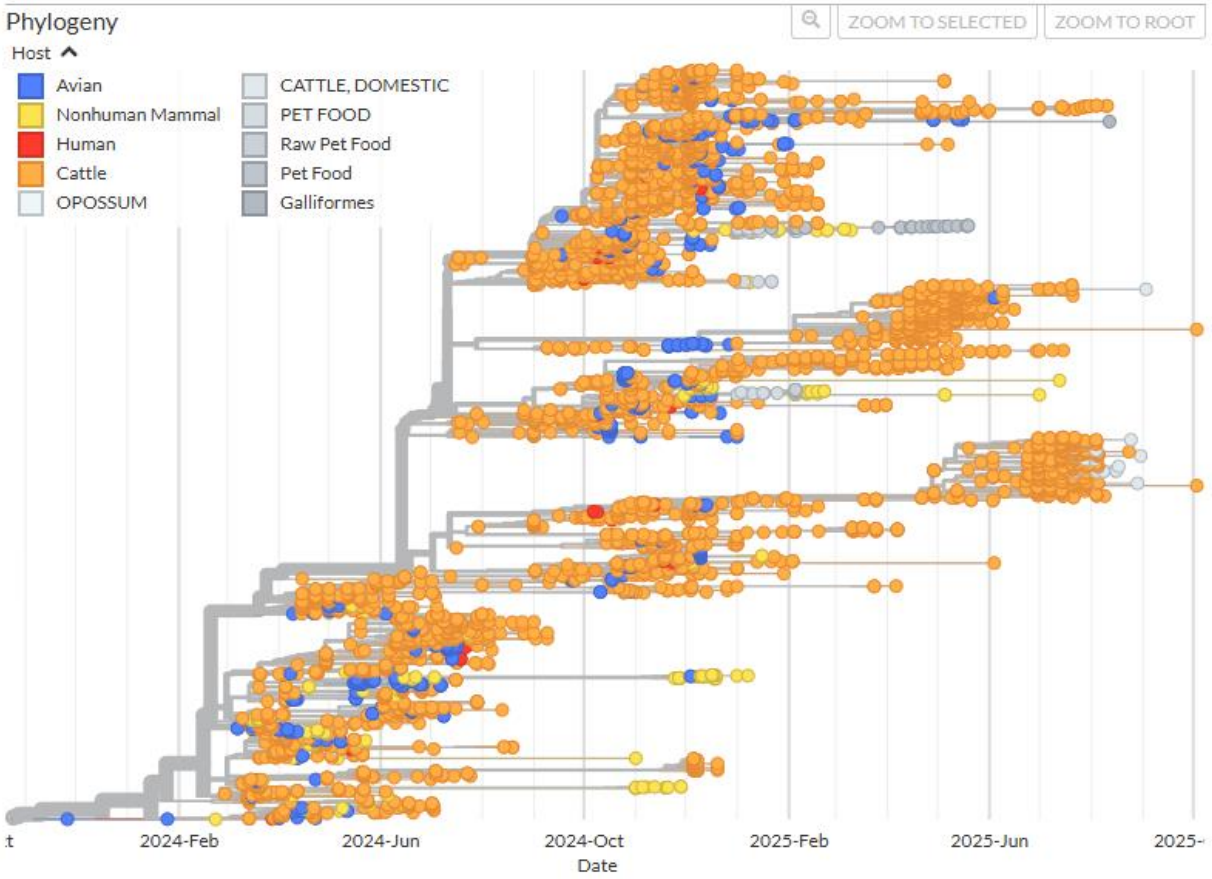
H5N1 influenza outbreaks have been repo
across 17 states in the US. Damage to the n
in milk were common features of the infe
virus initially invades the mammary glands, and no control strategy is currently available. Here, we found that cattle oral tissues support H5N1 virus binding and replication, and virus replicating in the mouth of cattle transmitted to the mammary glands of dairy cattle during sucking. We also found that an H5 inactivated vaccine or a hemagglutinin-based DNA vaccine induced sterilizing immunity in cows against challenges with different H5N1 viruses. Our study provides insights into H5N1 virus transmission and control in cattle.

