H5N1 Influenza A : What's happening in the cows?

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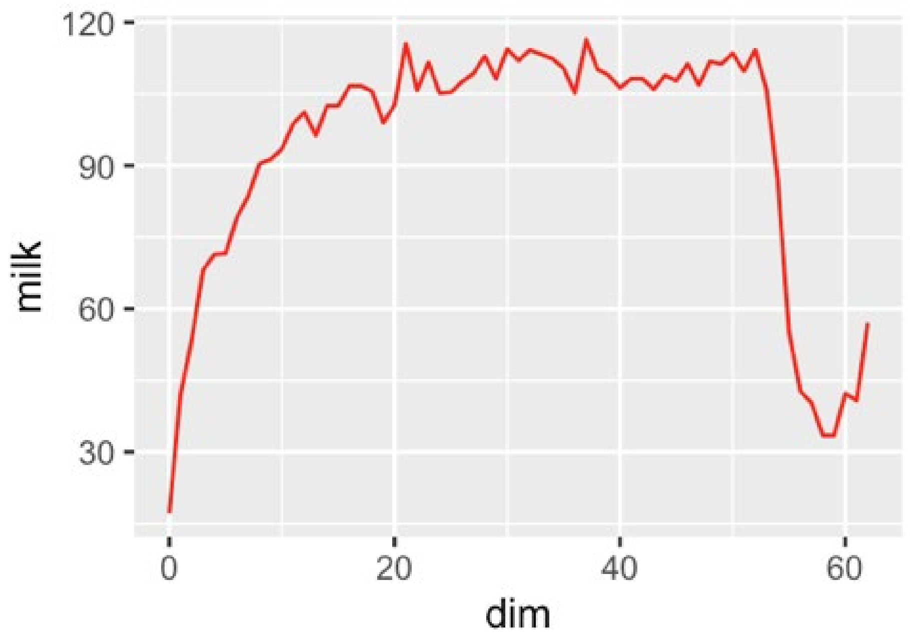
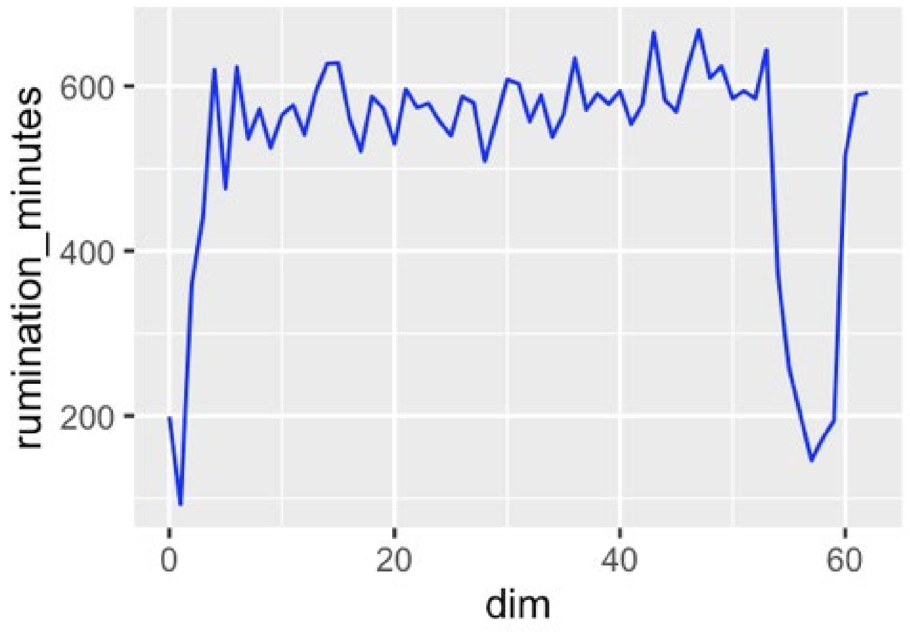
October 22, 2024

Clinical case definition

- Findings common to affected sites
  - Severe abrupt drop in feed intake, milk production
    - Older, mid-late lactation cows overrepresented
  - Marked decrease in rumination
  - Thick yellow milk (similar to colostrum) in a portion of cows

- Rapid increase in clinically ill cattle
  - ~15% of herd over 10 day period





Graphs courtesy of Dr. Pat Gorden, Iowa State

Research gaps: Infection

- Factors affecting disease severity in individual cows
  - Route of infection?
  - Stage of lactation/gestation?
- Intra-herd spread: Mechanical? Aerosol? Direct contact?
  - What can we do to impact transmission within an affected herd?
- Factors contributing to disease differences from site to site
  - Early outbreaks → almost no mortality
  - Recent outbreaks → significant mortality reported
- Long term impacts of infection: lifetime production, reproductive impacts

Influenza A PCR → Clinically affected cows

Specimen	Ct / Result
Milk	15.6 / Positive
Milk	16.0 / Positive
Milk	13.4 / Positive
Milk	17.8 / Positive
Milk	16.1 / Positive
Milk	12.3 / Positive
Milk	13.1 / Positive
Milk	14.2 / Positive

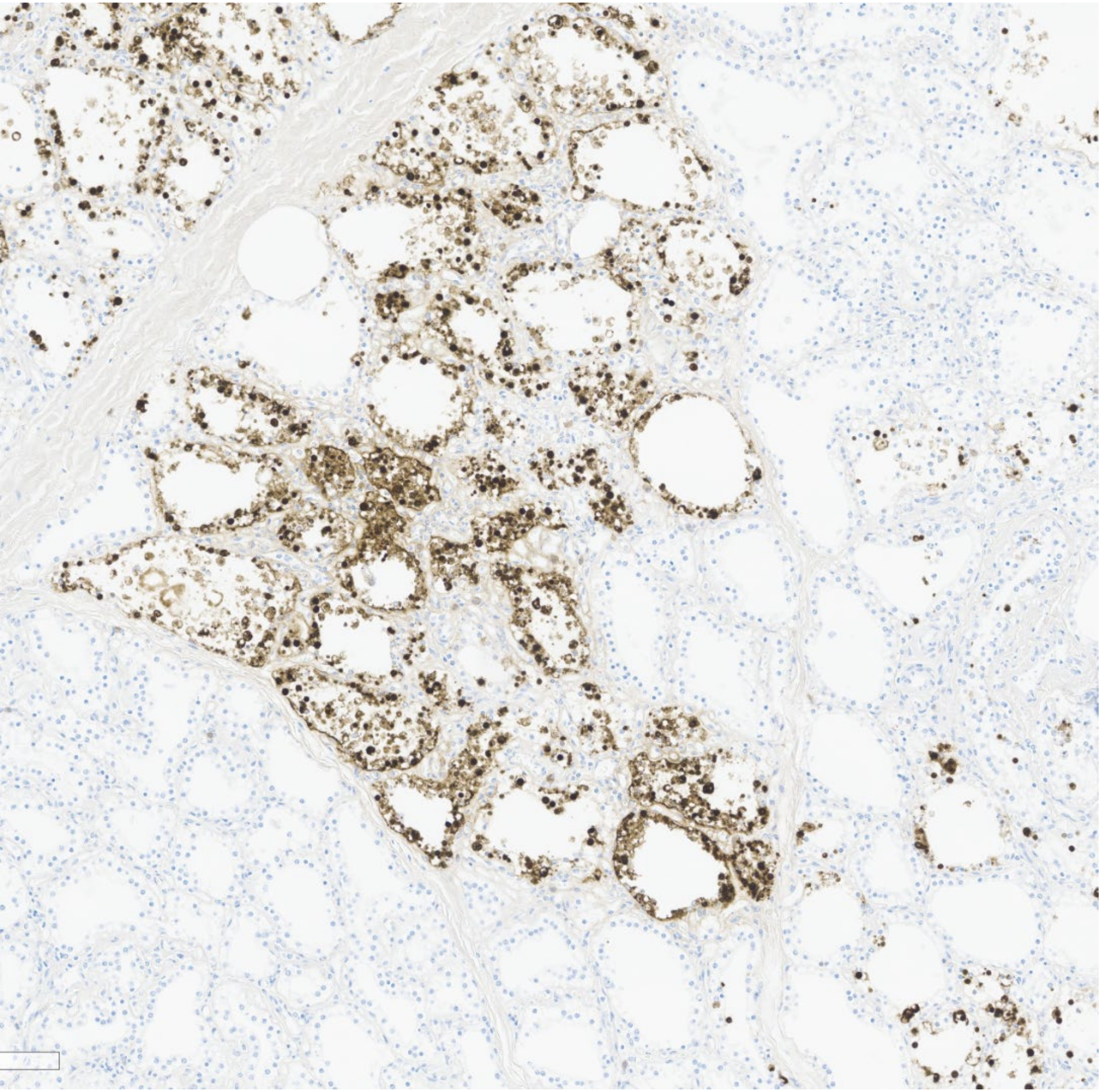
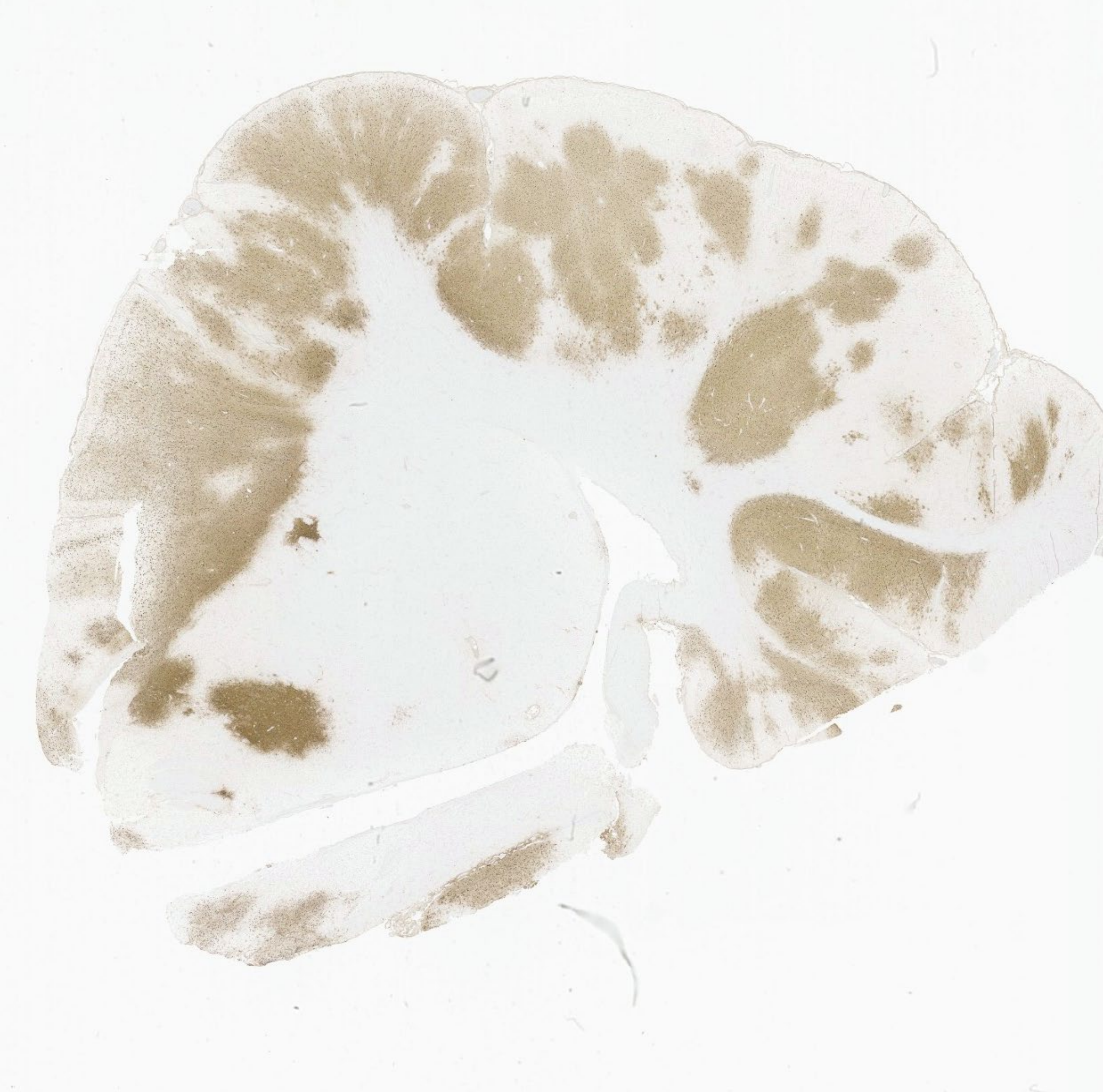
Specimen	Ct / Result
Milk	15.2 / Positive

