



EIC Avian Influenza Research Update

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EIC-Funded HPAI Research Identification & Selection Process

- ❑ Identification of research areas/needs
 - Input from academic experts
 - Consultation with EIC advisory board consisting of industry, government, and academia reps
- ❑ Distribution of RFP
 - Issued May 11, 2015 with a June 12 pre-proposal deadline (20 preproposals received, \$573,987)
 - 8 full proposals invited for peer review
 - 6 selected for funding (\$364,331)
 - ✓ ISU, UC-Davis, Auburn University, Hy-Line International



EIC-Funded HPAI Research Update: I

EIC 2016 Forum

Role of terrestrial wild birds, rodents and insects in spreading AIV to commercial layer operations

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Objective

- To determine whether small terrestrial wild birds (e.g., sparrows, starlings, finches), rodents, and/or insects can transmit AIV, including H5N2 HPAIV, between traditional wildlife reservoirs (i.e., waterfowl/shorebirds) and commercial layer operations, or among such farms.



(source: USDA)



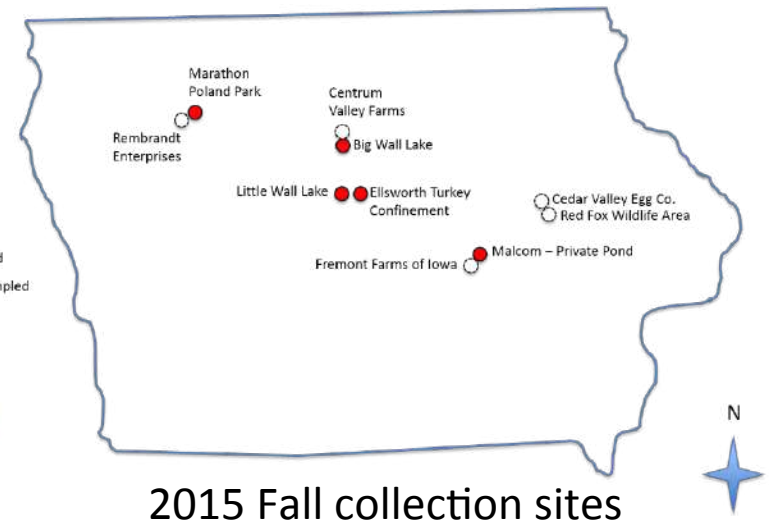
Study Plan



- Targeted surveillance for AIV infection in and on potential vectors (insects, rodents, and terrestrial birds) at locations likely to contribute to links between waterfowl and commercial layer operations within a proximity to wetlands



● Sampled
○ Not Sampled



Animal Catch & Sampling



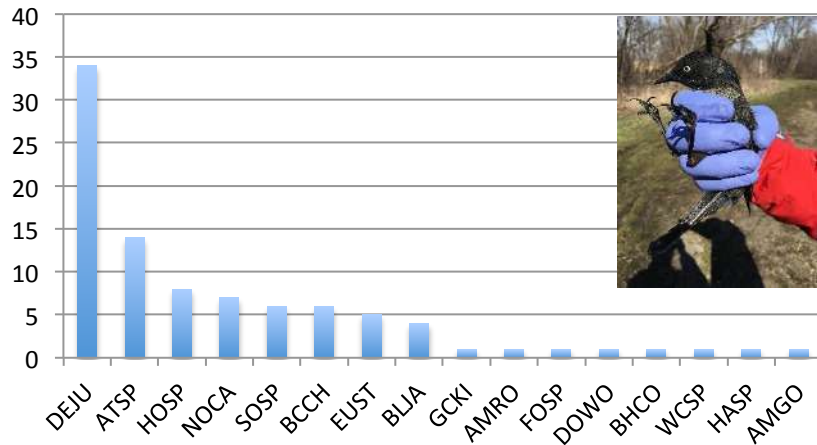
- Oropharyngeal swabs
- Cloacal or rectal swabs
- Feather/fur swab
- Blood



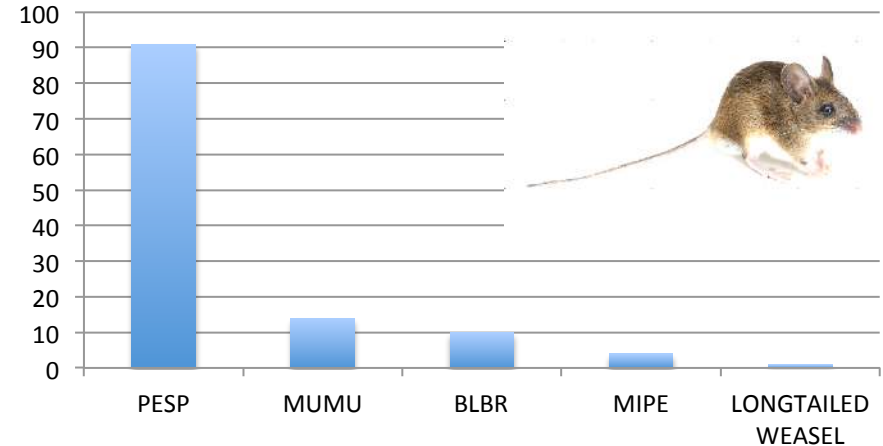
Catchment

- Totals = 101 birds and 120 mammals

Birds



Mammals



NOTE: Bird species captured varied depending on timing.

