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Molecular Epidemiology of H5 clade 2.3.4.4 in the US

US Department of Agriculture, APHIS VS STAS
National Veterinary Services Laboratories, Ames IA USA

AAVLD OCT 2015

Mia Torchetti presenting work on behalf of many... Mary Lea Killian, Kerrie Franzen, DongHun Lee, David Swayne, Hon Ip, Tom Deliberto, Andrew Fox, Phillip Riggs, Sherri Wainwright, Brian McCluskey, the amazing MN academics and industry veterinarians, to name just a few!

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Questions to ponder

- *How is this virus different than other viruses in North America?*
- *Where did it come from? Is it related to H5N1?*
- *What can the virus tell us?*
- *Do our assays work and what is new?*

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H5 in US prior to late 2014

- **North American lineage influenza A**
 - H5N2 LPAI historically one of the most common subtypes
 - LPAI to HPAI mutation in poultry
 - 1982-83 H5N2 PA
 - 2004 H5N2 TX – LBM; disease not severe, cleavage site consistent w/HPAI
 - North American lineage H5N8 LPAI – different virus from EA H5N8
- **Wild bird surveillance for Eurasian H5 2006-2011**
 - No Eurasian H5 detected in nearly 500,000 samples

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
Early detection in the US thanks to Wild Bird surveillance

Wild bird mortality event

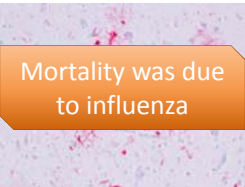


NOPI with

Mortality not due to influenza



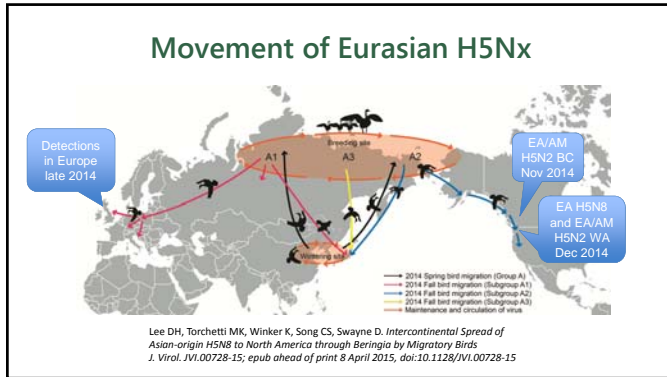
Captive gyrfalcon mortality



Mortality was due to influenza



fluA IHC of gyrfalcon A3465 brain
Courtesy of T. Baszler, WADDL

Ip HS, Torchetti MK, Crespo R, Kohrs P, DeBruyn P, Mansfield KG, et al. Novel Eurasian highly pathogenic influenza A H5 viruses in wild birds, Washington, USA, 2014. Emerg Infect Dis. 2015 May [date cited]. <http://dx.doi.org/10.3201/eid2105.142020>



Study and field observations

- Both viruses transmitted relatively poorly
- Transmission only occurred with H5N2 group at highest dose in turkey and quail
- Neurologic signs noted in clinical trials and in the field

EA/AM H5N2 pheasant
Okanogan Co., WA
Courtesy of L. Badcoe, WSDA

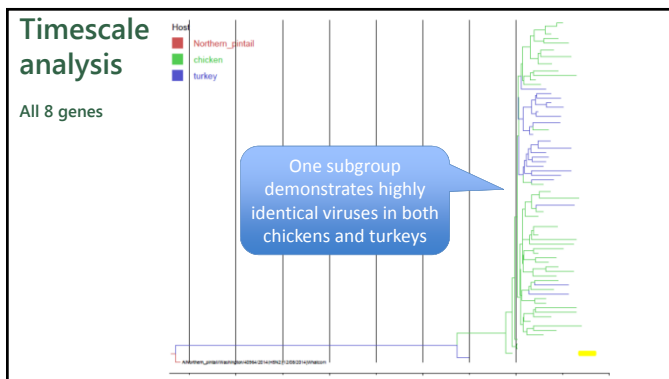
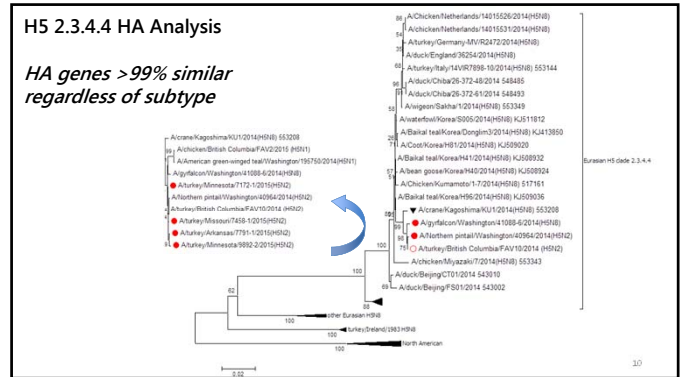
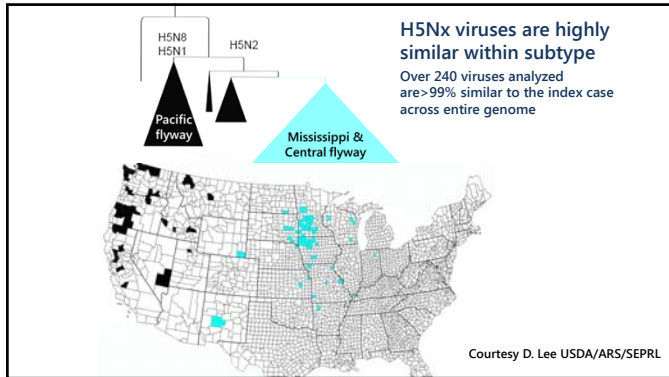
Courtesy of USDA/ARS/Southeast Poultry Research Laboratory

