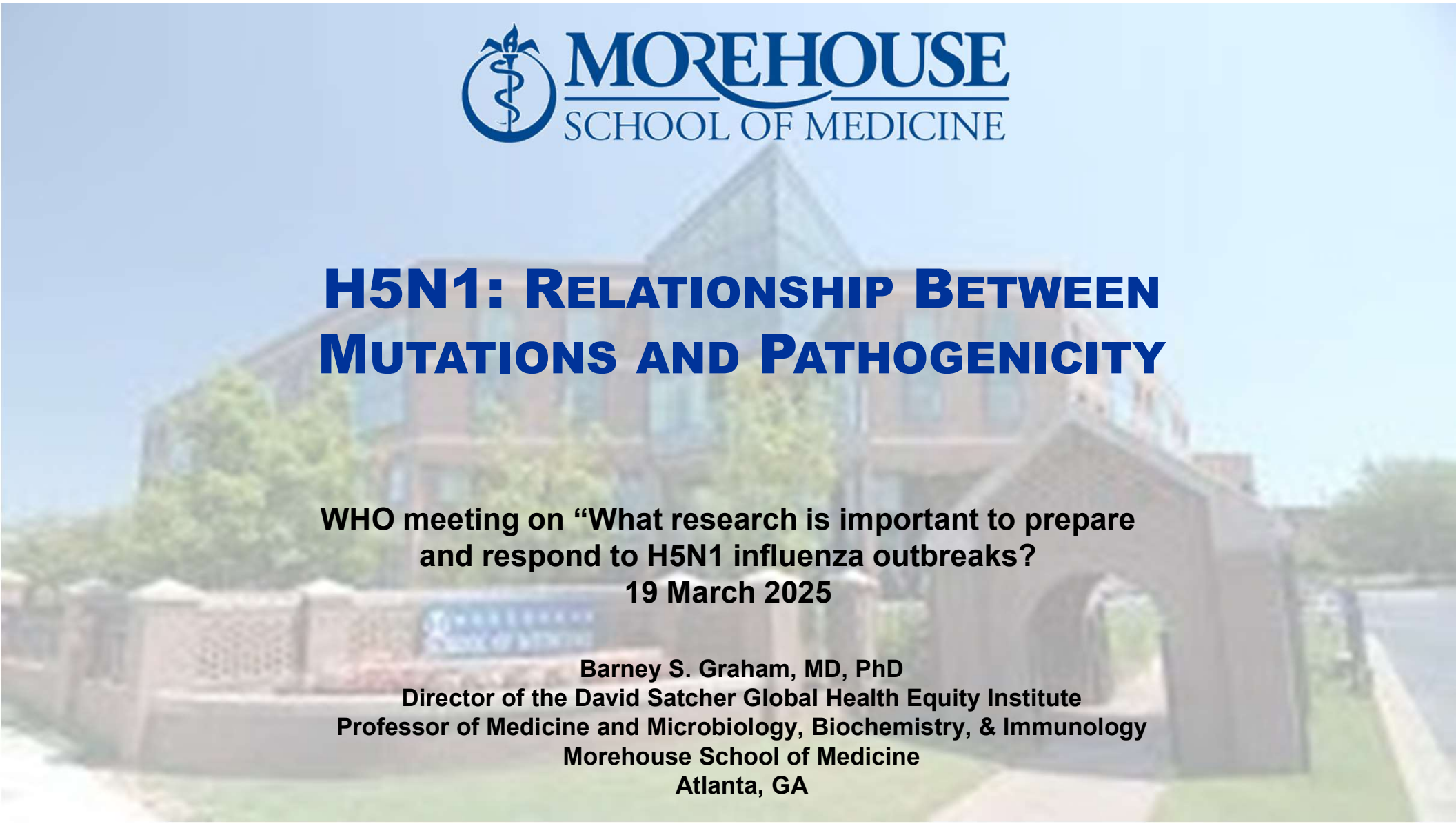
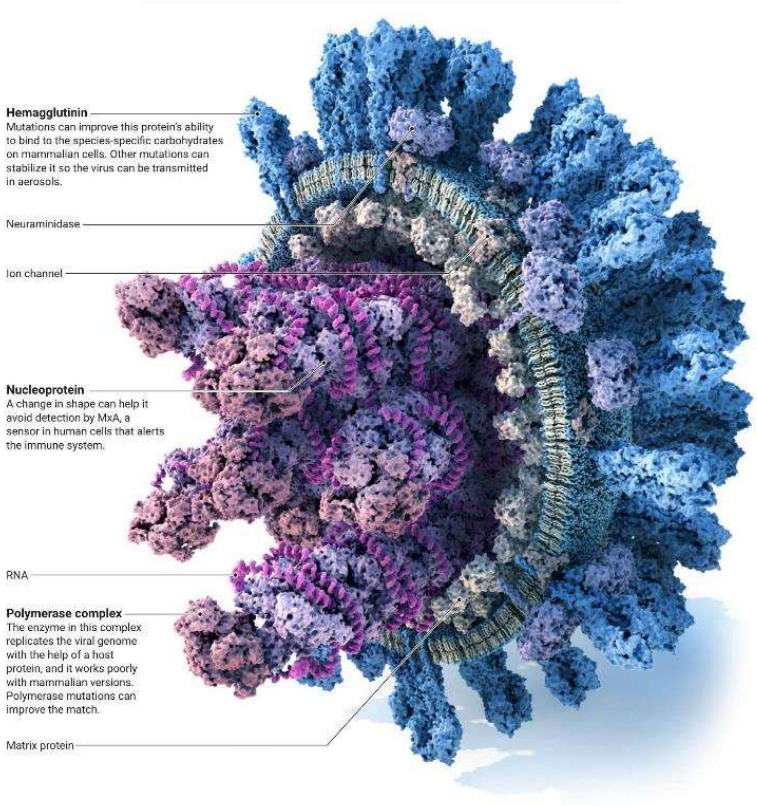


