มหาวิทยาลัยมหิดล
คณะสัตวแพทยศาสตร์

Bovine influenza A virus: A review for HPAI H5N1 virus infection in cattle

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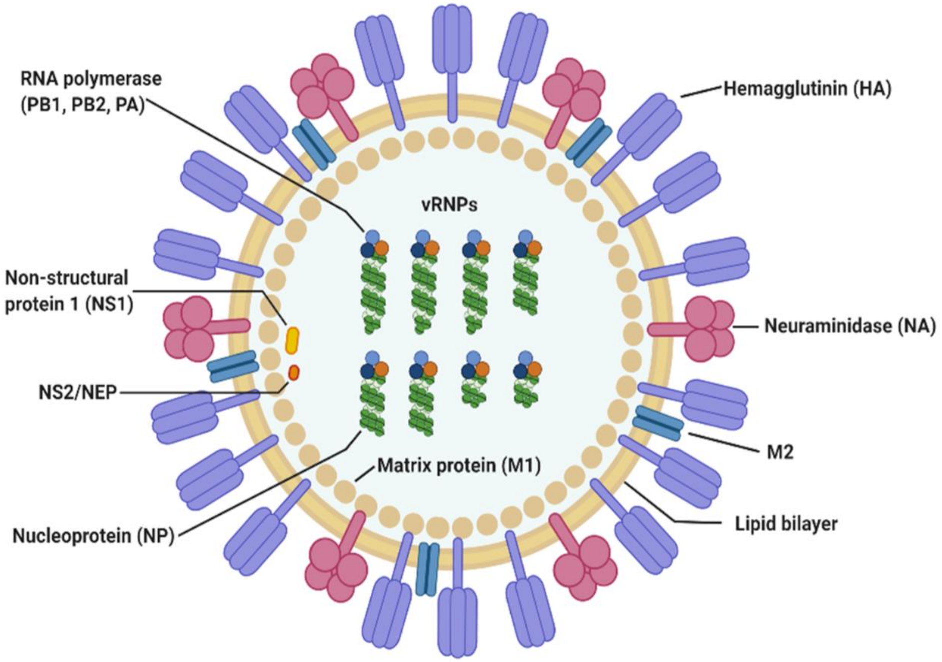
Influenza virus

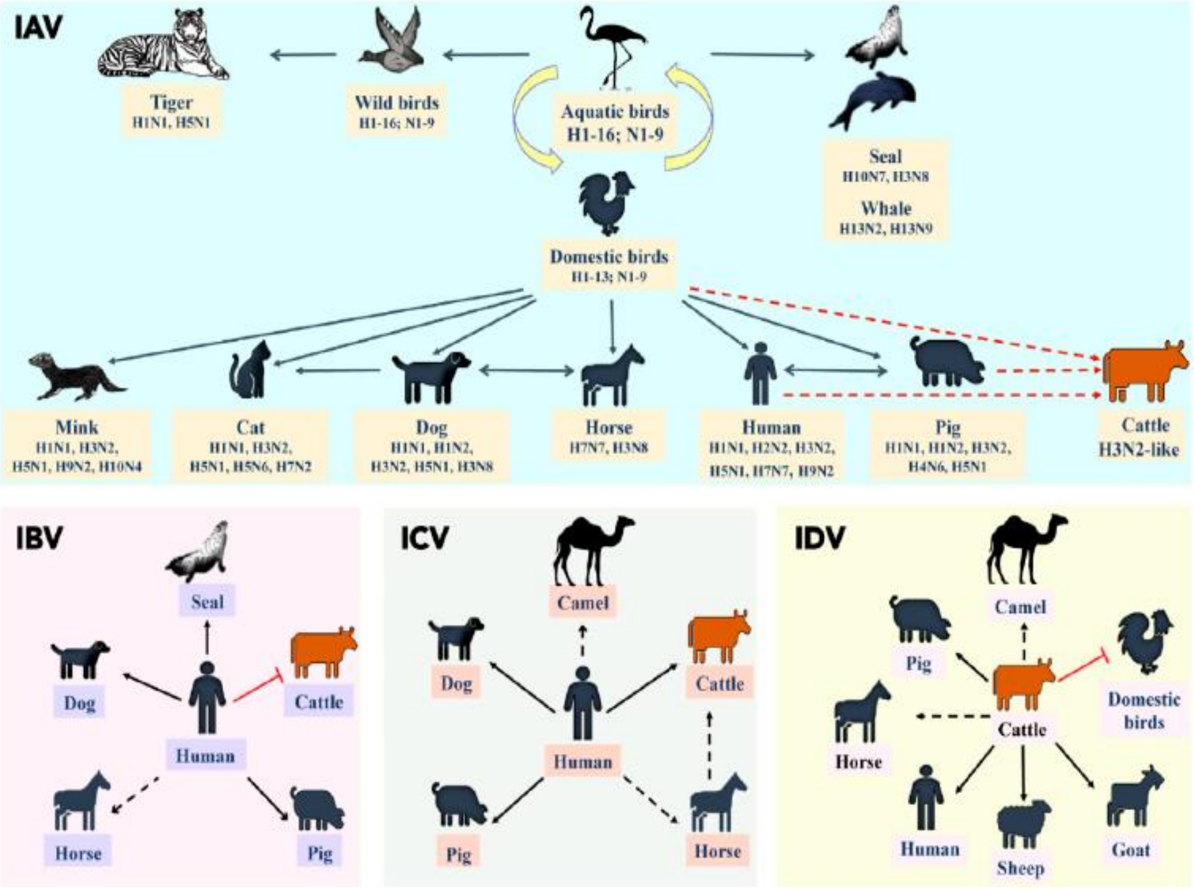
- Family Orthomyxoviridae consists of 7 genera
- Enveloped
- Negative-sense, ssRNA virus
- Segmented genome

(IAV:PB2, PB1, PA, HA, NP, NA, M, NS)

- IAV subtype H 1-19, N 1-11 Bat flu virus H17N10 (2009 in Guatemala), H18N11 (2012 in Peru)
- H19Nx in wild duck (Common Pochard (*Aythya ferina*)from Kazakhstan (2023)



Fereidouni S, *et al.*, Emerging microbes & infections. 2023



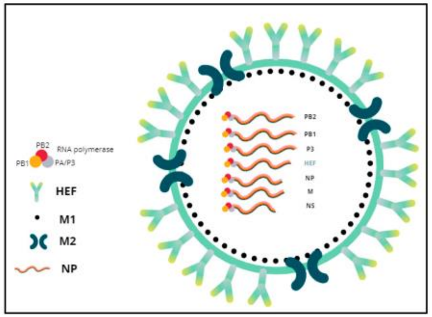
Influenza virus in bovine

- Ruminants were not previously considered to be the host species of influenza A virus (IAV)
- In 2011, an influenza C-like virus was isolated from pig, cattle in USA, Mexico, Asia (China, Japan) and Europe (France, Italy), then, classified by ICTV to be IDV

Sreenivasan CC, *et al.*, Viruses. 2019

Bovine Influenza Viruses

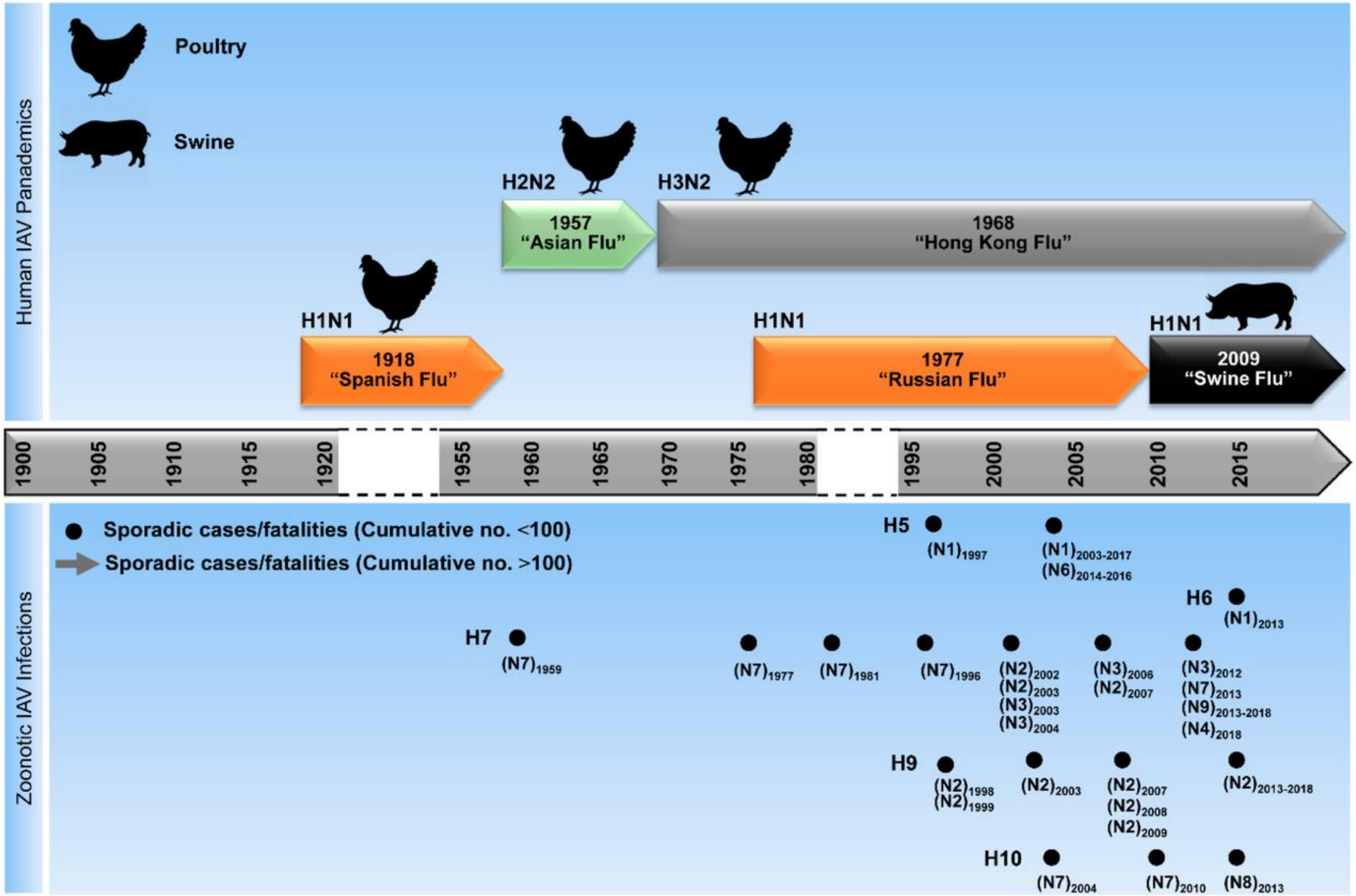
- Influenza C and D viruses are two of the many viruses and bacteria that can cause Bovine Respiratory Disease (BRD) in calves. BRD affects the respiratory system of young cattle.
- Historically, the first documented IAV case in cattle was reported back in 1949 where 160,000 cattle were infected in Japan by H1 and H3 subtypes
- The first isolated IAV from cattle was documented in Hungary in the 1960s



Ruiz M, *et al*., Viruses, 2022.

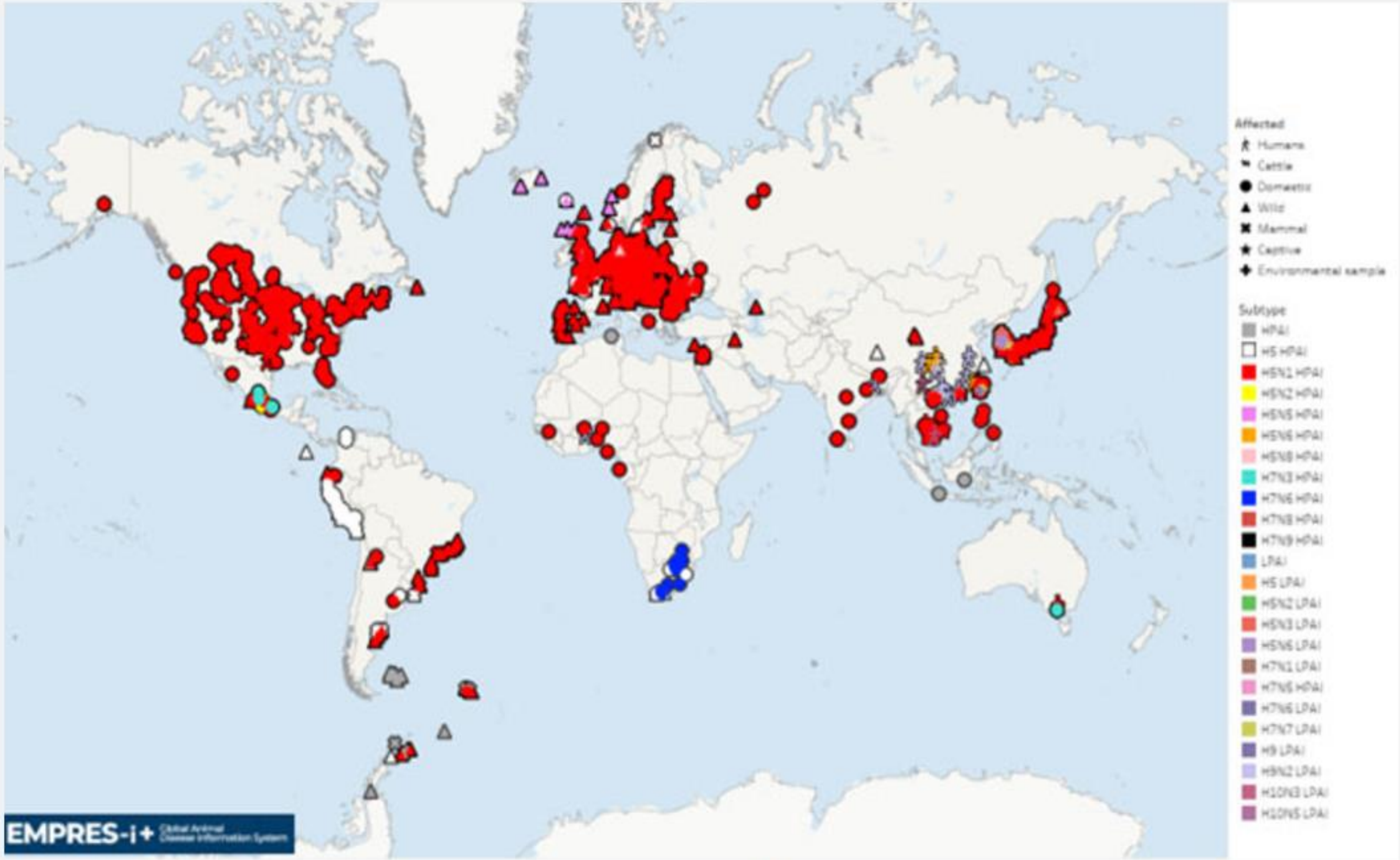
https://www.scientificamerican.com/article/this-cow-and-pig-influenza-virus-could-infect-humans-what-we-know-so-far/

