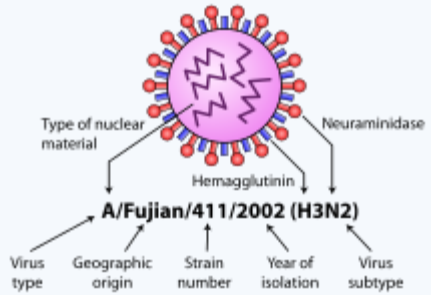
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Research Institute

Highly Pathogenetic Avian Influenza H5N1 outbreak in the U.S.

Michael Busch, MD, PhD
Director Emeritus, Vitalant Research Institute
Professor of Laboratory Medicine, UCSF

Presentation to IPFA/PEI Blood Safety and Surveillance Workshop 5-13-2025

Influenza A virus subtype H5N1



- Influenza A/H5N1 was first detected in 1959 after an outbreak of highly pathogenic avian influenza in Scotland, which infected two flocks of chickens. A/H5N1 is enzootic in many bird populations, and also panzootic (affecting animals of many species over a wide area).

- Earliest infection of humans by H5N1 was an epizootic (an epidemic in nonhumans) of H5N1 influenza in Hong Kong's poultry population in 1997. This outbreak was stopped by the killing of the entire domestic poultry population within the territory. Human infection was confirmed in 18 individuals who had been in close contact with poultry, 6 of whom died.

- Since then, avian A/H5N1 bird flu has become widespread in wild birds worldwide, with numerous outbreaks among both domestic and wild birds. An estimated half a billion farmed birds have been slaughtered in efforts to contain the virus.

- The segmentation of the influenza A virus genome facilitates genetic recombination by segment reassortment in hosts who become infected with two different strains of influenza viruses at the same time.[64][65]

- With reassortment between strains and relatively high mutation rate, an avian strain which does not affect humans may acquire characteristics from a different strain which enable it to infect and pass between humans – a zoonotic event. A/H5N1 in particular has infected a wide range of mammals and may be adapting to mammalian hosts.

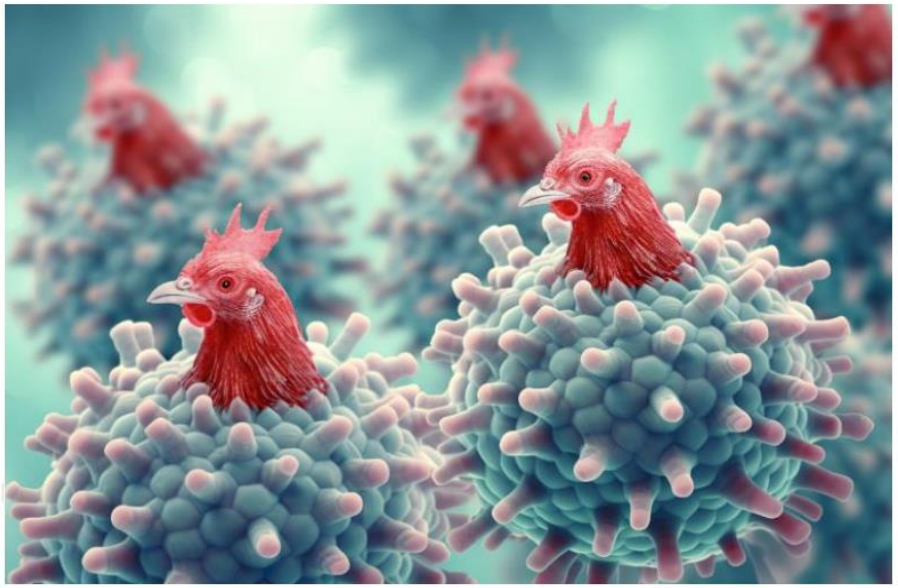
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Avian Influenza Background

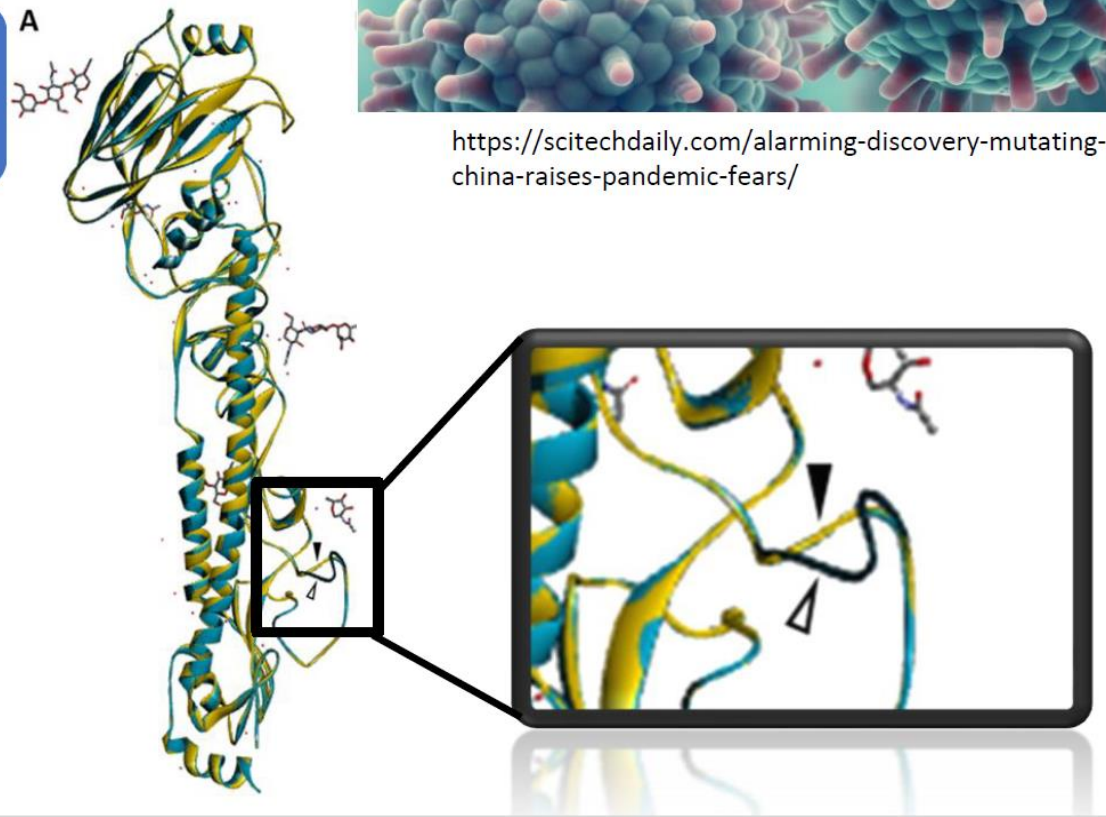
Isolated as early as 1959 (A/chicken/Scotland/59)

Exists as two different versions:

- Low Pathogenic Avian Influenza (LPAI) H5N1
- Highly Pathogenic Avian Influenza (HPAI) H5N1
- HPAI strains have a polybasic cleavage site in the HA which allows cleavage by furin-like proteases enabling the virus to grow outside of the GI and respiratory tract (other virulence factors may contribute as well)

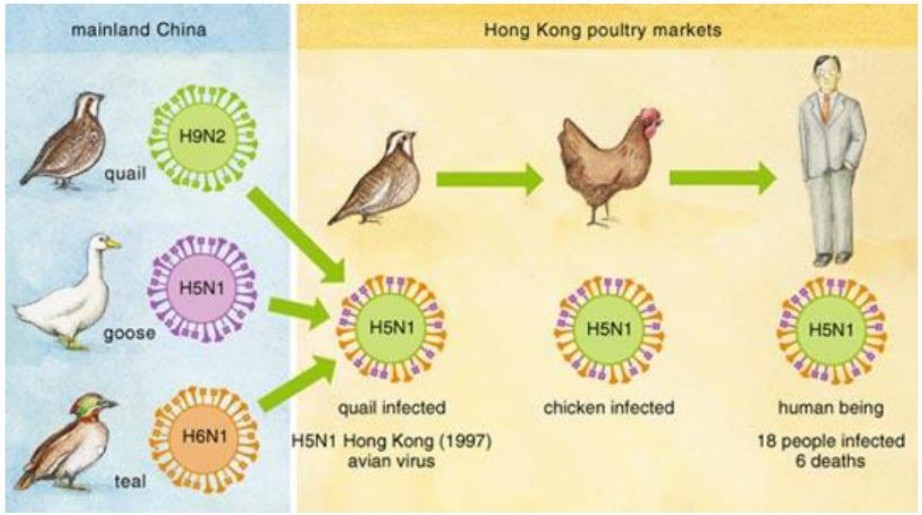


https://scitechdaily.com/alarming-discovery-mutating-bird-flu-in-china-raises-pandemic-fears/



First Human H5N1 cases in 1997 in Hong Kong

18 human H5N1 cases in Hong Kong, seven deaths, linked to HPAI H5N1 of the **A/goose/Guangdong/1/1996** lineage



https://people.scs.carleton.ca/~soma/biosec/readings/influenza/influenza-fig5.html

ELSEVIER
The Lancet
Volume 351, Issue 9101, 14 February 1998, Pages 472-477

Articles

Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus

Dr Eric CJ Claas PhD [a], Prof Albert DME Osterhaus DVM [a], Ruud van Beek MS [a], Jan C De Jong PhD [b], Guus F Rimmelzwaan PhD [a], Dennis A Senne MS [c], Scott Krauss MS [e], Prof Kennedy F Shortridge PhD [d], Prof Robert G Webster PhD [e]

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https://doi.org/10.1016/S0140-6736(97)11212-0

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ELSEVIER
The Lancet
Volume 351, Issue 9101, 14 February 1998, Pages 467-471