Eurasian H5Nx is different...

- First avian Eurasian H5 virus detected in North America
- Circulates as a highly pathogenic virus for poultry (no mutation needed)
 - Possibility of waterfowl-adapted virus to return assuming eradication of 2015 poultry viruses
- Index viruses better adapted to ducks than to gallinaceous poultry (turkey > chicken)
 - Longer mean death times, poor transmissibility
 - High infectious dose based upon laboratory studies
- Related to Eurasian H5N1 but the North American viruses currently represent low risk to human health

What can the virus tell us?

- Viruses sequenced by next generation methods
 - Consensus sequence data used to generate 3-gene and 8-gene (full genome) analyses
- Outcome of analysis may help inform epidemiologic investigations
 - Aids in detection of potential links

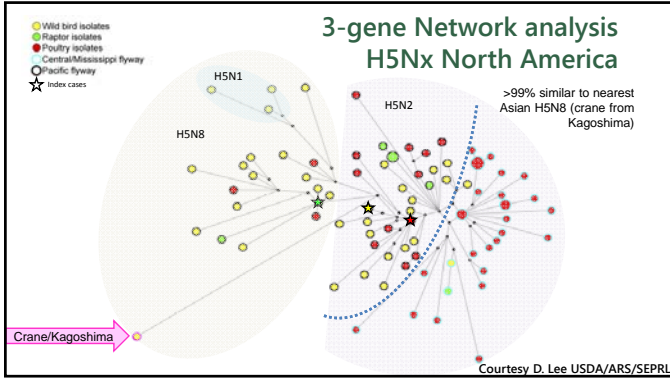


Network for dummies

- The oldest viruses are located at the "spine" ★
- Orientation of branch for visual only
- Length of branch is meaningful
 - Longer branches originating from the spine suggestive of point source or independent introduction
 - Shorter branches radiating from cluster suggestive of secondary or lateral spread

Recall:

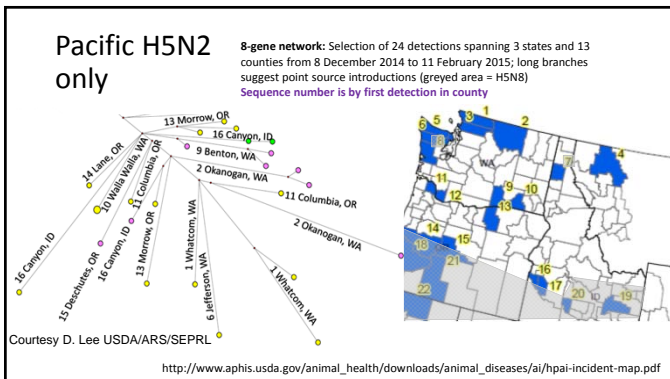
- All viruses are highly similar and all >99% identical to index (oldest) detections
- More wild bird viruses are available from the Pacific Flyway as compared to Midwest available for analysis