H5N1: Relationship Between Mutations and Pathogenicity

WHO meeting on "What research is important to prepare and respond to H5N1 influenza outbreaks?
19 March 2025

Barney S. Graham, MD, PhD
Director of the David Satcher Global Health Equity Institute
Professor of Medicine and Microbiology, Biochemistry, & Immunology
Morehouse School of Medicine
Atlanta, GA

Influenza Genome and Proteins

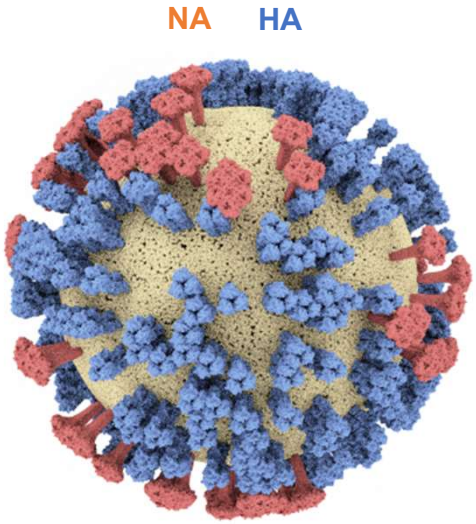


Adapted from Science

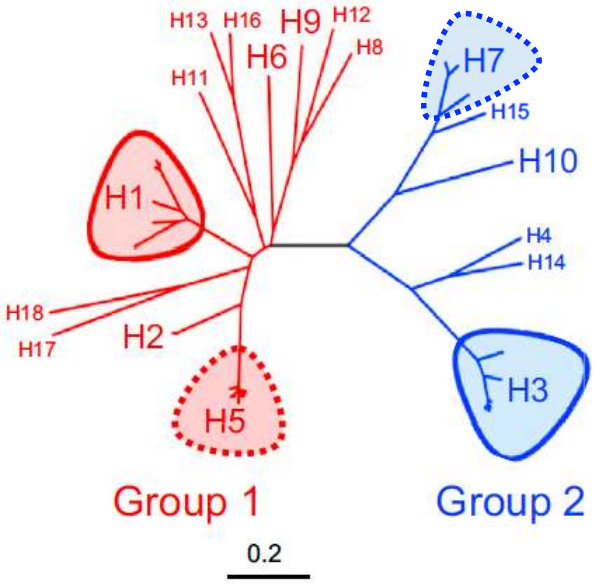
- Orthomyxovirus
- Negative-sense, single-stranded RNA genome
- 8 gene segments encoding 11 proteins
- Sialic acid receptor-dependent tropism

Influenza virus and hemagglutinin (HA)