Timeline of flu pandemics and epidemics caused by influenza A virus



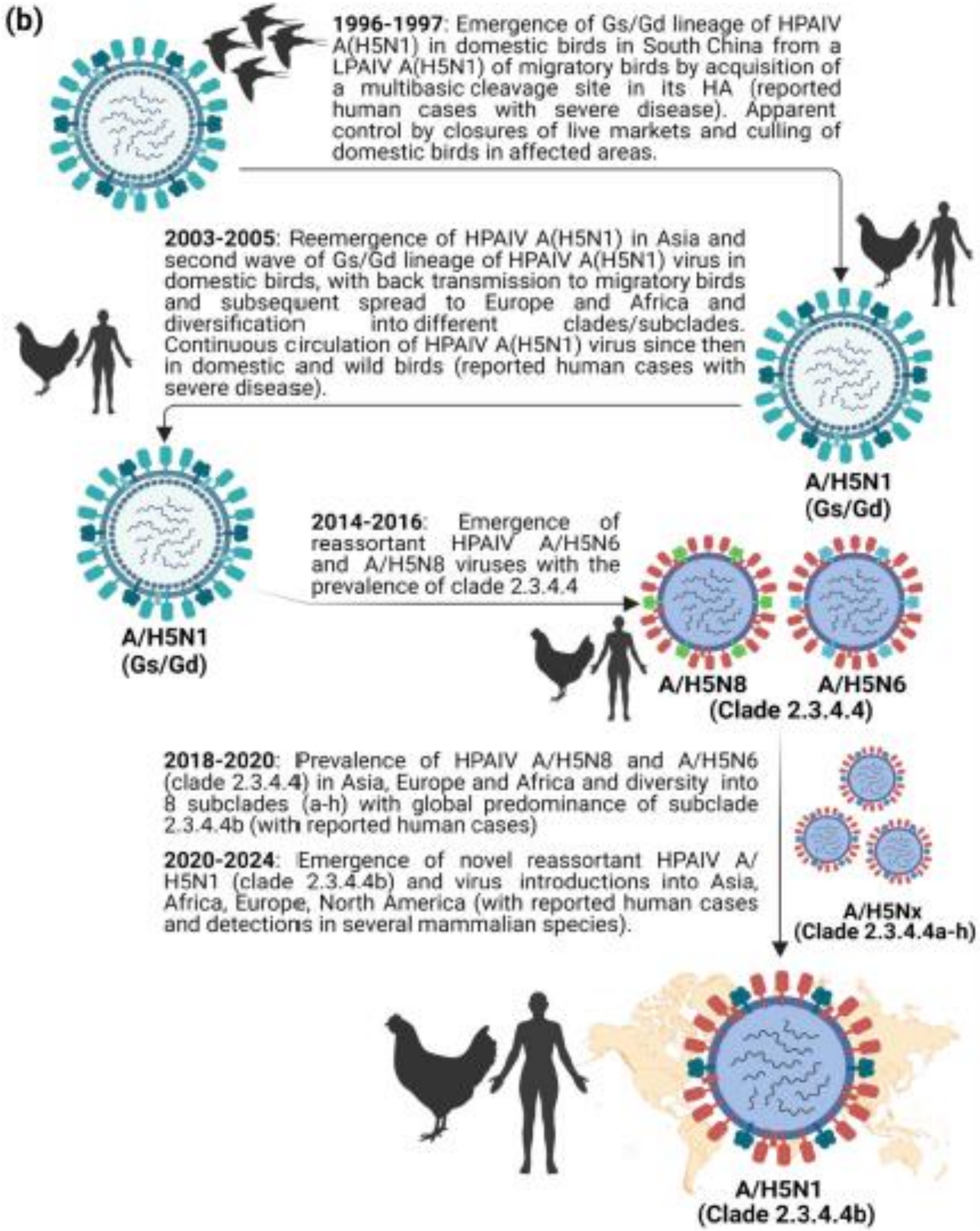
Ahmed Mostafa, Elsayed M. Abdelwhab, Thomas C. Mettenleiter, and Stephan Pleschka - mdpi.com/1999-4915/10/9/497/ htmhttps://en.wikipedia.org/wiki/Influenza_A_virus

Map 2. *Global distribution of AIV with zoonotic potential* observed in the period 1 October 2023 to 30 September 2024 (i.e. previous wave)*



Note: Symbols may overlap for events in similar geographic locations.

https://www.fao.org/animal-health/situation-updates/global-aiv-with-zoonotic-potential/en



1996-1997: Emergence of Gs/Gd lineage of HPAIV A(H5N1) in domestic birds in South China from a LPAIV A(H5N1) of migratory birds by acquisition of a multibasic cleavage site in its HA (reported human cases with severe disease). Apparent control by closures of live markets and culling of domestic birds in affected areas.

2003-2005: Reemergence of HPAIV A(H5N1) in Asia and second wave of Gs/Gd lineage of HPAIV A(H5N1) virus in domestic birds, with back transmission to migratory birds and subsequent spread to Europe and Africa and diversification into different clades/subclades. Continuous circulation of HPAIV A(H5N1) virus since then in domestic and wild birds (reported human cases with severe disease).

2014-2016: Emergence of reassortant HPAIV A/H5N6 and A/H5N8 viruses with the prevalence of clade 2.3.4.4

2018-2020: Prevalence of HPAIV A/H5N8 and A/H5N6 (clade 2.3.4.4) in Asia, Europe and Africa and diversity into 8 subclades (a-h) with global predominance of subclade 2.3.4.4b (with reported human cases)

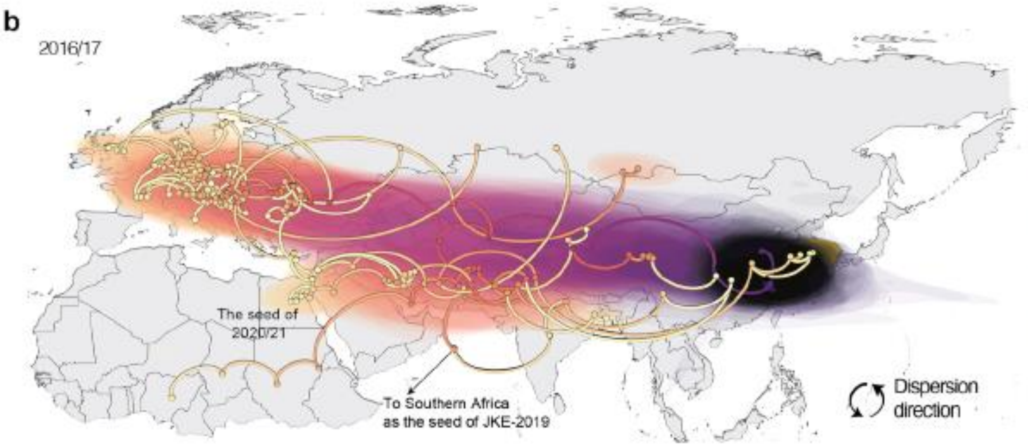
2020-2024: Emergence of novel reassortant HPAIV A/H5N1 (clade 2.3.4.4b) and virus introductions into Asia, Africa, Europe, North America (with reported human cases and detections in several mammalian species).

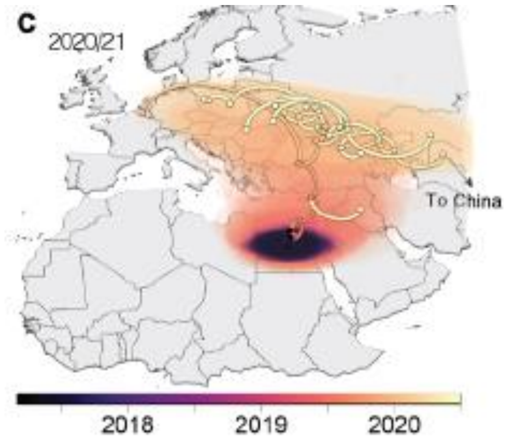
History of highly pathogenic avian influenza virus (HPAIV) H5N1 emergence in migratory birds in 1996 and key evolutionary occurrences until 2024

Mostafa A, *et al*.,. mBio. 2024 Nov 13:e02542-24.

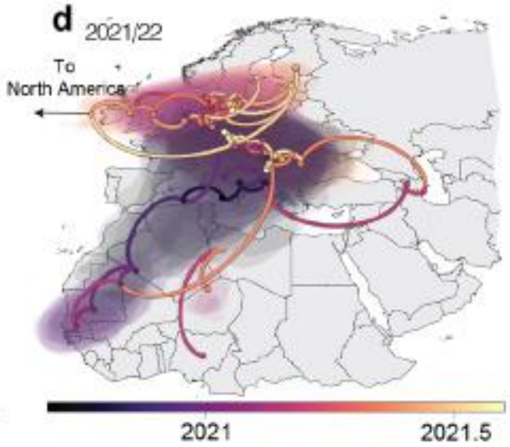
Evolution of clade 2.3.4.4b and early migration patterns of resurgence HPAI

Spread of H5N8 from mid-2014 to 2017

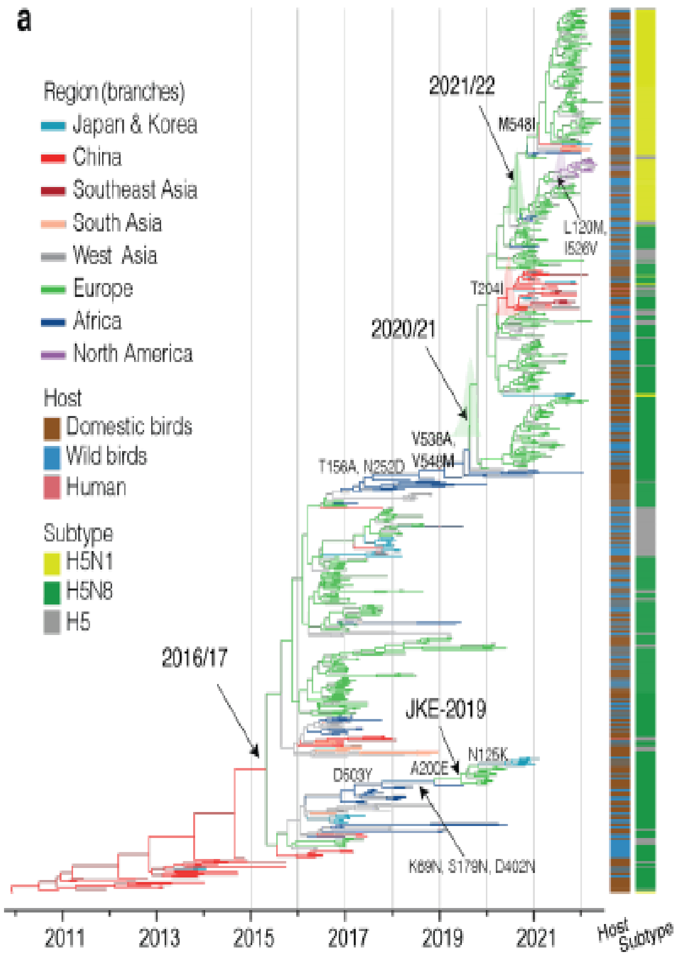




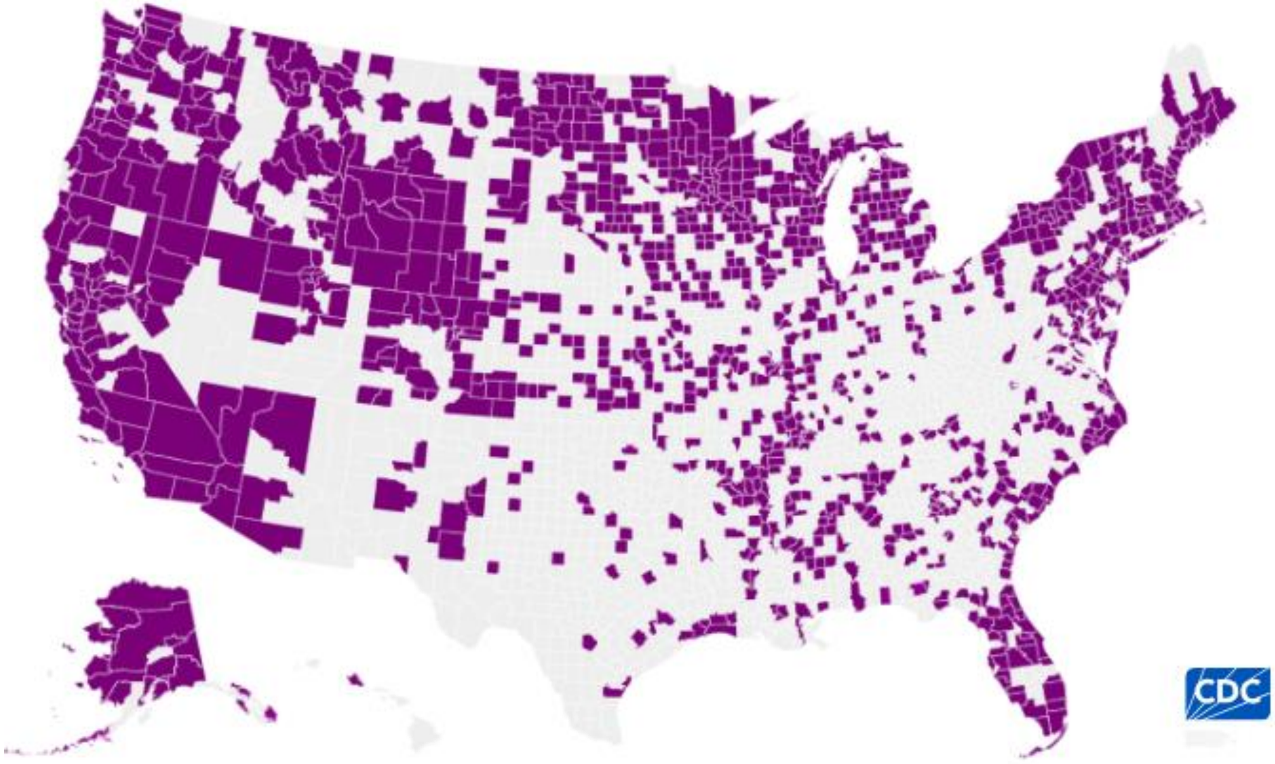
Early spread of H5N8 from 2017 to mid-2020



Early spread of H5N1 from mid-2020 to 2021



Xie R, *et al.*, Nature. 2023 Oct.



Birds Detected

10,619 (wild birds)

Birds Affected

111,176,176 (poultry)

Highly pathogenic avian influenza (HPAI) A(H5) viruses have been detected in U.S. wild aquatic birds, commercial poultry and backyard or hobbyist flocks beginning in January 2022.

Counties in which bird flu has been detected in wild birds

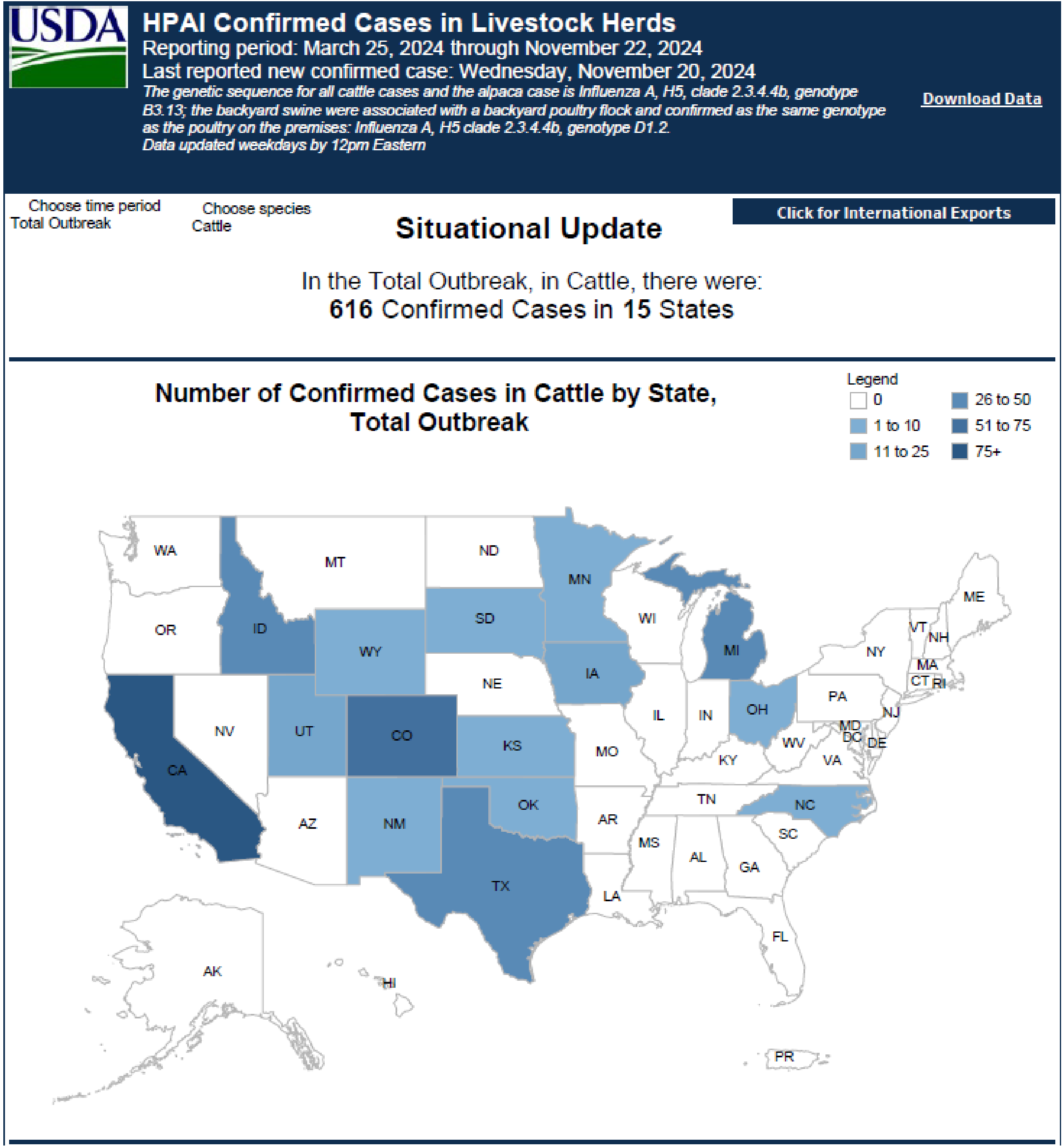
https://www.cdc.gov/bird-flu/situation-summary/data-map-wild-birds.html

HPAI H5N1 virus infection in US cattle

The 2024 H5N1 outbreak in US dairy cattle

- Starting in February 11, 2024, Texas dairy farmers noticed unexplained drops in milk production in lactating cattle and thick, yellow milk, which was later accompanied by dead cats on several farms.
- USDA first confirmed on March 25, 2024.
- All cattle viruses are belong to the H5N1 virus clade 2.3.4.4b genotype B3.13

Peacock T, et al., Nature. 2024 Sep 24:1-2.