Name Codes

Birds

- DEJU = Dark-eyed junco
- ATSP = American tree sparrow
- HOSP = House sparrow
- NOCA = Northern cardinal
- SOSP = Song sparrow
- BCCH = Black-capped chickadee
- EUST = European starling
- BLJA = Blue jay

Mammals

- PESP = Deer mouse
- MUMU = House mouse
- BLBR = Short-tailed shrew
- MIPE = Meadow vole

Testing Summary of Fall Migration Samples

- None of the samples (N=442) collected in fall 2015 were positive for influenza A virus by RT-qPCR.
 - OP/cloacal/rectal swabs → Inner swab/animal
 - Feather/Fur swab → outer swab /animal
- Serology testing is being done to look for evidence of previous exposure (i.e., antibodies).

Work in progress

- Spring migration sampling started in last week of March and will continue into May-June.





EIC-Funded HPAI Research Update: II

EIC 2016 Forum

Evaluation of alternative environmental sampling matrices for AI surveillance and stability in commercial poultry facilities



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Sample Matrices

Sample Types Used for Collection of Environmental Specimens

Cloth Booties

Feed

Swiffer Pads

Feathers

Water

Swabs in BHI

Environmental Sample Locations: Post-heat treatment (restock)

Environmental Sample Locations Collected from Poultry Houses during Restocking

Sample location	Number of Samples
Manure Pit	16
Egg Belt	42
Manure Belt	38
Water Drip Tray	16
Cage (bottom/top)	28
Feed Trough	32
House Floor (upstairs/downstairs)	39
Ventilation Fans	3
Corn/Feed	2

Results: Poultry House 1

Environmental Sample PCR and Virus Isolation Results from Poultry House 1					
Sample Type	No. of Samples	Sample Type	No. PCR Positive	PCR Ct Range	Virus Isolation
House Floor (Upstairs)	12	Cloth booties	7/12	29.2 - 36.8	Negative
House Floor (Downstairs)	11	Cloth booties	8/11	31.8 - 35.0	Negative
Egg Belt 1	10	Swiffer pad	9/10	30.3 - 36.4	Negative
Manure Belt 1	6	Swiffer pad	6/6	27.7 - 37.3	Negative
Cage	10	Swiffer pad	9/10	30.2 - 36.3	Negative
Cage (Top)	1	Swiffer pad	0/1	Negative	N/A
Cage (Bottom)	1	Swiffer pad	0/1	Negative	N/A
Ventilation Fans	3	Swiffer pad	1/3	36.7	Negative
Feed	2	Swiffer pad	0/2	Negative	N/A

Results: Poultry House 2

Environmental Sample PCR and Virus Isolation Results from Poultry House 2					
Sample Type	No. of Samples	Sample Type	No. PCR Positive	PCR Ct Range	Virus Isolation
House Floor	16	Synthetic swabs	9/16	32.5 - 39.2	Negative
Manure Pit	16	Synthetic swabs	7/16	31.6 - 36.4	Negative
Egg Belt 1	16	Synthetic swabs	16/32	33.0 - 37.1	Negative
Egg Belt 2	16	Synthetic swabs	9/16	32.5 - 38.9	Negative
Manure Belt 1	16	Synthetic swabs	10/16	35.2 - 37.9	Negative
Manure Belt 2	16	Synthetic swabs	10/16	32.3 - 39.0	Negative
Water Drip Tray	16	Synthetic swabs	6/16	34.2 - 38.2	Negative
Cage	16	Synthetic swabs	6/16	34.6 - 37.9	Negative
Feed Trough 1	16	Synthetic swabs	8/16	32.6 - 39.3	Negative
Feed Trough 2	16	Synthetic swabs	5/16	34.2 - 37.5	Negative

Summary of Environ. Sampling

- Sample types
 - Multiple sample types are applicable for environmental monitoring
 - Cloth booties, Swiffer™, Synthetic swabs
 - Comparable PCR results
- Sample locations
 - Alternative sample locations are comparable
 - Manure belt, cages, feed troughs, egg belts, etc.
 - House floors and manure belts demonstrated lower Ct' s in this sampling
 - PCR results are comparable across sample types
 - Overall average Ct values 32.5 – 36.7 across all sample locations
- Virus Isolation
 - PCR positive environmental samples are negative for live virus
- Additional work is in progress