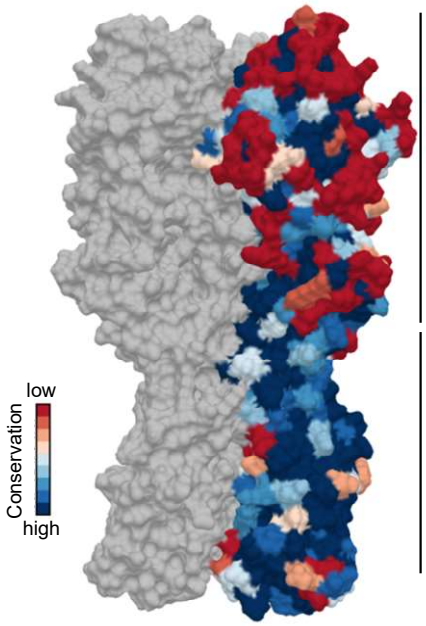
Influenza virus



Influenza A virus HA subtypes



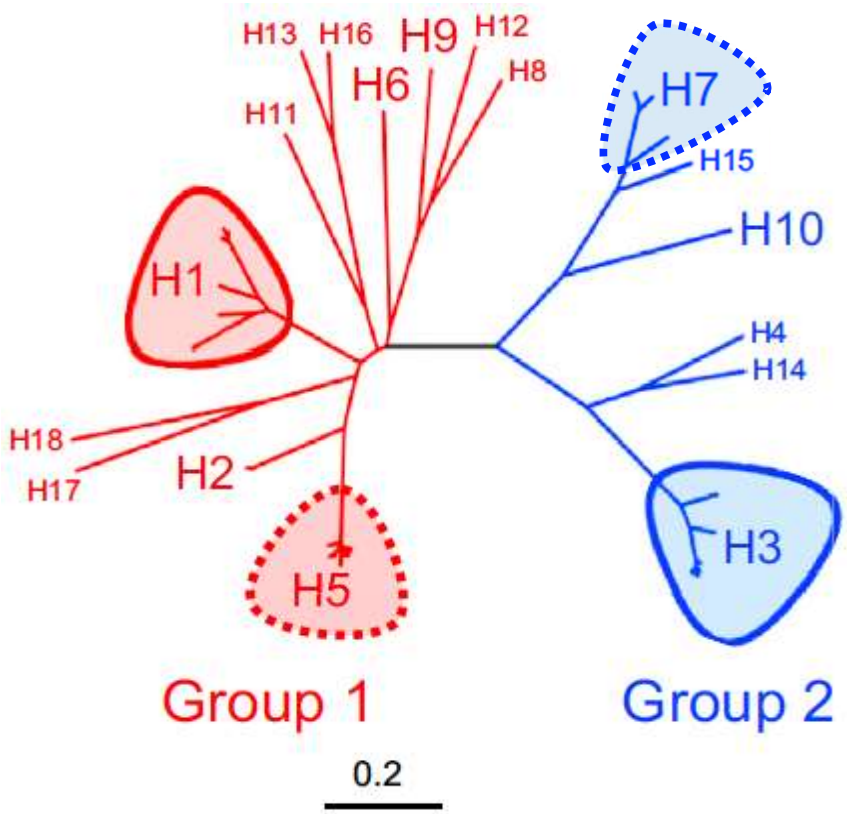
Hemagglutinin (HA)



Head
- Immunodominant
- Hypervariable
- Contains highly neutralizing epitopes

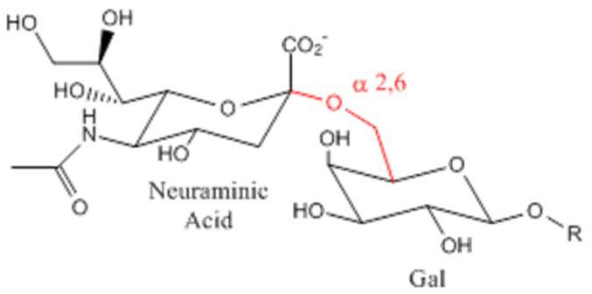
Stem
- Conserved within and across subtypes
- Contains neutralizing epitopes

Influenza A Tropism



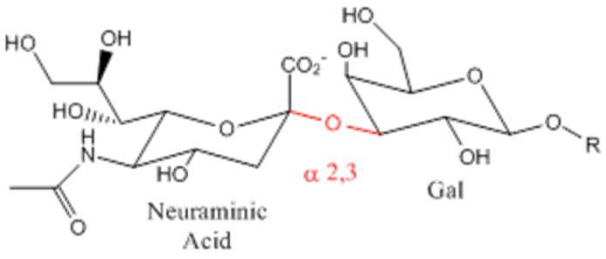
N-acetyl neuraminic acid (Neu5Ac) or sialic acid