Articles

Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus

Prof KY Yuen MRCPath [a], PKS Chan MRCPath [b], Dr M Peiris FRCPath [a], DNC Tsang MRCPath [c], TL Que MRCPath [c], KF Shortridge PhD [a], PT Cheung FRCP [a], WK To FRCA [c], ETF Ho FRCA [c], R Sung FRCP [b], Prof AFB Cheng FRCPath [b], Members of the H5N1 study group

Show more

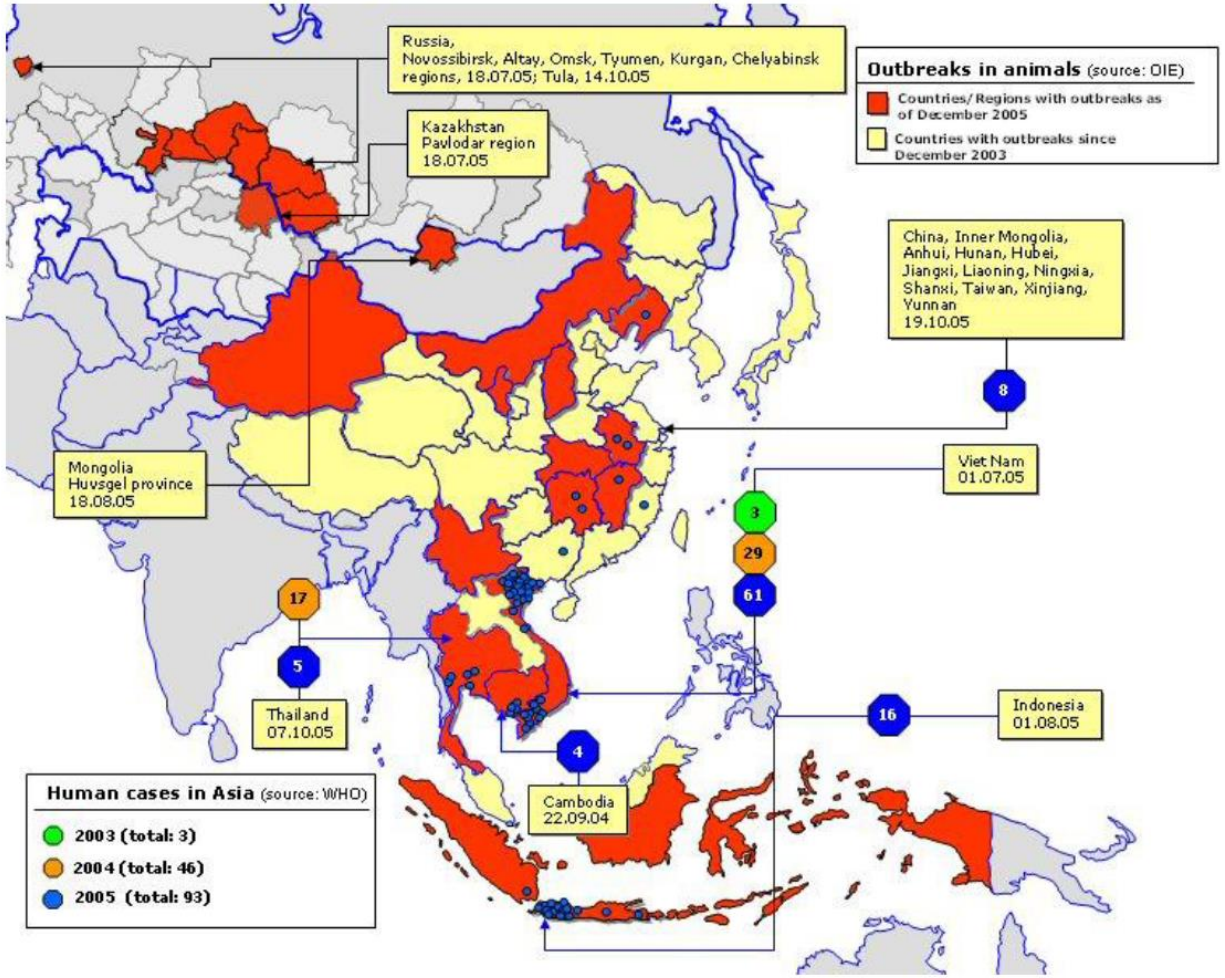
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https://doi.org/10.1016/S0140-6736(98)01182-9

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Return of HPAI H5N1 in 2003

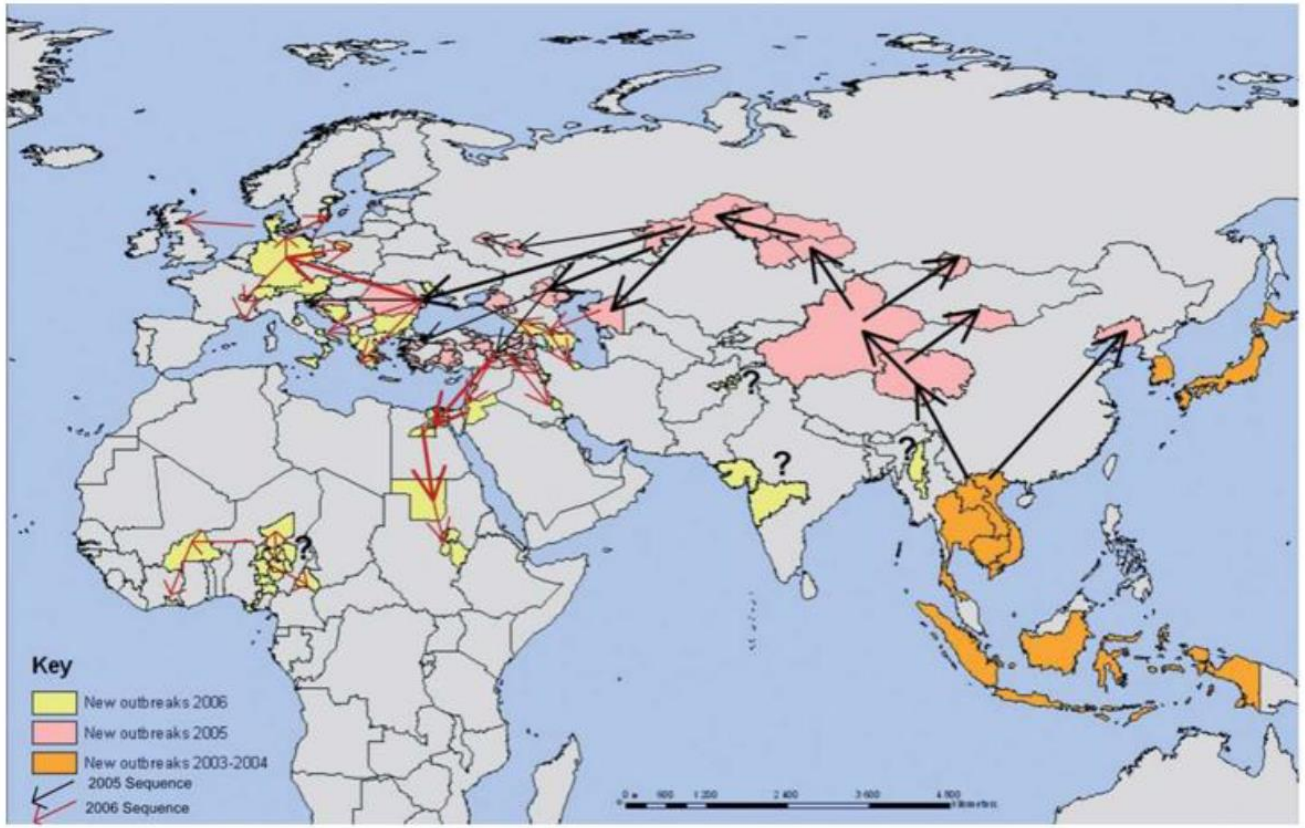


https://en.wikipedia.org/wiki/Global_spread_of_H5N1



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Spread across Eurasia and Africa via flyways



https://doc.woah.org/dyn/portal/index.xhtml?page=alo&aloId=30872

EMERGING INFECTIOUS DISEASES

Emerg Infect Dis. 2008 Apr; 14(4): 600–607.
doi: 10.3201/eid1404.071016

RESEARCH

Wild Ducks as Long-Distance Vectors of Highly Pathogenic Avian Influenza Virus (H5N1)