Specimen	Ct / Result
Milk	16.9 / Positive

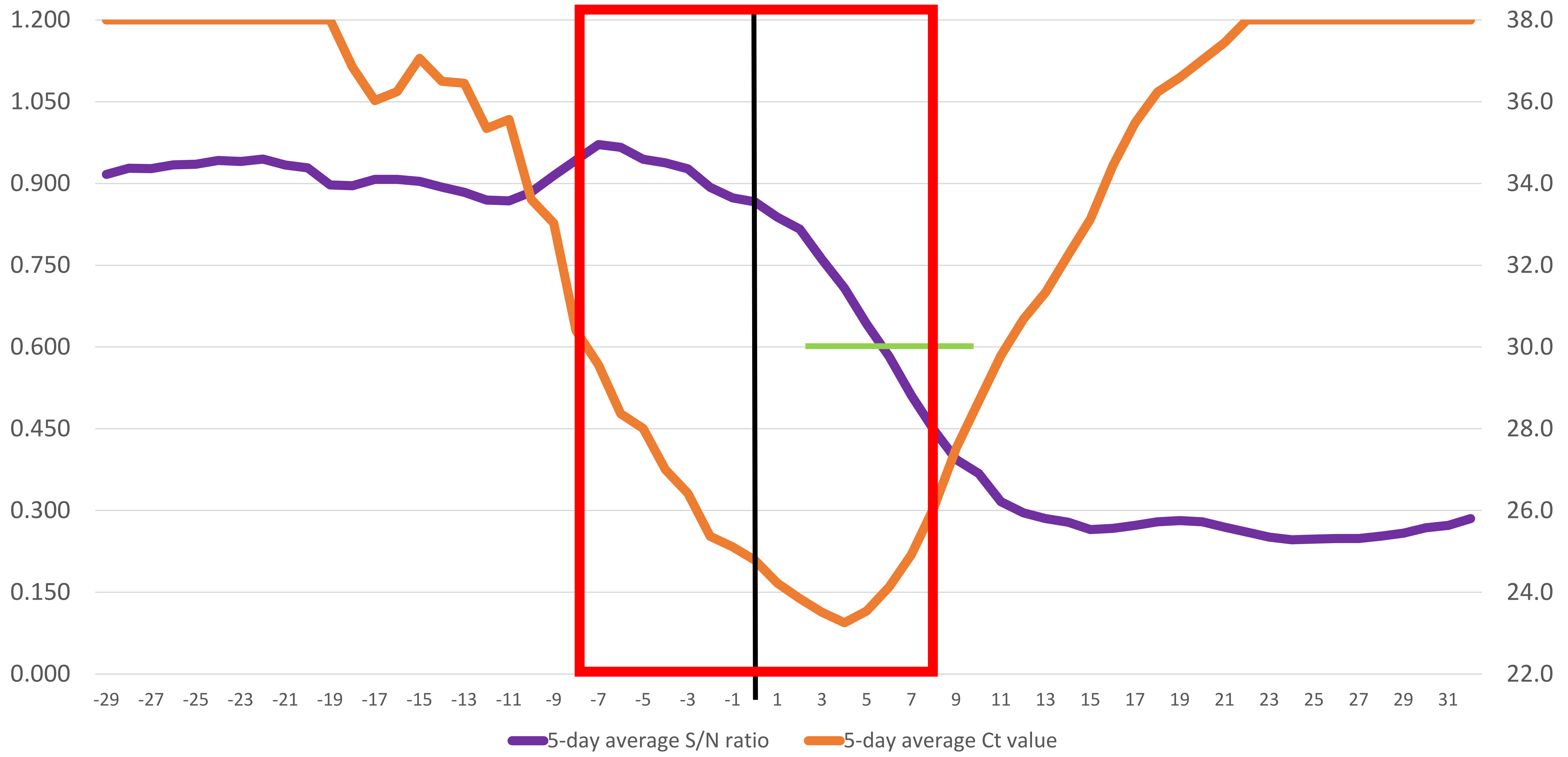
Specimen	Ct / Result
Milk	15.5 / Positive
Milk	21.7 / Positive
Milk	18.4 / Positive
Milk	25.8 / Positive
Milk	17.2 / Positive

Specimen	Ct / Result
Mammary	16.2 / Positive

Specimen	Ct / Result
Milk	15.5 / Non Negative
Milk	15.6 / Non Negative
Milk	14.9 / Non Negative
Milk	15.2 / Non Negative
Milk	13.9 / Non Negative
Milk	16.6 / Non Negative
Milk	>=40 / Inconclusive
Milk	13.1 / Non Negative
Milk	14.6 / Non Negative
Milk	>=40 / Inconclusive
Milk	17.3 / Non Negative
Milk	16.5 / Non Negative
Milk	14.7 / Non Negative
Milk	>=40 / Inconclusive
Milk	18.7 / Non Negative
Milk	20.9 / Non Negative
Milk	17.8 / Non Negative

Bulk tank milk during an outbreak



Research gaps: Transmission

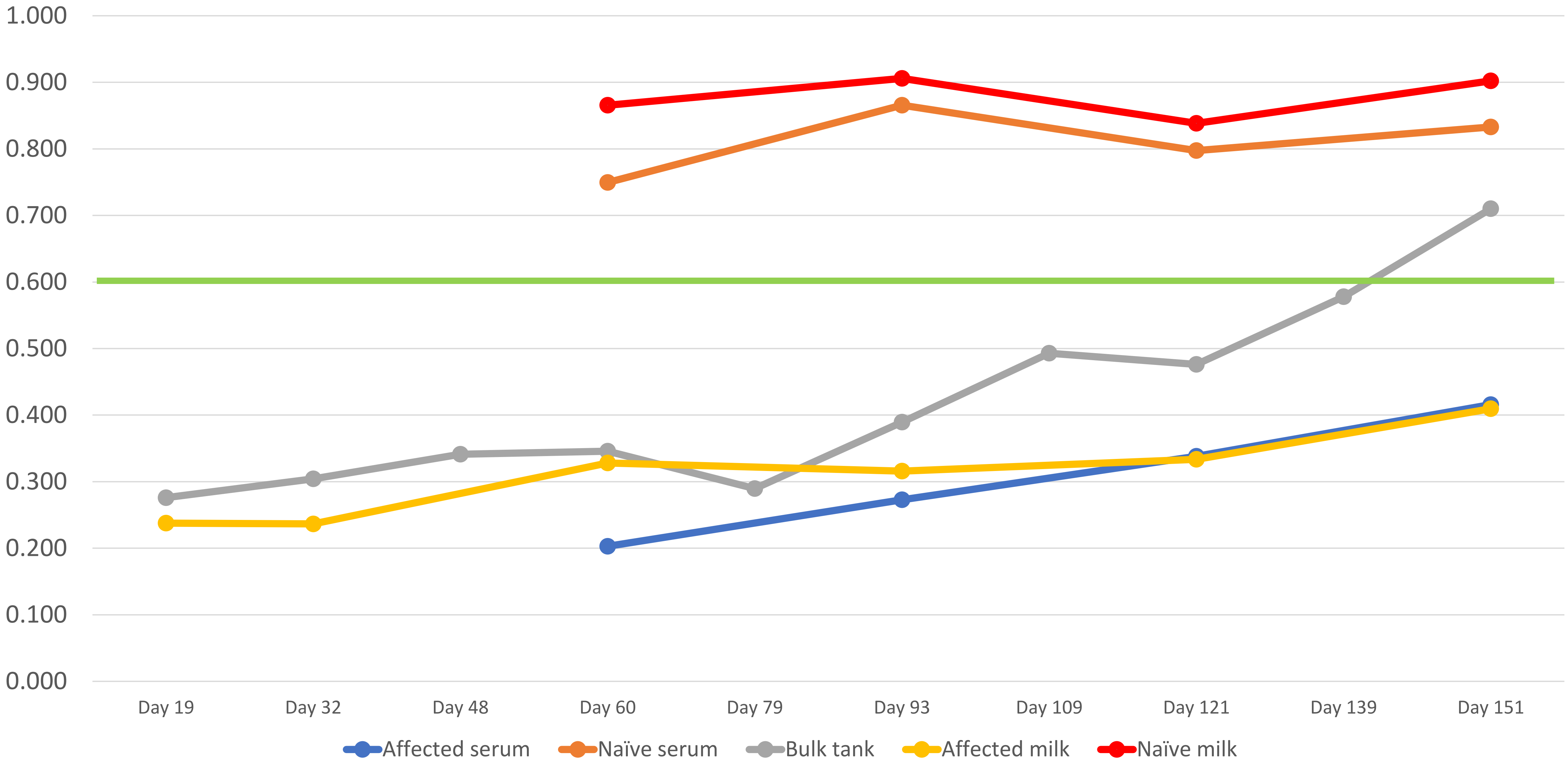
- Where is the virus now, where has the virus already been, and where isn’t the virus?

