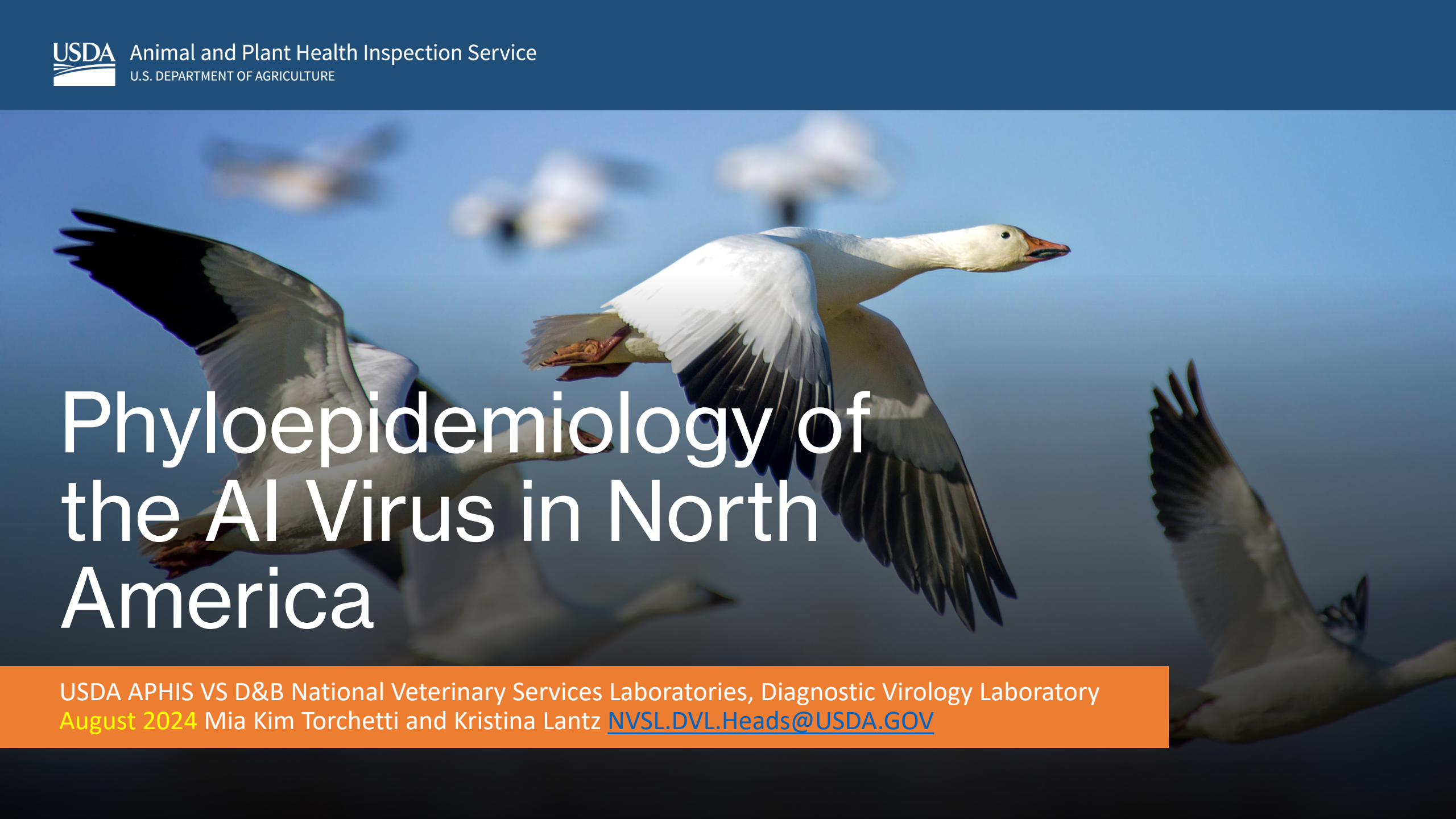




Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

A photograph of several white swans in flight against a clear blue sky. The swans are captured in various stages of their wing strokes, with some in sharp focus and others blurred in the background, creating a sense of movement and depth.

Phyloepidemiology of the AI Virus in North America

USDA APHIS VS D&B National Veterinary Services Laboratories, Diagnostic Virology Laboratory
August 2024 Mia Kim Torchetti and Kristina Lantz NVSL.DVL.Heads@USDA.GOV

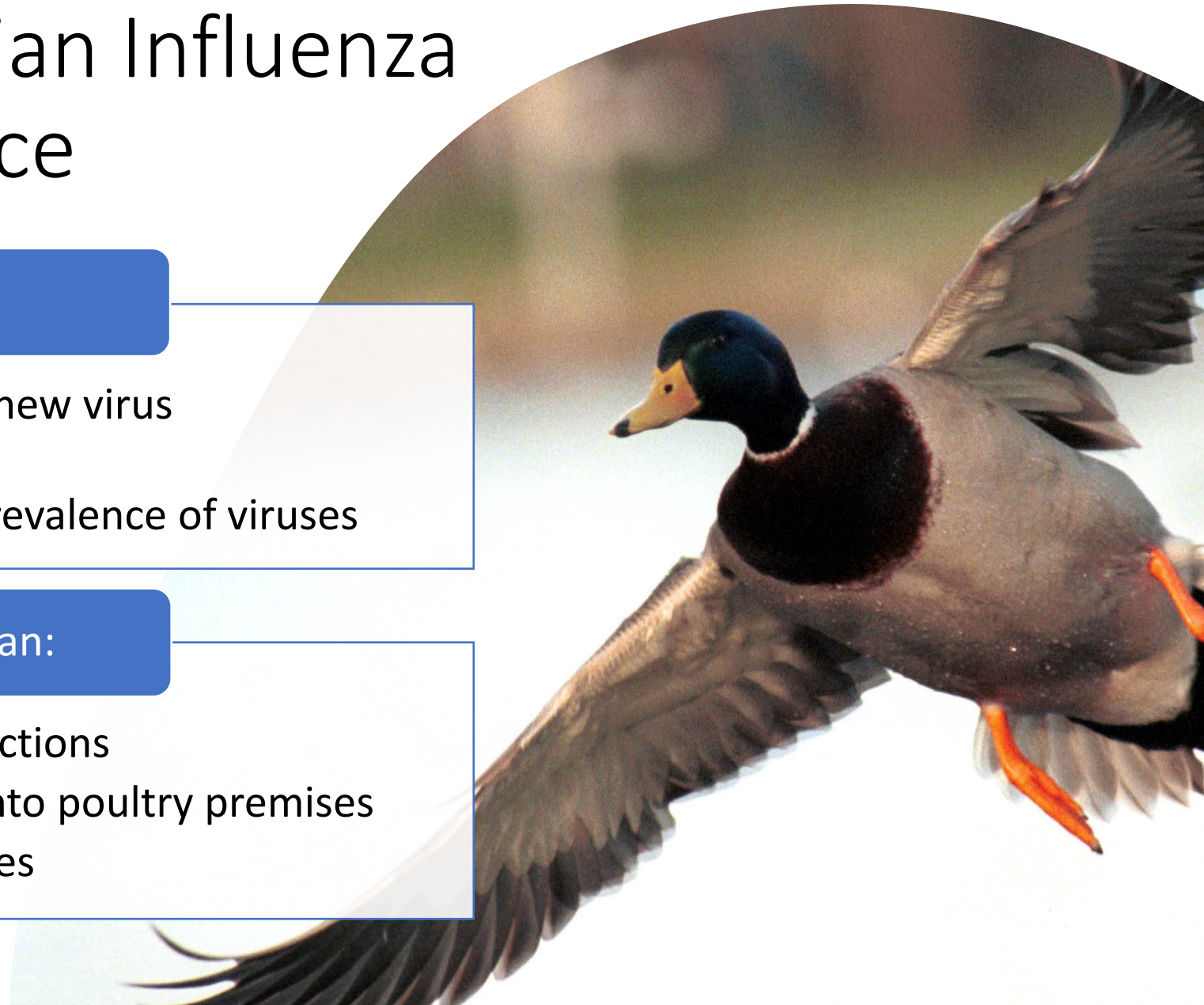
Wildlife Services Avian Influenza Wild Bird Surveillance