EIC-Funded HPAI Research Update: III

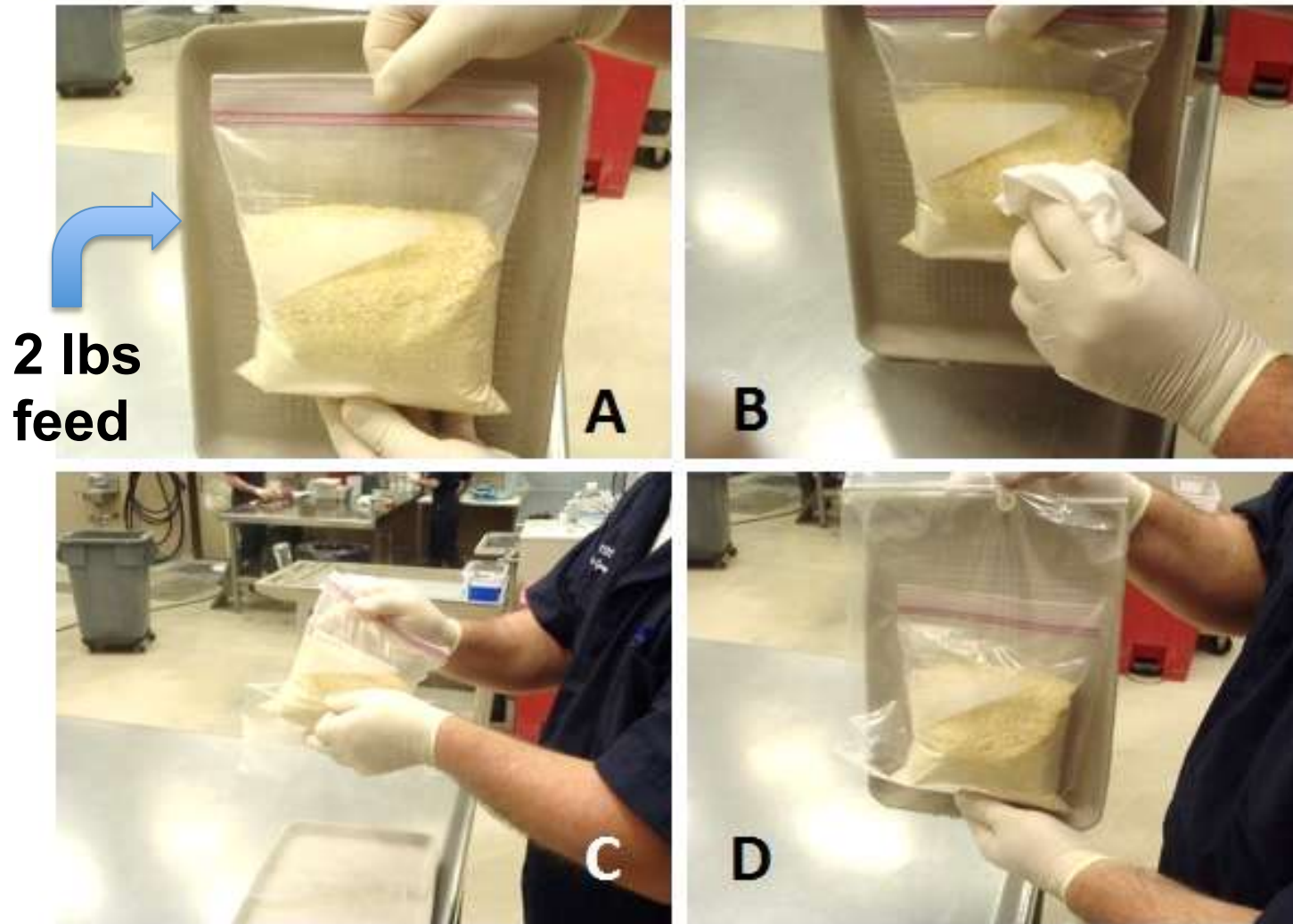
EIC 2016 Forum

Evaluation of feedstuffs for the presence of AIV collected from feed mills and poultry farms and their role in spreading AIV

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Feed Sampling



Feed Study – during outbreak

Type of Sample Collected	Numbers of Samples
Shell corn	4
Ground corn post-roller mill	2
Ground corn post-hammer mill	1
Formaldehyde-treated mash (mixer)	2
Formaldehyde-treated mash (transport truck)	1
Formaldehyde-treated mash (at auger)	1
DDGS	1
Egg shells	2
Wheat midds	2
Layer mash at feed bins	2
Layer mash at feed trough/carts	7
Feed mill dust (dust collecting system)	1