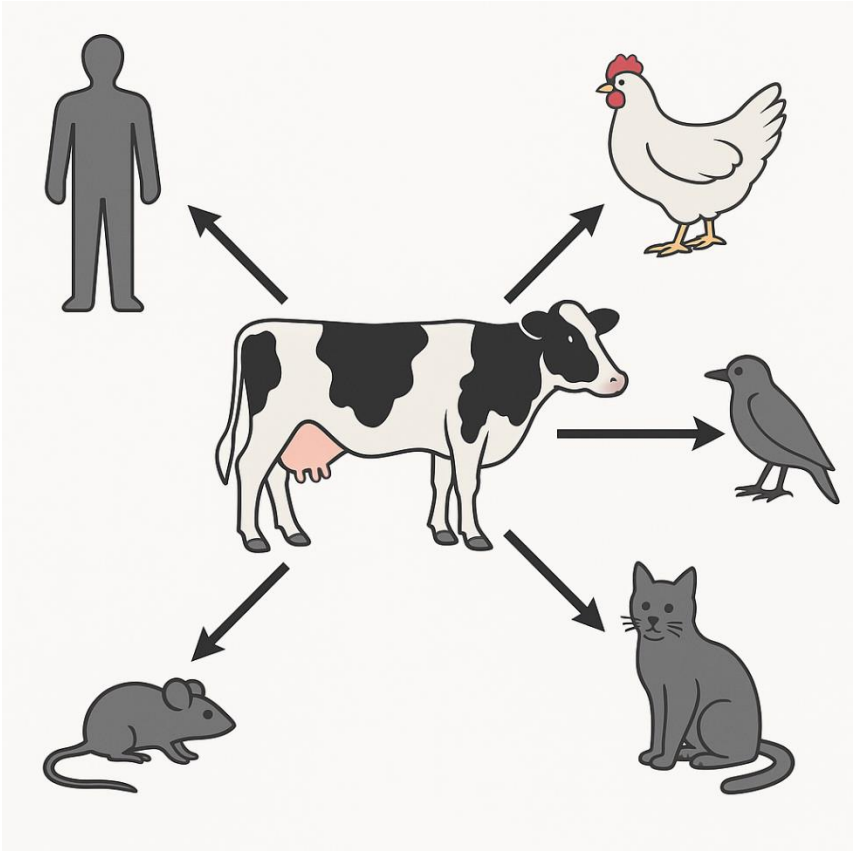


In dairy cattle, H5N1 spread appears to be driven primarily by:
-milking practices;
-a milk-mediated mammary–oral transmission route, closely linked to suckling behaviours
-no efficient respiratory cow-to-cow transmission

“Continuum”

Cornelis Escher. Aereo di riempimento II, 1957. Litografia. mm. 370 x 315





https://nextstrain.org/avian-flu/h5n1-cattle-outbreak/genome

Genotype B3.13 has not been identified only in dairy cattle premises but also across multiple poultry premises and other mammals such as a number of cats, racoons and alpacas..and even humans with 41 cases reported associated with exposure to diary herds

HPAI: Challenging dogma

Paradigm «Mammals are dead-end hosts for HPAI»

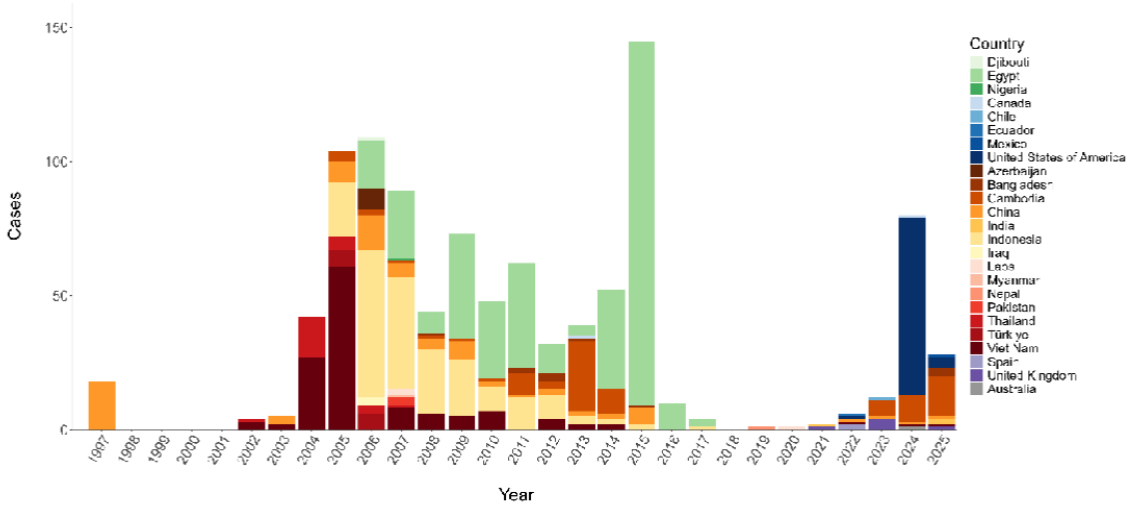
New challenge:

1-Mammals are no longer dead-end hosts

2-New paths (e.g. new bridging species) for virus dissemination (other than poultry and wild birds)

3-New ecological niches for virus evolution and potential changes in risk profile also for humans

Distribution of reported human cases of A(H5N1) virus infection by year of onset or detection and reporting country from 1997 to 7 March 2025



Avian influenza overview June–September 2025

efsa JOURNAL

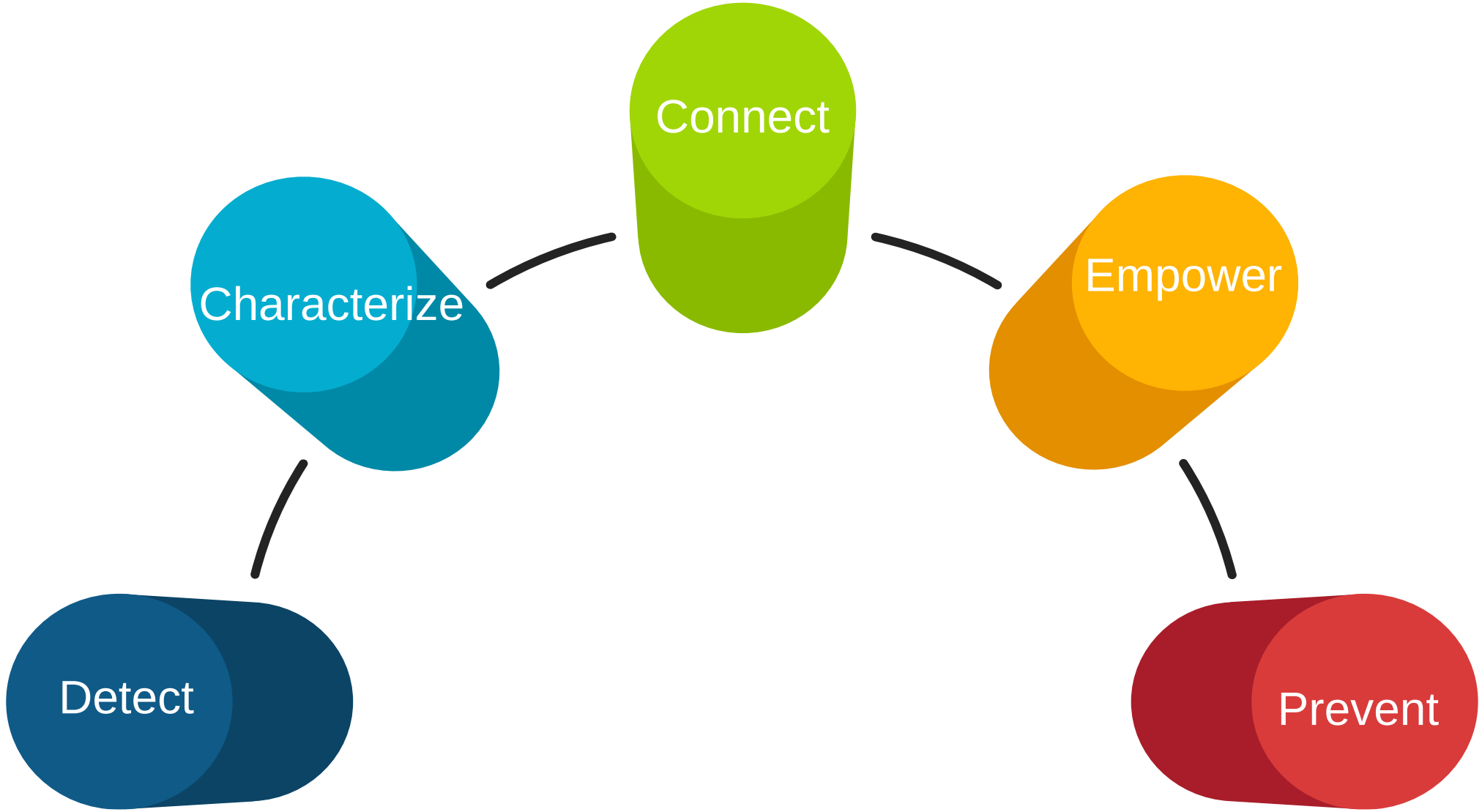
Year	Cases	Deaths	Case fatality rate
2003	4	4	100%
2004	46	32	69.6%
2005	98	43	43.9%
2006	115	79	68.7%
2007	88	59	67.0%
2008	44	33	75.0%
2009	73	32	43.8%
2010	48	24	50.0%
2011	62	34	54.8%
2012	32	20	62.5%
2013	39	25	64.1%
2014	52	22	42.3%
2015	145	42	29.0%
2016	10	3	30.0%
2017	4	2	50.0%
2018	0	0	0%
2019	1	1	100%
2020	1	0	0%
2021	2	1	50.0%
2022	6	1	16.7%
2023	12	4	33.3%
2024*	81	4	4.9%
2025	29	11	37.9%
Overall	992	476	48.0%

https://www.chp.gov.hk/en/resources/29/332.html

The more birds and mammals it infects, the more opportunities H5N1 has to mutate to further expand its host range