USDA

HPAI Confirmed Cases in Livestock Herds
Reporting period: March 25, 2024 through November 22, 2024
Last reported new confirmed case: Wednesday, November 20, 2024
The genetic sequence for all cattle cases and the alpaca case is Influenza A, H5, clade 2.3.4.4b, genotype B3.13; the backyard swine were associated with a backyard poultry flock and confirmed as the same genotype as the poultry on the premises: Influenza A, H5 clade 2.3.4.4b, genotype D1.2.
Data updated weekdays by 12pm Eastern

Choose time period
Total Outbreak

Choose species
Cattle

Situational Update

In the Total Outbreak, in Cattle, there were:
616 Confirmed Cases in 15 States

Number of Confirmed Cases in Cattle by State, Total Outbreak

Legend
☐ 0
🟦 1 to 10
🟦 11 to 25
🟦 26 to 50
🟦 51 to 75
🟦 75+

HPAI H5N1 virus infection in US dairy cattle

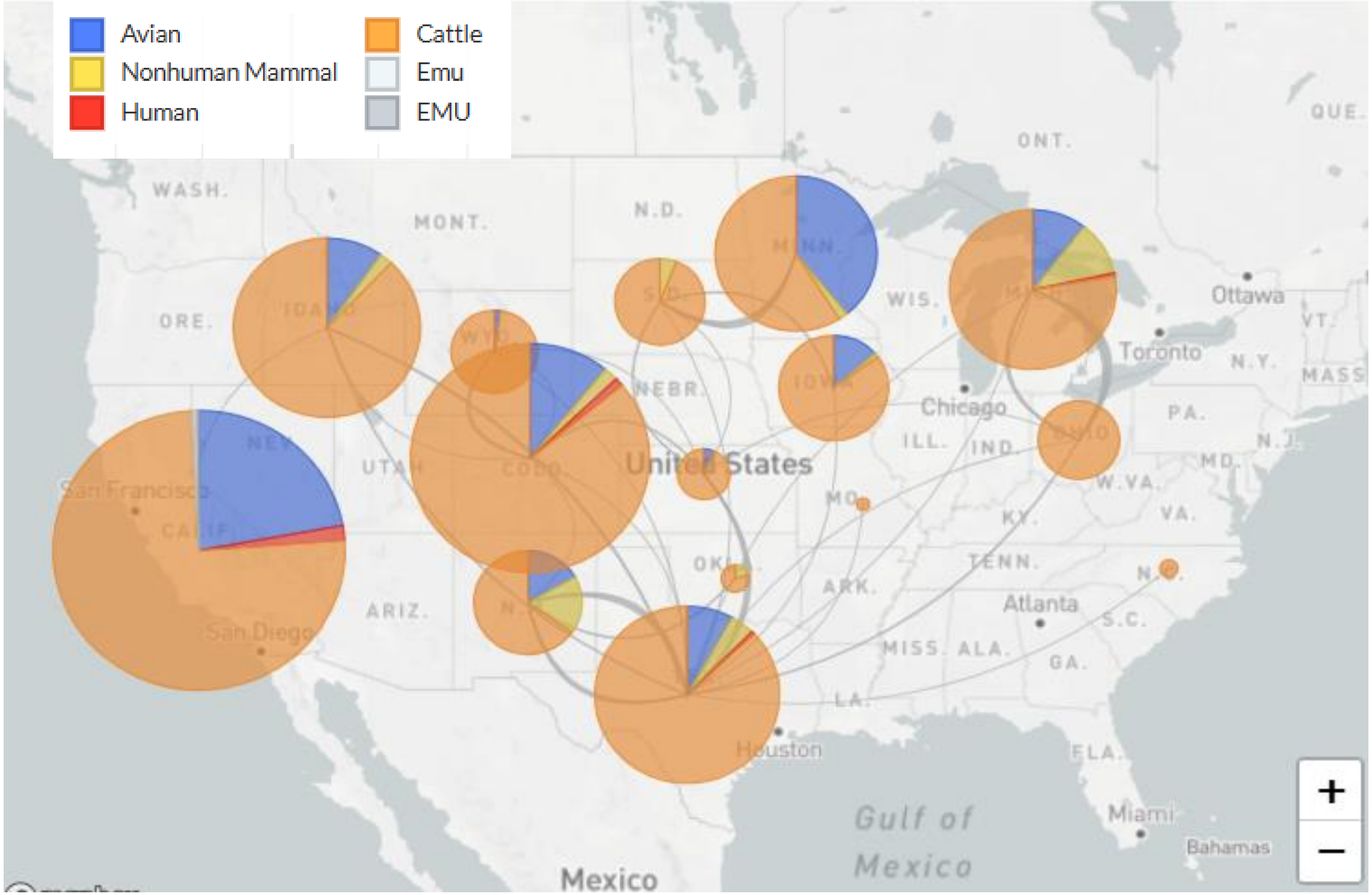
https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/hpai-confirmed-cases-livestock

The 2024 H5N1 outbreak in US dairy cattle

- The bovine B3.13 outbreak originated in Texas and rapidly spread to additional states (15 total as of Nov 2024, Texas, New Mexico, Oklahoma, Colorado, Kansas, Idaho, Wyoming, South Dakota, Michigan, Iowa, Minnesota, Ohio, and North Carolina, California, Utah)

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

H5N1 virus transmission pattern of the recent outbreak in the USA



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

Clinical sign in dairy cattle

- Affected dairy cattle presented with decreased feed intake, decreased rumination time, mild respiratory signs (clear nasal discharge, increased respiratory rate and laboured breathing), lethargy, dehydration, dry/tacky faeces or diarrhoea, and milk with abnormal yellowish colostrum-like colour, thick and sometimes curdled consistency.
- Morbidity 3% and 20%
- Mortality above average (low at 2% or less)

Caserta LC, *et al*., Nature, 2024.

Clinical presentation of HPAI H5N1 infection in dairy cattle

Clinically affected animals presenting clear nasal discharge and involution of the mammary gland/udder

Milk presents a yellowish colostrum-like color and appearance (top panels), or coloration varies from yellowish to pink/brown. The Curdling of milk is visible in some samples.

Caserta LC, *et al*., Nature, 2024.

The 2024 H5N1 outbreak in US dairy cattle

- In April-May 2024, more than one-third of retail pasteurized milk samples from 12 US states contained H5N1 genetic fragments that present no danger to humans, but indicate the widespread distribution of the virus in dairy cattle.

Peacock T, et al., 2024, Tarbuck N et al., 2024

Bird flu causes blindness in cats that drank milk from infected cows at Texas dairy farm - sparking fears the virus is evolving

- **More than half of the cats at a dairy farm died within 3 days of being infected**
- **Farmers first noticed that cows were producing strange milk - and less of it**
- **Cats infected with H5N1 went blind, lost coordination, and started circling**
- **READ MORE: What you can and CAN'T eat amid America's bird flu outbreak**

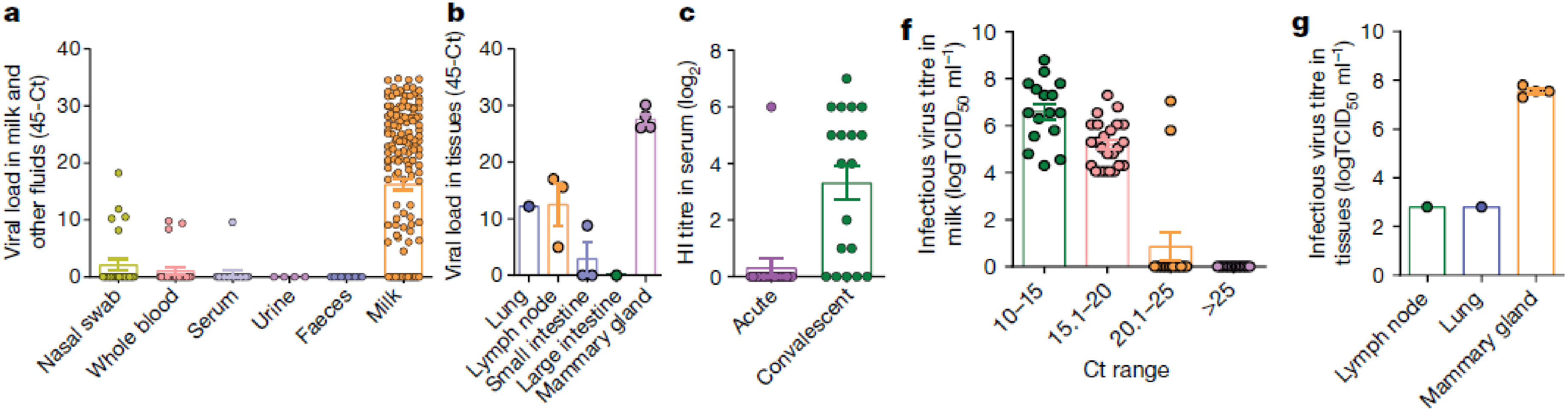
By PETER HESS FOR DAILYMAIL.COM

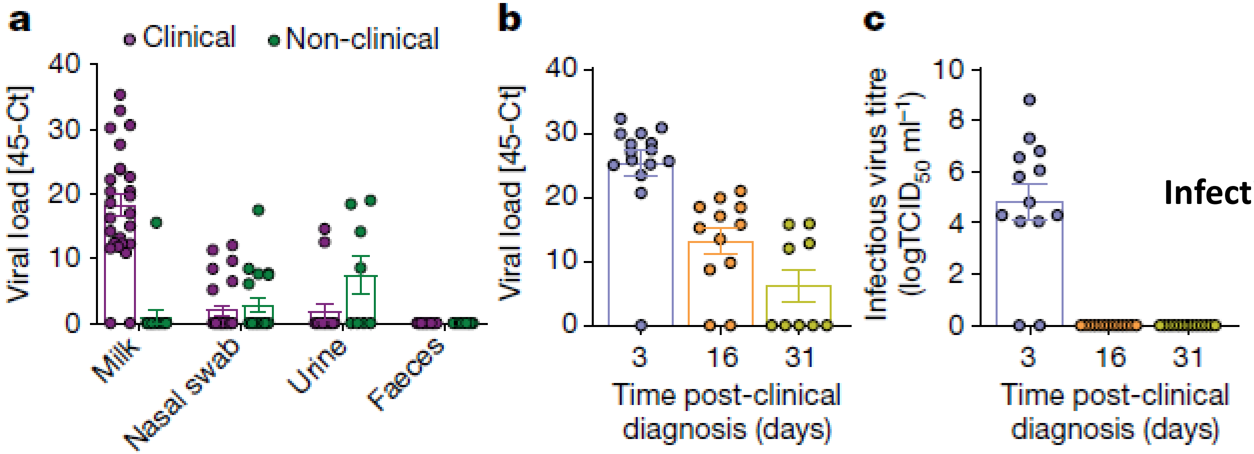
PUBLISHED: 17:20 GMT, 30 April 2024 | **UPDATED:** 17:41 GMT, 30 April 2024

The US Centers for Disease Control and Prevention (CDC) announced the cases this week, revealing at least 24 cats on a Texas farm experienced depressed mental states, body stiffness, loss of coordination, blindness, circling, and excessive mucous from their eyes and noses.

https://www.dailymail.co.uk/sciencetech/article-13366941/bird-flu-symptoms-cats-blind-raw-milk-texas.html

HPAI H5N1 detection and isolation of from infected dairy cattle



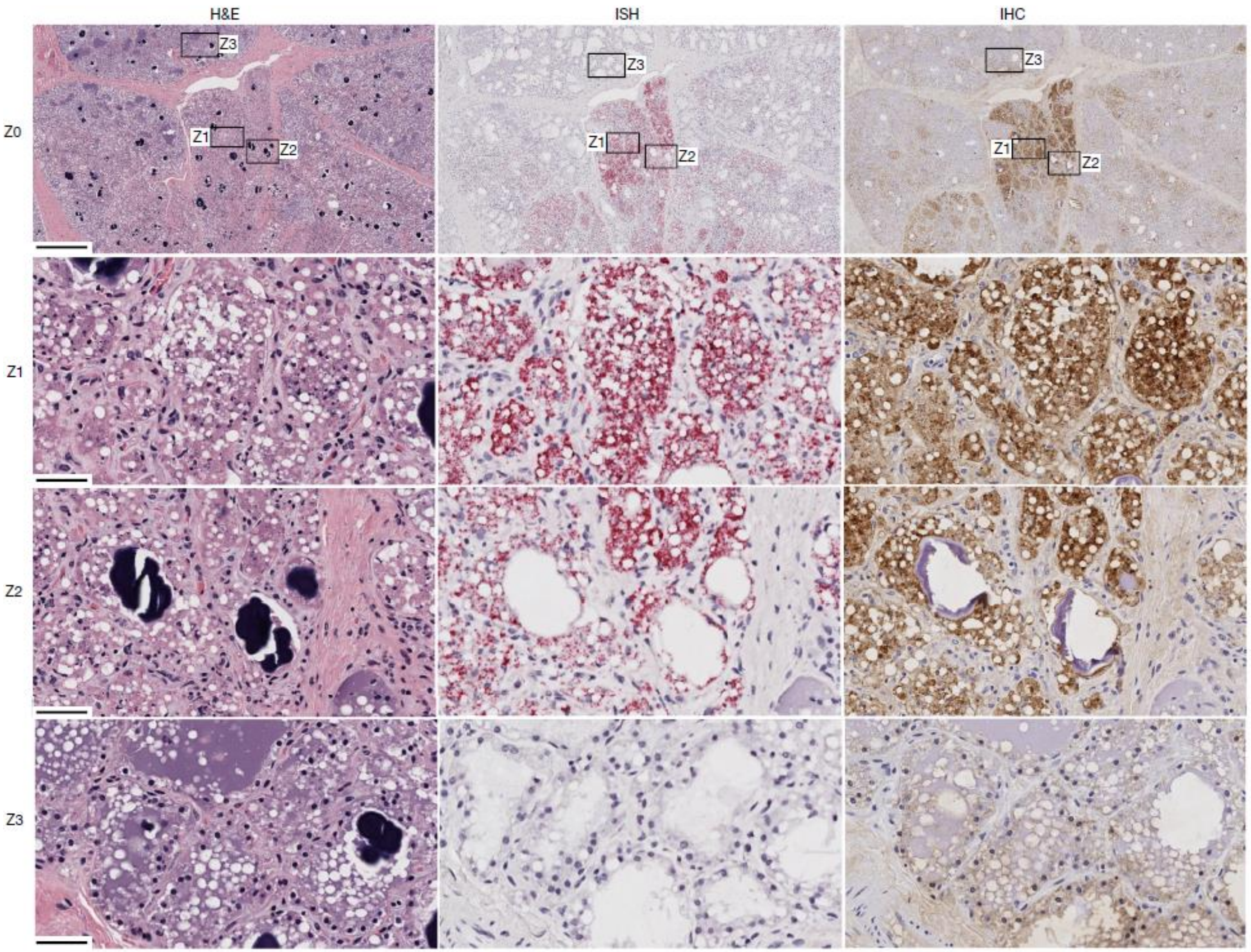


Infectious HPAI virus in milk

Virus shedding patterns

Caserta LC, *et al.*, Nature, 2024.

Detection of HPAI H5N1 in dairy cattle mammary gland tissue



- In situ hybridization (ISH; middle panels) targeting the IAV matrix gene
- Immunohistochemistry (IHC; right panels) targeting the IAV matrix protein

Caserta LC, *et al.*, Nature, 2024.