Wild bird detections:

- Act as early warning system for new virus introductions
- Identify new areas of risk and prevalence of viruses

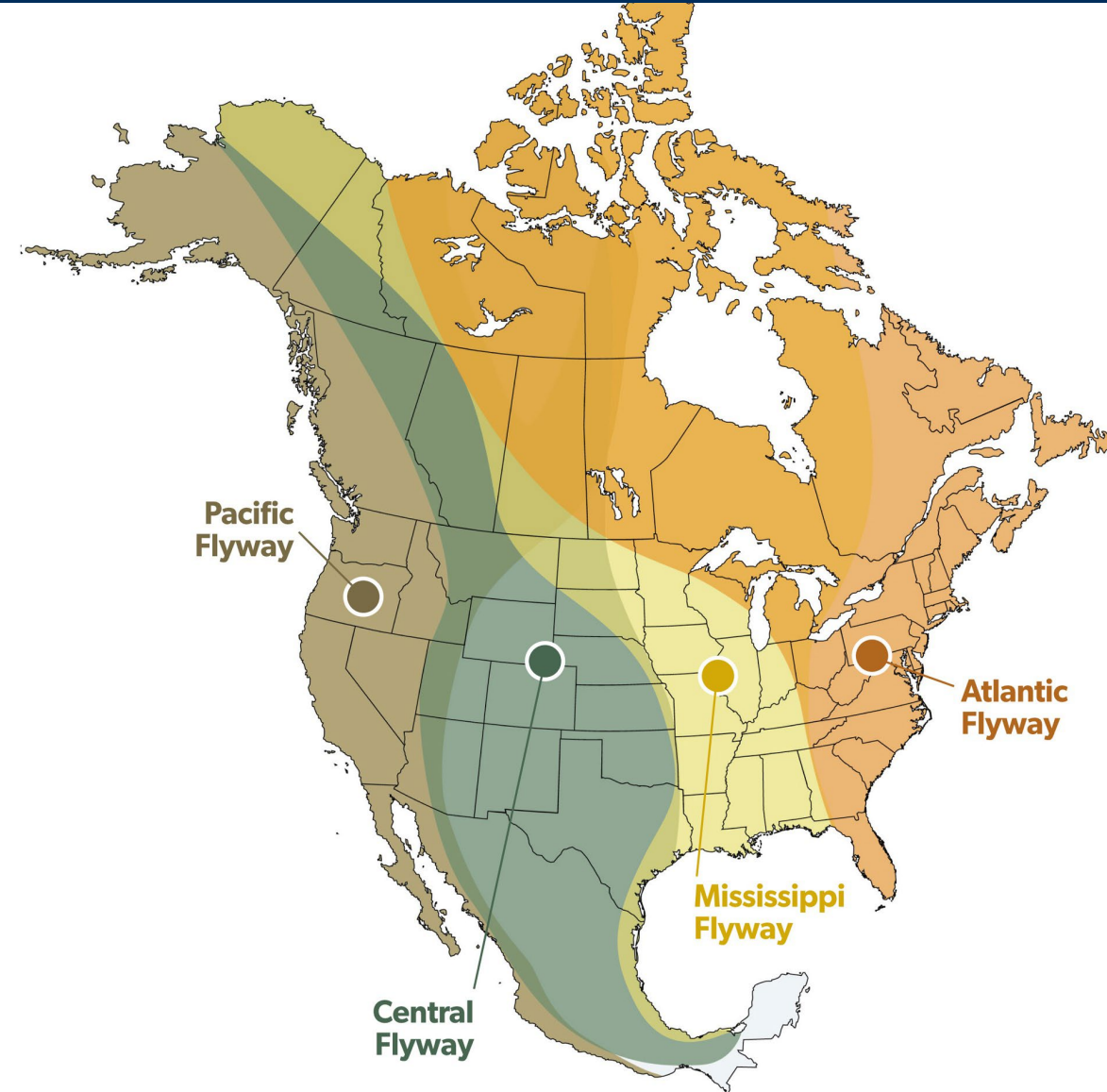
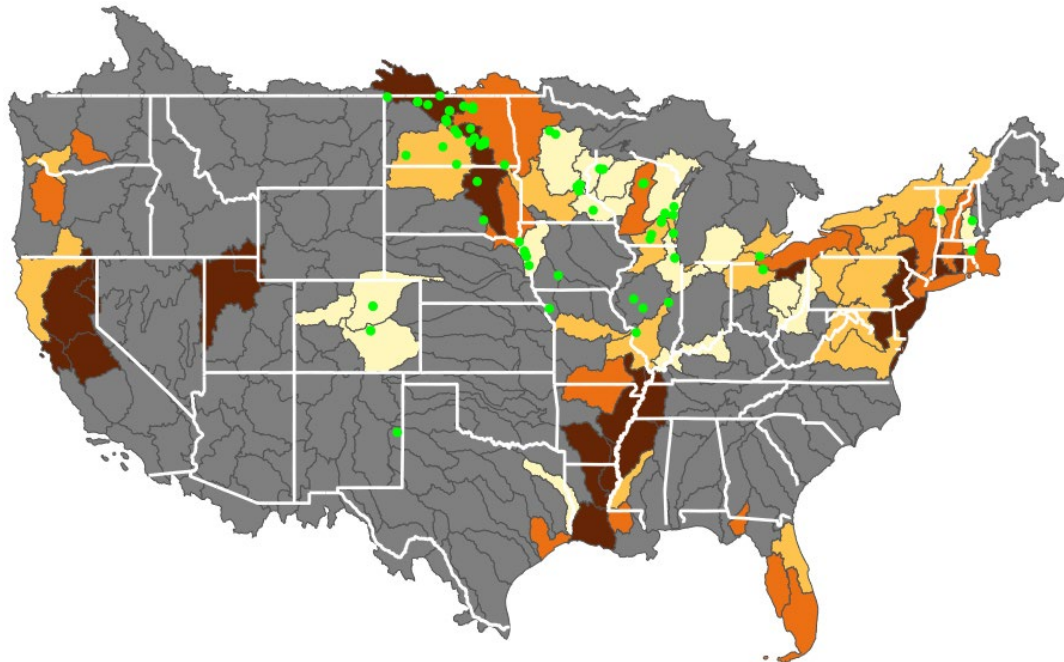
Wild bird genetic sequence data can:

- Provide context for poultry detections
- Identify points of introduction into poultry premises
- Identify new viruses/viral changes