Chapter 2: Expect the Unexpected

Staying Prepared Requires a One Health Approach



Detect

Change in virus ecology: new surveillance needs

New biological and environmental matrices

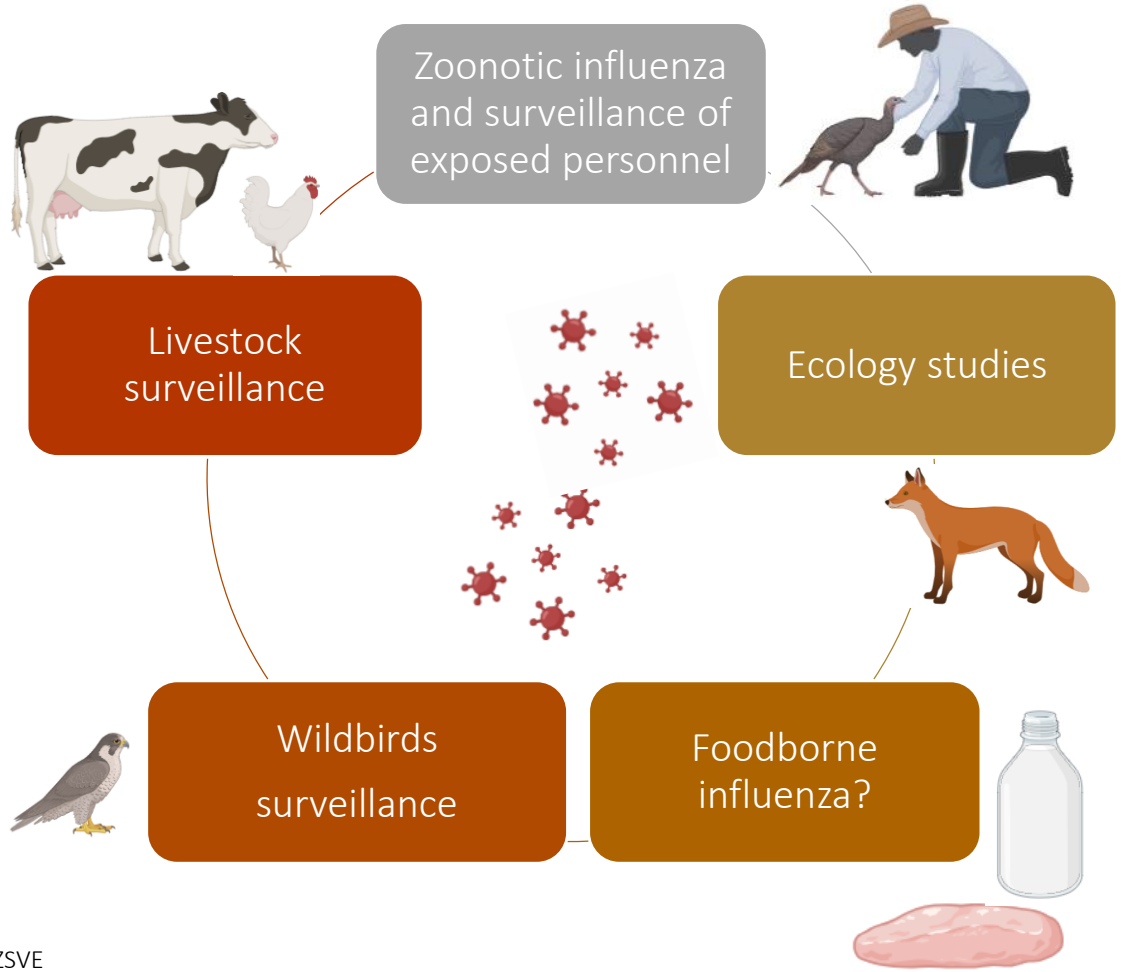
Surveillance stipulated by law (mainly commercial holdings)