Human



alpha 2,6

Avian, some Swine



alpha 2,3

H5N1 Influenza virus in Cattle

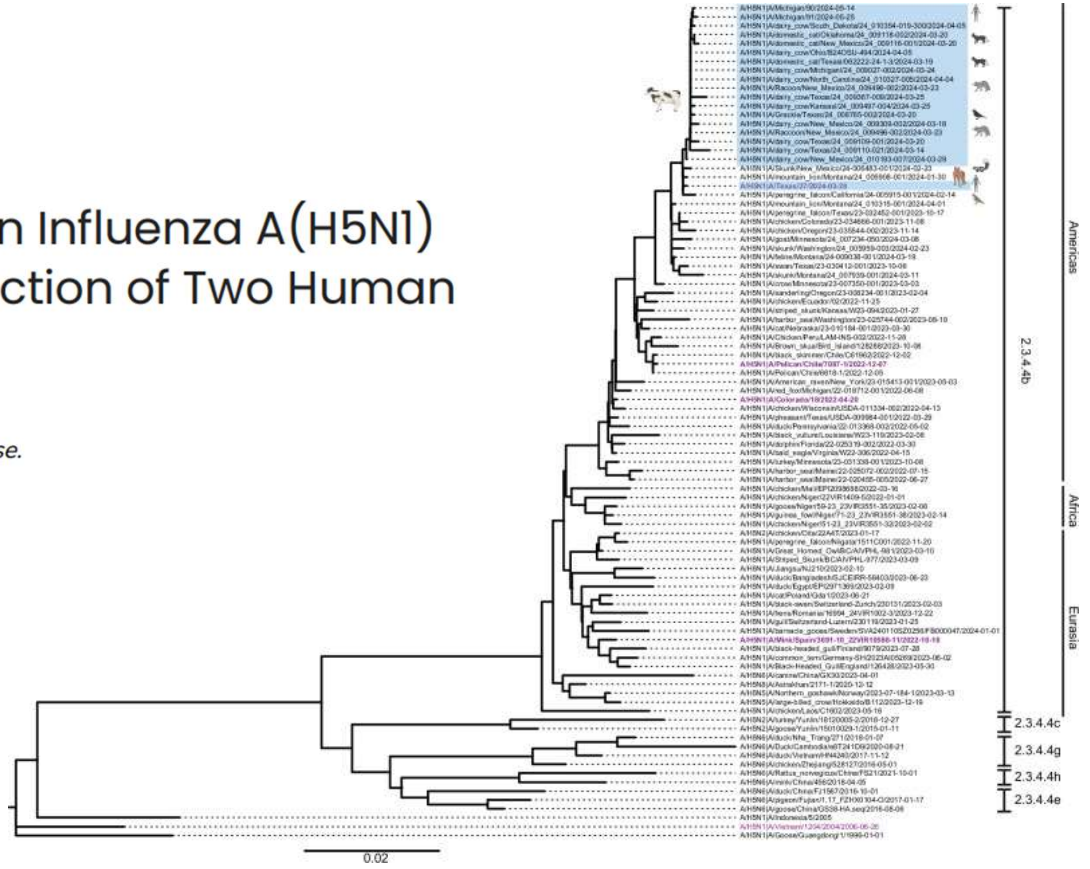
Morbidity and Mortality Weekly Report (*MMWR*)

Outbreak of Highly Pathogenic Avian Influenza A(H5N1) Viruses in U.S. Dairy Cattle and Detection of Two Human Cases — United States, 2024