Sialic Acid Receptor Specificity in Mammary Gland of Dairy Cattle

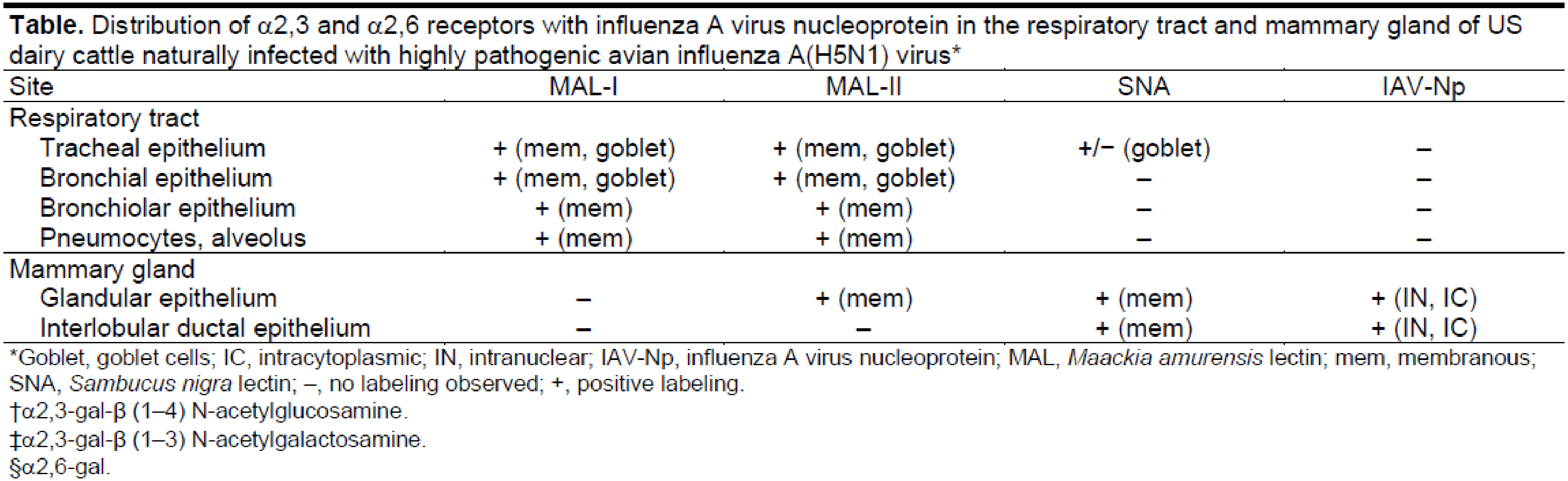


Table. Distribution of α2,3 and α2,6 receptors with influenza A virus nucleoprotein in the respiratory tract and mammary gland of US dairy cattle naturally infected with highly pathogenic avian influenza A(H5N1) virus*

Site	MAL-I	MAL-II	SNA	IAV-Np
Respiratory tract				
Tracheal epithelium	+ (mem, goblet)	+ (mem, goblet)	+/− (goblet)	–
Bronchial epithelium	+ (mem, goblet)	+ (mem, goblet)	–	–
Bronchiolar epithelium	+ (mem)	+ (mem)	–	–
Pneumocytes, alveolus	+ (mem)	+ (mem)	–	–
Mammary gland				
Glandular epithelium	–	+ (mem)	+ (mem)	+ (IN, IC)
Interlobular ductal epithelium	–	–	+ (mem)	+ (IN, IC)

*Goblet, goblet cells; IC, intracytoplasmic; IN, intranuclear; IAV-Np, influenza A virus nucleoprotein; MAL, *Maackia amurensis* lectin; mem, membranous; SNA, *Sambucus nigra* lectin; –, no labeling observed; +, positive labeling.

†α2,3-gal-β (1–4) N-acetylglucosamine.

‡α2,3-gal-β (1–3) N-acetylgalactosamine.

§α2,6-gal.

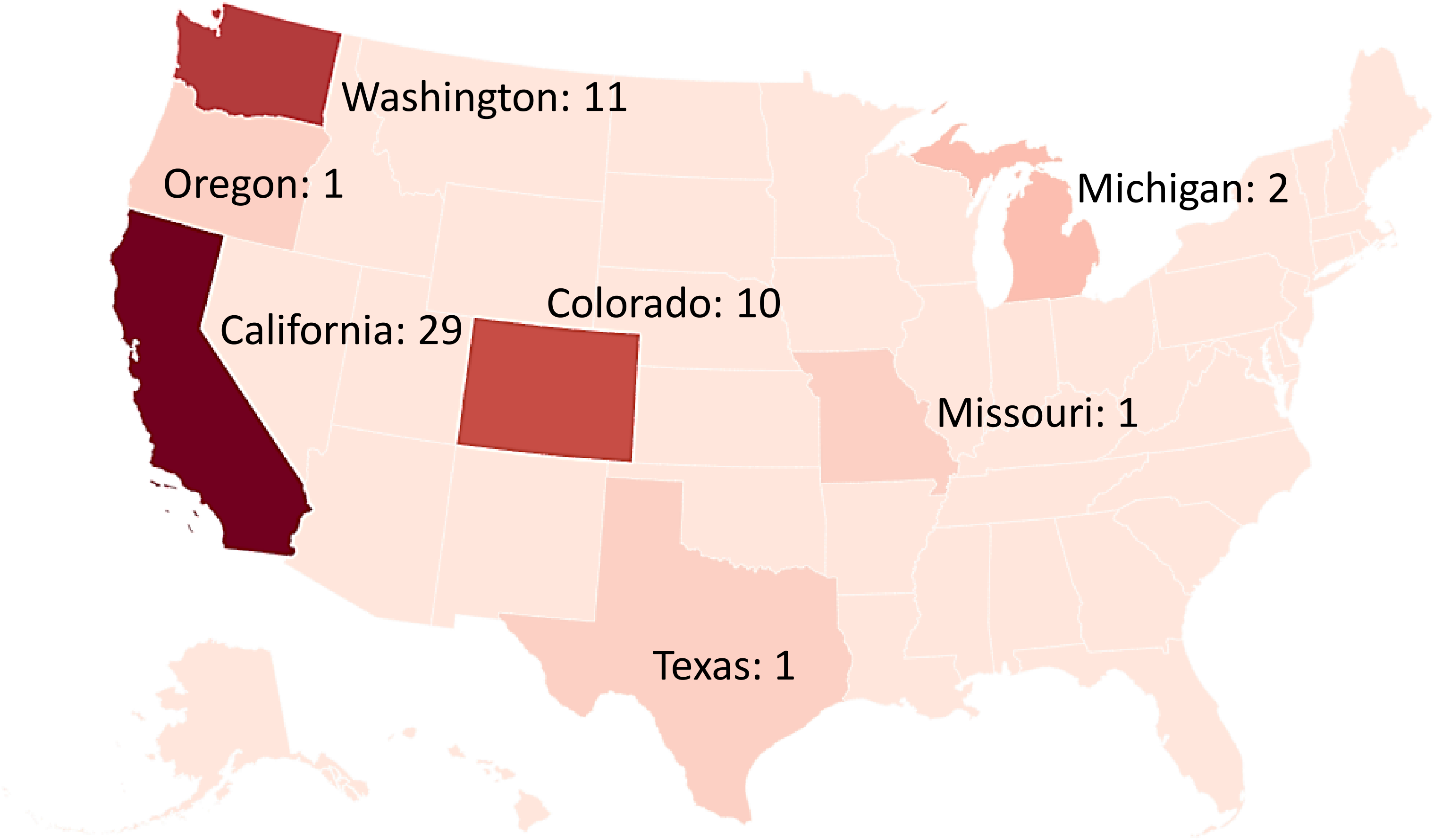
Nelli RK, *et al.*, Emerging Infectious Diseases. 2024

Human cases of bird flu infection in USA, 2024

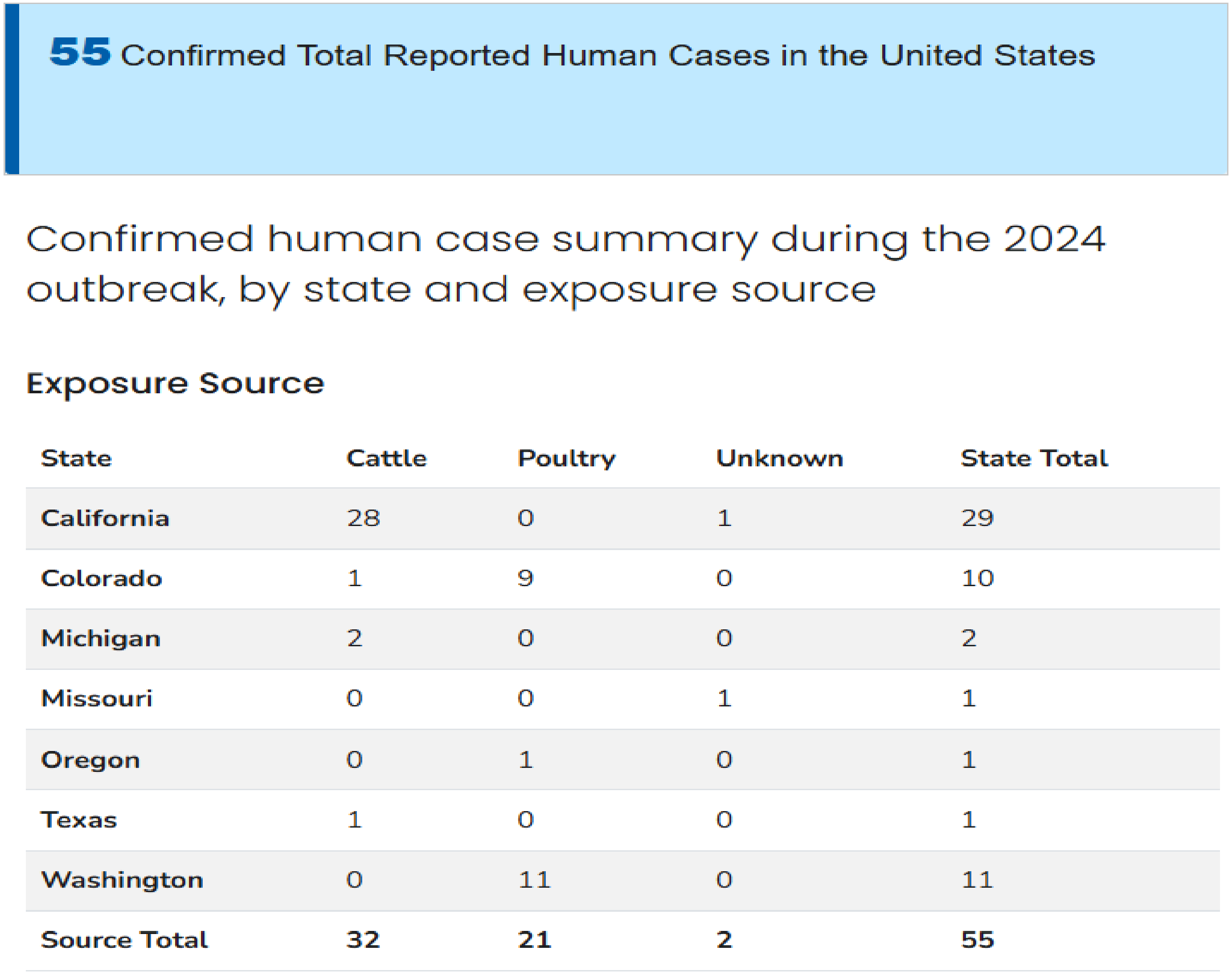
- Since April 2024, CDC, working with state public health departments, has confirmed avian influenza A(H5) virus infections in 55 people in the United States.
- The 28 cases are dairy farm workers in California

https://www.cdc.gov/bird-flu/situation-summary/index.html

Human cases of bird flu infection in USA, 2024



Adapt from: https://www.newsweek.com/bird-avian-flu-map-us-states-1987366



55 Confirmed Total Reported Human Cases in the United States

Confirmed human case summary during the 2024 outbreak, by state and exposure source

Exposure Source

State	Cattle	Poultry	Unknown	State Total
California	28	0	1	29
Colorado	1	9	0	10
Michigan	2	0	0	2
Missouri	0	0	1	1
Oregon	0	1	0	1
Texas	1	0	0	1
Washington	0	11	0	11
Source Total	**32**	**21**	**2**	**55**

https://www.cdc.gov/bird-flu/situation-summary/index.html

Human cases of bird flu infection in USA, 2024

- These farm workers all described mild symptoms, many with eye redness or discharge (conjunctivitis).
- Some workers who tested positive in Washington reported some mild upper respiratory symptoms. None of the workers were hospitalized.

Figure 1. Conjunctivitis with Subconjunctival Hemorrhage in Both Eyes.

Human case from Texas outbreak

Uyeki TM, et al., New England Journal of Medicine. 2024 May 3.

Human cases of bird flu infection in USA, 2024

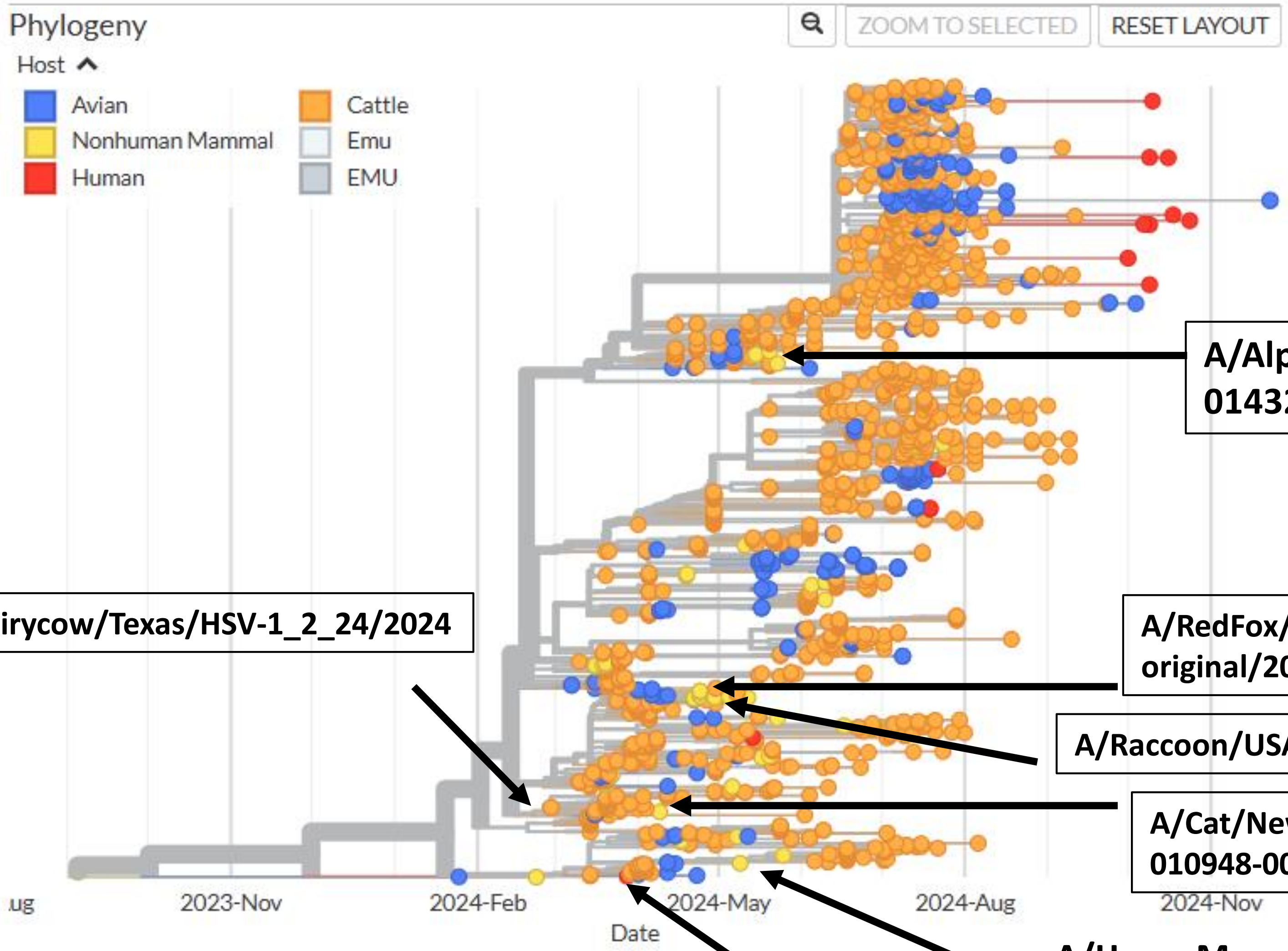
- CDC continues to recommend oseltamivir for treatment of patients with H5N1 and for post-exposure prophylaxis of close contacts of confirmed H5N1 patients and those with higher risk exposures to animals infected with H5N1 viruses.

https://www.cdc.gov/bird-flu/situation-summary/index.html

Full genome analysis of the ongoing influenza A/H5N1 cattle outbreak in North America

Built with nextstrain/avian-flu. Maintained by Louise Moncla and the Nextstrain team. Data updated 2024-11-23. Enabled by data from USDA, Andersen Lab and GenBank.