Surveillance in wild birds

Surveillance in domestic and wild mammals and the environment

AI molecular diagnostic methods

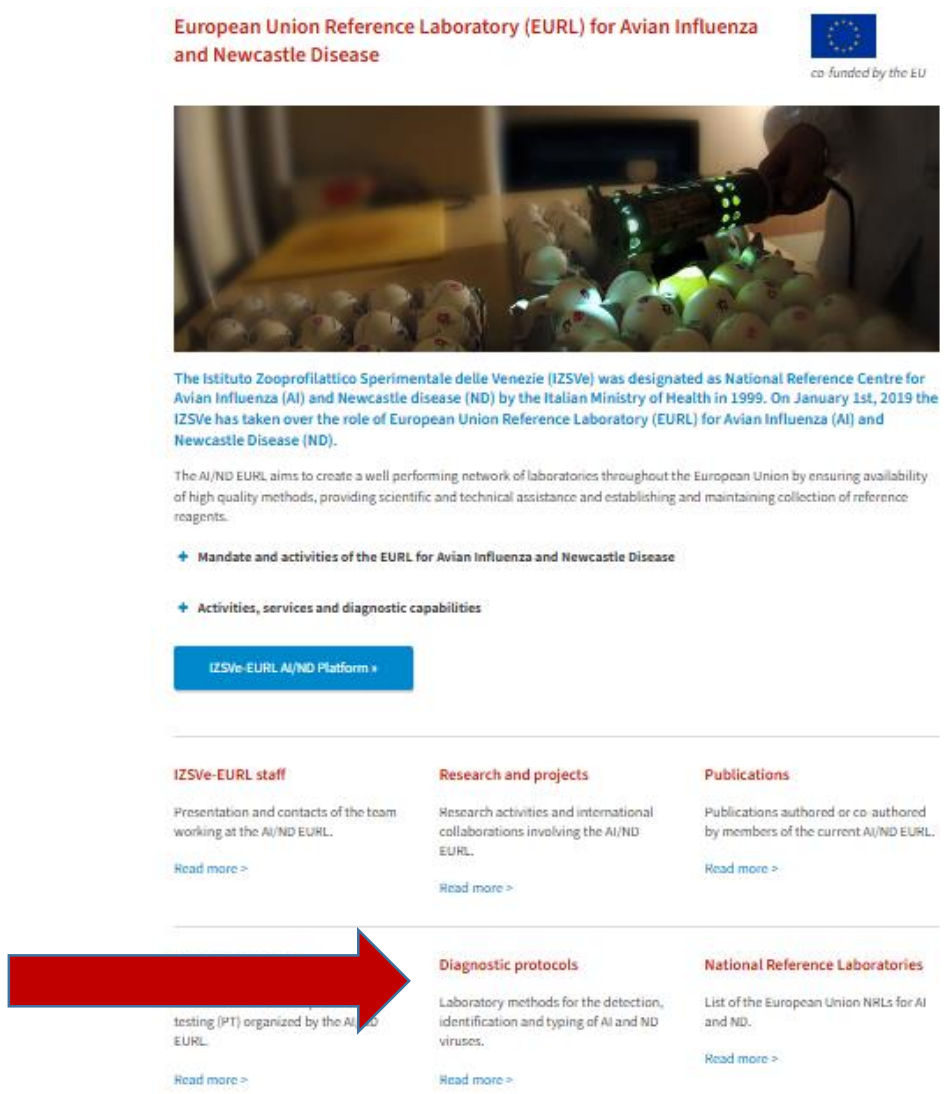
Changes in AI ecology require to extend the field of application of diagnostic methods designed for birds, to new matrices:



- LoD determination and repeatability in human swabs, bulk milk, and milk from cows with mastitis
- Analytical specificity to assess any cross-reactivity with mammalian viruses
- Diagnostic specificity employing specimens from wild and domestic mammals, and experimental samples

AI molecular diagnostic methods

SOPs and declaration of validation available at:
https://www.izsvenezie.com/reference-laboratories/avian-influenza-newcastle-disease/diagnostic-protocols/



European Union Reference Laboratory (EURL) for Avian Influenza and Newcastle Disease

co-funded by the EU

The Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) was designated as National Reference Centre for Avian Influenza (AI) and Newcastle disease (ND) by the Italian Ministry of Health in 1999. On January 1st, 2019 the IZSVe has taken over the role of European Union Reference Laboratory (EURL) for Avian Influenza (AI) and Newcastle Disease (ND).

The AI/ND EURL aims to create a well performing network of laboratories throughout the European Union by ensuring availability of high quality methods, providing scientific and technical assistance and establishing and maintaining collection of reference reagents.