- How is Influenza spreading from affected farms?

- How is it getting onto other farms <u>and</u> into cattle?

- How much does transmission risk vary during an outbreak?

IAV NP ELISA → antibody over time



Research gaps: Immunity

- Test development/capacity required to assess protective immunity
- What degree of protective immunity is achieved through natural infection?
  - Are there differences in immunity generated by different routes of infection?
- How long does any protective immunity last?

Everyone needs to keep in mind…..

- Opportunities → Bulk tank surveillance testing
  - Most useful sample for virus detection is collected from <u>every lactating cow</u> on <u>every dairy farm</u> in the country <u>2 or 3 times every day</u>
  - Sample from every load already collected by certified individuals

- Challenges → Logistics of researching this agent in lactating cattle
  - BSL-3 facilities → pathogenesis/cow-to-cow transmission
  - Field research on affected farms/in affected regions

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Dairy calves and lactating cows inoculated with HPAI H5N1 clade 2.3.4.4b

Baker, A.L., Arruda, B., Palmer, M.V. *et al. Nature* (2024).

https://doi.org/10.1038/s41586-024-08166-6

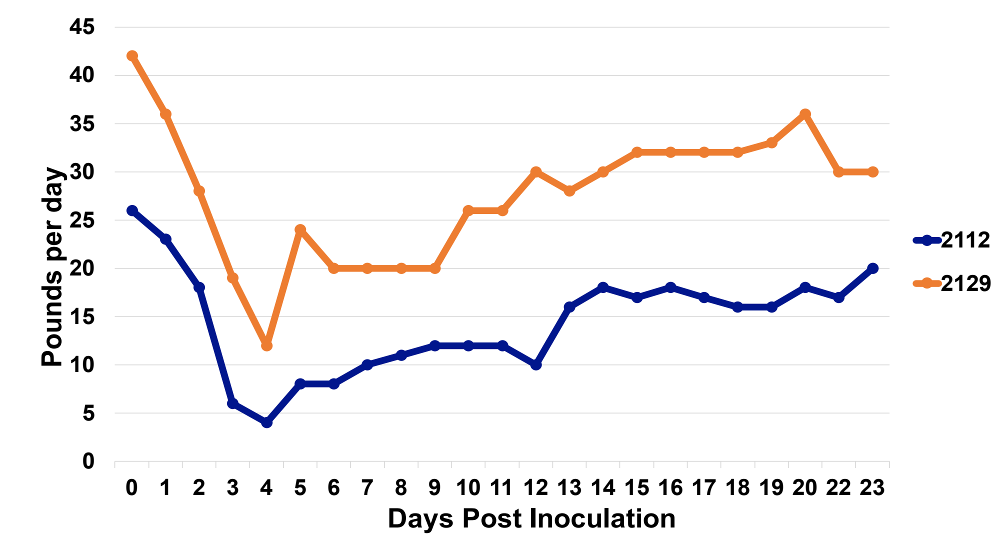
Virus and Prion Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture; Ames, Iowa, 50010, USA.

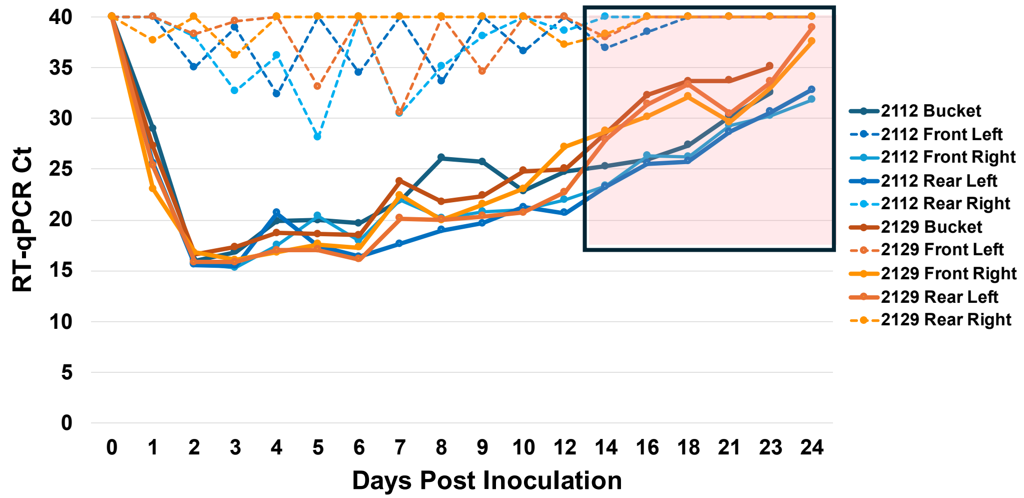
National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, United States Department of Agriculture; Ames, Iowa, 50010, USA.

Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, Iowa, 50010, USA.

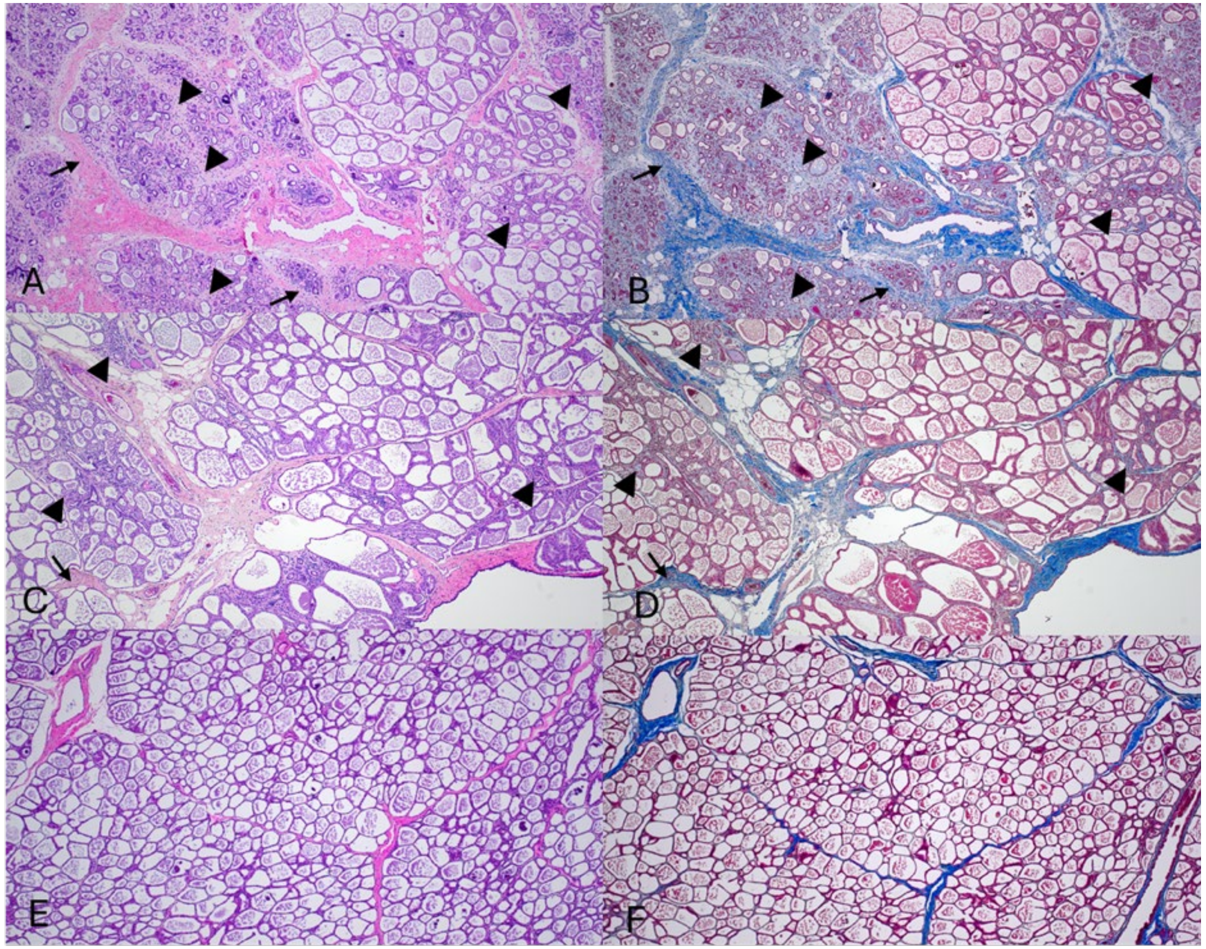
Milk Production Loss and Viral RNA Detection