Showing 1940 of 1940 genomes sampled between Jan 2024 and Nov 2024.



A/Alpaca/ID/24-014328-011/2024

A/dairycow/Texas/HSV-1_2_24/2024

A/RedFox/MI/24-013016-004-original/2024

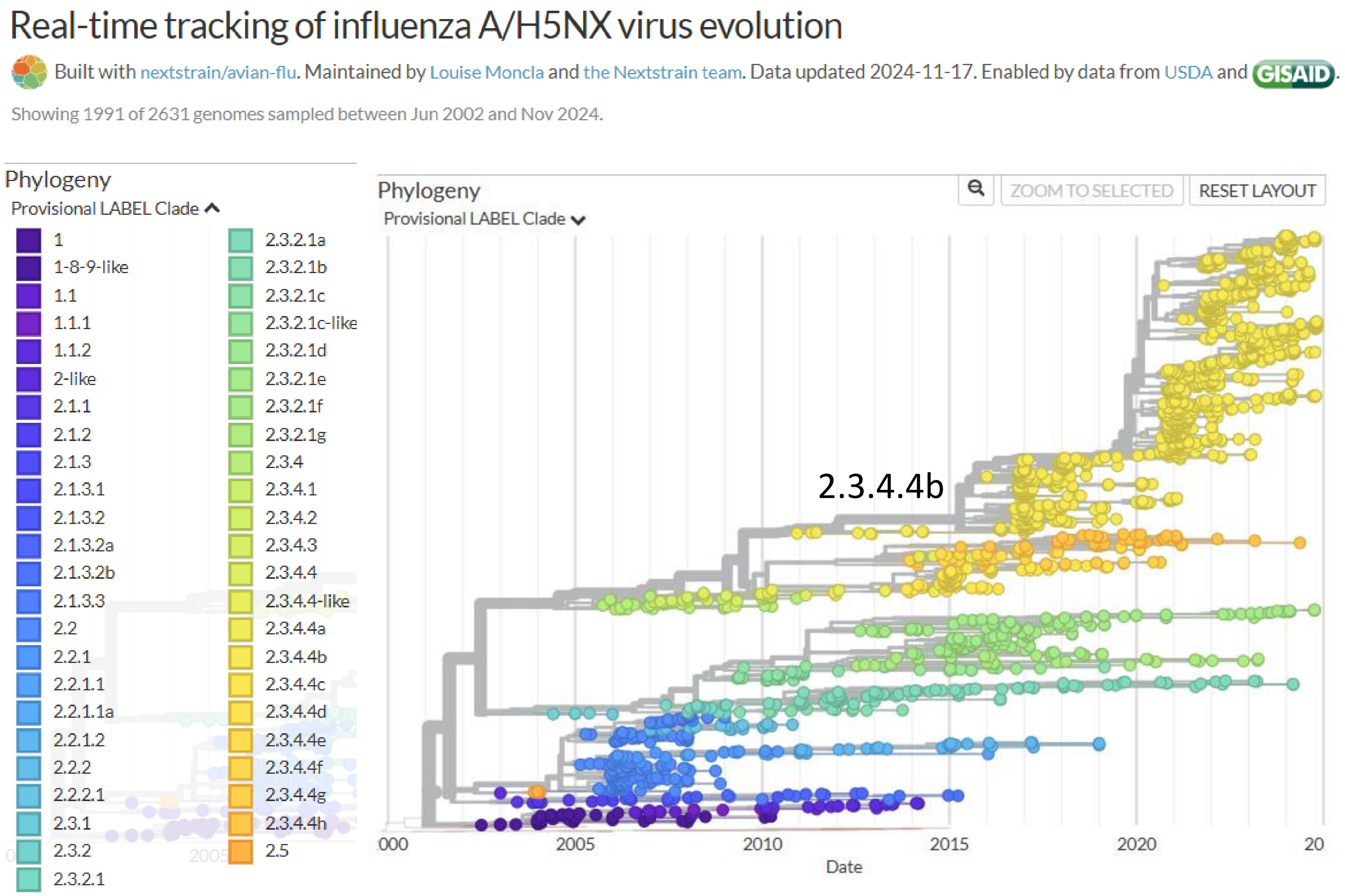
A/Raccoon/USA/24-011659-002/2024

A/Cat/NewMexico/24-010948-001original/2024

A/HouseMouse/NM/24-014782-003-original/2024

A/Texas/37/2024

https://nextstrain.org/avian-flu/h5n1-cattle-outbreak/genome?s=A/RACCOON/USA/24-011659-002/2024



Real-time tracking of influenza A/H5NX virus evolution

Built with nextstrain/avian-flu. Maintained by Louise Moncla and the Nextstrain team. Data updated 2024-11-17. Enabled by data from USDA and GISAID.

Showing 1991 of 2631 genomes sampled between Jun 2002 and Nov 2024.

https://nextstrain.org/avian-flu/h5nx/ha/all-time?c=h5_label_clade

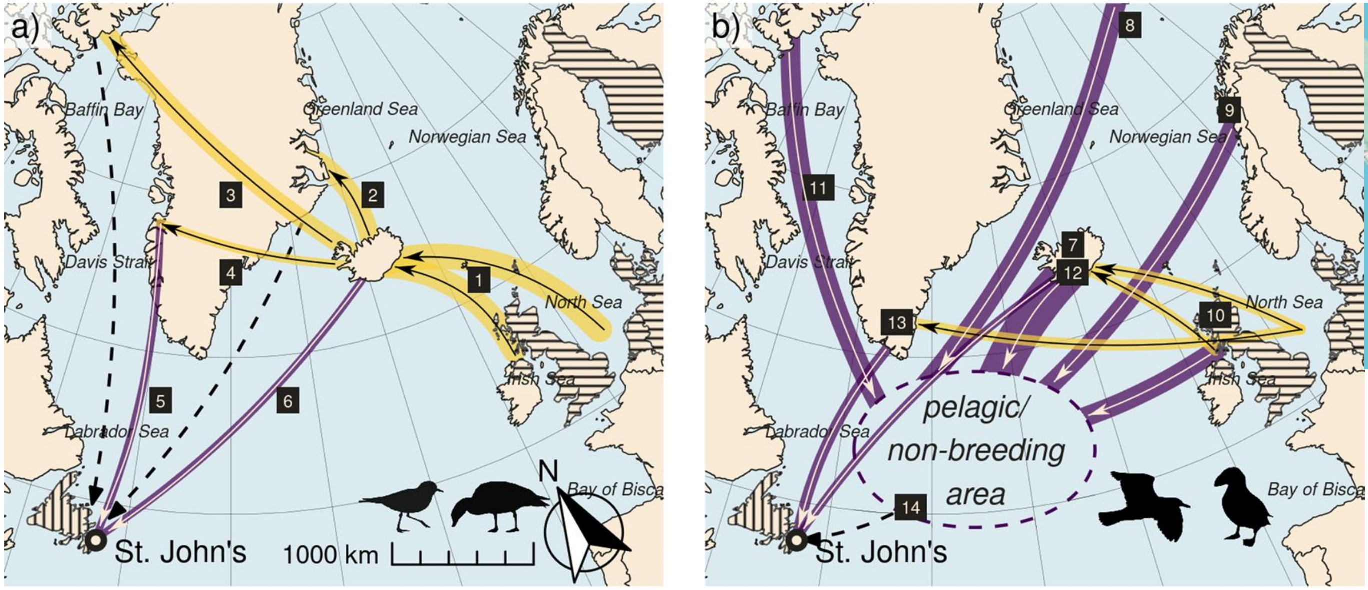
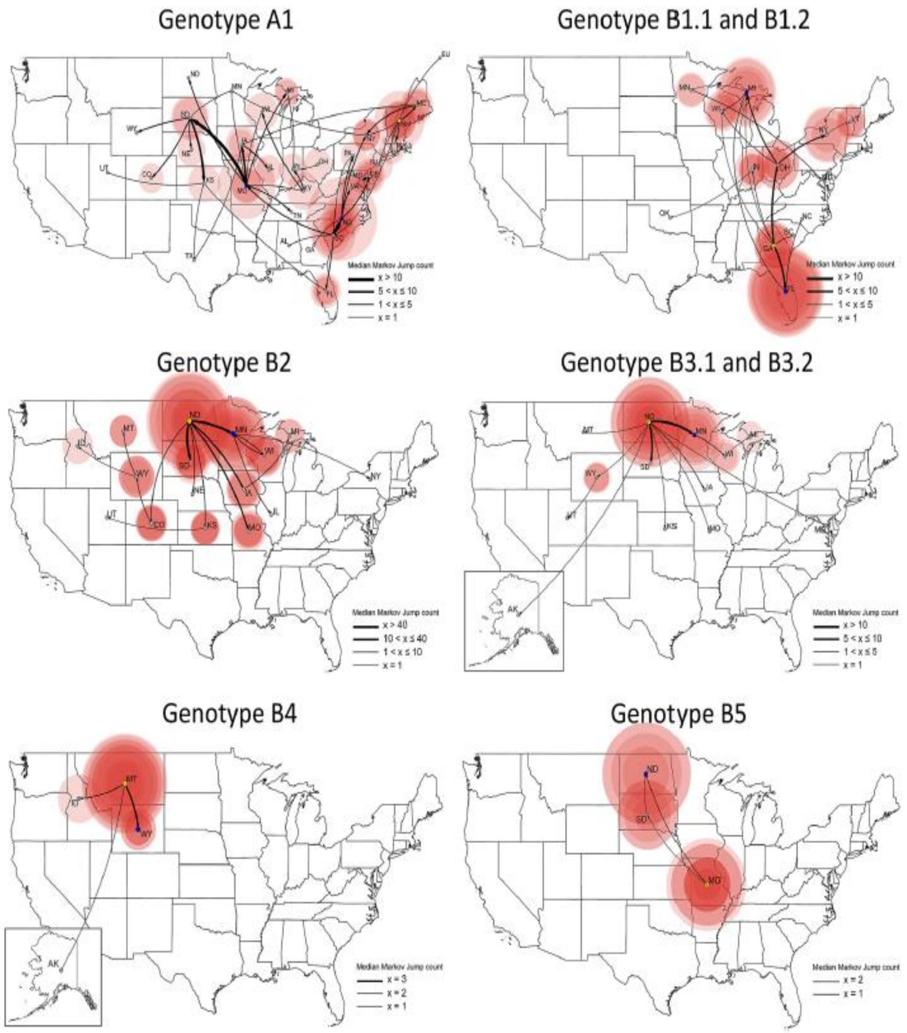


Figure Maps of transatlantic migration. Putative virus transmission pathways between Europe and Newfoundland via migratory waterfowl/shorebirds

- **In December 2021**, there was a die-off of domestic birds on an exhibition farm in St. John's, a city on the Avalon Peninsula of the island of Newfoundland, on the Atlantic coast of Canada. The virus was identified as a clade 2.3.4.4b H5N1 virus

Caliendo V, *et al*., Scientific reports. 2022 Jul 11;12(1):11729.



Spatial diffusion of US H5N1 clade 2.3.4.4b viruses

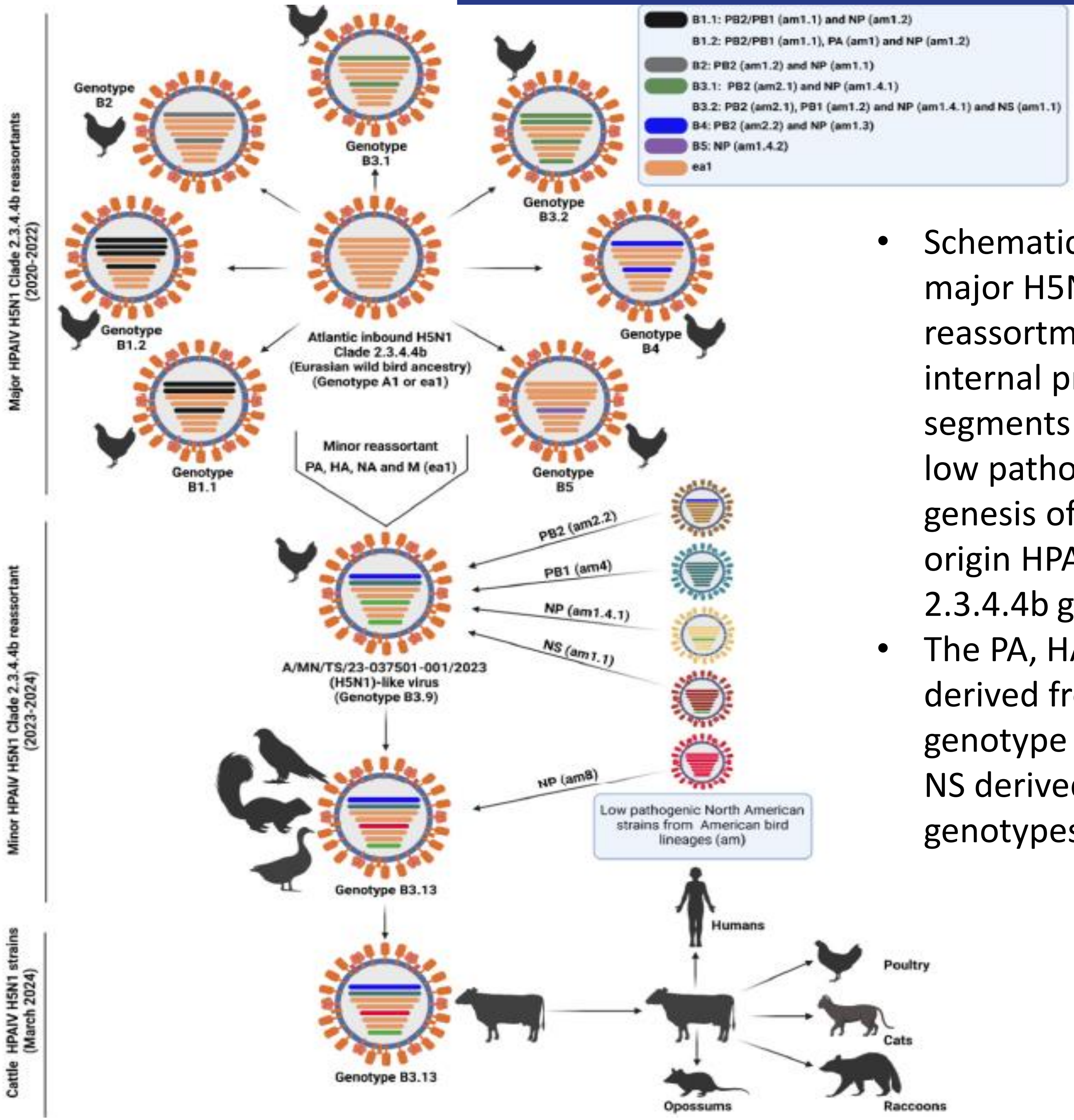
Youk S, *et al.,* Virology. 2023 Oct 1;587:109860.