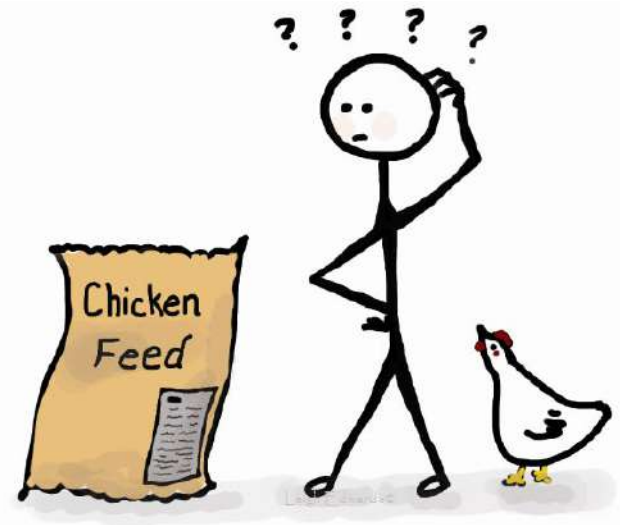
Feed Study (cont'd)

Summary of + PCR and VI results

PCR Positive sample type	Ct value	Virus isolation result
Feed mill, egg shells	39.7	Negative
Egg shells (raw ingredient)	38.1	Negative
Barn – feed trough 1	30.3	Negative
Barn – feed trough 2	31.1	Negative
Barn – feed trough 3	32.2	Negative
Barn – feed trough 4	32.3	Negative
Barn – feed corners	30.5	Negative
Feed mill – meat and bone meal	37.9	Negative

Summary of Feed Study

- All **PCR positive** samples had virus isolation performed and were all negative for AIV.
- Next step, use negative samples and spike feed for temporo-thermal virus survivability study at various temperatures.
- Question to answer:
Could contaminated feed be a potential source for AIV? Bioassay study is being considered.



Acknowledgements

Derek Houston, PhD



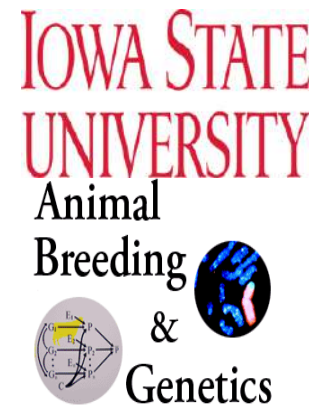
Shahan Azeem, DVM, MS



Coady Lundy



Baoqing Guo, DVM, PhD



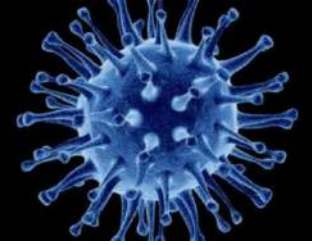
EIC-Funded HPAI Research Update: IV

EIC 2016 Forum

Identifying the Genetic Basis for Resistance to Avian Influenza in Commercial Egg Layer Chickens

Wioleta Drobik, Anna Wolc, Janet Fulton,
Jack Dekkers

Background



- ▶ Highly Pathogenic Avian Influenza (HPAI) has had a devastating impact on the poultry industry
 - ▶ H7N3 outbreak in Mexico in 2012 caused 70-85% mortality
 - ▶ H5N2 outbreak in the US in 2015 caused over 99% mortality
- ▶ **Scientific hypothesis:** Surviving birds contain natural genetic **variation** that make them resistant to HPAI.
- ▶ **Objective:** Identify genomic regions associated with resistance to HPAI