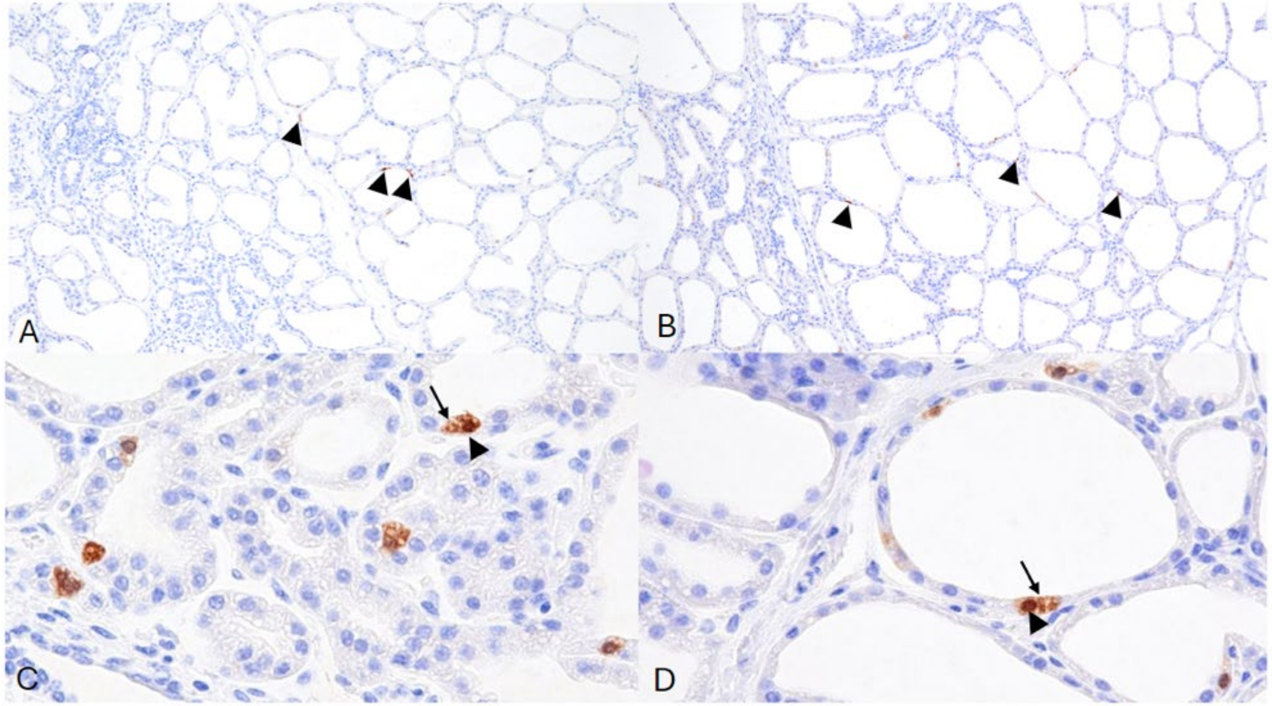
- Milk production loss and viral RNA detection were observed in a similar time course.
- Correlation between RT-PCR Ct values and milk color & consistency changes, loss in production, & rumen motility
- Although viral RNA detected until 24 days post infection, virus isolation was negative after **12 DPI**.
  - These timepoints are of interest to understand the immune response in the mammary glands of inoculated and un-inoculated quarters.





Mammary gland lesions & presence of viral antigen at 24 DPI





Summary

- These first studies demonstrated:
  - Respiratory infection is possible but mild in immature heifers.
  - Mammary inoculation resulted in rapid clinical signs, loss in milk production, and high levels of virus in milk from inoculated quarters. Mammary tissue damage may impact subsequent lactation.
  - No evidence of systemic infection or trafficking between mammary glands or organ systems.
  - Our results are consistent with Halwe et al. (2024) Nature.
- These results and reports from field investigations support the hypothesis that the lactating cow and mammary inoculation may be a primary mode of infection and transmission.
- Although mild and transient, respiratory infection is possible and may play a role in the epidemiology (nose to nose or **milk** to nose).
- Both routes induced systemic immune response. Understanding local and systemic immunity and duration of immunity is critical.
- Further experiments addressing knowledge gaps critical to disease control are dependent upon establishing a robust and replicable challenge models.

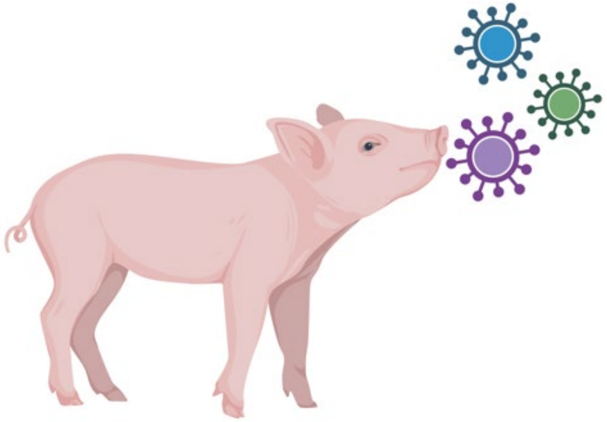
Ongoing studies and gaps

- ☑ Investigate kinetics of mammary lesions and replicate clinical observations of first study. Investigate 3 doses of inoculum in 3 quarters per cow.
- ☑ Investigate transmission.
  - Transmission between cows through milking equipment.
  - Respiratory + oral contact with mammary inoculated cows and infected milk.
  - Oronasal exposure to calves fed infected milk.
  - Phylogenetic links between farms and evolution of virus.
- ☑ Understanding immunity.
  - Evaluate the immune responses in convalescent cattle (3 weeks post exposure, necropsy DPI 7).
  - Mammary inoculation of convalescent cattle.
  - Oronasal exposure of calves with milk containing antibodies and virus from post-inoculated cows.
- ☑ Vaccine studies.
  - Test mRNA H5 vaccine in calves.
  - Test additional H5 vaccines.

Weaned pigs inoculated with HPAI H5N1 clade 2.3.4.4b

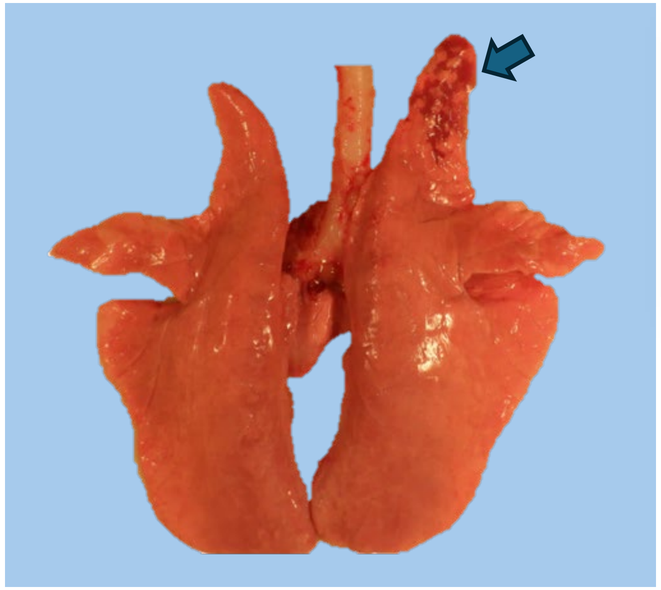
Bailey Arruda, Amy L Baker, Alexandra Buckley, Tavis K Anderson, Mia Torchetti, Nichole Hines Bergeson, Mary Lea Killian, Kristina Lantz

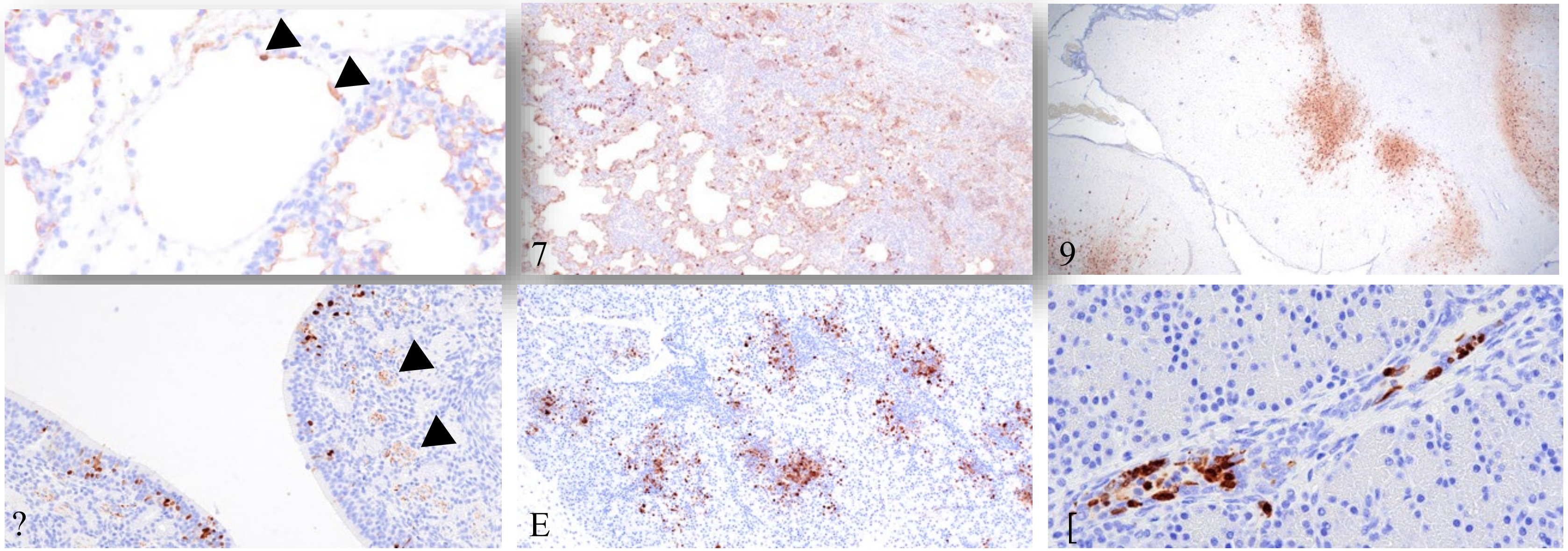
Emerg Infect Dis. 2024 Apr;30(4):738-751. doi: 10.3201/eid3004.231141