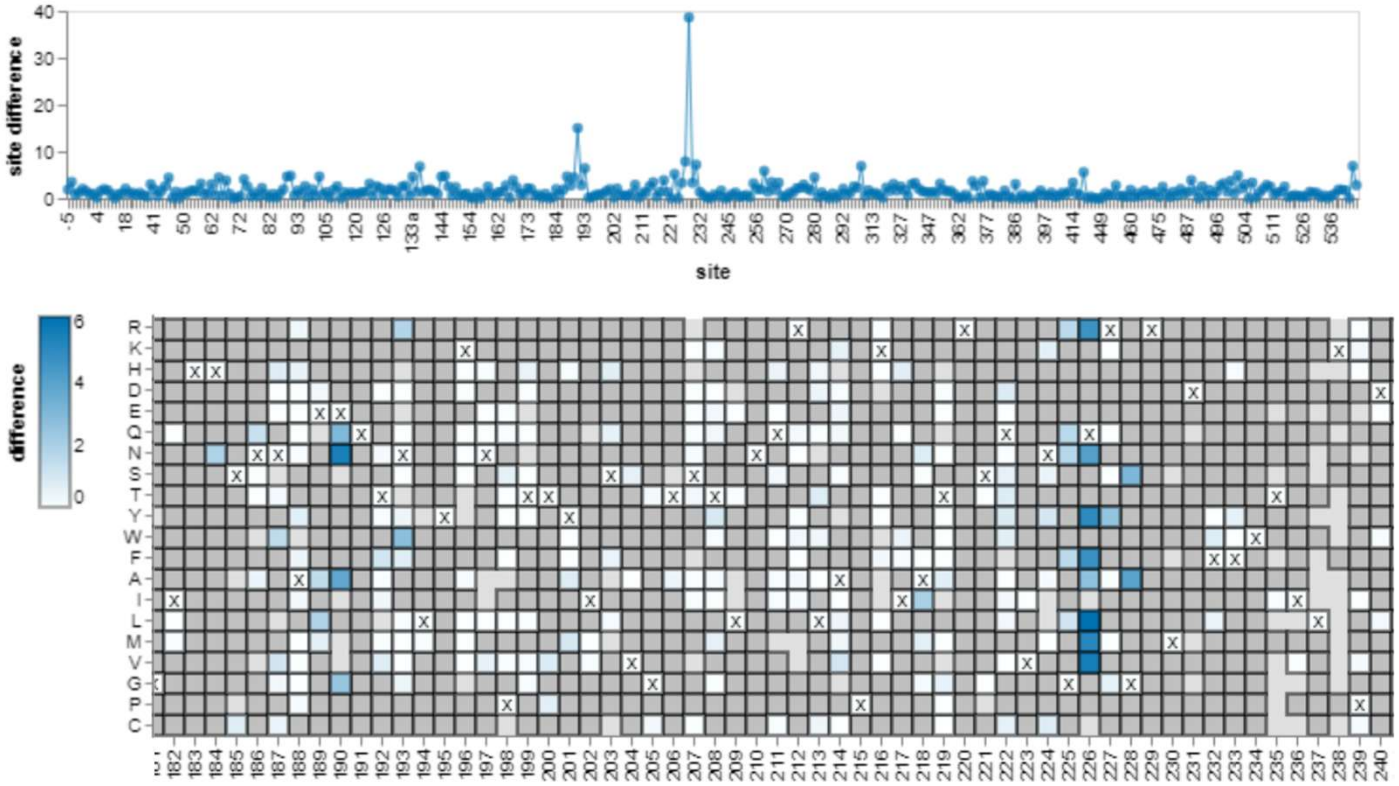
Weekly / May 30, 2024 / 73(21);501–505

On May 24, 2024, this report was posted online as an MMWR *Early Release.*

Clade 2.3.4.4b
Genotype B3.13 (now D1.1)
PB2 protein has E627K mutation



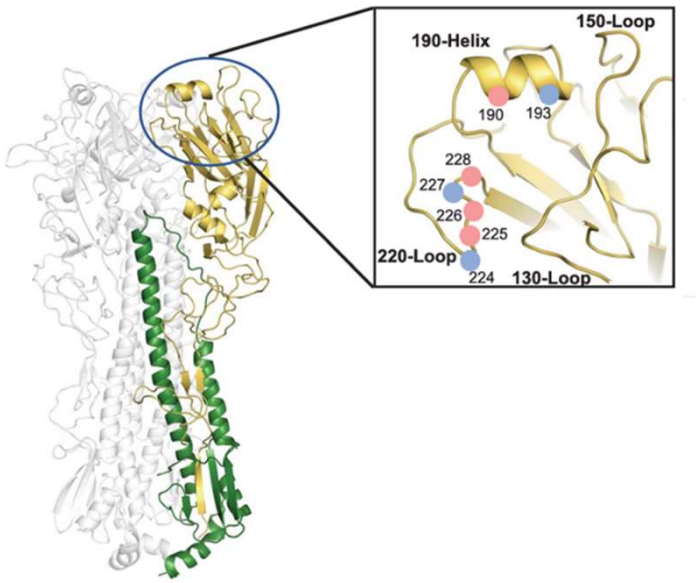
What would it take for H5 to bind α2,6 sialic acid?



Q226L E190 Y98

Dadonaite B. et al. (2024) Deep mutational scanning of H5 HA to inform influenza virus surveillance. BioRxiv, DOI 10.1101/2024.05.23.595634

Single point mutations associated with increased binding to α2,6 sialic acid



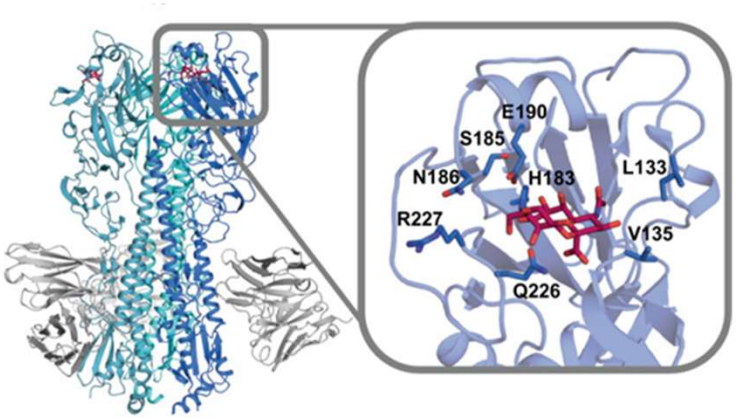
A single glutamine to leucine mutation at residue 226 of the **bovine** H5N1 hemagglutinin was sufficient to enact the change from avian to human specificity.