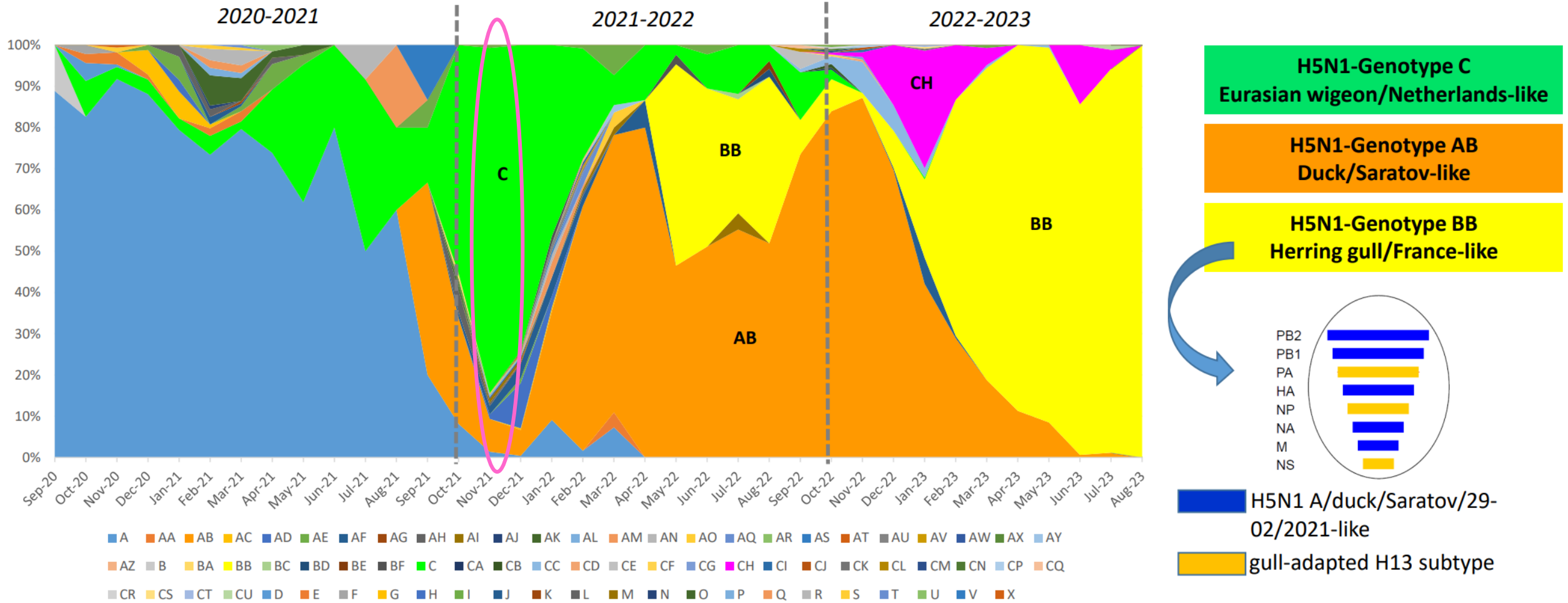


Routine Wild Bird Surveillance

- Apparently healthy birds – dabbling ducks
- Seasonal sampling
- 41,711 sample target (2023-2024)
 - Four flyways
 - 49 states with targets
 - Opportunistic sampling in HI, PR, USVI



Temporal dynamics of the virus genotypes in Europe: 2020-2023



Genotype C was introduced into the Atlantic flyway (per EURL genotyping nomenclature)

[Presentazione standard di PowerPoint \(izsvenezie.com\)](http://izsvenezie.com)



- H5N1 clade 2.3.4.4b was detected in the Atlantic flyway of the United States in December of 2021; thousands of detections in wild bird species, mammals and more than 1100 detections in domestic poultry followed.
- The virus rapidly re-assorted with North American avian influenza viruses as it spread westward across the US.
- There have been six virus introductions to date along both Atlantic and Pacific flyway, however, the majority of the reassortants have occurred from the A1 genotype.

January 2022

- Inaugural use of a 2.3.4.4b specific PCR assay on wild bird surveillance samples
 - Immediately detected the virus in an American Widgeon sample from South Carolina, collected December 30th of 2021.
- The first poultry detection in the Atlantic flyway soon followed in Indiana in early February 2022
- Utilization of a panel of PCR tests for influenza A, the 2.3.4.4b clade and N1 allowed for rapid detection of the virus, confirmation of cleavage site required sequencing
 - Addition of pathotyping PCR assay allowed for PCR based confirmation of the clade, neuraminidase, and cleavage site in ~6 hours from sample receipt

Whole Genome Sequencing

- Routine whole genome sequencing of Avian Influenza viruses already in place prior to the initial detection
- Utilization of influenza universal primer amplification
 - Sequencing by high throughput short read Illumina platform
- Analysis by segment utilizing IRMA pipeline, <https://wonder.cdc.gov/amd/flu/irma/>
 - Detection of mixed sequences, sequence coverage and quality
 - Assignment of hemagglutinin and neuraminidase type, as well as Eurasian versus North American origin of other segments

vSNP analysis for clade 2.3.4.4b HPAI

- Hemagglutinin (HA) – cleavage site determines HPAI vs LPAI
 - This is the determining segment for inclusion in analysis
- Utilizes the full genome for comparison, with the ability of fine resolution comparison of more closely related sequences
- Sequence sources
 - Wild bird surveillance and mortality events
 - Poultry detections

Hicks et al. *BMC Genomics* (2024) 25:545
<https://doi.org/10.1186/s12864-024-10437-5>

BMC Genomics

SOFTWARE

Open Access

vSNP: a SNP pipeline for the generation of transparent SNP matrices and phylogenetic trees from whole genome sequencing data sets



Jessica Hicks¹, Tod Stuber^{1*}, Kristina Lantz¹, Mia Torchetti¹ and Suelee Robbe-Austerman¹

Understanding SNP Tables

	A/Fancy_Ck/NL/FAV33/2021_NA:703	A/Fancy_Ck/NL/FAV33/2021_NA:434	A/mallard/Nevada/AH0190913/2021_NP:120	A/Fancy_Ck/NL/FAV33/2021_NA:392	A/Fancy_Ck/NL/FAV33/2021_NA:232	A/Blue-Winged_Teal/Alberta/39/2020_PB2:285	A/Fancy_Ck/NL/FAV33/2021_NA:423	A/Fancy_Ck/NL/FAV33/2021_HA:1540	A/Fancy_Ck/NL/FAV33/2021_HA:268	A/Fancy_Ck/NL/FAV33/2021_NA:1103
root	C	C	C	A	T	G	C	C	C	T
22-012093-001_WILD_BIRD_PEFA_2022-04-15_IA	T	T	T	G	A	T	T	C	C	T
	T	T	T	G	A	G	C	T	T	T
	T	T	T	G	A	G	C	T	T	W
	T	T	T	G	A	G	C	T	T	W

Identifying potential for common source or lateral transmission

	C	A	A	A	T	G	A	G	G	T	A	A	G	C
Wild Bird	T	G	A	A	T	G	A	G	G	T	A	A	G	C
Premises 1	T	A	A	A	Y	G	A	G	G	T	A	A	G	C
	T	A	A	A	Y	G	A	G	G	T	A	A	G	C
Premises 2	T	A	G	G	T	G	A	G	G	T	A	A	G	C
	T	A	R	R	C	R	R	G	G	T	A	A	G	C
Premises 3	T	A	A	A	C	A	G	A	G	T	A	A	G	C
	T	A	A	A	C	A	G	A	G	T	A	A	G	C
Premises 4	T	A	A	A	C	A	G	A	A	G	A	A	G	C
	T	A	A	A	C	A	G	A	A	G	T	A	G	C
Unrelated Prem	T	A	A	A	C	A	G	A	A	G	W	A	G	C
	T	A	A	A	T	G	A	G	G	T	A	G	A	C
	T	A	A	A	T	G	A	G	G	T	A	G	A	C
	T	A	A	A	T	G	A	G	G	T	A	G	A	C
	T	A	A	A	T	G	A	G	G	T	A	G	A	C



Analysis allows us to infer directionality but need epi to understand how it occurred