Summary: Similarities and Some Important Differences

©╦Ï řτ f?	Genotype	Adaptation marker	Replication LRT	Nasal detection	Fecal detection	Transmission	Clinical signs	Neurologic disease	Lung lesions	PCR detection outside the respiratory tract
Turkey/MN	B2.1	No	Yes	No	NA	No	No	No	Yes	NA
Bald Eagle/FL	B1.1	No	Yes	No	NA	No	No	No	Yes	NA
Raccoon/WA	B2.1	E627K	Yes	Yes	NA	Yes	No	No	Yes	NA
Red fox/MI	B3.2	E627K	Yes	Yes	NA	Yes	No	No	Yes	NA
TurkeyVult/WY	B3.2	D701N	Yes	Yes	Yes	Yes	Mild	Yes	Yes	Yes (brain/pancreas)
TurkeyVult/UT	B3.2	E627K	Yes	Yes	Yes	No	Mild	No	Yes	Yes (brain/pancreas)
TurkeyVult/CA	B3.2	T271A	Yes	Yes	Yes	Yes	Mild	No	Yes	Yes (pancreas)





Ongoing studies and gaps

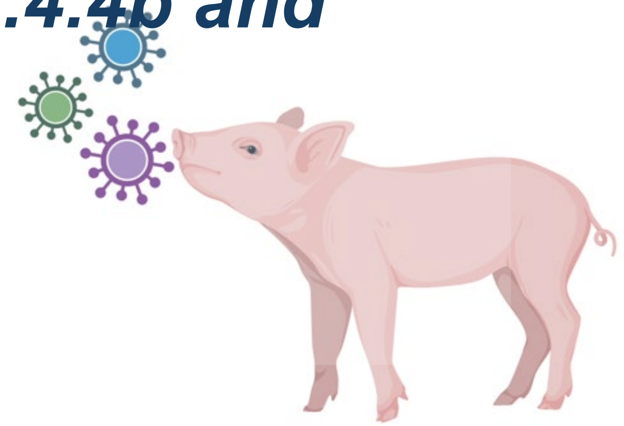
☑ • **Inoculate pigs with additional genotypes of H5N1, including B3.13, with and without mammalian adaptation markers.**

☑ • **Vaccine studies with mRNA H5 vaccine in pigs.**

☑ • **Serologic surveillance of feral swine for antibodies against H5N1 and endemic swine strains.**

• **Evaluate if sow mammary tissue supports H5N1 infection (*ex vivo, in vivo*).**

• ***Reassortment potential between H5N1 clade 2.3.4.4b and endemic swine strains.***



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Acknowledgments

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- Iowa State University VDPAM advisors
- NADC and NVSL technical staff

- Additional support:

CEIRR CDC

Will H5 influenza cause a human pandemic?

Jesse Bloom
Fred Hutch Cancer Center / HHMI

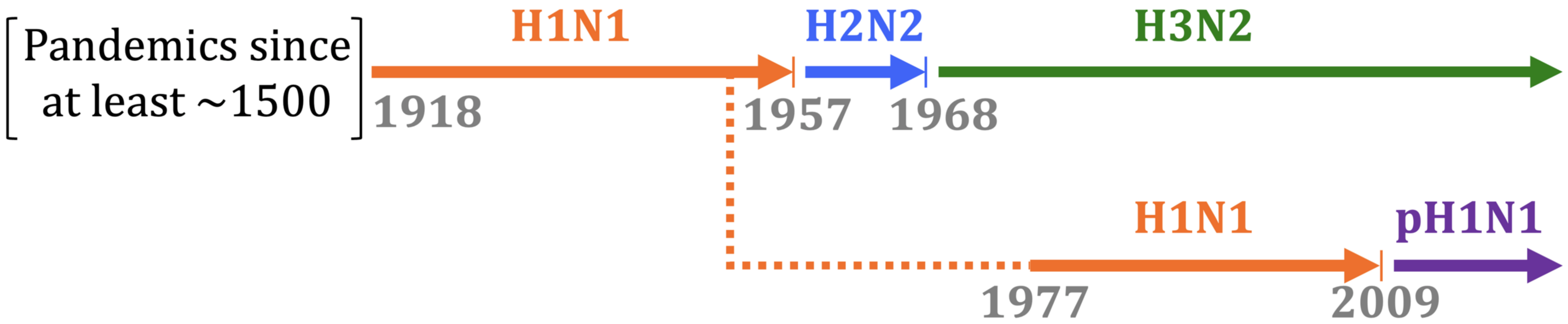
These slides: https://slides.com/jbloom/nasem-h5

"It's tough to make predictions, especially about the future."

- Yogi Berra

Historical precedent

Animal influenza strains have caused human pandemics in past



Influenza pandemics have occurred for at least 500 years (Morens et al, 2010). Most recently:

1918: animal virus (*maybe* from birds?) jumped to humans (dos Reis, 2009)

1957: avian virus reassorted HA / NA / PB1 with human strain (Palese, 2004)

1968: avian virus reassorted HA / PB1 with human strain (Palese, 2004)

1977: inadvertant human release of strain from ~1950s (Burke & Schleunes, 2024)

2009: swine virus jumped to humans (Smith et al, 2009)

But there are also many animal strains that never adapted to humans

In 1872, influenza caused major outbreaks in poultry and horses, but likely never spread in humans beyond sporadic cases (Morens & Taubenberger, 2010)

There are multiple influenza strains in pigs that so far have only caused sporadic human cases (Anderson et al, 2021)

Influenza has caused substantial outbreaks in dogs without infecting humans (Parrish, 2015)

Lessons from historical precedent

There would be precedent for H5N1 adapting to cause a human pandemic...

But there would also be precedent for it never adapting to transmit in humans...

What molecular changes might adapt H5N1 to transmit in humans?

Molecular properties thought to promote human transmissibility

Viral polymerase functions well in mammalian cells (Long et al, 2019)

HA binds human receptors (Matrosovich, 2000; Ayora-Talavera, 2009)

Higher HA stability (Imai, 2012; Herfst, 2012)

Nucleoprotein resistant to MxA and BTN3A3 (Manz et al, 2013, Pinto 2023)

Appropriately balanced HA-NA activity (Yen, 2011)

Probably other adaptations that are not well understood

Are there currently signs of H5N1 gaining adaptations?

Yes — Viral polymerase functions well in mammalian cells (Halwe et al, 2024)

No (at least so far) — HA binds human receptors (Santos et al, 2024; Chopra et al, 2024)

Higher HA stability (Peacock et al, 2024)

Unknown (at least in public literature to date) — Nucleoprotein resistant to MxA and BTN3A3

Appropriately balanced HA-NA activity

Probably other adaptations that are not well understood

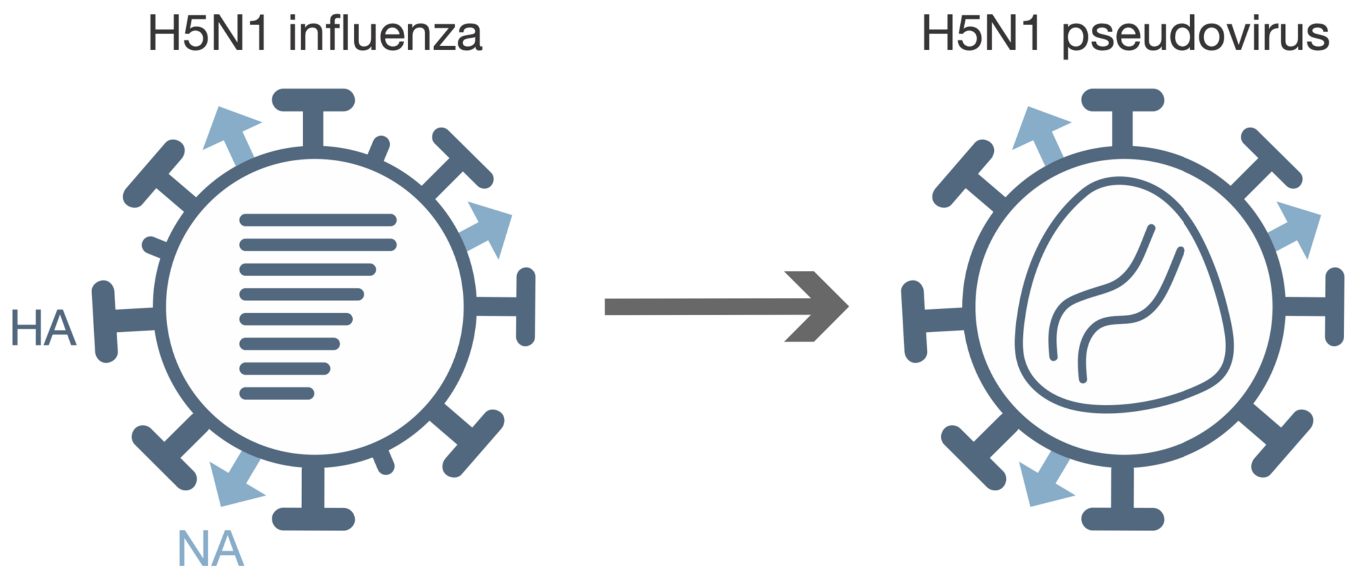
How to identify if strains have acquired adaptations

Test transmission in ferrets or growth in human airway cultures. Experimental gold standard---but slow, low throughput, and requires high biosafety. (Restori et al, 2024)

Test HA for binding to different glycans. Very informative, but only about receptor specificity. (Chopra et al, 2024)

Deep mutational scanning to test how all mutations affect key properties. High throughput, but may not capture epistasis or all relevant properties. (Dadonaite et al, 2024)

My lab specifically has used deep mutational scanning to measure effects of all mutations to H5 HA