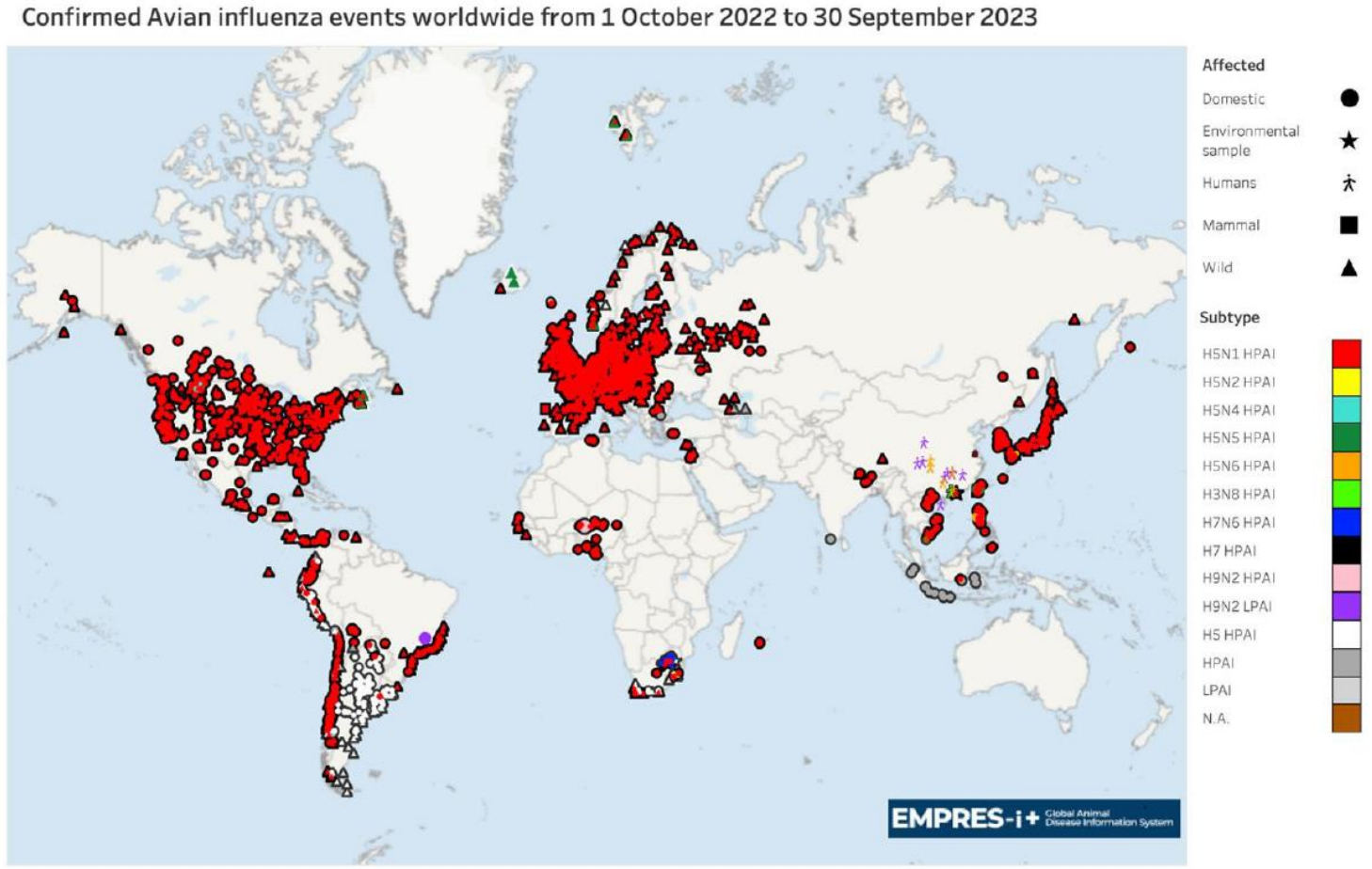
Juthatip Keawcharoen,* Debby van Riel,* Geert van Amerongen,* Theo Bestebroer,* Walter E. Beyer,* Rob van Lavieren,* Albert D.M.E. Osterhaus,* Ron A.M. Fouchier,* and Thijs Kuiken*



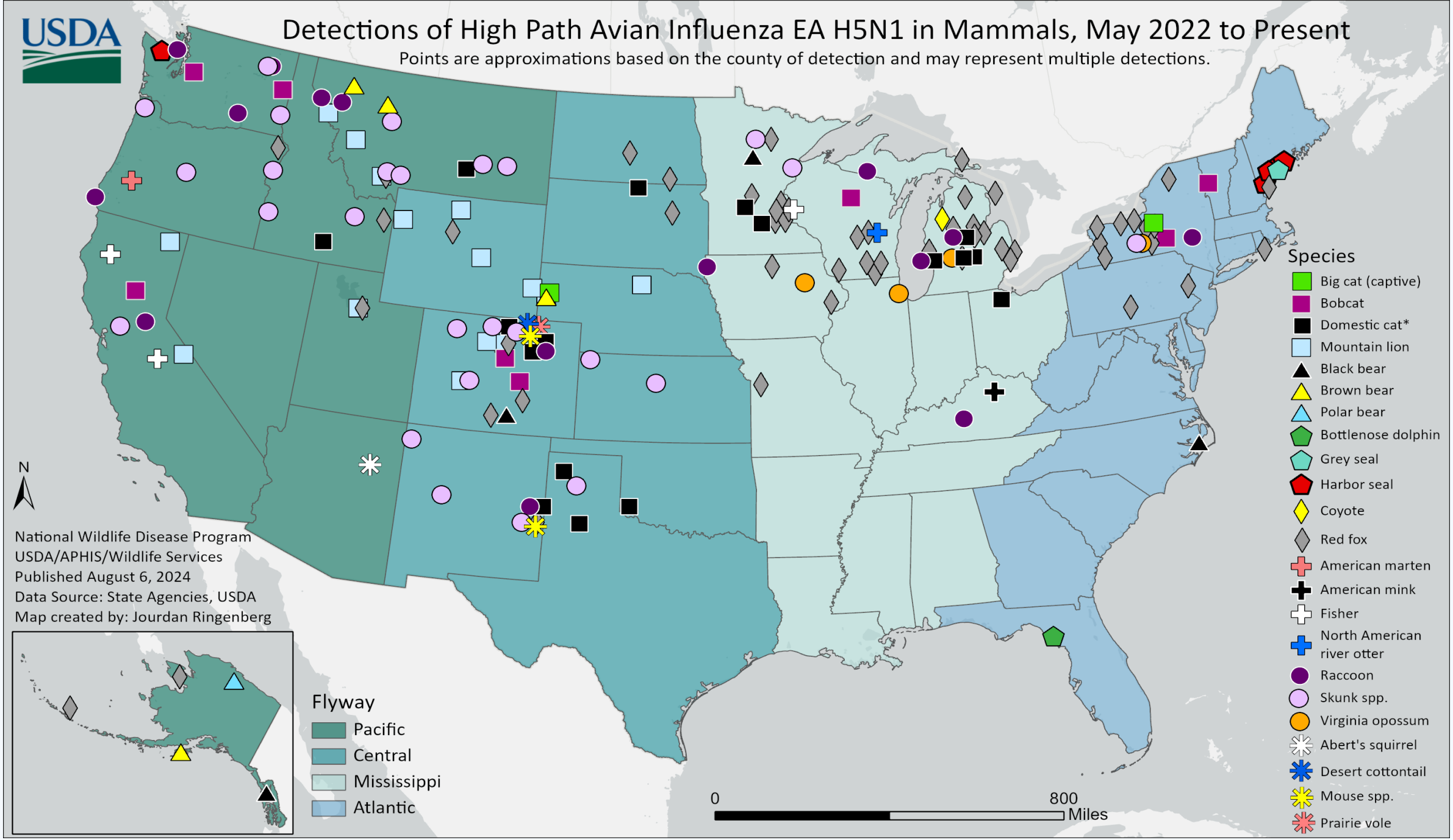
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Current spread of clade 2.3.4.4b H5N1

- Starting in 2020, H5N1 of the 2.3.4.4b clade spread across Europe
- In December 2021, H5N1 arrived in North America via wild birds
- Throughout 2022, H5N1 spread across North America, and later South America and has now reached Antarctica
- Frequent dead-end infections in mammals that feed on birds
- Suspected mammal to mammal transmission in fur farms in Europe and in marine mammals in South America
- A handful human cases, mostly mild, with two exceptions



Emergency Prevention System (EMPRES-i); Food and Agriculture Organization of the United States (FAO)



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

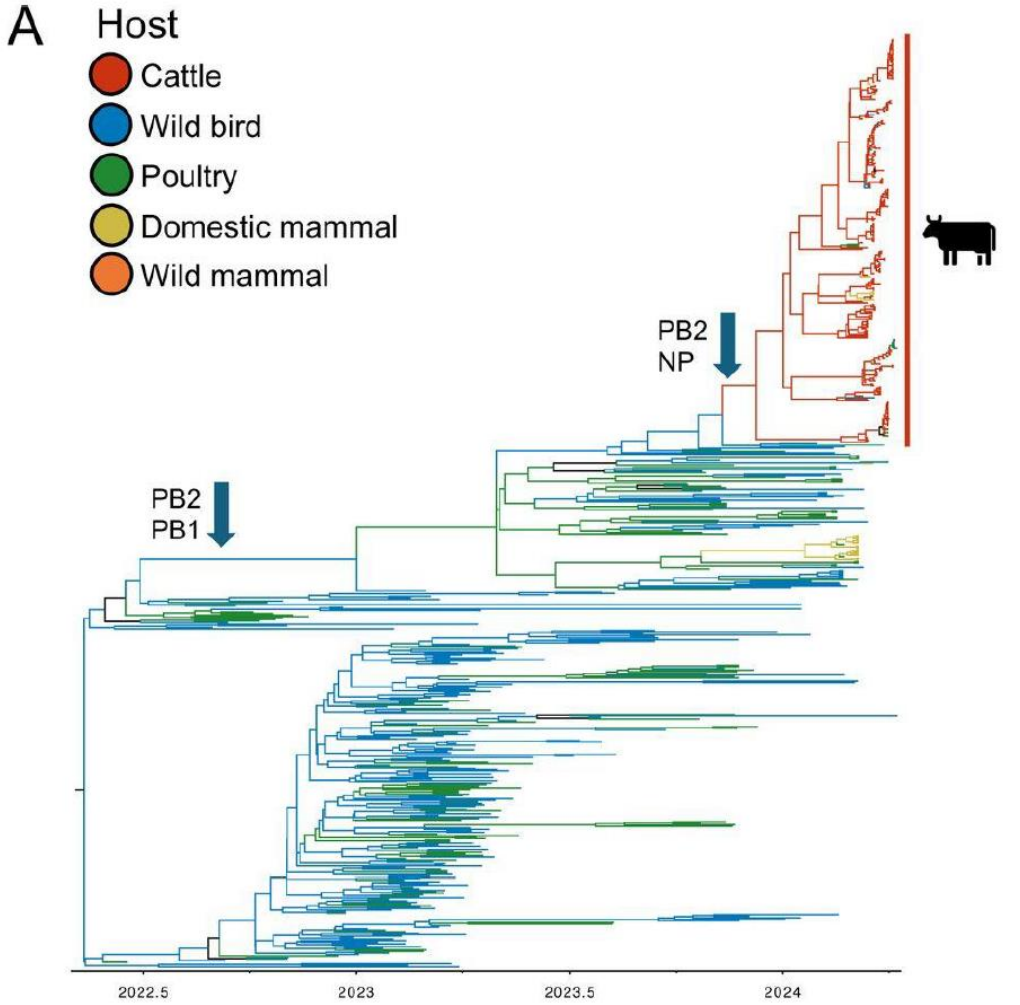
H5N1 spread into US dairy cattle herds

The New York Times

Bird Flu Spreads to Dairy Cows

U.S. regulators confirmed that sick cattle in Texas, Kansas and possibly in New Mexico contracted avian influenza. They stressed that the nation's milk supply is safe.