Material

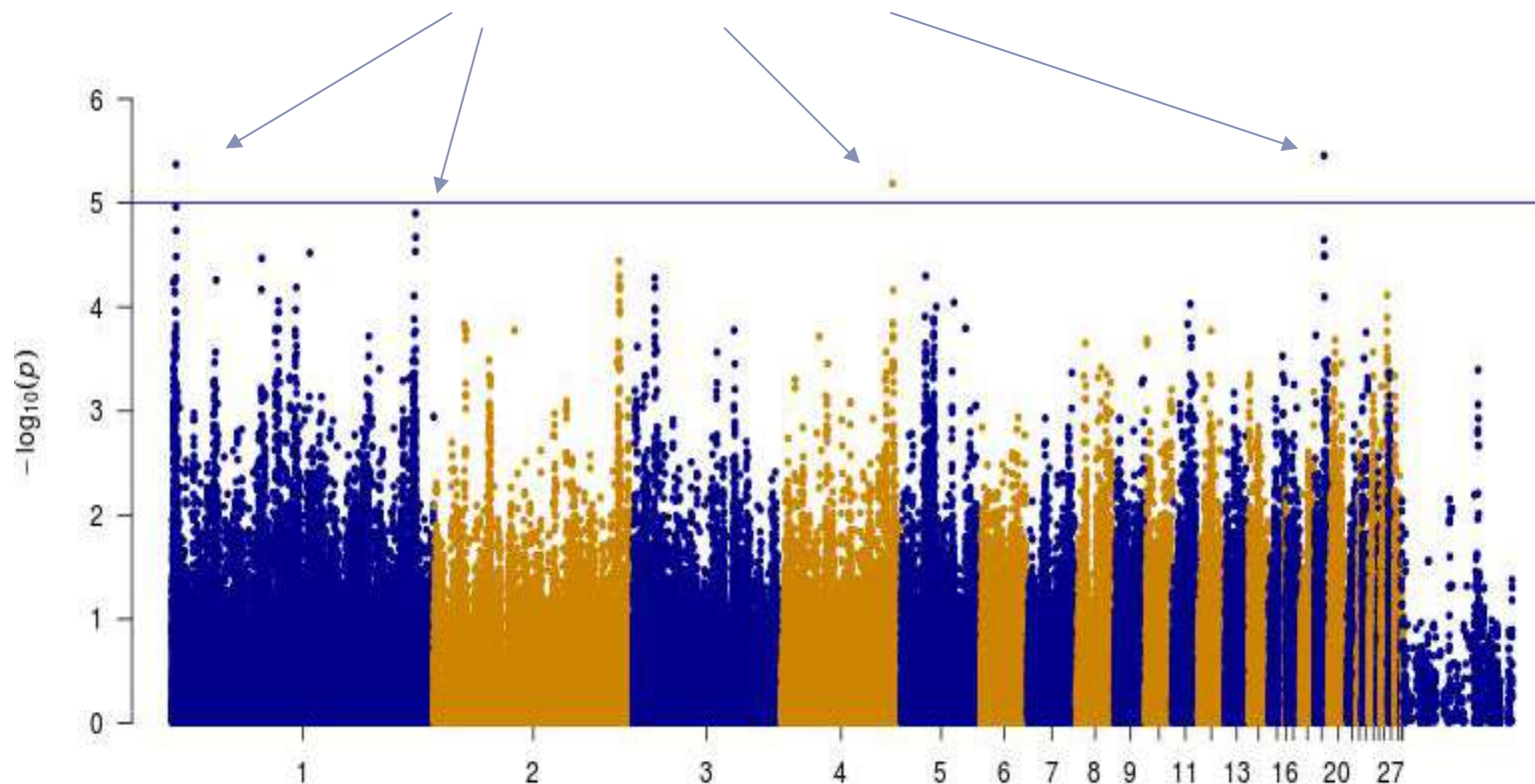


- ▶ DNA was extracted from blood samples of
 - ▶ 460 survivors and 95 controls from Mexico outbreak
 - ▶ 247 survivors and 317 controls from Iowa outbreak representing 4 genetic groups
- ▶ Genotypes at 600,000 locations across the genome were obtained using the Affymetrix SNP chip.
- ▶ Statistical analysis was performed to identify regions across the genome that differ between survivors and controls.



Results

- ▶ Preliminary analyses identified several regions that appeared to be associated with resistance to HPAI
 - ▶ Strongest associations were on chromosomes **1**, **4** and **19**.



Summary (Genetic Resistance Study)



- ▶ Several genomic regions were identified as potentially associated with resistance to Avian Influenza, but none with a very large effect.
- ▶ Different genetic regions were identified to be associated with resistance to H7N3 vs H5N2 virus.
- ▶ Different genetic regions were identified by genetic group.
- ▶ Identification of genomic regions associated with HPAI and further analysis of genes located in these regions can:
 - ▶ provide targets for genetic selection for resistance to HPAI,
 - ▶ identify candidate genes involved in viral resistance mechanisms,
 - ▶ provide targets for vaccine development.





Understanding New HPAI Viruses Affecting the U.S. Poultry Industry and their Persistence

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Huaijun Zhou³, Rodrigo A. Gallardo¹**

¹Poultry Medicine Program, School of Veterinary Medicine

²California Animal Health and Food Safety Laboratory

³College of Agriculture, Department of Animal Sciences

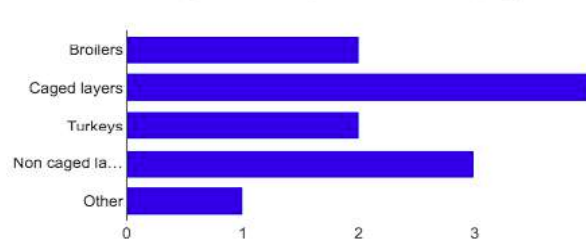
University of California, Davis

Objectives

- a) Assess biosecurity measures to impede introduction of pathogens, such as AI, to poultry premises by a survey re: management of feces, bedding & footbath;
- b) Determine and compare the full genome of HPAI (H5N8) and LPAI (H6N2) before and after mixed with organic materials (feces, bedding material, and footbath contents), looking for mutations in the viral gene suggestive of adaptation to poultry and persistence in the environment;
- c) Assess persistence of the virus (viability) in bedding, feces & footbaths maintained with different procedures.