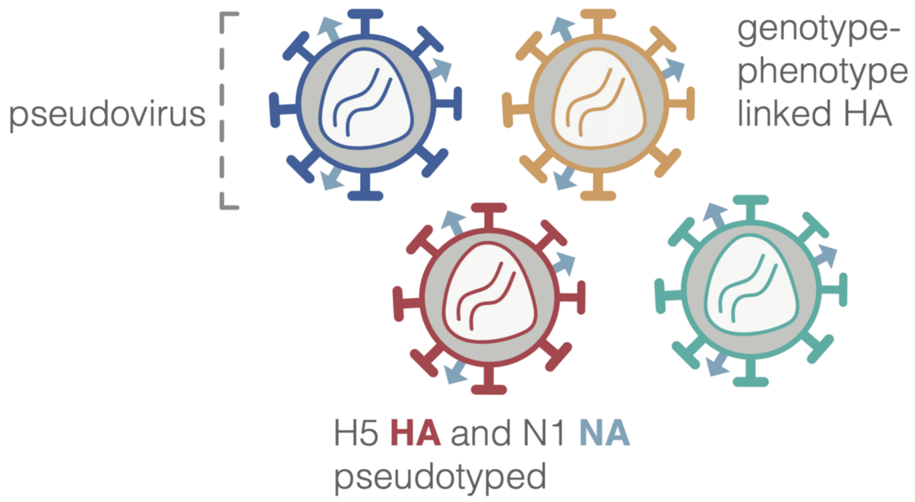


- BSL 3 virus

- Lentivirus that displays HA and NA
- BSL 2 virus

Dadonaite et al, 2024

Deep mutational scanning allows study of all HA mutations in a pooled library



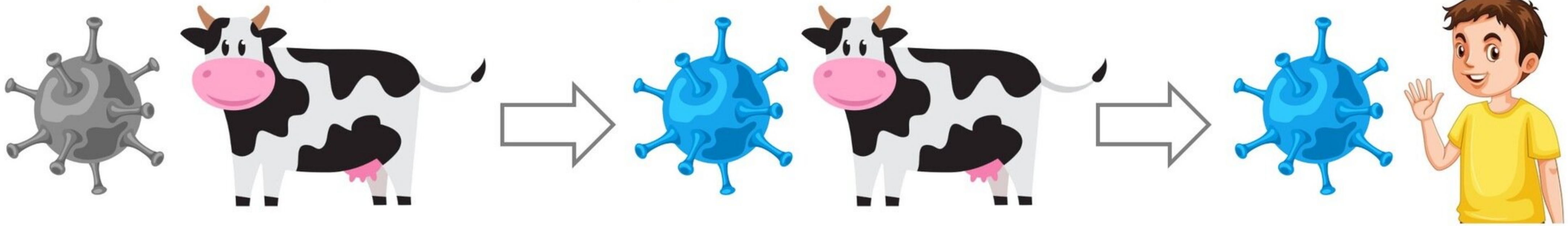
- Saturated deep mutational scanning library
- WHO recommended H5 vaccine seed strain
- effects of ~10,600 amino acid mutations in HA
- Suitable for biosafety level 2
- Ability to measure effects of ***all mutations*** to HA in a limited number of experiments

For data, see: https://dms-vep.org/Flu_H5_American-Wigeon_South-Carolina_2021-H5N1_DMS/

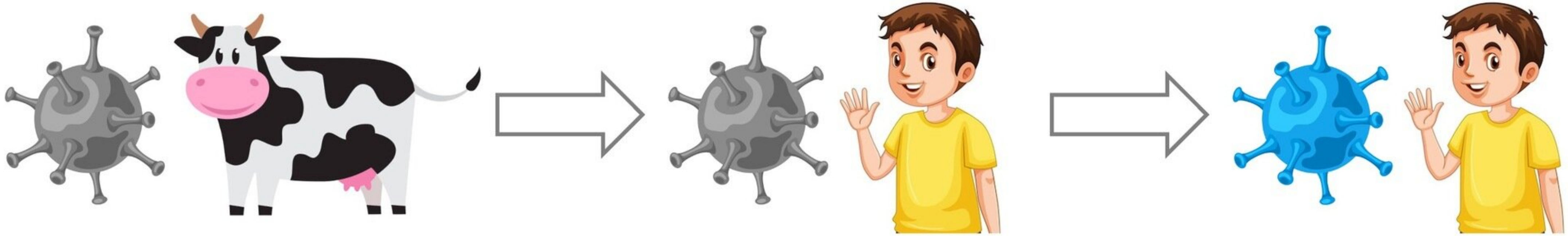
How adaptive mutations might evolve

(These scenarios are **not** mutually exclusive)

Scenario 1: **adaptive mutation(s)** evolve in cattle and then transmit to humans



Scenario 2: **adaptive mutation(s)** evolve in humans after sporadic infection

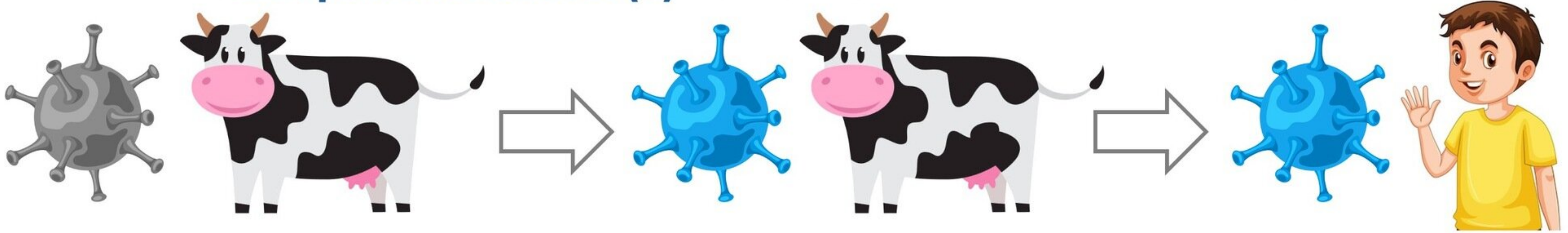


Scenario 3: reassortment with seasonal human strain, although this would still require **adaptive mutation(s)** in HA (it is presumed a pandemic requires the new HA)

How adaptive mutations might evolve

Cattle mammary gland may not select for viruses that use human receptors, which could reduce chance of scenario 1 (Carrasco, 2024)

Scenario 1: **adaptive mutation(s)** evolve in cattle and then transmit to humans



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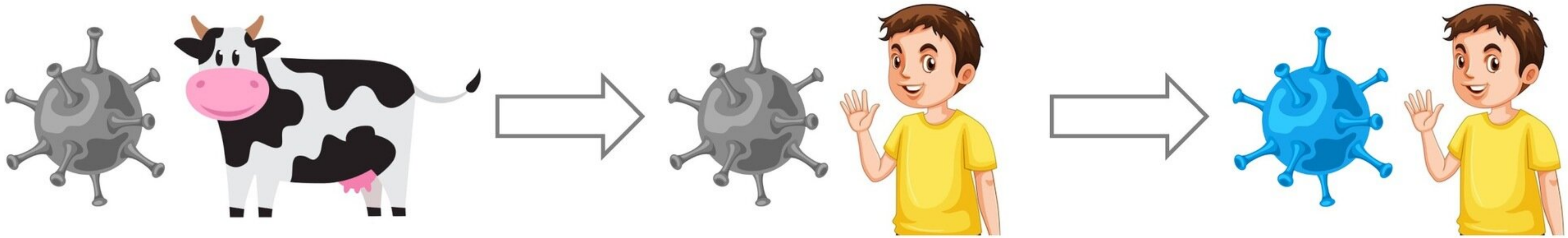


Scenario 3: reassortment with seasonal human strain, although this would still require **adaptive mutation(s)** in HA (it is presumed a pandemic requires the new HA)

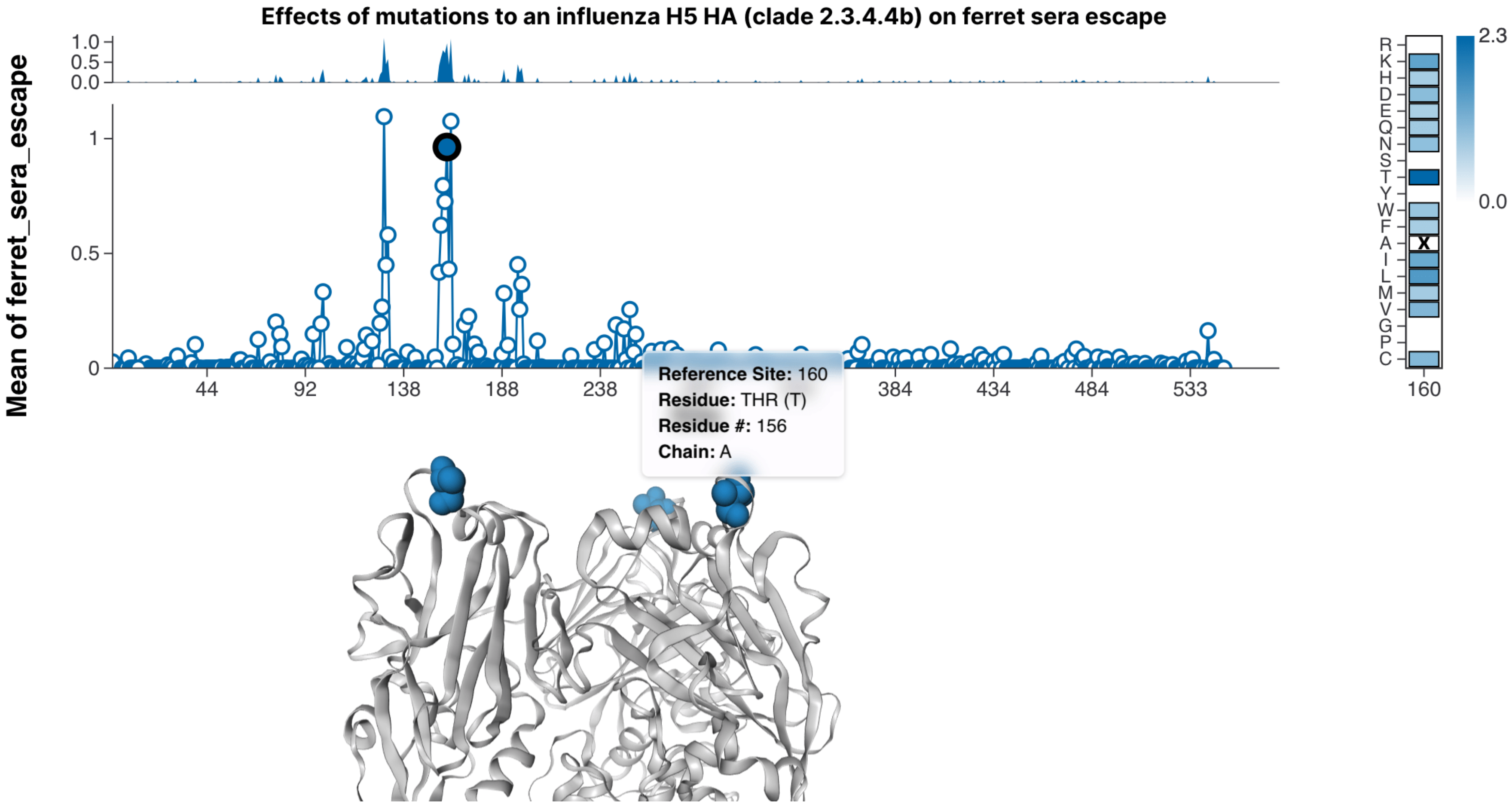
How can science help us be prepared **if** there is a pandemic?