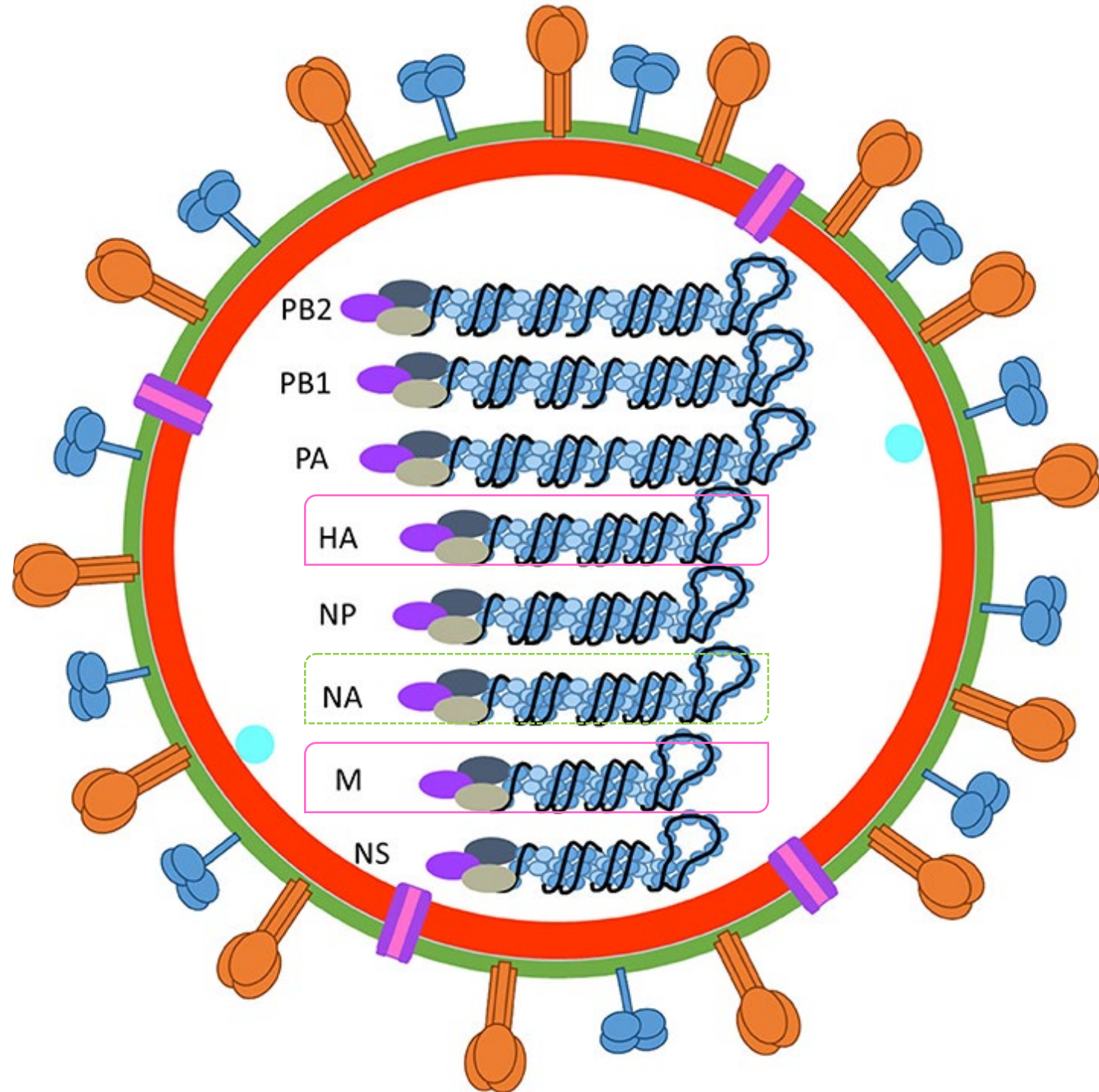
root	G	T	A	G	T	A	G	C	G	A	G	C	C	G	G	A	T	C	C
PREM1_CHICKEN_2022-02-21	A	G	G	A	C	G	A	T	A	A	G	C	C	G	G	A	T	C	C
PREM1_CHICKEN_2022-02-21	A	G	G	A	C	G	A	T	A	A	G	C	C	G	G	A	T	C	C
PREM1_CHICKEN_2022-02-21	A	G	G	A	C	G	A	T	A	A	G	C	C	G	G	A	T	C	C
PREM2_CHICKEN_2022-03-07	A	G	G	A	C	G	A	T	A	A	G	C	C	G	G	A	T	C	C
PREM2_CHICKEN_2022-03-07	A	G	G	A	C	G	A	T	A	A	G	C	C	G	G	A	T	C	C
PREM2_CHICKEN_2022-03-07	A	G	G	A	C	G	A	T	A	A	G	C	C	G	G	A	T	C	C
PREM3_CHICKEN_2022-03-03	A	G	G	A	C	G	A	T	A	A	G	C	C	G	G	A	T	C	C
PREM3_CHICKEN_2022-03-03	A	G	G	A	C	G	A	T	A	A	G	C	C	G	G	A	T	C	C
PREM3_CHICKEN_2022-03-03	A	G	G	A	C	G	A	T	A	A	G	C	C	G	G	A	T	C	C
PREM4_CHICKEN_2022-03-08	A	G	G	A	C	G	A	T	A	A	G	C	C	G	G	A	T	C	C
PREM4_CHICKEN_2022-03-08	A	G	G	A	C	G	A	T	A	G	G	C	C	G	G	A	T	C	C
PREM4_CHICKEN_2022-03-08	A	G	G	A	C	G	A	T	A	A	A	T	C	G	G	A	T	C	C
PREM3_CHICKEN_2022-03-03	A	G	G	A	C	G	A	T	A	A	G	C	Y	G	G	A	T	C	C
PREM2_CHICKEN_2022-03-07	A	G	G	A	C	G	A	T	A	A	G	C	R	G	A	T	C	C	
PREM5_CHICKEN_2022-03-04	A	G	G	A	C	G	A	T	A	A	G	C	C	S	A	T	C	C	
PREM5_CHICKEN_2022-03-04	A	G	G	A	C	G	A	T	A	A	G	C	C	S	A	T	C	C	
PREM3_CHICKEN_2022-03-03	A	G	G	A	C	G	A	T	A	A	G	C	C	G	R	T	C	C	
PREM3_CHICKEN_2022-03-03	A	G	G	A	C	G	A	T	A	A	G	C	C	G	R	T	C	C	
PREM3_CHICKEN_2022-03-03	A	G	G	A	C	G	A	T	A	A	G	C	C	G	R	T	C	C	
PREM3_CHICKEN_2022-03-03	A	G	G	A	C	G	A	T	A	A	G	C	C	G	R	T	C	C	
PREM3_CHICKEN_2022-03-03	A	G	G	A	C	G	A	T	A	A	G	C	C	G	R	T	C	C	
PREM3_CHICKEN_2022-03-03	A	G	G	A	C	G	A	T	A	A	G	C	C	G	R	T	C	C	
PREM3_CHICKEN_2022-03-03	A	G	G	A	C	G	A	T	A	A	G	C	C	G	R	Y	C	C	
PREM4_CHICKEN_2022-03-08	A	G	G	A	C	G	A	T	A	A	G	C	C	G	R	T	Y	C	
PREM4_CHICKEN_2022-03-08	A	G	G	A	C	G	A	T	A	A	G	C	C	G	G	T	T	T	



Less straightforward involving many premises in a short span of time

HPAI Reassortment

- North American wild bird virus segments
 - Re-assorted viruses detected in wild bird samples collected in early February of 2022 followed by detections in poultry in the Midwest by mid March
 - Multiple combinations of PB2, PB1, PA, NP, and NS segments
 - Very limited H5Nx reassortment to date



Adjusting to a changing virus

- Reassortment presented a problem for vSNP analysis
 - Reassorted North American virus segments are significantly different from the 2.3.4.4b virus
 - Poor assembly, artificial “jumps” in genetic distance
- Used BLAST match to identify appropriate North American segments for NP and PB2
- Developed a second vSNP analysis
 - All samples with 2.3.4.4b HA go into a vSNP analysis
 - If they have Eurasian PB2, they go into “Turkey”
 - If they have a North American PB2 and NP, they go into “Mallard”
- Developed a set of segment specific NJ trees
 - Used to compare other reassorted segments to establish relatedness between virus with and without re-assortment