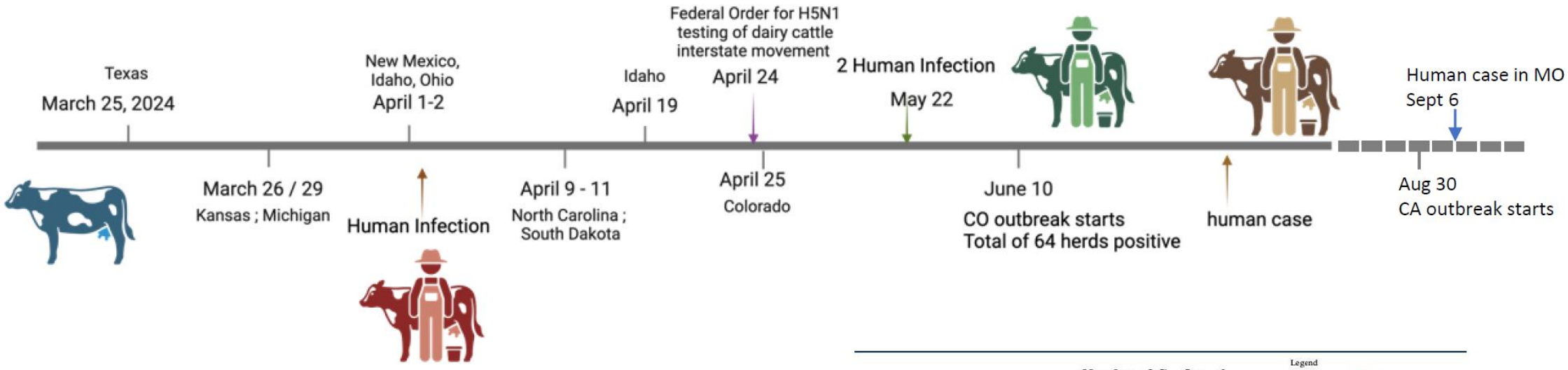




https://www.biorxiv.org/content/10.1101/2024.05.01.591751v1.full.pdf

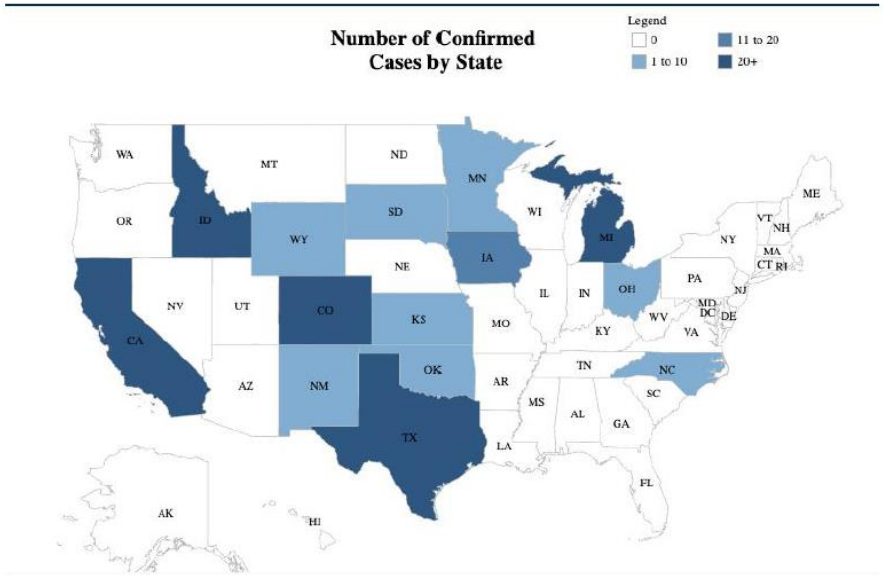
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Highly Pathogenic Avian H5N1 Influenza Virus in Cattle



Ongoing, multi-state outbreak

- **928** dairy herds affected as of 1/16/2024
- **16** states with outbreaks in dairy cows
- Last 30 days: 49 new confirmed cases in 2 states



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

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H5N1 virus from cattle – characteristics summary

- These viruses are **primarily avian viruses**
- **No consistent changes have been observed that would increase transmission to and among people**
- Alpha 2,3 sialic acid receptors (**avian virus-type**) are abundant in dairy cattle mammary tissue as well as in the respiratory tract.
- **Genetic analysis**
  - H5 clade 2.3.4.4b, **genotypes B3.13 and D1.1**
  - No known markers for reduced susceptibility to antiviral medicines, except in one virus from cow with a change in NA (T438I) associated with reduced susceptibility to (NAIs)
  - Humans likely to have limited immunity
- **Antigenic analysis**
  - Viruses **well covered by existing CVVs**

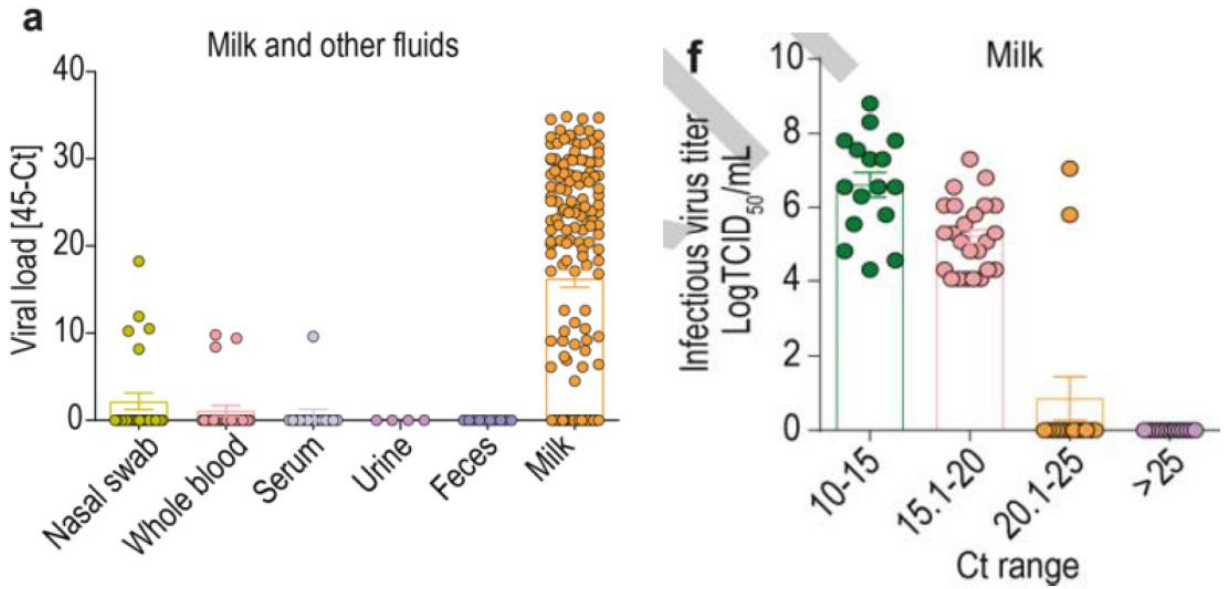
World Health Organization

https://www.cdc.gov/flu/avianflu/spotlights/2023-2024/h5n1-analysis-texas.htm
Uyeki TM et al.. Highly Pathogenic Avian Influenza A(H5N1) Virus Infection in a Dairy Farm Worker. N Engl J Med. 2024 May 3