If there is a pandemic, vaccines will be most important countermeasure

One way to ensure vaccines are available quickly is to prepare **candidate vaccine viruses**, or even stockpile vaccines.

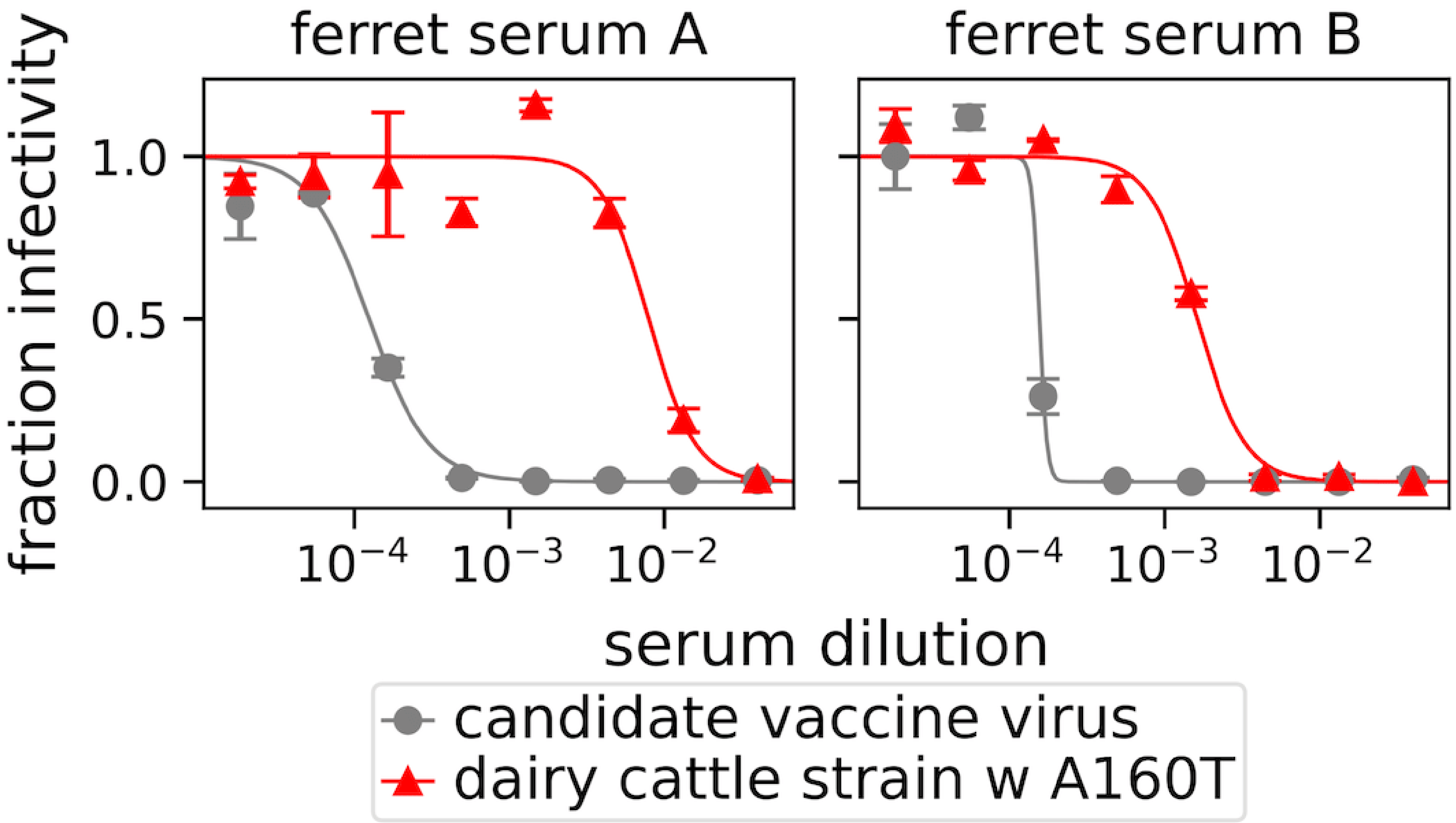
But as influenza HA acquires mutations, these candidate vaccines can become poorly matched to the actual viruses of concern.

Example: A160T mutation escapes antibodies from current candidate vaccine



Deep mutational scanning data from Dadonaite et al (2024)

A160T reduces neutralization by ferret sera, and is in a recent human case from Missouri



Dadonaite et al (2024)

We need to ensure we can rapidly produce well-matched H5 vaccines

Carefully track antigenic changes in HA and update candidate vaccine viruses accordingly

Develop vaccine platforms that can be rapidly tailored to new strains (Furey et al, 2024)

"It's tough to make predictions, especially about the future."

- Yogi Berra

But scientific research can help:

1. Understand how influenza has evolved in the past
2. Identify and monitor for potential adaptations to humans
3. Ensure well-matched vaccines could be produced quickly

Using genomic data to study H5N1 in US dairy cattle

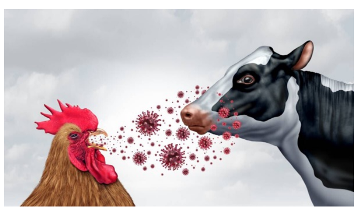
Martha Nelson, Division of Intramural Research, NIH

These views are my own and do not reflect the views of the NIH or US government.

NIH National Library of Medicine
National Center for Biotechnology Information

Genomic data can be used to:

- Track the ongoing evolution of B3.13 as it host-switches between cattle, poultry, humans, and wildlife.



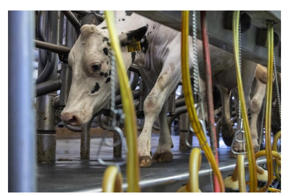
- Evaluate the effectiveness of NPIs in controlling virus transmission from a One Health perspective

H5N1 control in the United States relies on NPIs

Poultry

Dairy Cattle

Testing
Quarantine
Biosecurity
Contact tracing
Mandatory testing prior to interstate movement



Influenza vaccines only used in humans, swine and horses in the US

Research Gap #1: Are interventions in cattle working?

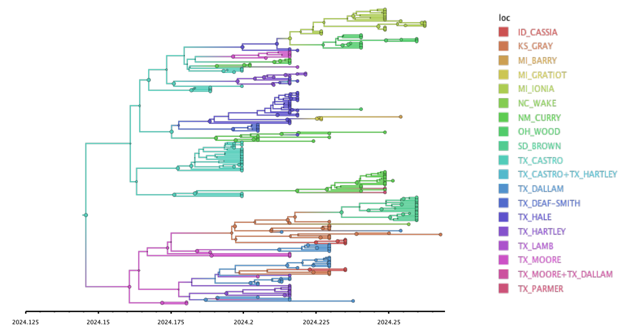
Did the national pre-movement testing requirement (April 2024) reduce H5N1 spread between states?

USDA Orders Testing Of Dairy Cattle Before Interstate Transport

Published: April 24, 2024 - United States Department of Agriculture

WASHINGTON, April 24, 2024 – To further protect the U.S. livestock industry from the threat posed by highly pathogenic H5N1 avian influenza, USDA is sharing a number of actions that we are taking with our federal partners to help us get ahead of this disease and limit its spread.

How does H5N1 spread between counties and states?



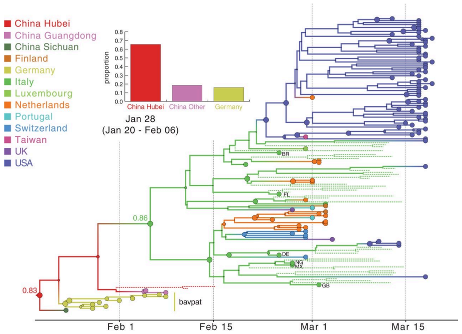
Time-scaled phylodynamic trees quantify the rate of H5N1 dispersal between locations over time using genomic data

Data needs: case counts, genomes sequences, spatial-temporal metadata

Phylodynamic analyses of SARS-CoV-2 showed where NPIs worked

Same methods could be applied to study H5N1

Wuhan, China, 2020



Worobey et al., 2020 Science

NPIs (intensive contact tracing + testing) successfully controlled Europe's 1st outbreak in Munich, Germany

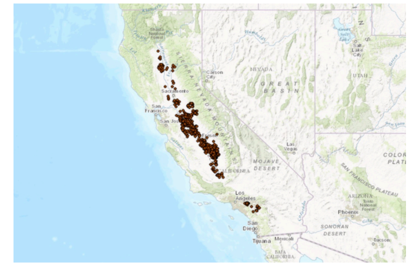
H5N1, 2024

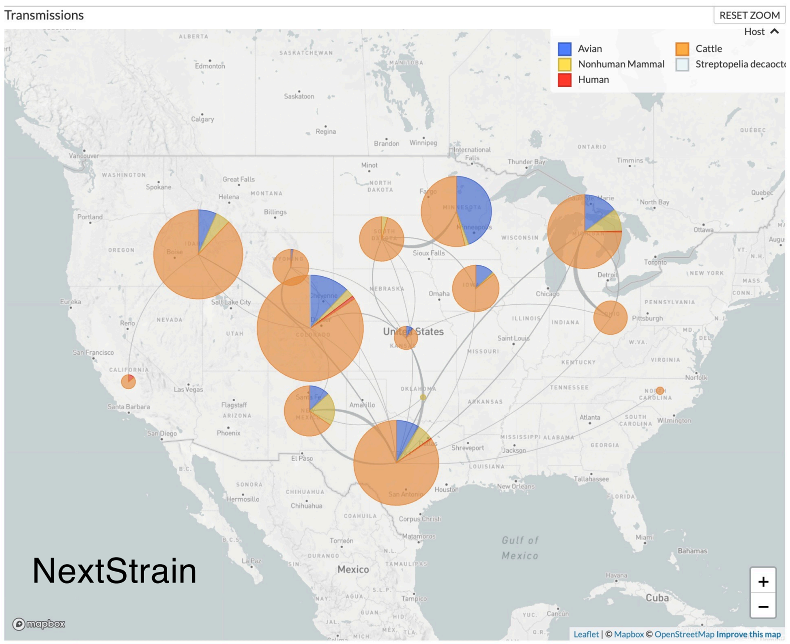
How well are different control strategies working to contain H5N1 in cattle?