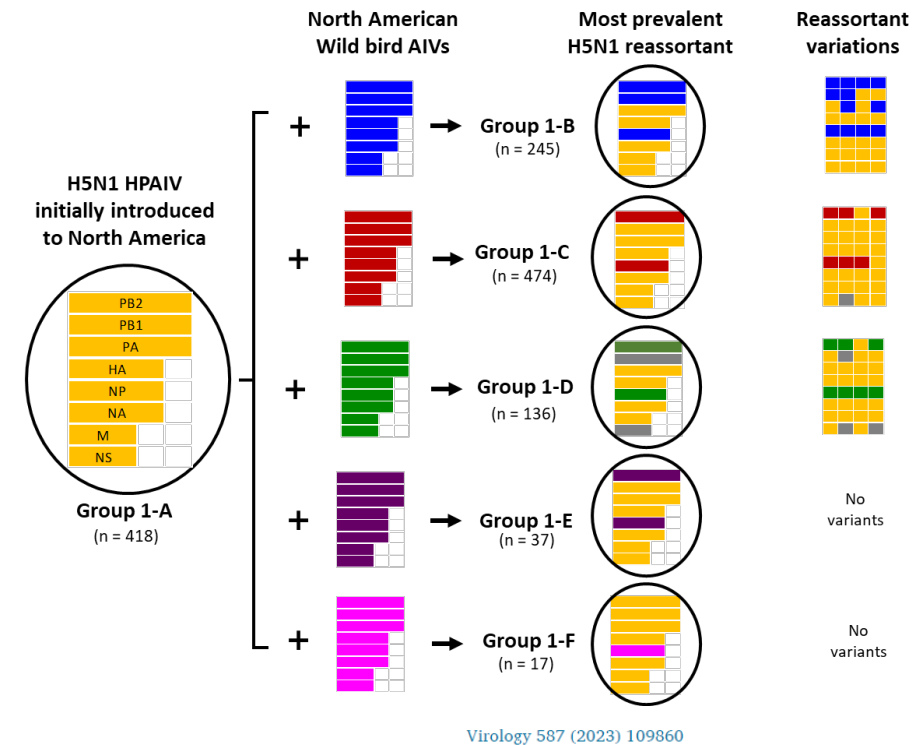
**Example highlighting point source transmission - 2 separate premises
3 different virus introductions**

- Left ~0.5 km from the lake
 - Un-reassorted (native constellation)
 - 2 gene reassortment
- Right ~0.1 km from the lake
 - 4 gene reassortment unrelated to the other two



Understanding re-assorted viruses

- The need quickly arose to classify reassortants of this virus in North America
- A genotyping scheme was developed based on unique re-assortment events
- Developed a tool to rapidly assign genotype, GenoFLU
<https://github.com/USDA-VS/GenoFLU>



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Virology

journal homepage: www.elsevier.com/locate/virology



H5N1 highly pathogenic avian influenza clade 2.3.4.4b in wild and domestic birds: Introductions into the United States and reassortments, December 2021–April 2022

Sungsu Youk^{a,f}, Mia Kim Torchetti^b, Kristina Lantz^b, Julianna B. Leno^c, Mary Lea Killian^b, Christina Leyson^a, Sarah N. Bevins^c, Krista Dilione^c, Hon S. Ip^d, David E. Stallknecht^e, Rebecca L. Poulson^e, David L. Suarez^a, David E. Swayne^a, Mary J. Pantin-Jackwood^{a,*}

^a Southeast Poultry Research Laboratory, U.S. National Poultry Research Laboratory, Agricultural Research Service, USDA, Athens, GA, USA

^b National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, USDA, Ames, IA, USA

^c Wildlife Services National Wildlife Disease Program, Animal and Plant Health Inspections Service, USDA, Fort Collins, CO, USA

^d United States Geological Survey, National Wildlife Health Center, Laboratory Services Branch, Madison, WI, USA

^e Southeastern Cooperative Wildlife Disease Study, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

^f Microbiology Laboratory, Department of Medicine, College of Medicine, Chungbuk National University, Chungbuk, South Korea

Genotypes wax and wane

- Migratory bird GenoFlu genotypes by overall percent, dates of detection, and flyway distribution as of 29 February 2024.
- Includes detections in wild migratory birds, poultry species and non-dairy mammals; only one sequence per poultry premises was included to represent the premises.
- For dates, shading is white for oldest and green for more recent dates in the column.
- **NOTE:** This table excludes genotype B3.13 that was first detected in the Central flyway and for which only four early viruses are available prior to the event in dairy.

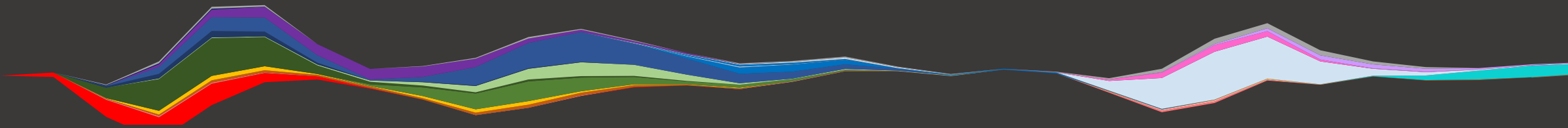
Genotype	First detected	Last detected	Flyway distribution (initial detection in bold)	Overall percent (n=6440)
A1	Dec-21	Dec-23	ATL>MISS CEN PAC	5.4
B1.1	Jan-22	Dec-23	ATL>MISS CEN	9.7
A2	Feb-22	Feb-24	ATL>MISS	2.8
B1.2	Feb-22	Dec-23	ATL>MISS CEN	4.0
B2.1	Mar-22	Sep-23	MISS>CEN PAC	10.2
B2.2	Mar-22	Nov-23	MISS>CEN PAC ATL	4.6
B3.1	Mar-22	Nov-23	MISS>CEN PAC southern ATL	1.5
B3.2	Mar-22	Jan-24	MISS>CEN PAC ATL	20.4
B5.1	Mar-22	Jul-22	CEN MISS	0.3
A3	Apr-22	Feb-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	May-23	MISS CEN>northern ATL	1.3
B3.6	Nov-22	Feb-24	CEN MISS>PAC ATL	10.8
‡A6	Jan-23	Feb-24	northern ATL	0.2
B3.7	Sep-23	Feb-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	Feb-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

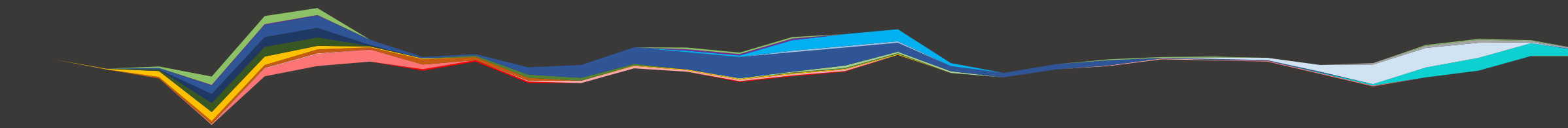
* genotype only detected in LBM flocks during 2023

Poultry Genotypes

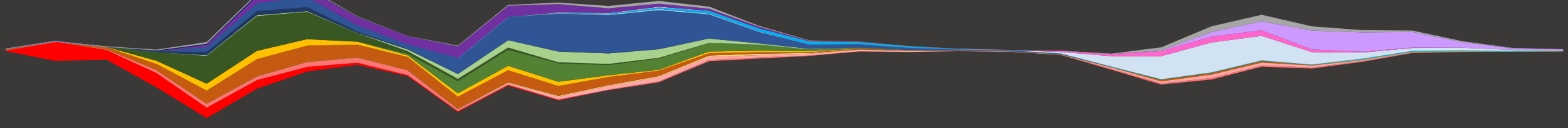
Stream Graphs by species group, month/year and genotype