+ Mandate and activities of the EURL for Avian Influenza and Newcastle Disease

+ Activities, services and diagnostic capabilities

IZSVe-EURL AI/ND Platform »

IZSVe-EURL staff
Presentation and contacts of the team working at the AI/ND EURL.
Read more >

Research and projects
Research activities and international collaborations involving the AI/ND EURL.
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Publications
Publications authored or co-authored by members of the current AI/ND EURL.
Read more >

testing (PT) organized by the AI/ND EURL.
Read more >

Diagnostic protocols
Laboratory methods for the detection, identification and typing of AI and ND viruses.
Read more >

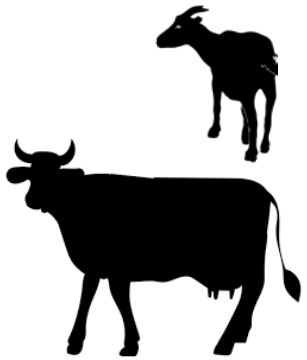
National Reference Laboratories
List of the European Union NRLs for AI and ND.
Read more >

Protocol	Laboratory	Download
Isolation and characterization of Avian influenza viruses using SPF embryonated chicken eggs and haemagglutination inhibition test *(Released on 25/01/24)*	IZSVe	SOP VIR 005
Isolation and characterization of Newcastle disease viruses using SPF embryonated chicken eggs and haemagglutination inhibition test *(Released on 12/10/21)*	IZSVe	SOP VIR 007
Influenza - Neuraminidase Inhibition Test	APHA	PDF
Detection of antibodies to type A influenza virus by agar gel immunodiffusion assay (AGID) *(Released on 23/02/23)*	IZSVe	SOP IMM 063
Detection of subtype-specific antibodies to Avian influenza virus by haemagglutination inhibition test *(Released on 29/12/20)*	IZSVe	SOP IMM 064
Detection of antibodies to Newcastle disease virus by haemagglutination inhibition test *(Released on 30/12/20)*	IZSVe	SOP IMM 065
Sample preparation and nucleic acids isolation for the detection and typing of Avian influenza virus and Avian Orthoavulavirus type 1 by molecular methods *(Released on 14/10/25)*	IZSVe	SOP VIR 1000
Detection of type A Avian influenza virus by real time RT-PCR (Heine et al., 2015; Laconi et al., 2020) *(Released on 14/10/25)*	IZSVe	SOP VIR 018
Detection of type A influenza virus by real time RT-PCR (Nagy et al., 2021) *(Released on 07/04/21)*	IZSVe	SOP VIR 1003
Detection of Eurasian H5 Avian influenza virus by real time RT-PCR (Slomka et al., 2007) *(Released on 08/10/24)*	IZSVe	SOP VIR 143
Detection of Eurasian H5 Avian influenza virus by One-step RT-PCR and Sanger sequencing of the hemagglutinin cleavage site (Slomka et al., 2007) *(Released on 14/10/25)*	IZSVe	SOP VIR 125
Detection of Eurasian H7 Avian influenza virus by real time RT-PCR (Slomka et al., 2009) *(Released on 09/04/21)*	IZSVe	SOP VIR 144
Detection of Eurasian H7 Avian influenza virus by real time RT-PCR (Van Borm et al., 2010 modified) *(Released on 07/04/21)*	IZSVe	SOP VIR 1001
Detection of Eurasian H7 Avian influenza virus by One-step RT-PCR and Sanger sequencing of the hemagglutinin cleavage site (Slomka et al., 2007) *(Released on 14/10/25)*	IZSVe	SOP VIR 126
Detection of H9 Avian influenza virus by real time RT-PCR (Panzarin et al., 2022)*(Released on 17/06/22)*	IZSVe	SOP VIR 014
HA and NA subtyping of Avian influenza virus by real-time RT-PCR (Hassan et al., 2022; James et al., 2018; Hoffmann et al., 2016) (Released on 08/10/24)	IZSVe	SOP VIR 1004
Avian Influenza virus (AIV) typing by reverse transcription PCR using a universal primer set *(Released on 06/19)*	IZSVe	PDF
Detection of Avian Orthoavulavius type 1 (AOAV-1) by real time RT-PCR (Sutton et al., 2019) *(Released on 28/09/23)*	IZSVe	SOP VIR 151
Detection and pathotyping of Avian Orthoavulavirus type 1 (AOAV-1) by one-step RT-PCR and Sanger sequencing of the fusion protein cleavage site (De Battisti et al., 2013) *(Released on 09/04/21)*	IZSVe	SOP VIR 063
Detection and pathotyping of Avian Orthoavulavirus type 1 (AOAV-1) by one-step RT-PCR and Sanger sequencing of the fusion protein cleavage site (Kant et al., 1997) *(Released on 29/09/23)*	IZSVe	SOP VIR 1002
Molecular pathotyping of Orthoavulavirus javaense (OAV-J) by real-time RT-PCR and Sanger sequencing (Fortin et al., 2023) *(Released on 14/10/25)*	IZSVe	SOP VIR 1006