Genotype	First detected	Last detected	Flyway distribution (initial detection in bold)	Overall percent (n=6983)
A1	Dec-21	Dec-23	**ATL**>MISS CEN PAC	5.4
B1.1	Jan-22	May-24	**ATL**>MISS CEN	9.7
A2	Feb-22	Mar-24	**ATL**>MISS	2.8
B1.2	Feb-22	Dec-23	**ATL**>MISS CEN	4.0
B2.1	Mar-22	Sep-23	**MISS**>CEN PAC	10.2
B2.2	Mar-22	Nov-23	**MISS**>CEN PAC ATL	4.6
B3.1	Mar-22	Nov-23	**MISS**>CEN PAC southern ATL	1.5
B3.2	Mar-22	Jul-24	**MISS**>CEN PAC ATL	20.4
B5.1	Mar-22	Jul-22	**CEN MISS**	0.3
A3	Apr-22	May-24	**PAC**	2.9
B4.1	Apr-22	Apr-23	**CEN**>PAC	6.4
B1.3	Jun-22	Dec-23	**ATL**>MISS CEN	7.6
‡A4	Sep-22	Nov-22	**northern PAC**	0.1
‡A5	Oct-22	Mar-23	**northern ATL**	0.2
B3.3	Oct-22	Sep-23	**MISS CEN***	0.5
B3.4	Nov-22	Apr-23	**MISS**>CEN	0.4
B3.5	Nov-22	Mar-24	**MISS CEN**>northern ATL	1.3
B3.6	Nov-22	Jul-24	**CEN MISS**>PAC ATL	10.8
‡A6	Jan-23	Mar-24	**northern ATL**	0.2
B3.7	Sep-23	May-24	**PAC**>CEN MISS ATL	1.8
B3.10	Oct-23	Jan-24	**northern PAC**	0.1
B3.11	Oct-23	Dec-23	**CEN**	<0.1
C1.1	Oct-23	Oct-23	**MISS**	<0.1
C2.1	Oct-23	Jul-24	**ATL**>MISS CEN	5.1
B3.8	Nov-23	Dec-23	**CEN**	0.1
B3.9	Nov-23	Dec-23	**ATL**>MISS CEN	0.2
B3.12	Nov-23	Feb-24	**CEN MISS**	0.1
Minors	n/a	n/a	n/a	3.1

‡ wild birds only　　* genotype only detected in LBM flocks during 2023

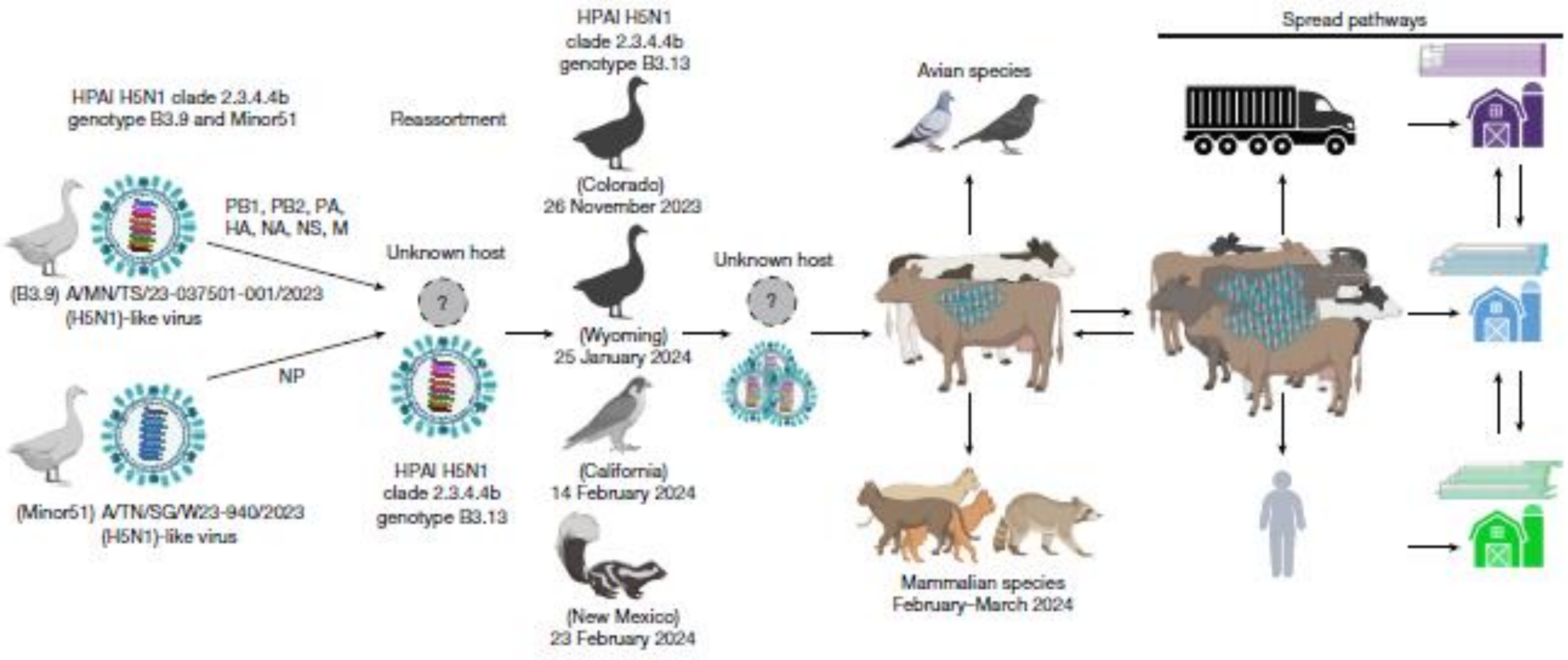
Migratory bird GenoFlu genotypes by overall percent, dates of detection, and flyway distribution as of July 2024.

https://github.com/USDA-VS/GenoFLU?tab=readme-ov-file



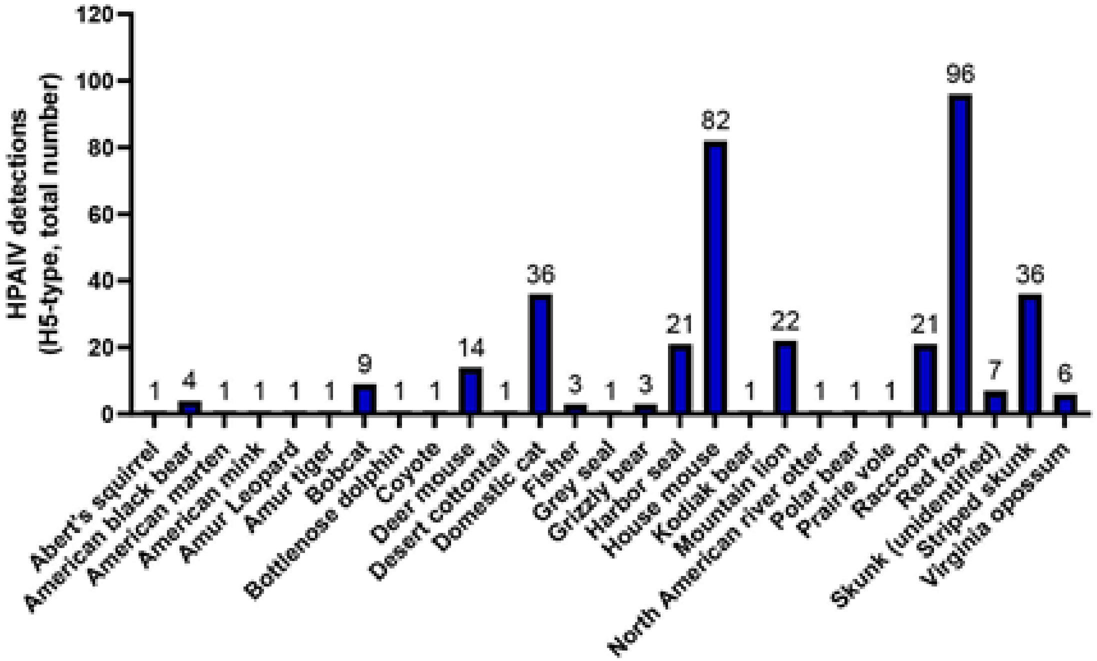
- Schematic representation of major H5N1 clade 2.3.4.4b reassortment events with the internal proteins encoding segments from North American low pathogenic strains and genesis of the reassortant cattle-origin HPAIV H5N1 strain clade 2.3.4.4b genotype B3.13.
- The PA, HA, NA and MP are derived from the European genotype and PB2, PB1, NP and NS derived from North American genotypes

Mostafa A, *et al.*, mBio, 2024



Genotype B3.13 emerged sometime in the fall of 2023 with only four detections in wild species prior to the detections in cattle (one goose in Colorado, one goose in Wyoming, one raptor in California, and one skunk in New Mexico).

Caserta LC, *et al.*, Nature, 2024. Jul 25:1-8.



The United States Department of Agriculture (USDA) has frequently reported infections with the HPAIV H5N1 clade 2.3.4.4b in **373 mammals representing 27 animal species** in **32 states** between May 2022 and August 2024

Mostafa A, *et al.*, mBio, 2024

How is the virus transmitted?

- The route of virus transmission into cattle is not clear yet but is likely to involve milking devices

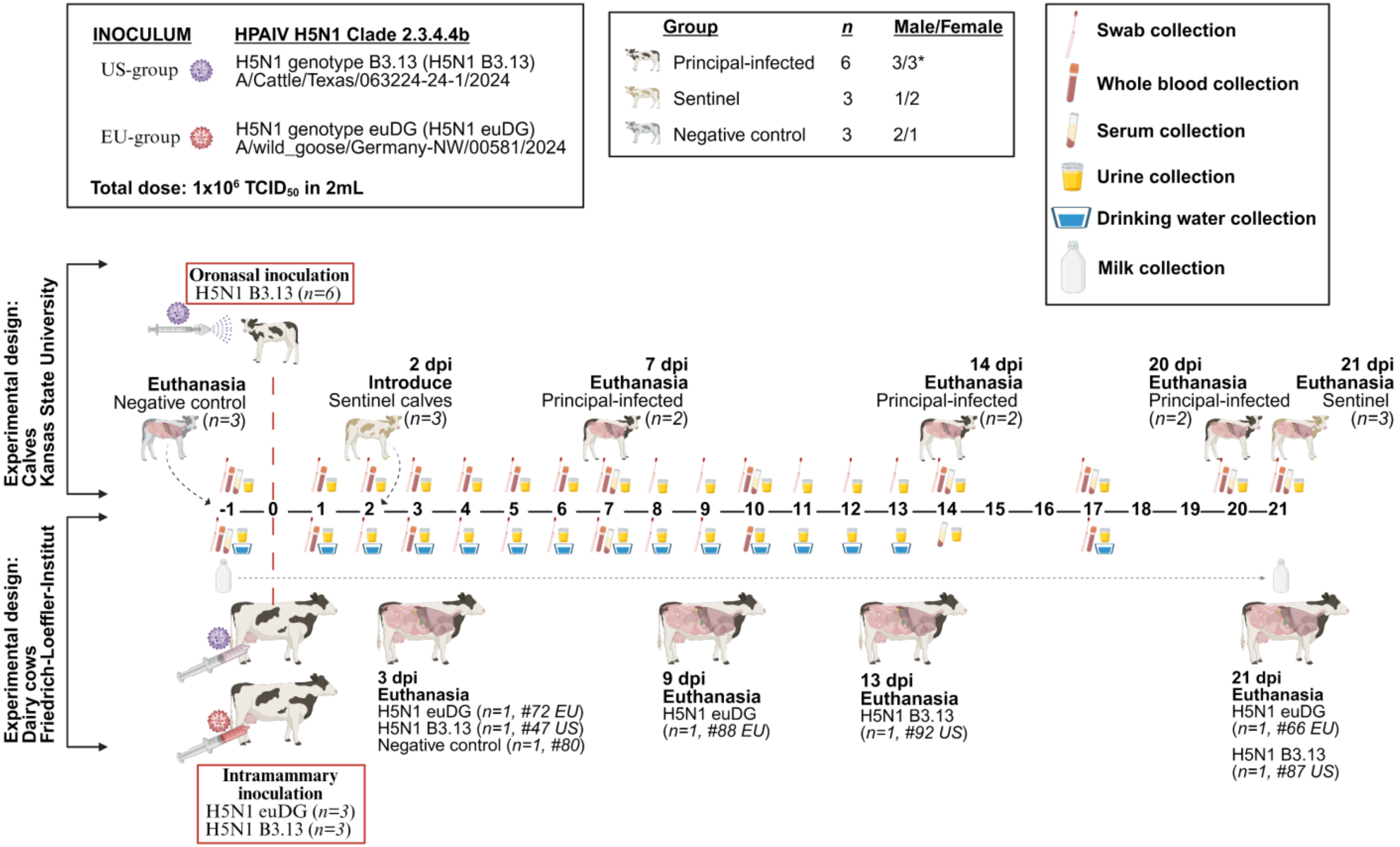
https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/livestock

How is the virus transmitted?

- Contaminated milking machinery is thought to be an important mode of H5N1 transmission between cattle from the same
- However, respiratory tract infection has not been ruled out.

https://www.feedstuffs.com/dairy/k-state-research-finds-bovine-h5n1-influenza-may-spread-via-milking

HPAI H5N1 experimental infection studies in cattle



INOCULUM **HPAIV H5N1 Clade 2.3.4.4b**

US-group: H5N1 genotype B3.13 (H5N1 B3.13)
A/Cattle/Texas/063224-24-1/2024

EU-group: H5N1 genotype euDG (H5N1 euDG)
A/wild_goose/Germany-NW/00581/2024

Total dose: $1x10^6$ TCID$_{50}$ in 2mL

Group	n	Male/Female
Principal-infected	6	3/3*
Sentinel	3	1/2
Negative control	3	2/1

Swab collection
Whole blood collection
Serum collection
Urine collection
Drinking water collection
Milk collection

Experimental design:
Calves
Kansas State University

Oronasal inoculation
H5N1 B3.13 (*n=6*)

Euthanasia
Negative control (*n=3*)

2 dpi
Introduce
Sentinel calves (*n=3*)

7 dpi
Euthanasia
Principal-infected (*n=2*)

14 dpi
Euthanasia
Principal-infected (*n=2*)

20 dpi
Euthanasia
Principal-infected
(*n=2*)

21 dpi
Euthanasia
Sentinel
(*n=3*)

Experimental design:
Dairy cows
Friedrich-Loeffler-Institut

Intramammary
inoculation
H5N1 euDG (*n=3*)
H5N1 B3.13 (*n=3*)

3 dpi
Euthanasia
H5N1 euDG (*n=1, #72 EU*)
H5N1 B3.13 (*n=1, #47 US*)
Negative control (*n=1, #80*)

9 dpi
Euthanasia
H5N1 euDG
(*n=1, #88 EU*)

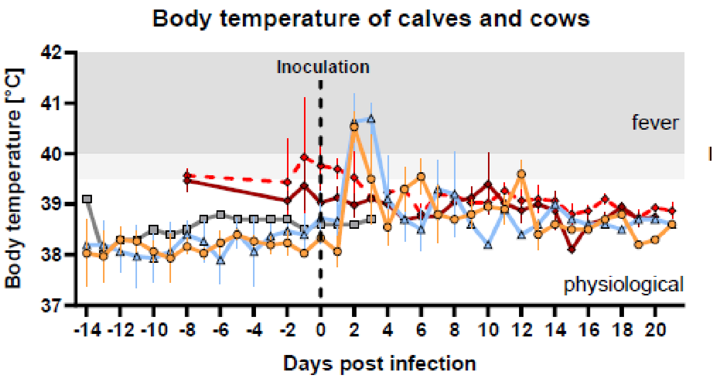
13 dpi
Euthanasia
H5N1 B3.13
(*n=1, #92 US*)

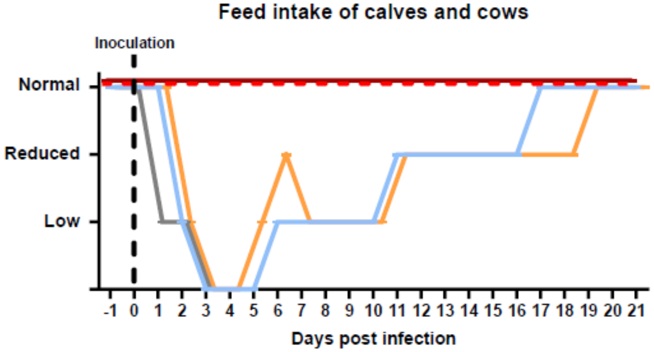
21 dpi
Euthanasia
H5N1 euDG
(*n=1, #66 EU*)

H5N1 B3.13
(*n=1, #87 US*)

Halwe NJ, *et al.*, bioRxiv. 2024:2024-08.

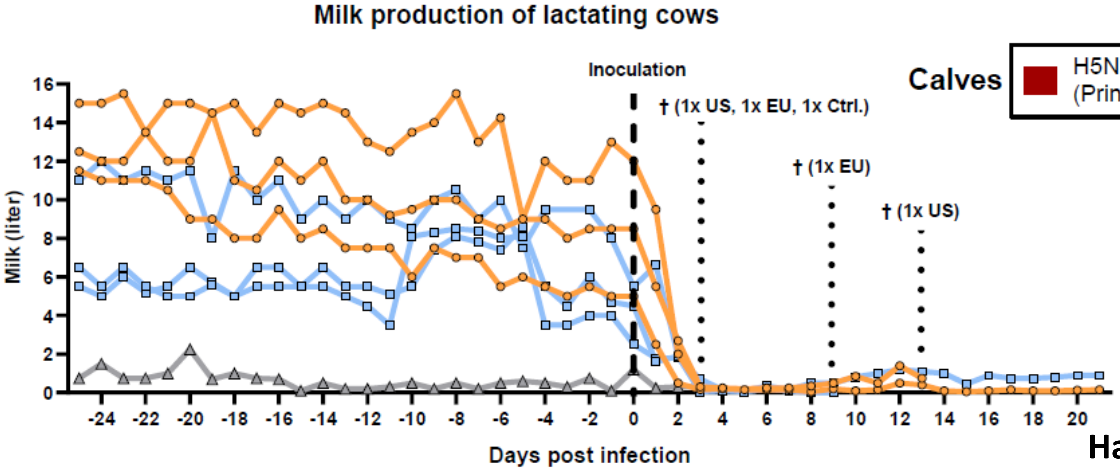
HPAI H5N1 experimental infection studies in cattle





Cows 🟧 H5N1 B3.13 (US-group) 🟦 H5N1 euDG (EU-group) ⬜ Ctrl.