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Pacific Flyway Findings

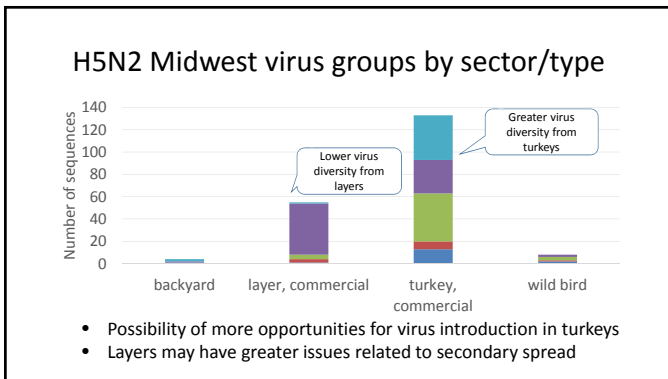
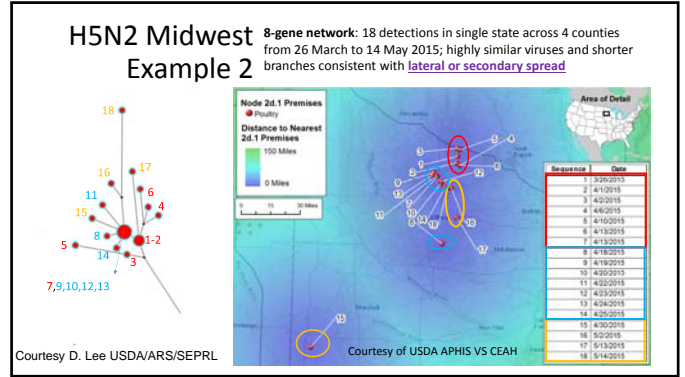
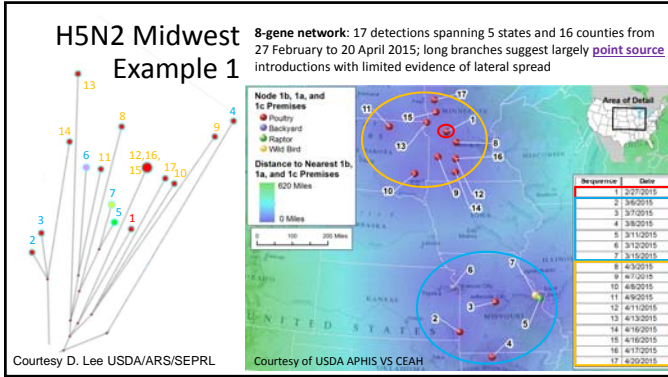
- 3 different subtypes detected; the H5N2 viruses predominated in wild birds
 - There were no H5N2 detections in commercial poultry
- H5N8 was detected in both poultry and wild bird populations in the Pacific flyway
 - The H5N8 viruses have wholly Eurasian gene constellations except two from OR (Jan2015) with two North American internal genes (PB1,PA)
- Long branches observed by network analysis for all H5Nx viruses in the Pacific flyway are suggestive of point source introductions
 - Findings are consistent with both the movement of the virus in wild bird flyways and the low infectivity in gallinaceous poultry



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Midwest EA/AM H5N2 Findings

- The Midwest viruses cluster into two major groups; wild bird-type viruses present in each
- Some subgroups span several states and counties and contain long branches suggestive of point source introductions with limited evidence of lateral spread
- Data for other subgroups contain evidence for point source introductions and lateral spread concurrently
 - MN viruses from turkeys – more subgroups = higher virus diversity
 - IA viruses predominantly layers – 85% in single subgroup



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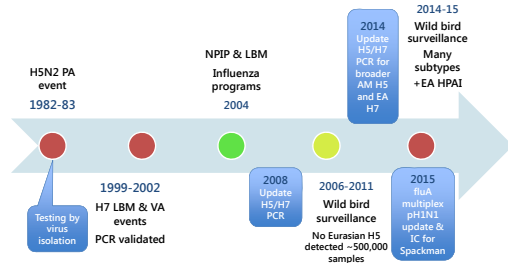
What does the virus tell us?

- Viruses are >99% identical across entire genome (by subtype; >240 viruses)
- Pacific data suggests largely point source introductions
- With one exception (Feb2015) the initial March 2015 Midwest events were detected from south to north
- Midwest H5N2 viruses from turkeys were more genetically diverse than those from layers
- Early Midwest data suggested point source followed by concurrent point source and lateral or secondary spread
- Late Midwest data indicates largely lateral or secondary spread

Considerations moving forward

- Current surveillance approach may not work equally well for all production types
 - May be related to poor transmissibility of this virus
 - May lead to misconception that the virus suddenly appears and rapidly kills when introduction was likely many days before
- More samples improve early detection
- **NEED to move to early indicators [environmental] that are *not* dependent on disease detection in a single bird**

fluA Assay updates



fluA Real-time PCR Assays

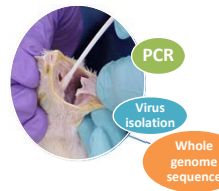
- Influenza A (IAV)
 - Targets all subtypes
 - Addition of pH1N1 primer (Spackman 2009)
 - Life Tech IAV kit licensed
- H5 and H7
 - Subtype specific, does not differentiate HP from LP
 - H5 EA/AM 2014
 - H7 EA/AM 2014
 - PT for 2008 assays accepted
- ❖ Centrifugation step no longer required for PCR
- Any fluA detection on poultry samples should be forwarded to NVSL
- Wild bird samples from which H5/H7 RNA is detected should be forwarded to NVSL