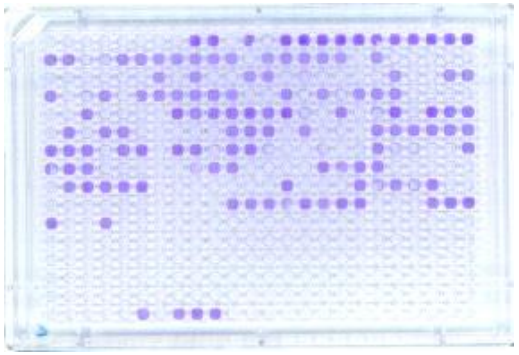
What about serosurveillance?

Optimize the performance of **serological diagnostic assays**.

Provision of reference reagents to the EU NRLs to render them independent: serum from cattle immunised with an inactivated HPAI H5N1 (clade 2.3.4.4b) virus for use as a **positive control serum.**



IZSVe (WOAH/EU RL) tested thousands of dairy cattle and small ruminants from high risk areas for the presence of antibodies against H5N1.



All sera tested were negative.

Real Time Sequencing and use of bionformatic tools: a diagnostic imperative

Characterize

How dangerous is this virus for mammals?

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

MARKERS OF INTEREST

- Zoonotic potential
- Increased virulence
- Resistance toward antiviral drugs

Giussani E, et al. Virus Evol. 2025 Mar 7;11(1): doi: 10.1093/ve/veaf011. PMID: 40093844; PMCID: PMC11908534.

Phenotype characterization to evaluate zoonotic/virulence potential

Rapid phenotyping tools for HPAI in a one health context

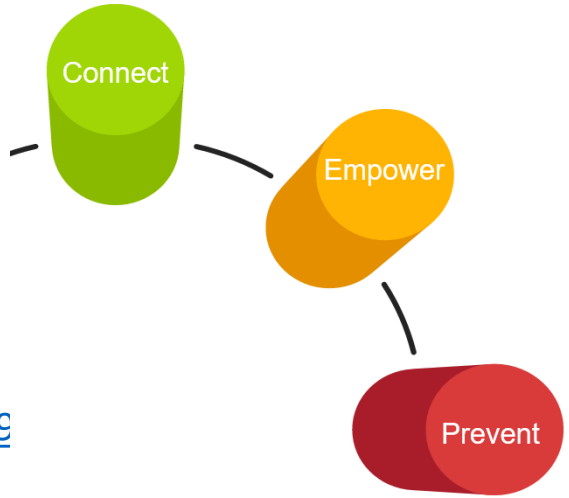
Cell-based infectivity and replication assays

- Use of continuous cell lines (Calu-3, Caco-2)
- Primary airway epithelial cells from birds, cattle and humans (ALI cultures)

Organoids and explant models

- Avian and human respiratory organoids
- Ex vivo explants to study tissue tropism and replication kinetics

Cross-sector collaboration in action



Scientific Opinions:

-Drivers for a pandemic due to avian influenza and options for One Health mitigation (https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2024.8735) (https://doi.org/10.2903/j.efsa.2025.919

-Preparedness, prevention and control related to zoonotic avian (https://www.efsa.europa.eu/en/efsajournal/pub/9191)

-Risk posed by the HPAI virus H5N1, Eurasian lineage goose/Guangdong clade 2.3.4.4b. genotype B3.13, currently circulating in the US

-Vaccination of poultry against highly pathogenic avian influenza – part 1 and 2. https://doi.org/10.2903/j.efsa.2024.8755 https://doi.org/10.2903/j.efsa.2023.8271

ECDC, EFSA, DG Sante and EURL: **Avian Influenza simulation exercise (SIMEX) on avian influenza,** Brussels on the 10th and 11th December 2024

Cross-sector collaboration in action

World Organisation for Animal Health

Food and Agriculture Organization of the United Nations

TECHNICAL ACTIVITIES

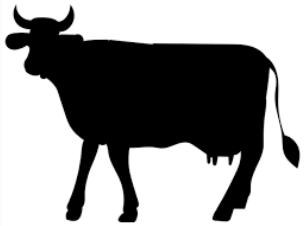
Avian Influenza

2025

- OFFLU statement on the development of a global consensus H5 Influenza genotyping framework (26 February)

2024

- OFFLU statement on HPAI in dairy cows (9 April)
- Public health resource pack for countries experiencing influenzas in animals (20 March)
- Updated OFFLU Statement on high pathogenicity avian influenza in dairy cows (13 May)
- Diagnostic guidance: HPAI dairy cattle (13 May)
- OFFLU avian influenza teleconference for sharing epidemiological and genetic information (12 December)