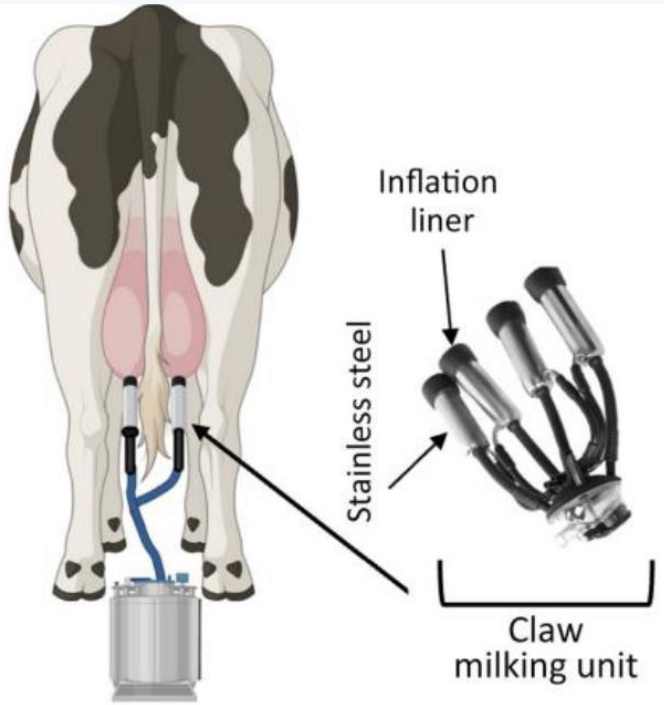
HEALTH EMERGENCIES programme

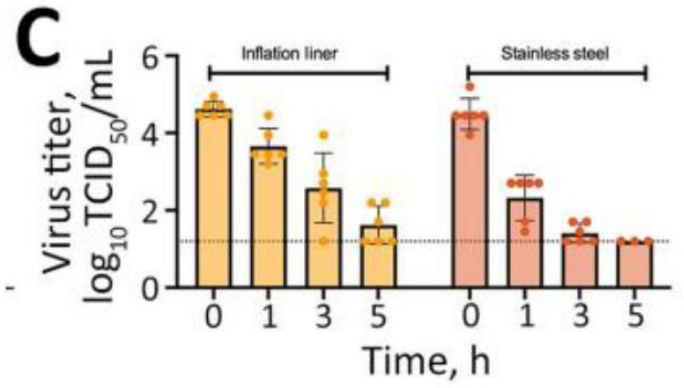
H5N1 is present in high titers in milk

- H5N1 infection reduces milk production
- Virus replicates in mammary glands
- Infectious virus is secreted into milk at high titers
- Raw milk from infected cow causes infections in mice
- Pasteurization kills of infectious virus but genome is now widely present in the US milk supply
- Cow-to-cow transmission likely mostly through milking equipment



Caserta *et al.*, Nature, 2024





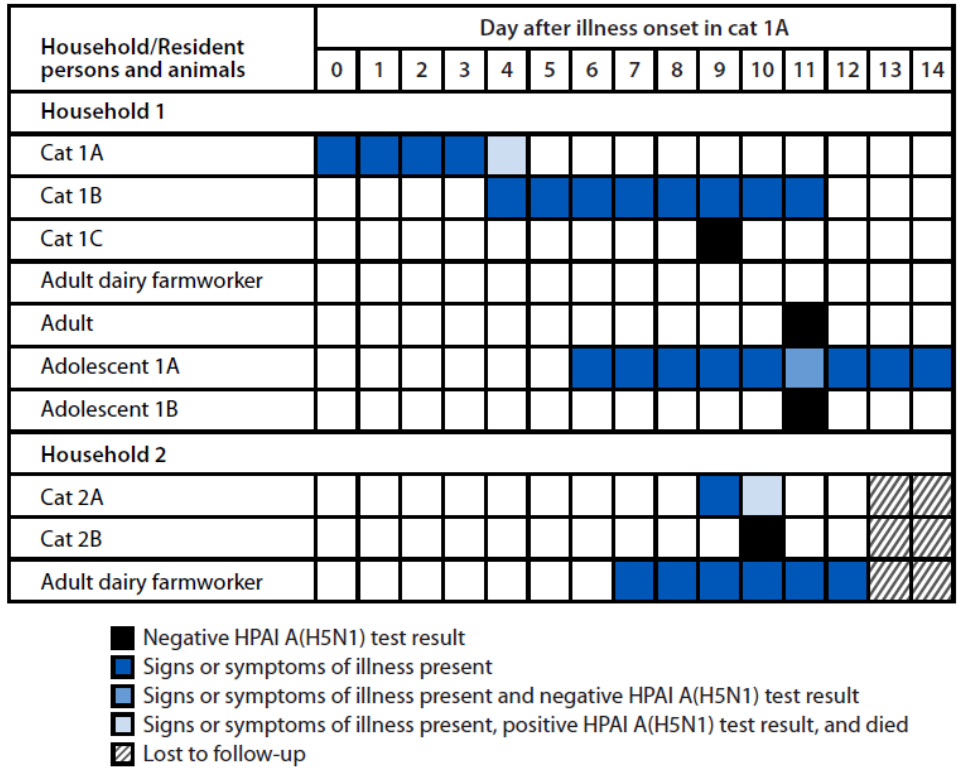
Le Sage *et al.*, EID, 2024

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Highly Pathogenic Avian Influenza A(H5N1) Virus Infection of Indoor Domestic Cats Within Dairy Industry Worker Households — Michigan, May 2024

Ramya Naraharisetti, PhD[1,2]; Meghan Weinberg, PhD[2]; Becky Stoddard[3]; Mary Grace Stobierski, DVM[2]; Kimberly A. Dodd, DVM[4]; Nora Wineland, DVM[5]; Mathew Beal, DVM[4]; Jennifer Morse, MD[3]; Samantha Hatter, DVM[4]; Dodd Sledge, DVM[4]; Katelynn Youatt, DVM[4]; Joseph Coyle, MPH[2]; Jevon McFadden, MD[2,6]; Timothy M. Uyeki, MD[7]; Lizette O. Durand, VMD, PhD[7]

FIGURE. Testing, signs, and symptoms in cats[*,†] and household members[§,¶] of domestic indoor cats infected with highly pathogenic avian influenza A(H5N1) virus, clade 2.3.4.4b genotype B3.13 (N = 2) — Michigan, 2024



Summary

What is already known about this topic?

Outdoor cats on U.S. dairy farms have been infected with highly pathogenic avian influenza (HPAI) A(H5N1) virus; infection has not been reported in indoor cats.

What is added by this report?

HPAI A(H5N1) virus was detected in two indoor domestic cats with respiratory and neurologic illness that lived in homes of dairy workers but had no known direct exposure to HPAI A(H5N1)–affected farms. Both dairy workers declined testing; other household members received negative test results for influenza A.

What are the implications for public health practice?

Veterinarians in states with confirmed HPAI A(H5N1) in livestock should consider obtaining household occupational information, testing for influenza A viruses, and wearing personal protective equipment when evaluating companion cats with respiratory or neurologic illness. Suspected cases should be reported to public and animal health officials.

REVIEW ARTICLE

Open Forum Infectious Diseases

Avian Influenza Virus Infections in Felines: A Systematic Review of Two Decades of Literature

Kristen K. Coleman[1,2] and Ian G. Bemis[1,2]

RIPT

Highly Pathogenic Avian Influenza A(H5N1) Virus Infections in Humans

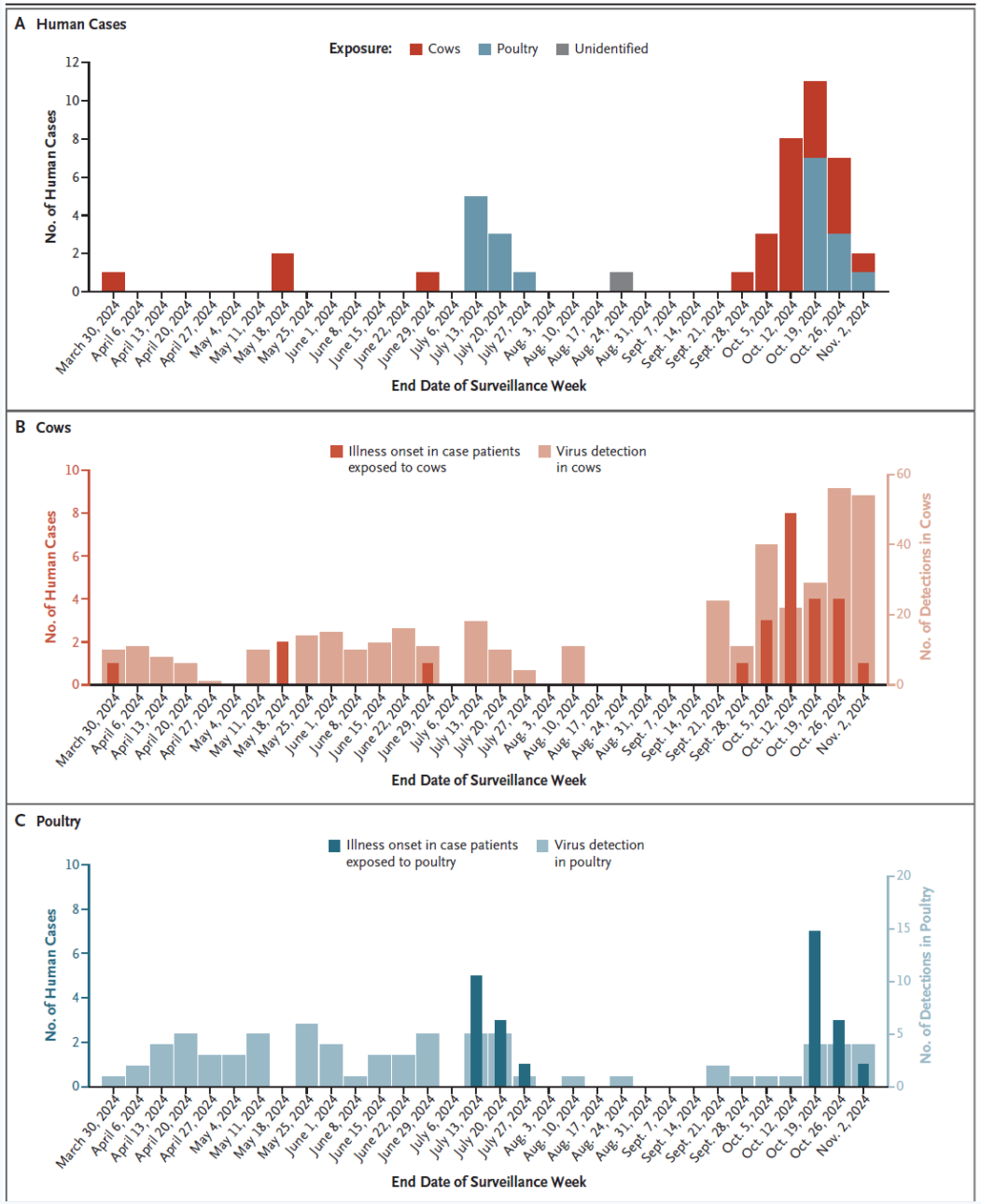


Table 3. Laboratory Results According to Specimen Type among 45 Case Patients with Highly Pathogenic Avian Influenza A(H5N1) Virus Infection Who Had Exposure to Infected Animals.*