Poultry Industry Survey

1. What birds do you keep? (Check all that apply)



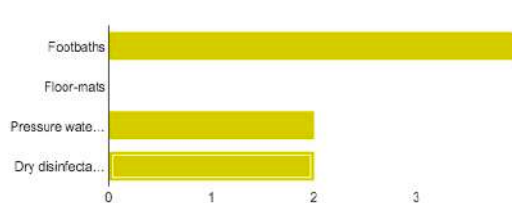
Broilers	2	22.2%
Caged layers	4	44.4%
Turkeys	2	22.2%
Non caged layers	3	33.3%
Other	1	11.1%

2. How many birds do you keep on your ranch?



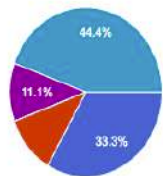
Less than 50,000	1	11.1%
50,000 to 200,000	2	22.2%
200,000 to 500,000	4	44.4%
More than 500,000	2	22.2%
Other	0	0%

8. What do you use for disinfecting boots: (Check all that apply)



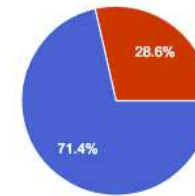
Footbaths	4	44.4%
Floor-mats	0	0%
Pressure water / disinfectant for boots	2	22.2%
Dry disinfectant powder	2	22.2%

15. How often do you prepare new disinfectant?



Daily	3	33.3%
Every 2 days	1	11.1%
Every 3 days	0	0%
Every 4 days	0	0%
At longer intervals	1	11.1%
No regular intervals; when footbath / floormat / powder gets dirty	4	44.4%

29. Do you use any product to treat the litter between flocks?



Yes	5	71.4%
No	2	28.6%

Conclusions

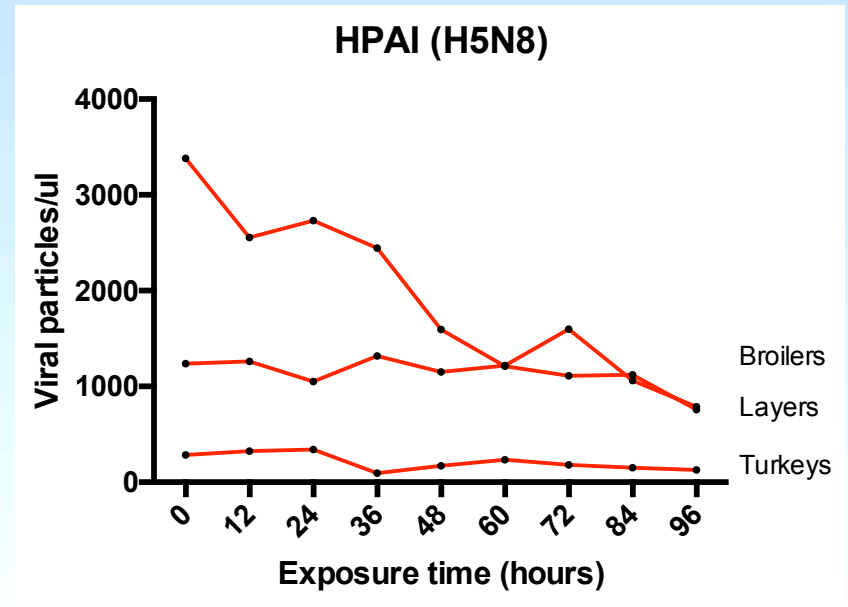
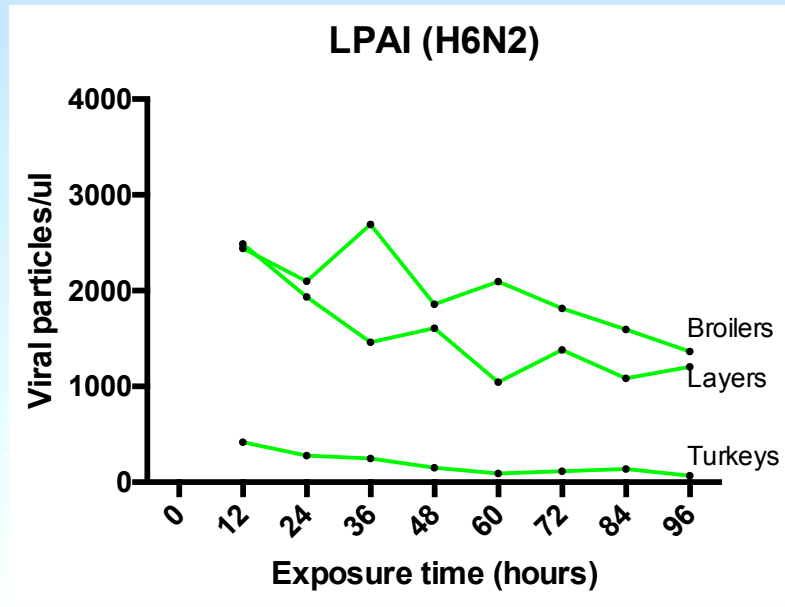
1. Wide representation including broilers, caged layers, turkeys and pastured poultry
2. Biggest players in the State were included
3. Dry disinfectants seem to be popular
4. No fixed protocol for footbaths
5. Litter amendment is used