Interpretation of Results

- PCR detects viral RNA from live and inactivated virus
- Ct values of 35 and higher are considered suspect for Poultry (OIE definition)
- Likelihood of isolation of virus decreases with increasing Ct value

Reporting changes

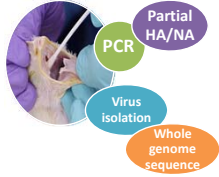
NVSL moving to DETECTED/NOT DETECTED + Ct value



- Partial reports are generated as soon as PCR results are ready – additional partial reports may be generated for reporting of partial HA/NA sequence findings when needed
- Final report contains outcome of VI and whole genome analysis

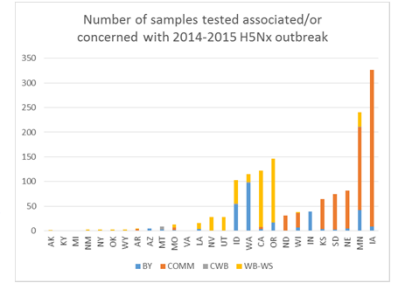
Fastest route for confirmation

Submit duplicate samples to NAHLN and NVSL in parallel

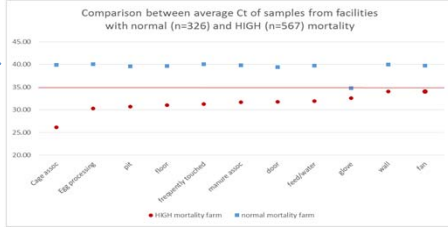
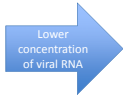


- Ct from NAHLN Lab PCR allows NVSL to target samples for direct partial HA/NA sequence for rapid subtype/pathotype *where sufficient RNA is present*

- **Timely and accurate information is crucial**
 - Animal location
 - Consider barcoding
 - Send 10-4 and tracking info ahead of samples
- nvsl.dvl.avian@aphis.usda.gov



Environmental sample testing of HPAI affected layer facilities (n=934) Sampling locations graphed by average Ct using influenza A PCR



Data courtesy of Drs. Jill Nezworski, Jeff Erickson, Julie Kelly, and Carol Cardona of the Mid-Central Research and Outreach laboratory, UMN, Willmar MN.

Updates to Environmental Sample Testing

- Sample collection guidance updated with feedback from UMN and poultry veterinarians
 - Facilities vary widely within and across production sectors: it important for those collecting samples to determine sampling appropriate to the facility
- Testing guidance included use of PCR with an internal control as an adjunct to virus isolation for post-cleaning environmental testing
 - Challenges with reagent supply – alternatives required
 - Work to include internal positive control in Spackman 2009 fluA assay is underway

Media updates

- BHI – new plastic tube for blue cap 3ml with no abx for up to 5 OP/CL swabs from any domestic species
- BHI – black cap glass tube 5.5 ml WITH abx for up to 11 OP swabs from gallinaceous poultry or environmental samples
 - Antibiotic containing inoculum was provided as a stop gap measure during the outbreak



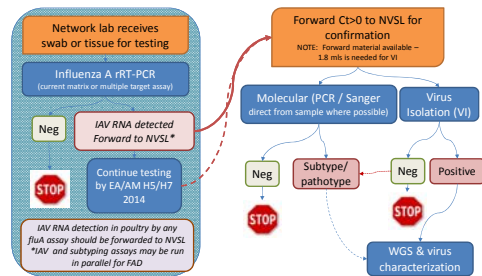
5.5 ml BHI w/ abx

Please use one 10-4 form per state

Form 4 for Select Agent identifications are tied to NVSL accession number

- Multiple preliminary results and final results may be generated from a single accession – one state per 10-4 ensures delivery to appropriate officials as each AD/state vet will only get the results corresponding to their state and aids timely response
- Separate samples from different states and label bags to correspond to each 10-4 (marked the bags and 10-4s both "A", "B"...)

Network Algorithm for Influenza A Molecular Workflow **UPDATED**



* Only NVSL can confirm the presence of a suspected Foreign Animal Disease in the US
As of Sept-15

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Special thanks to ARS-SEPRL, USDA APHIS CEAH, APHIS-WS, USGS-DOI, CDC, the ICG and field staff, the Avian Team, Poultry industry veterinarians, and many, many more...