Specimen Type	Exposure to Poultry		Exposure to Dairy Cows		Overall	
	Specimen Collected	Positive Specimen	Specimen Collected	Positive Specimen	Specimen Collected	Positive Specimen
	no./total no.	*no./total no. (%)*	*no./total no.*	*no./total no. (%)*	*no./total no.*	*no./total no. (%)*
Conjunctival	16/20	14/16 (88)	25/25	22/25 (88)	41/45	36/41 (88)
Nasopharyngeal	12/20	7/12 (58)	24/25	5/24 (21)	36/45	12/36 (33)
Combined nasal–oropharyngeal	3/20	2/3 (67)	19/25	7/19 (37)	22/45	9/22 (41)

The Emerging Threat of H5N1 to Human Health

Michael G. Ison, M.D.,[1] and Jeanne Marrazzo, M.D., M.P.H.[2]

N ENGL J MED 392;9 NEJM.ORG FEBRUARY 27, 2025

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H5N1 Bird Flu Surveillance and Human Monitoring
Weekly Snapshot for Week Ending May 3, 2025

Situation summary of confirmed and probable human cases since 2024

Confirmed Cases | Probable Cases

State or territory

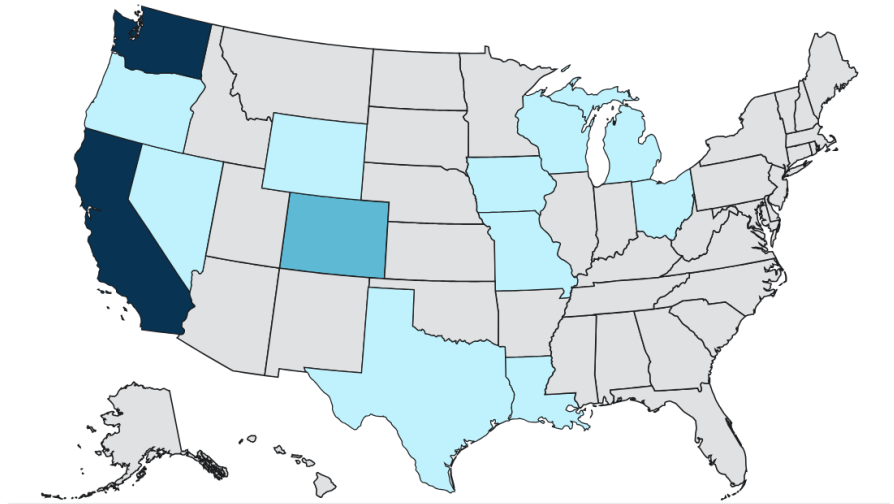
National

National Total Cases: 70

Cases	Exposure Source
41	Dairy Herds (Cattle)*
24	Poultry Farms and Culling Operations*
2	Other Animal Exposure†
3	Exposure Source Unknown‡

NOTE: One additional case was previously detected in a poultry worker in Colorado in 2022. Louisiana reported the first H5 bird flu death in the U.S.

*Exposure Associated with Commercial Agriculture and Related Operations
†Exposure was related to other animals such as backyard flocks, wild birds, or other mammals
‡Exposure source was not able to be identified