Experimental Design

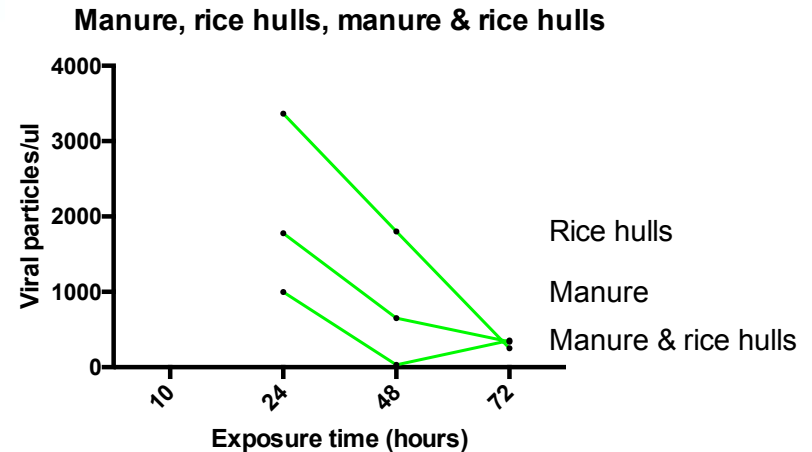
(bedding material and feces)

- Layer feces, broiler and turkey bedding as a substrate
- Spiked with $10^{5.75}$ EID₅₀/ml (HPAI or LPAI)
- Measurement of viral presence (qRT-PCR) and viability (virus isolation in SPF eggs) at 0, 12, 24, 36, 48, 60, 72, 84, and 96 hours post exposure
- Environmental temperature fluctuation daily: 4.9°C (20.4 to 25.3°C)
- Sealed containers used (to reduce humidity effect)

Litter effect on LPAI (H6N2) and HPAI (H5N8)



Effects of manure and rice hulls on LPAI recovery



Experimental Design (dry footbath **pilot experiment**)



Rice hulls were spiked
• $10^{5.75}$ EID₅₀/ml



Rubber boots were impressed in the spiked rice hulls



Boots were treated with the dry footbath

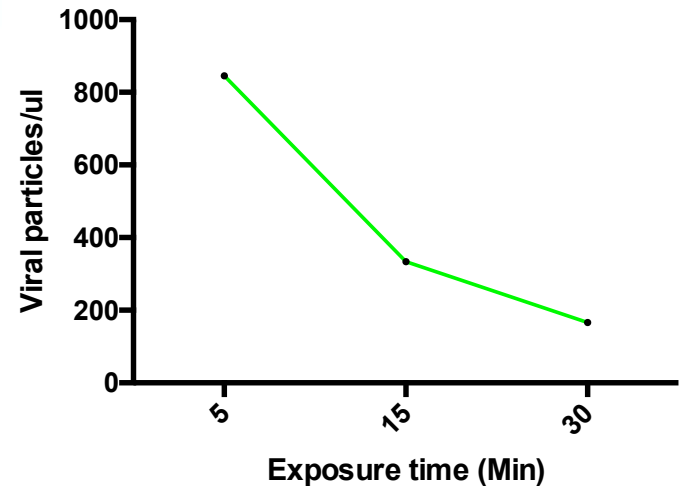


Samples of adhered boot material were collected



Samples were processed for RT-PCR and virus isolation

Dry footbath effect on LPAI (H6N2)



Results and Next Steps

- Based on viral particles detected by RT-PCR,
- ✓ Turkey bedding material seems to be detrimental on HP and LPAI persistence;
- ✓ Rice hulls seem to have a “protective effect” on the spiked AI;
- ✓ Dry footbath reduces but does not eliminate viral particles obtained from boot treated samples.
- Next steps are to determine the viability of the RT-PCR detected viral particles and run the final foot bath trials.