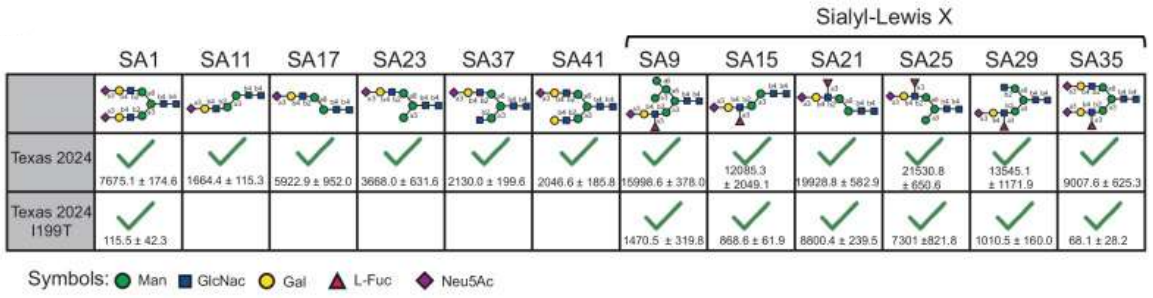
In H5N1 **avian** viruses from live poultry markets N193 residues bound exclusively to alpha-2,3-sialylglycopolymers (avian-type receptors), whereas viruses with K193 acquired alpha2,6-sialylglycopolymers (human-type receptors).binding

Lin TH, Zhu X, Wang S, Zhang D, McBride R, Yu W, Babarinde S, Paulson JC, Wilson IA. A single mutation in bovine influenza H5N1 hemagglutinin switches specificity to human receptors. Science. 2024 Dec 6;386(6726):1128-1134. doi: 10.1126/science.adt0180. Epub 2024 Dec 5. PMID: 39636969.

Wen F, Yang Y, Li Y, et al. Novel human-type receptor-binding H5N1 virus in live poultry markets, China. Lancet Microbe 2024; published online Dec 10. https://doi.org/10.1016/j.lanmic.2024.101049.

T199I increases breadth of sialylated glycan binding





T199I mutation increases binding breadth

Good MR, Fernández-Quintero ML, Ji W, Rodriguez AJ, Han J, Ward AB, Guthmiller JJ. A single mutation in dairy cow-associated H5N1 viruses increases receptor binding breadth. Nat Commun. 2024 Dec 30;15(1):10768. doi: 10.1038/s41467-024-54934-3. PMID: 39737954; PMCID: PMC11685663.