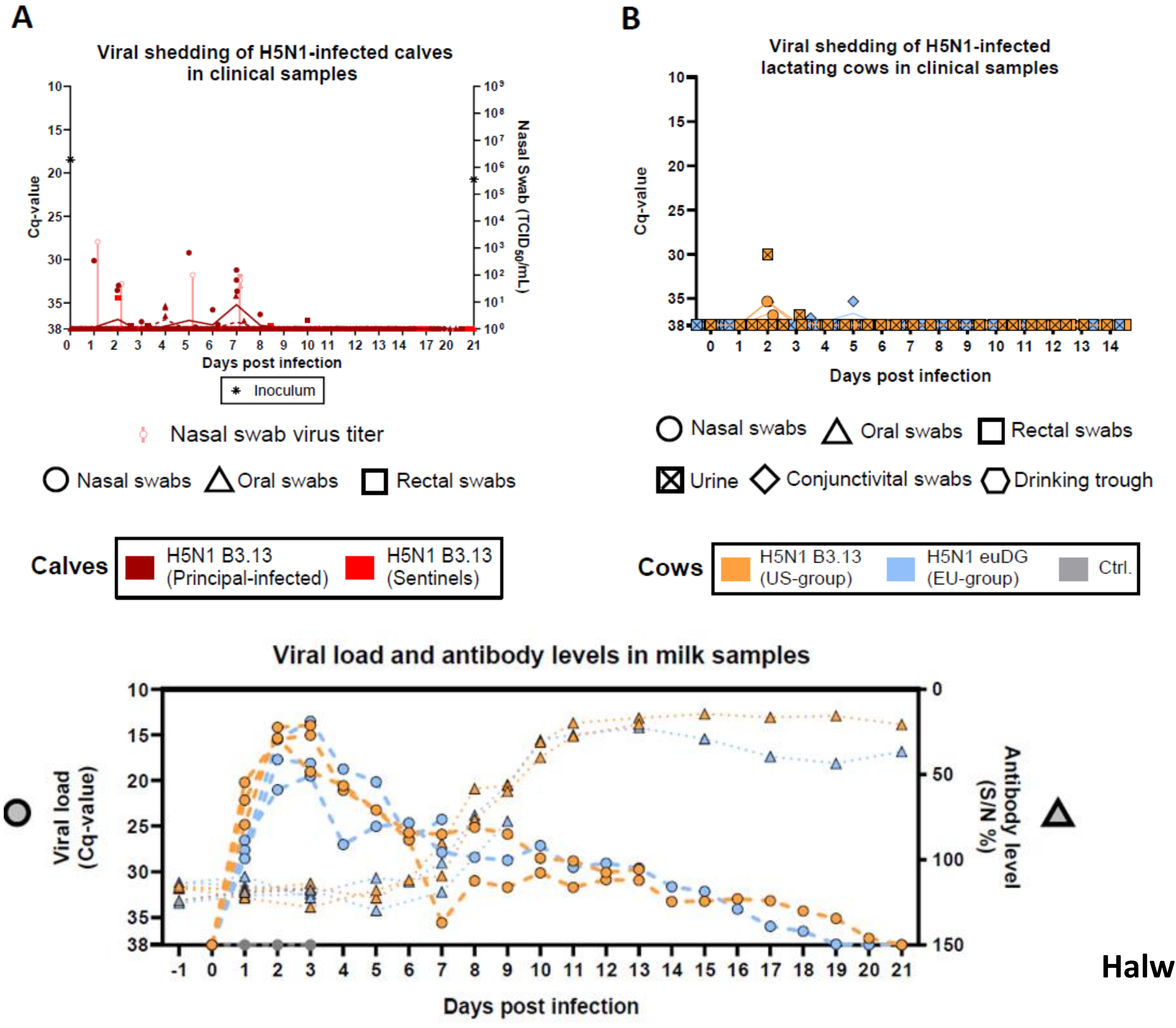
Calves 🟥 H5N1 B3.13 (Principal-infected) 🟥 H5N1 B3.13 (Sentinels)



clinical outcomes following infection with HPAIV H5N1 clade 2.3.4.4b isolates

Halwe NJ, *et al.*, bioRxiv. 2024:2024-08.

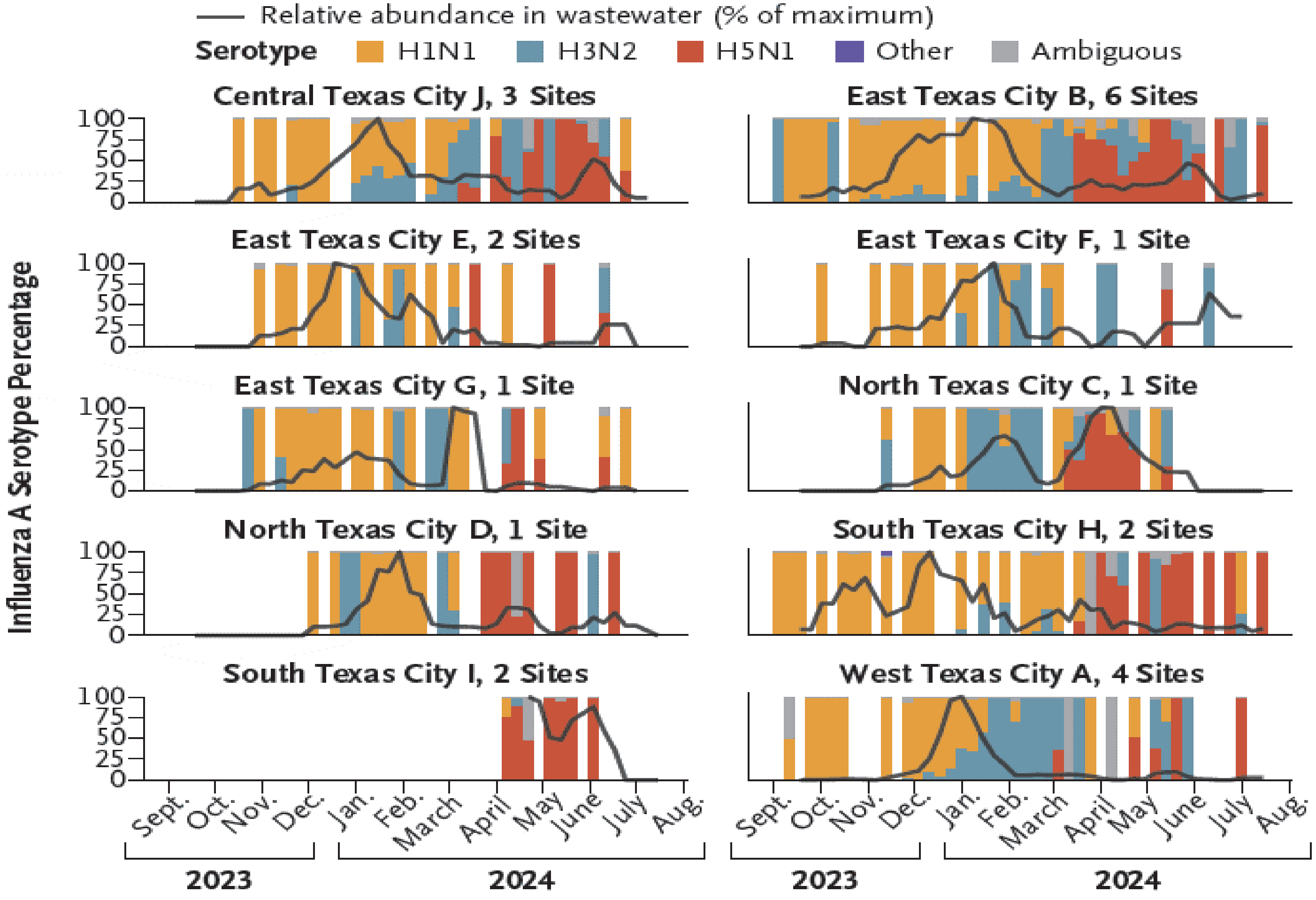
HPAI H5N1 experimental infection studies in cattle



- Inoculation of the calves resulted in moderate nasal replication and shedding with no severe clinical signs or transmission to sentinel calves.
- In dairy cows, infection resulted in no nasal shedding but severe acute mammary was observed

Halwe NJ, *et al.*, bioRxiv. 2024:2024-08.

Influenza A virus detection in wastewater of cities in Texas



In samples from March 4 through July 15, 2024, H5N1 was detected in 10 of 10 cities, 22 of 23 sites, and 100 of 399 samples

Tisza MJ, et al., New England Journal of Medicine. 2024

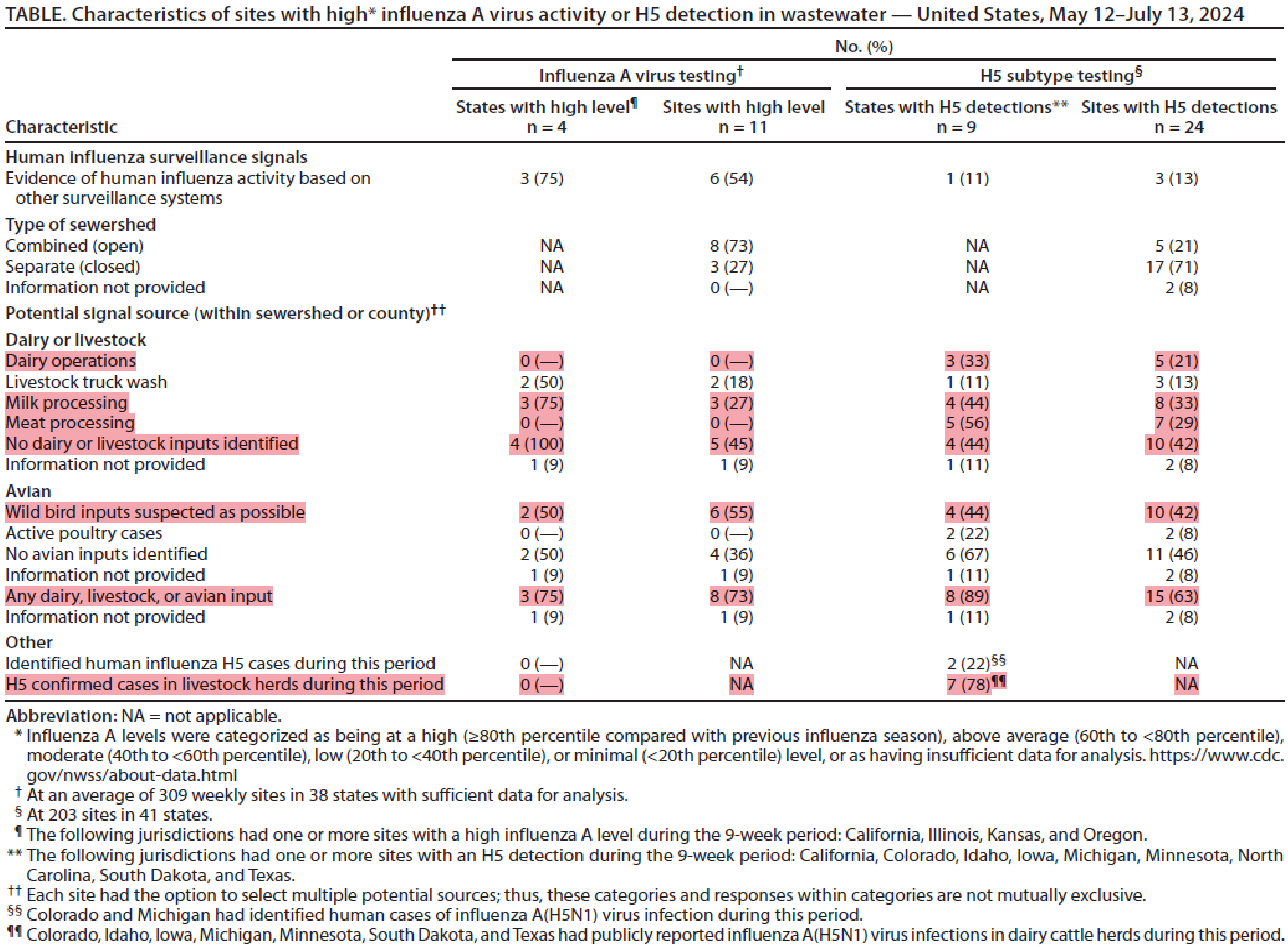


TABLE. Characteristics of sites with high[*] influenza A virus activity or H5 detection in wastewater — United States, May 12–July 13, 2024

Characteristic	No. (%)			
	Influenza A virus testing[†]		H5 subtype testing[§]	
	States with high level[¶] n = 4	Sites with high level n = 11	States with H5 detections[**] n = 9	Sites with H5 detections n = 24
Human influenza surveillance signals				
Evidence of human influenza activity based on other surveillance systems	3 (75)	6 (54)	1 (11)	3 (13)
Type of sewershed				
Combined (open)	NA	8 (73)	NA	5 (21)
Separate (closed)	NA	3 (27)	NA	17 (71)
Information not provided	NA	0 (—)	NA	2 (8)
Potential signal source (within sewershed or county)[††]				
Dairy or livestock				
Dairy operations	0 (—)	0 (—)	3 (33)	5 (21)
Livestock truck wash	2 (50)	2 (18)	1 (11)	3 (13)
Milk processing	3 (75)	3 (27)	4 (44)	8 (33)
Meat processing	0 (—)	0 (—)	5 (56)	7 (29)
No dairy or livestock inputs identified	4 (100)	5 (45)	4 (44)	10 (42)
Information not provided	1 (9)	1 (9)	1 (11)	2 (8)
Avian				
Wild bird inputs suspected as possible	2 (50)	6 (55)	4 (44)	10 (42)
Active poultry cases	0 (—)	0 (—)	2 (22)	2 (8)
No avian inputs identified	2 (50)	4 (36)	6 (67)	11 (46)
Information not provided	1 (9)	1 (9)	1 (11)	2 (8)
Any dairy, livestock, or avian input	3 (75)	8 (73)	8 (89)	15 (63)
Information not provided	1 (9)	1 (9)	1 (11)	2 (8)
Other				
Identified human influenza H5 cases during this period	0 (—)	NA	2 (22)[§§]	NA
H5 confirmed cases in livestock herds during this period	0 (—)	NA	7 (78)[¶¶]	NA

Abbreviation: NA = not applicable.

[*] Influenza A levels were categorized as being at a high (≥80th percentile compared with previous influenza season), above average (60th to <80th percentile), moderate (40th to <60th percentile), low (20th to <40th percentile), or minimal (<20th percentile) level, or as having insufficient data for analysis. https://www.cdc.gov/nwss/about-data.html

[†] At an average of 309 weekly sites in 38 states with sufficient data for analysis.

[§] At 203 sites in 41 states.

[¶] The following jurisdictions had one or more sites with a high influenza A level during the 9-week period: California, Illinois, Kansas, and Oregon.

[**] The following jurisdictions had one or more sites with an H5 detection during the 9-week period: California, Colorado, Idaho, Iowa, Michigan, Minnesota, North Carolina, South Dakota, and Texas.

[††] Each site had the option to select multiple potential sources; thus, these categories and responses within categories are not mutually exclusive.

[§§] Colorado and Michigan had identified human cases of influenza A(H5N1) virus infection during this period.

[¶¶] Colorado, Idaho, Iowa, Michigan, Minnesota, South Dakota, and Texas had publicly reported influenza A(H5N1) virus infections in dairy cattle herds during this period.