Mammal Genotypes

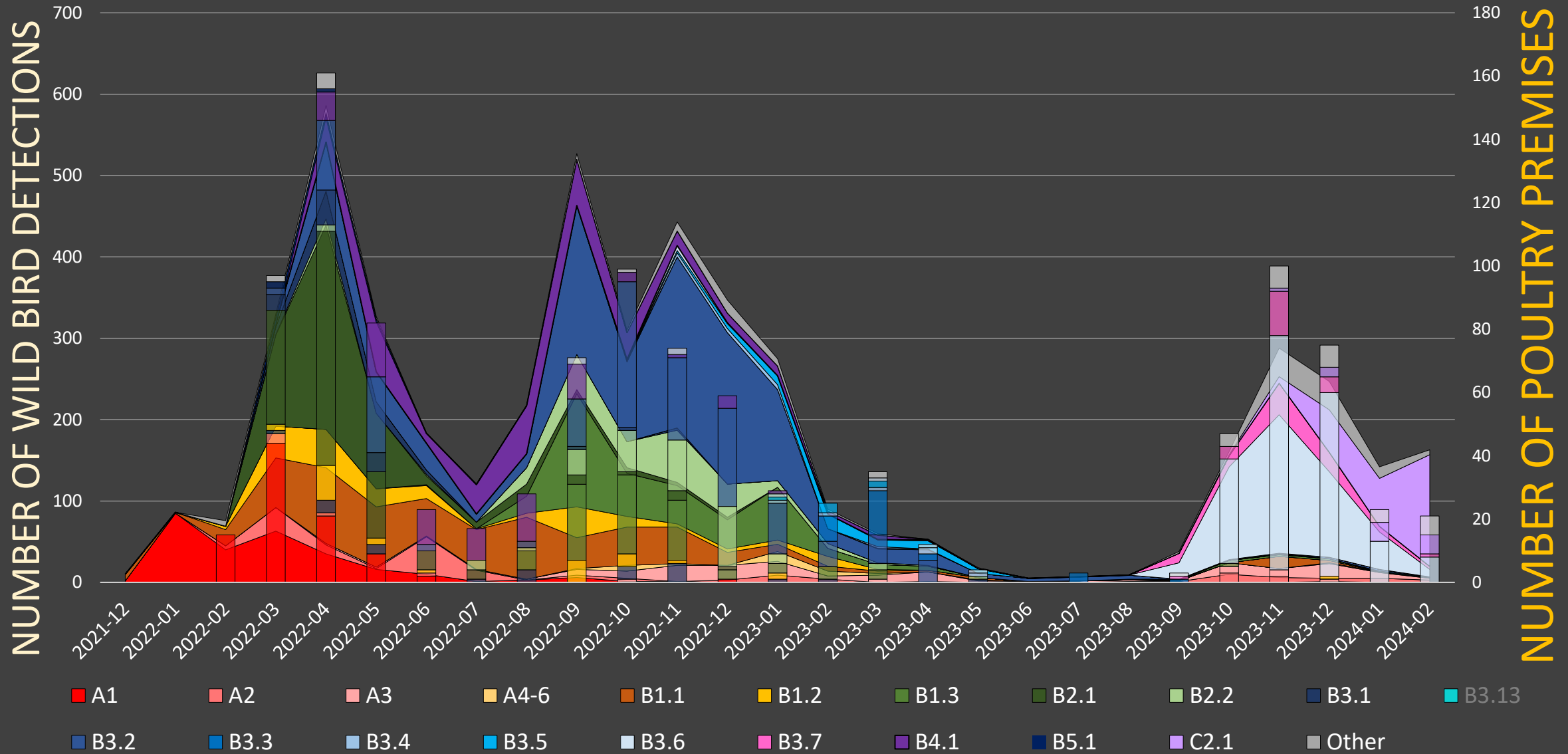


Wild Bird Genotypes



Wild Birds by Genotype Poultry Detections Overlaid

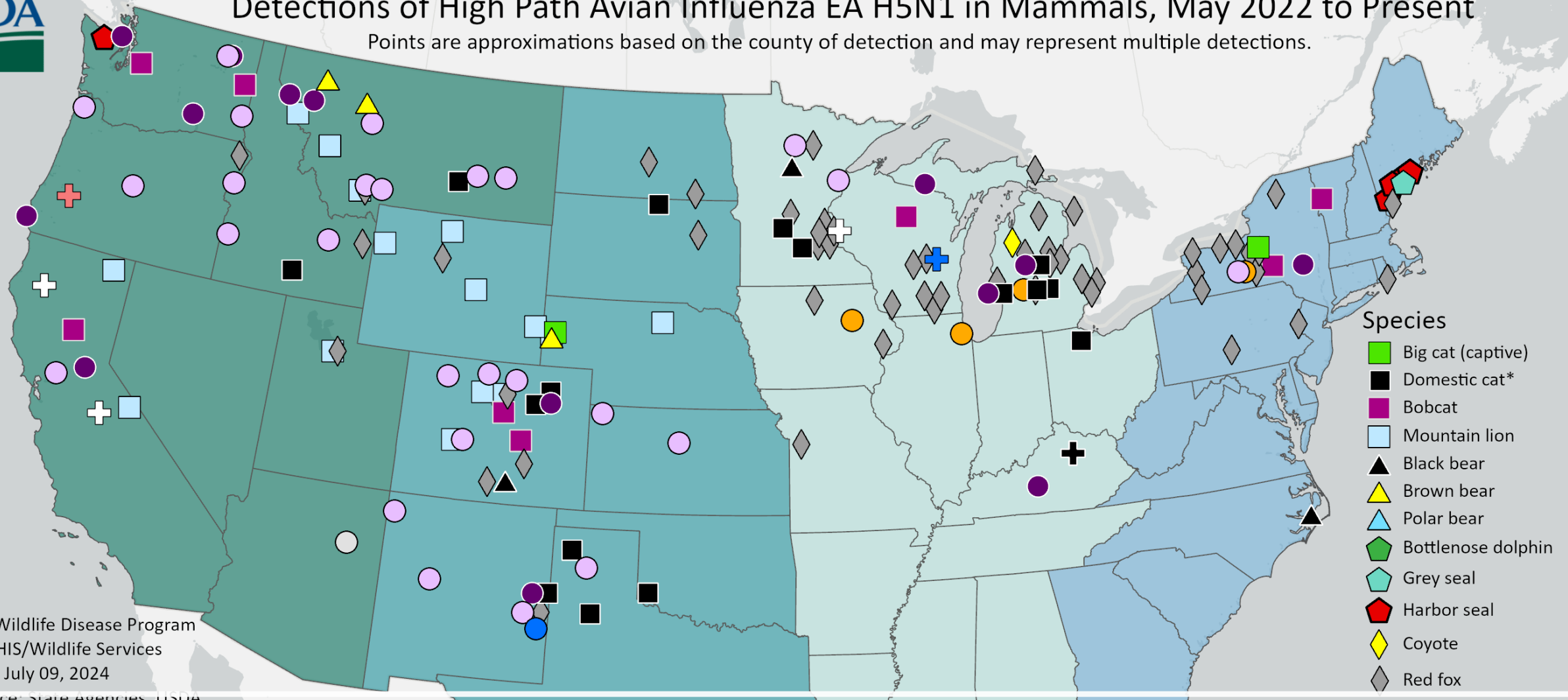
December 2021 - February 2024





Detections of High Path Avian Influenza EA H5N1 in Mammals, May 2022 to Present

Points are approximations based on the county of detection and may represent multiple detections.



- Species**
- Big cat (captive)
 - Domestic cat*
 - Bobcat
 - Mountain lion
 - ▲ Black bear
 - ▲ Brown bear
 - ▲ Polar bear
 - ◊ Bottlenose dolphin
 - ◊ Grey seal
 - ◊ Harbor seal
 - ◆ Coyote
 - ◆ Red fox

- + American marten
- + American mink
- + Fisher
- + North American river otter
- Abert's squirrel
- House mouse
- Raccoon

Detections across all flyways

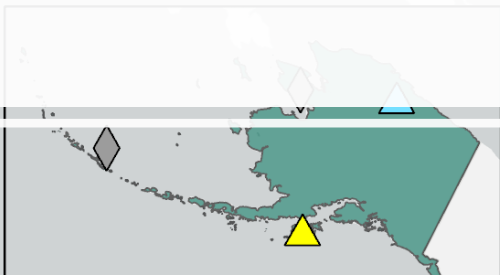
- Flyway**
- Pacific
 - Central
 - Eastern



National Wildlife Disease Program
 USDA/APHIS/Wildlife Services
 Published July 09, 2024

Data Source: State Agencies, USDA

Map created by: Jourdan Ringenberg



Adaptations

- Monitoring of sequences for PB2 markers associated with mammalian adaptation
 - E627K, D701N, T271A, and M631L
- Monitoring of mammalian detections for signs of:
 - Ongoing transmission
 - Sustained transmission of virus with these mutations
 - Spillover of mammalian adapted viruses back into wild bird or poultry populations





- The recent spillover of the virus into dairy cattle and subsequent spread to more than 140 herds in 12 states to-date presented yet more diagnostic challenges to track the spread of this highly clonal genotype and the need to closely monitor for development of mammalian adaptation.