Research Gap #2: How did B3.13 reach California?

How did other states get infected?

Is the high death rate in California caused by virus evolution?





<u>How does B3.13 persist in US dairy cattle?</u>

Is B3.13 maintained in the US by a metapopulation model?

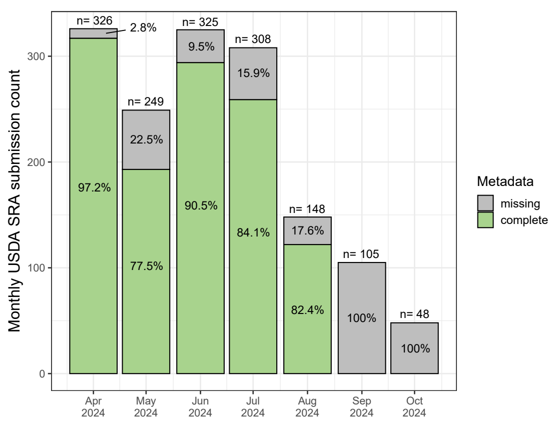
Or is there a permanent main source population that repeatedly seeds new outbreaks in other locations?

Data needs: real-time genomic surveillance with spatial-temporal metadata

The Missing Data Problem

Number of H5N1 genome sequences from cattle in California: 0

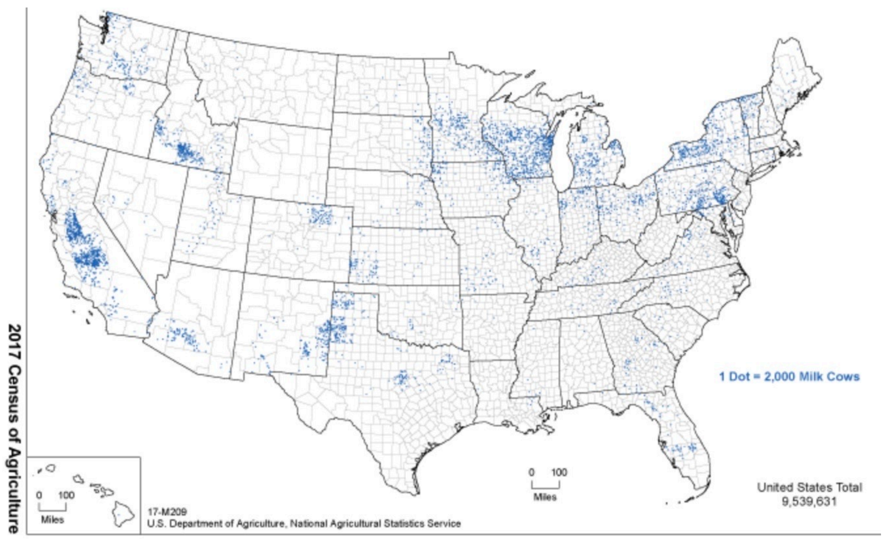
Since late August, over 100 herds in CA have been infected.



Critical sample data (date of collection, US state) is missing for many genomes, including all from September and October

Research Gap #3:
Which US states are at risk for future B3.13 outbreaks?

Need a national database for livestock movements



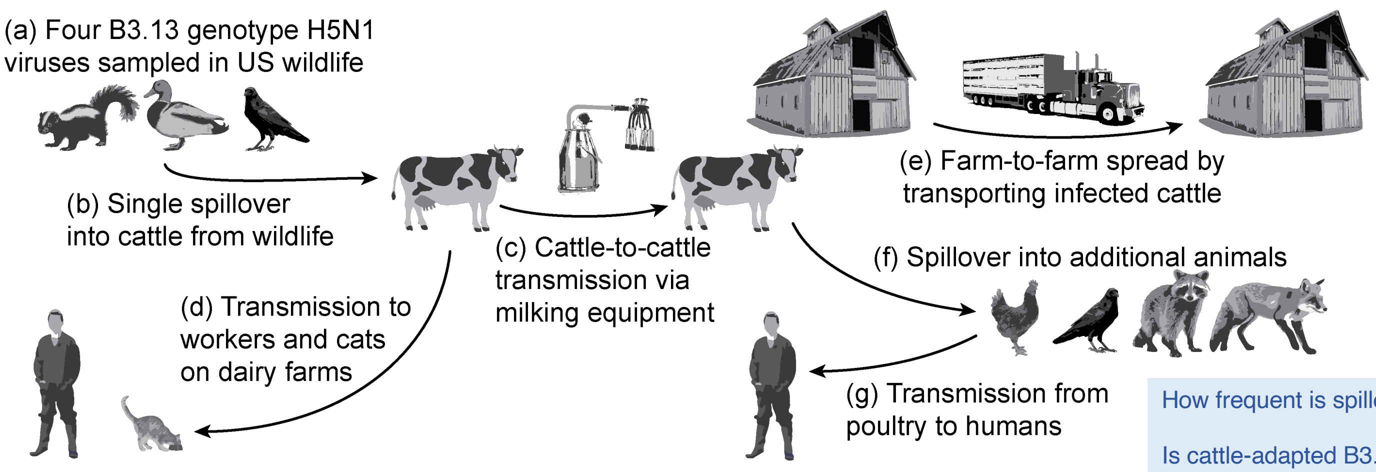
Does the direction of cattle movements predict the direction of virus movements?

Based on cattle movement networks, which states are at highest risk for future outbreaks?

Data needs: genomes, metadata, data on live animal movements

Research Gap #4: How is the ecology of B3.13 changing?

Is the virus spreading in wildlife? Or poultry?



How frequent is spillover?

Is cattle-adapted B3.13 cryptically transmitting in wild birds?

Is B3.13 evolving into a "generalist" virus?

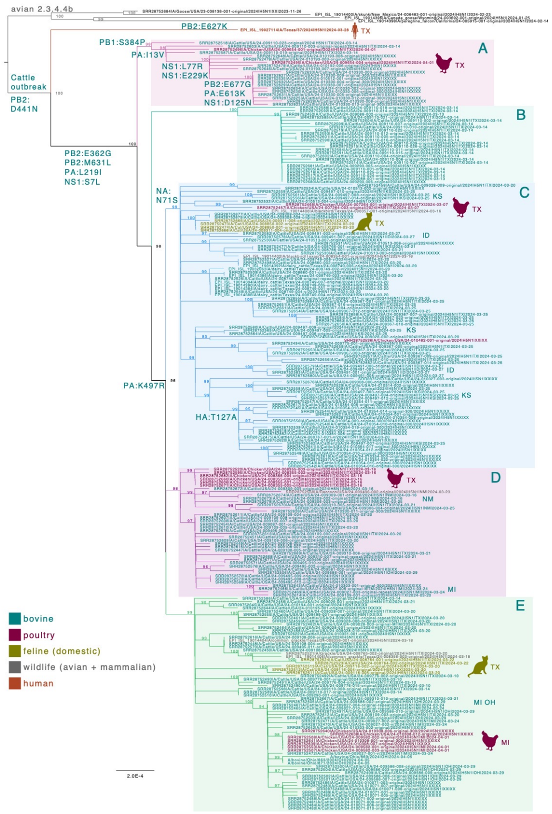
Peacock et al., 2024 Nature

Data needs: testing (PCR, serology) and genome sequencing using a One Health approach

Research Gap #5:
How is H5N1 evolving as it transmits between cattle, poultry, and humans?

Are adaptive mutations being selected for?

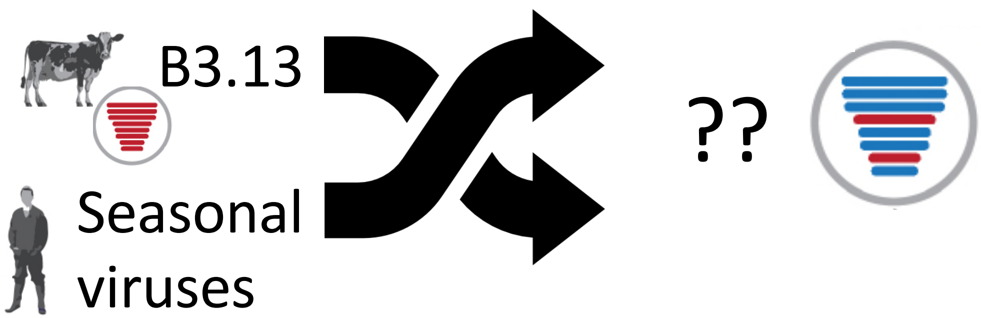
Is the California strain more pathogenic?



Data needs: real-time genomic surveillance with spatial-temporal metadata

Research Gap #6:

What is the reassortment potential of B3.13?



Data needs: increased surveillance in swine and humans

Many diverse lineages circulating in swine for B3.13 to (theoretically) reassort with