2023

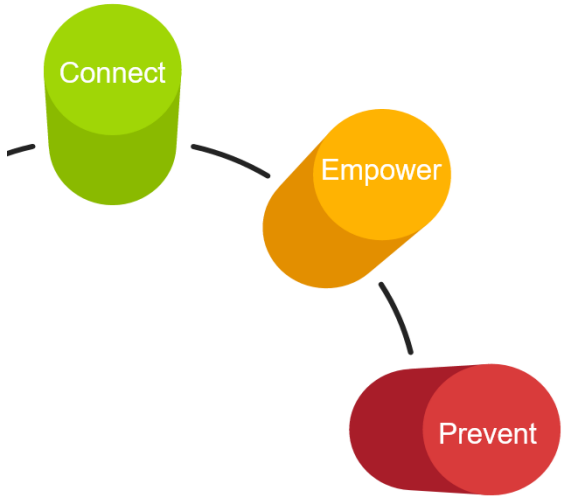
- Continued expansion of HPAI H5 in wildlife in South America and incursion into the Antarctic region (OFFLU statement) (21 December)
- OFFLU call to discuss Avian Influenza in the Latin America and Caribbean Region (9 November)
- OFFLU statement on HPAI H5 in wildlife in South America (23 August)
- Scientific Task Force on Avian Influenza and Wild Birds statement on H5N1 HPAI in wild birds (July)
- OFFLU statement on Infections with Avian Influenza A(H5N1) virus in cats in Poland (28 June)
- Global consultation on highly pathogenic avian influenza (HPAI) (2-4 May)
- OFFLU Statement on high pathogenicity avian influenza caused by viruses of the H5N1 subtype (17 March)
- OFFLU call to discuss Avian Influenza events in mammals (2 March)

2022

- OFFLU update of wild bird AI events in Canada, UK, Israel and other Europe countries (12 January)
- Influenza A Cleavage site update 2022 (4 January)

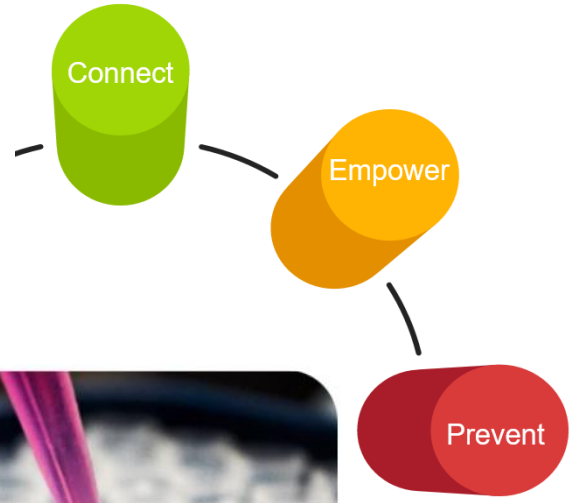
2021

- OFFLU zoom call for avian influenza global situation update summary (8 November)



OFFLU Summary Report from the WHO Influenza Vaccine Composition Meeting

September 2025



Avian Influenza Matching (AIM) Pilot

The goal of OFFLU Avian Influenza Matching (AIM) project (OFFLU-AIM) is to provide improved information on antigenic characteristics of avian influenza viruses to support vaccination programs against avian influenza.

Learn More ↗

WHO Vaccine Composition Meeting (VCM)

The need to update candidate vaccine viruses which may be prepared as part of the pandemic response for zoonotic influenza is assessed during the VCM. OFFLU brings important data from the animal health community.

Learn More ↗

Animal data feed into **VCM** (selection of candidate vaccine viruses for zoonotic risk), while **AIM** aligns **poultry vaccine** antigens with field strains

One Health in Action:
Key gaps and priorities

- **Data are not constantly fully integrated** – animal, human and environmental information should flow into a single, real-time One Health risk picture.

- Limited One Health use of **Proficiency testing schemes** in the human health sector rarely include zoonotic H5 strains, and **reference materials reflecting current animal-origin viruses** are often missing.

One Health in Action:
Key gaps and priorities

•**Surveillance:** big gaps in wild birds and mammals (dairy cattle, companion animals, wildlife) and in **many low-resource settings** (limited info from multiple continents.. International cooperation needs to be supported.. and be aware that there are other viruses we also need to keep under control such as H9N2, H3N8…)

•**Management of the Wild-Domestic Interface**:
-management strategies that favor spatial and ecological separation of wild birds and poultry habitats.
-continue surveillance of wildlife populations to inform and adapt conservation plan

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