Meeting the diagnostic challenges of a new host



Testing of a new sample matrix – milk



Understanding the host transmission dynamics, viral evolution, and spread

Where to sample, when to sample, how to sample
How to interpret sequence



Higher resolution monitoring of markers for mammalian adaption

Flumina <https://github.com/flu-crew/Flumina>.



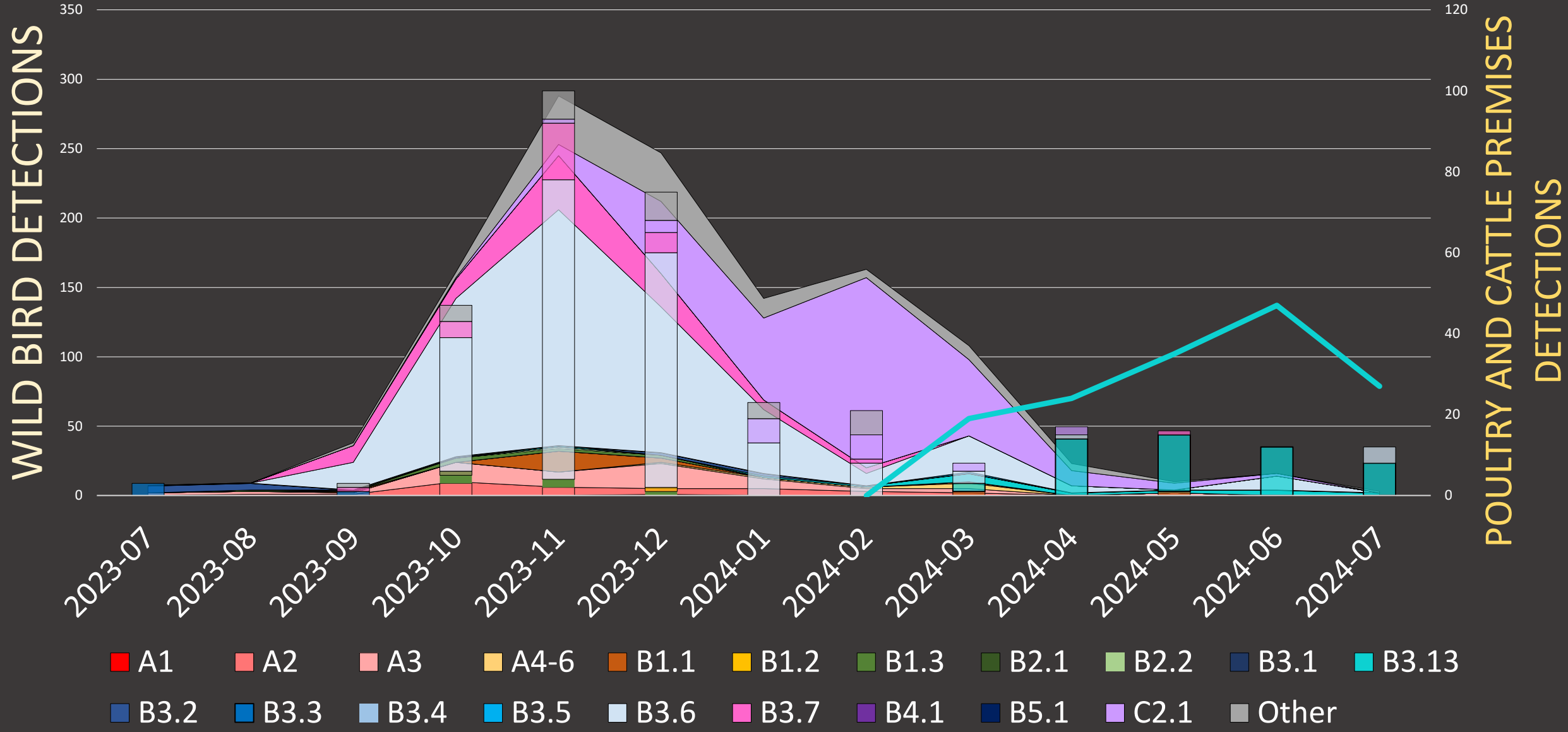
Detection of antibody

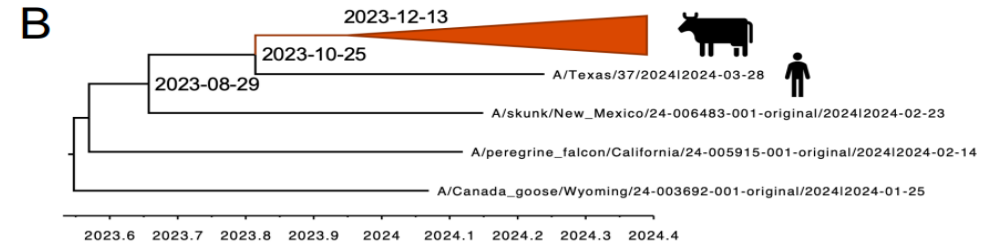
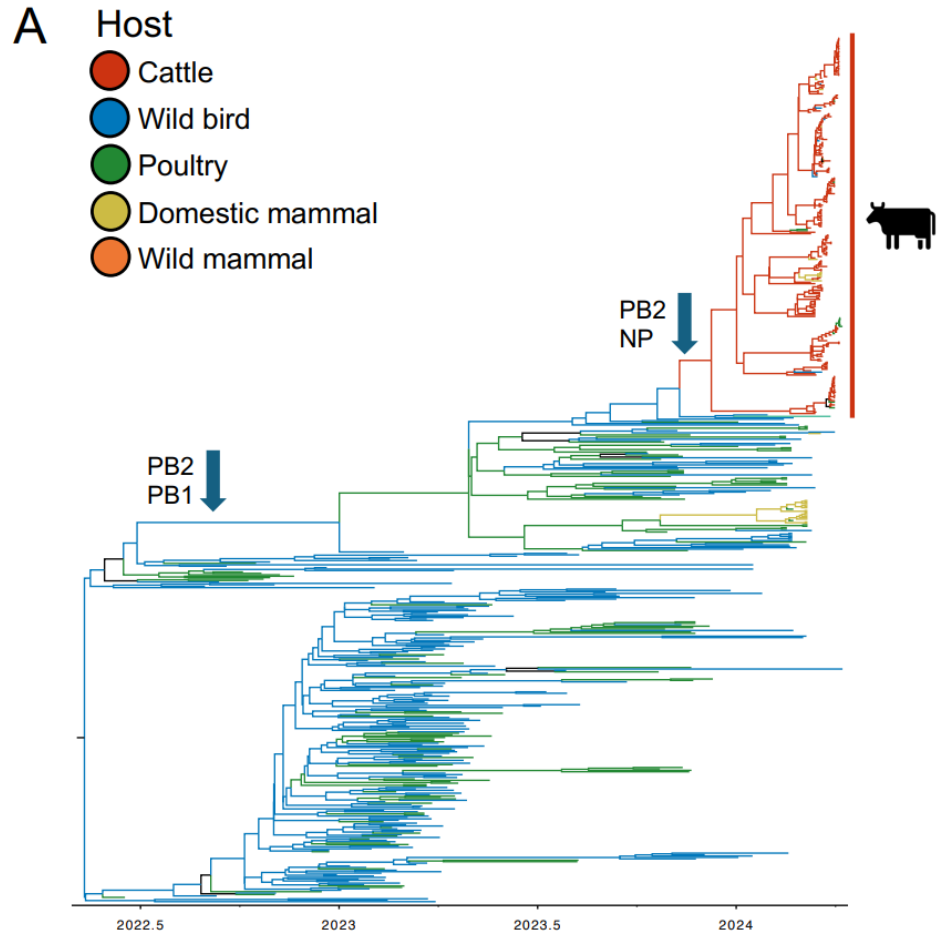
Adaptation of commercial ELISA for testing cattle serum and milk