The NEW ENGLAND JOURNAL of MEDICINE

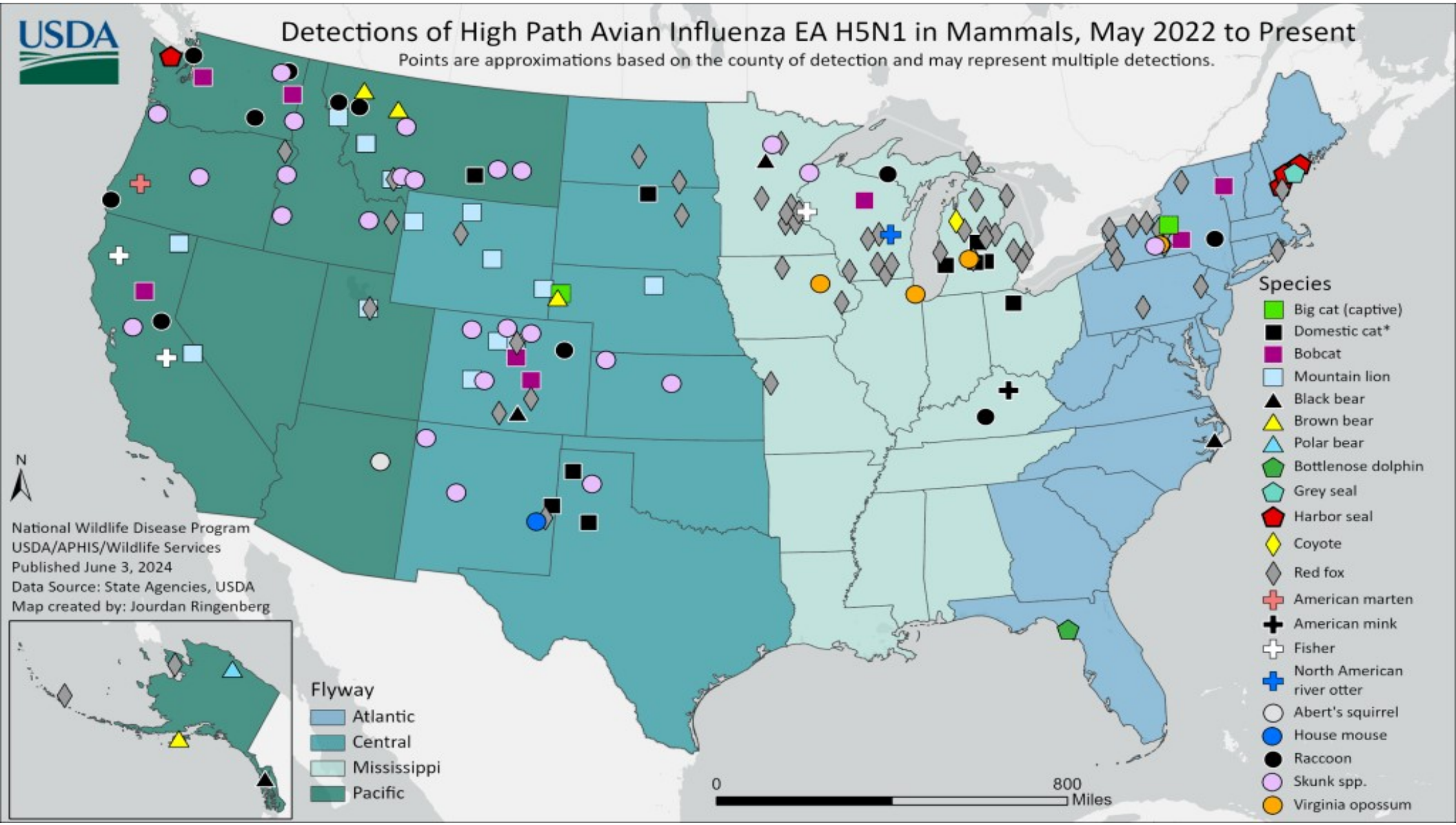
CORRESPONDENCE

Critical Illness in an Adolescent with Influenza A(H5N1) Virus Infection

Published December 31, 2024 | N Engl J Med 2025;392:927-929 | DOI: 10.1056/NEJMc2415890 | VOL. 392 NO. 9

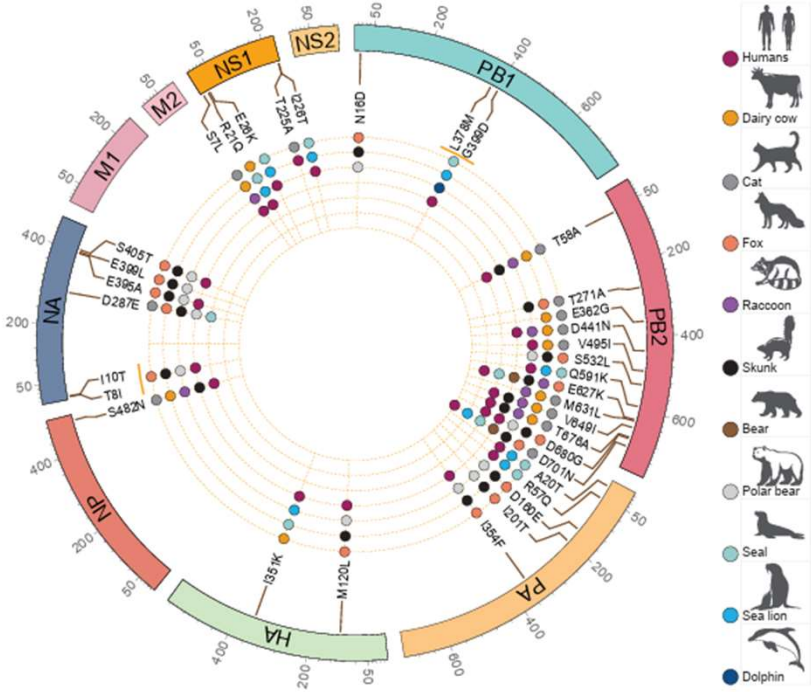
Jassem AN, Roberts A, Tyson J, Zlosnik JEA, Russell SL, Caleta JM, Eckbo EJ, Gao R, Chestley T, Grant J, Uyeki TM, Prystajecky NA, Himsworth CG, MacBain E, Ranadheera C, Li L, Hoang LMN, Bastien N, Goldfarb DM.