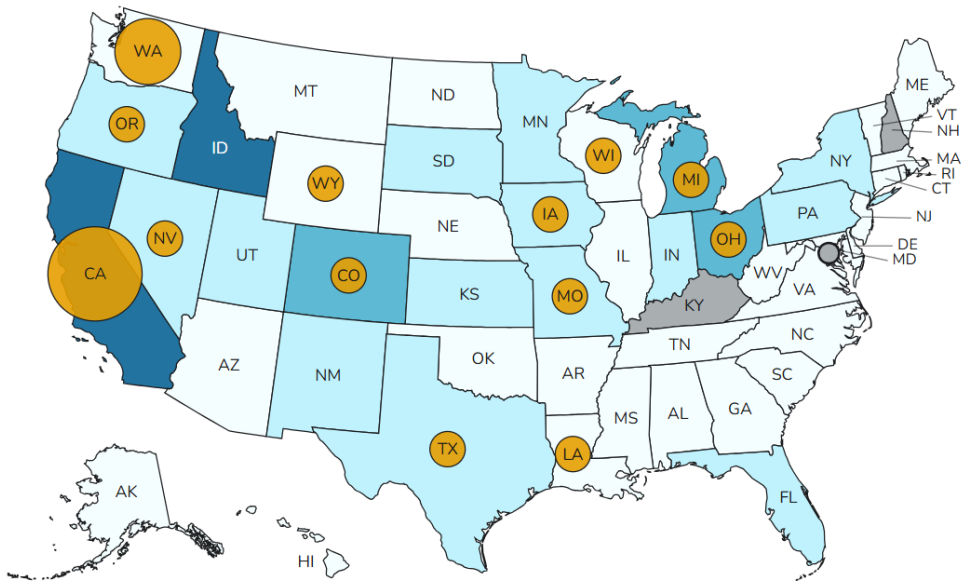
Total cases

0 | 1-5 | 6-10 | >10

0 | 1-10 | 11-50 | 51-100 | >100

Total human cases exposed to any animal

1-10 | 11-20 | >20

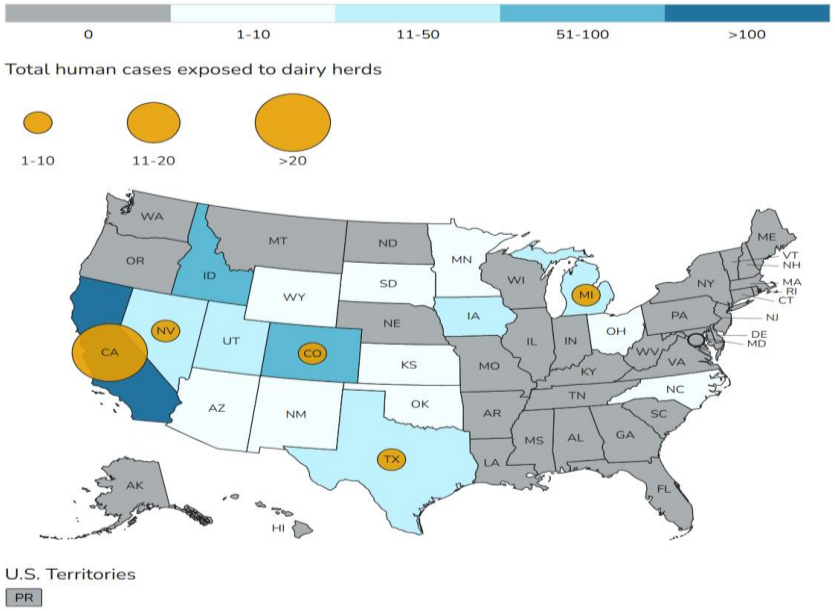


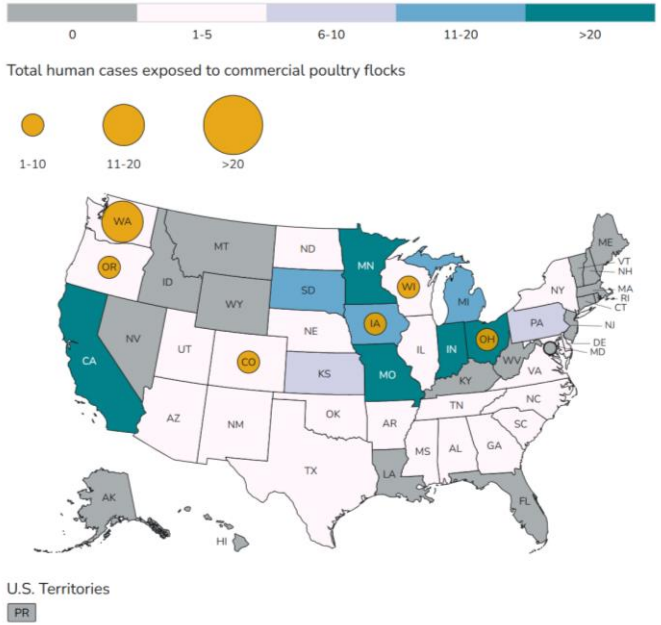
U.S. Territories

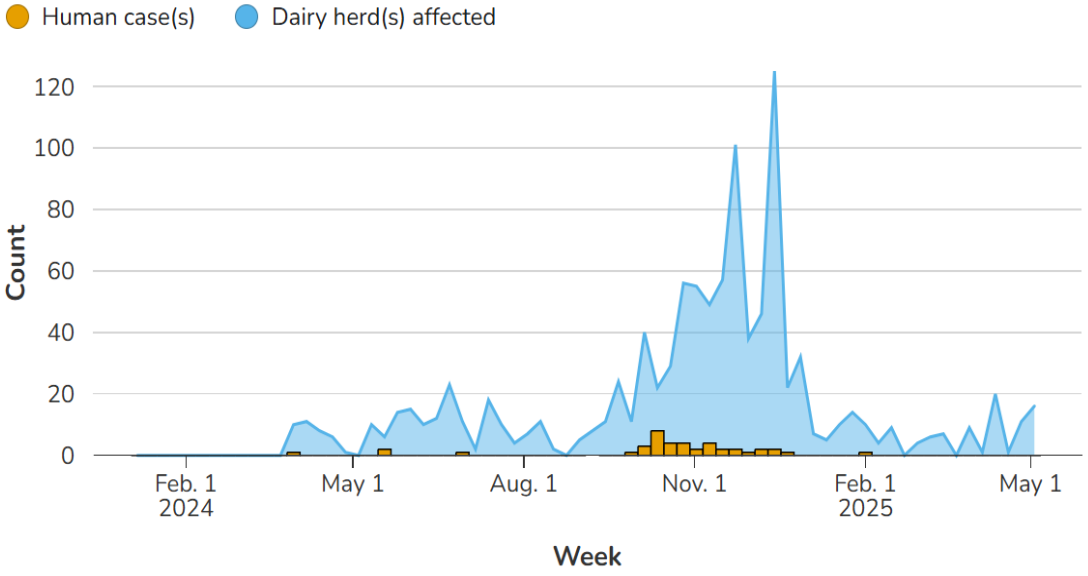
PR

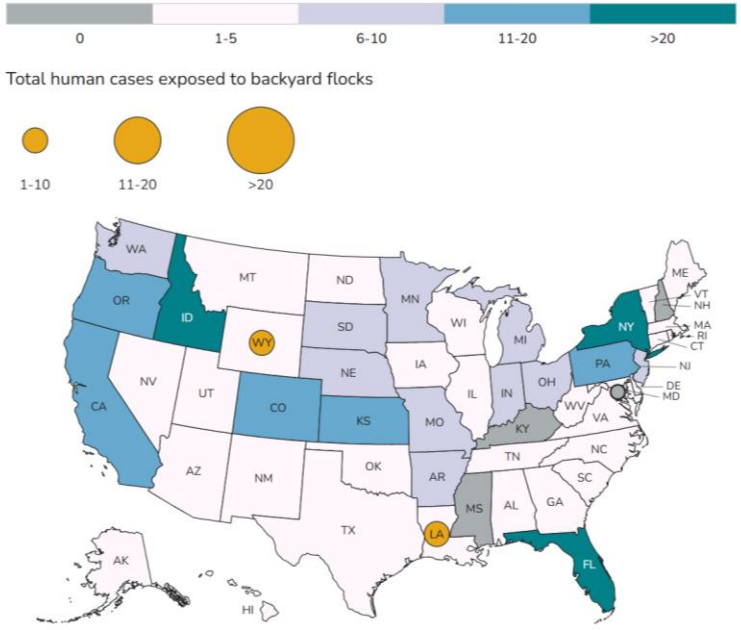
H5N1 Bird Flu Surveillance and Human Monitoring

Weekly Snapshot for Week Ending May 3, 2025









Enhancing the response to avian influenza in the US and globally

From 2024 through April 2025, there have been confirmed cases in 995+ dairy cow herds, 168+ million birds, 1650+ flocks, and at least 70 humans with the epizootic now present in all 50 states and human cases in 13 states and Canada

Detections in Animals

- **169,264,441** poultry affected as of 5/12/2025
- **51** jurisdictions with outbreaks in poultry

- **1,053** dairy herds affected as of 5/9/2025
- **17** states with outbreaks in dairy cows

- **13,001** wild birds detected as of 5/7/2025
- **51** jurisdictions with bird flu in wild birds

The Lancet Regional Health - Americas 2025;■: 101100

What research is important to prepare and respond to H5N1 influenza outbreaks?

19 March 2025 13:00 – 19:15 CET

Virtual meeting on Wednesday, 19 March 2025 from 13:00 to 19:15 CET (Geneva time)

OBJECTIVES

The objectives of this meeting are to:

- Identify the knowledge gaps and priority research questions related to the development and evaluation of medical countermeasures to prepare for and respond to H5N1 outbreaks
- Outline regulatory pathways for mRNA vaccines and other novel approaches
- Review other therapeutics, including monoclonal antibodies and long-acting antivirals

EXPECTED OUTCOMES

- Outline a roadmap for research and development
- Promote international collaboration among various researchers and research institutions

[1]Pathogens prioritization: a scientific framework for epidemic and pandemic research preparedness (who.int)

World Health Organization

HEALTH EMERGENCIES programme

Main areas of WHO response and readiness

- **Surveillance**
  - Human-animal interface
  - Genetic and antigenic characterization of influenza viruses with WHO CCs and H5 reference labs
  - Risk assessment: WHO, Tripartite, GISRS, internal
- **Communications**
  - Briefings (webinars, journalists, MS briefings, technical partners, regions) and information products (EIS, DON), updated Q&A, etc.
- **Clinical care**
  - Updated influenza management guidelines
  - Updating WHO IPC ARI guidelines update to strengthen IPC measures while protecting health workers and patients
- **Community protection**
  - PHSM, food safety, RCCE, Vaccination demand/uptake, etc.
- **Medical countermeasures and benefits**
  - PIP, CVVs, i-MCM, Antiviral preparedness, etc.
- **Partner coordination**
  - Coordinating with animal health partners (FAO, WOAH, OFFLU, UNEP) and Food Safety colleagues, GOARN, other global agencies

World Health Organization

HEALTH EMERGENCIES programme

Summary of current situation

- Over 900 detections of **A(H5N1) in humans** reported from over 20 countries since 2003
  - **Sporadic** with direct or indirect **exposure** to infected animals or contaminated environments
  - Infections **can be severe** and even fatal
  - Available genetic sequences of the virus from the human cases similar to those from local **animals**
  - No sustained human-to-human transmission
- Avian influenza A(H5N1) viruses, especially those of clade 2.3.4.4b, continue to **diversify genetically** and **spread geographically**
- **This is a oncerning, evolving situation**
- **Prompt & thorough investigations** are critical, **early identification** of any unusual events that could signal person-to-person transmission of the virus and **timely reporting** are key to optimal outcome of response
- **Advancing pandemic influenza preparedness** at national, regional and global levels
- **Advancing research and development for H5N1**
- **Global collaboration and coordination are key to optimal outcome**
  - Sharing of sequences and viruses from humans and animals is critical
  - GISRS is an effective foundation for surveillance, preparedness and response

World Health Organization

HEALTH EMERGENCIES programme

•Nature Published: 28 October 2024

Transmission of a human isolate of clade 2.3.4.4b A(H5N1) virus in ferrets

Abstract

Since 2020, there has been unprecedented global spread of highly pathogenic avian influenza A(H5N1) in wild bird populations with spillover into a variety of mammalian species and sporadically humans[1]. In March 2024, clade 2.3.4.4b A(H5N1) virus was first detected in dairy cattle in the USA, with subsequent detection in numerous states[2], leading to more than a dozen confirmed human cases[3,4]. In this study, we used the ferret, a well-characterized animal model that permits concurrent investigation of viral pathogenicity and transmissibility[5], in the evaluation of A/Texas/37/2024 (TX/37) A(H5N1) virus isolated from a dairy farm worker in Texas[6]. Here we show that the virus has a remarkable ability for robust systemic infection in ferrets, leading to high levels of virus shedding and spread to naive contacts. Ferrets inoculated with TX/37 rapidly exhibited a severe and fatal infection, characterized by viraemia and extrapulmonary spread. The virus efficiently transmitted in a direct contact setting and was capable of indirect transmission through fomites. Airborne transmission was corroborated by the detection of infectious virus shed into the air by infected animals, albeit at lower levels compared to those of the highly transmissible human seasonal and swine-origin H1 subtype strains. Our results show that despite maintaining an avian-like receptor-binding specificity, TX/37 exhibits heightened virulence, transmissibility and airborne shedding relative to other clade 2.3.4.4b virus isolated before the 2024 cattle outbreaks[7], underscoring the need for continued public health vigilance.

Wang *et al. BMC Infectious Diseases* 2014, **14**:192
http://www.biomedcentral.com/1471-2334/14/192

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Highly pathogenic avian influenza A virus (H5N1) can be transmitted in ferrets by transfusion

Xue Wang[1*], Jiying Tan[1], Jiangqin Zhao[1], Zhiping Ye[2] and Indira Hewlett[1*]

Abstracts

Background: Highly pathogenic avian influenza A virus has been shown to infect organs other than the lung, and this is likely to be mediated by systemic spread resulting from viremia which has been detected in blood in severe cases of infection with avian H5N1 viruses. The infectivity of virus in blood and the potential for virus transmission by transfusion has not been investigated.

Methods: Using a susceptible ferret animal model, we evaluated viremia and transmission by blood transfusion. Blood was collected on day 2, 4, 6, and 10 post-infection (or before death) from donor ferrets infected with either low dose ($1.0 \times 10^{2.6}$ EID_{50}/ml) or high dose ($1.0 \times 10^{3.6}$ EID_{50}/ml) of H5N1 virus, A/VN/1203/04 and transfused to recipient animals.

Results: Viremia was observed in 2/12 (16.67%) recipients that received blood from donor ferrets infected with low dose and 7/12 (58.33%) recipients who received blood from high dose infected donors. 1/12 (8.3%) low dose recipients and 6/12 (50%) high dose recipients died within 11 days after transfusion. Increased changes in body weight and temperatures were observed in high dose recipients, and high levels of viral RNA were detected in recipient ferrets after transfusion of blood from the early viremic phase, which also correlated with adverse impact on their survival.

Conclusion: These data suggest that highly pathogenic avian influenza A virus, H5N1, is transmissible by blood transfusion in ferrets. Low levels of viremia were detected around the time of onset of symptoms and later in ferrets infected with highly pathogenic H5N1 virus. These findings may have implication for pathogenesis and transmissibility of H5N1.