Louis S, *et al.,* Morbidity and Mortality Weekly Report. 2024

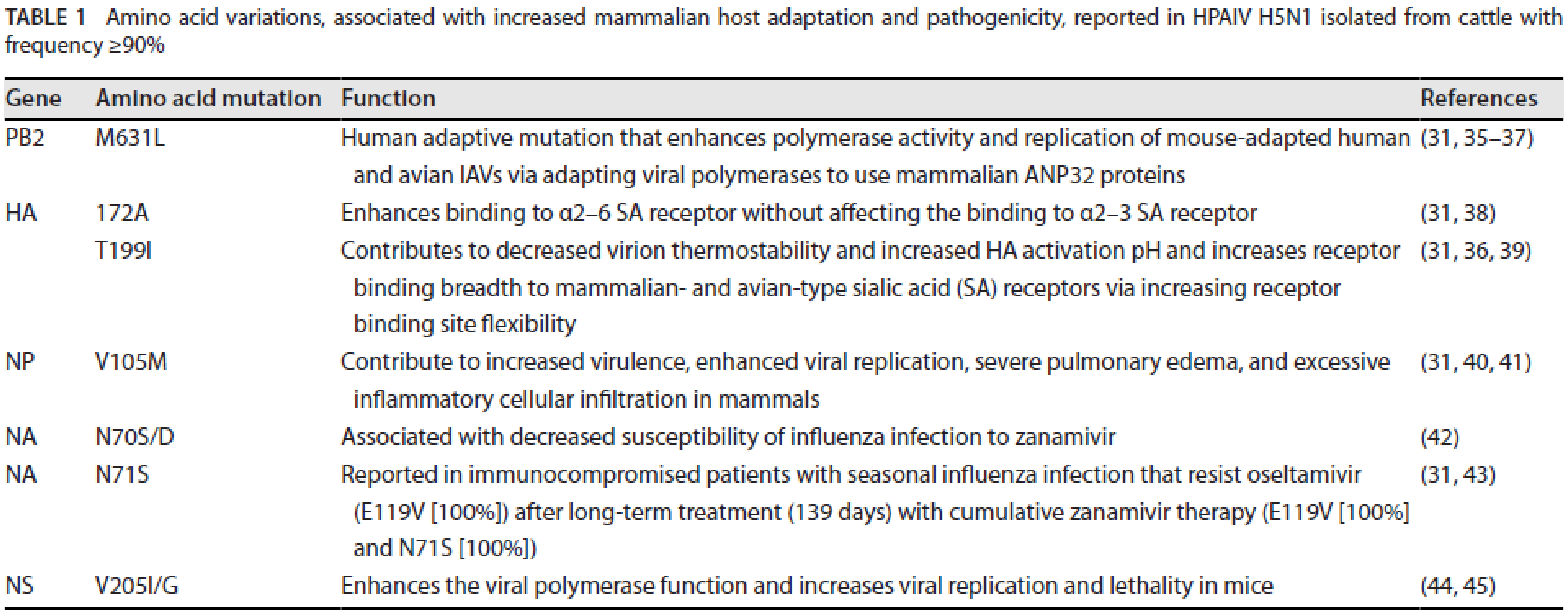


TABLE 1 Amino acid variations, associated with increased mammalian host adaptation and pathogenicity, reported in HPAIV H5N1 isolated from cattle with frequency ≥90%

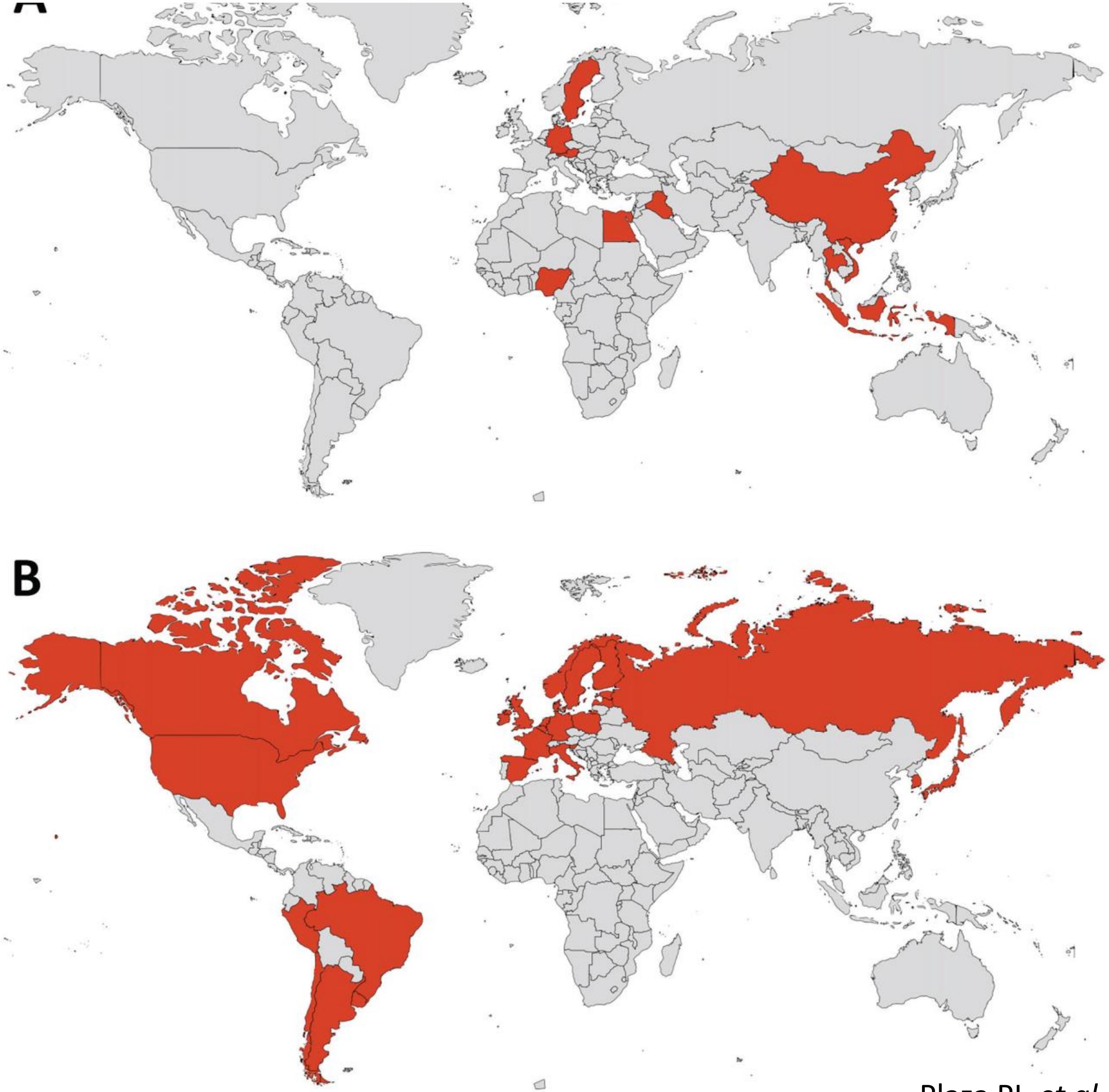
Gene	Amino acid mutation	Function	References
PB2	M631L	Human adaptive mutation that enhances polymerase activity and replication of mouse-adapted human and avian IAVs via adapting viral polymerases to use mammalian ANP32 proteins	(31, 35–37)
HA	172A	Enhances binding to α2–6 SA receptor without affecting the binding to α2–3 SA receptor	(31, 38)
	T199I	Contributes to decreased virion thermostability and increased HA activation pH and increases receptor binding breadth to mammalian- and avian-type sialic acid (SA) receptors via increasing receptor binding site flexibility	(31, 36, 39)
NP	V105M	Contribute to increased virulence, enhanced viral replication, severe pulmonary edema, and excessive inflammatory cellular infiltration in mammals	(31, 40, 41)
NA	N70S/D	Associated with decreased susceptibility of influenza infection to zanamivir	(42)
NA	N71S	Reported in immunocompromised patients with seasonal influenza infection that resist oseltamivir (E119V [100%]) after long-term treatment (139 days) with cumulative zanamivir therapy (E119V [100%] and N71S [100%])	(31, 43)
NS	V205I/G	Enhances the viral polymerase function and increases viral replication and lethality in mice	(44, 45)

- Different mutations which most likely facilitates viral entry and replication, respectively, in the new mammalian host cells

Mostafa A, *et al*., mBio, 2024

Why H5N1 clade 2.3.4.4b should be concerned?

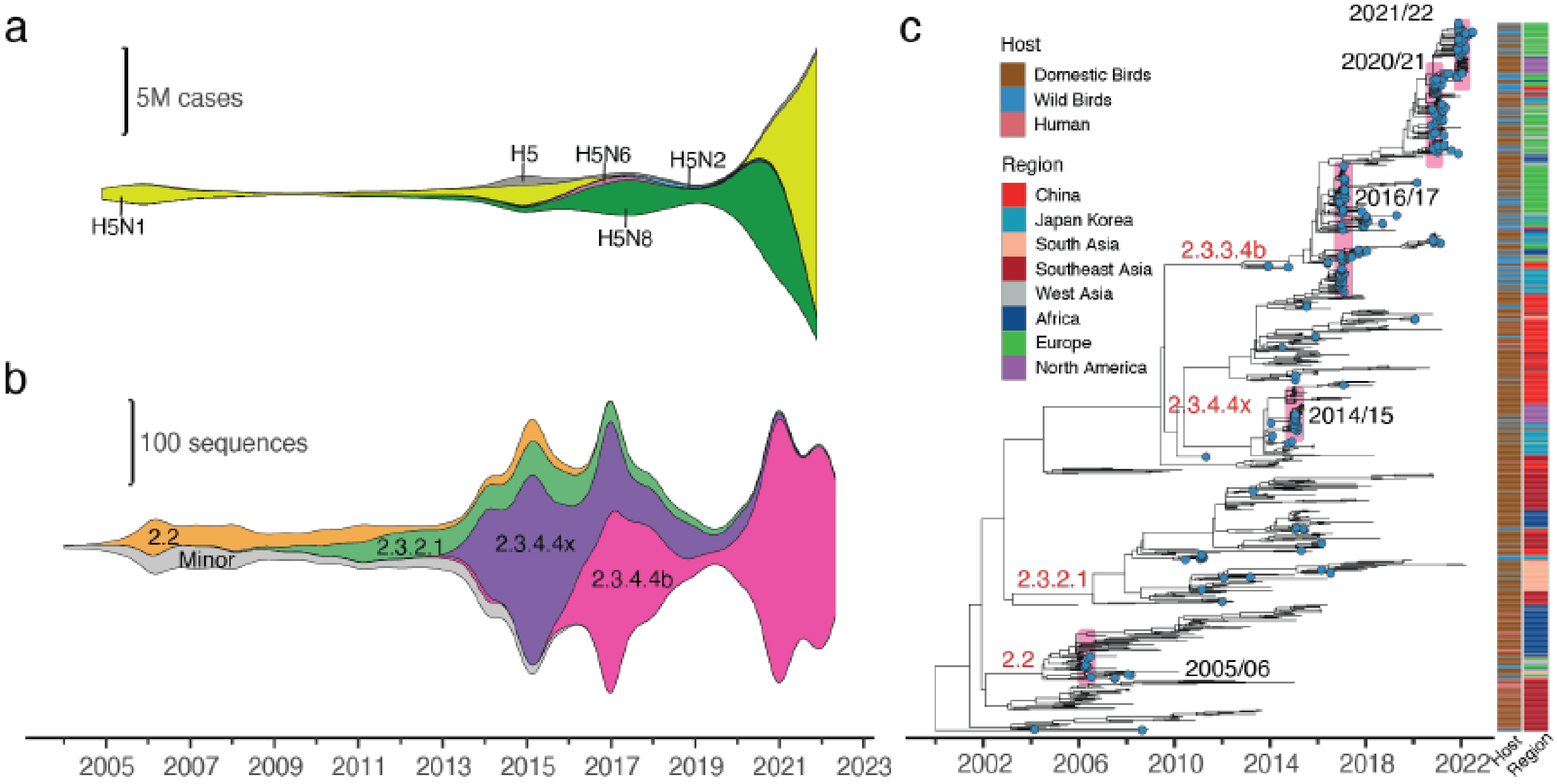
Patterns of Mammal Infection with H5N1 Virus Worldwide



Geographic location of mammal species affected by highly pathogenic influenza virus A(H5N1) in previous waves of infection, 2003–2019 (A), and in the current panzootic, 2020–2023 (B).

Plaza PI, *et al*., Emerging Infectious Diseases, 2024

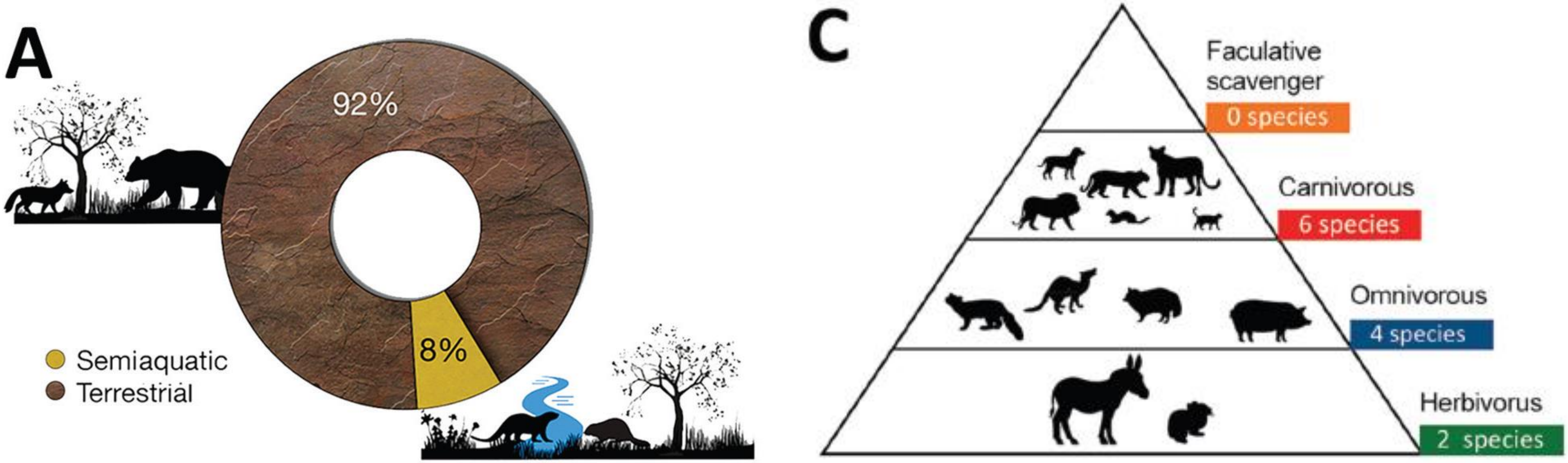
Dynamic changes in HPAI H5 subtypes and clades



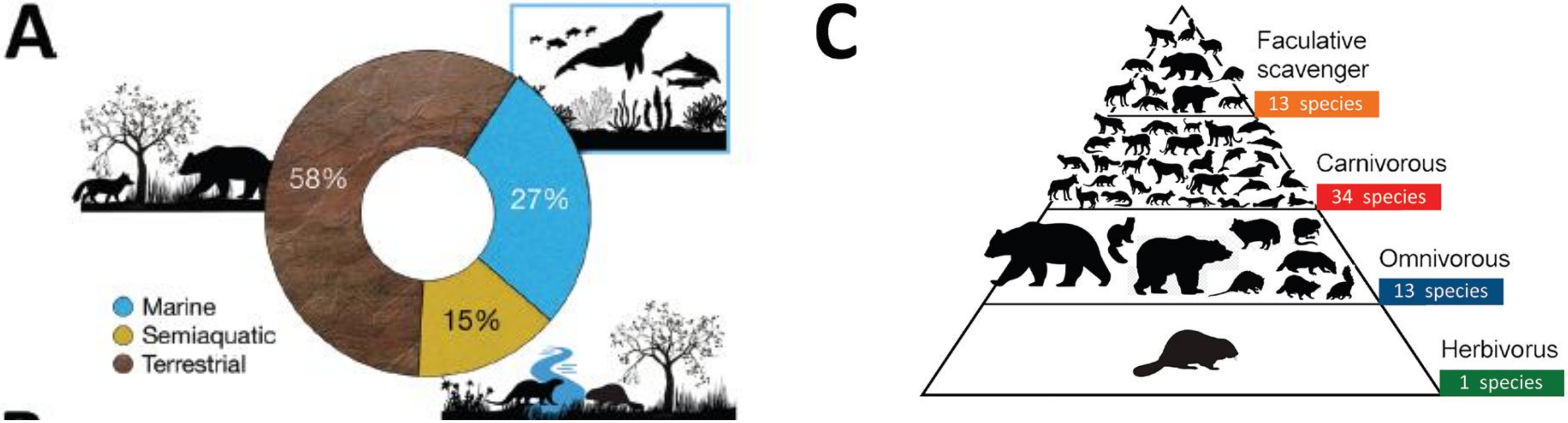
Xie R, *et al.,* Nature. 2023 Oct.

Patterns of Mammal Infection with H5N1 Virus Worldwide

H5N1 in previous waves of infection (2003–2019)



H5N1 panzootic (2020–2023)



Plaza PI, *et al.*, Emerging Infectious Diseases, 2024

Conclusions

- There is concern that the outbreak of H5N1 avian influenza in cows continues, mainly in the US
- There is the possibility that animals, including mammals, could become a permanent reservoir for HPAI virus subtypes, including H5N1, with the risk of mutations that may increase viral virulence in birds, mammals, and humans.

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Thank you