Wild Birds by Genotype

Poultry and Cattle Detections Overlaid

July 2023 - July 2024





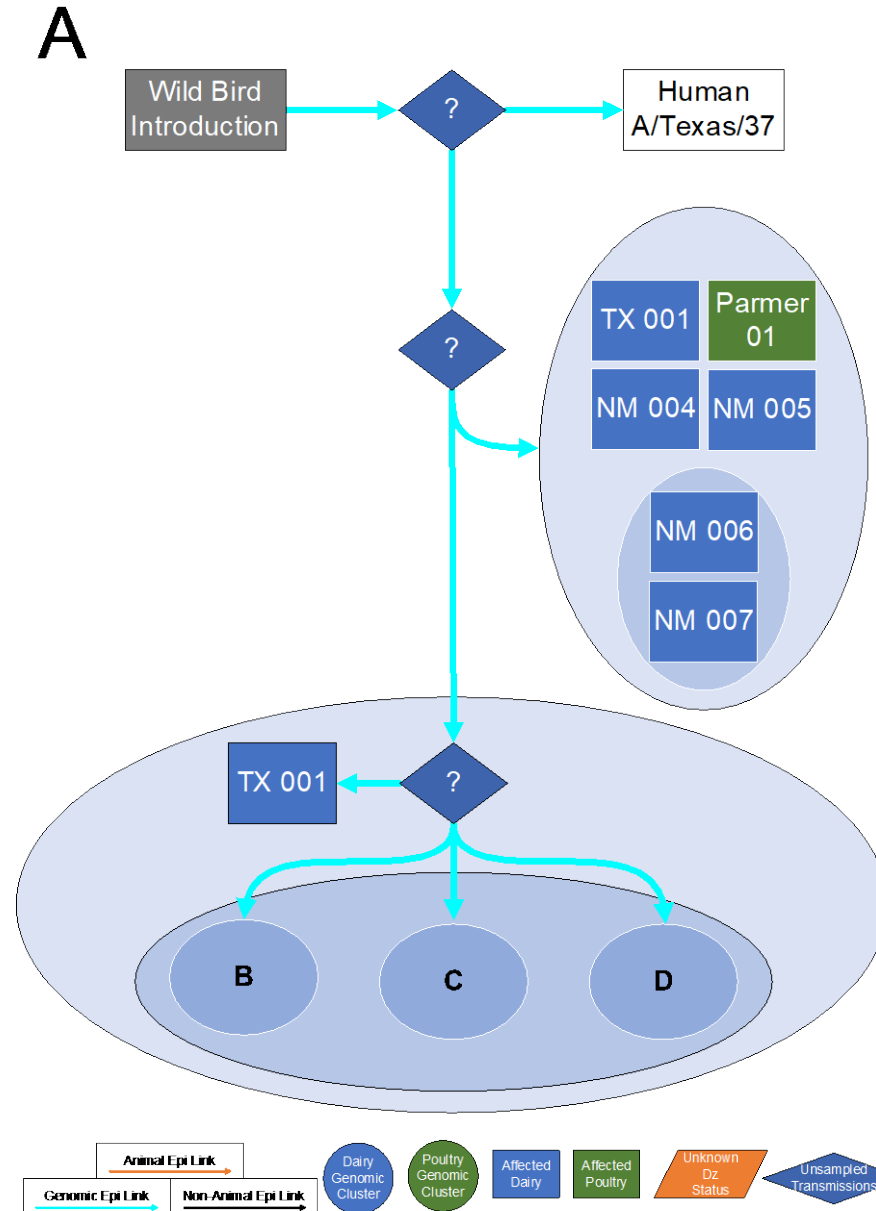
Human-associated virus branches ancestrally to known cattle samples suggesting an earlier unsampled source

Nguyen et al. [Emergence and interstate spread of highly pathogenic avian influenza A\(H5N1\) in dairy cattle | bioRxiv](#)

Animals are moved... and the virus too

Field Epidemiologic Investigation Services, and the National Veterinary Services Laboratories, USDA-APHIS

Nguyen et al. [Emergence and interstate spread of highly pathogenic avian influenza A\(H5N1\) in dairy cattle](#) | bioRxiv

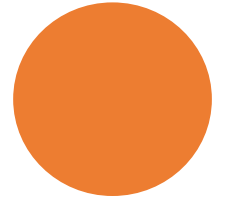


Key findings for B3.13

- The virus genotype found in dairy cattle emerged in wild birds in the fall of 2023.
- A single spillover event likely occurred in late winter of 2023 followed by limited local circulation which preceded the first evidence of cattle falling ill in January/February 2024.
- A small number of molecular markers were identified in virus populations that have potential to lead to changes in how the virus behaves and its transmission potential in mammals.
- Continued transmission of H5N1 within dairy cattle increases the risk for infection and subsequent spread.
- USDA Wildlife Services conducted an enhanced Spring surveillance April to June 2024 among species such as raptors, peridomestic birds, and agency removals of peridomestic animals, finding less than 1% H5N1 detections and no B3.13 among 5000 samples.
- Surveillance in migratory waterfowl resumed mid-July after the breeding season.

Other spillovers

- In March 2024, several newly kidded farmed goats developed acute neurological signs prior to death. The goat herd shared the pasture and water source with a flock of chickens and ducks, which had been recently depopulated due to HPAI.
 - Genotype 3.6 (related to B3.13)
 - Nannies and aborted fetuses tested negative
 - *Coxiella burnetii* also diagnosed in HPAI positive neonates
- In May 2024, HPAI stillbirths and abortions were reported among alpacas on an Idaho farm. The alpacas on the farm shared the same pasture and water source with infected chickens and ducks, which were depopulated.
 - Genotype B3.13
 - Adults and fetuses tested positive for HPAI
 - Investigation of comorbidities has not identified other agents



Detections of Highly Pathogenic Avian Influenza

Last Modified: June 21, 2024

The United States has the strongest avian influenza surveillance program in the world. Through our ongoing wild bird surveillance program, APHIS collects and tests large numbers of samples from wild birds in the North American flyways.

It is not uncommon to detect avian influenza in wild birds, as avian influenza viruses circulate freely in those populations without the birds appearing sick. In addition to monitoring for avian influenza in wild bird populations, APHIS monitors for the virus in commercial and backyard birds.

With the recent detections of the Eurasian H5 strain of highly pathogenic avian influenza (HPAI) in wild birds and domestic poultry in the United States, bird owners should review their biosecurity practices and stay vigilant to protect poultry and pet birds from this disease. APHIS is working closely with State partners on surveillance, reporting, and control efforts.



Confirmations in Commercial and Backyard Flocks



Detections in Wild Birds



Detections in Mammals



Detections in Livestock

The U.S. Department of Agriculture, Food and Drug Administration, Centers for Disease Control and Prevention, and State veterinary and public health officials are investigating cases of highly pathogenic avian influenza (HPAI) in dairy cows.

HPAI in dairy cattle main page



Acknowledgments



- We are grateful for the dedicated people and institutions working hard to combat this virus including the DVL team!
- Many thanks to our NAHLN and state partners as well as other academic institutions and states that continue to contribute to surveillance in wildlife.
- This analysis is possible thanks to our collaboration with Wildlife Services, ARS Southeast Poultry Research Laboratory, and our colleagues at the Center for Epidemiology and Animal Health.



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- Mia Kim Torchetti
- Suelee Robbe-Austerman
- Kristina Lantz
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