Keywords: H5N1, Transfusion, Viremia, Ferrets

H5N1 Serosurvey of US Blood Donors in Locations with Dairy Cattle Epidemic Activity and Human Infection Cases

Aim 1. Evaluate and validate Influenza seroplex assays for detection of antibodies to HPAI H5N1

- Evaluate multiplexed array technologies including multiple H5 and N1 antigens corresponding to the current H5N1 strain spreading U.S. dairy cattle and poultry.
- Establish influenza pseudovirus neutralization assays to confirm array findings and characterize neutralization antibody titers in sera from donors identified on the seroplex assays as reactive for H5N1.
- Collaborate with NIAID/CDC influenza lab teams to perform live virus neutralization testing as an additional confirmatory assay.

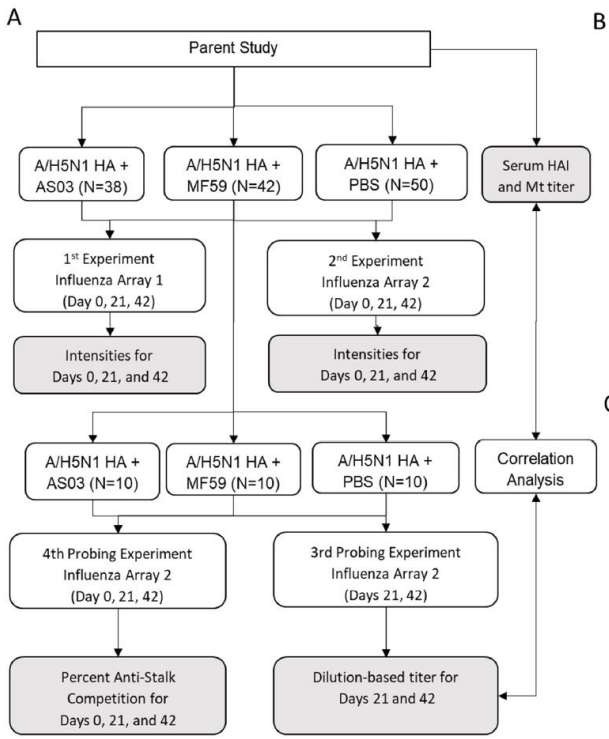
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The antibody landscapes against Group 1 and 2 influenza virus hemagglutinin following AS03 and MF59 adjuvanted H5N1 vaccination

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Figure 1 Study Overview. A: Study design B: Protein array antigen composition C: HA Subtype relationship, H5 trimer structure, MiniHA binding used for assessing percent anti-stalk competition.

A

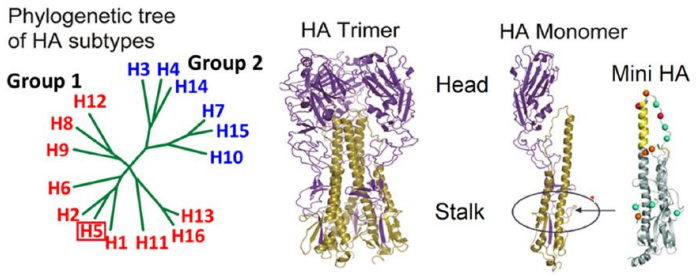


B

Influenza Array 1							Total HA
Type	Source	HA1	HA2	HA1+HA2	NA	NP	
H5 HA	SinoBio	33	2	41	2	0	76
	Felgner	3	0	3	0	0	6
non-H5 HA	SinoBio	29		49	2	1	78
	Felgner	57	2	60	0	0	119
	Total	122	4	153	4	1	279

Influenza Array 2							Total HA
Type	Source	HA1	HA2	HA1+HA2	NA	NP	
H5 HA	FLK	0	0	6	2	0	6
	SinoBio	33	2	43	2	0	78
	VB	0	0	1	0	0	1
non-H5 HA	FKL	1	0	74	24	0	75
	SinoBio	29	0	50	2	1	79
	Total	63	2	174	30	1	239

C



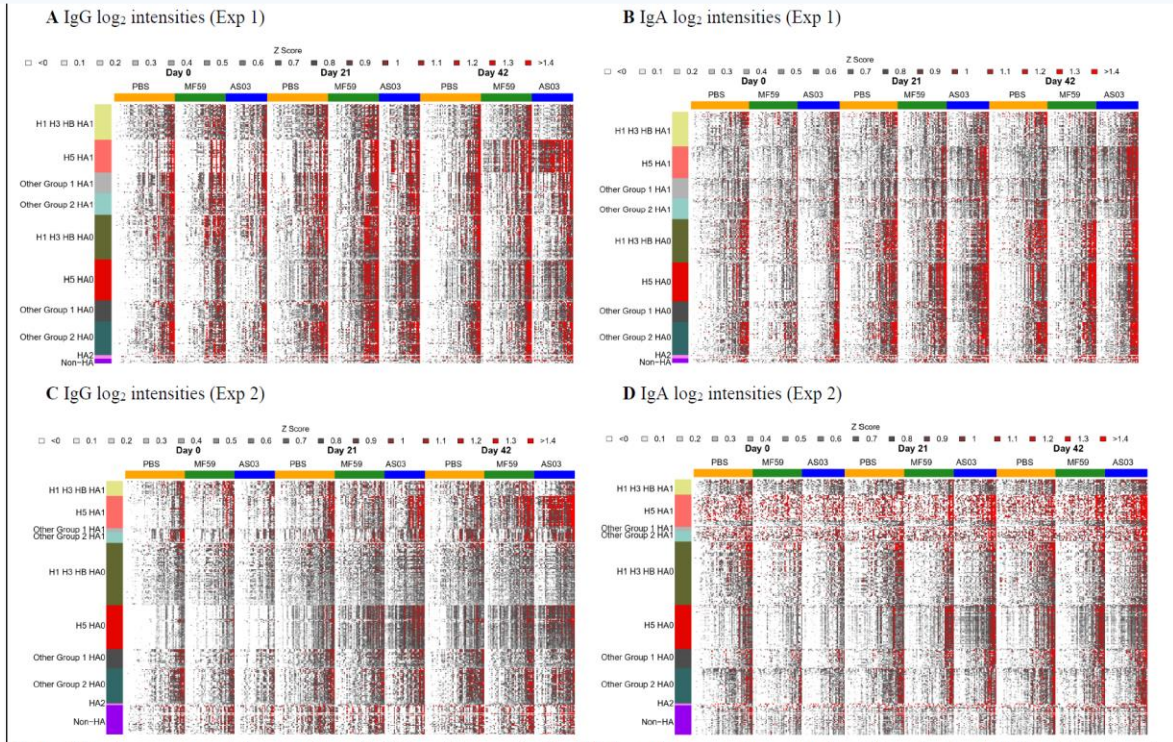


Figure 2 Heatmaps summarizing experiment 1 & 2 protein array IgG and IgA results by vaccine group, day, HA molecule, and HA subtype. A and B: log_2 fluorescent intensity results by HA subtype for Experiment 1 IgG and IgA antibodies, respectively. C and D: Corresponding results for Experiment 2. Each column represents one sample from a subject, and each row represents an antigen. The antigens are organized into groups, headgroup (HA1) or full-length molecule (HA0). The HA subtypes were grouped as H1/H3/FluB, H5, Other Group 1, and Other Group 2. Fluorescent intensities were background corrected, log_2 transformed, and standardized across rows (z-score, mean=0, variance=1). In red: intensities strongly increased compared to the mean log_2 intensity, in dark grey: moderately increased compared to the mean in white decreased relative to the mean. Samples were ordered by increasing reactivity

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Aim 2. Execute blood donor serosurveillance study in highest impacted outbreak states

- Execute two waves of blood donor based serosurveillance collection and testing in Q1 2025 and Q4 2025:
  - 6,000 donation-derived samples from CO/WY, TX & CA
  - First wave: cross-sectional in design to include first time and repeat donations with diverse demographics (oversampling for Hispanic or younger donor populations)
  - Second wave: re-sample donors from first wave to generate longitudinal serology data.
  - Execute an electronic survey, including occupation and symptoms, of all sampled donors, and would also be able to contact donors who test Ab-reactive for more detailed epidemiological investigation and follow-up sample acquisition.

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Aim 3. Perform plasma donor serosurveillance study to confirm findings and refine dynamics of viral and immunologic parameters in high frequency source plasma donors

- Execute plasma donor based serosurveillance collection and testing sampling 6,000 donation-derived samples from CO, TX and CA
- Capture serial archived donations from positive donors to perform viral RNA and antibody testing to characterize dynamics of acute infection stages and antibody persistence.

Convalescent Blood and Plasma Donors for Generation of Immunotherapies

- Convalescent plasma for direct transfusion (analogous to CCP)
- Convalescent plasma for fractionation and production of hyperimmune IVIG
- PBMC for isolation of B cells to generate hybridomas for pathogen-specific mAbs
- Collaboration with Gigagen/Grifols to produce recombinant polyclonal Ab therapies

GigaGen Awarded U.S. BARDA Contract to Develop Recombinant Polyclonal Antibody Therapies for Botulinum Neurotoxins and an Additional Biothreat

The contract will provide an initial commitment of $19.6 million and up to $135.2 million over a six-year period, supporting drug manufacturing and phase 1 trials for the two programs.

GigaGen's recombinant polyclonals are part of Grifols' robust innovation strategy and commitment to delivering the next generation of antibody drugs for patients and healthcare professionals.

The award, valued at up to $135.2 million, is to develop a recombinant polyclonal antibody therapy for botulinum neurotoxins (BoNT) and a second biothreat of interest to the agency that will be determined at a later time.

Thanks to Dr. Marion Lanteri for assistance with presentation

Thanks to you for your attention