E190D - 28% allele frequency
Q226H - 35% allele frequency


USDA
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
Skunk spp.
Virginia opossum
Flyway
Atlantic
Central
Mississippi
Pacific
N
0
800
Miles
National Wildlife Disease Program
USDA/APHIS/Wildlife Services
Published June 3, 2024
Data Source: State Agencies, USDA
Map created by: Jourdan Ringenberg

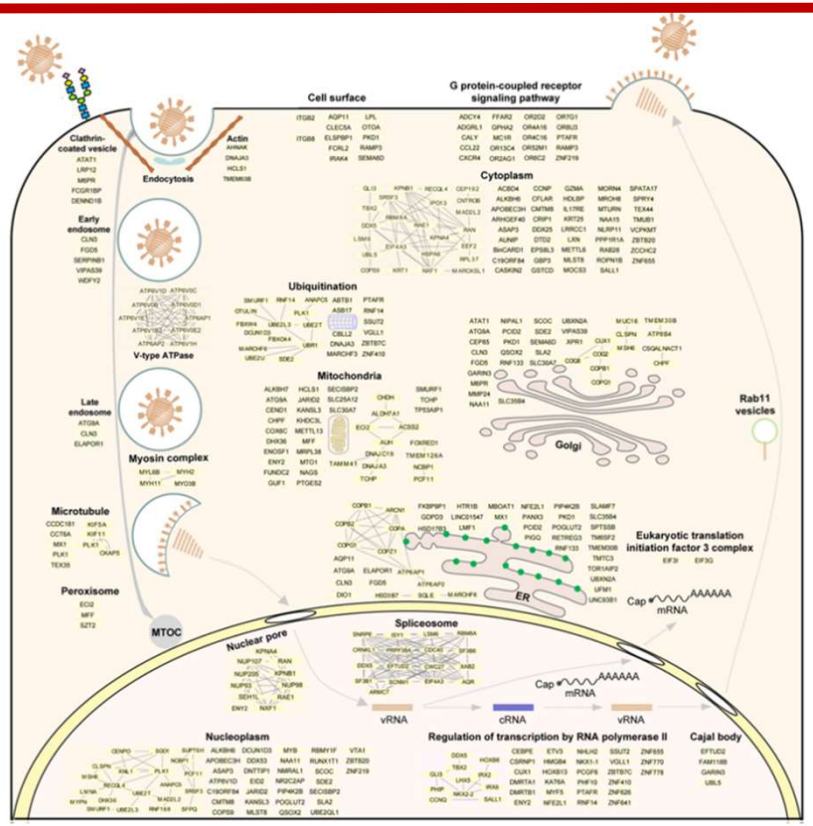
H5N1 in Mammals

Pardo-Roa C, Nelson MI, Ariyama N, Aguayo C, Almonacid LI, Gonzalez-Reiche AS, Muñoz G, Ulloa M, Ávila C, Navarro C, Reyes R, Castillo-Torres PN, Mathieu C, Vergara R, González Á, González CG, Araya H, Castillo A, Torres JC, Covarrubias P, Bustos P, van Bakel H, Fernández J, Fasce RA, Johow M, Neira V, Medina RA. Cross-species and mammal-to-mammal transmission of clade 2.3.4.4b highly pathogenic avian influenza A/H5N1 with PB2 adaptations. Nat Commun. 2025 Mar 6;16(1):2232. doi: 10.1038/s41467-025-57338-z. PMID: 40044729; PMCID: PMC11882949.



Misra S, Gilbride E, Ramasamy S, Pond SLK, Kuchipudi SV. Enhanced Diversifying Selection on Polymerase Genes in H5N1 Clade 2.3.4.4b: A Key Driver of Altered Species Tropism and Host Range Expansion. bioRxiv [Preprint]. 2024 Aug 19:2024.08.19.606826. doi: 10.1101/2024.08.19.606826. PMID: 39229076; PMCID: PMC11370473.

Pathways associated with H5N1 replication in mammalian cells



May need to accumulate multiple mutations in:

1) HA for tropism and entry
2) PB2 polymerase or other proteins involved in replication
3) Proteins that inhibit Type 1 IFN induction and effector functions
4) Proteins that inhibit other cellular restriction factors involved in virus entry, assembly, or release

… and may need multiple reassortment events to make human-to-human spread of respiratory disease feasible.

What type of surveillance can be done to target the animals most likely to be mixing vessels for reassorted viruses?

Wang G, Jiang L, Wang J, Li Q, Zhang J, Kong F, Yan Y, Wang Y, Deng G, Shi J, Tian G, Zeng X, Liu L, Bu Z, Chen H, Li C. Genome-wide siRNA library screening identifies human host factors that influence the replication of the highly pathogenic H5N1 influenza virus. mLife. 2025 Feb 24;4(1):55-69. doi: 10.1002/mlf2.12168. PMID: 40026577; PMCID: PMC11868839.