EIC-Funded HPAI Research Update: VI

EIC 2016 Forum

The role of litter beetles, water, and rodents in avian influenza virus transmission

Joseph J. Giambrone and Kenneth S. Macklin
Auburn University

Objectives

- 1) Determine if darkling beetles can transmit AIV
- 2) Determine if AIV can become incorporated into biofilm in poultry house's waterlines
- 3) Determine if rodents can be infected and transmit AIV

Stability of AIV in Feed

AIV, isolated from hunter-killed ducks in Florida, was grown in chicken embryos.

AIV was inoculated unto poultry feed and incubated at room temperature and examined for an AIV gene at 1-5 day intervals using a standard molecular test.

All inoculated feed samples were positive for the AIV gene at all intervals.

Implication: The AIV gene was stable and remained detectable in the feed for up to 5 days.

Feed samples containing the AIV gene will be tested to determine if they contain live virus.

Beetle Study

Darkling beetle behavior was examined in Petrie dishes in the lab. After 24 hours they consumed non-contaminated feed and, when satisfied, migrated out of the dishes.

They will be placed in AIV contaminated feed to determine if the insects can harbor live virus.



Beetle Study: Implication

If feed can harbor live AIV, can beetles given AIV contaminated feed become infected and harbor live AIV?

If so, feed additives need to be developed to inactivate the AIV as well as implementation of improved farm insect control measures to prevent chickens from consuming AIV infected beetles.

AEB- and USDA-Funded HPAI Research Update



- ***Air and environmental sampling of infected poultry barns*** (H. Xin, Y. Zhao, K.J. Yoon, B. Guo)



Findings:

- Virus was found in dust of HPAI positive barns during dry cleaning, though *not* viable.
- Some environmental samples were tested positive.



AEB- and USDA-Funded HPAI Research Update



- **Assessment of HPAI virus airborne transmission** (H. Xin, Y. Zhao, G. Takle, D. Schmitt)



Outbreak date	Type	Minnesota (3/4-6/5)							Iowa (4/12-6/15)													
		d-1	d-2	d-3	d-4	d-5	d-6	d-7	d-1	d-2	d-3	d-4	d-5	d-6	d-7	d-1	d-2	d-3	d-4	d-5	d-6	d-7	
4/12/15	Turkey		X	X	X	X																	
4/16/15	Turkey							X															
4/19/15	Layer	X	X				X	X					X										
4/24/15	Layer		X	X		X	X	X						X	X								
4/24/15	Layer		X	X		X	X	X						X	X								
4/25/15	Turkey			X	X	X	X		X	X	X	X	X	X									
4/26/15	Pullets	X		X	X	X	X	X	X	X					X								
4/26/15	Layer	X							X	X	X				X								
4/27/15	Turkey	X	X			X	X	X		X	X	X	X	X		X							
4/28/15	Layer	X	X	X			X	X			X	X	X	X			X						
4/28/15	Turkey	X	X	X			X	X	X	X		X	X	X			X						
4/28/15	Breeder	X	X	X		X	X	X						X		X							
4/29/15	Layer	X	X	X	X			X		X			X	X	X					X			
4/29/15	Turkey	X	X	X	X				X	X	X			X						X			
4/29/15	Layer	X	X	X	X				X	X	X	X	X	X						X			
4/30/15	Turkey	X	X	X	X	X					X		X	X							X		
4/30/15	Layer	X							X	X											X		
4/30/15	Turkey	X	X	X	X				X	X				X							X		
...	...		X	X	X	X	X	X	X	X	X	X	X	X									X

Preliminary Findings:

- There were days when air originating from ‘old’ inflected sites passed through ‘new’ inflected sites (within 7 days prior to confirmed outbreak).

AEB- and USDA-Funded HPAI Research Update



- *Evaluation of hen house air filtration* (Xin & Zhao)



Status: Ongoing, plan to monitor throughout different seasons.



Acknowledgements

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- American Egg Board
- Egg Farmers of Canada
- Egg Industry Center
- United Eggs Allied
- USDA Exploratory Program

Cooperation by

- Egg and turkey producers in Iowa
- State Vet's Office



Other HPAI Research

(Funded by USPEA, per Dr. John Glisson)

- *Eliminating infectious virus with heat* (**Dr. David Swayne**, USDA Southern Poultry Research Lab, Athens, GA)
- *Eradication of AI virus in feed with Anitox Termin-8[®]* (**Dr. Haroldo Toro**, Auburn University)
- *Evaluation of physiological responses of laying hens to euthanasia methods* (**Dr. Ken Anderson**, NC State University)